Abstracts accepted for publication only

Pathogenesis

R2249 Effect of a probiotic dairy product containing *Lactobacillus casei* Shirota on the gut microbiota in irritable bowel syndrome: a randomised, placebo-controlled, double-blind study

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Objectives: It has been hypothesized that disturbances in the intestinal flora could be a factor in the onset and persistence of IBS complaints. The aim of the study was to analyse the effect of *Lactobacillus casei* Shirota (LcS) on the faecal flora of IBS patients.
Methods: In a randomised, placebo controlled, double blind study, IBS patients fulfilling the Rome criteria II were included. Patients took 2 bottles daily for 8 weeks, containing either LcS or placebo. Faecal samples were collected before intervention (week −2/0,S1), at the end of intervention (week 6/8,S2) and during follow-up (week 14/16,S3). Microbial populations (*Bacteroides*, anaerobes, bifidobacteria, coliforms, clostridia, and lactobacilli) were enumerated using quantitative plating. Total bacterial counts were performed using FISH. Bacterial DNA was analysed by quantitative Real-Time PCR for the detection of *Atopobium* spp., *Bacteroides-Prevotella-Porphyromonas* group, *Clostridium coccoides-Eubacterium rectale* group, *Clostridium leptum* subgroup, *Clostridium difficile*, *Clostridium perfringens*, *Desulfovibrio desulfuricans* group, and *Bifidobacterium* spp. Statistical analysis was performed using Mann-Whitney U tests or Paired Wilcoxon tests. $P < 0.05$ was considered statistically significant.
Results: No significant differences in total bacterial counts were seen between the treatment and placebo group. Using quantitative plating, no significant differences were seen for the different bacterial groups between both groups. Significantly lower bacterial counts were seen in both groups for clostridia for S2 compared to S1 and S3. In the placebo group, significantly higher amounts of bifidobacteria and lactobacilli were detected for S2 compared to S1. Using qRT-PCR, significantly lower counts for *D. desulfuricans* group were seen in the placebo group compared to the treatment group. In the treatment group significant differences were seen only for S2 compared to S3, whereas in the placebo group, no significant differences were detected for the different bacterial groups at the different sampling points. Analysis at the IBS subtype level revealed differences in microbiota between the different subtypes.
Conclusions: Although significant changes in the microbiota were seen during intake of LcS in the treatment group, differences in microbial populations between both groups were not significant. Interestingly, specific findings are likely to be associated with a specific IBS subtype rather than with IBS in general.

R2250 H1N1 virus infection: review of chest radiographic findings

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Objective: To asses the chest radiography findings of patients with H1N1 Influenza virus infection.
Material and Methods: Review of radiological findings of 110 patients with confirmed influenza A virus infection admitted to two Hospitals in Cantabria, Spain. The radiological findings were characterized by pattern of opacities, lung distribution and, presence or absence of pleural effusions or hiliar or mediastinal adenopahies.
Results: The initial radiography was abnormal in 39 (34%) patients, but only 28 (25%) were related exclusively to the influenza A virus infection. Characteristic imaging findings in these 28 patients included: parenchymal consolidation 68%, consolidation plus ground glass 25% and ground glass opacities in 7%. Seventeen patients had a diffuse distribution of the opacities, in 61% it was bilateral, and lower and middle zones were those most frequently affected. No pleural effusions or pathological mediastinal lymph nodes were seen in this initial radiography. Patients with abnormal chest X-ray were more frequently men, smokers, had dyspnoea, pleuritic pain and diarrhoea.
Conclusions: Although more of patients with influenza virus A infection showed normal chest X-ray, those with abnormal chest radiographies, characteristically showed areas of consolidation with or without ground glass affectation, bilateral and diffuse distribution, and predilection for lower and middle zones. Pleural effusion or mediastinal adenopahty were not findings seen in the initial radiography.

R2251 Surveillance of drug resistance of *Mycobacterium tuberculosis* patterns in clinical sputum samples

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Introduction: Chest diseases tb reference lab is responsible for first line anti tuberculosis drug testing and so this formed the backbone of the study based on routine drug susceptibility testing. These drugs are widely used in the world and tested for in rounds of proficiency testing among reference laboratories. Drug resistance was probably the result of a selective process by which non susceptible mycobacteria are singled out through the elimination of the susceptible majority. Therefore the need to determine the susceptibility and resistance patterns of mycobacterium tuberculosis in 830 clinical samples sent for culture in the period between 2007 and 2010.
Objective: To determine the drug resistance patterns for mycobacterium tuberculosis in clinical samples.
Methodology: This was a retrospective descriptive study conducted at chest diseases laboratory between 2007 and 2010 and used data from the data base 830 tuberculosis (tb) positive cultures of clinical samples from new and retreatment patients using proportion methods were tested. Data of the drug resistance survey conducted during this period was excluded.
Results: The total number of cultures examined was 830 between 2007 and 2010, showing drug resistance to one or more drugs in mycobacterium tuberculosis strains from newly diagnosed and previously treated patients in a continuing sample surveillance.
Total resistant strains was 209 (25.1%), mono resistance to streptomycin 22 (2.6%), isoniazid 33 (3.9%), rifampicin 27 (3.2%), ethambutol 2 (0.2%).
Resistance to two drugs included rifampicin and isoniazid [multi drug resistance 49 (5.9%)], streptomycin and isoniazid 10 (1.2%), streptomycin and rifampin 25 (3.01%), isoniazid and ethambutol 07 (0.8%), streptomycin and Ethambutol 01 (0.1%), Rifampicin and Ethambutol 01 (0.12%).
Resistance to three drugs included streptomycin + isoniazid + rifampcin 36 (4.3%), isoniazid + rifampicin + ethambutol 0 (0%) streptomycin + isoniazid + ethambutol 07 (0.8%).
Resistance to all four drugs included 0.7 (0.8%).
Conclusion: Resistance to rifampicin and isoniazid (mdr) 5.9%) showed a rising trend and threatening to be a problem. Mono resistance to isoniazid (3.9%) was in rising proportions.
Resistance to ethambutol and rifampicin and isoniazid 0%) marked the least trend.

R2252 Changes in biostructure of the microflora in adolescents with inflammatory bowel disease

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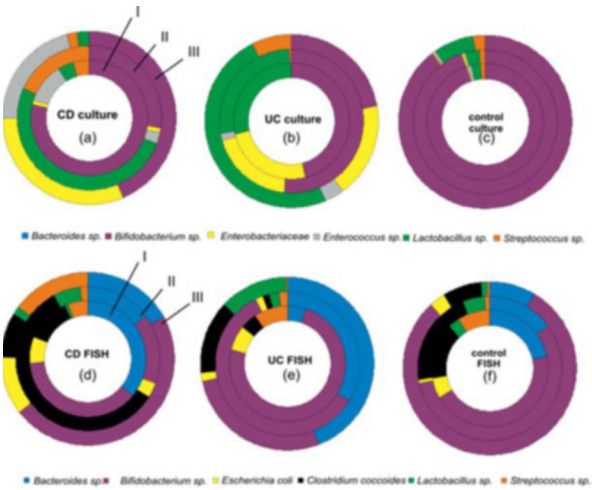
Objectives: The commensal bacterial flora of the gastrointestinal tract plays an important role in pathogenesis of inflammatory bowel disease (IBD). We examined what is structure of bacterial microflora in the colon in adolescents with Crohn's disease (CD), ulcerative colitis (UC) and control group.
Methods: Faecal samples were collected in three fractions from patients with CD (n = 22), UC (n = 12) and control (n = 24) during preparation to colonoscopy which was based on cleaning of the gastrointestinal tract with: first normal saline enema, oral administration of laxative sodium phosphate (0.6–0.8 ml/kg) and repeated normal saline enema until clean, at least three enemas in whole preparation procedure. After each preparatory step the large bowel intestinal contents were collected, resulting in three fractions I, II and III. Samples were examined using a quantitative culture technique and fluorescent in situ hybridization (FISH) method.
Results: Quantitative composition of the bacterial flora was different in the consecutive three faecal fractions of the study groups (Fig. 1a,b,d,e) but in patients from the control group the composition of the bacterial flora in the consecutive fractions was similar (Fig. 1c,f). Statistical analyses showed that the total distribution of the studied bacterial taxons in the contents, in all three faecal fractions in the given disease group, as well as in the control group were characteristic for the studied patient group and statistically significant ($p < 0.0001$). Generally, in the results obtained using the culture methods, the biggest differences were noted for *Bifidobacterium* genus, which numbers decreased in the consecutive, but remained stable and high in the control group. The above differences were also demonstrated using FISH, but using hybridization the percentages of bifidobacteria in the studied groups were not as high (Fig. 1). Moreover, it showed that in the first faecal fraction (transient planktonic flora) there is no statistical significance for any of the analyzed bacterial groups, both using the culture methods or FISH, but significant results were demonstrated in the II and III fractions for specific bacterial groups.
Conclusions: Distribution of the bacterial flora in the colon is layered, what can be named biostructure. It means that one may identify planktonic microflora and the flora which comes in direct contact with the mucus covering the colonic epithelium and exerts action on it.



Comparison of average, percentage bacterial composition in 1 g of fecal matter collected from patients in the CD, UC and control groups. (a-c) culture method; (d-e) FISH; I - first fecal fraction most distal from the colonic mucosa, II - second fraction closer to the colonic mucosa, III - third fraction, in direct contact with the colonic mucosa. Distribution of studied bacterial groups in all variants (a-f) were characteristic and statistically significant ($p<0.0001$, Chi square test).

R2253 SEM observations: pathogenesis of *Candida* infections in a human skin model

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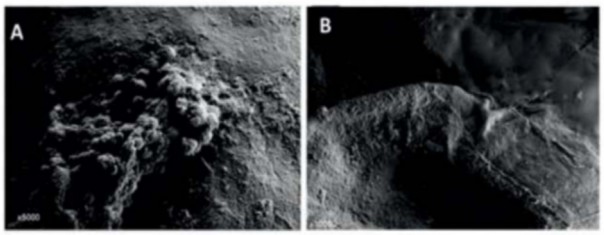
Candida albicans, is regarded as the most common agent in fungal infectios, but other *Candida* species have become a significant cause of infection. Scanning electron microscope (SEM) observations were used to analyse the capability of *C. albicans*, *C. tropicalis*, and *C. parapsilosis* to adhere to our human skin model which mimics the human skin in vivo. The skin sections were inoculated with low (104 ml-1) and high (106 ml-1) concentration of the yeasts for 1 and 5 days. The cells were fixed with glutaraldehyde and post fixed with o5o4, dehydrated in serial alcohol concentrations and viewed by SEM.
All three yeasts tested adhered to the skin but *C. albicans* covered the entire skin surface to a higher extend than *C. tropicalis* and *C. parapsilosis*, at the low concentration as well as the high one. Mucin like material coated the blastocoridia mainly in *C. albicans*. After 5 days of incubation all *Candida* species have shown biofilm formation. These results may give a partial explanation for the predominance in cutaneous pathogenicity of *C. albicans* and explain why almost any *C. albicans* is isolated from the skin.



Scanning electron micrographs (SEM) of *Candida albicans* biofilm (A), normal skin (B).

R2254 Rapid identification of five *Candida* species using Yeast Traffic Light PNA Fish

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Objectives: Candidemia are serious causes of morbidity and mortality, particularly in the immunocompromised hosts. Although *C. albicans* (CA) is still the most prevalent *Candida* spp. (CS) isolated from blood/fluid culture bottles (BFCB) at UCLA, other CS that are less susceptible to fluconazole (FLU) constitutes approximately 40% of all candidemias. IDSA guidelines recommends selection of initial anti-fungal therapy based on species identification, therefore, rapid identification of CS to guide appropriate empiric therapeutics is warranted. This study evaluated the performance of a rapid assay to identify CA, *C. parapsilosis* (CP), *C. tropicalis* (CT), *C. krusei* (CK), and *C. glabrata* (CG) directly from positive BFCB.
Methods: From March to November 2010, the Yeast Traffic Light PNA FISH™ kit (AdvanDx) was performed directly on yeast positive BFCB. Peptide nucleic acid fluorescent in situ hybridization (PNA FISH) was also performed on a number of yeast isolates cultured on Sabouraud agar (SAB). The assay targets the rRNA sequences of the five CS appearing as green, yellow, and red for CA/CP, CT, and CK/CG, respectively. CA/CP and CK/CG are differentiated by germ tube (GT) and rapid trehalose assimilation. Conventional identification methods were performed in parallel for confirmation.
Results: In the study period, PNA FISH was performed on a total of 120 positive culture bottles and 11 SAB. 131 (100%) of yeast specimens tested were correctly identified by PNA FISH as CA/CP (24/31), CT (13), CK/CG (7/16) or other yeast (no fluorescence). 91 (69%) of the isolates were definitively identified using the kit and no cross-reactivity was seen with the other 34 specimens, including *C. dubliniensis*, a fluconazole resistant (R) yeast commonly misidentified as CA. Of note, PNA FISH identified a mixed infection with CA and CG,

an important finding since 40% of CG isolated at UCLA is R to FLU (UCLA Antibiogram, 2009) and empiric therapy with an echinocandin is preferred. Conventional methods would have been solely reported CA from positive GT. Susceptibility testing by broth microdilution was performed on 43 isolates and 4/9 (44%) CG was R to FLU.
Conclusions: The Yeast Traffic Light PNA FISH™ kit identified specific CS, directly from BFCB (1.5 h) or from colonies (24 h), with 100% sensitivity and specificity. Moreover, one mixed infection was successfully detected. The ability to rapidly identify the majority of CS is pertinent in selecting appropriate anti-fungal therapy.

R2255 Environmental pH changes, but not the LuxS signalling pathway, regulates SpeB expression in M1 group A streptococci

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Objectives: The autoinducer-2/LuxS signaling pathway participates in quorum-sensing in diverse bacterial species. In group A streptococcus, LuxS has been shown to be involved in regulating expression of several important virulence factors. Streptococcal pyrogenic exotoxin B (SpeB) is a cysteine protease that has important roles in group A streptococcus pathogenesis. In the present study, whether the autoinducer-2/LuxS signaling pathway is participated in regulating speB expression in clinical prevalent M1 strains were analyzed.
Methods: The speB expression of clinical M1 isolates A-20, GAS 602, and their luxS isogenic mutants in different culture conditions were analyzed by Northern hybridization and real-time RT-PCR. In addition, the protease activities of these strains were analyzed by skim-milk agar plates.
Results: We found that the supernatant harvested from an overnight culture stimulated M1 strains to express speB. However, mutation of the luxS gene in M1 strains or treating M1 strains with luxS mutant culture supernatant did not affect speB expression, indicating that the LuxS pathway is not involved in regulation of speB expression in M1 strains. In addition, we found the acid property of culture broth can stimulate M1 strains to express speB in the same LuxS-independent manner.
Conclusion: These results indicate that speB expression in M1 strains is induced by environmental pH changes, but is not regulated by the LuxS signaling pathway.

R2256 Role of *Chlamydia* infection in the pathogenesis of vegetative state

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Objectives: The treatment of patients in vegetative state is not sufficiently elaborated. The primary injury of the brain leads to autoaggresive damages of nervous tissue, suppressing immune response and inflammatory process. The aim of the current research was to study the special features of im-mune status of the patients in vegetative state and the role of chlamydia infection in its mainten-ance.
Methods: 32 patients in vegetative state (23 males and 9 females aged from 14 to 58 y.) with the period of unconscious state from 3.5 months to 2 years were studied. All patients were examined using complex neurological, neuroradiological, electrophysiological and immunological methods. DNA, culture and serological methods were used for the diagnostics of chlamydiosis.
Results: Diffuse atrophic processes without typical signs of demyelization as well as glucose hypometabolism in different parts of brain were revealed with neuroradiological methods in all patients. In most patients were found the pathological changes in immune status: decreased number of CD4+ cells (24 out of 32), disimmunoglobulinemia (30 out of 32), distortion of phagocytes function (30 out of 32) and decrease of interferon production (29 out of 32). Specific antibodies against *C. trachomatis* or *C. pneumoniae* were found in 25 patients, 10 of them had cHSP 60 IgG. Five strains of *C. trachomatis* were isolated on McCoy cells from cerebrospinal fluid. In 4 cases of autopsy there were found lesions due to *Chlamydia* with revealing the pathogen both by PAS staining and immunofluorescence. All patients with confirmed chlamydiases received specific antibacterial therapy, which allowed to achieve notable improvement of communicative activity of patients. This positive dynamics was observed during antibacterial treatment also later (in 6 month–2 years). Improvement of glucose metabolism in some areas of the brain was proved as well. According to "Glasgow Outcome Scale" in 6 months–2 years good recovery was noted in 21% of patients.
Conclusion: The latent inflammatory immunopathological process associated with chlamydia infection is present in patients in vegetative state and should be taken in consideration during the examination and treatment of these patients.

R2257 Herpes simplex virus inhibits spermatogenesis in murine testis organotypic culture

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Objectives: Herpes simplex virus (HSV) is considered to contribute in etiology of the male infertility, but the pathogenesis remains unclear. Investigation of spermatogenesis is crucial for understanding the role of infectious agents in male fertility disorders. Short-term culturing of mice germ cells obtained from prepuberal mice before 10 days postpartum (dpp) is suitable for analysis of spermatogonia differentiation into spermatocytes, while pups testis before 19 dpp can be used for modeling meiotic process in vitro. The aim of this study was to develop organ culture system suitable for investigation of HSV influence on murine spermatogenesis.
Methods: Testicular fragments from 9 dpp (Group I), 17 dpp (Group II) pups and 20 weeks-old (Group III) DBA mice were cultured at 33ºC for 6 days on the liquid-gas interface. DMEM supplied with 10% serum, vitamins, insulin, transferrin, glutamine and pyruvate was used for culturing. HSV-infected and uninfected fragments were analyzed by morphological, virological and immunocytochemical methods.
Results: In Group I after 4 days of culturing we observed primary spermatocytes in 19% of uninfected tubules, comparing with 8% in infected ones ($p < 0.05$). In Group II pachytene spermatocytes differentiated into round spermatids in 100% of intact tubules, while HSV-infected cells passed through meiosis in 60% of tubules ($p < 0.001$). In Group III we found decrease in number of tubules, containing spermatocytes and round spermatids comparing with that of the control (38% vs 63%, $p < 0.001$). Viral load in Group I was 22-fold higher than in Group II and 40-fold higher than in Group III. Quantity of germ cells containing viral antigens in Groups I, II, III was 90%, 70% and 5%, respectively.
Conclusion: Culture system developed allow to investigate discrete steps of normal spermatogenesis and abnormalities caused by infectious factors. It was found that HSV disturbs spermatogonia differentiation and meiosis and as a result population of spermatocytes and round spermatids declines. Dramatic increase in viral yield and number of infected cells in pups testis comparing with adult mice can be explained by the absence of hemato-testicular barrier until 15–17 dpp.

R2258 *Vibrio fluvialis*-stimulated secretion of interleukin-8 by INT407 cells

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Objectives: *Vibrio fluvialis* is one of the causative agents of acute diarrhoeal infection in Kolkata. However, mechanisms of pathogenesis of this bacterium are not understood clearly as they did not harbour any of the known virulence genes. In this study, we demonstrate that *V. fluvialis* strains can induce secretion of IL-8 from INT407 human intestinal epithelial cells as detected by reverse-transcribed mRNA PCR and the gene product secretion by IL-8 ELISA.
Methods: The cell-free culture supernatants of *V. fluvialis* isolated from acute diarrhoeal patients were assayed on INT407 cells. The infected

and non-infected cells were incubated for different time periods. The supernatants of culture medium collected at different time intervals were lysed in Trizol and used in the IL-8 ELISA and RT-PCR. cDNA was prepared from RNA by using the SUPERSCRIPT™ and RT-PCR was performed by using the cDNA.

Results: We have shown that *V. fluvialis* strains can induce IL-8 from INT407 cells. Incubation of the epithelial cells with *V. fluvialis* significantly increased the levels of IL-8 mRNA in a time-dependent manner, indicating that the induction of IL-8 production by *V. fluvialis* occurred at the mRNA level. In, RT-PCR, IL-8 mRNA expression was evident in INT407 cells as early as 2 h and up to 24 h and G3PDH demonstrated that the expression of transcripts for this constitutive enzyme was unaffected (Fig). In ELISA, the IL-8 level of INT407 cells was examined at various time intervals with *V. fluvialis* infection. IL-8 secretion increased in up to 8 h (~755 pg/ml) (Fig. 1), and the amount of secreted protein declined to 520 pg/ml at 24 h, indicating that the event occurs in 4–8 h post infection.

Conclusion: We demonstrate that *V. fluvialis* isolated from the diarrhoeal patients can induce secretion of IL-8 by the INT407 cells. It is interesting to note that these strains did not harbor any of the known virulence gens markers associated with diarrhoea. Most these patients also had blood in their stools. Recent studies have suggested that IL-8 secretion may be an early signal for the acute inflammatory response following bacterial infections. Our data suggest that secretion of IL-8 by intestinal epithelial cells exposed to *V. fluvialis* may be the initial signal for the acute inflammatory response. We are also examining to elicit the cytokine induction of other inflammatory response in intestinal epithelial cell lines with following exposure to *V. fluvialis*.

G3PDH

IL-8

Fig: IL-8 mRNA induction in intestinal epithelial cell line Int407 following infection of V. fluvials strains. A representative agarose gel analysis shows different strains expression of IL-8 (lower band) and the control (G3PDH, upper bands) genes as determined by gene-specific co-amplification of both the genes by three independent RT-PCR experiments.

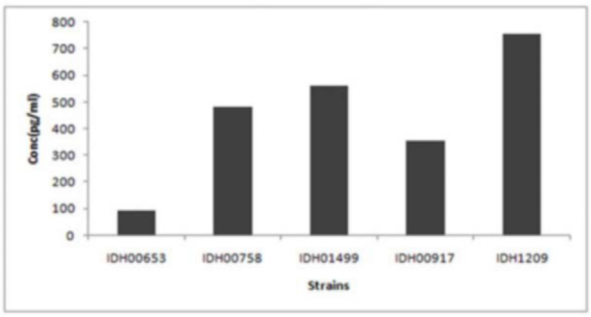

Fig:1 Induction of IL-8 mRNA in Int407 by different strains of *V. fluvialis*.

R2259 Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* isolates in patients with cystic fibrosis

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Objectives: *Pseudomonas aeruginosa* accounts for about one half of all pulmonary infections of cystic fibrosis (CF) patients. Once established, are difficult to eradicate even with intensive antibiotic treatment, and becomes predominant with age and predicts shortened CF survival. It is important to monitor the antibiotic susceptibility pattern of *P. aeruginosa* isolates to reduce morbidity and mortality in patients with cystic fibrosis.

Methods: Patients with CF (median age 6/2 years) referred to Alzzahra Medical Center in Esfahan between June 2003 and March 2008 were included in the study. The diagnosis of CF was based on both clinical and laboratory parameters. Fifty-nine CF patients were followed up monthly in the Alzzahra Medical Center for evidence of *P. aeruginosa* colonization of the respiratory tract. Antibiotic sensitivity of the mucoid morphotype and nonmucoid morphotype was performed by using the Kirby Bauer method and the following antibiotics; gentamicin, ciprofloxacin, piperacillin, amikacin and ceftazidime (Hi Media, padtan teb). *P. aeruginosa* ATCC 27853 served as the control strains.

Results: Antimicrobial susceptibility of the 21 *P. aeruginosa* CF isolates was investigated by using 5 antibiotic and the resistance rates according to NCCLS guidelines were as follows: Amikacin (9.5%), Gentamicin (9.5%), Ciprofloxacin (14.2%), Piperacillin (19%) and Ceftazidime (86%) (Fig. 1). The result showed that the majoritiy of *P. aeruginosa* isolates were the most resistance to ceftazidime (86%). Figure and Table will show at the full text.

Conclusions: Out of 11 resistance cases to at least one of four antibiotics (GM, AN, PIP and CP), 9 (81.8%) isolates were mucoid that is higher than in compared with two nonmucoid isolates (18.1%). 19 isolates (90.5%) were found sensitive to gentamicin and amikacin. The 85.7% of patients (6/7) harbored the isolates that was displayed resistance to at least 2 antibiotics had repetitive hospitalization (at least two time). The isolates of *P. aeruginosa* showed meaningful difference between drug resistance to ceftazidime in compared to other antibiotics (PIP, CP, AK, GM).

R2260 The culturability and virulence of *Legionella* cells during desiccation

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Objectives: Legionellosis is a serious and sometimes fatal form of pneumonia caused by the *Legionella* species. Most *Legionella* species are found in aquatic environments where they have the ability to reside and multiply in aquatic free-living amoeba. Some of species, such as *Legionella longbeachae*, have the ability to grow in soil and potting composts. The bacteria are transmitted to humans by aerosols from natural and human-made aquatic environments or, in the case of *L. longbeachae* infection, through exposure to contaminated potting soil. The objective of this study was to assess the culturability and viability of *Legionella* exposed to desiccation. Also, the role of *Acanthamoeba castellanii* in possible resuscitation of viable but non-culturable (VBNC) *Legionella* after desiccation was tested.

Methods: For desiccation study, bacteria were prepared in sterile water and $5\times20\,\mu l$ of bacterial suspension (~10^8 cfu/ml) were transferred in 96 wells plates and dried for 1 hour under laminar flow hood. The plates with dried *Legionella*, as well as plates with bacterial suspensions (wet conditions) were stored at room temperature. Bacteria were rehydrated and cultivated at different time points on BCYE agar. Bacterial viability was assessed with the Bacterial Viability Kit LIVE/DEAD® BacLight™ dying before fluorescent microscopy. At the same time, *Legionella* cells exposed to desiccation were added to *A. castellanii* monolayers and incubated for 48 hours.

Results: Both *Legionella* species could be cultivated on BCYE agar only 24 hours after desiccation. Using fluorescent microscope viable *Legionella* cells could be detected up to 15 days of desiccation so bacteria entered viable but non-culturable (VBNC) state. Our data show that although *L. pneumophila* become non-culturable after desiccation, co-culture with amoeba resuscitates the VBNC bacteria, with subsequent intracellular proliferation within *A. castellanii*.

In conclusion, *L. pneumophila* and *L. longbeachae* exposed to desiccation loss their culturability but remain viable and are able to infect and proliferate in *Acanthamoeba castellanii*.

R2261 BCG carrying ICL gene on plasmid shows increased survival in mice

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Objectives: Tuberculosis is one of the most persistent human diseases. No new anti-TB drugs have been introduced in the past 40 years, even though their development becoming increasingly important to face new challenges posed by multidrug resistant and extensively drug resistant strains and by acute infection with *Mycobacterium tuberculosis* of HIV positive patients. Isocitrate lyase (ICL) plays a pivotal role in the persistence of *M. tuberculosis* in inflammatory macrophages and was found to be important in virulence of many microbial pathogens.
Methods: TB-icl gene was amplified by polymerase chain reaction (PCR) and cloned into the *E. coli*-mycobacterium shuttle plasmid pMV262 to obtain recombinant shuttle plasmids pTB-icl. The plasmid was electroporated into *Bacillus* Calmette-Guérin (BCG).
To compare the survival BCG and recombinant BCG (rBCG) were injected to Apob(tm2Sgy)Ldlr(tm1Her/J) mice intraperitoneally either with the rBCG or with BCG 10^6 colony forming units (CFU) in a 100 microliter total volume. Mice were sacrificed 10, 14, 21, 35 days after the treatment. The titer of rBCG and BCG were determined from homogenized lungs and spleens. Copy number of icl was determined by qPCR.
Results: The titer of rBCG was 7 times higher than in BCG and after 21 days only rBCG could be demonstrated in both organs. Spleens were 3 times larger in rBCG infected mice. The copy number of this construct was 29 in rBCG while in BCG was 1.
Conclusion: These data suggest that ICL plays a very important role in the survival of mycobacteria and it gives potential to this enzyme as a drug target against infections.

R2262 Role of cytokines in patients with arthritis and low back pain brucellosis

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Objectives: Cytokines are secreted as the molecular products of different cells in particular T-Lymphocytes which contribute in the processes of immune responses. Therefore, the study may provide valuable information regarding the function of cell mediated immunity (CMI) in this disease.
Methods: This study was performed in the medical centers of Mashhad University of Medical Sciences. 50 patients with Brucellosis & 50 normal samples were took part in this investigation. Diagnosis of the disease was based on case history, clinical examinations & serological tests.
Results: The levels of TNF-α, IL-2, IFN-γ, IL-4 & IL-10 in serum were measured using ELISA method. The levels of the random variables IL-2 & IL-10 were tested by Kolmogorov Smirnov test & shown to be normal ($p < 0.05$). Other cytokines including IL-4, IFN-γ & TNF-α of patients serum were not normal. The results indicated that the levels of IL-10 in the sera of patients had increased ($p < 0.001$). IFN-γ level had increased in patients ($p < 0.05$). The level of IL-2 & IL-4 & TNF-α in the sera of patients did'nt show any appreciable difference with those of normal controls ($p > 0.1$). Kruskal-Wallis test showed a significant difference in IFN-γ in both groups.
Conclusion: Our results would suggest a definite role of IFN-γ & IL-10 in sera of the patients with arthritis & low back pain Brucellosis.

R2263 Frequency of virulence genes among different species of *Shigella* isolated from Peruvian children under 2 years of age

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Objective: The aim of this study was to describe the presence and frequency of 10 virulence genes in *Shigella* strains isolated from Peruvian children under 2 years of age, with and without diarrhoea.
Methods: Ninety-nine *Shigella* spp. isolates were tested; 77 from diarrhoea cases and 22 from healthy controls, from a cohort study in Lima, Peru. The isolates were biochemically and serologically identified as 66 *S. flexneri*, 16 *S. boydii*, 14 *S. sonnei* and 3 *S. dysenteriae*.
The following virulence genes were studied by PCR: gene of virulence related to serine protease autotransporters (SPATEs) (sigA, pic, sepA, sat), Enterotoxins ShET 1 y ShET2 (set1A, set1B y sen) and genes related to invasiveness (icsA, virA and ipgD).
Results: 75% of the strains analyzed presented sen, 69% sigA and 65% sat genes. The most frequently genes found in diarrhoea cases were icsA and virA (71%), sen (70%), and sat (68%); otherwise, the most frequently in control cases were sen (95%), virA and icsA (91%), and sigA (86%). sigA was most frequently in diarrhoea cases of *S. sonnei* (100%) and control cases of *S. flexneri* (93%) ($p < 0.05$).
The genes studied were found frequently in *Shigella flexneri* strains. The only strains that presented set1A y set1B in this study were *S. flexneri*, 48% (25/52) diarrhoeae cases and 64% (9/14) control cases. No one case of *S. boydii*, *S. sonnei* and *S. dysenteriae* presented sepA gen. 100% of *Shigella sonnei* amplified sigA, 71% (10/14) sen, 64% (9/14) icsA, and 57% (8/14) virA and ipgD, in this group no one strain amplify sat. virA, ipgD and icsA were present in all the three strains of *S. dysenteriae* studied.

R2264 Phenotypic adaptation of *Pseudomonas aeruginosa* in two different microenvirenments

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Objective: *Pseudomonas aeruginosa* may show adaptive pathogenicity according to microenviroment conditions. In this study, effect of minimum inhibitory concentrations (MICs) and sub-MICs of gentamicin (GN) and imipenem (IMP) treatment on *P. aeruginosa* which infected HEp-2 and RD were determined.
Material and Methods: Potential phenotypic changes in *P. aeruginosa* were determined by alkaline protease enzyme synthesis, quorum sensing systems which las and rhl responses and biofilm formation. Cellular changes of HEp-2 (epithelial cell line) and RD (muscles cell line) were determined by apoptosis, necrosis and nitric oxide responses following infected by *Pseudomonas aeruginosa* PAO1 (lasI) and *P. aeruginosa* PAO1 (wild type) strains (multiplicity of infection; m.o.i: 100:1) which were treated by MIC, % 50 MIC and % 25 MIC concentrations of GN and IMP following the infections, respectively.
Results: Biofilm formation was detected in both cell lines following infection by PAO1 (wild type) under the effect of all concentrations of IMP whereas it was not detected in HEp-2 cell line under the effect of 50% MIC and 25% MIC of GN. Biofilm formation was detected in both cell lines which were infected by PAO1 (lasI) strain under the effect of IMP and GN. The las system of the PAO1 (lasI) took place at MIC of GN in RD cell lines and under the effect of 50% MIC and 25% MIC of IMP in HEp-2 cell lines (confirmed by PCR). The rhl system of the PAO1 (lasI) and these las and rhl systems of the PAO1 (wild type) took place at MICs and sub-MICs concentrations of GN and IMP in both cell lines. In PAO1 (wild type) and PAO1 (lasI) infected HEp-2 and RD cell lines under the effect of GN and IMP concentrations showed apoptosis at a rate of ninety percent. Nitric oxide responses were higher in PAO1 (lasI) infected HEp-2 and RD cell lines, whereas alkalen protease responses were positive in all groups.
Conclusions: Bacterial protein synthesis inhibitor and bacterial wall synthesis inhibitor antibiotics create different microenvironments following infected by different strains of *P. aeruginosa*. Cellular vitality and secondary messenger responses of both cell lines changed while bacterial phenotypic changes occurred also. This adaptation ability shows us the real picture of the antibiotic-bacteria-microenvironment interactions in translational clinical studies also.

R2265 Antimicrobial activity of copper alloys compared to aminoglycosides against multidrug-resistant bacteria

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Objectives: The emergence of multi-resistant bacteria against powerful antibiotics and their transborder transmission constitute some of the most threatening public health problems of the recent years. Metallic copper alloys have recently attracted attention as a new antimicrobial agent, able to minimize environmental contamination by pathogenic bacteria.
We investigated the efficacy of a copper alloy (Cu63%-Zn37%) in relation to aminoglycosides.
Methods: Multi-resistant bacteria, isolated from blood culture of patients with signs of infection or fever appearing any time after 8 days of admission into ICU, were selected. The samples were cultured onto suitable culture media and bacterial isolates were identified using standard methods. The isolated bacteria were: *Escherichia coli*, *Klebsiella* spp, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecium*.
Resistance genes were detected for up to 10 antibiotics, evaluated with VITEK 2 System (bioMérieux) for identification and antimicrobial susceptibility test and the Minimum Inhibitory Concentration (MIC) to determine the antimicrobial activity of a material against specific bacteria and to control the activity of new antimicrobiological factors were recorded and compared.
In the above procedure we used coupons (1x1cm) of copper alloys containing (Cu63%-Zn37%) and Aminoglycoside discs.
Susceptibility testing was conducted by Kirby Bauer disc diffusion method on "Miller Hinton Agar" in which an aminoglycoside disc was placed at 3cm distance from the copper coupon. This procedure was followed for each isolated pathogen.
Results: After 24-hour and 48-hour incubation periods, the inhibition zone bacteria around the antibiotic disc as well as around the copper coupon were recorded.
The inhibition zones of antimicrobial copper were equal to those of aminoglycisides for *E. coli* and *Klebsiella* spp, were smaller for *Pseudomonas aeruginosa*, and *Enterococcus faecium* and larger for *Staphylococcus aureus* and *Acinetobacter baumannii* respectively.
Conclusion: The copper alloy exhibited antimicrobial activity against all multidrug resistant bacteria studied. The inhibition zone of our specific cooper alloy was increased, compared to that of aminoglycosides for *Staphylococcus aureus* and *Acinetobacter baumannii*. It should be noted that the reduction of bacterial load in a hospital where antimicrobial copper is used, leads to drastic reduction of antibiotic use.

R2266 Decidual inflammatory mediators: implication in pre-term labour with intrauterine infection

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Background: Increased risk of preterm labour has been linked to cervicovaginal infection. Bacteria colonising the lower genital tract may ascend to the gestational tissues, triggering an inflammatory response mediated primarily by pro-inflammatory cytokines, matrix metalloproteinases and prostaglandins.
Objective: The aim of this study was to determine if decidual cells produce inflammatory mediators implicated in preterm labour during intrauterine infection.
Methodology: Fetal membranes were obtained after caesarian section from 10 healthy women with normal pregnancies at term with no evidence of labour, and from 10 women with preterm labour and confirmed intrauterine infection [*Ureaplasma urealyticum* (2), group B streptococci (3), *Gardnerella vaginalis* (3), *Escherichia coli* (2)]. Decidua was scrapped and decidual cells were obtained and cultured overnight. Secretion of anti-inflammatory cytokines (IL-2, IL-4, IL-10), pro-inflammatory cytokines (IL-6, IL-8, IL-1b and TNF-a), and matrix metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9) was evaluated in the supernatants using the Bio-Plex System. Additionally, prostaglandin E2 (PGE2) was measured by ELISA.
Results: We found a significant increase of pro-inflammatory cytokines and a significant decrease of anti-inflammatory cytokines in supernantants from decidual cells obtained from women with preterm labour and intrauterine infection compared with women with normal pregnancies. Likewise, secretion of MMP-1, MMP-8, MMP-9 and PGE2 was significantly higher.
Conclusions: These results suggest that decidual cells promote a pro-inflammatory microenvironment during intrauterine infection leading to preterm labour.

Animal models incl. experimental treatment

R2267 Clinical experience of use of taurolidine in varied complex clinical cases

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Background: Taurolidine citrate [Taurolock™] is licensed as a medical device. Literature reveals its predominant use as a venous line locking system to prevent catheter line infections. Taurolidine has antimicrobial, antimycotic, antiadherent and antiendotoxic properties. The antimicrobial properties extend across multiresistant organisms. We present here clinical experience of taurolidine use in four very complex varied cases and an overview of taurolidine use at Blackpool Victoria Hospital.
Methodology: Case notes review of 10 cases over 2-years [including the complex cases] were undertaken.
Results: All cases were discussed between primary consultant and Microbiologist. The patients included were treated in a variety of hospital settings:including cardiology ward, cardiac intensive care unit, cardiac high care, general surgical ward, surgical high care, intensive treatment unit and general medical ward.
The 10-cases included 6-males [av age 59.2-years] and 4-females [av age 57-years]. All cases were discussed between primary consultant and Microbiologist. 70% [7/10] use as a line lock was for new (long term) hickman lines following removal of previous infected line. 30% was for salvage of colonised lines. Linelock use included haematology patients on chemotherapy as well as surgical patients on total parenteral nutrition. Average duration of use was 18-doses. 77.8% of patients received simultaneous systemic antibiotics. In 33.3% of cases salvage of colonised line with taurolidine failed and infected line was removed. In 66.7% there was successful outcome. 88.9% of patients were discharged home after successful outcomes.
Complex cases: A complex surgical case with enterocutaneous fistula, over a dozen episodes of candidaemia, multiple central line infections had a successful outcome with taurolidine locked hickman line.
Two complex thoracic cases with *Pseudomonas* infection had a successful outcome following taurolidine pleural irrigation.
Complex case of aortic root graft re-infection with pseudomonas underwent mediastinal irrigation before VAC dressing. Cases to be presented.
Conclusion: Successful outcomes with taurolidine-citrate, licensed as a medical device, finds its place in several case reports. Our complex cases indicate its potential outside of its use as a line lock to prevent infections in long term venous catheters. Further large randomised studies on taurolidine citrate are required. Cases to be presented.

R2268 The polyclonal anti-TNF immune Fab AZD9773 neutralises human TNF-a biological activity in vivo

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Objectives: AZD9773, comprising ovine-derived polyclonal anti-TNF-a Fab fragments, is intended for intravenous infusion to treat severe sepsis and septic shock. As AZD9773 is highly specific for human TNF-a, preclinical in vivo studies were undertaken to assess its effect on recombinant human TNF-a (rhTNF-a)-induced lethality in D-galactosamine

(D-gal)-sensitised mice and lipopolysaccharide (LPS)-induced endotoxaemia in humanised TNF-a transgenic (Tg1278/TNF–/–) mice (Tgx mice), respectively. D-CytoFab, an earlier experimental version of AZD9773, demonstrated to have a clinical benefit in patients with severe sepsis and septic shock, was included as a comparator.
Methods: rhTNF-a-induced lethality: female BALB/c mice received intraperitoneal (i.p.) rhTNF-a (5.9×10^5 IU/mouse), D-gal (18 mg/mouse) and 0–110 U/mouse D-CytoFab or AZD9773. Mouse survival was monitored for 36 h. Endotoxemia: Tgx mice received high (845 U/kg) and low (84 U/kg) i.p. doses of AZD9773 or D-CytoFab 2 h prior to LPS challenge (10 mg/kg, i.p.) and were sacrificed 2 h post-LPS challenge, with terminal bleeds for AZD9773/D-CytoFab and serum cytokine analyses. AZD9773 (0–850 U/Kg, delivered i.p. 2 h pre- i.p. LPS dose) effects on human TNF-a and a large panel of serum murine cytokines were determined 2 h post-LPS challenge. AZD9773, D-CytoFab, human TNF-a, and mouse cytokines were determined by enzyme-linked-immunosorbent assays.
Results: AZD9773 and D-CytoFab comparably (based on equivalent unit dosing) protected BALB/c mice from human TNF-a induced mortality. In the Tgx mice, high-dose AZD9773 or D-CytoFab significantly reduced LPS-induced serum human TNF-a and murine IL-6, 2 hours post-LPS ($p < 0.01$). Similar effects on cytokine suppression were observed when AZD9773 and D-CytoFab serum levels were unit normalised. LPS challenge in Tgx mice resulted in the expression of 42 cytokines. Pre-treatment (LPS –2 h) with AZD9773 resulted in a dose-related exposure (measured 4 h post-i.p. dose). AZD9773 doses of 850 and 425 U/Kg resulted in statistically significant reductions in 29 and 22, respectively, out of the 60 cytokines measured (including human TNF-a, IL-2, IL-4 and IL-6).
Conclusion: AZD9773 has been shown to be an effective anti-TNF-a agent in two separate human TNF-a mouse models. AZD9773 biological activity did not significantly differ from that of D-CytoFab.

R2269 Hyperbaric oxygen and medical ozone as adjuvant therapy in a murine model of streptococcal myositis

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Severe streptococcal infections lead to serious tissue damages. Myositis is an example of these conditions. Systemic antibiotic therapy is the principle course of treatment in necrotizing soft tissue infection. Clinical trials have also shown Hyperbaric Oxygen (HBO) therapy to be effective in streptococcal myositis patients. Ozone (O3), known as a cytotoxic gas, has recently been shown to support immune system by activating oxidative mechanisms and increasing pro-inflammatory cytokines.
In this study, we compared the effectiveness of antibiotherapy, HBO and O3 application on bacterial growth and wound recovery in a murine model of streptococcal myositis.
Forty-five male Sprague-Dawley rats were divided in five groups. Myositis was caused by inoculation of 0.15 ml 0.5 McFarland streptococcus pyogenes to thigh region in the four groups other than the Sham group. Normal saline was given to the rats in one of the four groups as a control, whereas other three groups were treated by penicillin G (98 mg/kg in 0.25 ml intraperitoneally 2X1), HBO (100% oxygen, 2,5 Atm. 90 min. 2X1) and ozone (1 mg/kg 1X1 intraperitoneally).
At the seventh day, histological and bacterial investigations were done on soft tissue specimens taken from thigh region. There was significant difference in response to therapy in all of the three groups compared to control group ($p < 0.05$). Antibiotic and ozone groups were also more successful than HBO ($p < 0.05$). Ozone group yielded better results than that of antibiotic group ($p < 0.05$).
Our results conclude that Ozone therapy should be used in addition to antibiotic treatment in serious infections such as myositis.

R2270 Limited value of MRI to detect apoptotic injury of the hippocampus in experimental meningitis

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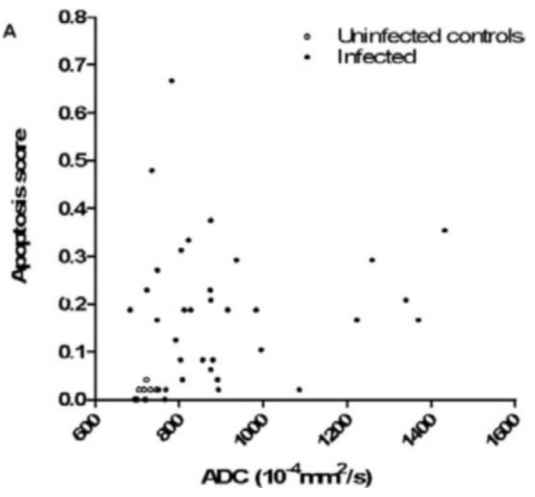
Objective: Increased neuronal apoptosis in the hippocampus is a well described brain pathological characteristic for pneumococcal meningitis and is associated with learning difficulties in experimental models of the disease (1). The present study hypothesized that in vivo measurement of water diffusion (apparent diffusion coefficient – ADC) in the hippocampus of rats with pneumococcal meningitis could disclose the risk of neuronal apoptosis.
Methods: Meningitis was induced by injecting rats i.c. with 106 CFU/ml *S. pneumoniae* serotype 3 (n = 35) or beef broth (n = 8). The study comprised of four experimental groups: I. meningitis; II. meningitis with attenuated bacteremia (treated with specific antipneumococcal antibodies); III. meningitis with early onset bacteremia (additional i.v. injection of pneumococci); IV. broth injected controls (2).
MRI images were acquired 28 and 38 hours after infection using a SISCO 4.7T imaging system. T1W, T2W, quantitative diffusion and dynamic MRI measurements were performed.
After imaging brains were harvested for histopathology. ADC maps were calculated from images acquired with b-values of 0, 185, 740 and 1665 s/mm^2. Regions of interest were drawn around the hippocampus on 2 consecutive coronal brain slices and a mean ADC measured with MIPAV (fig. B). On histopathological specimens, neurons with apoptotic morphology in the dentate gyrus of the hippocampus were scored. Image- and specimen analysis was performed blinded to the experimental design. For statistical analysis of correlations $p < 0.01$ was considered significant.
Results: No association between hippocampus ADC and apoptosis scores was found either including (Spearman Rank, Rho = 0.38, p = 0.011) or excluding the very homogenous uninfected control group (Rho = 0.019, p = 0.92), fig. A. High ADC values was found in the hippocampus of infected rats, and was significantly increased among all groups of infected rats compared to uninfected controls except the attenuated bacteremia group (Kruskal-Wallis, p = 0.0001, Dunn's post-test $p < 0.05$). Similarly, apoptosis scores were significantly increased among infected rats compared to uninfected controls (p = 0.0002), fig. C.
Conclusion: In vivo hippocampus oedema measured by ADC was not correlated to the rate of neuronal apoptosis. For the evaluation of apoptotic injury in the hippocampus, histomorphometry remains the gold standard.



R2271 Efficacy of ertapenem, zosyn and tygacil in a mixed infection rat intra-abdominal abscess model with ESBL *E. coli*

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Objectives: Complicated intra-abdominal infections (IAI) are among the most common infections in general surgery with morbidity and mortality rates as high as 59% and 21%, respectively. There is also an increasing prevalence of resistant Enterobacteriaceae in these nosocomial infections. The present study was conducted to evaluate the in vivo efficacy of ertapenem (ERT), Zosyn® (ZSN) and Tygacil® (TYG) in an experimental intra-abdominal infection model in rats with *Bacteroides fragilis* (Bf) and an extended spectrum β-lactamase (ESBL) producing *Escherichia coli* (Ec) expressing CTX-M-15.
Methods: Cultures were grown on appropriate media and diluted in either brucella broth for Bf or trypticase soy broth for Ec. Inoculums of 10^7 CFU for Bf and 10^5 CFU for Ec were added to a size 1 gelatin capsule filled with sterile rat fecal contents. Capsules were surgically implanted into the lower left quadrant of the abdomen of male Sprague-Dawley rats and sutured closed. Treatment was administered intravenously and initiated 4 hrs after implantation then continued twice-a-day (bid) for three days with either 50 mg/kg ERT, 700 mg/kg ZSN or 25 mg/kg TYG. Abscesses were aseptically removed 18–24 hrs after the last administered dose, plated on appropriate media and conditions to determine bacterial counts for both the Bf and Ec isolates.
Results: Susceptibility testing on the Bf and Ec isolates used in this model demonstrated MICs of 0.03 and 0.03 μgmL for ERT, 0.5 and 16 μgmL for ZSN and 0.125 and 0.25 μgmL for TGY, respectively. Abscesses from the untreated control group exhibited mean log10 CFU burdens of 8.86 for Bf and 9.44 for Ec on day 5. ERT treatment resulted in mean log10 CFU reductions of 2.43 for Bf and 3.89 for Ec as compared to the untreated controls. ZSN and TYG were less efficacious with log10 CFU reductions of 0.12 and -0.13 for ZSN and 0.23 and 0.40 for TGY against the Bf and Ec isolates, respectively.
Conclusions: The increased prevalence of ESBL producing Enterobacteriaceae and their role in complicated IAI points to the need for more effective therapies against these antibiotic resistant bacteria. Carbapenems, such as ertapenem, remain efficacious for serious infections such as IAI and further testing is warranted to optimize dosing regimens for maximal efficacy and determine the potential for selection of antibiotic resistance.

R2272 Efficacy of levofloxacin and gentamycin against a uropathogenic strain of *Escherichia coli* in a mouse urinary tract infection model

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Objectives: Due to the continued need to evaluate antibiotics for the treatment of urinary tract infections (UTIs), a mouse UTI model was developed by our lab to determine the efficacy of antibiotics against uropathogenic *Escherichia coli* (UPEC). Here, we describe the efficacy of levofloxacin and gentamicin against a susceptible UPEC isolate in the animal model.
Methods: Female C3H/HeJ mice were placed on 5% glucose water 6 days prior to infection. Anesthetized animals were transurethrally infected with a strain of *E. coli* that is uropathogenic but antibiotic susceptible (gentamicin and levofloxacin MICs of 0.5 and 0.06 μgmL, respectively) at 8.9 log10 CFUs per animal. Beginning 4 days after infection, gentamicin and levofloxacin were dosed subcutaneously (s.c.) twice daily for 3 consecutive days. Kidneys, bladders, and urine were collected 18 hours after the final dose, and the tissue homogenates (kidneys and bladders) and urine were plated for CFU counts. Efficacy was determined by comparing the mean CFU of treated groups to untreated controls.
Results: The mean CFU counts in the kidneys and bladders of untreated control animals increased from 5.4 and 5.2 log10 at 4 days post-infection to 6.2 and 6.7 log10 after 7 days, respectively. The urine counts for untreated controls (3.5 log10 CFU) remained unchanged on days 4 and 7. When compared to 7-day untreated controls, levofloxacin dosed s.c. at 0.03 to 2 mg/kg over three days decreased mean log10 CFU counts in kidneys by 2.0 to 2.8, bladders by 3.1 to 3.7, and urine by 0.1 to 0.8. Similarly, s.c. doses of gentamicin ranging from 0.125 to 8 mg/kg over three days effectively reduced mean log10 CFU counts in kidneys and bladders by 2.1 to 3 and 1.3 to 3.4, respectively. Additionally, the urine counts in gentamicin treated animals were maximally decreased by 2.5 mean log10 CFU when compared to 7-day controls.
Conclusion: The mean CFU counts in the kidneys, bladders, and urine of 4- and 7-day control groups suggested that a stable, ascending UTI was established in the animal model. Dose responses for levofloxacin and gentamicin were observed, and while similar efficacy results were seen in the kidneys and bladders, a greater reduction in urine counts was observed for gentamicin treated animals. These results suggest that the described mouse model can be useful in evaluating and comparing antibiotics that treat UTIs caused UPEC.

R2273 An invertebrate model of infection to evaluate virulence in *A. fumigatus*

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Objectives: The impact of acquired resistance mechanisms on *Aspergillus fumigatus* in terms of virulence and fitness is not yet well understood. Virulence of fungal pathogens has traditionally been determined using lethal infections in mammalian hosts such as mice or rats (the gold standard) although some have used an invertebrate host, which has demonstrated almost complete correlation with mice. Here we describe the use of *Galleria mellonella* as an alternative model to assess differences in virulence between *A. fumigatus* wild-type strains and different derived isogenic single mutants. Furthermore in vitro growth rates of the different mutants were compared.
Method: A total of 9 *A. fumigatus* strains were studied, including the parental strain (wild type for the gene encoding the azole target cyp51A) and 8 different derived mutants strains with altered cyp51A alleles (non-synonymous point mutations at codons 54, 98 and 220 in the Cyp51Ap), which confer changes in antifungal drugs susceptibility. Ten larvas of *G. mellonella* per group were infected with each strain (104 and 105 ufc/larva) in duplicate, incubated in plastic containers at 37ºC and monitored daily for survival. Survival among groups was compared using Mantel-Cox tests (GraphPad Software Inc, La Jolla, CA, USA). Growth kinetics assays in solid (Potato Dextrose Agar and Minimal Medium) and liquid media (RPMI2%glucose) were also performed in order to better characterize the growth rate of these strains. Differences between growth parameters were calculated and compared.
Results: Under the conditions used in this study, all the *A. fumigatus* strains (wild type and mutants) were able to kill *G. mellonella* larvae equally. All strains caused <15% or <30% mean survival of larvae 6 days post injection of 105 and 104 ufc/larva respectively. In addition, no significant difference in virulence between groups were detected between the inocula size tested ($P = 0.9$ and $P = 0.3$ respectively). Results from the in vitro growth experiments showed no significant differences in radial growth rate of the wild type compared to the mutant strains. The kinetic evaluation of growth over time in liquid RPMI medium also supported these finding.
Conclusions: Mutations involving cyp51 genes of *A. fumigatus* which confer changes in susceptibility to azoles are not associated with significant alterations of virulence. *G. mellonella* could provide a reproducible infection model for *A. fumigatus* in screening studies.

R2274 Infectivity of *Aerococcus urinae* in an ascending urinary tract infection mouse model

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Objective: *Aerococcus urinae* has recently been associated with urinary tract infection (UTI). It resembles staphylococci in Gram stain and α-haemolytic streptococci on blood agar. Strains have been recognized worldwide, isolated from 0.3–0.8% of urine specimens and found resistant to several antibiotics, used for treating UTI. Also, strains have been isolated from blood from patients suffering from urogenic bacteraemia/septicemia, often complicated with endocarditis. However, the pathogenic potential of *A. urinae* in UTI has been questioned. We therefore examined the pathogenic potential of *A. urinae* strains in a UTI mouse model.
Materials: Six *A. urinae* strains were used; one blood-culture strain and five urine strains. Outbred female albino mice (Ssc-CF1 mice) were used. Trans-urethral inoculation of anesthetized mice was done with a plastic catheter, which was gently inserted to the top of the bladder and the bacterial suspension injected. Urine from each mouse was collected by gentle compression of the abdomen. Mice were afterwards killed by cervical dislocation, and organs (bladder, kidneys) were removed aseptically and homogenized in PBS. The CFU/ml of urine, per bladder, or per kidney were determined after 18 to 24 h of incubation at 35ºC (lower detection limit was 50 CFU/ml).
Results: Three strains, were used to optimize conditions. These were overnight growth on 10% DBA, inoculum volume of 200 µl, 2 days observation period and an inoculum of 2×10^8 CFU/ml. Afterwards 3 additional strains were included. Presence of bacteria were most pronounced in the urine and to a lesser extent in bladder and kidney tissue. All strains could be isolated from either urine, bladder and/or kidney tissue after two days incubation, though a difference in concentration of bacteria/ml and number of mice colonized/infected was observed (Table 1). Prolonged infective episodes were not observed (2 strains; 1–2–3–4–7–14–21 days). A septicemic phase was not detected by culture of blood drawn at various periods after inoculation (2 strains; 1–3–6–24h; 2–3–7–14–21 days).
Conclusion: All 6 strains of *A. urinae* examined could mostly be detected from urine and kidney tissue, while bladder infection was less obvious than seen for *E. coli*. This indicates a present but low pathogenic potential of the strains in this model. A systemic spread of the bacterium did not take place and the infection established was selflimiting.

Biofilm

R2275 Activities of tigecycline and vancomycin

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Objective: Today, intravenous catheters, the most common cause of bloodstream infections, led to high mortality, prolonged hospitalization, and increasing costs. Staphylococci constitute 75–90% of all catheter-related infections. Coagulase-negative staphylococci are responsible for 35–50% of these infections and more adhesive to plastic catheters than other microorganisms. Methicillin-resistant *Staphylococcus aureus* (MRSA) is in the second place (15–20%) of the most common factors after the coagulase-negative staphylococci. Treatment options for catheter infections related to MRSA are restricted. Tigecycline is a broad-spectrum, well tolerated, injectable and new glycylcycline antibiotic. In this study, in an in vitro MRSA biofilm model, we aimed to compare the efficacy of tigecycline and vancomycin.
Methods: Silicone disks were incubated in human plasma for 24 hours and then in the suspension of MRSA for 24 hours for biofilm formation. Disks were exposed tigecycline and vancomycin for 24 hours and 5 days of 4-hour daily in the model of antibiotic lock therapy. After incubation, the disks were placed into saline solution for 15 minutes. Finally, the tubes were vortexed and from each tube, 100-µL of the solution was inoculated onto 5% sheep blood-Trypticase soy agar plates. The plates were incubated overnight at 37ºC and the colonies were counted.
Results: Colony forming unit counts of MRSA decreased from 100 000 cfu/mL to 510 cfu/mL in the tigecycline group and from 100 000 cfu/mL to 3 800 cfu/mL in the vancomycin group. The difference between the vancomycin and tigecycline groups was statistically significant ($p < 0{,}001$). In antibiotic lock therapy tests, bacterial growth was not detected on second day in tygecycline group and on fifth day in vancomycine group.
Conclusion: Under in vitro conditions, tigecycline is more effective against MRSA biofilms than vancomycin. This result suggests that tigecycline can be a good treatment of choice for catheter-related infections. However, its introduction to clinical use requires further clinical studies.

R2276 Evaluation of biofilms formation of standard fluconazole-resistant strain of *Candida albicans* on PVC and catheters coated with TiO_2 nanoparticles by XTT assay

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Objective: *Candida albicans* is the major fungal pathogen of immunocompromised Patients. Nowadays fungal infections especially candidiasis have significantly increased. Limitations in treatment of fungal diseases include drug resistance and side effects due to search for a new antifungal agents and using replacement new compounds such as nanoparticles. In this study fungal biofilms formation of Fluconazole resistant strain of *C. albicans* on PVC (Polyvinyl Chloride) and Catheter coated with TiO_2 (Titanium dioxide) nanoparticles was evaluated.
Methods: TiO_2 nanoparticles were synthesized with sol-gel method and hydrolysis of precursor Titanium tetrachloride and were assessed with Scanning Electron Microscope (SEM). Small pieces of PVC and Catheter were prepared (1 cm^2) and coated with dip coating method. Candidal biofilms formation was formed in 12-well tissue culture plates. Standard Fluconazole resistance strain of *C. albicans* (ATCC 76615), was used for the biofilms forming yeasts and cell suspension regulated at 1×10^6 cells/ml. The yeast cells were incubated for 2 h at 370C in 1 ml of 1% BSA then 80 µl of *C. albicans* cell suspension was inoculated. The yeast cells were allowed to adhere to the surface at 37ºC for 90 min. After this time the discs were gently submerged with 4 ml of YNB medium. The plates were incubated at 370C for 48 h. After incubation, fungal biofilms formation samples were evaluated by standard XTT reduction assays and SEM.
Results: Concentration of TiO_2 solution was 7.03 mg/ml 5.63×10^{20} particles/ml. Morphology and diameter properties of the TiO_2 nanoparticles with SEM showed that nanoparticles were spherical with diameter between 40–65nm. Optical density of survival cells in coated samples was less than control samples (not treated) and was determined for PVC 0.158± 0.07 and Catheter 0.142± 0.01 in comparison of controls 0.253± 0.04 and 0.359± 0.05 in order. Data of treated samples showed significant difference with control samples ($p < 0.05$). Also the images of SEM showed that fungal biofilms didn't form in coated samples.
Conclusion: According to our findings, coatings of TiO_2 were showed suitable antifungal activity in comparison of other antifungal agent. Thus overall these results indicate that a potential therapeutic application of TiO_2 and further investigations need that it could be represent candidates in elimination of Fluconazole resistance strains of *C. albicans* in field of medical especially in medical devices.

R2277 Detection of biofilm formation among the clinical isolates of *Acinetobacter baumannii*

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Introduction: *Acinetobacter baumannii* has emerged as a significant nosocomial pathogen, particularly in intensive care units. Ability of this organism to form bofilms puts a further strain on the helath care system. *Acinetobacter baumannii* growing in a biofilm are associated

with chronic and recurrent human infections and highly resistant to antimicrobial agents.
Objective: We have conducted this study to detect the biofilm formation among *Acinetobacter baumannii* isolated from a tertiary care hospital.
Materials and Method: The study was carried out at the Department of Microbiology, Army Medical College/National University of Sciences and Technology, Pakistan, from June 2010 to November 2010. A total of 110 *A. baumannii* isolated from various clinical specimens were investigated for biofilm production. Isolates were identified by standard microbiological procedures (Gram's stain appearance, colonial morphology, catalase test, cytochrome oxidase reaction, motility, API 20NE). Isolated organisms were subjected to tissue culture plate method and tube method for biofilm detection.
Results: From the total 110 clinical isolates of *A. baumannii*, the tissue culture plate method detected 22.7% as high, 41% moderate and 36.3% as weak or non producers of a biofilm. The tube method correlated well with the TCP method for identifying strong biofilm producers, but it was hard to differentiate between moderate, weak and non-biofilm producers due to the changeability in observed results by different observers.
Conclusion: Frequency of biofilm forming *A. baumannii* is high in our set up. The tissue culture plate method is an accurate and reliable method for detection of biofilm formation in *A. baumannii*. Tube method cannot be suggested as general screening test to identify biofilm producing isolates. TCP is an easy to do method which can be advised for detection of resistant bacteria.

R2278 First results of bacteriological examination of bladder wall in patients with recurrent lower urinary tract infections and interstitial cystitis/painful bladder syndrome

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Objectives: The aim of our study was to examine bladder wall in patients with recurrent lower urinary tract infections (RLUTI) and interstitial cystitis/painful bladder syndrome (IC/PBS) for the presence of infectious agents to improve diagnosis and treatment of these diseases.
Materials and Methods: In 2010 in our clinic 33 women aged 19–53 years with RLUTI (n = 23) and IC/PBS (n = 10) had undergone cystoscopy with biopsy and subsequent pathological and microbiologic examination. The bladder mucosa was cultured for allocation of pure culture and assessment of antibiotic resistance. The ability of isolated cultures to form a biofilm on abiogenic media was evaluated by bacteriscopy and bacteriologically (analyzer "BioTrac 4250").
Results: Microflora in bladder wall was isolated in the majority of patients (n = 32) with severe symptoms despite the lack of bacteriuria in a greater proportion of patients with RLUTI (n = 17) and IC/PBS (n = 9) with inflammation and dysplasia. Mixed infection took place in 6 cases of IC/PBS. The representatives of the family Micrococcaceae (*Staphylococcus* spp., *Kocuria* spp.) and gramnegative nonfermentative rod-shaped bacteria of the genera *Burkholderia*, *Flavimonas*, *Brevundimonas*, *Acinetobacter* were elucidated. A monoculture was allocated in 3 patients. The microflora growth was $1\times10^{3-}1\times10^{5}$ CFU/ml in all 23 patients with RLUTI. The same bacteria as well as *Pseudomonas*, and *Proteus mirabilis* were allocated. Monoculture was also isolated from three patients. Micrococci genus *Kocuria*, *Staphylococcus* and *Acinetobacter* demonstrated the ability to form biofilms in vitro.
Conclusions:
1. Identification of pathogens in the bladder wall of patients with IC/PBS confirms the role of infection in the etiology and pathogenesis of this disease.
2. Both pathology and microbiology examinations of biopsy samples should be performed in patients with RLUTI.
3. Etiological treatment of patients with RLUTI should include biofilm penetrating drugs.

R2279 Quartz crystal microbalance – a useful tool for evaluation of *Pseudomonas aeruginosa* biofilm formation

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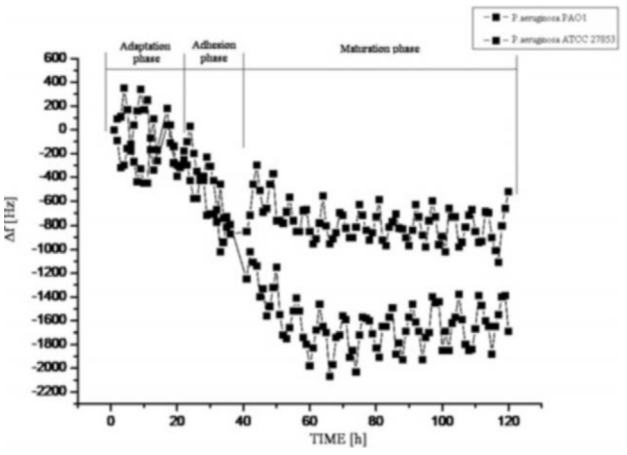
Objectives: Persistent of bacterial biofilm type growth attached to the biotic or abiotic surfaces is known problem for a few decades. There are two main groups of methods used for evaluation of bacterial community – colorimetric or fluorescence assays and various types of microscopy visualization. In this paper authors present a new tool for analyzing bacterial biofilm growth, based on crystal oscillators: quartz crystal microbalances (QCM). Authors tested the method for two biofilm model Gram-negative strains *Pseudomonas aeruginosa* ATCC 27853 and *Pseudomonas aeruginosa* PAO1.
Methods: QCM disc inside liquid cell was sterilized in an autoclave before experiments. For the experiments, refreshed bacterial strains were suspended in a Mueller Hinton broth (Becton Dickinson and Company, Cockeysville, MD, USA) to an optical density equal to the McFarland No. 5 (10^8 cells per milliliter). The culture was diluted 100-fold and one milliliter of culture containing 10^6 cells was added to the flow cell containing QCM disc. Culture was measured in a QCM system for 5 days at 37ºC. As controls crystal violet assay and AFM visualization was performed following to the presented technique.
Results: In the biofilm type of growth three phases are identified: adhesion, maturation and dispersion of biofilm structures. In our experiment additional phase – adaptation of the QCM disc to gold surface was observed (Fig. 1). A rapid increase of frequency shift for *P. aeruginosa* ATCC 27853 was reported after 20 hours of incubation. Maximum of frequency shift (3000 Hz) for this strain was observed in 55th hour of the experiment. The similar phases of the biofilm growth characteristics were noticed for *P. aeruginosa* PAO1 strain. The maturation phase of the biofilm structure was observed in the 60th hour after start of the test. Maximum frequency shift for PAO1 strain had a value of ca. 1000 Hz. During the experiment (5 days) the culture medium was not refreshed and QCM discs were not prepared and fixed before measurements.
Conclusions: We have shown that quartz crystal microbalance can be considered as a new useful tool for the evaluation of bacterial biofilm growth. Using this technique it is possible to recognize three phases of biofilm formation. Our results proved that presented measurement system can be applied as a convenient method for real-time observation of bacterial biofilm formation.



R2280 Phenotypical and genetical analysis of biofilm formation by coagulase negative staphylococci

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Coagulase-negative staphylococci (CoNS) are major nosocomial pathogens. The most important property of CoNS is their ability to form

biofilm on the surfaces of foreign bodies introduced (implanted) into the organism. The accumulative phase of biofilm formation is linked to the production of polysaccharide intercellular adhesin (PIA), which is synthesized by icaADBC-encoded proteins. The accumulation associated protein (AAP) encoded by aap is important genetic determinant of slime production.
The purpose of this study was to investigate strains of CoNS using phenotypical and genotypical methods.
Methods: 49 clinical group specimens of CoNS were isolated from surgical wounds (10), blood culture (18) and intravenous catheters (21). A control group of 12 CoNS specimens from nose epithelium of healthy people was included. Appearance of icaA and aap genes was tested by PCR. The Congo Red agar screening phenotypical method was evaluated for rapid and accurate detection of slime production by coagulase negative staphylococci. The microtiter plate assay described by Christensen et al. with modifications was used in parallel.
Results: Both genes icaA and aap positive were detected in 9 (50%) of 18 blood samples, 2 (20%) of 10 surgical wound samples, 6 (28,6%) of 21 catheter samples. In control group (12) only one of CoNS isolate was positive for aap gene. Congo Red agar (1% glucose) demonstrated that correlation between icaA, aap and specific phenotypical appearance was only in 8 (13,1%) of 61 CoNS samples. In this study, the ability of biofilm formation was evaluated in 31 samples using microtiter plate (96-well cell+ plate). Positive biofilm formation was detected in 8 (53,3%) of 15 central venous catheter and peripheral venous catheter samples, and in 5 (31,2%) of 16 blood samples.
Conclusions: The data indicate that icaA and aap genes along with phenotypical analysis (microtiter plate) are reliable methods for biofilm detection in coagulase negative staphylococci. The Congo Red agar (1% glucose) screening could not be recommended for analysis of biofilm forming CoNS, due to high rate of heterogeneity of results.

R2281 Effects of human polymorphonuclear neutrophils alone or in combination with three antipseudomonal antibiotics against *Pseudomonas aeruginosa* biofilms

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Objectives: *P. aeruginosa* (PA) is the main cause of chronic airway infections in cystic fibrosis (CF) patients. CF airway bacteria are rarely eradicated by antibiotics due to their ability to form biofilms (BFs). Little is known about the antimicrobial activities of host phagocytes and of antipseudomonal antibiotics (AAs) against PA BFs. We aimed to examine the in vitro activities of 3 AAs [Amikacin (AMK), Ceftazidime (CEF) and Ciprofloxacin (CIP)] alone and in combination with PMNs against PA BFs and compare these activities to those of their planktonic (PL) counterparts.
Methods: Six CF clinical isolates of PA, either susceptible to AMK (AMKS), CEF (CEFS), CIP (CIPS) or resistant to AMK (AMKR), CEF (CEFR), CIP (CIPR) were used. All isolates were grown by incubation in cation-adjusted Mueller-Hinton broth in 96-well flat-bottomed plastic plates under constant shaking for 48h at 37ºC in order to form BFs. PMNs from healthy donors at an effector-to-target (E:T) ratio of 1:10 or 1:20 were incubated further for 24h alone or in combination with 2, 8 or 32mg/l of AMK, CEF or CIP. Percent damage of BF or PL was assessed by XTT assay. Synergy was concluded when the observed bacterial damage was significantly higher to the expected sum of damages; whereas, antagonism was defined when the observed bacterial damage was significantly lower to the expected sum of damages. ANOVA (n = 6) with Dunnett's test was performed.
Results: PMNs damaged BFs and PL in an E:T ratio-dependent pattern; however, PMN-induced damage of BFs was significantly lower than that of PL even when PMNs were combined with each of the 3 AAs. BF or PL damages of susceptible isolates were relatively higher than those of resistant isolates. Synergy was observed when PMNs (at 1:10) were combined with AMK (8 or 32mg/l) for both AMKR and AMKS isolates in BF and PL cells. Antagonism was observed when PMNs (at 1:20) were combined with CEF (2, 8 or 32mg/l) for AMKR, with CEF (8mg/l) for AMKS, CIP (32mg/l) for CIPR and CIP (2 or 8mg/l) for CIPS.
Conclusions: PA BFs are more resistant than PL to the activities of PMNs, of AAs alone and of the combinations of PMNs with the 3 AAs. Synergy is exhibited between PMNs and AMK against BFs of PA, while antagonism is exhibited between PMNs and CEF or CIP. Even high concentrations of AAs alone or in combination with PMNs do not achieve eradication of PA, suggesting a possible mechanism of PA persistence in the respiratory tract of CF patients.

R2282 Biofilm-associated eye infections: an evaluation of the published evidence

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Objective: Biofilm-associated eye infections are increasingly being recognized as a diagnostic and therapeutic problem. We sought to systematically review the relevant published literature on the clinical characteristics of these infections.
Methods: We searched in PubMed and Google Scholar to identify articles that provided clinical data on patients with eye infections associated with the presence of bacterial or fungal biofilm, documented by electron scanning microscopy.
Results: We identified 12 articles involving 15 cases of documented biofilm-associated eye infection. The infectious syndromes in regard were infectious crystalline keratopathy (6 cases), keratitis (4 cases), endophthalmitis (2 cases), corneal abscess (1 case), conjunctivitis (1 case), and conjunctivitis with scleritis and corneal perforation (1 case). All 15 patients had prior ophthalmologic surgery. In 8 of the 15 patients, the infection was associated with the presence of a foreign body; the foreign body was a corneal suture (3 cases), intraocular lens (3 cases), punctual plug (1 case) and soft contact lens (1 case). In the remaining 7 of the 15 included cases, the biofilm-associated eye infection developed on native tissue; the presence of a biofilm was documented in corneal biopsy specimen in 5 cases, extracted corneal tissue in 1 case, and extracted bacterial concretion in the remaining case. Various causative pathogens were isolated. Treatment was eventually successful in all cases. In those with a foreign body, cure was achieved only after foreign body removal. Regarding the 7 cases with no foreign bodies, keratoplasty was required in 3 cases and evisceration of the eye 1 case.
Conclusion: Our findings suggest that biofilm-associated eye infections can develop on both foreign bodies and native tissue. Most of the published clinical cases refer to infections of the anterior hemisphere of the eye and particularly the cornea. Yet, this could be related to the diagnostic problems of the documentation of a biofilm. Further studies are need to better appreciate the particular characteristics of these infections.

Antimicrobial pharmacokinetics, pharmacodynamics, general pharmacology

R2283 Therapeutic drug monitoring of clindamycine in osteo articular infections: influence of body-weight on the pharmacokinetic variability

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Rationale and Objective: Clindamycin (CD), usually active against *Staphylococcus aureus*, has been recommended as one of the antibiotic to be used in combination (with vancomycine, rifampicin or quuinolone) in osteo articular infections by several infectious diseases societies. Our objective was to determine if the usual dosage of CD of 600 mg TID were adequate to obtain sufficient concentrations in bone.
Method: A prospective study included all patients treated with IV or oral CD from 11/2008 to 11/2010, for an osteo articular infection. CD peak concentrations were measured 30 min after IV infusion or 1.5 h after oral

dose. CD trough concentrations were measured between 7 and 9 h. The target residual concentration in blood was set to 2 mg/L, considering that the breakpoint for *S. aureus* is 0.5 mg/L, that the bone penetration of CD is only around 30%, and that CD displays time-dependant activity.
Results: Values are given as mean (first & third quartile). 58 patients, 31 male, 27 female, mean age 56 y-o, were included. The dosage of 600 mg TID of clindamycin led to a mean dose reported to weight 27.2 mg/kg/d (22.5–30). With this dose, the mean CD trough concentration was 2.06 mg/L (0.60–3.15) and 63.5% of the dosages were <2 mg/L. The CD mean peak concentration was 7.86 mg/L (5.78–9,33). Oral or IV regimen did not lead to significant differences in CD peak concentrations (8.62 vs 6.87 mg/L; $p = 0.09$) as well as CD trough concentrations (2.19 vs 1.93 mg/L, $p = 0.73$). A significant correlation was evidenced between the daily dose reported to weight and CD trough concentration ($p = 0.04$, analysis of covariance, with time of measure as covariable). A trough concentration over the target concentration of 2 mg/L was obtained in 44% of the patients having a daily dose >25 mg/kg/d and in 25% of the patients with a dose <25 mg/kg/d.
Conclusion: A clear relation was evidenced between CD through concentrations and CD daily dose reported to weigh. The daily dosage of clindamycin should not be uniform in all patients but reported to weight and should reach at least 25 mg/kg/d in order to reach sufficient concentrations in bone.

R2284 Moxifloxacin concentrations in plasma and pancreatic pseudocyst fluid of patients with chronic pancreatitis

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Objectives: To estimate the concentrations of moxifloxacin (MOX) after single intravenous injection in blood plasma and pancreatic pseudocyst fluid samples obtained from patients with chronic pancreatitis.
Materials and Methods: A total of 26 blood samples and 27 pancreatic pseudocyst fluid samples were obtained from 26 surgical patients with chronic pancreatitis complicated with pancreatic pseudocyst in 2008–2010. Blood samples were taken by venous puncture and pancreatic pseudocyst fluid samples were collected by ultrasound controlled transcutaneous aspiration or during abdominal operation at 3 hours after a single intravenous injection of moxifloxacin (400 mg). MOX concentrations were determined using a reversed-phase high-performance liquid chromatography (RP-HPLC) assay. The chromatographic separation was achieved on Symmetry C18 column (3.9x150 mm) using a mixture of 15% acetonitrile, 85% 50 Mm ammonium chloride, 7 Mm tetrabutylammonium hydroxide (pH 3.0 adjusted with citric acid) as the mobile phase with isocratic system at a flow rate of 1 ml/min. Fluorescence detection was employed with excitation at 287 nm and emission at 465 nm. Gemifloxacin was used as internal standard. Clinical samples were prepared by mixing aliquots of plasma or pseudocyst fluid with equal volumes of acetonitrile and diluting the supernatant obtained after centrifugation 2-fold in water. The assay was validated by analyzing spiked quality control samples and showed accuracy and precision of ±3%, and linearity of determination in the range of 0.125–4 mg/l with correlation coefficient of $\geqslant 0.9998$.
Results: The MOX concentrations in pseudocyst fluid samples of patients ranged from 0.04 to 1.98 (mean±SD = 0.57±0.41) mg/l. The corresponding MOX concentrations in plasma samples were 0.86 to 2.45 (mean±SD = 1.67±0.39) mg/l.
Conclusion: The concentrations of moxifloxacin in pancreatic pseudocyst fluid achieved 3 hours after a single 400 mg iv injection were generally lower than the corresponding concentrations in plasma but exceeded the epidemiological cut-off MIC values for most bacterial pathogens reported by EUCAST. These data indicate that moxifloxacin may potentially be effective for perioperative prophylaxis in patients with chronic pancreatitis.

R2285 Risk factors to obtain low colistin peak-plasma concentrations in patients with multidrug-resistant Gram-negative bacterial infections

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Objectives: Colistin use has remerged for the treatment of multidrug resistant Gram-negative (MDR-GNB) infections but there is a lack of information about the clinical and demographic patient's characteristics that could modify colistin plasma concentrations. The aim of the study was to assess the risk factors to obtain a colistin peak plasma concentrations (Cmax) lower than 1 mcg/ml in patients with MDR-GNB infections treated with colistimethate sodium (CMS) at dosages used in clinical practice.
Methods: Observational prospective pharmacokinetic study performed in hospitalized patients with MDR-GNB infections treated with CMS. Colistin Cmax (30 minutes after the end of the infusion) at steady-state was obtained in all the studied patients and quantified using a validated HPLC method. Data collected: age, gender, body mass index (BMI), Acute Physiology and Chronic Health Evaluation II score (APACHE II), gromerular filtration rate at the beginning of CMS treatment (GFR), presence of sepsis (SEP) or septic shock (SHOCK), total plasma proteins (TPP), human serum albumin (ALB), CMS dose, colistin Cmax and concomitant administration of vasoactive drugs and/or diuretics (VASO/DIUR). In univariate analysis the differences between patients with colistin Cmax higher or equal to 1 mcg/ml and those with Cmax lower than 1 mcg/ml were assessed.
Results: 36 patients. Mean values: age 65.8 (SD 15.7) years; 30 (83.3%) men; BMI: 25.0 (SD 5.3) kg/m^2; APACHE: 9.5 (SD 4.6); 23 patients (63.9%) with GFR higher or equal to 80 ml/min; 19 (52.8%) SEP; 2 (5.6%) SHOCK; TPP: 5.5 (SD 0.90) g/dl; ALB: 2.7 (SD 0.5) g/dl; CMS dose: 5.1 (SD 2.2) mg/kg; Cmax: 1.2 (SD 0.8) mcg/ml and VASO/DIUR: 14 (38.9%). Differences between patients with Cmax higher or equal to 1 mcg/ml versus those with Cmax lower than 1 mcg/ml are shown in table 1.
Patients with a GFR higher or equal to 80 ml/min presented a 7.150 times higher risk to obtain a colistin Cmax lower than 1 mcg/ml. Additionally, younger age was correlated with the achievement of lower colistin Cmax.
Conclusions: Younger patients with good renal function treated with colistin for MDR-GNB infections present a higher risk to obtain peak plasma levels far from the cut-off values for colistin bacterial susceptibility, regardless of their gender, BMI and CMS administered dose. In those patients the efficacy of colistin should be narrowly followed and colistin plasma concentrations during treatment should be monitored.

R2286 The pharmacokinetics/pharmacodynamics of meropenem in patients with febrile neutropenia and bacteraemia

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Objectives: The aim of this study was to compare the probability of target attainment (PTA) and cumulative fraction of response (CFR) for meropenem between administration by bolus injection and a 3 h infusion in febrile neutropenic patients with bacteremia.
Methods: The study was a randomized three-way crossover in eight febrile neutropenic patients with bacteremia. Each subject received meropenem in three regimens consecutively: (i) bolus injection of 1 g every 8 h for 24 h; (ii) a 3 h infusion of 1 g every 8 h for 24 h; and (iii) a 3 h infusion of 2 g every 8 h for 24 h. The Monte Carlo simulation was performed to determine the probability of attaining a specific pharmacodynamic target at various regimens. The CFRs were determined for each regimen against each population of *E. coli*, *Klebsiella* spp., *P. aeruginosa* and *Acinetobacter* spp.
Results: The PTA for three regimens are listed in the table.
The predicted CFRs for PTA achieving a target of 40% T>MIC for *E. coli* following the administration of meropenem by a bolus injection of 1 g every 8 h, a 3 h infusion of 1 g every 8 h and a 3 h infusion of 2 g

every 8 h were 97.03%, 99.88% and 99.96%, respectively. The predicted CFRs for PTA achieving a target of 40% T>MIC for *Klebsiella* spp. were 97.07%, 99.92% and 100%, respectively.

Conclusion: A 3 h infusion of 2 g of meropenem every 8 h resulted in the highest PTA rates. The three regimens of meropenem had high probabilities of achieving optimal impact against *E. coli* or *Klebsiella* spp.

MIC (mcg/mL)	PTA 40% *T*>MIC			PTA 60% *T*>MIC			PTA 80% *T*>MIC		
	Bolus injection	3 h infusion 1 g	3 h infusion 2 g	Bolus Injection	3 h infusion 1 g	3 h infusion 2 g	Bolus injection	3 h infusion 1 g	3 h infusion 2 g
1	99.7	99.99	100	84.02	99.93	100	49.01	87.01	97.56
2	97.07	99.92	100	56.74	98.12	99.95	22.88	57.95	87.18
4	75.7	99.24	99.96	20.16	73.73	98.06	3.97	17.56	59.77
8	17.83	78.79	99.24	0.37	9.26	74.76	0	0.1	16.82

R2287 Urinary pharmacokinetics and bactericidal activity of finafloxacin (200 and 800 mg) in healthy volunteers receiving a single oral dose

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Introduction and Objectives: Finafloxacin is a novel 8-cyano-fluoroquinolone under investigation for treatment of urinary tract infections. In contrast to other fluoroquinolones its antibacterial activity is enhanced in an acidic environment. An acidic environment is frequently found during infectious processes and in urine. The aim of this phase 1 study was to investigate urinary concentrations as well as urinary bactericidal titers (UBT) at different pH levels in healthy volunteers.

Material and Methods: Urinary concentrations and UBTs of finafloxacin 200 and 800 mg single doses in 6 healthy volunteers were measured up to 48 hours. Urinary concentrations were measured by a HPLC-MS/MS method. UBTs were determined for a reference strain and 9 selected clinical uropathogens at the pH of native, acidified (pH 5.5) and alkalinized (pH 8.0) urine.

Results: Mean maximum urine concentration of 200 and 800 mg finafloxacin was 69.3 mg/L (0 to 2 hours) and 150 mg/L (4 to 8 hours). Median UBTs were between 0 and 1: >2,048 and were in general agreement with MIC of strains and urinary pH values. UBTs in alkaline urine were significantly lower compared with those in native or acidic urine except for *Enterococcus faecalis*.

Conclusions: Finafloxacin exhibited significant bactericidal activity against susceptible uropathogens. The urinary bactericidal activity of finafloxacin was enhanced in acidic urine and significantly lower in alkaline urine.

Mechanisms of action and resistance

R2288 Osteomyelitis associated to CTX-M-15-producing *Aeromonas hydrophila*: first description in Europe

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Objective: We describe, to the best of our knowledgement, the first case of CTX-M-15-producing *Aeromonas hydrophila*.

Methods: A 37-year-old woman was hospitalised in May of 2009 for an open fracture grade III of the right tibial pilon. External fixator were placed and *Pseudomonas aeruginosa* and *Staphylococcus aureus* resistant to methicillin (SARM) were isolated from the wound. The patient got better and two months later, the patient was discharged with a treatment consisting on levofloxacin+rifampin due to a new isolation of SARM in the wound. In March of 2010, the patient returned to the hospital with an acute clinical pictures of suppuration through an ankle fistula. A surgery resection of the distal end of the tibia, affected by osteomyelitis, was done. The wound and surgical bone tissue specimens were processed.

Results: Gram-negative bacillus was isolated from both, wound exudates and bone samples. The isolate was identified as *A. hydrophila* by both phenotypic and genotyopic methods. The isolate showed resistance to ampicillin, cefazolin, cefotaxime, cefepime, gentamicin, tobramycin and ciprofloxacin; decreased susceptibility to amoxicillin/clavulanic acid, ceftazidime and aztreonam; and susceptibility to cotrimoxazole, amikacin, piperacillin/tazobactam and carbapenems. Clavulanic acid recovered the activity of cefotaxime and ceftazidime. The patient was successfully treated with meropenem+amikacin. Standard PCR conditions were used to amplify the genes blaTEM, blaSHV, blaCTX-M and blaOXA; sequence analysis of PCR products obtained identified TEM-1 and CTX-M-15 ESBL, no PCR products were obtained using the remaining primers. This isolate had also the aac(6′)-Ib-cr and the aac(3)-IIa aminoglycoside resistance genes.

Conclusions:

1. We present the first known case of osteomyelitis associated to a CTX-M-15-producing *A. hydrophila* isolate carrying also the blaTEM-1 gene, as well as genes conferring resistance to aminoglycosides, aac(3)-IIa, and to aminoglycosides and ciprofloxacin, aac(6′)-Ib-cr.
2. This report confirms the high dissemination capacity of the plasmids carrying CTX-M-15, and other antibiotic resistance determinants, not only between *Escherichia coli* or *Klebsiella pneumoniae*, as demonstrated, but also between other Gram-negative species as *A. hydrophila*.

R2289 Antimicrobial resistance of *Escherichia coli* clinical isolates causing neonatal sepsis

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Objectives: Neonatal risk factors for invasive bacterial disease and its diagnosis and therapy remain an important medical problem. Neonatal meningitis and septicemia caused by *Escherichia coli* and *Streptococcus agalactiae* are still major health problems in industrialized countries. The objective of the present work was to evaluate the antimicrobial resistance of *E. coli* strains causing early and late neonatal sepsis and to compare them with *E. coli* strains isolated from healthy neonates.

Methods: Sixty-seven *E. coli* strains were included in the study (27 from early neonatal sepsis, 40 from late neonatal sepsis and 28 from healthy neonates). Samples from healthy neonates were obtained from ear and pharyngeal swabs. Minimal inhibitory concentrations were determined using the MicroScan-Negative MIC Panel Type 37 (NM37, Siemens). Detection and characterization of determinants of resistance, integrons, and gyrA and parC mutations were carried out by PCR and sequencing.

Results: No statically significant differences were found in the antimicrobial resistance between strains from early and late neonatal sepsis. However, resistance to chloramphenicol and piperacillin was significantly more frequent among strains collected from sepsis than from healthy neonates ($p = 0.05$ and 0.0004, respectively). No differences were found among the resistance to the antimicrobial agents used to treat late neonatal sepsis (amikacin, ceftazidime and imipenem). Only two strains were found to carry BLEAS, one from early neonatal sepsis, which was CTX-M14 and one from late neonatal sepsis (CTX-M15.) Fifty-eight percent of strains from late neonatal sepsis were resistant to tetracycline showing a high variety of resistance genes (tetA, B, C, D and G) whereas only 37% of strains from early neonatal sepsis were resistant to this antimicrobial agent. Twenty strains (30%) presented class-1 integrons with different combination of gene cassettes. Finally, among the eight strains resistant to ciprofloxacin, four presented mutations in the amino acid codons Ser83Leu and Asp87Asn from the gyrA gene, two only Ser83Leu and one Asp87Lys. Of these, five also presented a mutation in the parC gene (Ser80Ile) and one also a mutation in the amino acid codon Gly84Val.

Conclusions: *E. coli* strains causing neonatal sepsis were more resistant to the antimicrobial agents studied than the strains collected from

healthy neonates except in those related to ciprofloxacin and gentamycin resistance.

R2290 Mechanisms of macrolide resistance in Polish *Campylobacter jejuni* and *C. coli* strains isolated from chicken meat, 2006–2009

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Objectives: Macrolides such as erythromycin and azithromycin have been used as drugs of choice for the treatment of human campylobacteriosis. Studies conducted in Poland during 2003–2005 did not find macrolide resistance in *Campylobacter* isolates of chicken origin.
The aim of this study was to examine the prevalence and genetic background of macrolide resistance in Polish strains of *C. jejuni* and *C. coli* isolated from chicken carcasses during 2006–2009.
Methods: A total of 306 chicken meat specimens, obtained in selected supermarkets in Warsaw from 2006 through 2009 were studied. Isolation and identification of *Campylobacter* sp. were performed according to the International Organization for Standardization guideline 10272. Susceptibility to erythromycin was determined by the E-test and the disk diffusion method. The PCR-RFLP and sequencing were used for the detection of A2074C and A2075G mutations in the 23S rRNA gene. Modifications in the L4 and L22 ribosomal proteins were analysed using PCR and sequencing. Inhibition of the efflux pump was determined on Mueller-Hinton agar plates supplemented with phenyl-arginine-b-naphthylamide (PAbN).
Results: A total of 208 *Campylobacter* strains were isolated (52 *C. jejuni* and 156 *C. coli*). Nineteen isolates (9.1%) were found resistant to erythromycin by the disk diffusion method, but only 12 (5.8%) were resistant by the E-test method. The A2075G mutation in the 23S rRNA gene was identified in 8 of 12 isolates displaying high-level resistance to erythromycin, whereas the A2074G mutation was found in 1 isolate. Sequence analysis of rplD and rplV genes of erythromycin-resistant strains showed modifications in the L4 and L22 proteins only in few isolates. The efflux pump inhibitor (PabN) increased the susceptibility to erythromycin in 3 resistant isolates.
Conclusions: Erythromycin-resistance in Polish *Campylobacter* isolates of chicken origin is mediated mainly by A2075G and A2074G mutations in the 23S rRNA gene, whereas modifications in the L4 and L22 proteins, and the efflux pump have only modest impact on macrolide-resistance. The risk of transmission of resistant strains from animals to humans via food chain requires constant monitoring of resistance in *Campylobacter* clinical isolates.

R2291 Impact of the new EUCAST breakpoints and rules on β-lactam susceptibility reporting for ESBL-producing enterobacteria

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Objectives: Production of extended-spectrum β-lactamases (ESBLs) is the most important mechanism of resistance to β-lactam antibiotics among Enterobacteriaceae. Based on the 2010 EUCAST criteria, susceptibility results for penicillins, cephalosporins, and monobactams should be reported as found in strains that produce ESBLs, when using the lowered breakpoints. Our study aimed to evaluate the impact of the new EUCAST breakpoints and rules on β-lactam antibiotic susceptibility reporting for ESBL-producing enterobacteria.
Methods: We studied 262 ESBL-producing Enterobacteriaceae collected at the Manzoni Hospital (Lecco, Italy) from January 2009 to June 2010: *Escherichia coli* (n = 195), *Proteus mirabilis* (n = 50), and *Klebsiella pneumoniae* (n = 17). Identification and antimicrobial susceptibility were determined using the Vitek2 automated system (bioMérieux, Marcy l'Etoile, France). ESBL production was assessed using phenotypic tests and confirmed by molecular methods.
Results: In 64/262 cases (24.4%), at least one of expanded-spectrum cephalosporins (cefotaxime, ceftazidime, cefepime) showed an MIC value ⩽1 mg/L, thus being reported as susceptible according to current EUCAST criteria. In 4/64 cases, these microorganisms had been obtained from blood cultures. Similar percentages of low values were found in *E. coli* (25.1%), *P. mirabilis* (22%), and *K. pneumoniae* (23.5%), although some features differed depending on ESBL type. Concerning tested drugs, ceftazidime (37/262, 14.1%) and cefepime (40/262, 15.3%) showed similar values, whereas cefotaxime rarely had an MIC ⩽1 mg/L (7/262, 2.7%).
Conclusions: Our data demonstrate that the adoption of the new EUCAST interpretive criteria will result, in a high number of cases, in categorization of ESBL producers as susceptible to expanded-spectrum cephalosporins. In absence of outcome data demonstrating efficacy of expanded-spectrum cephalosporins in these cases, it would seem advisable to keep informing clinicians about ESBL production in clinical isolates from serious infections.

R2292 *Aeromonas hydrophila* resistant to carbapenems: discrepancy in the results between E-test and microdilution method

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Objectives: *A. hydrophila* harbours several chromosomal β-lactamase (CepH, AmpH and ImiH or CphA) that are regulated by blrA/blrB and under selective pressure of certain antibiotics can be overexpressed jointly. CphA is a metallo-β-lactamase (MBL) with moderate carbapenemase activity. The objective is to communicate the observed discrepancy in carbapenems MIC values of clinical isolates of *Aeromonas hydrophila* determined by E-test and commercial microdilution system.
Methods: Three strains of *A. hydrophila* isolated from CSF, BAS and faecal specimen respectively were tested for antimicrobial activities of imipenem, meropenem and ertapenem using commercial microdilution system (Wider, Soria-Melguizo, Spain) and E-test (AB Biodisks) following manufacturer's indications. Phenotypical detection of ESBL, AmpC, and modified Hodge's test were done. CphA gene PCR and rep-PCR were also performed.
Results: All the strains were considered carbapenems resistant by commercial microdilution system and susceptible by E-test (0.5 McFarland). MIC's values are shown in Table 1. E-test for imipenem was repeated using a higher inoculum (4 MacFarland), obtaining MIC's values of 8 mg/L. Neither ESBL nor Amp C were detected, and modified Hodge's test were negative. The cphA gene was identified in all strains. The three isolates showed different rep-PCR patterns.
Conclusions: The MIC values obtained by microdilution do not correlate with those obtained by the E-test method. Presence of MTB expression encoded by cphA gene is better detected using higher inocula with commercial microdilution systems than E-test. The bacterial density in many infections is higher than that used in conventional MICs determinations so, for *Aeromonas* spp. in vitro studies, particularly E-test, is important to increased the inocula at least 4 McFarland to obtain accuracy results.

A.hydrophila	IMIPENEM		MEROPENEM		ERTAPENEM	
strains	Etest	Wider	Etest	Wider	Etest	Wider
1	2 mg/L	>8 mg/L	0.5 mg/L	>8 mg/L	0.5 mg/L	>4 mg/L
2	⩽1 mg/L	>8 mg/L	⩽2 mg/L	>8 mg/L	⩽0.5 mg/L	>4 mg/L
3	2 mg/L		⩽1 mg/L		⩽0.5 mg/L	

R2293 Comparison of antagonistic activity of lactic acid bacteria strains isolated from humans and food

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Objectives: Lactic Acid Bacteria (LAB) species are common bacteria in the environment. Often, LAB are isolated, among others, from food, human colon but foremost from human vagina. *Streptococcus agalactiae* (GBS) is one of the coexistent components of the vaginal microflora and it can trigger newborn infection. Metabolites with antagonistic properties

of LAB protect against multiplication of pathogenic microorganisms as well as of GBS. Therefore, the aim of the study was to assess of sensitivity of GBS strains in relation to capsular polysaccharides of GBS to antagonistic activity of chosen LAB strains isolated from vagina and food.

Methods: The antagonistic properties of *L. plantarum* (n = 3), *L. fermentum* (n = 2), *L. gasseri* (n = 2), *L. rhamnosus* (n = 2) originating from the vagina of healthy women and control bacteriocin-producing strains such as *L. plantarum* C11, *L. sakei* DSMZ 6333, *L. lactis* ATCC 11454 and *L. rhamnosus* GG ATCC 53103 were tested. In the antagonism study, 26 strains of GBS from human sources belonging to Ia, Ib, II, III or V serotypes were used as indicator bacteria. Antagonism between LAB and GBS was tested in a mixture of fluid 24 h cultures and the results were determined quantitatively by serial dilutions in 3 time intervals (10 min, 2 h, 4 h).

Results:

1. Most investigated strains of LAB have an ability to inhibit the growth GBS within 2 h after application.
2. *L. plantarum* strains were the most effective LAB strains isolated from vagina against GBS, on the other hand *L. fermentum* showed the weakest inhibitory activity against GBS strains.
3. The *L. plantarum* C11 control strain isolated from fermented cucumber, displayed comparable antimicrobial activity against GBS as *L. plantarum* strains originating from vagina.
4. Statistically significant relationship was confirmed for sensitivity of GBS serotypes to vaginal LAB and control LAB effect. GBS strains with serotype III were the least sensitive to LAB activity and the most sensitive were GBS strains belonging to serotypes V and II.
5. The control strains isolated from food, such as *L. sakei* DSMZ 6333 and *L. lactis* ATCC 11454 did not show antimicrobial activity against GBS strains.

Conclusions: LAB control strains isolated from food were less effective against GBS than LAB strains isolated from vagina. More studies are needed to elucidate the antibacterial property of vaginal *L. plantarum* as the producer of antimicrobial substances and the opportunity of its application as a probiotic preparation.

R2294 Detection of OXA-48-encoding plasmid in a clinical strain of *Enterobacter cloacae* isolated in Spain

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Objectives: The global spread of ESBLs has driven therapeutic choice towards carbapenems and lead to emergence of carbapenem resistance mechanisms. Carbapenemases now represent a public health challenge. Class D OXA-48 is one of the few members of this family with carbapenem-hydrolyzing activity. They are involved in outbreaks in various geographical regions, in particular in countries from the eastern and southern Mediterranean region. We described the first strain of *Enterobacter cloacae* with a plasmid-encoded bla OXA-48 gene in Spain.

Methods: In September 2010 an *Enterobacter cloacae* strain with reduced susceptibility to imipenem was isolated from sputum specimen corresponding to a 77 years-old man with global chronic respiratory failure exacerbation. Antimicrobial susceptibility tests were determined by a commercial microdilution system (Wider, Soria Melguizo) following manufacturer's indications. MICs of carbapenems were also performed by using the E-test method, and interpreted according to the CLSI standard. Phenotypic detection of carbapenemases was determined using modified Hodge's test (MHT). Molecular analysis of plasmid-encoded β-lactamases genes was performed using Check Carba ESBL (Check-Points, Hain Lifescience), a new molecular rapid system based in microarray platform with objective analysis by software.

Results: The strain was resistant to all the tested cephalosporins, including cefepime, and MICs by Etest for imipenem and meropenem were 2 mg/L and 8 mg/L respectively. MHT was positive. Results by Check-Point Carba ESBL showed the simultaneous detection of bla OXA-48, bla CTX-M9 and bla SHV-12 like.

Conclusions: The class D β-lactamases OXA-48 conferring decreased susceptibility to carbapenems has been reported from *Klebsiella pneumoniae* and *Escherichia coli*. However, its origin seems to be in *Shewanella* and other environmental Enterobacteriaceae. We report the first detection of OXA-48 in a clinical strain of *Enterobacter cloacae* in southern Spain. Since this plasmid confers a low level of resistance to carbapenems, clinical laboratory detection of OXA-48-producing strains may be difficult and can be very useful the molecular detection by rapid methods as microarray systems.

R2295 *Klebsiella pneumoniae* carbapenemase in Ontario, Canada: continuous surveillance of a worldwide disseminated β-lactamase

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Objective: The first *Klebsiella pneumoniae* carbapenemase (KPC) was detected in Ontario, Canada, in 2008 at the Ontario Public Health Laboratories (OPHL), the provincial reference laboratory in antimicrobial resistance. Since then, the OPHL has been performing a continuous surveillance of carbapenemase-producing Enterobacteriaceae in the province. The goal of this study was to characterize the blaKPC positive isolates received at the PHL during the period of April 2008-September 2010.

Methods: Antimicrobial susceptibility profiles of the blaKPC-positive isolates (confirmed by PCR) were determined using Etest (Clinical and Laboratory Standards Institute guidelines, 2010). Molecular screening of other β-lactamase genes in these KPC-producing isolates was performed by PCR (blaTEM, blaSHV, blaOXA-1-like, blaCTX-M groups 1, 2 and 9, blaVEB, blaPER, blaGES blaOXA-48-like, blaIMP, blaVIM, blaNDM-1, and 6 groups of blaAmpC genes). The molecular typing of the blaKPC positive isolates was determined by pulse-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). The blaKPC genetic platforms were analyzed by PCR-cartography.

Results: 15 clinical isolates (1 per patient) were detected as blaKPC positive during the study period: 4 from 2008, 3 from 2009, and 8 from 2010. The isolates (all *K. pneumoniae*) were submitted by 6 different hospital laboratories, most of them from the Great Toronto Area (n = 13). The susceptibility profile is shown in the Table. Broad MIC ranges for ertapenem (3 to ⩾32 μg/ml), meropenem (1 to ⩾32 μg/ml) and imipenem (1.5 to ⩾32 μg/ml) were observed. As expected, all the strains were positive for blaKPC (mostly blaKPC-2) and blaSHV. Thirteen of them were also positive for blaTEM, and 2 for blaOXA-1-like. No other β-lactamase genes were detected. All the isolates were closely related by PFGE (the profiles exhibited >80% similarity). By MLST, 13 isolates belonged to the sequence type (ST) 258, and the 2 remaining to the ST437, which is closely related to ST258 (6 identical loci and the 7th – tonB – differing by 4 point mutations). By PCR-cartography, blaKPC genes were identified on Tn4401 transposon, variants a (11 isolates) and b (4 isolates).

Conclusion: Since August 2008 to September 2010, only 15 multidrug resistant *K. pneumoniae* KPC-producers were detected at OPHL. According to the typing data, the slow dissemination of blaKPC in Ontario is consequence of a clonal spread of ST258 or related.

Table. Antimicrobial susceptibility profiles of the 15 *K. pneumoniae* positive for bla_{KPC} genes.

Antibiotics	MIC (μg/ml)														
	GN25	GN26	GN27	GN66	GN178	GN203	GN346	GN446	GN447	GN458	GN460	GN515	GN517	GN524	GN542
Ampicillin	⩾256	⩾256	⩾256	⩾256	⩾256	⩾256	⩾256	⩾256	⩾256	⩾256	⩾256	⩾256	⩾256	⩾256	⩾256
Cefoxitin	⩾256	48	32	256	256	64	24	32	48	64	32	48	24	24	24
Ceftazidime	⩾256	⩾256	⩾256	⩾256	⩾256	⩾256	128	192	⩾256	256	⩾256	⩾256	12	12	192
Cefotaxime	64	32	32	128	256	256	24	32	32	48	32	48	6	6	32
Cefepime	64	16	16	96	256	48	12	16	24	24	12	24	4	4	12
Ertapenem	⩾32	12	16	⩾32	⩾32	32	12	24	16	32	12	8	8	3	6
Meropenem	32	3	8	16	⩾32	12	2	6	6	6	3	6	2	1	1.5
Imipenem	12	4	12	⩾32	⩾32	32	3	24	12	6	2	6	1.5	1.5	1.5
Amikacin	24	48	48	24	96	64	48	192	96	64	64	192	12	12	48
Gentamicin	12	8	6	8	3	2	12	3	2	2	2	3	1.5	1.5	12
Tobramycin	24	24	24	24	64	32	48	96	48	32	32	64	24	24	32
Ciprofloxacin	⩾32	⩾32	⩾32	⩾32	⩾32	⩾32	⩾32	⩾32	⩾32	⩾32	⩾32	⩾32	⩾32	⩾32	⩾32
Tetracycline	4	6	4	6	6	6	6	6	6	6	8	8	192	128	4

R2296 Correlation between rifampicin consumption and resistance among *Acinetobacter baumannii* causing infections at a university hospital

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Objectives: To study the evolution of resistance to rifampicin by *A. baumannii* in the years 2008 and 2009, and consumption of rifampicin during the years 2007 and 2008 and analyse their correlation.
Methods: Retrospective ecological study conduced in a teaching hospital, with two centers, General Hospital and Traumatology Hospital, which was analysed a) the use of rifampicin since January 2007 to December 2008, by determining the defined daily dose (DDD/100 patient-days); and b) the development of resistance to rifampicin by *A. baumannii* since January 2008 to December 2009, by number of clinical isolates of rifampin resistant *A. baumannii*/1000 patient-days. (MIC >16 mg/L according to the French Society of Microbiology). These tests have been performed globally and inpatient areas. We analyzed the difference between consumption, MIC_{50} and MIC_{90}, in the mentioned period, by Mann-Whitney test. The evolution of the rate of resistance was tested by X2 test. Finally we have analysed the association between consumption of rifampicin and the resistance rate by Spearman Rho test.
Results: The consumption of rifampicin in 2007 and 2008 was 0.85 and 1.11 DDD/100 patient-days in the Hospital. In intensive care units, 0.43 and 2.37 DDD/100 patient-days. Medical and surgical services was 0.88 and 1.01 DDD/100 patient-days in the same years. During the years 2008 y 2009 were identified 171 and 310 strains of *A. baumannii* in clinical samples.
Conclusions: The data suggest the existence of correlation between the rifampicin consumption and resistance among *A. baumannii* causing infections at ICUs.

	UCI	Medical and surgical services
Consuption of rifampicin (DDD/100 patient-days)		
2007	0.43	0.88
2008	2.37	1.01
MIC 50 /MIC 90		
2008	2/6	2/32
2009	2/32[1]	4/32[2]

1. p=0.002 r=0.17
2. p=0.5

R2297 A nonsense mutation in MutS associated with a hypermutable phenotype in a clinical isolate of *Enterococcus faecalis*

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Introduction: Mutations in the DNA Mismatch Repair (MMR) genes, mutSL (equivalent to hexAB locus of pneumococci), have been associated with hypermutability phenotypes in various bacterial species and may play a significant role in the antibiotic resistance emergence.
Objectives: The mutator frequency of 233 consecutive non-redundant *Enterococcus faecalis* isolates collected in a 2001 extra-hospital survey was investigated.
Methods and Results: To detect mutators, a first approach was based on an agar diffusion method using a fosfomycin disk, and the identification of hypermutable strains was achevied by counting the "squatter" colonies growing in the inhibition zone after 24 h of incubation. Four strains (Ef1468, Ef1473, Ef1497 and Ef1591) with more than 50 Colony Forming Units within the fosfomycin inhibition area were considered as mutators. Their mutation frequency was evaluated on agar medium with or without rifampicin. The rates were comprised between 2.2×10^{-5} to 7.3×10^{-6} (SD, 2.4×10^{-5} to 7.3×10^{-6}), i.e. at least 100-fold higher than five other tested strains taken at random in the collection, including the *E. faecalis* strain ATCC 29212 (frequency, $<10^{-9}$). The mutators were isolated from urine (Ef1468, Ef1473 and Ef1497) or sperm fluid (Ef1591). The analysis of mutSL was investigated for Ef1497 by PCR amplification and sequencing experiments. Five (A321T, A323S, A397E, K415N, I800S) and three (A437V, A440E, A495T) amino acid (aa) substitutions in MutS (858 aa) and MutL (710 aa) respectively, were identified in comparison to the reference sequence (GenBank accession no. AE016830). In addition, a nonsense mutation of the arginine at position 341 led to the inactivation of MutS; this mutation was associated with the hypermutability of Ef1497.
Conclusions: The mutator frequency in a clinical population of *E. faecalis* was 1.7%. In a previous study, we have shown that linezolid mutants emerged more readily from Ef1497 than from *E. faecalis* ATCC 29212 (Ba et al., 2010, Antimicrob. Agent Chemother., 54:1443–52). Hypermutation capability of strains should be detected by the simple agar diffusion method using a fosfomycin disk, prior to a prolonged antibiotic monotherapy treatment. Our study gives the first evidence of an association of a MMR gene inactivation with a mutator phenotype in a clinical *E. faecalis* strain.

R2298 Class 1 integrons and associated resistance gene cassettes among enteropathogenic *Escherichia coli*

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Objectives: Infectious diarrheal diseases is recognized as the second cause of mortality among infectious diseases in children less than five years. Enteropathogenic *Escherichia coli* (EPEC) play an important role as a causative agent of children diarrhea. Integrons are DNA elements known to carry genetic cassettes responsible for antibiotic resistance. The objective of this study was to investigate the presence and resistance gene cassette content of class 1 integron among Enteropathogenic *E. coli* isolated from patients referred to Tehran hospitals.
Methods: In this study, 300 stool samples were collected and Enteropathogenic *Escherichia coli* was detected using biochemical and serological methods and confirmed by PCR amplification of eae, stx1 and stx2 genes. Isolates with eae+/stx1–/stx2– genotype were defined as EPEC and subjected to further analysis. Class 1 integron was investigated with primers specific for the conserved integrase gene (int). The variable resistance gene cassettes were amplified and sequenced.
Results: During the study period 28 cases of Enteropathogenic *Escherichia coli* strains was detected and serotype O86 and O127 were appeared as dominant serotypes. Twenty two strains (79%) appeared to harbor class 1 integron among which the variable region was defined to be dfrA7, aadA1, dfrA1/aadA1, dfrA12/aadA2 which related to amplicons of approximately 750, 1000, 1700 and 2000 bp in sizes, respectively.
Conclusion: The presence of class 1 integron in 79% reveals that, this genetic element plays a very important role in the transfer of antibiotic resistance among the strains studied and may transfer resistance to aminoglycosides and trimetoprim to other Gram-negative bacteria.

R2299 Is colistin resistance in *Klebsiella pneumoniae* associated with mutations in the BasRS two component regulatory system?

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Objectives: Colistin resistance in *Salmonella enterica* has been associated with mutations in the PmrAB two component regulatory system that controls the composition of the outer membrane lipopolysachharide. The mechanism of recently described colistin resistance in *Klebsiella pneumoniae* clinical strains has not been elucidated. BasRS, the homologue system to PmrAB in *K. pneumoniae*, was studied.
Methods: Colistin resistant *K. pneumoniae* strains isolated in clinical and surveillance specimens of 10 patients, who were on colistin treatment, as well as sensitive strains isolated from the same patients

before colistin use, were included in the study. Exact MICs for colistin were determined by the E-test strip. Sensitive and resistant isolates were epidemiologically studied by repetitive extragenic palindromic (REP)-PCR methodology. The basR and basS genes from the respective resistant and susceptible isolates were amplified by PCR and sequenced. The primers used were designed by WebPrimer to amplify a 653 bp internal fragment of BasR (5′-TGT-CGA-TGT-TGT-TAG-CCA-GCA-3′, 5′-AGT-CAT-TGA-AGA-CGA-TGC-GCT-3′)and a 1097 bp internal fragment of BasS (5′-TCA-ATG-GGT-GCT-GAC-GTT-CT-3′, 5′-TGG-CTC-TGT-TTG-CAA-CTG-3′). The amplified DNA's were sequenced on both DNA strands, by Eurofins MWG (Ebersberg, Germany) and analyzed for aminoacid substitutions by the BLAST program (National Center for Biotechnology Information, Bethesda, US).
Results: Only clonally related pairs of isolates (susceptible and resistant) were included in the study. MICs of the colistin resistant isolates ranged between 16 and 48 mg/L while susceptible isolates had MICs of 0.38 to 1mg/L. One colistin resistant strain with an MIC of 48 mg/L harboured a substitution of glycine to serine (G → S) at codon position 53 in the basS gene compared to the susceptible strain isolated from the same patient. In all other cases no significant differences were observed between the genes from the susceptible and resistant strains.
Conclusion: The mutation observed in the basS gene of one resistant isolate has already been described in the homologue gene pmrA of a colistin resistant laboratory strain of *Salmonella enterica*. However mutations in the two component regulatory system BasRS in clinical isolates do not explain most of the cases of colistin resistance in *Klebsiella pneumoniae* strains.

R2300 Mutations in penicillin-binding protein 1 is responsible for high-level methicillin resistance in a *Staphylococcus lugdunensis* isolate

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Objective: To investigate the mechanism of high-level methicillin-resistance in a *Staphylococcus lugdunensis* isolate.
Materials and Methods: The microorganism was isolated from blood culture of a patient with endocarditis. Identification to the species level was performed by the Vitek 2 System and was verified by a molecular method based on fbl gene. Susceptibility testing to various antimicrobial agents was assessed by the Vitek 2 system, while the MICs values were determined by Etest. The production of penicillinase was performed by the nitrocephin disk test, while the presence of mecA gene was detected by PCR. In order to detect mutations in PBP1 and PBP4 genes, amplification followed by sequencing analysis was done and the results were compared with those obtained from a clinical *S. lugdunensis* strain susceptible to oxacillin (MIC: 0.75 mg/L).
Results: The isolate, despite the high-level resistance to oxacillin (MIC: 256 mg/L), did not carry the mecA gene, while no overproduction of penicillinase or mutation of PBP4 were detected. However some mutations were found in PBP1: an alteration in the 482 position (L482P) and an insertion in 570 position of four aminoacids (SAYG). These mutations were not found in the susceptible strain. As β-lactams bind to the penicillin-binding motif KTG in the transpeptidase region, that is located in 582 position, alterations in or around the motif possibly confer resistance due to reduced affinity to β-lactams.
Conclusions: This is the first report for mutations in PBP1 resulting in high-level methicillin-resistance in a clinical *S. lugdunensis* isolate.

R2301 Molecular characterisation of fluoroquinolone-resistant *Escherichia coli* causing septic diarrhoea in calves in Italy: emergence of a multi-resistant O78 clonal group

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Objectives: The emergence of antibiotic-resistant epidemic clones among animals should be monitored since clonal relatedness among certain O serogroup strains (i.e. *E. coli* O78) capable of causing disease in different hosts, humans included, has been shown and, although different molecules are employed, cross-resistance exists among fluoroquinolones used in human and veterinary medicine.
An increased incidence of enrofloxacin-resistant *E. coli* associated with septic diarrhoea in calves was recently observed in northern Italy (from 14.3% in 2002 to >30% in 2007). The aim of this study was to investigate this phenomenon.
Methods: A total of 47 consecutive *E. coli* isolates exhibiting reduced susceptibility to enrofloxacin (intermediately resistant or resistant) causing septic diarrhoea in calves from 45 large-scale farms during 2006–2007, were studied. Phylogenetic group, antibiotic susceptibility and O serogroup were determined with RAPD and PFGE typing providing additional discrimination.
Results: The majority (97.8%) of microorganisms (46/47) carried resistance to two or more additional drugs with the pattern: fluoroquinolone–ampicillin–co-trimoxazole–tetracycline–gentamicin–thiamphenicol being the most represented (24/47; 51.0%). Plasmid-mediated extended-spectrum and AmpC β-lactamases including plasmid-mediated fluoroquinolone resistance genetic determinants were not detected. Third generation cephalosporins emerged as the most active antimicrobial agents tested (97.9% of susceptible strains).
Conclusion: Overall, 37 different RAPD profiles and 18 different O serogroups could be distinguished among the typable strains indicating a substantial heterogeneity and suggesting the occurence of several independent selection events. However, approximately one fourth (11/47) of the strains belonged to serogroup O78 and PFGE revealed that the majority (7/11) of these were clonally related, indicating the selection of a O78 clonal group.
Since animals have been suggested as a possible source of serogroup O78 *E. coli* infections in humans, further studies are needed to clearly determine the zoonotic potential of these strains.

R2302 Molecular characterisation of plasmids encoding CTX-M-15 β-lactamases from *Klebsiella pneumoniae* strains in the Moroccan community

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Objectives: The Morocco, like other countries worldwide, has a growing problem with CTX-M β-lactamase producing Enterobacteriaceae. The present study sought to characterize blaCTX-M-15-containing plasmids associated with *Klebsiella pneumoniae* CTX-M-15 isolates recovered in Moroccan community.
Methods: We characterized the multidrug resistance region sequences of three plasmids that encode CTX-M-15 β-lactamases in *Klebsiella pneumoniae* strains isolated in three Moroccan cities; Casablanca (plasmid A), El Jadida (plasmid B) and Settat (plasmid C). Conjugative mating was attempted on agar. MICs were determined by E-Test method. Antibiotic resistance genes and integrons were identified by PCR and sequenced.
Results: Conjugative transfer of cefotaxime resistance was achieved in all strains. blaCTX-M-15 was carried by a 125 kb plasmid. In addition the plasmid A harboured the following 6 antibiotic resistance genes conferring resistance to seven antibiotic classes: blaOXA-1, blaTEM-1, aac(6′)-Ib-cr, catB4, tet(A), and the qnrB genes; the plasmid B carried blaTEM-1 consistently, also blaOXA-1, catB4, aac(6′)-Ib-cr, and tet(A). By contrast plasmid C carried blaOXA-1, catB4, aac(6′)-Ib-cr, and tet(A). The blaCTX-M-15 gene presented the following genetic environment: ISEcp1-blaCTX-M-15-orf477 and blaTEM-1b -TnpA-ISEcp1-blaCTX-M-15-orf477.
Conclusions: To our knowledge, this is the first description of genetic environment of CTX-M-15 gene in *K. pneumoniae* from a Moroccan community. The plasmids encoding CTX-M-15 conferred similar multi-drug resistance phenotypes, suggesting that they may share a similar genetic scaffold. Both shared features with plasmids encoding CTX-M-15 β-lactamases in *Escherichia coli* from Canada.

R2303 Macrolide resistance and in vitro selection of antibiotic resistance in different human isolated *Lactobacillus* strains

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Objectives: Spreading of antibiotic resistance is of concern due to the increasing rate of isolation of multiresistant pathogens. Since commensal bacteria can transfer determinants of resistance to pathogens, studies on resistance should include lactic acid bacteria, especially those intended for use as probiotics. We performed this study aiming to evaluate the capability of some antibiotics to select for resistance in lactobacilli.
Methods: Strains of *Lactobacillus acidophilus* (n = 13), *Lactobacillus plantarum* (n = 9), *Lactobacillus crispatus* (n = 6) and *Lactobacillus casei* (n = 12) isolated from human feces were included.
Amoxicillin/clavulanate, erythromycin and tetracycline were tested.
Minimum Inhibitory Concentrations (MICs) were measured with the microdilution broth method. Susceptibility was determined according to breakpoints established by EFSA.
The frequency of spontaneous mutations was calculated as the number of colonies grown on antibiotic-containing agar plates per inoculum.
Selection of resistance was performed at 4× and 8× MIC and peak serum concentration (Cmax). Susceptible isolates were serially subcultured onto agar plates containing a linear gradient of each antibiotic. Bacteria were exposed to ten consecutive passages on antibiotic-gradient plates, then to ten passages on antibiotic-free plates. Acquisition of resistance was defined as >4-fold increase in MIC.
Stable mutants with reduced susceptibility to erythromycin were analysed by PCR to detect the presence of erm and mef genes.
Results: Resistance to macrolides was observed in 16 strains (8 of them harboured the ermB gene) and to tetracycline in 11 strains; all strains were susceptible to amoxicillin/clavulanate.
Frequencies of mutation of susceptible strains (n = 26) were lower at 8× than at 4× MIC. Tetracycline showed the highest frequencies of mutations.
After multi-step selection an increase in MICs was generally observed. Such change was stable only in some strains. All tested antibiotics could select stable resistance in all species. Molecular characterization of resistant mutants did not lead to detection of erm nor mef genes.
Conclusions: Our results suggest that a decrease in susceptibility following exposure to antibiotics might occur in some lactobacilli. So, evaluation of the ability to acquire resistance to common antibiotics should be performed in parallel with investigations on the presence of resistance determinants in strains intended for human and animal use.

Table 1 - Selection of resistance in antibiotic-susceptible lactobacilli of human origin

Strains of human origin	Erythromycin-resistant mutants*			Amoxicillin/clavulanate-resistant mutants *	Tetracycline-resistant mutants *
	erm	*mef*	other		
***L. acidophilus* (n=8)**	0	0	7	6	5
***L. plantarum* (n=6)**	0	0	6	4	5
***L. casei* (n=8)**	0	0	7	5	5
***L. crispatus* (n=4)**	0	0	3	2	2

* after multi-step selection of resistance

R2304 The active component, which mimics the role of β-lactam antibiotics in the β-lactam antibiotic-induced vancomycin-resistant MRSA

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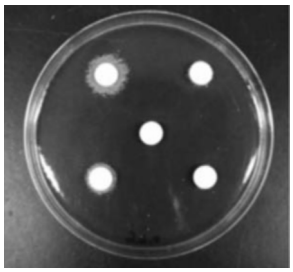
Background: Patients who suffer from MRSA infection are often coinfected with Gram-negative bacteria, and they are likely to be treated with a combination of vancomycin (VAN) and β-lactam antibiotics. However, this combination therapy causes the emergence of VAN-resistant MRSA, designated as β-lactam antibiotic-induced VAN-resistant MRSA (BIVR), which traps a large amount of VAN and lower free drug concentration in the mi-lieu. β-Lactam antibiotics inhibit peptidoglycan synthesis and promote the expression of autolysin resulting in the release of large amounts of peptidoglycan fragments into the extracellular milieu. We hypothesized that peptidoglycan fragments generated by the action of β-lactam antibiotics and autolysins were incorporated into the cytoplasm for recycling and promote synthesis of nascent lipid-II, an analogy to the case in *Escherichia coli*. Thus, we searched active compound(s), which mimics the role of β-lactam antibiotics in the induction of the BIVR phenomenon.
Method: The BIVR strain K744 was used for the indicator cells in the BIVR assay. The compounds tested for the induction of BIVR phenomenon were either prepared from peptidoglycan fragments or the synthetics. The compounds to be tested were impregnated on a paper disc with a diameter of 8 mm, and that was placed on the VAN agar plate streaked with the K744 cells. The plates were incubated overnight. If the test compound was active, the indicator BIVR cells grow around a paper disc forming a hollow, of which diameter was measured. The non-BIVR cell show no growth zone due to the presence of VAN.
Results: The purified muropeptide, GlcNAc-MurNAc-L-Ala-D-isoGln-L-Lys-(Gly4)-D-Ala-Gly2 and GlcNAc-1, 6-N, O-diacetyl-MurN-L-Ala-D-isoGln-L-Lys-(Gly4)-D-Ala-Gly2 at 75 μg per disc yielded 17.5 and 11.5 mm, respectively, of the K744 growth zone. The Gly4 peptide was not essential for the activity. The BIVR inducing activity was undetectable by GlcNAc-MurNAc-L-Ala-D-isoGln, MurNAc-L-Ala-D-isoGlu-L-Lys and L-Ala-D-isoGlu-L-Lys.
Conclusion: We concluded that the active compound was composed of the GlcNAc-MurNAc glycan chain and the L-Ala-D-isoGln-L-Lys-D-Ala-Gly2 peptide.



R2305 Report of transmission of VIM-producing *Enterobacter cloacae* in a university hospital in Ireland

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Objective: Emergence of carbapenem-resistant Enterobacteriaceae (CRE) has become a significant public health threat. Within this diverse group, VIM-producing *Enterobacter cloacae* is increasingly reported in countries such as Greece and Spain. In this report, we evaluate the evidence for transmission of carbapenem-resistant *E. cloacae* in a cardiothoracic intensive care unit (CT-ICU).
Methods: Clinical, infection control and microbiological data were collated. Clinical specimens were cultured by rountine methods. Patients (rectal swab) and the environment were screened for CRE. Isolates were identified by Vitek 2 and susceptibility testing was performed by CLSI disc diffusion. Evaluation for carbapenemase was conducted using the modified Hodge test (MHT), metallo-β-lactamase (MBL) Etest and synergy testing. PCR detection of carbapenemase genes and analysis of Xba1-restricted PFGE banding patterns were also performed.
Results: Carbapenem-resistant *E. cloacae* were cultured from sternal wounds of 2 patients within a 6-day period. Both patients had received broad-spectrum antibiotics but neither had a history of recent travel. Their respective stays in the CT-ICU had overlapped for 11 days. Both were treated with appropriate antibiotics while aggressive infection control measures were instituted. CRE was not isolated from other patients, the hospital environment or medical equipment at that time. The isolates were resistant to β-lactams (including carbapenems), gentamicin

and tobramycin, but susceptible to ciprofloxacin, amikacin and colistin. Phenotypic carbapenemase screening methods yielded positive but often inconsistent and subtle results. PCR confirmed the presence of blaVIM in both isolates, while PFGE demonstrated indistinguishable banding patterns.

Conclusions: This is the first report of the emergence of VIM-producing *E. cloacae* in Ireland. Sternal wound infections by such organisms were also not previously reported. Clinical and molecular data indicated that cross-transmission of carbapenem-resistant *E. cloacae* in CT-ICU was likely. Laboratory detection of VIM-producing Enterobacteriaceae remains challenging, while the need for active surveillance in high-risk patients warrants further consideration. The report highlights the therapeutic and infection control challenges posed by these organisms, and reiterates the importance of prudent antibiotic usage and aggressive infection control measures.

R2306 Integron carriage of ESBL-producing *Klebsiella pneumoniae* from bloodstream infections: a 2-year study

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Introduction: Integrons are mobile genetic elements capable of gene capture and expression via site-specific recombination and the action of a promoter. Integrons play a major role in the dissemination of antibiotic resistance genes and are commonly associated with members of the family Enterobacteriaceae.

Objectives: To find out the incidence and the classes of integron associated with ESBL producing *Klebsiella pneumoniae* isolated from blood stream infections.

Materials and Methods: This study was carried out on 256 *K. pneumoniae* isolates over a period of two years. Antimicrobial susceptibility was tested for 14 antibiotics. ESBL detection was done as per CLSI followed by a multiplex PCR. Integrase gene PCR was done to detect class 1, class 2 integrons; similarly for class 3 and class 4 integron using specific primers. Sequencing was done for representative number of strains.

Results: Out of the 256 isolates, 167 (65.2%) were ESBL producers. blaSHV (77.2%) and blaCTX-M (85.6%) were the most common. Of the 167 ESBL positive isolates, 121 (72.4%) carried class 1 integron; 51 (42.1%) isolates carried class 2 integron. Both class 1 and class 2 were found in 33 (27.2%) and none had class 3 or class 4 type. Sequencing and blasting results confirmed their identities. The drug resistant rates of integron positive isolates were 23% higher compared to integron negative strains.

Conclusions: A higher percentage of class 2 integrons association with ESBL strains is being noted for the first time from our region, also the co-existence of both class 1 and class 2 types increases the higher risk of multidrug resistant gene transfer rates. These findings strongly suggest that integrons have a major role in the dissemination of ESBL mediated resistance among nosocomial isolates of *K. pneumoniae*.

R2307 Prophages induction by mitomycin C in *Helicobacter pylori* clinical isolates and its association with resistance to antimicrobial agents

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Objective: The aim of this study was to detect the presence of prophages by the induction of its lytic cycle after culturing clinical isolates in the presence of low concentrations of mitomycin C and determine its association with the resistance to antimicrobial agents.

Methods: 47 *H. pylori* strains were studied. They were obtained from gastric biopsies following standard methodology. Strains were stored at −80ºC until used. TIGR 26695 was used as negative control as no prophage was detected in its genome. Amoxicillin, clarithromycin, rifampicin, ciprofloxacin, tetracycline, metronidazole resistance was determined by E-test following standard methodology.

For prophage induction, *H. pylori* strains were subcultured on recently prepared blood agar plates containing 5 ng/ml Mitomycin C, 10 mg/l vancomycin and 5 mg/l amphotericyn B. After 2 to 5 days incubation, plates were examined for the presence of growth inhibition plaques.

Results: Porphage induction was observed in 15 out of 47 strains (31.9%) (not in TIGR) by detection of inhibition plaques after culture on mitomycin C containing blood agar plates. The following resistance percentages were detected: 4.2% to amoxicillin, 44.7% to clarithromycin, 10.9% to rifampicin, 4.3% to ciprofloxacin, 2.1% to tetraciclin and 27.6% to metronidazole.

Percentage of resistance was analyzed in 2 groups (strains with or without prophages) Resistance was higher in the group with prophages for the following antimicrobial agents: amoxicillin (6,6% vs 3,1%), clarithromycin (53,3% vs 40,6%), ciprofloxacin (7,1% vs 3,1%) and metrronidazol (33,3% vs 25%). Resistance was lower in the group with prophages for the following antimicrobial agents: rifampicin (7,1% vs 12,5%) and tetracycline (0% vs 3,1%).

Conclusions: (1) Mitomycin C containing blood agar plates is an easy method to detect prophage carriage among *H. pylori* strains. (2) A high percentage of *H. pylori* strains showed prophage detected by mitomycin C induction and (3) the presence of these prophages seems to be associated with a higher percentage of resistance to the antimicrobial agents most frequently used to treat infection produced by *H. pylori*.

R2308 The first isolation and treatment of an OXA48 carbapenemase-producing *E. coli* in the UK

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Objective: To identify the resistance mechanism and determine appropriate antibiotic treatment for a patient whose blood and abdominal fluid isolated an *E. coli* resistant to ertapenem and the cephalosporins, with borderline imipenem and meropenem resistance. To highlight problems with detection and difficulties with treatment of multi drug resistant Gram negative organisms.

Methods: Following hospitalisation for an MI in Egypt, requiring ITU care in The Nile Hospital, the patient was repatriated via air-ambulance to Wythenshawe Hospital for cardiac rehabilitation and subsequently developed an acute abdomen secondary to cholycystitis. Laporotomy and cholecystectomy were carried out and cultures from blood and drain fluid isolated a resistant *E. coli*.

Routine in-house antibiotic susceptibility testing of the isolates was undertaken by VITEK. Resistance was confirmed by the Reference Laboratory who carried out MICs to cephalosporins, carbapenems, aminoglycosides, tigecycline and other agents by agar dilution. Potential carbapenemase activity was examined by a plate assay (Clover Leaf test), followed by enzyme identification by PCR.

Results: A pure growth of *E. coli* with reduced carbapenem susceptibility was isolated. MICs of carbapenems were ertapenem = 8, meropenem = 1 and imipenem = 2. The isolate was clover leaf positive for carbapenemase activity and PCR identified an OXA48. The isolate was pan-β-lactam-resistant due to a CTX-M, seen by the MIC of Cefotaxime potentiated from >256 to 1, whilst ceftazidime fell from 32 to 1. The isolate was sensitive to tigecycline, which the patient received for 10 days resulting in full recovery. A detailed travel history revealed visits to Tunisia, Spain and Portugal but no travel to Greece or Turkey, during the past 5 yrs.

Conclusion: OXA48 has not previously been identified in an *E. coli* in the UK. Detection of this carbapenemase is problematic as resistance to ertapenem may be the only indicator, yet the MIC of ertapenem can be readily raised by impermeability alone, making its use as a single maker uncertain. Dissemination of OXA48 has to date, been geographically and species restricted and not previously associated with *E. coli* or with travel to Egypt. Tigecycline concentrates well in the gall bladder and should be considered for sensitivity testing in these circumstances; however concerns remain around the achievement of adequate serum levels of the agent in patients with severe sepsis with a single confirmed pathogen.

Resistance surveillance

R2309 Study on drug resistance of *Mycobacterium tuberculosis* and mycobacteria other than tubercle bacilli strains to ofloxacin and ciprofloxacin isolated from patients admitted to research centre for TB and pulmonary diseases, Tabriz, Iran

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Objectives: Study and evaluation of effectiveness of second line drugs against mycobacterial strains has become more important in the past few years, particularly due to the outbreak caused by multi-drug resistant (MDR) strains of *Mycobacterium tuberculosis* (MT). The aim of this study was to evaluate the in-vitro susceptibility of *M. tuberculosis* (MT) and mycobacteria other than tubercle bacilli (MOTT) strains to the two main second line anti-mycobacterial agents (ciprofloxacin and ofloxacin).
Methods: In this study, in-vitro activities of ofloxacin and ciprofloxacin against totally 100 mycobacterial strains including 90 *Mycobacterium tuberculosis* strains (40 strains resistant and 50 strains sensitive to the first line drugs) and 10 mycobacteria other than tubercle bacilli strains (all strains resistant to the first line drugs) were investigated by proportional method on Lowenstein-Jensen (LJ) medium.
Results: Out of 90 *M. tuberculosis* strains, 50 strains that were sensitive to the first line drugs were diagnosed as susceptible to ofloxacin and ciprofloxacin. Of other 40 strains which were resistant to the first line drugs, only one strain was resistant to ofloxacin and 2 strains were found to be resistant to ciprofloxacin. Of 10 mycobacteria other than tubercle bacilli strains, 4 strains were resistant to ofloxacin and 3 strains were found to be resistant to ciprofloxacin.
Conclusion: The findings of this study showed that ofloxacin and ciprofloxacin could be effectively used against *Mycobacterium tuberculosis* strains and also mycobacteria other than tubercle bacilli strains.

R2310 *Staphylococcus aureus* faecal carriage among healthy humans in Spain. Detection of livestock-associated genetic lineages ST398 and ST133 in methicillin-susceptible isolates

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Objectives: To determine the rate of *S. aureus* faecal carriage in healthy humans in Spain and to perform the genetic characterization of recovered isolates.
Methods: Fecal samples of 96 healthy humans were recovered in La Rioja (Spain) during September-December 2010. Samples were inoculated into Mannitol-Salt-agar and ORSAB plates for *S. aureus* and methicillin-resistant *S. aureus* recovery, respectively. Isolates were identified by biochemical methods and nuc-gene PCR. Antibiotic susceptibility profile was determined by disk-diffusion method for 18 antibiotics. *S. aureus* isolates were submitted to spa- agr- and MLST typing. The presence of 9 antibiotic resistance genes (msrA, msrB, mphC, ermA, ermB, ermC, ermF, ant-4′, mupA) and 11 virulence factor genes (lukF/lukS-PV, lukE-lukD, lukM, tst-1, eta, etb, hlA, hlB, hlD, hlG, hlgv) were studied by PCR.
Results: *S. aureus* was recovered in 14 of 96 studied samples (14,6%), all of them being methicillin-susceptible (MSSA). A very high diversity of spa-types was detected among our isolates (t084, t002, t209, t012, t021, t216, t136, t3495, t571), types t084 and t002 being detected in two samples, each one. MLST was performed for isolates with spa-types t571 and t3495, and sequence types ST398 and ST133, respectively, were identified. Isolates were susceptible to most of antibiotic tested with some exceptions: erythromycin-clindamycin (3 isolates, with ermC with/without ermA+mphC), tobramycin and mupirocin (1 isolate with ant(4′) + mupA genes). All 14 MSSA were negative for lukF/lukS-PV genes encoding Panton-Valentine leucocidin. The lukDE gene was identified in 5 isolates. Interestingly, strain ST133 harboured the lukDE + lukM genes. Five isolates were tst-1 positives (35% of all MSSA). Others virulence factors detected were (number of isolates): hlA(14), hlB(9), hlD(14), hlG(6), and hlG2(8).
Conclusions: A high prevalence and a high clonal diversity of MSSA isolates were identified. As far as we know, this is the first detection of MSSA ST133 in faecal samples of healthy humans.

R2311 Antimicrobial resistance and vancomycin heteroresistance of MRSA strains from a tertiary hospital in Greece

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Methicillin resistant *S. aureus* strains cause a variety of infections that are sometimes difficult to treat and are spread both in hospital environment and community. Vancomycin heteroresistance (hVISA) is mainly observed among these strains.
Objectives: To detect antimicrobial resistance and hVISA prevalence of MRSA isolates and examine vancomycin MIC through time.
Methods: 155 MRSA non-duplicate isolates were isolated in the Clinical Microbiology and Infectious Diseases Laboratory during 1/1/2008 to 30/6/2010. Samples were:116 wounds and abscesses (74.8%), 29 blood (18.8%), 5 intravascular catheters (3.2%) and 5 fluids (4 synovial, 1 pleuritic) (3.2%). The majority of patients were hospitalised (33.5% internal medicine wards, 14.2% surgical wards, 7.1% ICU) while 45.2% were outpatients. Identification and susceptibility testing was performed by automated system (AS) (Phoenix BD) according to CLSI guidelines. All strains' MIC to vancomycin and teicoplanin was further tested by Etest (AB Biodisk). E-test macromethod was used to detect hVISA strains.
Results: Resistance rates of MRSA strains were: eryth 62%, clind 47.1%, cipr 45.8%, gent 31.6%, rif 29%, trim-sulf 14.2%, quin-dalf 2.6%, mup high-level 1.9%. MIC to fusidic ac was $\geqslant 4$ for 69% of the strains (only EUCAST brpoint: $R \geqslant 2$). Inducible MLSB phenotype was detected in 10% of the strains. There were no resistant strains to vancomycin, teicoplanin and linezolid. MICs to vanc and teic determined by E-test were higher than those of the AS. Vanc $\leqslant 1$ was detected in 97.4% (AS) and 33.5% (E-test) of the strains and teicoplanin $\leqslant 1$ in 94.8% (AS) and 81.9% (E-test) of the strains. MIC results by E-test were: vanc $MIC_{50} = 1.5$, $MIC_{90} = 2$ (range 0.5–3, one strain with MIC = 3 was not confirmed to be a VISA isolate by broth microdilution, nor was hVISA), teic $MIC_{50} = 0.75$, $MIC_{90} = 1.5$ (range 0.25–6). There was a transient increase ($p < 0.05$) in vanc MIC during 1/7/2008 to 30/6/2009.13 of 155 strains (8.4%) were hVISA. Among these 13 isolates, 8 were recovered from wounds and abscesses, 4 from blood, and 1 from intravascular catheter, while four of the patients were outpatients. Vanc MIC range was 0.75–2 (1 st = 0.75, 2 st = 1, 8 st = 1.5, 2 st = 2).
Conclusion: hVISA phenotype is not uncommon among MRSA isolates. Strains with vanc >1 should be tested for such a phenotype. Further studies using population analysis are needed to establish the prevalence of such strains, their antimicrobial resistance and infections clinical outcome.

R2312 ESBL-*Escherichia coli* fading away

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Objective: To study the evolution and pattern of consecutive urinary tract infections (UTI) caused by *Escherichia coli* in patients from the Comunitat Valenciana, Spain.
Methods: Urinary isolates were obtained from January 2007 to December 2008. Microbial identification and antimicrobial susceptibility testing were performed according to each laboratory standard procedures and conforming to CLSI antimicrobial susceptibility criteria, either by regular biochemical reactions and agar diffusion susceptibility tests or automated methods. Data were retrieved from the Comunitat Valenciana Microbiological Surveillance Network (RedMIVA), which daily compiles and analyzes information from 25 microbiology laboratories that manage more than 90% of the total population (5,029,601 people).

ESBL-*Escherichia coli* (EEC) was defined as an *E. coli* resistant to third generation cephalosporins but susceptible to amoxicillin clavulanate. Thus, most TEM, SHV and CTX-M-type enzymes (molecular class A enzymes or functional group 2be from the Bush-Jacoby-Medeiros classification) were taken into account.
ITU were considered different if time between two episodes was greater than 15 days or the isolate presented different susceptibility patterns affecting ESBL classification.
In vitro growth competitions were carried out fivefold by placing the same inoculum of two isolates of *E. coli* (EEC vs. *E. coli* with no antimicrobial resistance) into 10ml of thioglicolate (TG). Then, aliquots were sub-cultured every day for both re-growth in 10ml TG and colony identification.
Results: The total number of urinary isolates analyzed was 70,827 belonging to 49,304 different patients. Of these isolates, 5,161 (7.3%) were categorized as EEC. The number of different ITU was 64,472, 4,403 (6.8%) by EEC, and 1701 were discarded because they took place at the end of the study period during the fourth consecutive ITU.
The mean time from an EEC ITU to another EEC ITU was 43 days, while the mean time from an EEC ITU to a non-EEC ITU was 75 days. Growth competitions showed a greater than 99% decreased in the EEC population after 5 days.
Conclusions: Our data suggests that, given the time and in the absence of antimicrobial pressure, *E. coli* with fewer antimicrobial resistances will outgrow EEC, probably due to a better fitness of the isolate.

R2313 Antimicrobial susceptibility pattern among *Acinetobacter calcoaceticus*-baumannii complex in a Saudi Arabian hospital

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Objective: To examine patterns of antimicrobial susceptibility in *Acinetobacter calcoaceticus*-baumannii isolates to commonly used drugs at a tertiary care hospital in Riyadh, Saudi Arabia.
Methods: A retrospective study was carried out at King Fahad National Guard Hospital (KFNGH) between 2008 and 2010. Organisms were identified and tested by the automated identification and susceptibility system (MicroScan Walk away 96, Siemens®) and the Antibiotic susceptibility testing were confirmed by the Etest (AB Biodisk, Sweden). The procedural details interpretations were as recommended by the Clinical laboratory standards Institute (CLSI).
Results: Between 2008–2010, a total of 2552 isolates of *Acinetobacter baumannii* (*A. baumannii*) were available for analysis. The organism showed high rates of resistance to Pip-Tazo (81% of isolates), Imipenem (66%), Meropenem (83%), Gentamicin (68%), amikacin (65%), ceftazidime (82%), cefepime (70%), ciprofloxacin (80%) and colistin 19%. Multidrug resistance was observed in (65–75%) of *Acinetobacter* species isolates.
Conclusion: Antimicrobial resistance in *Acinetobacter baumannii* is a major emerging problem particularly in the intensive care unit, Strict infection control measures, judicious prescribing of antibiotics, antibiotic stewardship programs and antibiotic cycling should be adopted to control infections due to these bacteria in patients admitted to intensive care Continuous monitoring of antimicrobial susceptibility and strict adherence to infection prevention guidelines are essential to eliminate major outbreaks in the future.

R2314 Multicentre evaluation of in vitro activity of tigecycline against extended-spectrum β-lactamases and multidrug-resistant Enterobacteriaceae clinical isolates

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Objectives: Tigecycline is an antibiotic used in clinical setting where multidrug resistance (MDR) and extended spectrum β-lactamases (ESBL) production is prominent. Due to the constant potential of emergency of resistance, long term survey studies are needed. The aim of this study was to assess the activity of tigecycline against MDR and ESBL Enterobacteriaceae isolates during a period of ten years.
Methods: A total of 155 isolates, *Escherichia coli* (n = 92) and *Klebsiella pneumoniae* (n = 63), were collected at 5 hospitals in Portugal (1999–2010). Susceptibilities to antimicrobial agents were determined by disk diffusion and interpreted according to CLSI guidelines: tigecycline, imipenem, ciprofloxacin, gentamicin, amikacin, ampicillin, cefotaxime, ceftazidime, cefepime and amoxicillin/clavulanic acid. Tigecycline MICs were performed by Etest. The ESBLs were identified by PCR with specific primers for bla-CTX-M, bla-TEM and bla-KPC gene.
Results: According to the breakpoints proposed by CLSI, 25% of *K. pneumoniae* and 13% of *E. coli* ESBL-producing were nonsusceptible to tigecycline. Higher prevalence of intermediate susceptibility was found (*Klebsiella* 63% and *E. coli* 44%). Longitudinal analysis showed no increase in tigecycline resistance over the 10-years study period and was not influenced by the presence of CTX-M- and TEM-type enzymes. However for the first time in Portugal it was identified a KPC-3 *E. coli* resistant to tigecycline and nine *K. pneumoniae* isolates producing KPC-3 showed reduced activity (33%). MDR and ESBL-producing *E. coli* had tigecycline MIC_{90} ⩽3 mg/l and *K. pneumoniae* isolates had tigecycline MIC_{90} ⩽2 mg/l. The analysis of tigecycline MICs showed a poor correlation with values obtained and disk zone diameters, 38% of the MIC results are concordant. The discordant data occur mostly in the intermediate/susceptible zones.
Conclusion: Tigecycline showed good activity against MDR and ESBL isolates. However, the high frequency of isolates in intermediate category found and the discordant correlation between MICs and inhibition zone diameters, suggest that further selective pressure will lead to an overall reduced susceptibility to tigecycline in Enterobacteria. For instances, the tigecycline resistance found in KPC-3 isolates collected in 2010 may indicate an indiscriminate use of tigecycline. These results indicate that the use of tigecycline in hospital setting should be carefully controlled to avoid the emergence of resistance.

R2315 Comprehensive analysis of telavancin activity against staphylococcal isolates from Europe (2009–2010), including strains with reduced susceptibility to vancomycin

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Objectives: To assess telavancin (TLV) and comparator activities against staphylococci, including strains with decreased susceptibility (S) to vancomycin (VA) and teicoplanin (TE), from Europe (EU). TLV is approved in the United States (US) and Canada for the treatment of adults with complicated skin and skin structure infections (cSSSI). This drug is also under review for the treatment of complicated skin and soft tissue infections in EU and nosocomial pneumonia in the US and EU.
Methods: 3 868 *S. aureus* (SA) and 1 003 coagulase-negative staphylococci (CoNS) were collected from 33 sites in 12 countries, including Turkey and Israel. Isolates were submitted to a central laboratory and identification performed by standard algorithms and Vitek 2. Strains were S tested by CLSI methods (M07-A8, 2009). EUCAST criteria (2010) were applied, when available.
Results: SA were from SSSI (41.0%), bacteremia (35.3%), and respiratory tract infections (13.0%), while the majority (68.2%) of CoNS were from bacteremia. TLV ($MIC_{50/90}$, 0.12/0.25 mg/L) was 2-fold more potent than daptomycin (DA; $MIC_{50/90}$, 0.25/0.5 mg/L) and 4- to 8-fold more active than VA ($MIC_{50/90}$, 1/1 mg/L) and linezolid (LZ; $MIC_{50/90}$, 1/2 mg/L) against all SA. Similar results were noted for CoNS. 1.6 and 1.0% of SA had higher VA (>1 mg/L; all 2 mg/L) and TE MICs (2–8 mg/L), while 47.6 and 31.0% of CoNS had elevated VA (2–4 mg/L) and TE MICs (⩾4 mg/L), respectively. Rates of SA with VA MICs >1 mg/L varied among countries (0.7–5.1%), being lowest in Poland and highest in Switzerland. TLV ($MIC_{50/90}$, 0.25/0.5 mg/L) showed higher MICs (2-fold) against SA with decreased S to glycopeptides compared with strains with lower VA and TE MICs ($MIC_{50/90}$, 0.12/0.25 mg/L). No differences in the TLV $MIC_{50/90}$ values were noted against SA from different years, countries or infection sources ($MIC_{50/90}$, 0.12/0.25 mg/L). Consistent TLV ($MIC_{50/90}$, 0.12/0.25 mg/L)

activity was observed against CoNS, regardless of country of origin or glycopeptide S.

Conclusions: TLV exhibited higher potency (at least 2-fold) than direct comparators (VA, DA and LZ). SA with decreased S to glycopeptides showed higher TLV MICs, although all strains were inhibited (⩽0.5 mg/L) at concentrations below the FDA breakpoint for SA (⩽1 mg/L). The TLV activity against SA and CoNS suggests this drug as an option for staphylococcal infections, including those caused by strains with decreased S to glycopeptides.

Susceptibility profile[a] (no. tested)		MIC (mg/L) 50%	MIC (mg/L) 90%	Number (cumulative %) inhibited at telavancin MIC (mg/L) of[b]: ≤0.03	0.06	0.12	0.25	0.5
S. aureus	2009 (1 732)	0.12	0.25	0(0.0)	65(3.8)	**1160(70.7)**	478(98.3)	29(100.0)
	2010 (2 136)	0.12	0.25	2(<0.1)	91(4.4)	**1263(63.5)**	733(97.8)	47(100.0)
	VA MIC, 2 mg/L (60)	0.25	0.5	0(0.0)	1(1.7)	23(40.0)	**28(86.7)**	8(100.0)
	TE MIC, 2 – 8 mg/L (37)	0.25	0.5	0(0.0)	0(0.0)	6(16.2)	**23(78.4)**	8(100.0)
CoNS	2009 (515)	0.12	0.25	7(1.4)	65(14.0)	**299(72.0)**	128(96.9)	16(100.0)
	2010 (488)	0.12	0.25	8(1.6)	26(7.0)	**267(61.7)**	173(97.1)	13(99.8)
	VA MIC, 2 – 4 mg/L (477)	0.12	0.25	0(0.0)	13(2.7)	**264(58.1)**	187(97.3)	13(100.0)
	TE MIC, ≥4 mg/L (311)	0.12	0.25	0(0.0)	7(2.3)	**167(55.9)**	123(95.5)	14(100.0)

a. CoNS = coagulase-negative staphylococci. VA, vancomycin; TE, teicoplanin.
b. Modal MIC results are in bold.

R2316 Antibiotic susceptibility of *Escherichia coli* strains causing community-acquired urinary tract infection

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Escherichia coli is involved in 85% of community-acquired urinary tract infections (UTIs). For this reason, it is important to know its antimicrobial susceptibility patterns in order to give an appropriate empiric treatment of UTIs.

The aim of this study is to evaluate the antibiotic resistance of *E. coli* strains isolated in community-acquired UTIs, and to see the prevalence of extended-spectrum β-lactamase (ESBL) producing strains in the community.

Methods: 2598 *E. coli* isolates were collected from outpatients with clinical evidence of community-acquired UTIs during the period January to November 2010 in a hospital in Madrid, Spain. Urine samples were cultured in CLED agar and incubated at 37ºC for 48 hours. UTIs were defined as the culture of a single organism from a midstream urine specimen at ⩾105 colony forming units per milliliter. Identification and antibiotic susceptibility tests were made by the routinely used automated system MicroScan (Siemens). Additionally, susceptibility tests were performed by disk diffusion method according to standard procedures.

For the study, reduced susceptibility to 8 antibiotics commonly used in UTIs (amoxicillin, amoxicillin/clavulanic acid, cefuroxime, norfloxacin, ciprofloxacin, trimethoprim/sulfamethoxazole, fosfomycin and nitrofurantoin) was evaluated. In addition, extended-spectrum β-lactamase (ESBL) production was examined; a ratio of 8 or greater for the ceftazidime to ceftazidime-clavulanic acid minimal inhibitory concentrations (MICs) and the cefotaxime to cefotaxime-clavulanic acid MICs was considered ESBL-positive.

Table 1.- *E.coli* isolates from community-acquired UTIs presenting reduced susceptibility or resistance to antibiotics (January – November 2010)

NUMBER OF ISOLATES	AMOX	AMOX-CLAV	CFRX	NRFLX	CPFLX	T/S	FOS	NITRO
E.coli 2419	1375 (56,8%)	472 (19,5%)	140 (5,8%)	664 (27,3%)	661 (27,3%)	752 (31,1%)	99 (4,1%)	64 (2,6%)
ESBL *E.coli* 179 (6,9%)	179 (100%)	104 (58,1%)	179 (100%)	156 (87,1%)	154 (86%)	116 (64,8%)	49 (27,4%)	16 (8,9%)
TOTAL 2598	**1554 (59,8%)**	**576 (22,2%)**	**319 (12,3%)**	**820 (31,6%)**	**815 (31,4%)**	**868 (33,4%)**	**148 (5,7%)**	**80 (3,1%)**

AMOX= Amoxicillin; AMOX-CLAV= Amoxicillin-clavulanic acid; CFRX = Cefuroxime; NRFLX= Norfloxacin; CPFLX = Ciprofloxacin; T/S = Trimethoprim-sulfamethoxazole ; FOS = Fosfomycin; NITRO = Nitrofurantoin

Results: 2598 *E. coli* isolates were obtained, 179 of which (6,9%) produced an extended-spectrum β-lactamase. Isolates presenting reduced susceptibility to the different antibiotics evaluated are shown in the table attached.

Conclusion: This study shows the increasing prevalence of ESBL-*E. coli* in community-acquired infections, being almost 7% of the *E. coli* strains isolated positive for ESBL.

High resistance rates are obtained for quinolones (norfloxacin and ciprofloxacin) in both, ESBL and not ESBL producing *E. coli*. For this reason, these antibiotics should not be used for the empiric treatment of community-acquired UTIs in our country. However, low resistance rates are shown for nitrofurantoin and fosfomycin, in both ESBL and not ESBL *E. coli*, which suggests that these antibiotics may be a good option in the empiric treatment of UTIs.

R2317 High-level antimicrobial non-susceptibility rates among environmental bacteria isolated from Iztuzu Beach in Dalyan, Turkey

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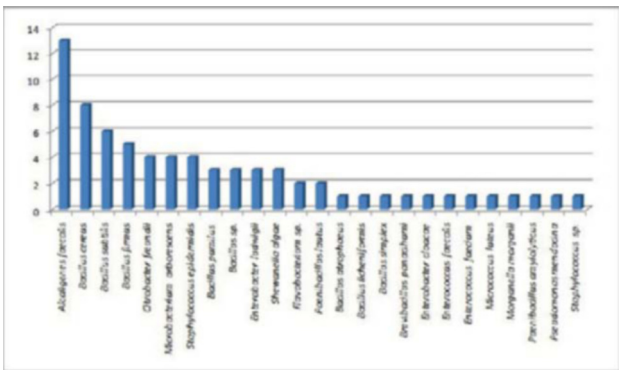
Introduction: Antimicrobial resistance level is high among pathogen bacteria especially hospitals. The aim of this study was to determine antimicrobial susceptibility levels of environmental bacteria from a national park in Turkey.

Materials and Methods: A total of 72 bacteria were defined by 16S rRNA sequencing (Figure 1). The antimicrobial susceptibilities of 72 bacteria isolated were investigated to penicillin, ampicillin, lincomycin, erythromycin, vancomycin, ciprofloxacin, gentamicin, tetracyclin, and chloramphenicol by agar diffusion MIC method. Strains with high MICs for lincomycin, chloramphenicol and penicillin were tested for inactivation by Gots' test. β lactamase positive strains were tested for ESBL production. All strains with high level MICs for gentamicin, tetracyclin, and chloramphenicol were studied for the presence of known gene by PCR. Gram positive bacteria were tested for the presence of erytromycin, and vancomycin resistanve genes. The following genes were tested for their presence: ermA, ermB, ermC, vanA, tetA, aac-aph and cat.

Results: Among isolates high MIC rates were 76.3%, 75%, 70.8%, 68%, 54.1%, and 47.2% for ampicillin, penicillin, gentamicin, chloramphenicol, tetracycline, and ciprofloxacin, respectively among all isolates. MIC values were evaluated for lincomycin, erythromycin and vancomycin among Gram (+) bacteria which showed 88.6%, 45.4%, and 15,9% high level MICs, respectively. Gots' test showed that, 35 isolates inactivates penicillin and only one isolate inactivates chloramphenicol. Among β-lactamase (+) strains one was ESBL producer. Of 20 isolates with high level erythromycin MICs 2 had ermB, 1 ermC, and 0 ermA. Of 51 isolate with high gentamicin MICs only 1 had aac-aph. Of 39 tetracyclin resistant isolates 4 had tetA and 2 had tetM genes but 33 were negative for both. Of 21 vancomycin resistant isolates 1 had vanA. The vanA (+) isolate was *E. faecium* and ST type of this strain was close to 17 with one allele difference.



Conclusions: Antimicrobial resistance is one of the main public health problem. The use of antimicrobials select resistance strains in hospitals but also the dispersion of antimicrobials to the nature may cause selection of resistance in the nature. Our study showed presence of high level antimicrobial non-susceptibility among environmental isolates in a natural park. The effect of environmental resistant strains on public health should be evaluated.

R2318 Increase of antibiotic resistance in *S. aureus* isolates from mastitis milk of Sicilian dairy farms: a seven-year survey

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Objectives: Antimicrobial agents are widely used in animal husbandry for treating infectious diseases and ensuring animal welfare and good quality food production. It is well known that antimicrobial usage can select for resistant forms of bacteria and resistant strains can be exchanged between humans, animals and other ecosystems. To the aim of monitoring resistance against selected antimicrobial agents commonly used in sheep producing milk, a survey was carried out on *S. aureus* strains isolated from milk of animals with mastitis problems in seven years from 87 farms.
Methods: 172 strains of *Staphylococcus aureus* were isolated and collected from milk during 2002–2008 in sicilian livestocks with big problems of ovine mastitis. The isolates in fact were used to prepare autogenous vaccine at the IZS of Sicily. They were tested for antimicrobial susceptibility by disk diffusion test (DDT) against the following agents: ampicillin (AMP 30mcg), amoxicillin+clavulanate (AMC 30mcg), enrofloxacin (ENR 5mcg), eritromycin (ER 15mcg), penicillin (P 10UI), tetracycline (TE 30mcg), vancomycin (VA 30mcg), oxacillin (OX 1mcg), oxytetracycline (OT 30mcg) according to CLSI guidelines.
Results: As shown in the table 1, the number of clones resistant to AMP, AMC, P and Te, OT, the antimicrobials commonly used in animal husbandry, increased during 2002 to 2008. The resistance to ER was also found (19–20%). A low level of OX resistance was detected, with a decrease from 2002–2005 to 2006–2008. A double increase in tetracycline resistence is shown and an substantial increase in OT was also observed.
The presence of mecA gene was investigated by PCR and resulted in a 10% of positive clones with similar percentages in the 2 considered periods. None of them showed resistance to VA and ENR.
Conclusion: The finding of a general increasing trend in antibiotic resistance against the selected drugs implies that the selective pressure has acted in the ecosystem favouring the espansion of resistant clones; for this reason is important the monitoring of drug resistance in food animals and a prudent usage of antibiotics in the veterinary field, to avoid major risk for increase drug resistance in the human population.

Table 1

2002-2005	OX	AMP	AMC	P	TE	VA	ER	ENR	OT
NUM R	5	31	27	32	17	0	14	0	21
TOTALE	73	73	73	73	73	73	73	73	73
%R	6,85%	42,47%	36,99%	43,84%	23,29%	0,v00%	19,18%	0,00%	28,77%

2006-2008	OX	AMP	AMC	P	TE	VA	ER	ENR	OT
NUM R	3	58	33	58	47	0	22	0	44
TOTALE	99	99	99	99	99	99	99	99	99
%R	3,03%	58,59%	33,33%	58,59%	47,47%	0,00%	22,22%	0,00%	44,44%

R2319 Evolving molecular epidemiology of Enterobacteriaceae resistant to expanded-spectrum cephalosporins in Italy: one-year results of a large, multicentre, observational study

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Objectives: Italy is among the countries reporting increasing resistance rates to expanded-spectrum cephalosporins (ESCs) in Enterobacteriaceae. Production of extended-spectrum β-lactamases (ESBLs) and AmpC-type β-lactamases (CBLs) remain the major mechanisms of ESC resistance. We report here on the molecular epidemiology of ESBL- and CBL-producing enterobacterial isolates collected during one-year in a large, multicentric study recently conducted in Italy.
Methods: Five Italian Medical Centres consecutively enrolled patients with infections caused by *Escherichia coli*, *Klebsiella pneumoniae* or *Proteus mirabilis* with reduced susceptibility to ESCs (cefotaxime and/or ceftazidime MICs >1 mg/L) during the period 2007–2008. ESBL production was confirmed using the double-disk synergy test. ESBL and CBL determinants were characterized by DNA hybridization and confirmed by PCR and sequencing. Clonal relatedness was investigated by RAPD.
Results: A total of 444 cases of infection caused by Enterobacteriaceae with reduced susceptibility to ESCs were enrolled (268 by *E. coli*, 74 by *K. pneumoniae* and 102 by *P. mirabilis*). ESBL production was confirmed in 396 (89%) of isolates, CBL production was observed in all ESBL-negative *P. mirabilis*. Among the ESBL producers, CTX-M-type enzymes were overall the most prevalent (80% overall, 91% in *E. coli*, 90% in *Klebsiella*, and 12% in *Proteus*) and mostly (95.8%) belonged in group 1 (CTX-M-1 and CTX-M-15). However, CTX-Ms of groups 2 (1.1%, CTX-M-2) and 9 (3.1%, CTX-M-14 and CTX-M-27) were also detected. Other ESBLs included TEM- and SHV-type variants (10% and 4.7% overall, respectively, including TEM-92, TEM-72 and SHV-12, SHV-2a). All CBLs belonged in the CMY-2 lineage, being totally represented by CMY-16. Clonal heterogeneity was observed among the ESBL producers of each species, although clonal expansion phenomena caused by CTX-M-producing *E. coli* ST131 were observed. All CBL-producing *P. mirabilis* isolates were clonally related with each other.
Conclusions: In comparison with previous studies, a remarkable evolution of the molecular epidemiology of ESC-resistant Enterobacteriaceae was observed in Italy. CTX-M-type enzymes have become the predominant ESBLs, with enzymes of group 2 and 9 also emerging, while CMY-2-like CBLs now significantly contribute to ESC resistance in *P. mirabilis*.

R2320 Further increase in carbapenem-, amikacin-, and fluoroquinolone-resistant isolates of *Acinetobacter* spp. and *P. aeruginosa* in Korea: KONSAR study 2009

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Objective: The increasing prevalence of carbapenem-, amikacin-, and fluoroquinolone-resistant *Acinetobacter* spp. and *P. aeruginosa* are of particular concern in Korea. We analyzed resistance trends of *E. coli*, *K. pneumoniae*, *Acinetobacter* spp. and *P. aeruginosa* in Korean hospitals (HOSP) and clinics.
Methods: Antimicrobial susceptibility was determined by participating laboratories (Labs) either by CLSI disc diffusion or commercial broth microdilution methods. The data collected from 24 HOSPs and two commercial Labs (CLAB) servicing secondary-care HOSPs and clinics were analyzed, excluding those from Labs with poor performance.
Results: Contrary to our expectations, typical nosocomial pathogens, *Acinetobacter* spp. and *P. aeruginosa*, comprised a large proportion of CLAB isolates. We were also surprised that most resistance rates were higher for CLAB isolates. Trends of resistance in 2007 vs. 2009 for HOSP isolates were: ceftazidime-resistant *E. coli* 8% vs. 17%, ceftazidime-resistant *K. pneumoniae* 29% vs. 33%; imipenem-resistant *Acinetobacter* spp. 22% vs. 51%; and imipenem-resistant *P. aeruginosa* 21% vs. 26%. A previous study showed that the majority of imipenem-resistant *Acinetobacter* spp. was mediated by OXA carbapenemase production. Among the HOSP *Acinetobacter* spp. isolates in 2007 and 2009, fluoroquinolone-resistance rose from 48% to 67%, and amikacin-resistance rose from 37% to 48%. Comparison of the data from six >1000-bed HOSP showed significantly higher imipenem-resistant rates among ICU isolates than non-ICU isolates: *Acinetobacter* spp. 74% vs. 53%, and *P. aeruginosa* 39% vs. 20%.
Conclusion: Ceftazidime-resistant *E. coli* and *K. pneumoniae* were also prevalent among CLAB-tested isolates. Imipenem-, amikacin- and

fluoroquinolone-resistant *Acinetobacter* spp. increased further in both HOSPs and CLABs. Imipenem-resistant isolates were more prevalent among ICU isolates. We are confronted with a worsening problem in treating *Acinetobacter* spp.-infected patients and controlling resistance.

R2321 Evaluation of tigecycline activity in clinical isolates

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Objectives: Resistance to antimicrobial agents is emerging in a wide variety of nosocomial-acquired pathogens. Tigecycline, a glycylcycline antimicrobial, is a newer treatment option for emerging single- or multidrug-resistant Gram-positive cocci (GPC) and Gram negative bacilli (GNB). The purpose of the present study was to determine the in vitro activity of tigecycline against clinical isolates of various pathogens.
Material and Method: A total of 3240 non-duplicate clinical isolates collected from hospitalized patients during 2009, were evaluated by the disk diffusion method. The isolates were classified as tigecycline susceptible and resistant based on FDA breakpoints. Isolates with intermediate resistance to tigecycline have been included in the resistant group.
Results: Tigecycline activity against all GPC and GNB isolates tested is shown in the table.
Conclusion: The present data show that tigecycline has excellent activity against all Gram positive and Gram negative organisms tested, with the important exception of *Acinetobacter* spp. that are showing alarming resistance rates to tigecycline in the area of Crete.

Microorganism	No of microorganisms	Resistance n (%)	Susceptibility n (%)
S. aureus	307	0	307 (100%)
Coagulase negative Staphylococcus	693	0	693 (100%)
Enterococcus spp	176	0	176 (100%)
S. agalactiae	26	0	26 (100%)
E. coli	984	8 (0.8%)	976 (99.2%)
Klebsiella spp	455	50 (11%)	405 (89%)
Enterobacter spp.	164	15 (9.1%)	149 (90.9%)
Serratia spp	57	4 (7%)	53 (93%)
Acinetobacter spp.	378	231 (61.1%)	147 (38.9%)

R2322 Susceptibility pattern of *H. pylori* after treatment failure

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Objectives: According to the Maastricht III Consensus, *H. pylori* test and treat is the strategy of choice in most *H. pylori* infected patients and PPI combined with amoxicillin and clarithromycin/metronidazole is recommendated as first line treatment. There is a growing concern regarding antibiotic resistance in *H. pylori*, which can result in treatment failure. The aim of this study was to evaluate the susceptibility pattern of *H. pylori* strains after treatment failure in Danish patients.
Methods: 50 clinically isolated *H. pylori* strains were susceptibility tested by E-test for amoxicillin, metronidazole, ciprofloxacin, levofloxacin, clindamycin, erythromycin, clarithromycin, rifampicin, tetracycline and meropenem.
The bacteria were grown and susceptibility tested according to the manufacturer.
Results: 74% of the strains were resistant to metronidazole, 54% were resistant to clindamycin and clarithromycin and 52% were resistant to erythromycin while 36% of the strains were resistant to all those 4 antibiotics at the same time. All strains were susceptible to amoxicillin, ciprofloxacin, levafloxacin, tetracycline and rifampicin.
Conclusion: This study shows a high rate of resistance to the most commonly used antibiotics and a high rate of multiresistant strains in patients with treatment failure. This suggests that antimicrobial susceptibility testing should be done after the first treatment failure.

R2323 Retrospective study of microbiological cultures during the last two years from intensive care unit

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Objectives: To study the results of cultures of patients, who had been hospitalized in intensive care unit (I.C.U.) during the last two years The specimens were from bronchial lavages, urine, rectum, central venous catheters, blood, traumas and pharyngeal smears.
Methods: The specimens were cultivated in chocolate, blood, McConkey No2 and Chapman agars. in 35–37º for 24 h. The identification and control of sensitivity were performed by mini API test and diffusion disk test by Kirby-Bauer according to the CLSI standards.
Results: From the 1145 cultures of the period (24/7/2009–24/7/2010), 815 were negative, and 330 were positive. From the 1350 specimens of period (24/7/2008–24/7/2009) 805 were negative and 545 positive. We isolated in the period (24/7/2008–24/7/2009) the following bacterial species *Pseudomonas aeruginosa* 104 (19,0%) *K. pneumoniae* 169 (31,00%) *Acinetobacter baumannii* 120 (22,0%) *E. coli* 28 (5,13%) *S. aureus* 32 (5,8%) *Stenotrophomonas maltophilia* 11 (2,01%), *Klebsiella oxytoca* 9 (1,6%) *Proteus mirabilis* 39 (7,15%) *Staphylococcus* spp 40 (7,3%) *Candida* spp. 146 (26,7%) *Candida albicans* 89 (16,3%) *Enterococcus* spp. 5 (0,9%). During the period (24/7/2009–24/7/2010),the following bacterial species were isolated: *Pseudomonas aeruginosa* 51 (15,4%), *K. pneumoniae* 33 (10%), *Acinetobacter baumannii* 12 (9,6%), *E. coli* 12 (3,6%), *S. aureus* 11 (3,3%), *Stenotrophomonas maltophilia* 11 (3,33%), *Klebsiella oxytoca* 9 (2,72%), *Proteus mirabilis* 4 (1,2%), *Staphylococcus* spp 17 (5.1%), *Candida* spp. 110 (33,3%) *Candida albicans* 21 (6,3%), *Enterococcus* spp. 10 (3,0%). The resistance to antibiotics for *K. pneumoniae* was the following: Ampicillin/Sulbactam 52%, Piperacillin/Tazobactam 42%, Amikacin22%, Meropenem 16%, Imipenem 15%. The resistance for *Acinetobacter baumannii* was the following: Amikacin 75%, Meropenem 74%, Imipenem 76%, Piperacillin/Tazobactam 80%, Ticarcillin/Clavulanic Acid 82%, Ampicillin/Sulbactam 84%, Colistin 0%.
Conclusions: The most common isolated microorganism from the Gram(−) negatives, during the period (2008–2009) was *K. pneumoniae* with second *A. baumannii*, while the period 2009–2010 first was *Pseudomonas aeruginosa* with second *K. pneumoniae*. First from the Gram(+) positive microorganisms, was *S. aureus*. Infections due to *Candida* spp. are an increasingly important complication in hospitalized patients in I.C.U. The resistance of *Acinetobacter baumannii* to antibiotics raises great concern.

R2324 Activity of ceftaroline against European isolates from complicated skin and skin structure infections collected in 2008–2009

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Objective: Ceftaroline (CPT) fosamil, the prodrug of the active compound CPT, is a broad-spectrum, parenteral cephalosporin recently approved in the USA for the treatment of acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia. The aim of this study was to determine CPT activity against complicated skin and skin structure infection (cSSSI) isolates from Austria, Bulgaria, Czech Republic, France, Germany, Greece, Ireland, Italy, Poland, Portugal, Russia, Slovakia, Spain, Switzerland, the Netherlands, Turkey and the UK.
Methods: 103 European centres submitted 2708 bacterial isolates causing cSSTIs in 2008–2009. These were re-identified and CPT (plus numerous comparator) MICs determined by CLSI broth microdilution at a central laboratory.
Results: Summary CPT MIC data are shown in the Table [cefuroxime (FUR) data are shown as a reference].

Conclusions: These data from a large collection of European isolates confirm the broad-spectrum activity of CPT against cSSSI pathogens. CPT is unique among clinically available cephalosporins, having good activity against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphylococci, in addition to moderate activity against Gram-negative bacteria.

Group/species (N)	Antibiotic	MIC (mg/L)			
		Min	Max	50%	90%
Beta-haemolytic streptococci (522)	CPT	<=0.001	0.03	0.004	0.015
	FUR	<=0.015	0.25	<=0.015	0.06
Viridans streptococci (75)	CPT	<=0.001	8	0.015	0.06
	FUR	<=0.015	>=32	0.25	2
Methicillin-resistant S. aureus (480)	CPT	0.015	2	0.5	2
	FUR	2	>=128	2128	>=128
Methicillin-susceptible S. aureus (470)	CPT	0.06	2	0.25	0.25
	FUR	0.25	>=128	1	2
Methicillin-resistant, coagulase-negative staphylococci (155)	CPT	0.06	>=128*	0.5	2
	FUR	0.12	>=128	4	>=128
Methicillin-susceptible coagulase-negative staphylococci (113)	CPT	0.015	0.5	0.06	0.25
	FUR	0.06	32	0.5	1
Enterobacter spp. (294)	CPT	0.03	>=128	0.5	>=128
	FUR	2	>=128	16	>=128
E. coli (393)	CPT	0.015	>=128	0.12	>=128
	FUR	0.5	>=128	4	>=128
K. pneumoniae (197)	CPT	0.008	>=128	0.12	>=128
	FUR	0.5	>=128	4	>=128

*Max MIC only seen against 1 S. capitis from Italy. All other coagulase-negative staphylococci had a CPT MIC of <= 4 mg/L.

R2325 Carbapenem-resistant klebsiella pneumoniae isolates in a Greek hospital

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Objectives: To evaluate the frequency and phenotype resistance to carbapenems of *K. pneumoniae* isolates from clinical samples in the General Hospital of Athens "PAMMAKARISTOS".
Methods: During one year period 105 strains of *K. pneumoniae* were isolated. Susceptibility tests were performed by Kirby-Bauer method, MIC determination by automated system (Microscan, Siemens) and E-test method, according to CLSI standards Phenotypic detection of VIM enzymes was based on CD synergy test with Meropenem and EDTA and of KPC enzymes on the combination of modified Hodge test and the CD synergy test with Meropenem and boronic acid.
Results: From a total of 105 *K. pneumoniae* isolates, 14 (14/105, 13.3%) exhibited reduced susceptibility to carbapenems (MIC range: 2–⩾16) and were isolated from cultures of urine (n = 8), blood (n = 2), wounds (n = 2), catheter tips (n = 1) and bronchial secretions (n = 1) of patients with haematologic malignancies (6), solid tumors (1), cardiovascular diseases (3) and surgical patients (4). Five strains (5/14, 35.7%) were KPC-producers, four (4/14, 28.6%) were VIM-producers, one (1/14, 7.2%) was co-producing both carbapenemases, two (2/14, 14.25%) produced KPC and ESBL and two (2/14, 14.25%) produced VIM and ESBL. The distribution of isolates was as follows: Internal Medicine unit (9), surgical units (4), Neurology (1). All strains were sensitive to gentamycin and tigecycline, 2 were resistant to tetracycline.
Conclusion: Phenotypic method is very helpful in detecting carbapenemase-producing *K. pneumoniae* isolates and in limiting the spread of multiresistant strains. All *K. pneumoniae* strains with reduced susceptibility to carbapenems should be monitored for production of VIM and/or KPC enzymes.

R2326 Distribution of bloodstream infection pathogens over time at an Australian tertiary hospital

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Objectives: Previous studies have demonstrated significant changes in the distribution of bloodstream infection (BSI) pathogens over time with Gram-positive pathogens becoming more prevalent than Gram-negatives since the 1970s. It is also recognised that there is a shift to Gram-negative pathogens with increasing age. This study describes the trends in BSI pathogens and antimicrobial susceptibility over 9 years at an Australian adult tertiary referral hospital.
Methods: Positive blood cultures from 1st January 2001 till 31st December 2009 were reviewed. Duplicate isolates (within 14 days of the primary culture) and patients <20 years of age were excluded. Patients in haematology (including bone marrow transplantation), respiratory (including lung transplant and cystic fibrosis), oncology and burns units were also excluded as they represented heterogeneous populations. Coagulase negative Staphylococci (CNS) were excluded as it was not possible to retrospectively differentiate between true infections and contamination.
Results: We found an overall decline in blood culture positivity rate from 9.48 to 4.80 per 1000 admissions. Similarly, the proportion of positive cultures also decreased from 2.6% to 1.5%. Overall, Gram-positive pathogens were isolated in 48.9% of patients (*S. aureus* 24.0%, *Enterococcus* spp. 7.1%, *S. viridans* 3.6%) compared to Gram-negative pathogens in 45.5% of patients (*E. coli* 20.3%, *Klebsiella* spp. 6.9%, *Pseudomonas* spp. 3.8%). There was a trend to an increasing proportion of Gram-negative pathogens during these 9 years. However, age specific proportions of Gram-positives and Gram-negatives remained similar, suggesting this relates to overall aging of the hospital population. The proportion of *S. aureus* isolates that were methicillin-resistant (MRSA) declined from 54.0% to 27.5%. In Gram-negatives, susceptibility to gentamicin and third generation cephalosporins by Enterobacteriaceae remained stable. Ceftazidime resistance also remained stable, suggesting no increase in extended spectrum β-lactamase (ESBL) producing organisms.
Conclusions: Our study demonstrated important trends in epidemiology of BSI pathogens over time and their impact on choices of appropriate antimicrobial therapy, particularly for the elderly hospital inpatient population. β-lactams, gentamicin and quinolones all remain effective antibiotics for our patient population.

R2327 *Acinetobacter* resistance to antimicrobial agents: a prospective study in Iran

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Objective: *Acinetobacter* species have become increasingly resistant to antibiotics over the past several years and currently present a significant challenge in treating these infections. They are important cause of nosocomial infections.
Methods: In a prospective study we evaluated 100 positive cultures of *Acinetobacter* from 100 patients in different wards of seven tertiary care hospitals in Tehran, Iran in a 6 months period in 2009. PCR was used to determine the species of *Acinetobacter*. E-test and Disk diffusion method was used to determine the resistance of isolated *Acinetobacter baumannii* and non-baumannii. Antimicrobial sensitivity to following antibiotics was analyzed: ceftazidime, cefepime, amikacin, imipenem, piperacillin-tazobactam, tigecycline and colistin.
Results: In our study 89% of isolated *Acinetobacter* was baumannii and 11% was non-baumannii. 70% of samples were isolated from male and 30% from female patients. The most incriminated wards were intensive care and burn units with a high prevalence of pneumonia and wound infection due to acinetobacter. The most frequently sites of infection were wound, respiratory tract, blood stream, urinary tract, cerebrospinal fluid and brain abscess. *Acinetobacter* was isolated from respiratory secretion in 38%, wound in 29%, tip of catheter in 14%, urine in 8%, blood in 4%, CSF in 4%, pleural fluid in 2% and brain abscess in 1% of samples. *Acinetobacter* was resistant to amikacin in 100%, to ceftazidime in 100%, to cefepime in 94.5%, to piperacillin-tazobactam in 83% and to imipenem in 64% of all the samples. Sensitivity to colistin was 100% and to tigecycline was 74.5% in our study.
Conclusion: Prevalence of cephalosporins and carbapenems resistant acinetobacter is high in our study. Colistin and tigecycline are best choices for treatment of acinetobacter. High prevalence of nosocomial

infections and presence of multidrug resistant *Acinetobacter* specially in ICU patients require developing new strategies for control of acinetobacter infection.

R2328 Resistance associated with extended-spectrum β-lactamases production by strains isolated from infections in neonatal intensive care units in Poland

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Objectives: Newborns in NICUs are at high risk for infections. The most important risk factors are invasive devices and frequent use of antimicrobials. The project aims to analyze epidemiological and microbiological infections with Gram negative etiology occurring in children with low birth weight (LBW) in a Polish NICUs.
Methods: Data collection was made prospectively from 01–12.2009 from a group of 910 LBW newborns. They were monitored from <1 500 g until discharge >1 800 g or death. Surveillance included main types of infections: bloodstream infections-BSI, pneumonia-PNU, necrotizing enterocolitis-NEC and others.
In 2009, 93 of Gram negative strains were isolated from 41 patients. 57 isolates were from PNU, 21 from BSI, 15 from others.
Species were determined in the Vitek automatic system, drug resistance was determined by disc diffusion method (according to EUCAST). Genes for β-lactamases SHV, TEM and CTX-M were identified with PCR.
Results: The incidence density per 1000 patient days was 27,1. The most common infection (regardless of the type of microorganism) was BSI: 43% of all and PNU: 38,8%.
Among the infections caused by Gram negative rods, the late onset infections (>2 days) dominated – 76%. 30% of them were PNU associated with MV, 8,45% with the use of NCPAP. 7% of BSI were associated with CVC and 4% with PVC.
The dominant species among the isolates were *E. coli* – 30, others were: *Enterobacter* sp. – 18, *Klebsiella* sp. – 24, *Serratia* sp. – 8, non-fermentative bacilli – 13.
Resistance to ceftazidime and cefotaxime was detected in 13% for EC, 31% for *Klebsiella*, 72% for *E. cloacae*. Resistance to 4 tested carbapenems was 6% for EC, 12,5% for PAR, the other strains were susceptible. All tested isolates were susceptible to tigecycline.
24 isolates of all (25,8%) showed the ESBL phenotype: *Enterobacter* 10, *Klebsiella* 8, *E. coli* 5 and *Serratia* 1. Only one ESBL+ isolate came from an early respiratory tract infection. 20 ESBL+ strains carried the blaCTX-M gene, 5 of them also had blaSHV and blaTEM genes. The remaining 4 ESBL+ strains did not had blaCTX-M gene but had both blaSHV and blaTEM.
Conclusion: Resistance due to production of ESBLs appears mainly in late infections. This has most likely occurred because of selection pressure imposed by the intensive use of broad spectrum antibiotics and/or because of plasmid mediated acquisition of resistance. One way to limit the spread of resistance would be the rational use of antibiotics for controlling ESBL+ strains infection.

R2329 Risk ranking predicted environmental concentrations of antimicrobial residues as a result of human consumption using regression analysis

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Objectives: The primary objective of this study was to determine leading factors influencing the presence of antimicrobial residues in the environment and to rank their predicted environmental concentrations (PEC).
Methods: A mechanistic model is presented for determination of antimicrobial presence in the environment (EXCEL 2003 with @Risk 5.0® add-on). The model was tested for six main groups of antimicrobials consumed in Europe: penicillins (PEN), β-lactams (BET), tetracyclines (TET), macrolides (MAC), quinolone/fluoroquinolones (Q/F) and sulfonamides/trimethoprim (S/T). The model simulates the release of antimicrobials into the environment by integrating the effects of antimicrobial use, metabolism, degradation, and dilution. Each input variable was unified and assigned a probability density to represent inherent uncertainty and variability in the parameter. The Monte Carlo simulation model resulted in probability distributions of PECs for each antimicrobial group. Using regression analysis, resulting PECs were ranked in relation to resistance potential, chronic and acute toxicity and hazard quotient (HQ).
Results: The model simulated the mean PEC of PEN, BET, TET, MAC, Q/F and S/T (Table 1). Degradation was the main input influencing PEN PEC, usage was foremost for BET and S/T, while metabolism was the most critical input for TET, MAC and Q/F PECs. Q/F expressed the highest rate of resistance formation potential (57%). BET expressed a moderate HQ (2.02) with all remaining antimicrobials expressing a low HQ. No antimicrobial group was predicted to express toxicity in the environment.
Conclusion: The observed results infer that current antimicrobial use can lead to levels in the environment which may increase resistance formation. The legislation of new antimicrobials should consider metabolism, as it will greatly influence levels emitted into the environment.
The in-depth understanding of antimicrobial presence in the environment is lacking; specifically, with regard to lower limits of minimum inhibitory concentrations acting as selectors for resistance formation. The use of ECOSAR® for antimicrobial toxicity reference values is limited as the bacteria targeted by antimicrobials are phylogenetically distinct from the cyanobacteria used to calculate the EC/LCsub50 values. The results and limitations presented here accentuate the need for further research into antimicrobials in the environment and the development of antimicrobial resistant strains.

Table 1: Monte Carlo simulation and regression analysis results of six main antimicrobial groups consumed in Europe.

Antimicrobial Group	PEC* (mg m^3 day)	Leading contributing factor	R^2	Resistance formation potential (%)	Chronic/Acute toxicity (%)	HQ#
Penicillin	0.43	Degradation	0.87	11	0	0.31
Beta-lactam	0.14	Use	0.66	47	0	2.02
Tetracycline	0.05	Metabolism	0.67	10	0	0.02
Macrolide	0.05	Metabolism	0.71	3	0	0.03
Quinolone/ Fluoroquinolone	0.02	Metabolism	0.58	57	0	0.86
Sulfonamide/ Trimethoprim	0.07	Use	0.82	5	0	0.08

* PEC, Predicted environmental concentration
# HQ, Hazard Quotient

R2330 In vitro activity of tigecycline against bacterial pathogens from Tigecycline Evaluation and Surveillance Trial (TEST Program 2010) in Hong Kong

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Objectives: Tigecycline is the first of the glycylcycline antimicrobial active against a wide range of bacterial pathogens. We have participated in the global Tigecycline Evaluation and Surveillance Trial (TEST) to study the in-vitro activity of tigecycline. This report summarizes the antimicrobial susceptibility of bacterial pathogens collected in 2010.
Methods: A total of 135 Gram-negative and 65 Gram-positive isolates were collected in the Prince of Wales Hospital. MICs were determined using microdilution trays purchased from TREK Diagnostics Systems, East Grinstead, UK and interpreted using CLSI guidelines.
Results: Tigecycline MIC_{90} were all smaller than or equal to 1mg/L against Gram-positive isolates including *Staphylococcus* spp. (n = 25), *Enterococcus* spp. (n = 15), *Streptococcus pneumoniae* (n = 15) and other *Streptococcus* spp. (n = 10). Tigecycline MIC_{90} were smaller than or equal to 2mg/L against most of the Gram-negative isolates including *E. coli* (n = 25), *Klebsiella* spp. (n = 25), *Enterobacter* spp. (n = 25), *Serratia* spp. (n = 10) and *H. influenzae* (n = 15) but with MIC_{90} = 8mg/L against *Acinetobacter* spp. (n = 15) and >16mg/L against *P. aeruginosa* (n = 20). The percentages of resistance of different antibiotics against

Gram-negative and Gram-positive bacteria are shown in Table 1 and 2 respectively. Shading denotes % resistance value smaller than or equal to 10%.

Conclusion: Tigecycline showed excellent activity against all Gram-positive and Gram-negative isolates but less active against *Acinetobacter* spp. and *P. aeruginosa*.

Table1: The percentages of resistance of different antibiotics against Gram-negative bacteria

Organism	N	AUG	P/T	LEVO	AXO	FEP	AMP	AMI	MIN	TAZ	TGC	MERO
E. coli	25	32	12	48	36	28	76	4	36	8	-	0
Klebsiella spp.	25	20	4	0	12	12	92	0	36	4	-	0
P. aeruginosa	20	-	15	25	95	25	-	0	-	20	-	10
Enterobacter spp.	25	100	28	4	36	0	100	0	44	32	-	0
Acinetobacter spp.	15	-	73.3	53.3	100	53.3	-	6.7	20	53.3	-	53.3
Serratia spp.	10	90	0	10	30	0	90	0	60	0	-	0
H. influenzae	15	0	0	-	-	-	40	-	-	-	-	-

AUG: Amoxacillin/clavulanic acid; P/T: Piperacillin/tazobactam; LEVO: Levofloxacin; AXO: Ceftriaxone; FEP: Cefepime; AMP: Ampicillin; AMI: Amikacin; MIN: Minocycline; TAZ: Ceftazidime; TGC: Tigecycline; MERO: Meropenem

Breakpoints not suggested by CLSI denotated by "-"

Table2: The percentages of resistance of different antibiotics against Gram-Positive bacteria

Organism	N	AUG	P/T	LEVO	AXO	LZD	MIN	VAN	AMP	PEN	TGC	MERO
Staphylococcus spp.	25	16	12	16	20	0	0	0	100	100	-	0
Enterococcus spp.	15	-	-	46.7	-	13.3	100	0	20	26.7	-	-
Streptococcus pneumoniae	15	20.0	-	0	40.0	-	-	-	-	46.7	-	46.7
Streptococcus spp.	10	-	-	0	-	-	-	-	-	-	-	-

AUG: Amoxacillin/clavulanic acid; P/T: Piperacillin/tazobactam; LEVO: Levofloxacin; AXO: Ceftriaxone; LZD: Linezolid; MIN: Minocycline; VAN: Vancomycin; AMP: Ampicillin; PEN: Penicillin; TGC: Tigecycline; MERO: Meropenem

Breakpoints not suggested by CLSI denotated by "-"

R2331 Invasive *Streptococcus mitis* and *Streptococcus oralis*: penicillin and macrolide resistance mechanisms in patients with haematological malignancies

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Objectives: Penicillin resistance concomitant with erythromycin resistance is a risk factor in the mortality of patients with *S. mitis* and *S. oralis* bacteremia. The aim of the study was to determine penicillin and macrolide resistance mechanisms in invasive *S. mitis* and *S. oralis* bacteremia strains isolated from patients that have haematological malignancies.

Methods: *S. mitis* (n:23) and *S. oralis* (n:17) isolates from blood cultures of patients (n:40) with haematological malignancies were included in the study. Isolates were identified with BD Phoenix system. Penicillin and erythromycin susceptibilities were performed with E-test. Vancomycin, clindamycin, cefotaxime, levofloxacin and linezolid susceptibilities were determined by broth microdilution. Penicillin and erythromycin resistance genes, pbp1a, pbp2b, pbp2x, ermB and mefA/E were amplified using PCR method. BamHI restriction enzyme was used for discrimination of mefA and mefE.

Results: Patient age range was 42–84. Twenty of fourty patients were women. Thirty of the patients have diagnosed as myelogeneous (n:22) and lymphoblastic leukaemia (n:8). Fifteen of the patients have died due to solid cancers. Fourteen (35%) and eighteen (45%) strains were resistant to penicillin (MIC $\geqslant$4 mg/L) and erythromycin (MIC $\geqslant$4 mg/L), respectively. Rate of resistance to clindamycin and cefotaxime were 32.5% and 22.5%. All the isolates were susceptible to vancomycin, levofloxacin and linezolid. Five of penicillin resistant isolates carried pbp2b and three carried pbp2x genes. Among erythromycin resistant and intermediate (n:23) isolates,16 exhibited ermB and 7 mefE genotypes.

Conclusion: Erythromycin resistance is highly due to ermB and followed by mefE genes and penicillin susceptibility can partially be explained by the presence of pbp2b and pbp2x genes among our isolates. It is important to be aware of high level resistance of penicillin and erythromycin in *S. mitis* and *S. oralis* in patients with haematological malignancies as an emerging threat since susceptible empirical antibiotics may not reduce overall mortality.

R2332 Trend of antimicrobial susceptibility of *P. aeruginosa* isolates from UTI and RTI of Japanese hospital participating in the levofloxacine surveillance group during 1994–2010

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Objectives: *Pseudomonas aeruginosa* has become ploblematic because of an outbreak of multidrug-resistant clone producing metallo-β-lactamases (MBLs). We have already been taken nationwide surveillance for FQ and other antimicrobials resistance against many bacterial clinical isolates since 1994 in Japan. In this study, we report surveillance data for *P. aeruginosa* isolates from patients with urinary tract infection (UTI) and with respiratory tract infection (RTI) collected between 1994–2010.

Methods: A total of 7,019 clinical isolates (3,232 isolates from UTI and 3,787 isolates from RTI) were collected from 92 centers participating in the Levofloxacin Surveillance group during 1994–2010 in Japan. Antimicrobial susceptibility testing by broth microdilution methods was based on Clinical and Laboratory Standards Institute (CLSI) guidelines that were updated annually as revised documents were published.

Results and Discussions:

1. UTI; The 'Susceptible' rate for levofloxacin has particularly increased over time (from 41.8% in 1994 to 74.2% in 2010). Although the cause of this increase is obscure, recent request for strict observance of the dosing period is probably implicated because drug use reviews do not show any decrease of the amount of levofloxacin used nationwide in the field of urology. Amikacin and ceftazidime also showed a gradual increase of the 'Susceptible' rate. The susceptibility for imipenem remained unchanged. The rate of 4 drugs resistant isolate to levofloxacin, ceftazidime, amikacin and imipenem was about 1% until 1998, however increased to 4.6% abruptly in 2000 and then shifted at the level of approximately 4%.
2. RTI; The 'Susceptible' rate to levofloxacin, amikacin, ceftazidime and imipenem has been maintained constantly at a level of approximately 80%, 97%, 88% and 70%, respectively. The rate of 4 drugs resistant isolate to levofloxacin, ceftazidime, amikacin and imipenem was in a moderate increase in comparison with that in UTI.
3. Metallo-β-lactamase; The rate of metallo-β-lactamase producing isolates from UTI and RTI was 8.0% in 2002, 7.2% in 2004 and 5.6% in 2007 (UTI), and 1.5% in 2002 1.0% in 2004 and 2.2% in 2007 (RTI), respectively.

R2333 Detection of extended-spectrum β-lactamases in clinical isolates of *Pseudomonas aeruginosa*

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Objectives: *Pseudomonas aeruginosa* is a leading cause of nosocomial infections worldwide. The infections can be particularly severe in patients with impaired immune systems. The infections of this agent are frequently difficult to treat because of both the natural resistance of the species and its ability to acquire further resistance mechanisms to multiple groups of antimicrobial agents. Resistance of *P. aeruginosa* strains to the broad-spectrum cephalosporins may be mediated by the extended-spectrum b-lactamases (ESBLs). The aim of this study was to determination of *P. aeruginosa* antibacterial resistance patterns and the prevalence of ESBLs producing strains as PER-1 and VEB genes.

Methods: In our study, a total of 106 clinical isolates of *P. aeruginosa* were studied. The isolates were collected from two university hospitals in Hamadan, Iran, during 7 month (2009), to assess the current levels of antimicrobial susceptibility. The susceptibility of the investigate

P. aeruginosa isolates to 12 antimicrobial agents was determine by the disc diffusion method on Mueller Hinton agar plates and was interpreted according to the Clinical an Laboratory Standards Institute (CLSI) recommendation. ESBLs producing strains have detected by combined disk test and the presence of PER-1 and VEB genes by PCR.
Results: The antibiotic resistance rates against the broad-spectrum cephalosporins and monobactames were: cefepime 97%, cefotaxime 92.5% ceftazidime 51%, and aztreonam 27%. Ciprofloxacin (91.5%), imipenem (84.9) and meropenem (82.07) were the most effective anti-pseudomonal agents. The results revealed that 94 (88.7%) of the isolates were multidrug resistant and 60 (58.25%) of the isolates were ESBL positive. Sixteen (26.6%), 9 (15%) and 3 (5%) strains among 60 ESBL-producing strains amplified blaPER-1, blaVEB and blaPER-1/blaVEB respectively.
Conclusion: This study highlights needs to establish antimicrobial resistance surveillance networks for *P. aeruginosa* to determine the appropriate empirical treatment regimen. Bacterial strains resistant to most classes of antibiotics will continue to emerge unless inappropriate uses of drugs are decreasing a continuous education of infection control practices maintained. The high prevalence of multidrug resistance and production of ESBLS in *P. aeruginosa* isolates in patient confirm that Protocols considering these issues should be considered in hospitals.

R2334 A trend of drug-resistant *Streptococcus pneumoniae* at King Chulalongkorn Memorial Hospital, Thailand

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Objectives: To determine the epidemiologic, clinical, and microbiologic data of infections caused by drug-resistant *Streptococcus pneumoniae* (DRSP) in adult patients.
Patients and Methods: A retrospective study of all adult patients with pneumococcal infections who were hospitalized at King Chulalongkorn Memorial Hospital, Bangkok, Thailand, was carried out from January 1, 2008 to December 31, 2009. In addition, a trend of prevalence of penicillin- and cephalosporin-non-susceptible *S. pneumoniae* (PNSP and CNSP) from 1997 to 2009 in our institute was analyzed.

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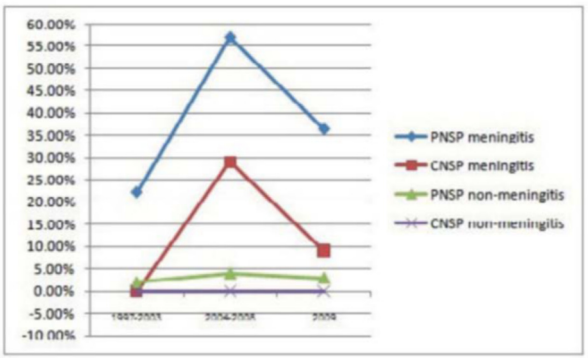
Table. A trend of penicillin and cephalosporin non-susceptible *Streptococcus pneumoniae*



Results: Of 86 pneumococcal isolates, there were 70 (81.40%), 5 (5.81%), and 11 (12.79%) patients with pneumonia, primary bacteremia, and meningitis, respectively. There were 56 (65.11%) males and 30 (34.89%) females with the mean age of 62 + 10 years (range 15–101 years). Of 70 patients with pneumonia, there were 67 (95.70%), 3 (4.30%), 0 (0%), 70 (100%), 0 (0%), and 0 (0%) of penicillin-susceptible *S. pneumoniae* (PSSP), penicillin-intermediate *S. pneumoniae* (PISP), penicillin-resistant *S. pneumoniae* (PRSP), cefotaxime-susceptible *S. pneumoniae* (CSSP), cefotaxime-intermediate *S. pneumoniae* (CISP), and cefotaxime-resistant *S. pneumoniae* (CRSP), respectively. Of 5 patients with primary bacteremia, there were 5 (100%) and 5 (100%) of PSSP and CSSP, respectively. Of 11 patients with meningitis, there were 7 (63.64%), 4 (36.36%), 10 (90.91%), 1 (9.09%), and 0 (0%) of PSSP, PRSP, CSSP, CISP, and CRSP, respectively. All isolates were susceptible to vancomycin. A trend of prevalence of meningitis caused by PNSP and CNSP from 1997 to 2009 in our institute is summarized in Table.
Conclusions: There is a relatively high prevalence of infections caused by PNSP and CNSP in adult patients in our institute. This emphasizes an urgent need to strengthen both appropriate use of antimicrobials and strict infection control measures to help reduce the occurrence of DRSP.

Molecular epidemiology of resistance genes, strains and serotypes

R2335 Penicillin and macrolide resistance mechanisms in invasive *Streptococcus pneumoniae* isolates before introduction of PCV7 in Ankara, Turkey

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Objectives: In this study, macrolide resistance mechanisms and the diversity of PBPs 2b, 2x and 1a of invasive *Streptococcus pneumoniae* (SP) isolates identified from patients admitted to Hacettepe Hospitals before the introduction of heptavalent pneumococcal conjugate vaccine (PCV-7) in Turkey between 1996 and 2008 was investigated.
Methods: Invasive SP clinical isolates were collected from children and adults admitted to Ihsan Dogramaci Children's Hospital and Adult Hospital of Hacettepe University. Antimicrobial susceptibility testing of all isolates were performed for six antimicrobial agents; penicillin (PEN), ceftriaxone (CRO), levofloxacin (LEV), erythromycin (EM), clindamycin (CD) and vancomycin (VAN) by broth microdilution method according to "Clinical Laboratory Standards Institute, CLSI". Serotypes were determined by Quellung reaction with specific antisera for SP. Isolates with MIC $\geqslant$0.125 mg/ml were examined for the PBP genes pbp2b, pbp2x ve pbp1a by PCR. Resistance genotypes of EM resistant isolates (MIC $\geqslant$0.5mg/ml) were determined by a multiplex erm(B)/mef PCR method for SP. RFLP analysis was done to differentiate mefA/E genes.
Results: Of the 182 nonduplicated pneumococcal isolates, 59 were obtained from children and 123 from adults. Of these, 32 were cerebrospinal fluid (CSF) and 150 were blood isolates. In 16 of CSF isolates (50.0%), MICs for PEN were $\geqslant$2 mg/ml. EM resistance (MIC $\geqslant$0.5mg/ml) was found in 23 (12.6%) isolates, of these 11 were resistant to both EM and CD. In EM resistant isolates, 10 (43.5%) had ermB gene, two (8.7%) had mef E gene. In 55 isolates with PEN MIC $\geqslant$0.125 mg/ml, 27 (49.1%) had pbp2x, pbp2x and pbp1a together, four (7.3%) pbp2x and pbp2b, three (5.5%) pbp2b, one pbp2x and pbp2b, one pbp2B and pbp1a and one pbp1a. In all isolates, resistance for LEV was 1% and 0 for CRO and VAN. In the paediatric age group, 17 different serotypes and in the adult group, 23 different serotypes were observed. The most frequent serogroups in both age groups were 6, 3, 23, 9 and 5.
Conclusions: Although there was no resistance for PEN among the blood isolates, PEN resistance was high among the CSF isolates. The major serotype associated with PEN resistance in CSF isolates was serotype 23F. Analysis of PBP genes showed predominancy in pbp2B. The predominant mechanism of macrolide resistance is associated with the erm(B) gene with strains showing co-resistance to clindamycin in the majority of cases.

R2336 Co-dissemination of antibiotic and copper resistance genes in large conjugative plasmids of enterococci from different ecological niches

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Objectives: tcrB, a copper resistance (CuR) gene, has been identified among enterococci from several geographical regions but data supporting its co-transference with genes encoding antibiotic resistance (ABR)

are scarce. Our goal was to analyze the occurrence of tcrB among enterococci from different ecological niches and to characterize the genetic context of this gene.
Methods: We analyzed 214 *E. faecalis*-Efl, 266 *E. faecium*-Efm, 23 *E. gallinarum*, 4 *E. casseliflavus*, 37 *E. hirae*, 97 Enterococci sp from hospitalized humans (H, n = 103); healthy humans (HV, n = 125), poultry (P, n = 129), piggeries environment/swine (PE, n = 232) and sewage/river (SR, n = 52) recovered from different Portuguese areas (1997–2007). Genes coding for CuR and ABR were searched by PCR. Mating assays were performed for 45 representative tcrB+ isolates in the presence of tetracycline, erythromycin, vancomycin or gentamicin, using different receptor strains. Clonality was studied by PFGE (SmaI)/MLST. Co-localization of tcrB and ABR genes was assessed by S1 PFGE hybridization.
Results: tcrB was detected in 15% (98/641) of isolates (26% PE, 15% SR, 11% HV, 9% P, 3% H; 59 Efm, 14 Efl, 25 other species). It was co-transferred with ABR genes (ermB-20; vanA-7; tetM-5; tetL-2; aac(6′)-Ie-aph(2″)-Ia-1). A polyclonal population was detected among tcrB+ representative isolates. Among Efm, 32 PFGE types corresponded to CC17 (ST18, ST393, ST431); CC5 (ST5, ST185, ST150) and also ST432, ST434 and ST432. Ten Efl PFGE types were detected. A few common strains were identified within and between niches (PFGE A-PE and H). tcrB was located on plasmids of 90–120kb in Efl and 120–300kb in Efm. The tcrB gene was located alone (5Efm PE, HV; 1Efl P) or with vanA (1Efm; PE), tetL (2Efm PE, HV), tetM (3Efm PE, HV), ermB (2Efm PE, SR; 2Efl PE, P), tetL+ermB (5Efm PE, HV), tetM+ermB (2Efm PE, SR), tetM+tetL (1Efl P), vanA+tetM+ermB (1Efm PE), tetM+tetL+ermB (6Efm PE, HV, SR). In 14 isolates tetM, tetL, ermB or aac(6′)-Ie-aph(2″)-Ia were located in other plasmids (25–90Kb), which co-transferred with those of tcrB (n = 4).
Conclusions: The co-transference of tcrB with other ABR genes located in the same or different plasmids suggest that the intensive use of copper in some niches might favour the selection of ABR enterococci. Both plasmid transfer and clonal expansion play important roles in the spread of tcrB.

R2337 Evaluation of L6 ribosomal protein alterations in fusidic acid-resistant *Staphylococcus aureus*: fitness cost and time-kill analysis

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Objectives: To evaluate fitness cost and activity by time kill experiments on three *S. aureus* (SA) clinical strains displaying elevated fusidic acid (FA) MIC values (4–8 mg/L) and L6 ribosomal protein (RP) alterations. L6 RP (encoded by fusE) has been recognized as a FA secondary action site and L6 mutations have been described in FA-resistant (R) SA small colony variant laboratory mutants.
Methods: Three SA clinical strains showing FA-R MIC values were identified in 2008 and 2009. Two strains were detected from one hospital during a surveillance study and one strain was isolated following FA therapy. MIC values were determined by CLSI broth microdilution method (M07-A8, 2009). Isolates were screened for known FA-R mechanisms by PCR and sequencing. Clonality was assessed by PFGE and spa typing. Growth rate studies were performed Q30 min for 10h. Time kill analyses were performed over 48h and tested FA at 8X MIC and at the clinical trough plasma level (80 mg/L).
Results: SA clinical strains showing FA-R (MIC, 4–8 mg/L) were screened for the presence of fusB, fusC, and fusD, as well as mutations in fusA, yielding negative results. All three SA showed fusE alterations. Two SA clinical strains were recovered from different patients in Michigan and possessed a 22 amino acid deletion in fusE starting at position 78 (compared to SA Mu50). These strains were shown to be identical by two typing methods, and belonged to USA300 clone. A third strain (FA MIC, 8 mg/L) recovered after FA therapy had a stop codon in L6 at position 77. This strain had identical PFGE and spa typing results as the pre-treatment isolate (FA MIC, 0.12 mg/L). SA displaying L6 RP alterations did not reach log phase in 10h (very slow generation times), while controls [NSR384 (USA300) and pre-treatment strain] demonstrated a mass doubling time of 90 min. Time kill studies showed rapid killing at the clinical trough plasma level for all FA-R clinical strains and controls.
Conclusions: L6 alterations have not been described in SA strains from patient infections. In this study, three SA clinical strains displaying L6 RP alterations were associated with modest elevation of FA MICs, but possess a remarkable difference in fitness cost. Furthermore, under experimental conditions, simulated FA plasma trough levels attained in vivo killed these R mutant strains.

R2338 Carbapenem-resistant *Acinetobacter baumannii* in burn unit from hospital in Krakow, Poland

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Objectives: Nosocomial infections remain the main cause of morbidity and mortality in burn patients. Infections caused by *A. baumannii* have emerged as a significant problem reported in burn units. *A. baumannii* strains are usually resistant to multiple antimicrobial agents including carbapenems which represent an important option for the treatment of *Acinetobacter* infections caused by multidrug-resistant isolates. The most common mechanism responsible for carbapenem-resistance are carbapenem-hydrolysing-β-lactamases belonging to molecular class D (OXA enzymes) and also may be associated with the presence of an insertion sequence (ISAba1). The aim of the study was detection of:1) OXA encoding genes; 2) presence of ISAba1.
Methods: A total of 32 *A. baumannii* strains were collected from 2007–2010 in burn unit (BU) of Specialized Hospital in Krakow, Poland. All strains selected to this study were carbapenem-resistant and were isolated from single patients. Identification and susceptibility testing were performed by VITEK-2 Compact (bioMérieux, Poland) according to CLSI criteria. Multiplex PCR described by Woodford et al. (2006) was applied for detection of OXA carbapenemases encoding genes (blaoxa-51-like, blaoxa-24-like and blaoxa-23-like). All strains were also tested for the presence of 549-bp fragment containing a portion of ISAba1.
Results: Antibiotic resistance rate to piperacillin was 100%, piperacillin-tazobactam 94%, ceftazidime 91%, cefepime 94%, imipenem 100%, meropenem 100%, gentamicin 100%, ciprofloxacin 100% but was only 3% to tobramycin and 25% to amikacin. All carbapenem-resistant isolates contained intrinsic gene encoding β-lactamase belonging to OXA-51-like group. The isolates were also found to encode blaOXA-23-like and blaOXA-24-like genes respectively in 28 (88%) and 2 (6%). ISAba1 insertion sequence gene was detected in all tested strains.
Conclusions: Carbapenem resistance in tested isolates might be associated with: 1) expression of acquired oxacillinases belonging to OXA-23-like and OXA-24-like groups; 2) extended expression of intrinsic oxacillinases belonging to OXA-51-like group supported by the presence of insertion sequence ISAba1.

R2339 Mutations in the quinolone resistance-determining region of gyrA gene of *Arcobacter butzleri*

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Objectives: *Arcobacter butzleri* has been associated with human infection. Fluoroquinolones are potential drugs for treatment. However, there is evidence of increasing resistance to these antimicrobial agents. This resistance may occur due to mutations in a quinolone resistance-determining region (QRDR) of the gyrA gene. Therefore the goal of this study was to look for the mutations associated with quinolone resistance in *Arcobacter* isolates.
Methods: Forty-four *A. butzleri* isolates and the reference strain *A. butzleri* DSM 8739 were used in this study. *Arcobacter* susceptibility to ciprofloxacin and levofloxacin was determined by disc diffusion tests (BD, USA) and E-test® strips (AB BIODISK, Sweden). As there is not any recommendation about antibiotic resistance of arcobacters, the disc diffusion breakpoints and the minimum inhibitory concentration (MIC)

values were determined as recommended by the Clinical and Laboratory Standards Institute (CLSI®) for campylobacters. All the isolates were subsequently analysed in order to determine the presence of mutations in the QRDR of gyrA gene. For that purpose, a 344-bp fragment of gyrA gene of *Arcobacter* spp. was amplified using F-QRDR (5′-TGG ATT AAA GCC AGT TCA TAG AAG-3′) and R2-QRDR (5′-TCA TMG WAT CAT CAT AAT TTG GWA C-3′) primers. Finally, PCR products were purified and sequenced on both strands by Sistemas Genómicos S. L. (Valencia, Spain).

Results: Among the 44 isolates tested, 31 were sensitive and the remaining 13 were considered to be resistant to both antibiotics. A disc diffusion zone of ⩽6 mm and a MIC value ⩾4 μg/mL indicates resistance. The MIC values with respect to ciprofloxacin and levofloxacin ranged from 0.064 to 0.38 μg/mL and 0.094 to 0.5 μg/mL, respectively for the sensitive isolates. The 13 resistant isolates presented MICs ranging from 8 to 32 μg/mL for both antibiotics. The sequencing of the 344-bp PCR product revealed a mutation in position 254 of the gyrA gene in the 13 resistant *Arcobacter* isolates. This C-254 to T mutation could be the cause of quinolones resistance as this change was absent in all the susceptible isolates.

Conclusion: This study shows high rates of quinolone resistance in arcobacters that was always associated to one mutation in the QRDR region of the gyrA gene. The increasing resistance to this class of antibiotics could be a public health concern as they have been reported to be the most commonly used and best performing fluoroquinolones against arcobacters.

R2340 Multidrug-resistant *Acinetobacter baumannii* blood isolates: evaluation of susceptible antibiotics, metallo-β-lactamase production and distribution of oxacillinase genes

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Objectives: The aim of this study was to evaluate antimicrobial susceptibility, metallo β-lactamase production (MBL) and dissemination of oxacillinase genes among multidrug resistant (MDR) invasive *Acinetobacter baumannii* isolates obtained in Hacettepe University Hospital between 2009–2010.

Methods: A total of 40 MDR *A. baumannii* invasive isolates were included in the study. Species identification was performed by use of BD Phoenix system. Susceptibility against piperacillin (PIP), tazobactam (TZP), amikacin (AN), gentamicin (GM), imipenem (IMP), meropenem (MER), cefotaxime (CTX), ceftazidime (CAZ), cefepim (FEP) ciprofloxacin (CIP), levofloxacin (LEV) was determined with broth microdilution method. Colistin (CL), tigecycline (TGC) and doripenem (DOR) susceptibility were performed by E-test. MBL production was determined by the combined disk test with IMP-0.1M EDTA. An increase in zone diameter of ⩾4 for 0.1M IMP-EDTA disks was considered positive for MBL production. OXA genes, 23-like, 24-like, 51-like and 58 were amplified by multiplex PCR assay.

Results: Twelve of the patients (30%)were from intensive care units (ICU). Colistin (97.5%), tigecycline (95%) were the most susceptible antibiotics tested. Carbapenem resistance rate was 95% (n:38) for IMP and MER and 90% (n:36) for DOR among the isolates. MIC_{90} values for the isolates were >64 μg/ml for PIP, >64/4 μg/ml for TZP, >32 μg/ml for AN, >8 μg/ml for GM, >8 μg/ml for IMP, >8 μg/ml for MER, >32 μg/ml for CTX, >16 μg/ml for CAZ, >16 μg/ml for FEP, >2 μg/ml for CIP, >4 μg/ml for LEV, 0.38 μg/ml for CL, 2 μg/ml for TGC, >32 μg/ml for DOR, respectively. Among 40 *A. baumannii* isolates, 2 (5%) yielded positive results for MBL production by 0.1M IMP-EDTA disk test. Oxacillinase genes were detected in 39 (97.5%) of the isolates. The occurence of OXA genes was: blaOXA-23 (n:27, 67.5%), blaOXA-58 (n:4, 10%) and blaOXA-51 (n:39, 97.5%). None yielded blaOXA-24.

Conclusion: This study shows that carbapenemase resistance is highly due to blaOXA-23 gene among our MDR *A. baumannii* invasive isolates. As the treatment alternatives gets restricted for MDR *A. baumannii*, reevaluation of carbapenems and other antibiotics is mandatory.

R2341 Molecular detection of class 1 integron associated antimicrobial gene cassettes in multidrug-resistant *Salmonella* serovars isolated in Iran

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Objective: High levels of multidrug resistance are normally associated with mobile genetic elements that encode specific resistance genes. Among these genetic elements are the integrons, which are structures that can integrate and express resistance genes. Integrons play an important role in the capture and expression of exogenous genetic material.

Methods: Eighty five epidemiologically unrelated clinical isolates of *Salmonella*.spp were collected from different provinces of Iran through 2008–2009. All isolates were serotyped comprising four serovars (A, B, C, D) and tested for the antimicrobial susceptibility for several antibiotic. All isolates were screened for the presence of class 1 integron using primers specific for intI1 gene. The gene cassettes inserted in the variable region of class 1 integrons were amplified using 5′-CS and 3′-CS primer pairs. The PCR products were extracted from agarose gel and purified with the High Pure PCR Product Purification Kit. According to the size of IVR amplified, one representative band of each group were sequenced and compared with the GenBank sequences using online BLAST software ww.ncbi.nlm.nih.gov/BLAST. Following this analysis, sequences were submitted to the EMBL/GenBank database.

Results: Forty isolates (47.05%) which were resistant to at least 4 antimicrobial agents considered as MDR *Salmonella* serovars. Of the 85 isolates, 58.82% (50 isolates) presented class 1 integrons. 68% (34 cases) of these isolates were multidrug resistance. PCR assays and DNA sequencing of internal variable regions (IVRs) of class 1 integron characterized four gene cassette arrays including dhfr7 (0.8 kb), aadA1 (1kp), blaP1 (1.2 kb),dhfr7-aadA1 (1.6 kb) with eight IVR distribution patterns in MDR isolates. The nucleotide sequences of aadA1 gene, the dhfr7 gene, the blaP1 gene, the dhfr1-aadA1 gene cassette and the aadA1 gene in the class 1 integrons have been deposited in the NCBI GenBank sequence databases under the accession numbers HQ132374,HQ132376,HQ132377,HQ132378, HQ132375 respectively.

Conclusion: High frequency of MDR *Salmonella* serovars demonstrates that antimicrobial selection pressure is widespread in our clinical settings. Detection of class 1 integron carrying gene cassettes which confer resistance to different classes of antibiotics such as aminoglycosides,β-lactams and trimethoprim confirms that integron-mediated antimicrobial gene cassettes are common in MDR *Salmonella* serovars isolated in Iran.

R2342 Molecular and phenotypical analysis of non-typable *Salmonella enterica* serovar typhimurium clinical isolates

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Objectives: The objectives of this study was molecular and phenotypical analysis of *Salmonella enterica* serovar Typhimurium clinical isolates, which did not react with the typing phages (nontypable).

Methods: A total 131 isolates were collected and analysed. The isolates were characterized using antimicrobial susceptibility testing, PCR determination of class 1 integrons and SGI1, DNA sequencing for class 1 integrons as well as macrorestriction analysing (PFGE) and biofilm production.

Results: A high frequence of multidrug resistance to antimicrobial agents (78.6%) was detected. Two multidrug resistance patterns were the most frequent in nontypable isolates: isolates with ampicillin, streptomycin, sulfizoxazole and tetracycline (R-type ASSuT) [38.9%] and isolates with ampicillin, streptomycin, sulfizoxazole, sulphamethoxazole, tetracycline and trimethoprim (R.type ASSuSxTTTMP) [19.8%] resistance patterns. Class 1 integrons were detected only in ten (7.6%) of the multidrug-resistant isolates. The results demonstrated the presence of both SGI1-borne resistance genes and class 1 integrons in three isolates only. After treatment of genomic DNA with XbaI, we observed

macrorestriction profiles which were grouped into ten patterns. Most of the isolates belonged to X1 (34.9%) and X2 (31.0%) PFGE patterns. With regard to biofilm production of isolates studied, all isolates were positive in different degree of production, except of two isolates.

Conclusion: This study shown that the nontypable S. Typhimurium isolates were characterized by multidrug antimicrobial resistance encoded by class 1 integrons in some of them. PFGE analysis suggested presence of the multiple clones of nontypable clinical isolates of this serovar.

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R2343 Emm types distribution of macrolide-resistant GAS from pharyngitis patients in Serbia

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Objective: Group A streptococci (GAS), are the most common cause of bacterial pharyngitis. Penicillin remains the first line therapy for GAS infections, but in the case of allergy, macrolides are used instead. However, resistance to these antibiotics among GAS population is in rise in many European countries. The distribution of resistance pheno/genotypes as well as the prevalence of different emm types among macrolide resistant strains varies considerably in different regions and with time.

Therefore, the aim of this study was to investigate phenotypes, genotypes and emm type distribution among macrolide resistant GAS isolates from pharyngitis patients in Serbia.

Methods: Fifty one GAS isolates exhibiting macrolide resistance were collected from various regions from Serbia in the period 2008–2009. Phenotypes of erythromycin resistance (M, iMLS and cMLS phenotype) were evaluated by triple disk diffusion test with erythromycin, clindamycin and spiramycin, as previously described. The corresponding resistance genes: mefA, ermA, and ermB were detected using PCR amplification. The emm genotypes were determined by PCR with "all M" primers following a previously published protocol by Podbielski and co-workers.

Results: Out of 51 GAS isolates, the majority (71%) harbored mef A, 25% had ermA, while, ermB was rarely encountered (4%). All strains with M phenotype had mef A gene. Out of 13 iMLS isolates, all were ermA positive, and one harbored additionally mefA gene. ErmB was present in 2 out of 3 strains with cMLS phenotype. Emm genotyping revealed the presence of 5 different emm types: emm 75 (47%), emm 77 (25%), emm 12 (24%), emm 28 (2%) and emm118 (2%). The relation of emm types and particular resistance mechanisms could be observed: all emm 75 type isolates were mefA positive and all emm 77 isolates were ermA positive. Emm 12 was encountered among isolates harboring mefA as well as in one ermA positive strain and one cMLS strain that harbored none of the tested genes. Erm B positive strains were of emm 28 and emm 118 types.

Conclusion: Our data show the predominance of efflux mediated resistance (mefA) and homogenous emm type distribution among macrolide resistant GAS in Serbia. Genotypes associated with resistance were mainly emm 75 and emm 77.

R2344 A comprehensive study on mechanisms of imipenem resistance in *Acinetobacter baumannii* clinical isolates in Taiwan

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Objectives: A total of 82 *A. baumannii* clinical isolates from two teaching hospitals in Taiwan in December of 2006 and 2009 were collected and examined in order to elucidate current resistance mechanisms.

Methods: The minimum inhibitory concentrations (MIC) of imipenem, ceftazidime, and ceftriaxone were checked by E-test analysis to these isolates. Primers specific for resistance genes (blaIMP-1, blaVIM-1, blaVIM-2, blaOXA-23, blaOXA-24, blaOXA-40, blaOXA-54, blaOXA-58, blaOXA-51, blaADC) and upstream regions of insertion sequences (ISAba1, ISAba2, ISAba3, ISAba4 or IS1008) were designed for PCR amplification and sequence identification. All 62 *A. baumannii* isolates in 2009 were genotyped by pulsed-field gel electrophoresis (PFGE). The 30-day mortality data of patients with isolates in 2009 were collected.

Results: The upstream ISAba1 was found in 53 isolates with blaOXA-23, including Tn2006 (ISAba1–blaOXA-23–ISAba1, n = 47) and Tn2008 (ISAba1–blaOXA-23, n = 6), and in 9 isolates with blaOXA-51-like. All these isolates expressed full resistance to imipenem (MIC >32) (Table 1). ISAba3-blaOXA-58-ISAba3 was found in 3 isolates and offered 2 isolates with resistance to imipenem (MIC >12). In the contrast, without upstream ISAba1, or ISAba3, isolates with OXA-type β-lactamases were all susceptible to imipenem. The upstream ISAba1 found in 50 isolates with blaADC-25 and the upstream ISAba1 or ISAba3 found in other 3 isolates with OXA-type β-lactamases offered these isolates with full resistance to both ceftriaxone and ceftazidime. Isolates with the combination of upstream ISAba1 or ISAba3 and OXA-type β-lactamases or blaADC-25 showed more resistant to imipenem, ceftazidime and ceftriaxone than those without such combination (all $P < 0.001$). Forty-one PFGE genotypes were found in 62 isolates in 2009. Tn2006 were found in 19 genotypes (46.3%) that is significantly more than ISAba1–blaOXA-51 (12.2%, 5/41) ($P = 0.001$). The 30-day mortality rate was significant lower in patients infected by isolates with ISAba1–blaOXA-51-like (0%, 0/6), compared with isolates with ISAba1–blaOXA-23 (50%, 17/34) ($P = 0.030$).

Conclusion: Displacing ISAba1–blaOXA-51 which was the main resistant mechanism to imipenem in *A. baumannii* in 1993–2007, Tn2006 with higher ability of spreading to different PFGE genotypes and correlation with higher mortality, became the predominant imipenem resistance mechanism in *A. baumannii* in Taiwan.

Table 1. Distribution of Class C or Class D β-lactamases genes and insertion sequences found in 82 *A. baumannii* isolates and their relationship with imipenem and ceftazidime resistance (The same distribution and resistance relationship found with ceftriaxone)

Class D or Class C β-lactamases genes and their insertion sequences		No. of antibiotics-resistant isolates/No. of the isolates with the sequence (Percentage of resistance, %)	
Class D β-lactamases genes	Class C β-lactamases genes	Imipenem -resistance	Ceftazidime -resistance
ISAba1–bla_{OXA-23}–ISAba1 (Tn2006) +	ISAba1–bla_{ADC-25}	44/44 (100)	44/44 (100)
$bla_{OXA-51-like}$ ISAba1–bla_{OXA-23}–ISAba1 (Tn2006) +	ISAba1–bla_{ADC-25}	3/3	3/3
ISAba1–$bla_{OXA-51-like}$ ISAba1–bla_{OXA-23} (Tn2008) +	ISAba1–bla_{ADC-25}	6/6 (100)	6/6 (100)
$bla_{OXA-51-like}$ ISAba1–$bla_{OXA-51-like}$	ISAba1–bla_{ADC-25}	6/6 (100)	6/6 (100)
ISAba3–bla_{OXA-58}–ISAba3+	ISAba1–bla_{ADC-25}	1/1 (100)	1/1 (100)
$bla_{OXA-51-like}$ ISAba3–bla_{OXA-58}–ISAba3 +	bla_{ADC-25}	1/2 (50)	2/2 (100)
$bla_{OXA-51-like}$ ISAba1–bla_{OXA-23}–ISAba1+	bla_{ADC-25}	1/1 (100)	1/1 (100)
$bla_{OXA-51-like}$ $bla_{OXA-51-like}$ only	ISAba1–bla_{ADC-25}	0/7(0)	7/7 (100)
bla_{OXA-23} + $bla_{OXA-51-like}$	ISAba1–bla_{ADC-25}	0/3 (0)	3/3 (100)
bla_{OXA-58} + $bla_{OXA-51-like}$	bla_{ADC-25}	0/1 (0)	0/1 (0)
$bla_{OXA-51-like}$ only	bla_{ADC-25}	0/8 (0)	0/8 (0)

R2345 Types of β-lactamases in pathogenic Enterobacteriaceae, resistant to ampicillin, in Saint Petersburg and north-west region of Russia

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Objectives: The aim of the present study was to determine the presence of different types of β-lactamases in pathogenic Enterobacteriaceae (*Salmonella* spp. and *Shigella* spp.) strains, resistant to ampicillin, isolated in Saint-Petersburg and North-West region of Russia.

Methods: Resistance to ampicillin was studied on Muller-Hinton agar (Oxoid, GB) by dilution techniques. Isoelectric focusing and PCR were used to reveal the presence of β-lactamases, their number and types in pathogenic Enterobacteriaceae species (51 *Salmonella* spp. and 62 *Shigella* spp. strains), resistant to ampicillin.
Results: MIC of ampicillin in 113 strains of *Salmonella* spp. and *Shigella* spp. varied from 32 to >512 mcg/ml: in 7.1% MIC was 32 mcg/ml, in 32.7% – 64 mcg/ml, in 53.1% – 128 mcg/ml, in 7.1% – >512 mcg/ml. All the strains produced one (77%) or more (23%) β-lactamases of different types. There were 4 types of β-lactamases with isoelectric points (pI) 5.4 (TEM-1), 5.7 (PSE), 7.0 (OXA-1) and 7.6 (SHV-1) detected. Predominant types of enzymes, responsible for the resistance to ampicillin, were OXA-1 (52.2%) and TEM-1 (46.0%). Frequency of other types of β-lactamases was lower: 17.7% for SHV-1 and 8.0% for PSE.
Totally ten spectrums of β-lactamases were detected. The prevalence of one enzyme production in the studied strains was revealed – OXA-1 (39.8% strains) or TEM-1 (25.7%). The combination of two enzymes – TEM-1+SHV-1 or TEM-1+OXA-1 produced 9.7% and 8.8% of strains respectively. The other combinations of enzymes were revealed only in 1–2 strains. Few strains produced only one enzyme – PSE or SHV-1 – 6.2% and 5.3% respectively. Only one strain (*S. enteritidis*) produced three types of enzymes (TEM-1+OXA-1+SHV-1).
Conclusions:
1. The resistance to ampicillin in *Salmonella* spp. and *Shigella* spp. in St. Petersburg and North-West region of Russia was mostly due to production of one type of β-lactamase.
2. The predominant types of β-lactamases were OXA-1 and TEM-1.
3. The most widespread combinations of these enzymes, detected in 18.5% strains, were TEM-1+SHV-1 and TEM-1+OXA-1.

R2346 Nasal Panton-Valentine leukocidin-positive *Staphylococcus aureus* carriage in skin and soft tissue infections

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Objectives: PVL toxin is commonly associated with community acquired-*S. aureus* isolates with a wide range of diseases including skin, soft tissue and pulmoner infections, as well. The aim of this study is to assess the prevalence of PVL toxin co-carriage both in wound and nasal samples.
Methods: Nasal and wound samples of 125 patients admitted to outpatient clinics were evaluated for PVL toxin carriage in the study period, between 2007–2008. Oxacillin, cefoxitin disk diffusion tests, oxacillin screen agar test and PCR methods were used for methicillin resistance detection. PVL and mecA gene detection was performed by PCR. Data were compared by the chi-square test, Fisher's exact test with SPSS 15.0. P values of <0.05 were considered statistically significant.
Results: Among 115 *S. aureus* (92 wound, 23 nasal swab) strains, 11 wound and one (4.3%) nasal *S. aureus* were defined as methicillin resistant *S. aureus* (MRSA). Nasal *S. aureus*, MSSA, MRSA prevalence were as; 18.4%, 17.6%, 0.8%. 14 patients were *S. aureus* carriers both in wound and nasal sample. 14 wound, 2 (8.7%) nasal sample were PVL toxin positive, none of which were MRSA. Among wound samples PVL positive strains were isolated from froncule (71.4%), abscess (21.4%) and carboncule (7.1%). PVL toxin carriage was found to be associated with fronculosis ($p < 0.05$) but statistically significant relationship was not identified between PVL positivity and age, gender. Among PVL positive nasal samples one was from froncule and the other from carboncule. Two patients with nasal PVL positive *S. aureus* were found to have PVL positive *S. aureus* in wound sample showing the similar antimicrobial susceptibility pattern (one was resistant only penicillin, the other penicilin and erytromycin).
Conclusion: PVL toxin carriage is not alarming in our country, but routine surveillance should be performed for methicilin resistance and PVL carriage for all isolates on available laborotories to prevent the dissemination of these isolates.

R2347 Eight-year surveillance of imipenem non-susceptible clinical isolates in *Klebsiella pneumoniae* in Taiwan

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Objective: The aim of this study was to investigate the mechanism of resistance and clonality among 29 imipenem-non-susceptible *K. pneumoniae* isolates collected between 2002 and 2009 from a nationawide surveillance.
Methods: Twenty-nine imipenem non susceptible *K. pneumoniae* isolates were obtained from seven medical centers and thirteen regional hospitals between 2002 and 2009. Laboratory investigation of the *K. pneumoniae* included antimicrobial susceptibility testing, resistance gene (include class A carbapenemaseIMI,SME,GES,KPC; class B metallo β-lactamase IMP, VIM, NDM; AmpC enzymes CMY,DHA; Class D oxacillinase, ESBL genes CTX-M, SHV,TEM) by PCR amplification and DNA sequencing, genotyping by pulse-field gel electrophoresis (PFGE), and outer membrane protein (OmpK35, OmpK36) analysis by SDS-polyacrylamide gel electrophoresis.
Results: Eleven isolates had blaIMP-8 alleles, whereas blaCMY-2 and blaDHA-1 alleles were detected in 1 and 11 isolates, respectively. All isolates had either blaCTX-3, blaCTX-M-15 blaCTX-M-14 or blaSHV-12 ESBL genes. SDS-PAGE of outer membrane proteins showed 22 isolates lack or greatly diminished expression of OmpK35 or OmpK36. Pulsed-field gel electrophoresis of XbaI-restricted genomic DNA revealed one closed related cluster among four blaIMP-8 positive isolates.
Conclusion: In conclusion, non-imipenem susceptible among *K. pneumoniae* in Taiwan were largely by class B metallo-β-lactamase IMP-8 and by production of plasmid-mediated AmpC β-lactamases along with lack or greatly diminished expression of OmpK35 and OmpK36 porins.

R2348 Molecular epidemiology of CTX-M-14 ESBL *E. coli* from cattle and humans in England and Wales

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Objectives: The molecular epidemiology of an outbreak CTX-M-14 ESBL *E. coli* on a dairy farm in Wales was investigated to determine the geographical dissemination, linkage to this farm following the dispersal of the cattle to other holdings and presence in the human population. Risk factors for CTX-M *E. coli* on cattle farms were also investigated.
Methods: Pat samples from cattle on selected farms were screened for CTX-M *E. coli* using Chromagar CTX and sequence typed (Randall et al., 2008). A contemporaneous, year long passive surveillance study was used to assess the geographical dissemination of CTX-M *E. coli* by the analysis of cattle and sheep diagnostic faecal samples ($n = 113$) submitted to local VLA regional laboratories in Wales. A network analysis approach was used to investigate the dissemination of the CTX-M-14 *E. coli* from the outbreak farm using the cattle tracing system. The CTX-M-14 isolate from the outbreak farm was compared to human CTX-M-14 isolates ($n = 19$) from Wales using a multiplex PCR (Cottell et al., 2011) for the plasmid (pCT) bearing the CTX-M-14 from that farm.
Results: Geographical analysis revealed that the CTX-M gene from veterinary sources was widespread in the study area and predominantly CTX-M-15 ($n = 7$) not CTX-M-14 ($n = 3$). The prevalence of CTX-M *E. coli* in the population of farms ($n = 17$) linked to the outbreak farm was 59% compared with 37% in the control farms ($n = 48$) and no statistically significant linkage was observed. However, there was a significant ($P < 0.05$; OR 4.4) association between the presence of CTX-M *E. coli* on cattle farms and the use of 3rd/4th, but not 1st/2nd, generation cephalosporins. Analysis of the human CTX-M-14 isolates revealed that some isolates from humans ($n = 3$) had similar plasmid backbone genes to pCT.
Conclusions: While the CTX-M gene was present in the study area there was no evidence for linkage of the CTX-M-14 to the outbreak farm. A

similar plasmid to that from the outbreak farm (pCT) was found in CTX-M-14 *E. coli* isolates from humans. The role of 3rd/4th generation cephalosporins in the selection of CTX-M *E. coli* on cattle farms is being further investigated.

R2349 Serotyping of *Streptococcus pneumoniae* strains isolated in Algeria

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Objectives: The aim of this work is the study of *Streptococcus pneumoniae* serotypes isolated in many algerian cities, their distribution by localisation site, and the evaluation of the theoretical coverage for conjugate vaccines.
Methods: Our study was performed on 256 strains of *Streptococcus pneumoniae*, collected from January 2001 to July 2010. These isolates came from various samples: CSF (Cerebrospinal fluid) (n = 104); blood (n = 36), ascitic, pleural, peritoneal and gastric fluid (n = 14); nasal, throat and ear swabs (n = 61); lung samples (n = 25) and finally, fistulae (n = 16). Among these strains, 45.6% came from children, of which 33.3% were under 5 years of age. The strains were identified using the usual tests. For serotyping, we use the latex particle agglutination (Statens Serum Institut Pneumotest-Latex), and the capsular swelling test using monovalent sera (Statens Serum Institut).
Results: The most common serotypes were: 14 (19,5%), 23F (9,7%), 6B (9,3%), 19F (5,4%) and serotype 1 (5%). The frequency of these serotypes in invasive sites in children under 5 years is 14 (31.25%), 23F (10.4%), 19F (8.3%), 6B (6.25%) and serotype 1 (4.2%). The theoretical coverage of invasive infections in children under 2 years is 61.5%, 69.2% and 76.9% for the 7-valent, 10-valent and 13-valent conjugate vaccines, respectively. This coverage represent 63.5%, 64.9% and 68.9%, respectively for each vaccine, against pneumococcus non susceptible of penicillin (PNSP).
Conclusion: The national consensus for the treatment and management of bacterial meningitis requires updating. Numerous publications describe the variation over time of circulating serotypes of *Streptococcus pneumoniae*. The differences noted between the two studies conducted in Constantine (1994) and Algiers (2000), and the present study we are presenting reveal the need to establish a pneumococcal infection monitoring program in Algeria. Effort is required to increase the number of samples and their quality in order to extend sampling for a more representative epidemiological study.

R2350 Activity of daptomycin against staphylococcal blood isolates. Glycopeptide tolerance and comparison of vancomycin MICs determined by broth microdilution and E-test

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Objectives: To assess the activity of daptomycin and fourteen comparator agents against staphylococcal blood isolates, to evaluate vancomycin and teicoplanin tolerance and the incidence of heterogeneous glycopeptide-intermediate (hGISA) isolates, and to investigate the presence of the cfr gene in the linezolid nonsusceptible strains.
Methods: The activity of daptomycin and fourteen comparators was evaluated against 702 staphylococcal blood isolates (316 methicillin-susceptible *Staphylococcus aureus* [MSSA], 187 methicillin-resistant *S. aureus* [MRSA], and 199 coagulase-negative staphylococci [CoNS]) recently collected in 41 Spanish medical centers. Vancomycin minimum inhibitory concentrations (MICs) determined by the Etest were compared with those obtained by the reference broth microdilution method.
Results: Daptomycin exhibited good activity against the majority of the isolates and only two (0.3%) isolates were nonsusceptible to this antibiotic. Nonsusceptibility to linezolid was observed in two MRSA isolates and in 16 CoNS. The cfr gene was detected in seven (38.8%) of these 18 isolates. Vancomycin and teicoplanin tolerance was 9.6% and 21.9%, respectively, in MRSA isolates. We detected the hGISA phenotype in 5.8% of MRSA isolates and in 0.3% of MSSA isolates. Vancomycin MICs by the Etest were slightly higher than those obtained by broth microdilution, mainly for *S. aureus*. Most of the differences in MIC observed between the methods were of only one dilution. Daptomycin retained activity against isolates that were not susceptible to linezolid, teicoplanin, or quinupristin-dalfopristin, and was highly active against hGISA isolates (daptomycin MICs, 0.25–0.5 μg/ml).
Conclusion: Daptomycin represents an alternative in the treatment of serious infections caused by multiresistant staphylococci.

R2351 Performance of a new modified NucliSENS EasyQ® MRSA assay with community-associated Danish MRSA strains

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Objectives: Several commercial assays have been developed for rapid detection of MRSA colonization with nasal swabs, but genetic variations within the SCCmec cassette poses a constant challenge to the primer design. NucliSENS EasyQ® MRSA (bioMérieux) is an amplification-based method which simultaneously detects the mecA gene and a specific cassette junction confirming the presence of the SCCmec cassette integrated in the *S. aureus* chromosome. A selection of different MRSA strains was previous tested with the first version of the assay and only 11 out of 15 strains were correctly identified as MRSA at that time (ECCMID 2010 – Ab. Nr. 2145). Among others the assay failed to detect clonal complex (CC) 398 (ST398) associated to livestock animals and constituting an emerging problem in both Denmark and The Netherlands. A rapid test for screening and identification of these livestock-associated strains could have important implications for surveillance and infection control initiatives. The objective is to evaluate the performance of a new modified version with the collection of community-associated Danish MRSA strains tested previously.
Method: MRSA isolates were obtained from residents in North Jutland, Denmark and were characterized genetically by spa typing and clustered into spa CC groups (www.ridom.de) as part of the Danish national surveillance program maintained by Statens Serum Institut. Cultures of MRSA strains were processed according to the manufacturer's guidelines for positive controls and analysed on the EasyQ instrument, a NASBA-based platform.
Results: 15 MRSA strains representing 10 different CCs [numbers in brackets]: CC1 [1], 5 [3], 8 [1], 22 [2], 30 [1], 59 [1], 72 [1], 88 [3], 97 [1], and 398 [1]) were all positive with the new version of the NucliSENS EasyQ® MRSA assay. These results supersede the ones previously obtained with the first version where 4 strains failed the test (CC5 [1], CC59 [1], CC97 [1], and CC398 [1]). The test was easy to perform and was conducted in 3 hours.
Conclusion: The NucliSENS EasyQ® MRSA assay is a very rapid test and easy to perform. In the current epidemiological situation in Denmark the assay should identify correctly the prevalent clonal complexes including CC398. An extensive trial with samples from community-dwelling Danes including persons in close contact with livestock animals is warranted.

R2352 Resistance to macrolides in *Corynebacterium striatum*

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Objectives:
- Analyze the activity of two macrolides (erythromycin and spiramycin) and clindamycin against 74 *Corynebacterium striatum* clinical strains isolated at the hospital Marqués de Valdecilla, Santander, between 2005 and 2009.
- Phenotypic detection of macrolide resistance.
- Detection of the gene(s) associated with the resistance phenotype.
- Mapping the region surrounding the resistance gene(s) to know if it is associated with a transposable element.
- Assay the transferability of the resistance gene(s) to *Escherichia coli*.

Methods: Susceptibility of *C. striatum* strains to erythromycin and clindamycin was analyzed by Etest (AB BIODISK, Solna, Sweden). For spiramycin the disk diffusion method was used. The type of resistance of the *C. striatum* strains was elucidated by the double disc diffusion test. The gene(s) associated with resistance were isolated by PCR with primers specific for ermX and mef genes. The region surrounding the resistance gene(s) in two representative *C. striatum* isolates was cloned by inverse PCR using primers designed from the sequence of their ermX alleles. The transferability of the resistance gene(s) from two *C. striatum* strains to *E. coli* was assayed by electroporation and conjugation.
Results: We analyzed a collection of 74 *C. striatum* from clinical specimens responsible for cases of wound infections, skin ulcers and pneumonia. 64 *C. striatum* were resistant to erythromycin, 64 to clindamycin and 62 to spiramycin; 62 were resistant to both erythromycin and clindamycin; 60 isolates were resistant to the three compounds. The gene ermX was detected in 62 of the 64 *C. striatum* resistant to erythromycin and clindamycin. This gene was also found in one isolate sensitive to the three compounds. Preliminary results indicate that in our *C. striatum* isolates the gene ermX is chromosomic and does not take part of transposon Tn5432.
Conclusion: Our study revealed a high incidence of resistance to two macrolides and clindamycin in *C. striatum* clinical strains isolated at hospital Marqués de Valdecilla, Santander, Spain. First results show that in our *C. striatum* collection the ermX gene is chromosomic and is not associated to transposon Tn5432.

R2353 IRT associated phenotypes might mask inhibitor-resistant CTX-M β-lactamases mutants

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Objectives: Unlike TEM and SHV, β-lactam plus β-lactamase inhibitor (BBLIs) resistant variants among CTX-M enzymes have not hitherto been described in the clinical setting. These variants may exist but their phenotypic patterns could be confused with those of IRT or IR-SHV. The objectives of this work were: i) to test if an automatic susceptibility testing system (Wider) and blind expert analysis might identify different CTX-Ms harbouring different laboratory-introduced mutations conferring increased MICs to BBLIs; ii) to compare the efficiency to detect IR-CTX-M enzymes when using CLSI or EUCAST breakpoints.
Methods: 14 constructions of hybrid plasmids containing 3 wild-type CTX-M genes and 11 CTX-M laboratory-obtained coding for enzymes resistant to inhibitors were transformed into 4 isogenic *E. coli* laboratory strains with porin membrane alterations: MKW505 (wild-type), KAEC5 (ompC::Kn), MH621 (ompF::lacZ) and KAECF5 (ompF::lacZ; ompC::Kn). Wild-type CTX-Ms were CTX-M-1, CTX-M-2 and CTX-M-14 and mutated CTX-M belonged to these CTX-Ms but carrying changes (S130G, S237G and K234R) that we previously described conferring increased BBLI MICs.
Results: All wild-type CTX-Ms into the four genetic backgrounds were correctly detected by Wider and expert personnel. Those six mutated CTX-Ms carrying S130G were classified by Wider as IRT (with and without overexpression of TEM) or ESBL (2 cases) while expert personnel as IRT (2), overexpression of TEM (1), overexpression of SHV (1) and ESBL (2). Those three mutated CTX-Ms-K234R and two CTX-Ms-S237G mutants were classified as ESBL with Wider and expert personnel. Using current breakpoints, reference CTX-Ms were always identified as ESBL, except KAECF5-CTX-M-1 which was classified as IR phenotype with EUCAST breakpoints. All variants were coincidently identified as IRT in CTX-Ms-S130G and as ESBL in CTX-Ms-K234R and CTX-Ms-S237G when using both CLSI and EUCAST breakpoints. Interestingly, CTX-M-14-K234R and CTX-M-35-S130G were classified as IR-ESBL when using EUCAST but not with CLSI breakpoints.
Conclusions: Not a single IR-CTX-M phenotype was detectable either by the automatic system or the blind expert analysis, suggesting that these IR-CTX-M variants could be overlooked in the clinical microbiology setting. When using current breakpoints, EUCAST detected more IR-CTX-M-ESBL phenotypes than CLSI. This result suggests that EUCAST must be considered as reference to detect these IR-CTX-M mutants in the future.

R2354 Carbapenemase-producing *Pseudomonas aeruginosa* in French intensive care units

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Objectives: A multicenter, prospective, observational study was conducted in June 2010 to determine the prevalence of carbapenemase-producing *P. aeruginosa* in patients hospitalized in French intensive care units (ICUs).
Methods: Thirty hospital bacteriology departments participated in this study. All of the non-duplicate ICU isolates of *P. aeruginosa* with imipenem MIC >4 mg/L were collected and sent to the coordinating center (Besançon). Drug MICs were determined by the microplate dilution method (Sensititre®). Carbapenemases were detected among the ceftazidime-resistant isolates (MIC >8 mg/L) by double disc synergy tests (ceftazidime-EDTA, imipenem-clavulanate) and PCR targeting carbapenemase-encoding genes from Ambler classes A, B and D.
Results: One hundred and five *P. aeruginosa* isolates were collected from 26/30 participating centres (median: 4 isolates; range: 1–10). Seven isolates (6.7%) from 5 hospitals harbored a class B metallo-β-lactamase (MBL): 1 VIM-1, 4 VIM-2, 1 VIM-4, and 1 novel IMP-type enzyme (IMP-29). Five of these isolates exhibited a high level resistance to the 3 carbapenems tested (MICs ⩾128 mg/L) whereas 2 strains (producing either IMP-2 or IMP-29) turned out to be moderately resistant to imipenem (MIC of 32 mg/L). No class A or D carbapenemases were found in the collection. Among the 98 remaining strains (93.3%), the decreased susceptibility to imipenem (MIC from 6 to 64 mg/L) was attributed to alteration or loss of porin OprD. In these impermeability mutants, the activity of meropenem and doripenem was generally better than that of imipenem (MICs from 2 to 16 times lower).
Conclusion: Seven % of the imipenem non-susceptible *P. aeruginosa* isolated in French ICUs produce a MBL. This prevalence is lower than in some other European countries. Nevertheless, the epidemic potential of these broad-spectrum β-lactamases should incite ICUs and microbiology laboratories to strengthen surveillance measures to prevent their spread.

R2355 AmpC β-lactamases in *Escherichia coli* and *Klebsiella pneumoniae* blood isolates

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Objectives: Detection of plasmid mediated AmpC β-lactamase producing organisms is important to ensure effective therapeutic intervention and optimal infection control. However, detection of these enzymes is a challenge for laboratories since there is no reliable method for the routine detection of plasmid mediated AmpC enzymes. This study was aimed to investigate the prevalence of plasmid mediated AmpC β-lactamases in *E. coli* and *Klebsiella* spp. in a university hospital in Turkey.
Methods: Among a total of 1317 blood *E. coli* and *Klebsiella* spp. isolated from the blood cultures of patients admitted to Hacettepe University Hospital between 2007–2010, 40 *K. pneumoniae*(n = 30) and *E. coli*(n = 10) isolates which were found to be resistant to cefoxitin (FOX) by BD Phoenix system were included in the study. The antimicrobial susceptibility of the isolates were determined by disk diffusion method according to the CLSI guidelines. AmpC and ESBL production were confirmed with combined disk diffusion test in combination with or without boronic acid (BA) and clavulanic acid (CA). Multiplex PCR was performed in all isolates for the detection of plasmid-mediated blaMOX, blaCIT, blaDHA, blaACC, blaFOX and blaEBC ampC genes.
Results: Of the 40 FOX resistant isolates, 32 (80%) were found resistant to FOX by disk diffusion method. The rates of susceptibility to imipenem, cefepime, aztreonam, ciprofloxacin, cefotetan, ceftazidime, cloxacillin, cefotaxime, cefpodoxime, amoxicillin-clavulanic acid and

amikacin were 90.0%, 27.5%, 47.5%, 50.0%, 75.0%, 42.5%, 97.5%, 25%, 30%, 2.5% and 92.5%, respectively. Phenotypic AmpC β-lactamase production was found in 15 (37.5%) and ESBL production in 18 (45%)of the isolates with cephalosporin/BA/CA and cephalosporin/BA disk diffusion test. Plasmid-mediated ampC genes; blaCiT and blaEBC was found in two *K. pneumoniae* isolates (6.7%), only one being phenotypically positive for AmpC production.
Conclusion: Plasmid mediated AmpC prevalence was 6.7% among blood *Klebsiella* isolates in our center. These results emphasized that clinical laboratories should consider testing the presence of plasmid mediated AmpC β-lactamases particularly in cefoxitin resistant *Klebsiella* spp. and continuous surveillance of these enzymes is necessary in terms of improved hospital infection control and therapeutic options. These data also highlighted the use of molecular analysis to verify the presence of plasmid-mediated AmpC in Gram negative pathogens.

R2356 Molecular characterisation and epidemiology of a carbapenem-resistant Enterobacteriaceae outbreak in a tertiary care centre, Istanbul

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Introduction: Bacteria from clinical and non-clinical settings are becoming increasingly resistant to conventional antibiotics. The increase in resistance of Gram-negative bacteria is mainly due to mobile genes on plasmids encoding carbapenem-hydrolyzing β-lactamases that can readily spread through bacterial populations. Here we describe a long lasting nosocomial outbreak of carbapenem-resistant Enterobacteriaceae strains expressing OXA-48.
Objectives: Between 2002 and 2010, we identified 42 Enterobacteriaceae strains that are resistant to any of the carbapenems in a 1500 bed university hospital. The aim of the study was to identify the resistance mechanisms for carbapenems in these strains and to check for clonal dissemination.
Methods: All the strains (29 *Klebsiella pneumoniae*, 6 *Escherichia coli*, and 7 *Enterobacter* spp.) were nonrepetitive clinical isolates from the Infectious Diseases and Clinical Microbiology Laboratory of Istanbul University, Cerrahpasa Medical Faculty. The strains were identified with the API 32GN system. Carbapenemase- and extended-spectrum-β-lactamase (ESBL)-encoding genes were identified by PCR experiments using previously designed primers for blaTEM, blaSHV, blaCTX-M, blaOXA-48, and blaVEB followed by sequencing. Isolates belonging to the same species were compared by pulsed-field gel electrophoresis. Transferability of β-lactamase genes were studied by conjugation experiments using an azide-resistant *E. coli* J53 as the recipient.
Results: All the carbapenem resistant strains isolated produced OXA-48, a class D oxacillinase with significant carbapenem-hydrolyzing activity. Many of the strains coproduced various β-lactamases (SHV-12, CTX-M-15 and TEM-1). Conjugation experiments were successful with *K. pneumoniae* isolates and all the transconjugants had decreased susceptibility to carbapenems. Isolates of the same species belonged to different pulsotypes.
Conclusion: The present work indicated that dissemination of the blaOXA-48 gene is not driven by the dissemination of a single clone but by dissemination of the blaOXA-48-carrying plasmid among Enterobacteriaceae during the last decade in our hospital. Since OXA-48 confers by itself a low level of resistance to carbapenems, clinical laboratory detection of OXA-48-producing strains may be difficult and the problem may actually be much bigger than it seems.

R2357 Are ready-to-eat salads an important vehicle of pathogenic and commensal bacteria resistant to antibiotics?

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Objectives: The increase demand for fresh fruits and vegetables is causing an expansion of the market share for minimally processed vegetables along with recognized food safety problems. We analyzed the microbiological quality of Portuguese ready-to-eat salads (RTS) and their role in the spread of bacteria carrying antibiotic resistance (ABR) genes.
Methods: RTS (n = 50; 7 brands; split or mixed leaves, carrot, cornmeal) were collected in 5 of the main supermarkets (2010). The evaluation of microbiological load and quality followed the international standard methods for counting aerobic mesophilic, coliforms, *Enterococcus* sp and detection of *Salmonella* sp or *Listeria monocytogenes*. Samples were also plated in different culture media with/without AB before and after a pre-enrichment step. ABR was studied by agar diffusion method (CLSI) and ESBL expression by double disk synergy test (DDST). Species were identified by PCR (Gram positive), API ID32GN or 16rRNA (Gram negative). ABR genes, integron types and *E. coli* phylogenetic groups were searched by PCR and clonality by MLST in specific isolates.
Results: A high number of RTS presented poor microbiological quality (86% for aerobic mesophilic, 74%-coliforms, 4%-*E. coli*), but no pathogens. Different ABR phenotypes and genotypes were seen to both Gram positive and Gram negative bacteria. *E. coli* detected in 13 samples (n = 26; phylogenetic groups A-7, B1–10, B2–1, D-8) presented resistance (%) to tetracycline (73; tetA and/or tetB), streptomycin (50; aadA), sulfametoxazole (46; sul1 and/or sul2), trimethoprim (46; dfrA1 or dfrA12), ampicillin (46; blaTEM), nalidixic acid (27), ciprofloxacin (8) or chloramphenicol (4). Two integron types (dfrA1 + aadA, dfrA12 + aadA) were detected in 11 isolates. Multidrug resistant *E. coli* (n = 2; D) belonged to the widespread ST69; the fumC alleles of other *E. coli* were highly diverse and identified as 8, 48, 65 and 100. DDST gave a positive test for 2 *Raoultella* sp (2 samples) carrying an ESBL identified as SHV2. Among enterococci (n = 108) ABR (%) was seen for tetracyclines (6; tetM and/or tetL), erythromycin (3; ermB), nitrofurantoin (1) or ciprofloxacin (1).
Conclusions: The present study positions RTS within the spectrum of ecological niches that may be reservoirs/vehicles for ABR bacteria/genes with clinical interest (e.g. *E. coli*-B2 or ST69; ESBL) being these findings worthy of attention as their spread to humans by ingestion cannot be dismissed.

R2358 Plasmid diversity among vancomycin-resistant and vancomycin-susceptible *Enterococcus* spp. (1991–2010)

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Objectives: Mobile genetic elements (MGE) of *E. faecalis* and *E. faecium* have been recently analyzed in detail, but little is know about MGE in other enterococcal species. The aim of the study was to search the plasmid diversity among unfrequent enterococcal species of *Enterococcus* recovered from different hosts in Spain for a 20 year period.
Methods: We studied 31 *Enterococcus* spp isolates recovered from hospitalized and healthy humans, animals and waste water (1996–2010). They included 17 *E. durans* (10 VSE, 7 vanA), 5 *E. hirae* (4 vanA and 1 vanB2), 7 *E. avium* (7 VSE), 2 *E. casseliflavus* (2 vanC)), Typing of van-Tns was performed by PCR based assays. Plasmid characterization included determination of size and content (S1-PFGE), and identification of relaxases (rel), rep initiation proteins (rep), or toxin-antitoxin systems (TA) by using PCR-typing methods, hybridization and sequencing.
Results: Isolates contained 1–3 plasmids/cell ranging from 25kb to 250kb. They belong to different families of: i) RCR (32% pEF1071, 6% pEFNP1), ii) small tetha replicating (6% reppCIZ, <5% reppEF418), Inc18 (48% reppRE25, 29% reppVEF, 10% repInc18) RepA_N (26%

reppAD1, <5% reppRUM, 48% reppLG1), and pHTβ-like (10%). Relaxases from plasmids pAD1, pLG1 and pRUM plasmids were rare (all <5%). TA systems ω-ε-ζ (52%), Axe-Txe (32%) and parpAD1 (16%) were detected. pUSA02, pAD1 and pLG1 were mostly detected among *E. durans* and *E. hirae*. pEF1071 pRE25, and pAD1 were predominant among vancomycin resistant isolates. All but one vanA isolates harboured the complete Tn1546 backbone. One *E. hirae* strain contained a Tn1546 truncated by a IS19-like insertion within vanX-vanY.
Conclusions: These data constitute the first report of MGE in *Enterococcus* spp. and demonstrates the presence of plasmids widely disseminated among *E. faecium* and *E. faecalis* among these species. Differences among species might mirror ecological background. Enrichment of certain plasmid groups among VRE suggests acquisition from an external source.

R2359 Molecular characterisation of *Enterococcus faecalis* plasmids from clinical isolates in Spain, 2001–2009

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Objectives: The population structure of *Enterococcus faecalis* (Efc) causing nosocomial infections is comprised of few clonal complexes as CC2, CC9 and CC87. Although resistance to first line antibiotics (aminoglycosides, macrolides, glycopeptides) is associated with either Tn and/or plasmids, little is known about the plasmid diversity among Efc. The aim of this study was to evaluate the plasmid content of Efc isolates from our institution in 2001 and 2009, situated in an area with low prevalence of enterococci resistant to glycopeptides and high levels of aminoglycosides.
Methods: We studied 68 Efc clinical isolates from infected and colonized patients (37 blood, 28 faecal, 3 wounds) recovered in 2001 and 2009. They are representative isolates of invasive and non-invasive strains from our hospital during both periods. Antibiotic susceptibility was determined by CLSI microdilution. Clonality was established by SmaI-PFGE and MLST. Plasmid characterization included determination of size and content (S1-PFGE), and identification of relaxases (rel), rep initiation proteins (rep) and toxin-antitoxin systems (TA) by PCR, sequencing and hybridization.
Results: Efc strains studied were classified in 47 PFGE-types and 38 STs which clustered into CC9 (n = 11, 16%), CC2 (n = 10, 15%), CC16 (n = 9, 13%), CC25 (n = 5, 7%) and CC21 (n = 4, 6%). They were highly resistant to erythromycin (72%), tetracycline (59%), streptomycin (47%), gentamicin (37%), ciprofloxacin (35%) and chloramphenicol (18%). All were susceptible to glycopeptides. Plasmid content of Efc isolates was variable (1–3/cell; 20–100 kb), those ranging from 40 to 60 kb being predominant. They belong to different plasmid families: i) RCR/thetha (15% reppS86/pAMa1; 21% relpS86/pAMa1); ii) repA_N (62% reppAD1, 10% reppAM373, 62% relpAD1, 32% relpCF10, 10% par) iii) Inc18 (18% repInc18, 22% reppRE25, 19% relpRE25, <5% relpEF1). Modules from small theta replicating plasmids (pCIZ2, pEF418 and pEF1071) were rarely found (<5%). Similar rep and rel content was observed among isolates for each CC. Mosaic plasmids containing rep and/or rel from different families were identified.
Conclusions: Most Efc isolates harboured mosaic plasmids associated with the repA_N family. The influence of particular plasmids or plasmid modules in the success of major Efc lineages and in the distribution of antibiotic resistance genes among different clonal lineages of the same or different enterococcal species remains to be established.

In vitro antibacterial susceptibility and drug interaction studies

R2360 In vitro fosfomycin activity against extended-spectrum β-lactamase producing *Escherichia coli* related urinary tract infections

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Objectives: Extended spectrum β-lactamase (ESBL) producing *E. coli* disseminated worldwide as an important cause of both nosocomial and community-acquired urinary tract infections (UTI). Increasing resistance rates to antimicrobials among these isolates limit the choice of treatment. The aim of this study was to evaluate invitro activity of fosfomycin in ESBL-producing *E. coli* isolates recovered from UTI.
Methods: Between November 2008-December 2010, 169 *E. coli* strains (115 outpatient, 54 inpatient; 47 male, 122 female; ranging 1–93 years) isolated from UTI were included in the study. Susceptibilities to fosfomycin (FF), amoxicillin-clavulanic acid (AMC), gentamicin (CN), amikacin (AK), piperacillin-tazobactam (TPZ), ciprofloxacillin (CIP), trimethoprim-sulphametoxasole (SXT), imipenem (IMP), ertapenem (ERT) and meropenem (MEM) were analysed using Kirby Bauer disk diffusion method according to CLSI. Double disk synergy test and combined disk diffusion tests with ceftazidime-clavulanic acid and cefotaxime-clavulanic acid were used to determine ESBL production. Two different periods were analysed for ESBL positivity and fosfomycin resistance: from November 2008-December 2009 and January-December 2010.
Results: The rates of ESBL producing strains isolated from inpatients and outpatients were 68% v 32%, respectively. Among these isolates, the resistance rate to fosfomycin was 3.0% (n = 5), and two (1.2%) isolates showed intermediate susceptibility. Nosocomial isolates displayed higher resistance rates for fosfomycin than community strains (7.4% v 2.6%, OR = 2.987; 95% CI = 0.64–13.84; p = 0.144). The prevalence of fosfomycin resistance rate among ESBL-producing *E. coli* increased from 1.4% in the first study period to 6.1% 2010 (p = 0.241). Additionally, ESBL-producing *E. coli* infections in community increased from 40.9% to 59.1% in 2010.
Conclusion: In the present study high susceptibility rates to fosfomycin was observed for ESBL producing *E. coli* strains suggesting that it may be a good alternative for the treatment of community acquired and nosocomial UTI related with ESBL producing *E. coli*. Additionally, SXT and ciprofloxacin resistance is in an alarming position among ESBL producing isolates.

Table. Antimicrobial resistance rates of *E.coli* strains (n=169) isolated from community and nosocomial urinary tract infections

Antimicrobial	(%) Resistance		P
	Community	Nosocomial	
AMC	31.3	40.7	
CN	52.2	61.1	
AK	13.9	25.9	
TPZ	23.5	20.4	<0.05
CIP	60.9	70.4	
SXT	60.9	51.9	
ERT	2.6	7.4	
FF	2.6	7.4	
IMP/MEM	1.7	9.3	0.035*

R2361 Use of ampicillin to predict susceptibility of ampicillin-susceptible but penicillin-resistant isolates of *Enterococcus faecalis* to other β-lactam antibiotics

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Enterococcus resistance to β-lactam antibiotics is a serious problem because it prevents bactericidal synergism obtained by association of these drugs with aminoglycosides. Currently, CLSI (Clinical and Laboratory Standards Institute) recommends that susceptibility to penicillin or ampicillin can predict susceptibility to amoxicillin, imipenem and piperacillin, but susceptibility to ampicillin should not be used to predict susceptibility to penicillin. Therefore, the aim of the present study was to determine the resistance profile of isolates ampicillin-susceptible, penicillin-resistant of *E. faecalis* to amoxicillin, imipenem and piperacillin. The resistance profile was determined by Etest (AB Biodisk, Sweden) and by disk diffusion and broth dilution methods, according to CLSI guidelines. A total of 59 *E. faecalis* isolates recovered from different clinical specimens of hospitalized patients were included in this study. All of them were susceptible to ampicillin and resistant to penicillin according to the results obtained by disk diffusion method using Oxoid disks. Ampicillin susceptibility was confirmed for all isolates by using Etest and broth dilution test, while penicillin resistance was confirmed for 44 (74.6%) and 36 (61.0%) of the 59 isolates according the results obtained by those two tests, respectively. Regarding to the other β-lactam antibiotics, 3 (5.1%) isolates were resistant to amoxicillin, 32 (54.2%) to imipenem and 37 (62.7%) to piperacillin by disk diffusion test. Using broth dilution test, there were no *E. faecalis* isolate resistant to amoxicillin, while 10 (16.9%) isolates were resistant to imipenem and 55 (93.2%) to piperacillin. In conclusion, we demonstrated that the in vitro results obtained for ampicillin by broth dilution test might accurately predict the in vitro susceptibility of amoxicillin. Differently, the results obtained for ampicillin using either disk diffusion or broth dilution tests were not concordant with that for piperacillin and imipenem. Therefore, our results suggest that it is necessary to reevaluate the use of ampicillin results to predict the in vitro susceptibility to piperacillin and imipenem.

R2362 Evaluation of daptomycin activity in *Staphylococcus aureus* isolates with decreased susceptibility to vancomycin

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Objectives: Daptomycin belongs to a new class of antibiotics and is approved for use in the treatment of complicated skin and soft-tissue infections (SSTI) caused by Gram-positive bacteria. *Staphylococcus aureus* is the most common Gram-positive microorganism isolated from patients with these infections. The aim of this work was to evaluate the activity of daptomycin in *Staphylococcus aureus* isolates with a vancomycin MIC of 1 μgmL.
Methods: During a two months period, thirty *S. aureus* with a vancomycin MIC of 1 μgmL were collected in the Hospital Infante D. Pedro EPE and included in this study. The strains were collected from SSTI, bloodstream and urinary tract infections. The isolates were identified by the Vitek 2 system (CLSI guidelines) and Advanced Expert System (VITEK 2 AES) (BioMérieux, Marcy L'Etoile, France). Vancomycin MIC was determined by the automated method and confirmed by Etest strips (AB Biodisk). Daptomycin Etest strips were used according to the manufacturer's instructions to determine daptomycin MIC.
Results: Among the thirty *S. aureus* isolates collected, twenty-two (73%) exhibited a metacillin resistant phenotype and eight (27%) were found to be metacillin sensitive. Most of the isolates were collected from bloodstream infections, followed by SSTI and urinary tract infections. Twelve isolates (40%) exhibited a MIC higher than 1 μgmL to daptomycin. The higher MIC obtained had a value of 3 μgmL and the lowest a value of 0.125 μgmL.
Conclusions: Daptomycin is not yet in use in the Portuguese hospitals, however and despite breakpoints for resistant phenotype are not established, this work shows that daptomycin can be an alternative to vancomycin, as therapeutic option for the treatment of *S. aureus* infections. Moreover, the decreased susceptibility to vancomycin exhibited by the metacillin sensitive isolates requires surveillance.

R2363 In vitro activity of tigecycline and colistin against multi-drug-resistant *Acinetobacter baumannii*

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Objectives: Tigecycline (TIG) is a member of the glycylcycline class of antibiotics with a broad spectrum of activity which includes several Gram-positive and Gram-negative bacteria. Colistin (polymyxin E) is a polymyxin antibiotic produced by certain strains of *Bacillus polymyxa* var. colistinus. Also, Colistin is a mixture of cyclic polypeptides colistin A and B. Colistin is effective against most Gram-negative bacilli and is used as a polypeptide antibiotic. It is one of the last resort antibiotics for multidrug resistant pathogens *Pseudomonas aeruginosa*, and *Acinetobacter*.
In this study we evaluate the in-vitro activity of TIG against Multidrug resistant *Acinetobacter* spp.
Methods: A total of 506 carbapenem resistant isolates of *Acinetobacter* spp isolated in our centre were studied Isolates were recovered from blood cultures, body fluids, catheters tips, pus, bronchial secretions and urine samples in a two year period, from January 2009 till November 2010. The identification and susceptibility testing was performed via the MicroScan Walkaway (Siemens). The TIG and Colistin susceptibility testing performed using Etest strips (AB Biodisc, Sweden) according to CLSI guidelines. The EUCAST Enterobacteriaceae breakpoints were used to interpret Tigecycline and colistin MIC results for *A. baumannii*.
Results: Of the 506 isolates, 420 isolates exhibit an MIC less the 4mg/L (Susceptible range) which represent 83.00%. There are 86 resistant isolates (16.99%). Of the resistant strains; there were 8 samples exhibit very high level of resistance MIC 256mg/L, and 12 isolates has MIC equal to 4mg/L and the rest fall between 6mg/L and 24mg/L. In our study, all these isolates have susceptibility to amikacin at 21%, to cefepime at 0.6%, to imipenem 2.00% and to colistin 98.00%.
Conclusion: Tigecycline maintains potent in vitro activity against highly resistant *Acinetobacter* spp. As for *A. baumannii*, there is a decreased susceptibility but it may be an alternative treatment option when other agents are excluded due to multidrug resistance. Due to the possibility of gaining resistance, it is essential for a hospital to remain aware of the susceptibility patterns of this new agent.

R2364 Effect of carbon dioxide on susceptibility testing of serogroup 19A *Streptococcus pneumoniae*

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Objectives: Routine use of susceptibility testing with disk diffusion and E-tests for detecting antibiotic resistance is essential for the treatment of pneumococcal infections. For 19 A *Streptococcus pneumoniae* there is an ongoing concern because increasing numbers of strains within this serotype exhibit high-level resistance to multiple drug classes. We investigated the effect of incubation under ambient atmosphere and the effect of carbon dioxide against 4 antimicrobials by using the E-test and disk diffusion test.
Methods: MICs and zone diameter of 50 serogroup 19A *Streptococcus pneumoniae* were determined against the antimicrobials erythromycin, azithromycin, clarithromycin and clindamycin. E-tests and disk diffusion tests were performed on two different media incubated at ambient atmosphere and 5% CO_2 at 35ºC. Interpretation of susceptibility were determined according the guidelines recommended by the CLSI and EUCAST. The MICs and zone diameter were read after 1 day of incubation at 35.

Results: Elevated MICs and smaller zone diameters were noted for the two different methods (CLSI and EUCAST) and all the tested antimicrobials in the presence of CO_2. Geometric mean MIC values generated by CLSI method increased in CO_2 by 1.85, 3.84, 1.98 and 1.78, and by EUCAST method 1.83, 3.39, 1.94 and 1.62 log2 dilutions for erythromycin, azithromycin, clarithromycin and clindamycin, respectively; the noted differences in smaller diameter zones (mm) were by CLSI method is 3.4, 7.1, 4.2 and 5.7 and by EUCAST method 4.8, 8.4, 5.0 and 6.3.
Conclusion: Incubation conditions have an effect on the MIC and disk diffusion results for *Streptococcus pneumoniae* serogroups 19A. We can expect that incubation conditions are also of influence for *Streptococcus pneumoniae* serogroups other than 19A. Further investigation is needed.

R2365 In vitro susceptibility of ceftobiprole, vancomycin and fosfomycin against recent clinical bloodstream isolates of methicillin-resistant *Staphylococcus aureus* in the General Hospital of Vienna

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Objectives: Since 1961 methicillin-resistant *Staphylococcus aureus* (MRSA) has spread worldwide and has become a leading cause of nosocomial infections. The aim of the present study was to evaluate the in vitro susceptibility of recent MRSA-isolates against the fifth generation cephalosporin ceftobiprole compared to the standard anti-MRSA agents vancomycin and fosfomycin.
Methods: We tested 103 recent clinical MRSA strains, isolated from the blood of septic patients hospitalized to the General Hospital of Vienna. A single isolate per patient was accepted. Minimal inhibitory concentrations (MICs) of ceftobiprole, vancomycin and fosfomycin were determined using broth microdilution method according to the guidelines of the Clinical Laboratory Standard Institutes (CLSI).
Results: The MIC_{50} and MIC_{90} values for MRSA were 2 μg/ml and 4 μg/ml for ceftobiprole, 1 μg/ml and 2 μg/ml for vancomycin, 4 μg/ml and 16 μg/ml for fosfomycin, respectively. According to the breakpoints established by CLSI and EUCAST, a single isolate was resistant to ceftobiprole, another isolate was resistant to vancomycin, whereas seven isolates showed in vitro resistance to fosfomycin.
Conclusions: Ceftobiprole and vancomycin demonstrated potent in vitro activity against MRSA blood-stream isolates with 99% susceptibility compared to less activity of fosfomycin with a MRSA resistance rate of 6.9%.

R2366 Prospective study of antimicrobial resistance of *H. influenzae* in Russia: trends in 2010

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Objectives: To estimate antimicrobial resistance of *Haemophilus influenzae* in different regions of Russia from 2003 to 2010.
Methods: This study was conducted in Central, Volga, Urals, Siberia regions of Russia from 2003 to 2010. Identification of the strains was done on the basis of colony morphology on chocolate agar, Gram stain, bacitracin resistance (10 IU). Susceptibility to 11 antimicrobials was performed according to CLSI with usage *Haemophilus* Test Medium broth prepared on the basis of cation-adjusted Mueller-Hinton broth with 13 ml of the hematin stock solution, 5 g of yeast extract and 3 ml of a nicotinamide adenine dinucleotide (NAD) stock solution (50 mg of NAD dissolved in 10 ml of distilled water, filter sterilized). Microtiter plates were incubated for 24 h at 35ºC and 5% CO_2. Breakpoints were those of Clinical and Laboratory Standards Institute (CLSI 2010).
Results: A total of 691 of non-duplicated clinical *H. influenzae* were included to the study for 2003–2010. Respiratory samples (sputum, BAL, sinus aspirate, middle ear and pleural fluid) were the the main sources of strains – 87.3% in 2003–2005 and 90.7% in 2006–2010.
The susceptibility testing results are presented in the Table.

Conclusions: Thus β-lactams (amoxicillin, amoxicillin/clavulanic acid, ceftibuten, ceftriaxone), azithromycin, clarithromycin, respiratory fluoroquinolones retain their high in vitro activity against haemophili, thus continue to serve as the first line of choice for treatment of infections caused by *H. influenzae* in Russia.

Antimicrobial	I,%	R, %	MIC, mg/l	I,%	R, %	MIC, mg/l
	2003-2005 (n=258)			2006-2010 (n=433)		
Amoxicillin	0.8	4.6	0.5	1,6	1,2	1
Amoxicillin/clavulanate	0	0	1	0	0	0.5
Ceftriaxone	0	0	0,03	0	0	0.03
Ceftibuten	0	0	0.06	0	0	0.125
Azithromycin	0	1.6	4	0	0	1
Clarithromycin	10.5	0	16	0.5	0	8
Tetracycline	2.3	2.7	0.5	0.5	3.3	0.5
Levofloxacin	0	0	0.015	0	0	0.03
Moxifloxacin	0	0	0.03	0	0	0.03
Chloramphenicol	0.4	4.3	1	0.9	2.8	0.5
Co-trimoxazole	12.4	17.4	4	8.7	24.1	16

R2367 In vitro activity of tigecycline against methicillin-resistant *Staphylococcus aureus*, vancomycin resistant *Enterococcus faecalis* and extended-spectrum β-lactamases producing *Klebsiella pneumoniae*

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Objectives: Tigecycline is a newly introduced glycylcycline with a broad spectrum activity against both Gram (+) and Gram (−) bacteria and anaerobes as well, that gained approval initially for treatment of complicated skin, soft tissue and intra-abdominal infections and latest for community acquired pneumonia. The objective of the present study was to investigate the in vitro activity of tigecycline against a range of resistant clinical isolates included MRSA, VRE and *K. pneumoniae*.
Methods: A total of 250 different clinical isolates were tested. The isolates included 50 MRSA strains, 50 VRE and 150 *K. pneumoniae* strains, of which 30 produced ESBL, 45 ESBL and KPC, 35 ESBL and MBL, 28 MBL, 8 KPC and 4 ampC b-lactamases. All strains were recovered from blood stream infections, skin and skin structure infections and community acquired lower respiratory tract infections, from patients treated in ICU (64%), Internal Medical Wards (28%) and Surgical Wards (8%) in a general hospital the last two years. Species identification and initially susceptibility testing of the isolates was performed using the Wider system (Soria), while phenotypic detection of the production of extended spectrum b-lactamases ESBL, ampC-b-lactamases, metallo-b-lactamases MBL and carbapenemases KPC, was performed by the double disk synergy test, the combined disk test, the two-sided E-test and the modified Hodge Test on M. H. agar. Tigecycline susceptibility was determined using the E-test method following the manufacturer's guigelines (bioMerieux, Sweden). Sensitivity and resistance breakpoints for tigecycline were determined according to FDA guidelines. *S. aureus* ATCC29213, *E. faecalis* ATCC29212 and *E. coli* ATCC 25922 were used as QC.
Results: Tigecycline MICs (mg/L) ranged from: 0.032–0.5 (MRSA), 0.032–0.25 (VRE), 0.38–12 (ESBL *K. pneumoniae*), 0.75–6 (ESBL+ KPC *K. pneumoniae*), 0.38–8 (ESBL+MBL *K. pneumoniae*), 0.5–8 (MBL *K. pneumoniae*), 0.75–8 (KPC *K. pneumoniae*) and 0.5–6 (ampC *K. pneumoniae*). MICs 50/MICs 90 were respectively 0.094/0.25, 0.064/0.125, 1.0/6.0, 1.5/4.0, 1.0/6.0, 1.5/6.0, 1.5/4.0 and 1.0/6.0.
Conclusions: The in vitro activity of tigecycline, according to the current breakpoints established by FDA, was considered active against all MRSA and VRE isolates. Furthermore it seems to provide a promising alternative choice for treatment of infections caused by several extended spectrum b-lactamases producing *K. pneumoniae*, as its sensitivity ranges overall between 70–85%.

R2368 **In vitro activity of doripenem against imipenem and/or meropenem non-susceptible isolates of *Pseudomonas aeruginosa* from a university hospital, Bangkok, Thailand**

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Objective: *Pseudomonas aeruginosa* is an important causative agent of nosocomial infections. Treatment of hospital acquired *P. aeruginosa* infection has become more difficult and complicated and usually required broad spectrum antibiotics such as carbapenem. Currently, the carbapenem resistant strains of *P. aeruginosa* are more recognized among hospital-acquired isolates. This has limited the choice of effective antibiotics. The objective of the study was to evaluate the in vitro activity of doripenem, a new anti-pseudomonal carbapenem against the imipenem and/or meropenem non-susceptible *P. aeruginosa*.
Method: A total of 171 clinically isolated *P. aeruginosa* which showed non-susceptible to either IPM or MEM were tested against doripenem. The MICs of imipenem, meropenem and doripenem were determined by broth microdilution using THANF customized panel (Trek, UK). The full range MIC of doripenem was tested with E-test method.
Results: The organisms were divided into groups according to the results of imipenem and meropenem as susceptible (S), intermediate (I) or resistant (R). The MIC_{50} and MIC_{90} of each group were compared. The overall MIC ranged from 1 to >32 μgml. The double resistant isolates (n = 59) revealed the MIC_{50} and MIC_{90} of doripenem at >32 μgml. In contrast, isolates with a combination of one resistance and one intermediate (n = 51) showed the MIC_{90} of doripenem at 4 μgml and the majority (82.35%) of these isolates had the MIC at the same point as MIC_{90}. Although the imipenem susceptible and the meropenem susceptible groups revealed the similar MIC_{90} at 4 μgml, the meropenem susceptible group tended to have lower doripenem MIC_{50} (2 μgml). This occurred in both imipenem susceptible and intermediate groups. The MICs of all isolates from double intermediate and the imipenem susceptible groups were at 4 μgml.
Conclusion: The above data of doripenem has showed that this new carbapenem could be an alternative choice for the treatment of imipenem/meropenem non-susceptible *P. aeruginosa* infectiom.

R2369 **In vitro efficacy of tigecycline, minocycline and colistin against multidrug-resistant *Acinetobacter baumannii***

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Introduction: *Acinetobacter baumannii* has now got itself established as a very common and difficult to treat nosocomial pathogen. Its ability to develop resistance against the major groups of antibiotics limits therapeutic options. Very few antibiotics can be reliably used against this resistant organism.
Objective: We have conducted this study to find out the efficacy of tigecycline, minocycline and colistin against MDR *A. baumannii*
Materials and Method: The study was carried out at the Department of Microbiology, Army Medical College/National University of Sciences and Technology, looking after an 1100 bedded tertiary care hospital. Routine clinical specimens were received from various wards. *A. baumannii* was identified using standard microbiological procedures. MDR was defined as isolates simultaneously resistant to aminoglycosides, carbapenems and fluoroquinolones Minimum inhibitory concentration was performed by using E-strips (AB-Biodisk) of colistin, minocycline and tigecycline for each isolate. Results were interpreted by using SPSS version 17.0.
Result: A total of 100 MDR *A. baumannii* were tested. Colistin found out to be the most effective among the three antibiotics tested. All the isolates were susceptible to colistin. Minocycline was better in activity than tigecycline against MDR *A. baumannii*. 94% isolates were susceptible to minocycline and 80% were susceptible to tigecyclien.
Conclusion: Colistin and minocycline, cost effective antibiotics, can be reliably suggested against infections caused by MDR *A. baumannii*. Emerging resistance against Tigecycline and its high cost hinders its use as first choice.

New antimicrobials

R2370 **A preliminary quantitative assessment of anti-*Helicobacter pylori* activity of xanthone derivatives**

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Objectives: The aim of the study was to determine anti-*H. pylori* activity of a series of 14 xanthone derivatives. They were substituted in different position in xanthone structure i.e. 2, 3, 4 and 6, 2 and 6 or 2 and 7. They possessed among other piperazine, pyridine, aminoalkanol, allyl, methylamine, ethylamine, alkoxy, fenoxy or carbamyl moiety, some of them had also chlorine atom(s) in their structure.
Methods: Disc diffusion procedure (Kirby-Bauer method) was used for primary screening of susceptibility *H. pylori* clinical strain isolated from a patient and ATCC 43504 *H. pylori* strain to the xanthone derivatives (in concentration 10 mg/ml).
In addition compounds giving an inhibition zone ranged from 11mm to 45 mm in diameter were chosen to quantitative assay to establish the lowest concentration that inhibits growth of the *H. pylori* strain (MIC). Bacterial suspension of *H. pylori* was adjusted to yeld approximately 1.0×10^8 CFU/ml and plated on Mueller Hinton agar with 5% horse blood and NAD plates (Oxoid). A stock solution (10 mg/ml) of each substance was appropriately diluted in DMSO to obtain the required concentrations of 1mg/ml, 200 μgml, 100 μgml and 50 μgml. Filter paper discs were placed on the inoculated agar surfaces and impregnated with 10 ul of each sample solution. Antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by the substances. The lowest concentration required for growth inhibition of *H. pylori* strain was regarded as MIC.
Result: The results were very similar for two examined strains. Most of the tested compounds were active against both. *H. pylori* strains at the concentration of 10 mg/ml (stock solution). Two substances exhibited more than 40 mm wide grown inhibition zone, 1 – 39 mm, 2 – from 29 to 30 mm, 7 – from 12 to 20,5 mm. 2 of the presented substances were classified as nonactive.
MIC of the xanthones ranged from 10 mg/ml to 50 μgml. Most potent activity with MIC value of 50 μgml was characteristic for 3 compounds.
Conclusion: Performed research on anti-*H. pylori* activity of the series of xanthone derivatives, it could be stated that: chlorine atom as well as allyl moiety are favorable, substitution in the xanthone structure in position 2 is more favorable than in position 3 and 4 and carbonyl group in a side chain is responsible for the partial loss of activity. Further studies will be necessary to investigate the effect of these active compounds.

Epidemiology of MRSA, VRE and other Gram-positives

R2371 **Changing molecular epidemiology of vancomycin-resistant enterococci in a Chinese hospital between 2003 and 2009**

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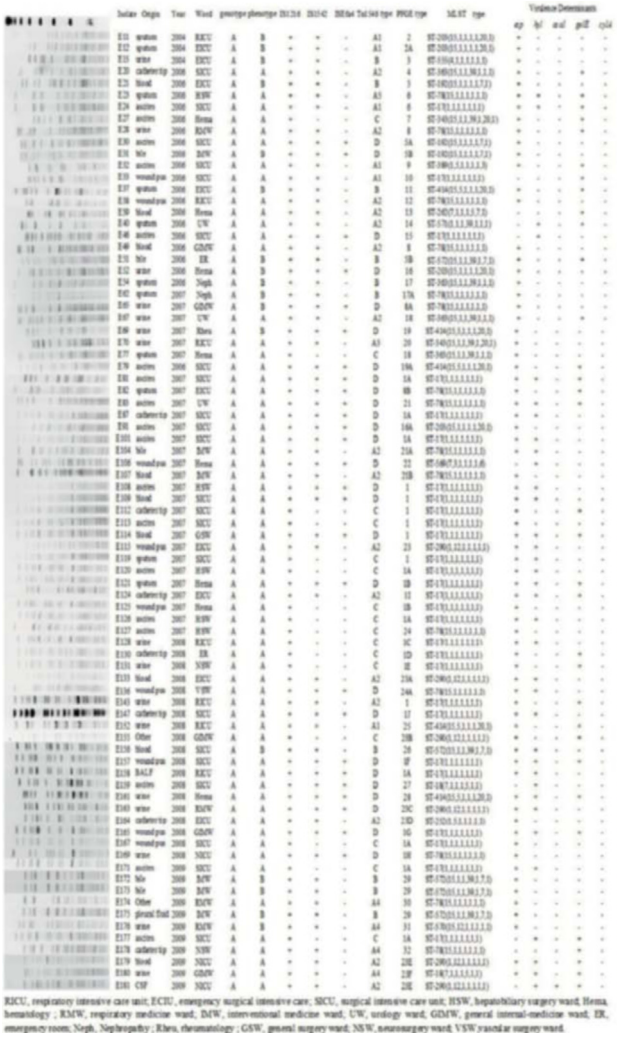
Objective: To investigate the molecular epidemiology of vancomycin resistant enterococci (VRE) in our hospital.
Methods: We examined a total of 98 clinical VRE isolates cultured from patients admitted to Beijing Chao-Yang hospital from June 2003 to December 2009. Minimal inhibitory concentrations (MICs) were determined by the agar dilution method. The vancomycin-resistant genes including vanA, vanB were amplified by multiplex PCR. Isolates were characterized by pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). The vanA gene cluster and the distribution of the virulence markers were investigated using PCR.
Results: Of the 98 VRE, there were 81 *E. faecium* and 17 *E. faecalis*. Amplification of vanA and vanB showed that the vanA gene was exclusive to *E. faecium* isolates while vanB gene was unique to *E. faecalis* isolates. Our results indicate that single clone of *E. faecalis*

(VREfs) is responsible for the vancomycin-resistance from 2003 to 2007. All 17 VREfs were genotyped as ST4. After 2007, VREfs was completely replaced by VREfm which has since been the predominant species in our hospital. Vancomycin-resistant *E. faecium* (VREfm) were found to have a polyclonal distribution and thus appear to have originated from several different sources (Fig. 1). VREfm isolates were heterogeneous in their vanA cluster types and in the presence of virulence genes. All VREfm isolates were ampicillin- and levofloxacin-resistant, and the majority (88.9%) of VREfm possessed the esp gene. MLST revealed five novel STs and one new allele in VREfm. We observed incongruence between genotype and phenotype in 21% of VREfm isolates.

Conclusion: Our findings indicate that the dissemination of the vanA-containing VREfm isolates might be due to either clonal dissemination or horizontal transfer of a resistance gene cluster, but the dissemination of the vanB-containing VREfs isolates support clonal dissemination. VRE appears to be in an evolutionary flux in our hospital.



RICU, respiratory intensive care unit; ECIU, emergency surgical intensive care; SICU, surgical intensive care unit; HSW, hepatobiliary surgery ward; Hema, hematology ; RMW, respiratory medicine ward; IMW, interventional medicine ward; UW, urology ward; GIMW, general internal-medicine ward; ER, emergency room; Neph, Nephropathy ; Rhea, rheumatology ; GSW, general surgery ward; NSW, neurosurgery ward; VSW vascular surgery ward.

R2372 Detection of Panton-Valentine leukocidin by real-time polymerase chain reaction in methicillin-resistant *Staphylococcus aureus* producers isolated in clinical samples

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Objectives: The aim of this study is to detect by real time polymerase chain reaction (RT-PCR) the Panton-Valentine leukocidin (PVL) produced by methicillin resistant *Staphylococcus aureus* (MRSA) isolated in clinical samples of patients in a University Hospital.

Methods: During 2008, 416 MRSA were isolated in clinical samples (56) or nasal swabs of an active surveillance (416) corresponding to patients joined in our hospital. The identification and susceptibility patterns were performed by VITEK II (BioMerièux) and MRSA were classified according to our pulse field electrophoresis (PFGE) patterns. RT-PCR for detection of PVL genes was performed in 56 MRSA isolated in clinical samples using lukS-PV and lukF-PV primers with iQTMSYBR®Green Supermix (BIORAD) in iQTM5 Real Time PCR detection system (BIORAD). All these 56 cases of MRSA were classified in hospital and community colonization or infection.

Results: In 2008, 56 MRSA were isolated in clinical samples (31 swabs, 10 blood, 7 bronchial aspirates, 3 sputum, 2 catheter, 2 abscess and 1 surgical swab). These isolates produced 20 (35.7%) hospital infections, 17 (30.4%) community infections, 16 (28.6%) hospital colonization, 2 (3.6%) community colonization and 1 (1.8%) contamination. RT-PCR detected 6 (12%) MRSA PVL producers, 5 (83.4%) were community infections (1 ST5-MRSA IVA clone, 1 ST22-MRSA-IV, 1 ST36-MRSA-II, 1 ST8-MRSA-IV and 1 ST88-MRSA-IV clone) and 1 (16.6%) was a hospital infection (1 ST5-MRSA-IVA clone). Of 5 community infections 4 (80%) were infections of tissue and soft skin and 1 (20%) was an infection of surgical site, the hospital infection was a primary bloodstream infection.

Conclusion: PVL is a cytotoxin that causes leukocyte destruction and tissue necrosis. In our hospital, a 12% of MRSA isolates in clinical sample were PVL producers and a high percentage was related with community infections associated to tissue and soft skin infections as in others studies but also a low percentage was related with hospital infections. According to MRSA clones, PVL is usually produced by community acquired MRSA (CA-MRSA) clones as we found in our study. RT-PCR is a rapid procedure to detect the presence of PVL MRSA producers isolated in clinical samples related to community infections.

R2373 Validation of Copan MRSA-B broth for MRSA detection by culture and by Xpert MRSA/SA/nasal assay

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Objectives: Enrichment broths are increasing the sensitivity of detecting MRSA when screening colonized patients prior hospital admission or to critical care wards. Copan produced a new Liquid Base Microbiology (LBM) device, the MRSA-B, consists of a 2 ml tube of enrichment broth and can be used for pooling samples for MRSA collected from different sites from the same patient. The objective of this study is to validated the Copan MRSA-B for the detection of MRSA and MSSA by culture and by Xpert MRSA_SA_NASAL assay (XMSN) (Cepheid).

Methods: Simulated samples were prepared by inoculating MRSA-B tubes with MRSA ATCC (43300) and MSSA ATCC (6538SA) strains and tested by culture and by XMSN assay. 0.5 McFarland dilution of MRSA and MSSA strains were prepared from fresh cultures and diluted to 0.5:104 and 0.5:105 in PBS. 200 ul of each dilution were used to inoculated 2ml sets of MRSa-B5 tubes and tested at 0 times and after 2, 4 and 6 hours incubation at 350 C. Aliquots of 100ul from each MRSA and MSSA inoculated tube were plated in Mannitol salt agar after all incubation times and conditions. Colonies were counted and recorded after 24 hours plate incubation at 350 C. To enhance the sensitivity of XMSN assay the 200 ul and the pellets generated from centrifuging 1.0 ml and 1.5 ml of broth were also tested. Negative vial broths were also tested. The XMSN assay testing was done as per package insert procedure.

Results: In the culture results for the final dilution of 0.5:105 there were change in the number of CFU/100 was noted up to 2 hours incubation with the MRSA and MSSA enrichment broth samples, but 9 and 27 fold increases after 4 and 6 hours for the MRSA but not for the MSSA. In the XMSN assay there was an increase in the 200 ul (and 1ml pellet) by 1.7 (4.4), 7.8 (3.4) and 11.8 (2.6) CT from 0T to 2, 4, and 6 hours incubation respectively. No MSSA was detected when testing for MRSA.

The MRSA dilution of 0.5:106 had very few colonies in culture up to 6 hours but the pellet of 1.5 ml of the broth after 2, 4, and 6 hours incubation there was an increase of 1.7, 3.6 and 4.8 in CT. There was no interference, inhibition or cross-reaction with MSSA in the XMSN assay.
Conclusions: The Copan MRSA-B enrichment broth increases the number of MRSA colonies 27 folds and 11 CT in the XMSN assay after 6 hours incubation. The sensitivity of the XMSN assay is increased when using the pellet from centrifuging 1.0 or 1.5 ml of the MRSA-B enrichment broth.

R2374 Which role for MRSA screening in the intensive care unit?

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Objectives: Several recent studies have shown that active surveillance for Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in ICU (Intensive Care Unit) patients may decrease MRSA transmission and/or infection, by isolation, decolonisation of positive cases and hand hygiene programs. A potential role for active MRSA surveillance in ICU, has been recently proposed, as a guide for antibiotic treatment in patients suspicious of being infected with MRSA, by using a care bundle approach.
Methods: From March 2009 through September 2010, a total of 376 patients were screened for MRSA nasal carriage at admittance to ICU. All nasal swabs were analyzed by molecular test (GeneXpert MRSA, Cepheid), able to amplify by real-time PCR the target sequence for MRSA at the SCCmec-orfX junction. The results were available in the LIS (Laboratory Information System) from specimen receipt in the laboratory, within the same day.
Results: Of the 376 patients admitted during the study period, 26 (7%) were colonized with MRSA and 350 were MRSA negative. During the ICU hospitalization, MRSA infection was more likely to develop in MRSA carriers (8/26, 30%) compared with non-MRSA carriers (1/350, 0.3%). Our data showed an increased relative risk for developing MRSA infection in the first group compared to the other, therefore a strongly association between MRSA colonization and subsequent MRSA disease.
Conclusion: Our data suggest that MRSA colonization in ICU patients is a risk for development of subsequent MRSA infections and that nasal MRSA screen appears a potential predictor of the infection, confirming the role of a documented MRSA colonization as a useful determinant for empirical MRSA antimicrobial coverage in ICU patients, in a care bundle approach.

R2375 Hospital-associated Methicillin-resistant *Staphylococcus aureus* in Germany: epidemic but not really multi-resistant

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Objectives: As National Reference Centre for Staphylococci we perform long term molecular typing on representative samples of Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from German hospitals and thus following the dynamics of emergence and spread of hospital associated MRSA (HA-MRSA) clones.
Methods: The respective isolates were sent to the Reference Centre for further characterisation. The antimicrobial susceptibility, the presence of several resistance and pathogenicity associated genes, the spa- and multilocus sequence-type were analyzed.
Results: From 1991 to 2000 the incidence of HA-MRSA increased from ~2% to >20%. During this time clonal lineages (ST247, ST228) disappeared, and among newly emerging clonal lineages ST225 and ST22 became particularly prevalent. These strains exhibit a less broad resistance profile: OXA, ERY, CLI, CIP, MFL (mecA, ermA/ermC, mutations parC, gyrA).
Resistance to other classes of antibiotics is still not frequent: (ST22/ST225) DAP 0,2/1,7%; FOS 0/0%; FUS 1,9/0%; GEN 0/4,8%; LNZ 0/0,5%, MUP 0/0%, RAM 1,7/4,5%; TGC 0/0%; TPL 0/0%; VAN 0/0%. Of particular interest are isolates exhibiting resistance to daptomycin and in parallel the GISA phenotype (not observed so far).
MRSA ST225 represent a subpopulation of MRSA CC5 which evolved from MRSA ST5 in the USA ~20 years ago and became epidemic in Central European hospitals after 2002 (Nübel et al., PLoS Pathog., 2010).
MRSA ST22, already known as "EMRSA 15" in the UK, became epidemic in Central Europe after 1996. It presents an epidemic subpopulation of *S. aureus*/MRSA ST22 spreading in hospitals. CA-MRSA ST22 (PVL-positive) represents a separate subpopulation (Kurth et al., unpublished data).
Obviously there is also an "import" of MRSA known to be epidemic in other European countries (e.g. ST36, UK; ST239, South-East-Europe; ST125, Spain). However, these cases are sporadic.
Also infections caused by CA-MRSA ST398 are rare so far (1,68%).
Conclusions: As shown by the results from the EARSS/Seqnet study particular HA-MRSA clonal lineages are obviously widely disseminated. However, in certain geographical areas a few lineages predominate. The reasons for different epidemiology in different environments are not known so far. The frequent resistance to macrolides/lincosamides and fluoroquinolones besides all β-lactames reflects the consumption volume of antibiotics in German hospitals. In cases of calculated therapy a number of options are left.

R2376 Antibiotic resistance profiles and molecular epidemiological characteristics of *Staphylococcus aureus* isolates in Changsha, south China

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Objectives: Increasing prevalence of *Staphylococcus aureus*, particularly methicillin-resistant *S. aureus* (MRSA) has been reported in China. The aim of the present study is to investigate the drug resistance characteristic, the genetic background and the molecular epidemiological characteristic of *S. aureus* in Changsha, south China.
Methods: Between December 2006 and December 2008, a total of 293 clinical isolates of *S. aureus* were collected from 11 representative hospitals in Changsha and then identified by Vitek-2 system. All the isolates were verified as *S. aureus* and MRSA by PCR amplification of femA and mecA gene respectively. K-B disk method was used to test drug sensitivity of *S. aureus* to 23 commonly used antibiotics. Chromogenic cephalosporin spot test was applied to detect β-lactamase. Pulsed-field gel electrophoresis (PFGE) was performed for genotypic and homologous analysis of 115 isolates randomly selected from the original 293 clinical *S. aureus* isolates.
Results: Among the 293 strains, 273 (93.2%) were β-lactamase test positive. Resistant rates to penicillin and ampicillin were the highest (both 96.6%). All the isolates were susceptible to tecoplanin, vancomycin and linezolid. All the stains exhibited 93 antimicrobial-resistant profiles to the 23 antibiotics tested. The PX1 type included 47 (16.0%) strains and the PX2 type included 35 (11.9%) strains, which accounted 28.0% (82/293) of all, were the primary epidemic antimicrobial-resistant profiles. 190 (64.8%) were MRSA and 103 (35.2%) were MSSA. The resistant rates of MRSA to 18 antibiotics were higher than MSSA ($P < 0.05$). The MIC range of OXA and FOX was 0.125 μg/ml–>256 μg/ml and 2 μg/ml–>256 μg/ml respectively. Both of their MIC_{50} and MIC_{90} were ⩾256 μg/ml. Of all the 115 clinical *S. aureus* isolates, 39 PFGE types were demonstrated. PFGE type A with the incidence of 48.7% (56/115), from 8 hospitals (8/11), was the predominant genotype. 55 (55/56) strains with PFGE type A were MRSA. The next was PFGE type L with the incidence of 4.3% (5/115). PFGE type A included 13 subtypes from A1 to A13, and the subtype A1 is the predominant type and epidemic clone with the incidence of 22.6% (26/115).
Conclusion: *S. aureus* in Changsha is multiple resistant to commonly used antimicrobial agents and has a high resistant rate to methicillin. PFGE type A outbreak at clinical isolates of *S. aureus* occurred in Changsha and A1 subtype is the predominant epidemic clone.

R2377 Risk factors for developing reduced vancomycin susceptibility and methicillin resistance among episodes of *Staphylococcus aureus* bacteraemia (SAB) in an intensive care unit

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Objectives: The aims of this study were to describe the vancomycin susceptibility levels of *Staphylococcus aureus* bloodstream infections (SAB) in critically ill patients, to know the prevalence of methicilin-resistant episodes (MRSA), to know their prognosis and finally to define clinical risk factors independently associated to the development of RVS and MR among ICU-SAB.
Methods: From 1995 to 2010, 503 patients with a clinically significant bacteremia were prospectively evaluated in an intensive care unit of a university hospital, specially SAB. MRSA (oxacilin MIC$\geqslant$4 μg/ml) and RVS (MIC >1 μg/ml) were defined by E-Test® method. Clinical and microbiological variables were studied. Several multivariate analysis were performed to determine clinical risk factors independently associated to the development of RVS and MR among ICU-SAB.
Results: Ninety (17.8%) of 503 ICU bacteraemias were caused by SAB and 32.2% of them was MRSA. RVS were detected in 48,8% of the cases. The global and related mortality rate for SAB was 53.3% and 18.8%. Global mortality rates were significantly higher in MRSA episodes (68.9% vs 45.9%; p = 0.04). whereas RVS episodes showed higher related mortality rates (27.2% vs 10.8%; p = 0.04). Age (OR 1.04; CI95% 1.01–1.08; p = 0.01), previous use of corticosteroids (OR 5.23; CI95% 1.08–25.2; p = 0.03), polimicrobial bacteraemia (OR 5.31; CI95% 1.22–23.1; p = 0.02) were factors independently associated to RVS episodes whereas the presence of phlebitis (OR 0.12; CI95% 0.02–0.76; p = 0.02) was a protective factor for these episodes. On the other hand only the previous use of corticosteroids (OR 4.53; CI95% 1.04–19.7; p = 0.04) and nosocomial origin (OR 6.71; CI95% 1.34–33.6; p = 0.02) were independently associated to MR episodes.
Conclusions: 1 in 3 of SAB in ICU was caused by MRSA and almost half presented RSV. Both MR and RVS episodes were associated to an increased mortality rates in ICU patients. Age, previous use of corticosteroids, polimicrobial bacteraemia, and the absence of phlebitis were identified as risk factors for developing RVS in ICU-SAB and only nosocomial origin and previous use of corticosteroids were related to MR episodes in this study.

R2378 A research for methicillin-resistant *Staphylococcus aureus* originated from foods: several methods for the detection, occurrence, enterotoxigenic characteristics, and epidemiologic typing method

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Objectives: The purpose of this study was a research for Methicillin-resistant staphylococcus aureus originated from foods based on pheno and genotypic methods.
Methods: 93 *S. aureus* isolates from 913 food samples were investigated for the detection of methicillin resistance using mecA specific PCR, oxacillin agar screen and disk diffusion, penicillin binding protein 2 (PBP2) latex agglutination, β-lactamase production and MIC tests. The MRSA strains were characterized by the antimicrobial susceptibility, production of type A-D staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin1 (TSST1), as well as biotyping and molecular typing based on the x-region of the protein A (spa) and the coagulase (coa) genes.
Results: Out of the 93 *S. aureus* isolates, eight were mecA positive while phenotypic tests showed a discrepancy in comparison with each other. The majority of MRSA isolates were PBP producers and Multi-resistant. All TSST1 producing MRSA were SE producers. Out of the 12 MRSA isolates, eight were belonged to human biotype; three belonged to NHS and one to bovine biotype. Amplification of polymorphic spa and coa genes revealed three and five distinct types, respectively.
Conclusion: Food is an important vector for transmission of antibiotic resistance to human. Some MRSA isolates recovered from different sources showed genetic and phenotypic similarity in the patterns obtained, suggesting that contamination with human origin has been occurred during food handling.

R2379 High diversity of Panton-Valentine leukocidin-positive MSSA clones circulating in Italy

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Objectives: Both MSSA and MRSA PVL-positive strains can cause serious and recurrent infections in subjects without risk factors living in the community. In this work we focus the attention on the characterization of the PVL-positive MSSA strains, responsible for outbreak and sporadic infections in Italy.
Methods: During the period 2005–2010, twenty-two PVL-positive MSSA were collected. The isolates have been sent to the National Laboratory by different Italian hospital laboratories on the basis of the type and the severity of the infections that appeared typical of PVL-positive strains.
The presence of nuc (species confirmation), PVL and toxic shock syndrome toxin 1 (TSST-1) genes were detected by PCR. Molecular typing techniques such as agr typing, spa typing and Multi-Locus Sequence typing (MLST) were performed. Clonal Complexes (CCs) were determined by using eBurst software.
Results: All the isolates were confirmed as PVL-positive MSSA. Fourteen isolates were from skin and soft tissue infections (SSTIs), 4 from nasal carrier, 2 from necrotizing pneumonia, one each from an ORL infection, sepsis and osteomielitis. One of these isolates was the strain responsible for an hospital-community outbreak in 2005. Molecular typing revealed that isolates belonged to all the four known agr alleles (from 1 to 4), to 16 different spa type and to 12 different sequence type (ST). Following eBurst analyses, the related STs were grouped in CCs except for ST1209 that was a singleton. The isolates belonged to 9 different genetic lineages. The most common were CC121/agrIV and CC30/agrIII (6 isolates each). The other lineages were: CC5/agrII, ST1209/agrIII and CC22/agrI (2 isolates each) and CC1/agrIII, CC78/agrIII, CC152/agrI and CC8/agrI (1 isolate each). Three strains were TSST-1 positive: both the ST1209/agrIII strains and one CC30/agrIII.
Conclusions: This study showed the great variety of the PVL-positive MSSA clones circulating in Italy, with CC121/agrIV and CC30/agrIII being the most commons. Differently from other countries, we did not find strains belonging to the common CC80/agrIII clone. A new lineage was found (ST1209/agrIII) which also was TSST-1 positive. Further studies should be done to evaluate the circulation and the characteristics of PVL-positive MSSA since it can cause serious infections similarly to its counterpart, PVL-positive MRSA.

R2380 Demonstration of the hypervirulent ST17 clone of *Streptococcus agalactiae* in Hungary

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Objectives: *Streptococcus agalactiae* (Group B streptococcus, GBS) is well established as a major human pathogen causing serious infections primarily in neonates but affecting also adults. The screening of pregnant women for GBS prior to delivery is performed in many European countries but has not been introduced in Hungary. In addition, no data on the clonal distribution and virulence of GBS has been reported in Hungary to date.
Methods: 110 strains of GBS, isolated from pregnant women or newborns, at the Central Bacteriology Laboratory of Semmelweis University between 2009–2010, were genetically characterised. The strains were identified by routine biochemical tests and by PCR detection of the GBS specific dltR gene. The serotypes of the isolates were determined with the Pastorex latex agglutination test which distinguishes

types I, II and III, and the genetic relatedness of the strains was determined by PFGE.
Results: In the examined period, out of all cervical or vaginal exudates, 20.7% proved to be GBS positive. 9.5% of the newborns were colonised (ears or throat), and only 0.4% had GBS in the blood. The hypervirulent ST17 clone was detected in 46.2% of GBS+ women and 35.6% of GBS+ newborns. Generally, serotype III was most prevalent (56%), type I was isolated in 18% (mostly from the ears of newborns) and type II in only 7%. The vast majority of the ST17 strains (36/58) were also of type III. The ST17 strains belonged to 4 clearly distinguishable PFGE clones, but were generally closely related to one another.
Conclusions: Serotype III was shown to be the most prevalent in neonatal colonisation or asymptomatic carriers also in Hungary. The ST17 clone of GBS identified by multi locus sequence typing (MLST) is recognised as a hypervirulent international clone associated mainly with invasive neonatal infections. ST17 comprised 46.2% of asymptomatic carriers among the pregnant women, and could be transmitted to the newborns at high rates, with a potential of causing severe neonatal infections. These results emphasize the necessity of a regular screening during pregnancy also in Hungary.

R2381 Surveillance of *Staphylococcus aureus* carriage in healthy young adults in Hungary

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Objectives: The carriage of pathogenic bacteria such as *Staphylococcus aureus* in healthy individuals is well known, with a 25–30% prevalence. In this study we surveyed the nasal carriage of students of a medical university, who already attend hospital wards, so they can be a potential source of infection. This is the first study of this kind in Hungary.
Methods: Eighty-eight *S. aureus* isolates were collected from the nasal passages of the students attending Semmelweis University, Budapest, Hungary. The species identity was confirmed by colony morphology, catalase test, Pastorex test (Bio-Rad) and nucA PCR. MRSA strains were screened by mecA PCR. The antibiotic sensitivity was determined by E-test, applying the EUCAST breakpoints. The genetic relatedness of 56 strains was examined by PFGE.
Results: Altogether 300 3rd-year students (205 Hungarian and 95 non-Hungarian) were sampled on a voluntary base. The overall *S. aureus* carriage rate was 29.3%, little higher among the Hungarians (31.7%) than in the non-Hungarian group (24.2%). On one occasion carriage of two unrelated strains was detected. Out of the 88 strains, only 2 carried the mecA gene, but these had oxacillin MICs of only 0.75 and 2 mg/L, respectively. The strains were fully sensitive to gentamicin, ciprofloxacin and vancomycin. There were 9 isolates with high-level (MIC $\geqslant$256 mg/L), and 3 with low-level (MIC = 12–32 mg/L) erythromycin resistance. Only 1 isolate was resistant to clindamycin. Based on the PFGE pattern, 3 clones comprised approximately half of the strains (15, 14 and 6 strains, respectively), but the rest were rather diverse.
Conclusions: The *S. aureus* carriage rate correlates well with international data. Luckily there were only 2 MRSA strains (2.3%). The one with the higher MIC (the EUCAST breakpoint is >2 mg/L) was highly resistant also to erythromycin, but the other strain was fully sensitive to all tested drugs. The other isolates were generally very sensitive, which is characteristic in the case of carried pathogens. The strains proved to be genetically more diverse than the usual MRSA populations, with only a few smaller clones, indicating the presence of several independent strains. This, and the dissimilarity of isolates within the same groups, indicate that there is no extensive exchange between the students flora.

R2382 Detection of pili in clinical and carried isolates of Hungarian *Streptococcus pneumoniae*

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Objectives: In 2006, presence of pili on the surface of *Streptococcus pneumoniae* (pneumococcus) was discovered and it was shown to enhance adhesion to epithel cells. In the case of pneumococcus, carriage in the nasopharynx (esp. children) is the initial step of subsequent invasion. According to the literature, 20–30% of strains possess pili, and these are restricted to certain serotypes, especially the so-called vaccine-types (i.e. included in the pneumococcal conjugate vaccines). In the present study, we wanted to determine the presence of pili in Hungarian pneumococcal strains, for the first time.
Methods: We have tested 100 clinical strains, isolated at different routine laboratories in Hungary between 2002–2008, as well as 156 strains that derived from healthy children attending day-care centres (2009–2010), all well characterised earlier. Boiled bacterial colonies were used as template. We have used previously described primers for the PCR detection of pili.
Results: Among the clinical strains, the pilus was present in 24 cases (24.0%). Seven of these derived from invasive infections, and the rest from respiratory specimens. Half of them came from small children (<4,5 y), and half from adults (41–84 y). The majority of the strains was resistant (R) to macrolides, and had elevated MICs to penicillin (0.25–1.5 mg/L). Their serotypes were: 6 (n = 11), 14 (n = 7), 19F (n = 3), 11A (n = 2) and 23F (n = 1). Among the carried isolates, only 15.4% (n = 24) were pilus positive. These were of the following serotypes: 6 (n = 10), 19F (n = 6), 14 (n = 3), 19A (n = 2), 15B (n = 1), 3 (n = 1) and 18C (n = 1). Although the majority of the carried strains was sensitive to antibiotics, nearly half of the pilus + strains was also R to macrolides and non-susceptible to penicillin. Out of the 24 children, only 2 were previously vaccinated with Prevenar.
Conclusions: The rate of pilus positive clinical strains correlates well with international data, but it was significantly lower in the carried strains. This suggests that pili are required more for invasion rather than merely for colonisation. Very probably there is no direct correlation between pili and resistance, but rather we found pili only in certain resistant serotypes. These were almost all (45/48) vaccine-types (with the dominance of serotype 6), except for 3 strains (11A or 15B). The conjugate vaccines seem to be quite effective in preventing colonisation with pilus positive strains, hence preventing subsequent invasion in the body.

R2383 Meticillin-resistant *Staphylococcus aureus* ST22 outbreak in a French neonatology unit

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Objectives: Meticillin-resistant *Staphylococcus aureus* (MRSA) has been diffusing in French ICUs and long-term care facilities. Here we report a MRSA outbreak in the Neonatology unit of our hospital and the emergence of the ST22 MRSA clone in French hospitals.
Methods: Patients admitted to the Neonatology unit of the HFME hospital in Lyon were screened weekly for MRSA carriage in the feces. Strains isolated on chromogenic agar for MRSA detection as well as MRSA isolated from various infections in neonates were fully characterized by using macro-array Staphy-Type from Clondiag (Alere), MLST typing and for the antibiotic susceptibility profile.
Results: From January to August 2010 the prevalence of MRSA carriage and infection among neonates admitted to our hospital was extremely low (2 MRSA strains were isolated from the feces and one MRSA strain was isolated from a surgical wound infection). During the first week of September, one MRSA strain was isolated from blood culture from one neonate who deceased from septicemia. From September to December no other MRSA infection was detected but MRSA gut-carriage was diagnosed in 23 other neonates. All 26 isolated strains displayed the same antibiotic susceptibility profile: oxacillin and levofloxacin resistant. Genotyping results showed that the strains belong to two different clones. The first 3 strains isolated (from January to August) were agr1, SCCmec type IV, harbored enterotoxin A gene and belong to the ST8 Lyon clone. The 23 MRSA strains isolated from September to December were agr1, SCCmec type IV, harbored enterotoxins C, L and collagen binding protein genes and belong to the ST22 EMRSA-15 clone.

Conclusions: Previous data account for MRSA diffusion in French hospitals, most isolated strains belonging to the ST8 Lyon clone. We report here a MRSA outbreak in a Neonatology unit and the emergence of ST22 MRSA clone in a French hospital. Our observation raises the question of the competition between these two MRSA clones. Further survey may clarify whether ST22 would displace the Lyon clone and spread more efficiently in our hospitals.

R2384 Molecular characterisation of methicillin-resistant coagulase-negative staphylococci isolated from patients of cardiovascular and neonatal centres in Moscow

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Objectives: Coagulase-negative staphylococci (CoNS) are now recognized as the aethiological agents of an important range of infections in humans. Most countries have reported an increase in CoNS infections in hospitalized patients that are resistant to methicillin and other antibiotics. Despite of it information of population structure and global epidemiology of *Staphylococcus epidermidis* and some other CoNS is scare. The goal of investigation was to analyze collection of CoNS from diverse clinical origins using molecular genotyping.
Methods: CoNS isolates were recovered from the blood (36), umbilical wound (10), conjunctivitis (33), urine (1), pulmonary tissue (2) in 2009–2010. Antimicrobial susceptibility was determined by standard methods. Species identification was carried out by amplification and sequencing of tuf gene. *S. epidermidis* isolates were analyzed by multilocus sequence typing (MLST) by protocol, as described by Thomas J.C. et al. (2007). MLST of *Staphylococcus haemolyticus* isolates was performed using original primers sets. Structural components of staphylococcal cassette chromosome mec (SCCmec) were tested by PCR additionally.
Results: Identification of CoNS at the species level indicated that *S. epidermidis* was the most common species with 46 isolates, followed by *S. haemolyticus* (18), *Staphylococcus hominis* (10), *Staphylococcus warneri* (7), *Staphylococcus pasteuri* (1). 69 (84.2%) isolates were methicillin-resistant. Among *S. epidermidis* isolates 24 ST were discovered, including 5 new. New alleles for 5 analysing genes were discovered two. ST59 was predominated (41.3%. isolates). *S. epidermidis* isolates carried SCCmec IV, composite variant SCCmec (mecB+, ccr1,+ccr2) or unusual patterns with two mec gene complexes (class A and class B). Among *S. haemolyticus* isolates 9 St were recovered. Isolates carried modified SCCmec type IV (ccr2+, comlex mec class B 1kbp), or elements SCCmecV (ccr5+). SCCmec in 42.7% isolates were not typable. The predominant SCCmec type found among *S. hominis* isolates was a new type with ccr1 and class mec typeA.
Conclusion: Only 4 species (*S. epidermidis*, S haemolyticus, *S. hominis*, *S. warneri* were prevalent (98.8%) among tested collection of coagulase-negative staphylococci. High level of genetic diversity of *S. epidermidis* and *S. haemolyticus* isolates were discovered. Isolates *S. epidermidis* ST59 were epidemic in neonatal center and aethiological agent of different forms of infectious process. CoNS may serve as reservoir of new types SCCmec.

R2385 Clinical and molecular features of methicillin-resistant *Staphylococcus aureus* causing bacteraemia in Andalucía, Spain

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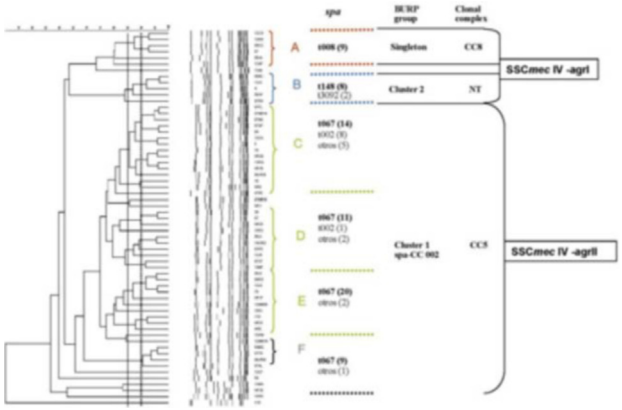
Background: Some changes in the molecular epidemiology of MRSA has been reported in Spain during the last decade. Whether the clinical features of MRSA bloodstream infections (BSI) are different according to molecular profiles of the isolates has not been extensively studied.
Methods: A multicenter cohort study of patients with BSI due to MRSA was performed from 2008 to 2010 in 10 hospitals in Andalucía, Spain. Antimicrobial susceptibility was assessed by broth microdilution and E-test. Isolates were characterized by PFGE after SmaI digestion, SCCmec typing, agr typing, spa typing with BURP analysis, and clonal complex assignment.
Results: 99 cases were included (data were collected retrospectively in 36 and prospectively in 64). Complete clinical data are currently available for 81; 51 (63%) were hospitalized in medical, 20 (25%) in intensive care units, and 10 (12%) in surgical wards. According to Friedman's criteria, acquisition was classified as nosocomial in 47 (57%), healthcare-associated in 28 (35%), and community in 7 (9%). The Charlson index was $\geqslant$2 in 65%, and 42% had a Pitt score >2. The most frequent source was an intravascular catheter infection (43%). BSI was considered as complicated in 26 patients (33%); 36 patients (44%) met $\geqslant$1 criteria for therapeutic failure; BSI-related mortality was 31% (25 patients). All 100 isolates were studied. There were 14 different antibiotic resistance patterns, with combined resistance to ciprofloxacin, tobramycin and erythromycin being the most prevalent (29%). By broth microdilution, MIC of vancomycin was <1 and 2 μg/ml for 84% and 6% of the isolates, respectively; by E-test, it was $\leqslant$1, 1.5 and 2 μg/ml in 56%, 39% and 5%, respectively. Regarding daptomycin, only 2% of the isolates showed a MIC >1 μg/ml by microdilution although all the isolates showed a MIC $\leqslant$0.5 μg/ml by E-test. Eighty percent of the isolates were grouped into 5 clusters by PFGE (groups A to E) with the following characteristics: A (9 isolates), spa t008, agrI, and SSCmecIV; B (9 isolates), spa t148 and t3092, agrI y SCCmecIV; clusters C, D and E (61 isolates), predominantly spa t067 and t002, agrII, and SSCmecIV. Acquisition, predisposing features, source of BSI, complicated BSI or mortality were similar among the 5 clusters.
Conclusion: Isolates of MRSA causing BSI in our area are grouped into 5 clusters, in which SSCmecIV, agrII and spa t067 predominated. We were unable to find significant epidemiological or clinical differences or trends among the clusters.



R2386 Molecular epidemiology and characterisation of CA-MRSA selected according to the CDC criteria

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Objectives: Community-associated MRSA (CA-MRSA) has become a worldwide phenomenon. In this study, we investigated the molecular epidemiology and the antibiotic resistance mechanisms of CA-MRSA selected from clinical specimens according to the CDC criteria.
Methods: 34 non-duplicated outpatient isolates were classified as CA-MRSA (July 2009 to July 2010) in our institution. Identification and susceptibility testing was carried out by WIDER® system and disk diffusion method. All isolates were genotyped by PFGE/SmaI and spa, SCCmec and agr typing. The presence of genes encoding PVL and resistance genes: methicillin (mecA), erythromycin (ER) and clindamycin (CC) (ermA, ermB, ermC and msrA), gentamicin

(GM), kanamycin, amikacin and tobramycin (TO) (aac(6′)-Ie-aph(2″)-Ia, ant(4′)-Ia and aph(3′)-III) and tetracycline (TE) were performed by PCR.
Results: A high diversity of PFGE patterns was observed in CA-MRSA. Resistance to ER, CC, TO, GM and TE was 52.9%, 14.7%, 73.5%, 26.4% and 8.8%, respectively. ER resistance genes detected were msrA (32.3%), ermC (8.8%), msrA+ermC (8.8%) and ermA (2.9%). The msrA gene was found in isolates with macrolide-streptogramine (MS) resistance phenotype and ermC, ermA or msrA+ermC genes in those with MLSBc or MLSBi resistance phenotypes. Aminoglycoside resistance genes detected were ant(4′)-Ia (61.8%), aac(6′)-Ie-aph(2″)-Ia (2.9%), aph(3′)-III (2.9%) and aac(6′)-Ie-aph(2″)-Ia+aph(3′)-III (5.9%). Five (14.7%) strains were PVL positive.
SCCmec type IVc was detected in 27 strains (79.4%), type V in 3 (8.8%) and 4 strains were type I, II, IVa and non-typeable, respectively. The agr type II was identified in 28 strains (82.3%), agr type I in 3 (12%), agr type III in 2 (5.8%) and one strain was non-typeable. The spa-type t067 was the predominant (64.7%) followed by t002 (8.8%). spa type t067 and t002, normally grouped in clonal-complex CC067 and CC5 (sequence-types ST125 and ST228), are responsible for more than half of nosocomial MRSA infections in Spain. Single strains were typed as t008, t019, t024, t116, t127, t314, t548, t1084 and t2220.
Conclusion: Five of 34 selected CA-MRSA carried PVL gene and corresponded to spa types classically community-associated: t024 (ST8-USA300), t019 (ST30), t008 (ST8-USA300), t314 (ST121) and t127 (ST1- USA400). The remaining 29 CA-MRSA corresponded to spa types more associated to the hospital environment what suggests the interchange of genetic lineages of MRSA among community and hospital niches.

R2387 No significant increase of colonisation rate but diversification of methicillin-resistant *Staphylococcus aureus* strains among children in the community

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Objectives: We compared methicillin-resistant *Staphylococcus aureus* (MRSA) strains' characteristics and colonization rates among children attending kindergartens in Tg Mures, Romania, in two study periods three years apart. We also examined the strains' relatedness with MRSA clinical isolates obtained from hospitalized patients.
Methods: Sampling was performed randomly and after the parents' informed consent. Nasal swabs were collected and placed in transport medium. After enrichment *S. aureus* strains were identified by conventional methods. Multiplex PCR was applied for the detection of nucA, mecA and lukS-lukF-PV genes. Pulsed-field gel electrophoresis was performed for determining clonal relations. Pulsotypes were compared to a database of clinical isolates recovered from hospitalized patients.
Results: The study was conducted in 16 kindergartens, in 2007 and 2010. During the first period 186 children out of 2900 were enrolled, while in the second period 189 children out of 2695. *S. aureus* colonization rate was 49% (40–57%, 95% CI) in 2007 and 44% (38–52%, 95% CI) in 2010. MRSA colonization rate was 2.3% (0–5%, 95% CI) and 3.4 (1.5–4.9%, 95% CI) in 2007 and 2010, respectively. All MRSA strains identified by conventional methods were confirmed by the molecular methods. None of the MRSA strains harboured genes encoding for Panton-Valentine leukocidin. MRSA strains isolated in 2007 belonged to the same clonal type. Five distinct pulsotypes were recorded in 2010. MRSA strains endemic in the hospital setting were not found in children. On the contrary, community strains were sporadically recovered among hospital clinical isolates.
Conclusion: There was no significant increase in MRSA colonization rate between the two periods, although a diversification of circulating strains was recorded. Hospital strains were not disseminated in children collectivity, while community strains were present among hospitalized patients.
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R2388 *Staphylococcus aureus* carriage in physicians attending three national meetings about infectious diseases

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SA (*Staphylococcus aureus*) SARM and/or SAMS)) carriage in physicians caring for weakened patients is not well documented and could be a concern. We investigated it among microbiologists, infectious diseases, hygiene and intensive care physicians attending their national annual meetings (namely JNI for infectious disease physicians and microbiologists, SFAR for intensive care physicians, and JNHH for hygiene physicians).
Methods: Volunteer physicians were recruited during the meetings. The anonymous screening (nasal swab) was performed using nasal MRSA/SA Xpert test (Cepheid), an automatized PCR test, feasible in less than 2 minutes, focusing on SCCmec (staphylococcal cassette chromosome), mecA (meticilline résistance gene), and spA (specific of SA species) targets.
Results: We performed 293 tests during SFAR meeting, 152 during JNI, and 120 during JNHH. 32% of volunteers in SFAR meeting were positive for SA, including 8% of MRSA, 34,2% and 2,4% in JNI, 27,5%, and 1,6% in JNHH. We did not reveal any difference according to the type of structure and number of patients' beds. 50% of SA carriers in JNI were in close contact with potentially weakened patients, and 51,2% in SFAR meeting. 73% of SA carriers identified during the SFHH meeting reported to be potentially in contact with patients at risk for MRSA acquisition, but they were not in charge of patients' care.
Conclusion: One third of volunteer physicians, essentially working closely with compromised patients, was positive for SA in a nasal screening test. The MRSA rate was small but the targeted decontamination in healthcare workers should be debated, due to its consequences for patients.

R2389 Presence of methicillin-resistant *Staphylococcus sciuri* in nasal swab specimens obtained from hospitalized patients and healthcare workers

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Although *Staphylococcus sciuri* is only occasionally isolated from humans, less is known about the carriage of this bacterium in humans, especially methicillin-resistant *S. sciuri*. The aim of the present study was to provide the analysis of carriage of methicillin-resistant *S. sciuri* in hospitalized patients and healthcare workers (HCWs).
Nasal swab were taken from 195 hospitalized and 105 HCWs at the Clinical Center of Serbia in Belgrade. Oxidase positive colonies of staphylococci were further identified as *S. sciuri* by BD Phoenix Automated Microbiology System. Methicillin resistance was confirmed by PCR for mecA gene. Susceptibility to antibiotics was performed by disk diffusion method in accordance to the CLSI recommendations. Determination of SCCmec types was done by previously described protocol for mec class and ccr type (Kondo et al., 2007). PFGE was performed as described previously (Bannerman et al., 1995).
Among 195 hospitalized patients and 105 HCWs, 12 (6.1%) and 4 (3.8%) respectively were colonized with methicillin-resistant *S. sciuri*. All 16 isolates (100%) were resistant or intermediate resistant to two or more antibiotics beside β-lactam antibiotics, i.e. they were multidrug resistant. All tested strains were susceptible to trimethoprim/sulfamethoxazole, vancomycin, linezolid, and pristinamycin, while 15 (93.8%) were resistant to gentamicin, 15 (93.8%) to kanamycin, 15 (93.8%) to tobramycin, 1 (6.2%) to erythromycin, 1 (6.2%) to clindamycin and the remaining 15 (93.8%) were intermediate resistant to clindamycin, 2 (12.5%) to ciprofloxacin, 1 (6.2%) was resistant to rifampin and 1 (6.2%) was intermediate resistant to rifampin, 1 (6.2%) to tetracycline, 4 (25%) to chloramphenicol, 1 (6.2%) to mupirocin, and 12 (75%) were intermediate resistant to fusidic acid. PFGE analysis revealed 11

pulsotypes within the population of methicillin resistant *S. sciuri* strains. Isolation of methicillin-resistant *S. sciuri* of the same PFGE cluster B, and E, from different individuals in different hospitals and different wards indicate successfuly spread of this bacterium. All isolated strains had mec type A and ccr type 3.
Carriage of methicillin-resistant *S. sciuri* among hospitalized patients and HCWs was determined to be surprisingly high, 5%,. Carriage was higher in hospitalized patients, 6.1%, than in HCWs, 3.8%. Isolated methicillin-resistant *S. sciuri* strains has different genotypic and phenotipyc characteristics.

R2390 Characterisation of *Staphylococcus* spp. isolated from wounds

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Objectives: Identify the presence of *Staphylococcus* spp in wounds of patients attended primary care units, in the city of Botucatu, the characterize the staphylococcal cassette chromosome mec (SCCmec).
Methods: This study included 103 isolates from 86 patients treated between March to November 2010. The samples were submitted for identification and detection of resistance by the diffusion method with oxacillin, cefoxitin, penicillin, levofloxacin, clindamycin, erythromycin, gentamicin, sulfametazol/trimethoprim, tigecycline, fusidic acid, quinupristin/dalfopristin, linezolid and vancomycin and the characterization of SCCmec by multiplex PCR in samples with mecA gene.
Results: Of the 103 samples studied, 61 (59.2%) were identified as *Staphylococcus* spp, these 41 (67.2%) were identified as *S. aureus* and 20 (32.8%) were identified as coagulase-negative staphylococci (CNS). The mecA gene was found in four (9.7%) isolates of *S. aureus* and 12 (60%) isolates of CNS. The characterization of SCCmec revealed for *S. aureus* 1 (25%) sample type 3 and 3 (75%) type II and to CNS, 8 (66.6%) samples were characterized as type III, 4 (33.3%) samples as type IV. The drug sensitivity test disk showed only 3 (5%) strains were sensitive to all drugs, 25 (41%) were resistant to only one drug, 12 (19.7%) strains were resistant to two drugs, 8 (13.1%) were resistant to three drugs and 13 (21.3%) strains were resistant to more than four drugs. All strains of CNS resistant to more than four drugs were positive to gene mec A, and two samples carrying the gene were sensitive to the oxacillin disc diffusion method, revealing heteroresistance.
Conclusion: The results from this study shows a high number of *Staphylococcus* resistant to oxacillin, with a predominance of staphylococcal cassette chromosome type III in CNS and type II in *S. aureus*, common in hospital strains and inserted in the community explained by being chronic wounds and patients with a history of multiple hospitalizations over of life. There were great similarities between strains of *Staphylococcus* found and the sensitivity of the drugs tested, when comparing samples found in the same basic health unit, which may indicate the presence of a possible clone of *Staphylococcus* in several patients. The results also call attention to the high rate of multidrug resistance found in strains isolated these patients with predominance of SCCmec type III which complicates the treatment these infections.

R2391 Monitoring of antibiotic resistance of *S. aureus* in patients with knee or hip joint replacement in 2007–2009

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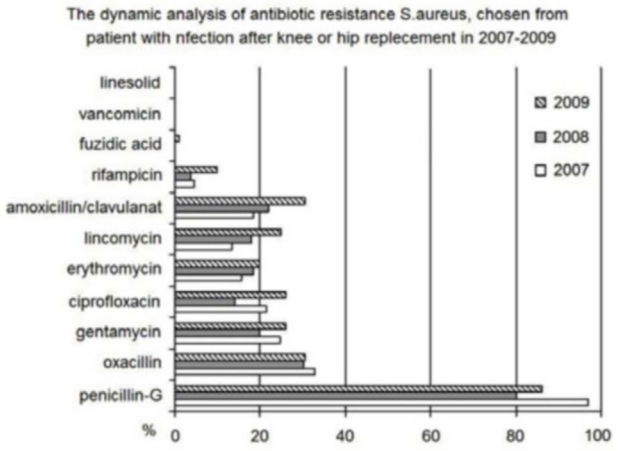
The incidence of an orthopedic implantrelated infection is 1.5% to 2.5% for primary knee or hip replacements and 3.2% to 5.6% for revision surgeries. The essential role in development paraprosthetic joint infections (PJI) belongs to *Staphylococcus aureus* (*S. aureus*). This bacteria has entered the spotlight as a globally pervasive drug-resistant pathogen. When dealing with prosthetic joint infections (PJI) there is often a need to start empirical antibiotic therapy. The constant monitoring of resistance chosen pathogen allows in good time to correct the schemes an antibiotic therapy that brings about increasing of its efficiency. The present prospective study was performed to evaluate the frequency of detection *S. aureus* as etiology factor of PJI and conduct the test of its antibiotic resistance. Methods. We examined the records of 938 isolates is chosen from tissue and fluid samples from 618 patients with PJI from 2007 to 2009. Antibiotic sensitivity *S. aureus* was tested by agar dilution method. Resalt. *S. aureus* was the most common pathogen in PJI during these years. The frequency of *S. aureus* was 42.0%, 38.8% and 38.1% accordingly for 2007, 2008 and 2009 year. The prevalence of methicillin-resistant *S. aureus* (MRSA) almost did not changed (32.8%, 30.3% and 30.4% for each specified year accordingly).
Figure 1 summarizes results of dynamic analysis antibiotic resistance *S. aureus* in PJI. None of these strains was vancomycin and linezolid resistant. More than 90% isolates were sensitive to fuzidic acid, trimethoprim-sulfamethoxazole, rifampicin and fosfomycin. Resistance rates of *S. aureus* to the antibacterial agents, respectively, were as follows: less than 20% isolates were resistant to erythromycin, lincomycin, more than 20% to ciprofloxacin, gentamycin, amoxicillin/clavulanate, The highest resistance rate of *S. aureus* was detected to to penicillin-G (more than 80%). Ciprofloxacin, lincomycin, amoxicillin/clavulanate, rifampicin show gradual reduction of activity against *S. aureus*.
Conclusions: *S. aureus* account for about 40% of cases PJI. MRSA was cultured in 1/3 of cases with no relevant difference whithin years. Vancomycin and linezolid are 100% effective against *S. aureus* and could be used for empirical therapy for orthopedic implantrelated infection in acute cases. The β-lactam antibiotics, fluoroquinolones and gentamycin can be used for staphylococcus infection therapy only in case of previously confirmed isolate sensitivity.



Epidemiology of MDR-Gram-negatives

R2392 Detection of ESBL-producing Enterobacteriaceae during 2008–2010, in Aveiro, Portugal

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Objectives: The emerging changes in the resistance phenotype of ESBL-producing Enterobacteriaceae are becoming a serious problem. The aim of the present study was to screen for ESBL-producers among *Escherichia coli* and *Klebsiella pneumoniae* isolates, recovered from patients in Hospital Infante D. Pedro, Aveiro-Portugal.
Methods: From January 2008 until December 2010 *E. coli* and *K. pneumoniae* ESBL-producing isolates were collected in the Hospital Infante D. Pedro, EPE, central Portugal. The identification and susceptibility profile of the isolates were performed with the Vitek2 system (according to CLSI guidelines) and Advanced Expert System (VITEK 2 AES) (BioMérieux, Marcy L'Etoile, France). ESBL producers were confirmed by Etest® (AB Biodisk) ESBL with Cefotaxime/Cefotaxime + Clavulanic acid and Ceftazidime/Ceftazidime + Clavulanic acid strips, according to

manufacturer's instructions. PCR, nucleotide sequencing and sequence analysis were employed to identify β-lactamases.
Results: From a total of 15843 clinical isolates, 222 *Escherichia coli* and 263 *Klebsiella pneumoniae*, with an ESBL phenotype were detected. The isolates were collected mainly from the urinary tract, blood and bronchial secretions. The Advanced Expert System identified 108 isolates in 2008, 175 in 2009 and 202 isolates in 2010 with ESBL-positive phenotype. 483 bacterial isolates were further investigated. The PCR revealed that CTX-M group was the most prevalent in both species.
Conclusions: The presence of ESBL in clinical specimens is becoming common in our region, however there were two tendencies occurring among the Enterobacteriaceae studied. The occurrence of ESBL-producing *K. pneumoniae* in our hospital has increased from approximately 20% (1:5) in 2008 to more than 50% (1:2) in 2010. On the other hand, the occurrence of ESBL-producing *E. coli* has decreased in the last year. Nevertheless the percentage of producing isolates is still considerable and requires surveillance. CTX-M is the dominating enzyme group in both *E. coli* and *K. pneumoniae*. The presence of ESBL's among the isolates highlights the importance of routine detection of ESBL producers. CTX-M-group is plasmid mediated and, therefore, can represent a dissemination problem, as these genes can be easily mobilised between strains or even species.

R2393 Incidence of ESBL-producing *E. coli* and *K. pneumoniae* strains from 2006 to 2010 in two German teaching hospitals

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Objectives: ESBL producing *E. coli* and *K. pneumoniae* strains are a serious problem as these strains often leave little therapeutic options especially in intensive care units. We analysed these strain in two big teaching hospitals in Germany to see whether their number is still increasing.
Methods: The two laboratories participate in the network using the automated BD PHOENIX-systems measuring MICs. The BD EPICENTER Data-Management-System is used for the evaluation of the data in the laboratory and for the transfer of the data for joint analysis. Copy strains are excluded. Quality control assays are routinely performed. For this study we analysed the ESBL production in all *E. coli* and *K. pneumoniae* strains isolated in a period from 2006 to 2010 with the associated BD-expert system, as the Phoenix shows excellent performance in ESBL detection (Maurine A. Leverstein-van Hall, et al.: J Clin Microbiol. 2002 October; 40(10): 3703).
Results: We looked at more than 34000 *E. coli* and *K. pneumoniae* strains for ESBL production (see table). With about 12% ESBL producing strains the incidence in *K. pneumoniae* strains was nearly identical in both laboratories. Two % higher were the ESBL producing strains in *E. coli* UTI isolates of laboratory B as compared to laboratory M.
We observed a steady increase in the number of ESBL producing strains in *E. coli* (6 to 15%) and *K. pneumoniae* (8 to 21%) from 2006 to 2009 only. In 2010 however the incidence decreased for 3% in *E. coli* and *K. pneumoniae*. The selected blood isolates for *E. coli* showed a permanent increase on a lower level until 2010 (3 to 9%).
Conclusion: As far as the incidence of ESBL producing strains is concernd, differences in both laboratories were negligible for *K. pneumoniae*. The incidence of ESBL producing *E. coli* strains was slightly higher in Lab B. *E. coli* blood isolates showed a lower rate of ESBL producing strains. The data might imply that we already changed the trend of increase, what needs careful observation.

		ESBL producing strains					
	number of strains	all strains	Lab B all strains	Lab M all strains	Lab B urine	Lab M urine	Lab B blood
E.coli	27211	9,26%	9,63%	8,56%	10,25%	8,39%	5,34%
K.pneumoniae	6791	12,18%	12,27%	12,01%	12,27%	12,01%	nd

R2394 Antimicrobial resistance in *Escherichia coli* and *Klebsiella pneumoniae* from Singapore: a proliferation of plasmids

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Objectives: This study was conducted to survey antimicrobial resistance trends and the distribution of resistance genes in *Escherichia coli* and *Klebsiella pneumoniae* isolates from Singapore.
Methods: Antimicrobial susceptibility data were collected from microbiology laboratories of five participating healthcare institutions. Categorical susceptibility was analysed by WHONET software, and converted into incidence-density rates based on inpatient-days. For detailed bacteriological testing, a small subset of bacterial isolates were collected from 2007–2009. Minimum inhibitory concentrations to multiple antibiotics were determined by microbroth dilution. Phenotypic screening in Enterobacteriaceae for extended-spectrum β-lactamases (ESBL), AmpC enzymes and metallo-β-lactamase enzymes was performed by disc diffusion. The genotypic distribution of ESBL, plasmid-borne ampC genes and plasmid-borne qnr genes (quinolone resistance) was determined by multiplex PCR. Clonal typing on a subset of *E. coli* and *K. pneumoniae* isolates was performed by pulsed-field gel electrophoresis (PFGE).
Results: Routine laboratory data demonstrated rising incidence-density of ceftriaxone and quinolone resistance in *E. coli*, but falling incidence-density for *K. pneumoniae*. Detailed susceptibility testing demonstrated that ESBL genes were present in 23% of *E. coli*, predominantly CTX-M (91%) and TEM (17%). The corresponding ESBL rate was 30% in *K. pneumoniae* isolates, predominantly CTX-M (82%) and SHV (13%) genes. Plasmid borne ampC genes were present in 8% and 5% of *E. coli* and *K. pneumoniae* respectively, with DHA-like and CMY-like genes detected. Plasmid-mediated qnr genes were detected in 10% and 23% of *E. coli* and *K. pneumoniae*, respectively. PFGE typing showed greater clonal diversity in ceftriaxone resistant isolates of *E. coli*, but some limited clonal clustering for ceftriaxone-resistant *K. pneumoniae* isolates.
Conclusion: Antimicrobial resistance to quinolones and third-generation cephalosporins was high in *E. coli* and *K. pneumoniae* isolates, with a high level of plasmid-mediated resistance genes. Resistance trends to quinolones and cephalosporins showed divergent results for *E. coli* as compared to *K. pneumoniae*.

R2395 Prevalence study of clinically significant Gram-negative pathogens depending on the consumption of antibiotics in two large cancer hospitals in Moscow, Russia

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Objective: To study prevalence of multidrug- (MDR) and pandrug-resistant (PDR) *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, and ESBL-producing *Klebsiella pneumoniae* and *E. coli* and to correlate the data with the consumption with cephalosporins (CEPH) and carbapenems in NN Blokhin Cancer Research Center (CRC) of Russia and Moscow Cancer City Hospital no. 62 (MCCH).
Methods: CRC has 1.120 beds vs 600 beds in MCCH. From 01.01.till 01.12.2010 (11 months) 17,320 and 19,548 cancer patients were treated in CRC and MCCH, and total numbers of bed-days were 349,654 and 184,218, respectively. Average number of bed-days per patient was 20,2 vs 13,6 in CRC and MCCH, and average number of pretreatment bed-days per patient was 6,4 vs 3,7 days, respectively. In both clinics identification and susceptibility testing was performed with VITEK-2 System.
Results: A total of 2,512 bacterial and fungal strains in CRC and 1,696 strains in MCCH were isolated. *P. aeruginosa* accounted for 10.3% (259/2512) in CRC vs 6.1% (103/1696) in MCCH ($p < 0.0001$). MDR-strains of *P. aeruginosa* were 23.2% (60/259) in CRC vs 11.6% (12/103) in MCCH ($p < 0.001$). PDR *P. aeruginosa* accounted for 49.4% (128/259) in the CRC vs 24.2% (25/103) in MCCH ($p < 0.0001$).

A. baumannii accounted for 8.8% (221/2512) in CRC vs 2.0% (34/1696) in MCCH ($p < 0.0001$) and MDR *A. baumannii* (sensitive only to colistin) accounted for 77.8% (172/221) in CRC vs 41.2% (14/51) in MCCH ($p < 0.0001$). The number of ESBL-producing *K. pneumoniae* accounted for 62.1% (74/119) versus 69.7% (76/104) ($p > 0.05$) and *E. coli* (ESBL) was 37.0% (94/254) vs 26.8% (39/145) ($p < 0.002$), respectively. The ratio of aforementioned strains to the number of patients in CRC was 2–4 times higher than in MCCH (except *K. pneumoniae*).
The consumption with carbapenems and 4th-generation CEPH was significantly higher in CRC: 13.9 vs. 0.8 DDDs/1000 patient-days (PD) for carbapenems; 55.4 vs. 8.6 DDDs/1000 PD for cefepime. On the contrary, consumption with 3rd-generation CEPH was higher in MCCH: 5.9 vs. 18.9 DDDs/1000 PD for ceftriaxone; 0 vs. 6.7 DDDs/1000 PD for ceftazidime. Total consumption with antipseudomonal CEPH was 55.4 vs. 15.3 DDDs/1000 PD, respectively.
Conclusions: The rate of MDR and PDR *P. aeruginosa* and *A. baumannii* (isolated from patients) was higher in CRC associated with the higher average number of bed-days and pretreatment bed-days and massive consumption with antipseudomonal antibiotics, including carbapenems. In MCCH ESBL-producers are the main problem.

R2396 Multi-resistant Enterobacteriaceae in a private hospital in London: mechanisms of resistance and epidemiology

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Objectives: The London Clinic delivers care to a mixed clientele of UK and International patients. It has an active Intensive Care Unit (ICU) and Oncology Unit with some stem cell but no solid organ transplantation. Observing a number of significant infections with Enterobacteriaceae sensitive only to amikacin and carbapenems, we prospectively examined first isolates from 24 patients collected over 8 months in 2009. We were concerned about the risk of cross infection and the frequency of such isolates and whether empirical therapy of febrile patients should be with a carbapenem rather than the more conventional piperacillin-tazobactam, ceftazidime or ciprofloxacin with or without gentamicin.
Methods: The 24 isolates were identified to species level and underwent detailed susceptibility testing using disc diffusion, agar dilution MIC determination and automated "Walkaway Microscan™" methods. PCR was then used to detect the presence of CTX-M groups, and *Escherichia coli* isolates underwent PCR for phylogrouping.
Results: Samples from outpatients, inpatients, and ICU grew 11 *Escherichia coli*, 9 *Klebsiella* spp. and 4 *Enterobacter cloacae* isolates. 33% of the isolates were from urine, 33% from wounds and sputa, 21% from ICU screening swabs and 13% from blood cultures. 11/24 isolates (3 *E. coli*, 5 *Klebsiella* spp, 3 *E. cloacae*) were from patients with contact with ICU. The patients from whom the 24 strains were isolated originated from the Middle East (13), UK (9) and Nigeria (2). The average interval from admission to detecting the first significant isolate was between 0 and 85 (mean 13, median 2 days). PCR showed that 20 of the 24 isolates belonged to CTX-M group 1, potentially CTX-M-15s by inference of the MICs. Care in ICU was a significant risk factor for the finding of CTX-M ESBL producing pathogens ($p < 0.05$). Phylogrouping of the 11 *E. coli* strains revealed 2 group A, 6 group B1, 3 pathogenic group D, and no pathogenic B2 strains.
Conclusions: MICs, PCR and phylogrouping of the 24 isolates suggest that despite the profile of sensitivity being consistently to only amikacin and carbapenem, that this profile was created by expression of heterogeneous resistance mechanisms, which would preclude concerns about cross infection. Further work to characterise the CTX-M group 1 ESBL cluster and molecular environment of these genes could clarify the resistance mechanisms and origins of these strains.

R2397 Colonisation and infection with multidrug-resistant Enterobacteriaceae in a neonatal intensive care unit: a surveillance study

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Objectives: Infections from Gram-negative bacteria are responsible for considerable morbidity and mortality in neonatal intensive care units (NICUs).
Surveillance study of multidrug-resistant (MDR) Enterobacteriaceae was performed in a 30-bed, university-affiliated, level III-IV NICU of a tertiary paediatric hospital in Athens, Greece.
Methods: All neonates, admitted to the NICU during Dec 2007–May 2009, with no MDR pathogens in their bacterial flora upon admission were included. Surveillance cultures for the detection of MDR bacteria consisted of throat and rectal swabs, taken upon admission and weekly thereafter until discharge. Culture of samples and identification of organisms were made by standard methods. Antimicrobial susceptibilities of isolates and phenotypic detection of ESBLs were determined according to the CLSI guidelines.
Results: The study included 493 neonates. During hospitalization in the NICU, 27 neonates (5.5%) were colonized with ESBL-positive *Escherichia coli*, 32 (6.5%) with ESBL-positive *Klebsiella pneumoniae*, 16 (3.2%) with ESBL-positive *Enterobacter cloacae* and 19 (3.9%) with AmpC-hyperproducing *E. cloacae*. Duration of hospitalization prior to colonization with the above pathogens was 7–111 days (median 31 d), 7–104 d (median 23 d), 6–57 d (median 16 d) and 5–248 d (median 17 d), respectively. Analysis of monthly incidence revealed: 1) a minor epidemic with ESBL-producing *E. coli* in Jan 2008 ($n = 11$); 2) a cluster of cases with ESBL-producing *K. pneumoniae* during Feb–Mar 2008 ($n = 7$) and a probable epidemic in Feb–May 2009 ($n = 10$); 3) a minor epidemic with ESBL-producing *E. cloacae* between Dec 2007 and Feb 2008 ($n = 10$); 4) a cluster of cases with AmpC-hyperproducing *E. cloacae* in Feb 2009 ($n = 5$). In total, seven episodes of invasive infection occurred (one due to ESBL-producing *E. coli* and six due to ESBL-producing *K. pneumoniae*).
Conclusion: Regular surveillance of colonization/infection with MRD pathogens permits prompt implementation of infection control measures, targeted at minimization of spread and avoidance of epidemics.

R2398 Outbreak of *Enterobacter cloacae* ESBL(+) colonisation in a neonatal intensive care unit in Greece

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Objectives: *Enterobacter cloacae* has emerged as an important nosocomial pathogen in neonatal units. The aim of this study was to investigate a massive colonization of neonates with ESBL producing *E. cloacae* in a 30-bed, university-affiliated, level II-IV Neonatal Intensive Care Unit (NICU) at a large pediatric hospital in Athens.
Methods: Routine surveillance cultures for the detection of multidrug resistant bacteria at the throat and rectum of the neonates at admission and weekly until discharge is a standard practice in our NICU. Environmental samples are collected during suspected outbreaks. Clinical and environmental samples are processed according to classic microbiologic methods and the susceptibility to antimicrobials are tested according to the CLSI guidelines. ESBLs are detected by phenotypic methods. Molecular typing of isolates is determined by PFGE after digestion of genomic DNA with XbaI.
Results: On August 2010 seven neonates were found colonized with ESBL producing *E. cloacae* as opposed to only 5 neonates found colonized during their NICU stay (NICU-acquired cases), and one neonate colonized upon admission (referred case) during the last 12 months. A nosocomial outbreak due to ESBL(+) *E. cloacae* was then highly suspected, and a one-day survey was performed on 2/9/2010. Twenty out of 26 neonates were found colonized with ESBL(+) *E. cloacae* reported. Environmental samples were all found negative.

Three neonates developed bacteremia due to ESBL(+) *E. cloacae* during the study period, but only one, suffering from serious underlying conditions died.
PFGE analysis of 10 epidemic strains plus 4 epidemiologically unrelated *E. cloacae* isolates proven >85% similarity among the epidemic strains, a fact consistent with nosocomial spread.
Strict infection control measures, such as hand hygiene, patient cohorting and shuting down the NICU for new admissions, were established. Continuous surveillance over the next weeks showed a steady decline in the number of colonized neonates, and on 16/11/2010 only 2 out of 18 hospitalized were found colonized.
Conclusion: Continuous surveillance was an important tool in early identifying the outbreak, whereas the implementation of proper infection control measures resulted in containment of the outbreak in a short period of time.

R2399 First identification of KPC-2 producing *Klebsiella pneumoniae* in the Czech Republic and in vivo selection of colistin resistance during the therapy

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Objectives: The occurrence of carbapenemase producing Gram-negative bacteria is of high clinical interest as they can hydrolyze most β-lactam agents including carbapenems recognised as antibiotics of choice in ESBL or acquired AmpC producers usually resistant to other antibiotics. The resistance to carbapenems, generally very rare in *Klebsiella pneumoniae* strains in the Czech Republic, is predominantly caused by an alteration of the cell wall, together with a production of extended-spectrum and/or AmpC β-lactamases. At the end of 2009, the first putative KPC-producing isolates of *K. pneumoniae* was submitted for confirmation to the National Reference Laboratory for Antibiotics of the National Institute of Public Health in Prague. The isolates were obtained from one patient.
Methods: MICs of 24 antibiotics were determined according to EUCAST recommendation. β-lactamases were identified by isoelectric focusing followed by PCR and sequencing of β-lactamase genes. The gene environment of blaKPC-2 was determined by PCR mapping and sequencing of the amplicons. Isolates were compared by PFGE after the restriction with XbaI endonuclease and multilocus sequence typing (MLST).
Results: The isolates of *K. pneumoniae* (ST258) were obtained from a wound swab, decubitus and urine from patient formerly hospitalized in a Greek hospital. The carbapenemase was identified as a KPC-2 with a gene located on Tn4401a traspozon variant. The resistance to colistin developed after 20-days long therapy with this drug, but after this single isolation, the KPC-2 producing strain disappeared.
Conclusion: This is the first description of the KPC-producing *K. pneumoniae* in the Czech Republic in patient with a travel history in Greece – the country with an endemic KPC. Despite the history of travel to the countries with the endemic KPC situation (Greece, USA, Israel) no import of KPC strain to the Czech Republic was noticed during past years.
This work reported here has been supported by the research project grants NS9717–4/2008 and NT11032–6/2010.

R2400 Nosocomial outbreak of *Pseudomonas aeruginosa* in adult inpatients: multidrug- vs. non-multidrug-resistant strains

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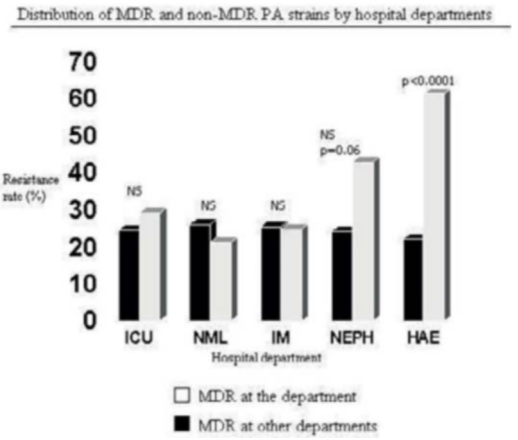
Objective: *Pseudomonas aeruginosa* (PA) is an emerging opportunistic pathogen that can easily exhibit multidrug-resistance (MDR). It is intrinsically resistant to many antibiotic classes and can readily acquire new mechanisms. Higher rates of death, prolonged hospitalization and rising costs have been associated with MDR-PA. Comparison of MDR rates among institutions is not always possible because there is no agreement to define MDR in PA. We use here a modified MDR criterion from Paterson et al. to make it more operational. The aim of this study was to compare epidemiological features between MDR and non-MDR-PA infection rates within an outbreak at a University Hospital in Madrid.
Methods: An observational retrospective study of 822 PA isolates from 369 adult inpatients was conducted for one year. All kind of samples were obtained. Colonization and infection were not distinguished. We studied 10 drugs: ceftazidime (CAZ), cefepime (CPE), piperacillin-tazobactam (TZP), imipenem (IMI), meropenem (MER), ciprofloxacin (CIP), gentamycin (GEN), tobramycin (TOB), amikacin (AKA) and colistin (COL). Susceptibility was tested by a broth microdilution method, using CLSI breakpoints to interpret. MDR was defined as a decreased susceptibility to two or more of the six following items: (1) CPE and/or CAZ; (2) IMI and/or MER; (3) TZP; (4) CIP; (5) AKA and/or [GEN and TOB]; (6) COL. Qualitative variables are expressed as proportions, compared using Chi-square test and quantified by odds-ratio (OR) with 95% confidence. P-values lower than 0.05 were considered significant.
Results: Globally, 296 (36.0%) isolates were considered MDR according to the MDR criterion. Among first isolates, 95 (25.7%) were MDR. No significant difference in gender was found between MDR and non-MDR-PA ($p = 0.17$). An increased rate of MDR-PA was shown in patients under 70 years (mainly in the 5th decade, $p = 0.03$). PA isolates were specially high at ICU, Neumology, Internal Medicine, Haematology and Nephrology departments, but only Haematology dpt. (OR = 5.55 [2.43–12.81], $p < 0.0001$) showed a statistically significant difference of MDR over the rest.
Conclusions: (1) A very high rate of MDR-PA isolates was found within the outbreak. (2) Although PA isolation rate is higher in males, no difference has been found between MDR and non-MDR. (3) Among patients infected by PA, young ones have a higher likelihood of being infected by a MDR-PA than older. (4) Haematology dpt. had the highest rate of MDR strains.



R2401 Genetic dynamics of the emerging *Salmonella enterica* serotype 4,5,12:i- (monophasic variant of *S. typhimurium*) in Portugal

P. Antunes, J. Mourao, L. Dias, L. Ferreira, L. Peixe (Porto, PT)*

Objectives: A marked increase in the prevalence of the serotype 4,5,12:i-, a monophasic variant of S.Typhimurium, has been noted worldwide in recent years. Our goal was to assess clonal relationships and to characterize antibiotic and/or biocide resistance in Portuguese isolates from different sources.
Methods: We studied 132 isolates (2002–2010) from humans (n = 115), food (n = 9), environment (n = 4) and piggeries (n = 4). The serotype was confirmed by PCR (fljA, fljB, hin and fljA-fljB). Antibiotic susceptibility to 10 antibiotics was tested by disk diffusion method (CLSI). Characterization of antibiotic and biocide resistance genes and

class 1 integrons were done by PCR, RFLP and sequencing. Clonality was established by MLST in representative isolates.
Results: All but 2 isolates (99%) were resistant to antibiotics of which 94% were multidrug-resistant (MDR, 2–8 antibiotics). They expressed resistance to sulfametoxazole (Su, 92%; sul1, sul2 and/or sul3), tetracycline (T, 91%; tetA and/or tetB), streptomycin (S, 88%; aadA and/or strA-strB), ampicillin (A, 67%; blaTEM), chloramphenicol (C, 45%; cmlA1 and/or floR), trimethoprim (Tr, 35%; dfrA12), gentamicin (G, 27%; aac(3)-IV), nalidixic acid (4%) or kanamicin (3%). Three major groups of isolates mostly associated with 3 genotypes could be identified: (i) ASSuT type (n = 48; blaTEM, strA-strB, sul2 and tetB), also carrying the pcoD copper efflux gene and assigned to the ST34 (SLV of ST19); (ii) MDR (3–8 antibiotics) type (n = 45), being more frequent the phenotype ACGSSuTTr (n = 27; blaTEM, cmlA1, aac(3)-IV, aadA, sul1-sul2-sul3, tetA and dfrA12;) carrying an unusual class 1 integron with estX-psp-aadA2-cmlA1-aadA1 associated to qacH, IS440 and sul3, and in addition a class 1 integron of 2000 bp (dfrA12-orfX-aadA2) or an empty one with qacEdelta1 and sul1 in the 3′CS, and belonging to the worldwide spread ST19 and DT104/U302 phagetype. (iii) CSSuTTr type (n = 15; cmlA1, aadA, sul3, tetB and dfrA12), carrying a class 1 integron with the dfrA12-orfF-aadA2-cmlA1-aadA1 gene cassettes associated to an unusual 3′ sequence region with qacH, IS440 and sul3 and belonging to ST19.
Conclusions: This is the first study characterising the *S. enterica* serotype 4,5,12:i- in isolates from Portugal. The spread of three MDR genotypes belonging to worldwide spread clonal lineages in association with biocide resistance determinants (pcoD, qacEdelta1 and/or qacH) might account for the recent emergence and success of this serotype.

R2402 Efficacy of fosfomycin in the extended-spectrum β-lactamase producing Enterobacteriaceae infections

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Objectives: To document fosfomycin susceptibility compared with other antibiotics used for urinary tract infections caused by extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella* spp.
Methods: A retrospective study of ESBL-producing *E. coli* and *Klebsiella* spp strains isolated from clinical samples of patients attended in the Hospital Universitario Miguel Servet (hospitalized and outpatients) over a five-year period (2005 to 2009) was performed.
Isolates were identified and tested for antibiotic susceptibility by microdilution system (MicroScan Walkaway® Siemens). ESBL production was confirmed by the double-disk diffusion method according to CLSI standards.
Results: Antibiotic susceptibility against fosfomycin was tested in 516 strains of *E. coli*, 47 of *K. pneumoniae* and 7 of *K. oxytoca*.
95.54% of *E. coli* ESBL-producing isolates were susceptible to fosfomycin. Similarly, 68.08% of *K. pneumoniae* isolates and 100% of *K. oxytoca* isolates were susceptible to fosfomycin.
Gentamicin susceptibility data was 88.57% of *E. coli* isolates, 39.21% of *K. pneumoniae* and 60% of *K. oxytoca* isolates. Most of the strains were ciprofloxacin resistant (80.81% of *E. coli* isolates, 77.27% of *K. pneumoniae* and 36.84% of *K. oxytoca*).
Cotrimoxazole shows similar resistance in the three species, 55.86%, 60% and 44.44% in *E. coli*, *K. pneumoniae* and *K. oxytoca* isolates respectively.
Conclusions: The high susceptibility rates and the absence of variation during the period study, indicates that fosfomycin can be considered an important oral treatment option in urinary tract infections by ESLB-producing strains.

R2403 Molecular characterisation of blaVIM-2-producing *Pseudomonas aeruginosa* clinical isolates from Portugal

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Objectives: *Pseudomonas aeruginosa* is an important etiological agent of nosocomial infections frequently exhibiting multidrug resistance. Metallo-β-lactamase (MBL)-producing strains are emerging worldwide, with VIM-2 exhibiting particular relevance in Europe. Dispersion of integrons carrying blaVIM-2, clonal spreading and the association of MBL genes with transposons may contribute to the observed dissemination rates. Our aim was to analyze the population structure of blaVIM-2-carrying *P. aeruginosa* clinical isolates.
Methods: From our collection of carbapenem-resistant *P. aeruginosa* isolates (2005–2008), derived from five hospitals in North and Centre of Portugal, all blaVIM-2-producing *P. aeruginosa* isolates were characterized (n = 12). Antibiotic susceptibility was determined by the agar diffusion method and E-test (CLSI). Class 1 integrons were characterized by PCR and sequencing. Clonal relatedness was studied by PFGE (XbaI and SpeI) and MLST (http://pubmlst.org/paeruginosa).
Results: All *P. aeruginosa* isolates showed a multidrug-resistant phenotype including resistance to imipenem (MIC >32 mg/L), aminoglycosides and ciprofloxacin. They carried blaVIM-2 as a gene cassette inserted in 5 different class 1 integrons: In56, In58, In100, In102 (ORF11, aacA4, aacC1, blaVIM-2) and In103 (aacA7, blaVIM-2, aacA4). In58 was the most frequent, found in 8 isolates. blaVIM-2 gene was found to be chromosomal and/or plasmid located.
All isolates were clonally unrelated corresponding to 12 PFGE types. On the other hand, 8 different sequence types (STs) were found: ST111 (n = 2), ST175 (n = 1), ST179 (n = 3), ST235 (n = 1), ST253 (n = 2), ST260 (n = 1), ST282 (n = 1), and ST815 (n = 1). No close relationships (single or double locus variants) were observed among these STs. Most blaVIM-2 producing strains were assigned to widespread STs as ST111, ST179 or ST235, which is the founder of the international Clonal Complex 11 containing MBL isolates.
Conclusions: This is the first report characterizing the population structure of VIM-2-producing *P. aeruginosa* isolates in Portugal. Our data indicate that these isolates belong to an extremely polyclonal population with no apparent common ancestry. Further studies are imperative to follow-up the relevance of specific clones in the dissemination of blaVIM2 carrying isolates.

R2404 Clinico-epidemiological characteristics of tularaemia cases: a tertiary hospital experience, Turkey

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Francisella (F.) tularensis, Gram-negative, aerobic, facultative intracellular bacterium have been distinguished to date: *F. tularensis* subsp. *tularensis*, *holarctica*, *mediasiatica* and *novicida*. *F. tularensis* has been detected in more than 200 animal species, with rabbits, hares, small rodents (e.g. voles, field mice) and semi-aquatic animals etc. Tularemia is a re-emerging disease in our country recently.
In here, we evaluated the clinico-epidemiological characteristics of our patients with tularemia.
Method: Prospective review of clinical records of patients diagnosed with tularemia admitted to our hospital in Ankara, from January to December 2010.
Case definition: Patient with suggestive clinical course and epidemiology (coming from the epizootic area) and positive serology (antibodies to *Francisella tularensis* >1/160).
Results: 22 patients (13males, 9 females) with a mean age of 41 years were included to the study. Nine patients were living in endemic areas of turkey and thirteen patients had travelled to the endemic areas. Among 21 cases, contaminated food or water were the most commonly noted exposures (ten patients were consumed spring water), and one had tick bite exposures Thirteen patient reported being exposed to a rat which lived around patients houses. The clinical symptoms period of patients ranged from 10 to 45 days. The final diagnosis of 20 patients was glandular tularemia, whereas two of them were oculoglandular tularemia. 4 had suppurated lymph nodes. Fourteen of the 22 patients were hospitalized (median duration: 4 days [range: 1–27 days]). History of antibiotic use was available for all patients. 18 of patients had been treated with a β-lactam or β-lactam/β-lactamase inhibitors antibiotics those are considered ineffective against tularemia.

Among 3 patients initially treated with streptomycin monotherapy and one of the patient was treated with streptomycin and ciprofloxacin combination. Epidemiological and clinical findings of patients were summarized in Table 1.

Conclusions: Tularaemia should be considered in the differential diagnosis of patients with fever, pharyngitis, conjunctivitis, and cervical lymphadenopathies. Hence, early diagnosis and treatment of tularaemia are important, every clinicians must be aware of diseases.

	Number (n)	Percent (%)
Gender(M/F)	13/9	
Age	41.6±16.2	
Fever	20	91
Sore throat	19	86
Headache	16	73
Chills	15	68
Lymphadenitis	14	64
Myalgia	14	64
Abdominal pain	13	59
Conjunctivitis	12	55
Weight loss	11	50
Loss of apetite	9	41
Similar symptoms in family	4	18
Environmental exposure	10	46
History of inappropriate antibiotic use	21	95

R2405 Multidrug-resistant *Acinetobacter baumannii*: study of clonally related isolates spread in an Italian single center institution over a four-year period (2007–2010)

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Objectives: *Acinetobacter baumannii* (Ab) is an opportunistic pathogen associated with severe hospital infections. In order to better control Ab spread, it's necessary to assess the multidrug resistance and implement measures to prevent the transmission in the critical divisions.

The aim was to control Multidrug-Resistant (MDR) Ab spread in S. Donato Hospital, Northern Italy. We investigated the epidemiology of MDR Ab clinical isolates, with a focus on the mechanisms of carbapenem and aminoglycoside resistance.

Materials and Methods: 106 non replicate Ab strains were collected between January 2007 and December 2010 from different samples; Ab strains identification and their susceptibility were performed by Vitek2 Compact automated system. Molecular identification at the species level was performed by PCR amplification of blaOXA-51-like allele. Susceptibility to imipenem (IP) was tested by standard disk diffusion method, also in presence of EDTA, and by Etest MBL in according to CLSI 2010 breakpoints. PCR analysis was performed for detection of resistance gene and genotyping was carried out by multiplex PCR and PFGE.

Results: Ab strains distribution in the wards was: 32.7% in Post-Surgery Intensive Care Unit, 30.6% in General Intensive Care and Stroke Unit, 10.2% in Pneumology, 8.2% in Cardiac Surgery, 8.2% in General Surgery, 6.1% in Oncology/Medicine, 2% in Endocrinology and 2% in Urology. All strains resulted resistant to cefotaxime, ceftazidime, cefepime, amikacin, gentamicin, tobramycin, levofloxacin, ciprofloxacin, IP and meropenem (MP). 49/106 were found to be carbapenem resistant. Isolates, showing amikacin MIC values ranging 2–8 μg/ml, displayed a double growth alone in disk diffusion test and exhibited heteroresistance to amikacin by Etest. IP and MP MICs were >16 μg/ml; all Ab strains were susceptible to colistin. OXA-23 carbapenemase and ArmA methylase production were the main mechanisms responsible of carbapenem and aminoglycoside resistance. The Ab strains belonged to a single clone and were related to pan-European clone II (RUH134). Multiplex-PCR showed that isolates belonged to the sequence type Group 1, related to European clone II.

Conclusion: Our data show that the spread of MDR Ab is becoming a substantial problem in our hospital; moreover, the results emphasize the need to adopt surveillance programs to prevent colonization and infection by MDR Ab in the hospital settings.

R2406 Incidence, evolution and antibiotic resistance of multidrug-resistant microorganisms in urinary tract infections: 2000–2009

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Objectives: To determine the epidemiology of urinary tract infections (UTIs) by multiresistant microorganism isolation in hospitalized patients and the community diagnosed at the Hospital Universitario (Albacete, Spain) from 2000 to 2009; to study antimicrobial susceptibility to the drugs commonly used in such infections.

Methods: 292,291 urine samples were processed for aerobic culture by standard methods from January 2000 to December 2009. Identification and antimicrobial sensitivity testing were performed with the commercial Wider® system (Soria Melguizo). The criteria proposed by the CLSI were followed to detect multidrug resistance mechanisms. We considered multidrug-resistant *Pseudomonas aeruginosa* (MRPA) with resistance to 3 or more antibiotics of the following: ceftazidime, carbapenems, quinolones and aminoglycosides; and multiresistant *Acinetobacter baumannii* (MRA) when the isolate was sensitive to only tigecycline and colistin.

Results: Of all the urine samples tested, 51,990 (18.0%) were positive. Of these, (1,240; 2.4%) some MDR microorganism was isolated, 1,102 (88.8%) were extended-spectrum β-lactamase-producing Enterobacteria (ESBL), 84 (6.8%) were meticilin-resistant *Staphylococcus aureus* (MRSA), 28 (2.2%) were MRPA and 26 (2.2%) were MRA. Within the ESBL-producing Enterobacteria we found 996 (90.3%) *Escherichia coli*, 90 (8.1%) *Klebsiella* spp. and 12 (1.0%) *Proteus* spp.

Multidrug-resistant isolates increased from 3 in 2000 to 274 in 2009 in both inpatients and the community. Of the 1,240 total patients, 1,188 (95.8%) were adults and 52 (4.2%) were children; 62.4% were women. The antimicrobial resistance rates of ESBL-producing enterobacteria were: nalidixic acid 77.6%, ciprofloxacin 64.8%, cotrimoxazole 60.0%, gentamicin 22.0%, nitrofurantoin 7.8%, fosfomycin 4.2% and amocillin-clavulanate 27.6%. No carbapenem resistant strains were found.

Conclusions: We observed an increase in the incidence of UTIs through multiresistant pathogens during the study period, both in inpatients and in patients with infections acquired in the community. Among inpatients, the highest incidence corresponded to the Internal Medicine and Geriatric Departments. ESBL-producing Enterobacteria were the most frequently isolated multiresistant organisms. Carbapenems and aminoglycosides could be the best choice for empiric treatement in hospitalized patients. Quinolones and cotrimoxazole should not be recommended due to the high resistance rates observed.

R2407 Frequency of extended-spectrum β-lactamase in *Escherichia coli* and *Klebsiella* spp. and *Proteus* spp. over a five-year period in a university hospital, Spain

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Objectives: To study the prevalence of extended-spectrum β-lactamase-producing (ESBL) *Escherichia coli*, *Klebsiella* spp and *Proteus* spp strains during the period 2005–2009.

Methods: A retrospective study of ESBL-producing *E. coli*, *Klebsiella* spp and *Proteus* spp strains isolated from clinical samples of hospitalized and outpatients attended in the Hospital Universitario Miguel Servet of Zaragoza (Spain) from 2005 to 2009 was performed.

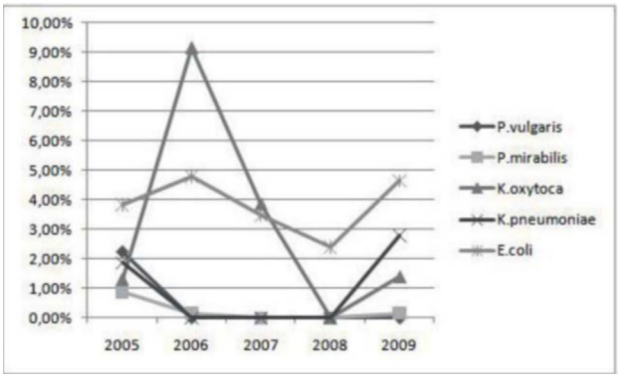
Isolates were identified and tested for antibiotic susceptibility by microdilution system (MicroScan Walkaway® Siemens). ESBL production was confirmed by the double-disk diffusion method according to CLSI standards.

Results: In our study 27887 *E. coli*, 4422 *K. pneumoniae*, 1042 *K. oxytoca*, 1852 *P. mirabilis* and 45 *P. vulgaris* strains were isolated. The prevalence of ESBL-producing Enterobacteriaceae is shown in table 1. ESBL-producing strains were more frequently isolated from

urine samples: 92.94% of *E. coli*, 64.10% of *K. pneumoniae*, 41.86% of *K. oxytoca* and 100% of *Proteus* spp strains.

Conclusions:

1. During 2009 isolation of EBSL-producing *E. coli*. been increased.
2. *E. coli* remains the most frequent isolated species with this characteristic.
3. The punctual increase in 2006 of ESBL *K. oxytoca* was due to an outbreak in an intensive care unit.



R2408 High-throughput DNA sequencing of resistance plasmids: a powerful approach to identify resistance and virulence features

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Introduction: High-throughput DNA sequencing of resistance plasmids allows a very quick determination of plasmid content. By this novel approach it is possible to identify the major characteristics of resistance and virulence and to trace the evolution of specific plasmid families, recognizing their basis for virulence, host range, transferability and stability.

We sequenced plasmids identified in *Klebsiella pneumoniae* (KP) of nosocomial origin that were i) untypable by PCR-Based Replicon Typing (PBRT); ii) relevant being characteristic of epidemic clones.

Materials and Methods: A procedure was devised to simultaneously pyrosequence multiple plasmids by the 454 GS-FLX Titanium, using MID-TAG barcoded primers in large volume emPCR (Roche, Italy). Mobile DNA elements associated with resistance genes, regions implicated in replication and plasmid host ranges, maintenance (toxin-antitoxin, plasmid partitioning and exclusion properties) and transfer were annotated.

Results:

i. The majority of the plasmids carrying blaSHV-12 gene are classified as untypable being negative to the most common replicons of Enterobacteriaceae. One of these plasmids belonged to the novel IncR plasmid family. This plasmid family was characterized by a novel replicase gene that we previously identified in a *Salmonella* Montevideo from The Netherlands associated to the qnrS1 gene (García-Fernández et al. 2009). The novel fully sequenced IncR-SHV-12 plasmid confers resistance to β-lactams and cephalosporines (blaSHV-12, blaTEM-1, blaOXA9), but also to mercuric ions, aminoglycosides (strA-strB, aacA4, aadA1) and sulphonamides and is negative for the conjugative transfer locus, suggesting in trans mobilization mediated by co-resident plasmids.

ii. The entire plasmid content of two KP ST258 strains (one multi-drug resistant and one susceptible) was determined. The resistant strain was positive for the carbapenemase KPC-3 located on plasmid pKpQIL, previously identified in ST258 from Israel (NC_014016). The susceptible ST258 contained virulence-like plasmids of the FIIK type that likely contributed to the successful spread of this clone, independently by the acquisition of antimicrobial resistance genes.

Conclusions: Since plasmids can be considered as "genomes" within the bacterial genome, a fully sequence approach may help to identify the characteristics enhancing the successful spread of specific plasmid families, but also the ability of plasmids to support the host survival and spread.

R2409 Extended virulence profile of major UPEC clones from different origins and geographical locations

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Objectives: Specific UTI-causing *E. coli* clones have been linked to the emergence and spread of AbR genes but the complete virulence profile of these UPEC clones has only been assessed for a few lineages.

Methods: Our collection includes 104 B2-ST131 (n = 29), D-ST393 (n = 12), D-ST69 (n = 10), D-ST405 (n = 11), A-ST10 complex (n = 18), A-ST23 complex (n = 13), B1-ST155 (n = 6) and B1-ST359 (n = 5) isolates, representing isolates from a 19 year period (1991–2010), different origins (62% hospital, 13.6% healthy volunteers, 13.6% animals, 8.7% outpatients and 1% environment) and locations (n = 89, 8 EU countries; n = 4 Korea; n = 11 USA). Of these, n = 46 were UTI-isolates and n = 60 were ESBL/AmpC producers. Clonal relatedness was assessed by PFGE/MLST and phylogroups were identified by PCR. Screening for 33 UPEC virulence factors (VFs) including adhesins, toxins, siderophores, polysaccharide coatings and others (PAI, usp, ibeA) was performed by PCR as described. ExPEC isolates were identified by a previously described criterium.

Results: fimH (90%), iutA (78%) and traT (71%) were found within all STs. The number of VFs varied: ST131 or ST393 (n = 18); ST69, ST405 or ST10 (n = 11–14); ST155 or ST359 (n = 7). Common virulence profiles were established for each UPEC clone rather than for UTI-causing isolates. ExPEC isolates (n = 55, 53%) belonged to all ST393, all ST69 and most ST131, which were enriched in kpsMTII, iutA and iha. ExPEC identified in nearly half ST23 and occasionally other STs had pap alleles (papEF, papGIII or papC) and/or kpsMTII besides iutA. ST393 and ST69 were also significantly enriched in pap alleles. Frequency of usp (53%), sat (55%) and fyuA (58%) was high, though with varible distribution: a) fyuA and sat among D and B2-EC and ST23/ST10, respectively; b) usp among ST131, ST10, ST23 and ST359 (54% of these were CTX-M-14 or CTX-M-15 producers). On the other hand, specific VFs were only found in particular STs: i) PAI (31%) mostly within ST131 and ST405; ii) cvaC (12%) was confined to ST23 complex, ST155 and ST359; iii) ibeA (4%) was only identified in ST131 non-CTX-M-15 producers (TEM-4, TEM-24 or CTX-M-1); iv) sfa/doc (3%) was found in ST155 and v) afa/dra (2%) was detected in ST131 isolates.

Conclusion: Consistent VFs profiles were found within STs, and differences could not be explained by origin, pathogenesis or AbR profile. A and B1 UPEC profile was linked to the presence of fimH (adhesion), iutA (iron uptake), traT (surface exclusion) colV (colicin) and/or usp.

R2410 Outbreak of ertapenem-resistant widespread Enterobacteriaceae clones in a Portuguese hospital

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Objectives: The emergence of carbapenem resistance among ESBL-producing isolates has been increasingly reported worldwide. We aimed to characterize a recent outbreak of ertapenem (ERT)-resistant Enterobacteriaceae isolates from a Portuguese hospital.

Methods: ERT-R (n = 15, MIC = 3–>32 μgml, IMI and MER susceptible) and ERT-S (n = 16, presumptive ESBL-producers) *K. pneumoniae* (KP, n = 21), *E. coli* (EC, n = 7), *E. asburiae* (Eas, n = 1), *E. aerogenes* (Eae, n = 1) and *E. cloacae* (Ecl, n = 1) isolates were studied (March-August 2010). β-lactamase production was evaluated by phenotypic tests, IEF, PCR (blaKPC, blaNDM, blaVIM, blaIMP, blaOXA, blaGES, blaAmpC, blaTEM, blaSHV and blaCTX-M genes) and sequencing. False positives obtained for carbapenemase production phenotypic

assays were confirmed spectrophotometrically. Strain identification and antibiotic susceptibility testing were performed by standard methods. Clonal analysis included PFGE, MLST and identification of *E. coli* phylogroups. Plasmid content and blaCTX-M-15 location were assessed by S1-PFGE. Presence of class 1 integrons, sul (sul1, sul2 and sul3) and qnr (qnrA, qnrB, qnrS) genes was searched as described. Porins (ompK35, ompK36, ompC, ompF, omp35 and omp36) were investigated by PCR and sequencing, and SDS-PAGE.

Results: Seven ERT-non-S clones (3 KP, 1 EC, 1 Eas, 1 Eae and 1 Ecl, MIC 4–>32 μgml), mostly producing ESBL/AmpC were detected. A MDR KP-ST15 epidemic clone (n = 19, 3 PFGE-types, 63% urology-transplant unit, sul2+) was the most frequently identified throughout the whole period and included ERT-S (n = 10, PFGE A and B) and ERT-R (n = 9, PFGE B and C, MIC 3–32 μgml) CTX-M-15 encoding isolates, bla within 50–70Kb plasmids. KP-ST14 and KP-ST45 (n = 1 each, MIC 12 μgml) were sporadic non-ESBL producers. A CTX-M-15-producing EC-B2-ST131 clone [n = 5 isolates/5 PFGE, ERT-R (MIC = 32 μgml) and ERT-S, different units] was detected at the end of the period (06/07.2010), bla located in 130Kb plasmids. One ACT-2 carbapenem-R Eae, one TEM-24-producing Ecl (MIC 4 μgml) and one Eae (MIC >32 μgml) clones were also identified (03/04.2010). Different porin alterations were observed among ERT-R clones. qnrB (1 KP, 1 EC), qnrA (1Ecl) and class 1 integrons (dfrA25, dfrA17-Delta-aadA5 and aacA4) were sporadically detected.

Conclusion: This study reveals a complex allodemic scenario associated with the emergence of ertapenem resistance among different ESBL/AmpC Enterobacteriaceae widespread clones in Portugal.

R2411 Characterisation and clonal analysis of *Klebsiella pneumoniae* sequence type 258 KPC-2 positive isolates responsible for a nosocomial outbreak in northern Italy

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Objectives: KPC are an important group of β-lactamases given their spreading in nosocomial settings among Enterobacteriaceae. KPC-2 and -3 are the wider disseminated, found mostly on plasmids in *K. pneumoniae*. KPC-positive *K. pneumoniae* (KPC-KP) have been reported in many European countries. After the first description of a KPC-KP in Italy in 2009 only few reports has been published. Here we report on the second Italian outbreak, discussing on clinical and molecular features.

Methods: 22 *K. pneumoniae* isolates collected during 2010 at the San Gerardo Hospital in Monza were analyzed as representative of 58 isolates involved in the outbreak. Antibiotic susceptibility tests were performed with Vitek 2. MICs for imipenem and meropenem were confirmed by Etest for isolates that were positive for carbapenemase production (detected by phenyl boronic acid test). Clonal distribution were assessed by rep-PCR, RAPD, PFGE and MLST. Selected isolates were subjected to PCR analysis and sequencing to detect the pattern of resistance determinants.

Results: The 22 investigated isolates showed a MDR phenotype being susceptible only to colistin, gentamicin and tigecycline. Results of genotyping techniques were all concordant and showed that all but one isolates were clonally related and belonged to ST 258. All isolates were confirmed to be blaKPC-2 positive by sequencing. In addition the investigated strains were positive for blaCTX-M-15 and all but one to blaSHV-12 ESBLs. 36 additional isolates showing the same phenotypic pattern were presumed to be related to the epidemic cluster.

Conclusion: Before November 2009 at the San Gerardo Hospital no KPC-KP strains has been isolated. During the studied period, 22 clonally related KPC-KP were obtained. Isolates belonged to ST 258 as the first described Italian KPC-KP. This clone is the major responsible of worldwide spreading of these resistance determinants. Molecular analysis demonstrated the presence in all isolates of blaKPC-2, and blaCTX-M-15 and in all but one of blaSHV-12 β-lactamase. The only KPC-KP isolate not clonally related to the outbreak clone, was positive for blaSHV-1 determinant and was obtained from a patient transferred from another Hospital. This different clone represent an exception in the Italian epidemiological scenario where all described KPC-KP belonged to ST258. The rep-PCR approach here described appear to be a rapid and robust technique to investigate clonal relationship of KPC-KP.

R2412 Colonisation sites of extended-spectrum β-lactamase-producing enterobacteria

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Objectives: Detection and isolation of patients colonised with ESBL-producing bacteria is an important measure to prevent transmission of resistant bacteria in hospitals. In a prospective study we wanted to determine the anatomical sites of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*.

Methods: Patients colonised or infected with ESBL-producing bacteria were included in a prospective study from November 2009 to November 2010. Rectal swab, urine culture, and throat swab were performed in each patient. Wound swabs were obtained in patients with wound, and sputum was collected in patients with productive cough. Sample collection was repeated every 3 months. Collected samples were inoculated on chromogenic agar selective for ESBL-producing bacteria. Disc diffusion method was done for *K. pneumoniae* and/or *E. coli* isolates to assess antimicrobial susceptibility profile for each isolate.

Results: 89 sample collections were done in 42 patients. Patients, 28 males and 14 females, were 24 to 94 years old (62 years on average). 4 patients were on immunosuppressive therapy, 5 had a chronic cardiovascular disease, 3 were diabetics on insulin, 2 patients had chronic kidney failure and 3 had a chronic respiratory illness. 26 patients were colonised with *K. pneumoniae*, 6 patients with *E. coli*, in 10 patients both were isolated. At least one sample was positive for ESBL-producing bacteria on 73 occasions. Rectal swab was positive on 68 (93.2%), throat swabs on 22 (30.1%) and urine cultures on 25 (34.2%) occasions. Sputum culture was performed 11 times, 6 (54.6%) were positive. Wound swabs were positive in 50% (9/18 samples taken). On 5 occasions (in 4 patients), in which rectal swab was negative, ESBL-producing *K. pneumoniae* was isolated only from urine. In 3 out of these 4 patients rectal swabs from initial sample collections were positive, together with urine, throat or wound swabs. 6 to 12 months later, however, urine was the only positive site, in 1 of the patients even on 2 subsequent occasions.

Conclusions: Our results have shown that rectal swabs are positive in most patients colonised with ESBL-producing *E. coli* and *K. pneumoniae*. We can assume that rectal swab is an appropriate method for routine screening for colonisation with *E. coli* and *K. pneumoniae* producing ESBL. However, in some patients the urine may at least transitory be the only positive site.

R2413 Epidemiology of bloodstream infections caused by *Enterobacter cloacae* isolates producing the VIM-1 metallo-β-lactamase in a neonatal unit of a tertiary Greek hospital

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Objectives: *Enterobacter cloacae* isolates, which produce metallo β-lactamases (MBLs) are regarded as an emerging clinical threat. Infections by such pathogens involving neonates remain uncommon. We analyzed clinical characteristics and outcomes of bloodstream infections due to VIM-producing *E. cloacae* in preterm neonates who were hospitalized in the neonatal unit of a tertiary hospital in Greece.

Methods: During a one-year period (Jan 2010-Dec 2010), neonates presented bloodstream infections due to *E. cloacae* which exhibited reduced susceptibility to carbapenems were studied. Bacterial isolates were identified by Vitek 2 (bioMérieux). MICs were determined by E-testing (AB Biodisk). Phenotypic testing was performed using the modified Hodge test, the combined EDTA disk test and boronic acid potentiation disk tests. PCR was used to screen for carbapenemase genes (blaVIM, blaIPM and blaKPC) as well as for ESBL (blaCTX-M,

blaTEM, blaSHV) and plasmid-mediated AmpC genes. DNA sequencing was used to identify the MBL type gene.
Results: During the study, five premature neonates had bloodstream infection due to carbapenem-non-susceptible *E. cloacae*. The isolates exhibited variable carbapenem MICs ranging for imipenem from 1–4 μg/ml, meropenem 1–⩾16 μg/ml and ertapenem 0.5–⩾8 μg/ml. All isolates remained sensitive to tigecycline and colistin, while two were resistant to gentamicin. Phenotypic tests were indicative of MBL production. PCR was used for the verification and identification of the carbapenemase; it confirmed the presence solely of the blaVIM gene in all isolates. DNA sequencing and BLAST search identifying blaVIM-1 gene. Of the 5 neonates, 4 were female and one male, their mean age of gestation was 27.6±2.5 w and their mean weight was 0.974±0.218 kg. Three of the 5 neonates had been exposed to carbapenems during their hospitalization prior to the reported bloodstream infection. Clinical records of these premature neonates were analyzed and the attributable mortality rate was 40%.
Conclusion: The study showed that premature neonates represent a group of patients especially vulnerable to life threatening infections due to MBL-producing *E. cloacae* isolates. Carbapenem MICs tend to be variable for these strains and early detection remains a challenge for the laboratory. The timely implementation of infection control strategies is an absolute necessity in order to prevent the spread of these MDR bacteria.

R2414 Dissemination of bla(DHA-1) genes by a putative ICE among isolates of Enterobacteriaceae

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Objectives: DHA-1, an inducible acquired AmpC β-lactamase, confers resistance to most β-lactams except cefepime and carbapenems. Since a previous study displaying the genetic context of a well characterized collection of 30 DHA-1 producers showed that donors and transconjugants of two of these isolates of *Escherichia coli* carried bla(DHA-1) on multiple plasmids as well as on the chromosome, one of these isolates was selected for further analyses to better understand the element mobilizing bla(DHA-1).
Methods: To check if results obtained by the first study were reproducible, we repeated the conjugation assays and picked up 25 independent transconjugant colonies, selected with ceftazidime and rifampin. The plasmid and chromosomal location of bla(DHA-1) in the parental and the 25 transconjugants selected was then investigated by S1 and Xba1 digestion of the entire DNA followed by pulsed field gel electrophoresis (PFGE) and probing with 32P labelled bla(DHA-1) probes.
Results: S1-PFGE and hybridisation analyses with bla(DHA-1) probe among the donor and the 25 transconjugant colonies analysed revealed that bla(DHA-1) genes were carried on plasmids in the donor and six of the 25 transconjugants tested, but also on the chromosome in all cases. Moreover, multiple hybridisation bands were observed when hybridising the Xba1-PFGE with bla(DHA-1) in all transconjugants.
Conclusions: Acquired AmpC β-lactamases has recently been described mobilised by integrative conjugative elements (ICEs). These elements are capable to transfer themselves from chromosome to chromosome via conjugation. As bla(DHA-1) was present on the chromosome of the donor and was transferred to the chromosome of all 25 transconjugants, we hypothesize that an ICE could be involved in the mobilisation the bla(DHA-1) genes in these *E. coli* isolates.

R2415 Interactive MLST database management using the BioNumerics® plugin, as illustrated on *Campylobacter*

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Multi Locus Sequence Typing (MLST) is a method to discriminate microbial isolates through the partial sequencing of selected housekeeping genes. The BioNumerics® software is widely used for the storage and analysis of MLST sequences. With the use of an adapted plugin tool, BioNumerics automatically assembles and processes sequence trace files, connects to the online MLST database, and retrieves corresponding allele numbers, sequence types as well as available clonal complex information. In the case of *Campylobacter jejuni* and *C. coli* (http://pubmlst.org/campylobacter/) 4378 profiles are available (24/02/2010) linked to 43 clonal complexes.
Using the BioNumerics® Minimum Spanning Tree (MST) we illustrate the interrelation of these clonal complexes. MST's are well-known in the context of mathematical topology. When a set of distances is given between n samples, a minimum spanning tree connects all samples in such a way that the summed distance of all branches of the tree is minimal. With the principle of maximum parsimony (MP), MST shares the idea that evolution should be explained with as few events as possible. In contrast to MP, a "classical" MST does not allow the creation of hypothetical nodes, relying on all states present in the sampled dataset to explain the relationships and evolution. Therefore, MST is only applicable in studies focusing on a very short evolutionary time frame (micro-evolution) and for complete datasets. The MST algorithm presented here allows the creation of hypothetical nodes when the total distance of the tree is reduced by a user-defined number of events. In the context of MLST, hypothetical nodes are usually missing allelic types for which a number of single locus variants (SLVs) are present in the dataset. Introducing hypothetical allelic types has proven to produce more reliable models in non-complete sampled datasets. Additionally, to select the MST that has the most probable evolutionary interpretation among multiple equivalent solutions (degenerate trees), BioNumerics® has editable priority rules for tie handling adopted from the BURST program (http://www.mlst.net).
The data flow will be illustrated through the processing of MLST trace files from *C. jejuni* and *C. coli* isolates. An UPGMA dendrogram for a mixture of MLST profiles of *C. jejuni* and *C. coli*, based on similarities calculated by the categorical coefficient, will be compared to a Minimum Spanning Tree obtained using the same data.

R2416 Molecular-epidemiological analysis of nososcomial carbapenem-resistant *Klebsiella* spp. by automated rep-PCR and MALDI-TOF

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Objective: Fast techniques for routine strain typing in the clinical microbiology laboratory are useful tools. We report the results of a comparative study between automated rep-PCR and whole-cell matrix-assisted laser desortion ionization-time of flight mass spectrometry (MALDI-TOF MS) for molecular-epidemiological analysis of nososcomial carbapenem-resistant *Klebsiella* spp.
Methods: Thirteen *Klebsiella* spp (9 *K. oxytoca* and 4 *K. pneumoniae*) nosocomial isolates that were VIM-1 metallo-β-lactamase producers were analyzed by automated rep-PCR (DiversiLab System) and MALDI-TOF MS AXIMA. Species identification was performed by Vitek 2 System and by MALDI-TOF. Antimicrobial susceptibility testing of isolated bacteria were assayed using Vitek 2 System and Etest. DNA array was used for detection of KPC and, TEM, SHV and CTX-M extended spectrum β-lactamase. The detection of blaVIM-1 gene was done by PCR and sequencing.
Results: All *Klebsiella* spp isolates except one *K. oxytoca* had the blaVIM-1 gene and all *K. pneumoniae* had, also, blaSHV gene associated to ESBL production. Rep-PCR using DiversiLab system showed higher discriminatory power than MALDI-TOF spectra analysis for strain typing.
Conclusions: The combined use of MALDI-TOF for species identification and DiversiLab System for clonal strain typing may be a useful tool for fast and accurate management of nosocomial outbreaks.

Antibiotic usage

R2417 European Surveillance of Antimicrobial Consumption (ESAC): trends in systemic azole consumption in hospital care in Europe

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Objectives: To describe trends in systemic azole consumption in hospital care in Europe from 2005 till 2009 in the context of increased prevalence of azole-resistant species.
Methods: Within ESAC (www.esac.ua.ac.be) adopting the anatomic therapeutic chemical (ATC) and defined daily dose (DDD) methodology, data on hospital use of all 14 antimycotics (12) and antifungals (2) for systemic use (ATC J02 and D01B), aggregated at the level of the active substance, were collected for 2005–2009, and use was expressed in DDD (WHO ATC/DDD, version 2010) per 1000 inhabitants per day (DID). Only countries for which validated data were available for at least three years, were included in the analysis.
Results: Data were available for 15 countries. Total systemic azole use in hospital care on average represented ⩾75% of the total hospital antimycotic and antifungal use in all countries except in Croatia (72%), Malta (68%) and Ireland (46%). In 10 out of 15 countries, total systemic azole use in hospital care increased on average >5% each year between 2005 and 2009. In France, it declined on average >5% each year. In Hungary, Ireland, Luxembourg and Slovenia no such changes were observed. In 2009, fluconazole was the most frequently used azole in hospital care in all countries except in Slovakia (ketoconazole). In 2009, azole consumption more than doubled in hospital care in Denmark, Finland, Latvia and Croatia compared to 2008.
Conclusion: Our study demonstrates a trend of increased systemic azole use in hospital care in Europe contrasted by a decreasing use in France. These trends are mainly driven by fluconazole use, and call for closer monitoring of antimycotic and antifungal use and resistance.

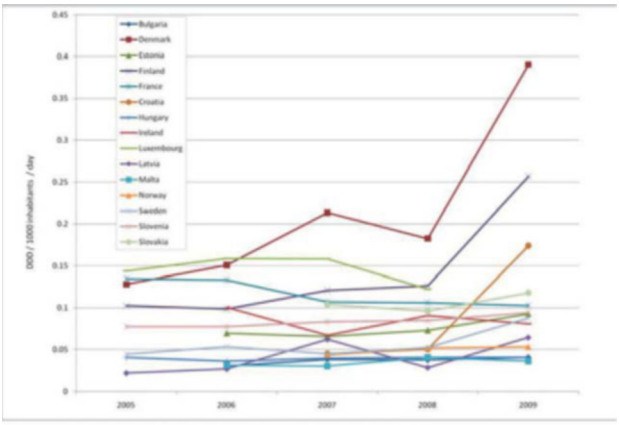

Figure: Consumption of azoles for systemic use (ATC J02AB & J02AC) in hospital care from 2005 till 2009 in 15 European countries

R2418 Stability and administration of cefotaxime in glucose 10% in continuous infusion in neonates

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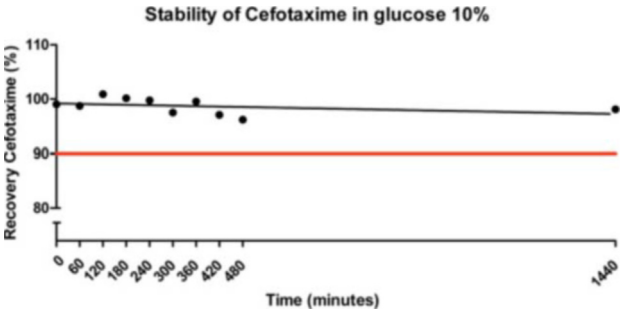
Objective: Cefotaxime is a widely used antibiotic in the treatment of neonatal infection. Dosing of β-lactam antibiotics by continuous infusion has been suggested to improve treatment outcome, as compared to intermittent infusion. However, continuous infusion is critical in neonates because of fluid and salt restriction. Glucose 10% is often used as a nutrient supply in neonates and could at the same time serve as a good solvent for cefotaxime. Yet, stability data of cefotaxime in glucose 10% are lacking, restraining pediatricians from continuous infusion of cefotaxime. We investigated the stability of cefotaxime in glucose 10%.
Method: Cefotaxime was dissolved in glucose 10% in a concentration of 80 mg/ml and stored at room temperature. Cefotaxime levels were measured every sixty minutes for eight hours and after 24 hours using a validated High Performance Liquid Chromatography (HPLC) method. All measurements were performed in duplicate. Values estimated for each sample at subsequent time points were calculated as percentages of the initial concentration. Recovery below 90 percent of the initial concentration was defined as significant loss, using worldwide standards.
Results: The relative concentration time curve is shown in figure 1. After 24 hours, the cefotaxime concentration declined to 98.1% (SEM 1.65), indicating that cefotaxime is stable for at least 24 hours in glucose 10%.
Conclusion: Even after 24 hours, cefotaxime does not decrease to the 90% limit of stability in a concentration of 80 mg/ml when dissolved in glucose 10%. Glucose 10% is a good solvent for cefotaxime allowing continuous infusion with changes of infusion bags only once a day. This facilitates optimal treatment, allows glucose supplementation and reduces nursing staff workload.



R2419 Congruence of suspected clinical diagnosis in the origin of the bacteraemia and final diagnosis. Is it important in the prognosis evolution of bacteraemia?

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Objective: Analysis of diagnostic accuracy of the suspected origins of the bacteremias and the prognostic influence of the type of microorganism, adquisition, empirical treatment and origin of bacteremia in the evolution to death.
Material and Methods: A retrospective study of bacteremia diagnosed during 2 years in a South Hospital in Madrid, analyzing suspected origin, final origin, acquisition, microorganism, empirical therapy and evolution to death.
Results: 326 bacteremia were analyzed. Congruence between diagnosis suspected/definitive origin of nosocomial bacteremia was 80,26% (61/76) with a difference of 19,7% (95% CI 10,7% to 28,6%), Sig: <0,001. Congruence between diagnosis suspected/definitive origin of community bacteremia was of 81,3% (192/236) with a difference of 18,6% (95% CI 15,6% to 30,6%), Sig: <0,001. Bacteremias of respiratory origin had a percent of apropriate empiric diagnosis of 93,5% while that of vascular origin, only 56%.
S. pneumoniae had a percent of apropriate empiric diagnosis of 91,6%, while non-fermentative Gram-negative bacilli 60% and coagulase negative *Staphylococcus* 52,1%.
Mortality was 2,95 CI 95% (1,01 to 4,29) fold higher in bacteremic inadequate empirical antibiotic treatment. The mortality in the group receiving inadequate antibiotic therapy was 19,7%. Difference between both groups was 9,1% CI 95% (0,8% to 19%) Sig: 0,042. The difference in mortality between the group with right and wrong suspected diagnosis of origin of bacteremia was 3,45% CI 95% (−0,6% to 13,5%) Sig: 0,47. OR 1,33 (95% CI 0,59 to 2,99).
Conclusions: Clinicians have a high degree of suspicion about correct original focus of bacteremia, higher in the respiratory origin and lowest in those of vascular origin, and these data are consistent with the type of microorganism. The degree of success does not affect the overall mortality of bacteremia but empirical treatment does it.

R2420 Colistin by inhalation: superior for the treatment of nosocomial pneumonia associated with multidrug-resistant *Pseudomonas aeruginosa* as compared to parenteral administration? A retrospective observational study

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Objective: To investigate the outcome of patients treated with colistin either by IV or inhalation therapy for nosocomial pneumonia associated with multidrug-resistant (MDR) *Pseudomonas aeruginosa*.
Methods: A retrospective study of 20 intensive care patients with a pneumonia associated with MDR *P. aeruginosa* receiving colistin sulphomethate sodium (Colistineb®) between 2007 and 2009 was performed. A strain was considered multidrug-resistant if it was resistant to at least 6 of the following antibiotics: piperacillin-tazobactam, ceftazidime, cefepime, meropenem, aztreonam, ciprofloxacin, and amikacin. The administration mode, predicted mortality based on the SAPS3 score, SOFA score at onset of the colistin treatment, clinical and microbiological response, and mortality during the episode of the infection were analysed. The non parametric Kruskal-Wallis and Fisher's Exact test were used for statistical analysis of respectively the predicted mortality/SOFA score and mortality rate.
Results: Six patients received colistin by inhalation only, 5 were treated only parenterally, and 9 by a combination of both administration modes. All patients received concomitant bètalactam therapy. The mean predicted mortalities were respectively 72%, 68%, and 69% ($p = 0.91$). SOFA scores at the onset of the treatment were also comparable ($p = 0.87$). Clinical response was favourable in all patients receiving colistin by inhalation (6/6) and in 40% (2/5) of the patients receiving colistin parenterally ($p = 0.06$). In the patients with colistin administered both via inhalation and parenterally, clinical response was favourable in 78% of the patients (7/9) ($p = 0.27$ as compared to the treatment group receiving colistin only parenterally). When all patients with inhalation therapy were compared to the group without inhalation therapy, a favorable clinical response was present in respectively 87% and 40% ($p = 0.06$). In none of the patients, the *Pseudomonas* spp. was eradicated from the follow-up cultures.
All patients in the parenterally treated group died. None of the patients receiving colistin by inhalation, and 3 of 9 patients of the combination group eventually died ($p = 0.002$ and $p = 0.03$ respectively as compared to the group receiving colistin only parenterally).
Conclusion: Aerosolized colistin could be beneficial as adjunctive treatment for the management of pneumonia associated with MDR *P. aeruginosa*.

R2421 A point-prevalence study on surgical antibiotic prophylaxis in a tertiary care hospital and evaluation of the use of the surgical prophylaxis guide

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Objectives: Inappropriate use of antimicrobial drugs is associated with increased hospital expenditure, emergence of resistant bacteria and unnecessary side-effects. Concerning this, antibiotic stewardship is among the main concerns of the hospital infection control programmes. A "surgical antibiotic prophylaxis guide" was established in our hospital in 2008. The aim of this study was to investigate the point prevalence of antibiotic prophylaxis in surgery clinics and to evaluate the appropriateness of the antibiotic use.
Methods: The study was conducted in the Ankara Training & Research Hospital, a 670-bed tertiary care teaching hospital. The hospital contains 23 operation rooms and over 15.000 operations are performed within a year. On the 16th of December 2010 infectious diseases consultants went to the operation rooms of eight surgery clinics (Plastics & reconstructive surgery, general surgery, ophthalmology, ear-nose-throat, orthopaedics, urology, obstetrics & gynaecology (O&G) and neurosurgery) and investigate the prophylactic antibiotic regimen before the surgeries. The duration of the intended use of antibiotics after the surgeries was also included in the questionnaire.
Results: Seventy-two of 196 hospitalized surgery patients were underwent different kind of surgeries. 50 of them (69.4%) were using prophylaxis. Cefazolin (45.8%), sulbactam-ampicillin (11.1%) and ceftriaxone (6.9%) were the most frequent antibiotics used for prophylaxis. In four patients (5.6%) combined antibiotics (metronidazole plus any antibiotics above) were used. 30.6% of the patients did not get any prophylaxis. The mean duration of the antibiotic use was 2.7 (1–22) days and the intended use of the antibiotic prophylaxis after the surgeries was 3.8 (1–10) days. 45 (62.5%) of the antibiotic prophylaxis regimens were inappropriate according to the guidelines. The reasons for the inappropriateness were as follows: wrong antibiotic (8.9%), wrong doses and duration (84.4%) and no antibiotics in spite of the need (6.7%).
Conclusion: The current study showed a high percentage of inappropriate surgical prophylaxis regimens in our hospital. The main problems about the inappropriate prophylactic antibiotic use were the dose and the duration (84.4%). The chosen antibiotic was 91.1% right. As conclusion we suggest that the hospital infection control programmes must include the follow up of the use of established guidelines, just introducing them seems to be not efficient.

R2422 Therapy approach and problems in treatment of tularaemia patients in Serbia

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Objectives: The first epidemic of tularemia in South-eastern Serbia happened in 1999. During a 10 years period, from 1999. till 2008, 151 patients were hospitalized and treated. The purpose of the study was to analyze and determine the best therapy choices in patients suffered from tularemia.
Methods: Before hospitalization 16.5% were not treated, 58.8% were inadequately treated, 10.3% were adequately treated and 14.4% unknown. An average duration of disease before hospitalization was 35 days. Antibiotic monotherapy was administrated to 53 patients: gentamicin (27 patients), ciprofloxacin (11), amikacin (8), streptomycin (5) and doxycycline (2 patients). Combined (polyvalent or successive) antibiotic therapy was administrated to 98 patients: gentamicin and ciprofloxacin (66 patients), gentamicin and doxycycline (18), ciprofloxacin and doxycycline (2), gentamicin, ciprofloxacin and doxycycline (12 patients).
Results: The biggest success in treatment (75% cured) was noticed in patients after combination of gentamicin, ciprofloxacin and doxycycline, then combination of gentamicin and ciprofloxacin (71%), then monotherapy of gentamicin (68%) and combination of gentamicin and doxycycline (67% cured). In comparison to number of treated patients, the biggest success in treatment (71%) had the combination of gentamicin and ciprofloxacin, then monotherapy of gentamicin (68%).
Outcome of medicamentosus treatment: 92 patients (61%) of 151 were totally cured. The complications happened in 59 patients (39%): 1. abscessing of lymph nodes with or without fistulisation at 36 patients (23.8), 2. relapse – 12 patients (8%), 3. both complications at 11 patients (7.2%) (graphic 1). The relapses included recidivante lymph node enlargement or intake more than one lymph nodes. Final outome after conservative and radical treatment was total curing of 143 patients (94.7%) and residual persistant lymphadenopathy at 8 patients (5.3%).
Conclusion: Reasons for appearance of complications during therapy of tularemic patients:
1. inadequate initial antibiotic treatment
2. late onset of adequate antibiotic therapy
3. possible appearence of resistant species of *F. tularensis* in the region of South-eastern Serbia.

Early, forehand treatment restrains the colliquation of lymph nodes, recurrence of disease and dissemination of infection. Early diagnosis and treatment are crucial for prevention of complication and complete curing.

R2423 Impact of an antimicrobial stewardship program in a second level hospital

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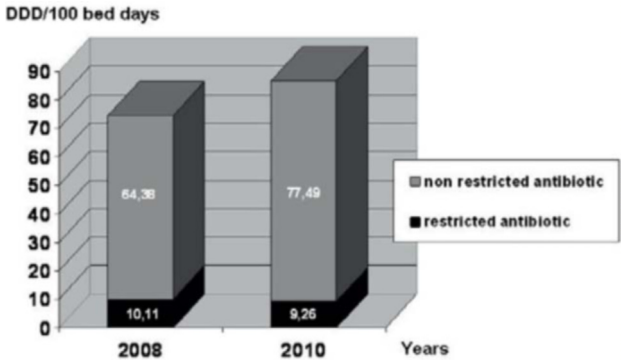
Objective: There is a variety of methods to improve antimicrobial appropriateness in hospitals. The aim of our study was to investigate the influence of an antimicrobial stewardship program on antibiotic use at the Hospital Sant Joan de Déu de Martorell.
Methods: The study was conducted in a 132 bed-hospital. Antibiotic consumption was monitored one year prior (2008) and one year during (2010) the antimicrobial stewardship program. During 2009, internal protocols were reviewed and actualized. The antibiotic working group included a clinical pharmacist, a microbiologist and an attending infectious disease physician who provided clinical backup. This group met once a week and checked the indication, dose, frequency and duration of restricted antibiotics (including carbapenems, voriconazole, vancomycin, piperacillin-tazobactam, linezolid, cefepime, aztreonam, liposomal amphotericin and amikacin). When needed, the group contacted the physician in charge in order to improve the treatment. Antibiotic utilization was measured in defined daily doses (DDD)/100 bed days. Multiresistant bacteria comprising multidrug resistant *P. aeruginosa*, methicillin resistant *Staphylococcus aureus*, extended spectrum β-lactamase and AmpC β-lactamase producing *Klebsiella* and *E. coli* strains were selected from all the cultures of *P. aeruginosa*, *Staphylococcus aureus*, *E. coli* and *Klebsiella* spp.
Results: Total antimicrobial consumption in 2008 was 74.5 DDD/100 bed days in front of 86.75 DDD/100 bed days in 2010. After implementation of the program, use of restricted antibiotics decreased from 10.11 DDD/100 bed days in 2008 to 9.26 DDD/100 bed days in 2010. Seventy three interventions were performed related to: antibiogram susceptibility (34), treatment duration (18), incorrect diagnosis (16) and wrong dose (5). Meropenem and piperacillin-tazobactam were the most overused antibiotics.
Multiresistant bacteria increased from 5.76% in 2008 to 7.45% in 2010.
Conclusions: Although total antimicrobial consumption and multiresistant bacteria increased in 2010, the implementation of a multidisciplinary antibiotic working group reduced the use of restricted antibiotics.



R2424 Fosfomycin and multi-resistant bacteria

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Objective: For several years, antimicrobial resistance rates among bacteria have continued to rise, with limited therapeutic options due to the shortage of new antimicrobial agents. This situation has led to renewed interest in old antibiotics such as fosfomycin. We evaluate efficacy and safety of fosfomycin on resistant bacteria especially in critically ill patients.
Method: Retrospective study evaluating the use and efficacy of fosfomycin as the only covering drug for 27 infections due to multi resistant bacteria.
Results: Twenty seven patients (15 males, 12 females, mean age was 60 year (range 18–81)). Indications for antibiotherapy was lung infections for 15 patients (53.5%), complicated urinary tract infections (UTI)for 8 (28.5%), abdominal infections for 3 (10.7%) and bacteraemia without onset for 1. Four (4/27) patients had positive blood culture, 18 (18/27) were admitted to intensive care unit (18/27) and 11 (11/27) presented septic shock. Infections were monomicrobial (85.7% n = 23) or plurimicrobial (14.3% n = 4). The main identified bacteria were *P. aeruginosa* (n = 22), then *Stenotrophomonas maltophilia* (n = 4). Mean global treatment duration was 16.8±7.0 days (lung infection 22±6.8, complicated UTI 14.0±2.13, and abdominal infection 19.6±9.1).
22 (85.7%) patients were cured and 5 died, 12 (12/15) patients with lung infection were cured, 2 (2/3) for abdominal sepsis, 8 (8/8) for complicated UTI. The patient with bacteraemia without onset died during first month after sepsis. No adverse events possibly due to fosfomycin were reported.
Concerning monomicrobial infection due to *P. aeruginosa*, 15 (15/16) patients have favourable outcome. Fosfomycin seems to be an excellent choice for infections due to resistant *Pseudomonas aeruginosa*.
Conclusion: Our study included a high number of critical situations with 67.9% of patients admitted to intensive care unit and 39.2% involving septic shock. Considering resistant bacteria and critical situations, the global outcome was attractive.
Parenteral fosfomycin combined with another antibiotic appears to be safe and effective treatment for severe infections caused by resistant bacteria sensitive only to this antibiotic. A further benefit is its low (~3%) and stable rate of resistance in countries in which it has been in use for a long time. This should be confirmed by further studies to substantiate this finding.

R2425 UK registry experience of the treatment of infective endocarditis with daptomycin

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Objectives: To describe the real world clinical experience of the use of daptomycin (DAP) for the treatment of infective endocarditis in the UK.
Methods: A retrospective non-interventional review of patients (pts) receiving DAP in 8 UK institutions participating in the European Cubicin® Outcomes Registry and Experience (EU-CORESM) during the first 2.5 years of the registry. Efficacy was evaluated by the investigator at the end of DAP therapy as cured, improved, failure and non-evaluable. Data were collected on demographics, antibiotic usage, microbiological and clinical outcomes and adverse events from pts treated between January 2006 and August 2009. Patients (pts) were categorised by severity (complicated and uncomplicated) and the anatomical site of the primary infection. All pts included in the registry had received at least one dose of DAP.
Results: 51 pts with a mean age of 61 yrs were treated. The majority of pts had significant underlying disease (86%). 55% had left-sided IE. Both sides were involved in 16%, 14% had right-sided and 14% foreign body involvement. Prior antibiotics had been received by 84% pts, with the reason for discontinuation of therapy being loss of susceptibility/resistance in 46% pts. Cultures were obtained for the primary infection in 94% pts. *Staphylococcus aureus* was the most frequently isolated organism (31%), followed by and coagulase-negative staphylococci (14%) and *Streptococcus* spp (8%) Heart valves were replaced in 22% pts, but there was no surgical intervention in the majority of pts (72%). Mean duration of therapy was 21 days and 63% of pts received concomitant antibiotics. DAP 6 mg/kg 24h was the most frequently used dose. Clinical outcomes were success, defined as 'cure plus improved' (73%), failure (4%) and non-evaluable (23%). Adverse events were reported regardless of study drug relationship and were experienced by 29% of pts. The mean time to clinical improvement was 6 days. Therapy was stopped because of an adverse event in 12% pts.
Conclusions: Despite the number of prior antibiotic failures and multiple comorbidites in these pts the overall clinical success rate in this UK population was 73%. There is emerging evidence that DAP is being used to treat infections caused by other Gram-positive species that are

of increasing importance in IE, particularly where treatment options may be limited. The data from the current series adds to the body of evidence for the efficacy of DAP in left-sided IE.

R2426 Nebulised antibiotics for the treatment of patients with non-cystic fibrosis bronchiectasis with chronic *Pseudomonas aeruginosa* colonisation

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Objectives: To describe the effect of nebulized antibiotics (NA) on hospital admissions and length of hospital stay in patients with non-cystic fibrosis bronquiectasis (BQ), chronically colonized by *Pseudomonas aeruginosa* (PA) during the last 5 years.
Methods: Retrospective case review of BQ patients with PA isolated from sputum at least twice during the year prior to NA administration and a minimum 6-month follow-up. Main outcomes were number of hospital admissions and length of stay before and after NA. Data on clinical, microbiological and functional changes and tolerability was also collected.
Results: Seventeen patients were included (9 men), median age 69.7 years, average follow-up 753 days (range 183–1824). Initial treatment: tobramycin (TOB) in 9 patients, colistin (COL) in 7 and gentamicin (GEN) in 1. Nine patients received more than 1 NA during follow-up, resulting in a total of 31 NA courses: 16 with COL (average dose: 2.875.000 U/day), 14 with TOB (average dose: 442.857 mg/day), 1 with GEN (320mg/day). Average treatment duration was 492.47 days (range 100–971).
Subjective clinical improvement after NA was described by 12 patients (70.5%) and microbiological eradication of PA was confirmed in 7 patients (41.2%), though there was 1 relapse. NA did not result in clinical improvement in 2 patients, though it was well tolerated. Three patients did not tolerate any of the NA administered (at least COL and TOB).
Table 1 shows the reduction in mean number of admissions/year and mean lenght of stay/year in days after NA onset for all patients. A statistically significant reduction is observed when PA is eradicated.
On the whole, 8 patients presented side effects at any time during treatment (47%), mainly bronchospasm (60%).
Side effects that prompted treatment interruption were observed during 15 NA courses: 6 with COL (40%), 8 with TOB (53.3%) and 1 with GEN.
Conclusions:

1. NA resulted in clinical improvement in 70.5% of BQ patients and in eradication of PA in 41.2%.
2. NA reduced number of admissions and length of stay per year in all patients. A statistically significant reduction was observed in patients that achieved PA eradication, in whom NA prevented nearly 2 admissions per year.
3. Side effects were observed in 47% of patients and 17.6% did not tolerate any NA.

Difference between number of admissions/year and hospital stay in days/year

		Period prior to NA (*)	Period after NA (*)	Difference between periods (**)	p (***)
All patients (n=17)	Admissions/year	2.24 (2.17)	1.5 (1.53)	0.73 (0-1.97)	0.17
	Length of stay/year (days)	31.35 (39.1)	16.58 (17.53)	14.7 (0-36)	0.22
Patients with eradication of PA (n=6)	Admissions/year	3 (1.67)	1.06 (1.27)	1.9 (0.3-3.5)	0.05
	Length of stay/year (days)	51.67 (54.4)	10.64 (12)	41 (0-97)	0.06

(*) Results expressed as mean and SD or CI 95% (**)
(***) Within subjects Paired T-TEST for comparing means.SPSS statistical package.

R2427 Trends in regional outpatient antibiotic prescription data and interventions in the Dutch-German EURSAFETY HEALTH-NET project

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Increasing prescription of broad-spectrum antibiotics is regarded to facilitate selection of multiresistant germs. Surveillance of outpatient antibiotic use might contribute to the efforts made for preventing the spread of Methicillin-resistant *S. aureus* (MRSA) within regional networks. In the EURSAFETY HEALTH-NET, this issue has been addressed by offering training sessions on MRSA and antibiotic awareness to general practitioners in cooperation with the Association of Statutory Health Insurance Physicians Westphalia-Lippe (KVWL) which represents the German part of the EUREGIO and other regions in North Rhine-Westphalia. Furthermore, in 2006, a possibility for reimbursement of MRSA eradication therapy in outpatients has been created. We present data comparing the use of selected antibiotics in outpatient care of medical doctors in the EUREGIO to those in other KVWL regions.
Regional data for outpatient prescription of antibiotics (based on Defined Daily Doses (DDD)) were collected by the KVWL and analyzed for the years 2002 to 2009. In order to compare the prescription of different antibiotics like fluoroquinolones or mupirocin in the EUREGIO to the whole KVWL region, we used the Cochrane Armitage Trend Test.
Altogether, a total of 12.2 DDD/day per 1,000 inhabitants (DID) were prescribed in 2002, followed by 12.9 DID, 12.9 DID, 14.4 DID, 13.8 DID, 14.4 DID, 14.7 DID and 14.8 DID from 2003 to 2009, respectively. From 2002 to 2009 the percentage of prescriptions of all antibiotics and of fluorochinolones decreased significantly in EUREGIO relating to the whole KVWL region. In contrast, the number of mupirocin prescriptions increased significantly more in EUREGIO than in KVWL region.
As desirable, the medical doctors in the EUREGIO region tends to more prudent antibiotic use. The increasing number of mupirocin prescription among outpatients reflects the growing demand and, facilitated by new refunding possibilities, the increasing implementation of MRSA eradication therapy in outpatient care. Regional trends which may reflect effects of interventions can be observed in routine data.

Molecular bacteriology

R2428 *Haemophilus influenzae* and *Haemophilus haemolyticus* identification and serotyping by molecular methods

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Isolates of the non-pathogenic *Haemophilus haemolyticus* from respiratory samples are often misclassified as non-typeable *H. influenzae* because bacteriological techniques are not sufficient to distinguish these two closely related species. The presence of *H. haemolyticus* among the *H. influenzae* isolates hampers studies on the pharyncheal flora and limits the use of Slide Agglutination SeroTyping (SAST), since *H. haemolyticus* may give false positive results.
Objectives: The development of a novel protocol for the distinction of the two *Haemophilus* species and the subsequent serotyping of *H. influenzae* isolates by molecular methods.
Methods: The protocol consists of two parts: a PCR to distinguish between *H. influenzae* and *H. haemolyticus* using the 7F3 epitope of the ompP6 gene, followed by PCRs on the bexA and capsular genes for serotyping.
Results: Among 386 isolates from vaccinated children, we found 18% *H. haemolyticus* isolates and 82% *H. influenzae*. The latter species was confirmed by the presence of the iga gene, a marker for *H. influenzae*. By SAST we found that all the *H. haemolyticus* isolates reacted falsely with the serotype a antiserum, while as expected the bexA and capsular genes were absent in these isolates.
Conclusions: The distinction of the two *Haemophilus* species by the ompP6 PCR and the subsequent serotyping of *H. influenzae* gives unequivocal results that will aid studies on the pharyncheal flora.

R2429 A 37 kDa/83 kDa RNase L isoform ratio could be used as a biochemical marker for chronic brucellosis patients

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Objectives: Chronic brucellosis (CB) and chronic fatigue syndrome (CFS) are characterized by a collection of non-specific symptoms and long-lasting fatigue. Of note, there are no objective clinical and diagnostic findings in both CB and CFS. A low-molecular-mass (37 kDa) isoform of RNAse L has been described in peripheral blood mononuclear cell (PBMC) extracts and the ratio of two isoforms of RNAse L (37 kDa/83 kDa) has been proposed as a potential biochemical marker of CFS (Tiev et al., 2003). To our knowledge, the RNase L has never been analyzed in CB patients.
The aim of this study was to determine the ratio of the 37kDa/83KDa isoforms in CB patients and compare the results with healthy well-matched volunteers.
Methods: The CB group consisted of 9 patients (5 women and 4 men; mean standard deviation (SD) age: 49±9 years) that had been diagnosed with brucellosis between five and 35 years prior to the study. Two of the CB patients had focal disease (one spondylitis and one multifocal motor neuropathy). The remaining 7 subjects had non-specific symptoms such as fatigue, malaise, arthralgia and/or myalgia. Five of the 9 CB patients had been diagnosed of CFS. The control group consisted of 7 matched healthy volunteers (4 women and 3 men; mean standard deviation (SD) age: 31±3 years). PBMCs were isolated by density-gradient centrifugation. Target proteins were probed using a Western Blot procedure.
Results: PBMC extracts from all subjects give rise to a major 83 kDa and a minor 37 kDa polypeptide band. The ratio of RNase L isoforms (37 kDa/83 kDa) of 0.4 used as a cut-off, allowed discrimination of CB patients from controls with high sensitivity (88.9%), specificity (85.7%), a positive prognostic value (88.9%) and a negative prognostic value (85.7%). The mean amount of the ratio of RNase L isoforms (37 kDa/83 kDa) in chronic brucellosis patients (0.84) was higher than in healthy subjects (0.22) ($P < 0.05$).
Conclusion: The chronic brucellosis patients are more likely to show a higher ratio of the two isoforms of RNAse L (37 kDa/83 kDa) than the healthy subjects. A high 37 kDa/83 kDa ratio of the RNase L could distinguish CB patients from healthy subjects.

R2430 Rapid molecular detection of *S. aureus* nasal colonisation among patients undergoing urgent surgery

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Objectives: Recent publications suggest that 20–30% of surgical site infections are caused by *S. aureus*, and half of these arise from endogenous flora. This study evaluates rapid molecular identification of *S. aureus* nasal carriage from patients undergoing urgent surgery.
Methods: The study was carried out in Tel-Aviv Medical Center from 9.5–30.11.2010. Patients were selected from different surgical wards: neurosurgery, thoracic, orthopedic, vascular and plastic surgery. Nasal swabs from the patients were collected using a double swab; one swab was used for molecular testing on GeneXpert (Xpert MRSA/SA Nasal Assay – Cepheid) and the other was used for confirmatory culture on solid (chromagar MRSA II and 5% sheep blood) and liquid medium (brain heart Infusion broth).
Results: 174 patients were sampled. 54 patients (31%) were found to be cariers of *S. aureus* by molecular testing: 49 (28%) MSSA, 5 (2.9%) MRSA. Compared to routine culture the sensitivity and specificity of the molecular testing were 94% and 95% respectively; the positive predictive value (ppv) and negative predictive value (npv) were 89% and 98%. 13 patients had invalid results on initial molecular testing. On repeated molecular testing (with the swab used initially for culture) only 3 had invalid results.
Conclusion: Xpert MRSA/SA Nasal Assay is capable of rapid and accurate detection of MSSA and MRSA nasal colonization. Further studies are needed in order to reduce invalid results.

R2431 Clinical usefulness of a PCRQ assay in a case of chronic brucellosis with multifocal motor neuropathy

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Objective: To test the clinical usefulness of a quantitative real time PCR (PCRQ) assay for the diagnosis of one chronic brucellosis (CB) case with multifocal motor neuropathy (MMN).
Patient and Methods: Blood, serum and a cerebrospinal fluid (CSF) sample from one patient with MMN were analyzed by a PCRQ assay to detect and quantify *Brucella melitensis* DNA (Navarro et al. Clin Infect Dis. 2006 May 1; 42(9): 1266–73).
Case report and Results: A 44-years-old man, veterinarian. In 1990 he pricked with a Rev-1 *B. melitensis* strain and experienced clinical features compatible with acute brucellosis with positive serology for *Brucella* spp. He received doxycycline (Dox) for 45 days. Eight months later he developed a MMN. In December 2002, he started treatment with rifampicin (RFP) for 40 days, followed by cyclophosphamide for 6 months, coinciding with improvement of the left hand mobility. In June 2006, he consulted the Internal Medicine Department because he considered that his neuropathy was related to the initial brucellosis episode. Serology was positive for the RB, Coombs (1/40) and Brucellacapt (1/40) tests. The PCRQ assay was positive in blood (461copies/ml) and negative in serum. In November 2002, he received a new treatment with Dox+RFP for 8 months, with transient improvement of symptoms and the electromyogram. During the 3 years follow-up, we analyzed 49 samples (24 blood, 24 serum and a CSF sample). The CSF sample was negative for both serology and the PCRQ assay. Three months after completion of the treatment, the patient resumed it for 6 months, because he considered that his clinical improvement was associated with antimicrobial therapy. During the second treatment period 10 samples (5 blood, 5 serum) were analyzed by PCRQ. Only one serum sample was positive with 1125copies/ml. Blood samples were negative. In the last year post-treatment follow-up, we analyzed 12 samples (6 blood, 6 serum) which were all negative by PCRQ. Conventional serology showed no detectable antibody titers and Brucellacapt test showed a titer of 1/40. Nowadays, the patient remains with severe loss of strength in his left hand and right foot and continues receving doses of intravenous IgG monthly.
Conclusion: This may be the first report of CB with MMN as neurological complication described, thanks to the high sensitivity and specificity of a PCRQ assay.

R2432 Cloning, expression and purification of truncated form of Flagellin, N-terminal (1–161), of *Pseudomonas aeruginosa* in *Escherichia coli*

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Introduction: *Pseudomonas aeruginosa* is an opportunistic bacterium that has high antibiotic resistance. Immunoprophylaxy and immunotherapy may be considered as desirable ways for control and treatment of *Pseudomonas aeruginosa* infections. Flagellin is a main virulence factor. There is high homology and cross reaction among flagellin of *Pseudomonas aeruginosa* strains. N-terminal domain of flagellin plays an important role in attachment to TLR5. It also may play an important role in the induction of protective immune responses. This study was aimed to produce rN-terminal flagellin(1–161). In future, its immunological effect would be evaluated on animal and compared with native flagellin.
Materials and Methods: The coding sequence of flagellin N-terminal of *Pseudomonas aeruginosa* 8821M was isolated by PCR and cloned into pET28a vector. *E. coli* BL21 (DE3) strain was used as an expression host. The recombinant protein was purified by Ni-Sepharose resin. The

Antigenicity of protein was evaluated by western blot analysis by using of antibody against native flagellin.
Results: Expression of flagellin gene in the host produced considerable amount of protein. Single band of the recombinant protein was observed in SDS-PAGE after purification that indicates high pure protein. Result of western blot analysis showed that antibody against native flagellin can identify recombinant protein.
Conclusion: Pure recombinant N-terminal flagellin(1–161) was produced and further characterized by native antibody of flagellin by our methods. It would be expected the recombinant protein induce immunological responses against flagellin in an animal model.

R2433 Development of a new diagnostic tool for the detection of *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* in a duplex real-time PCR

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Objectives: *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* are important and common causes of community-acquired pneumoniae. The highest incidence of *C. pneumoniae* and *M. pneumoniae* infections is among schoolchildren 5 to 14 years old. Symptoms may be mild, nonproductive persistent cough, malaise, and fever, but more severe illness occurs when the lower respiratory tracts is affected, given rise to acute bronchitis and pneumoniae. The agents causing respiratory infections are difficult to distinguish clinically, since many bacterial and viral infection share clinical features, including symptoms. It is therefore important to find a sensitive and effective way to identify these agents and propose the appropriate antibiotic therapy. Currently culture and serological confirmation of the diagnosis of *C. pneumoniae* and *M. pneumoniae* are difficult and time-consuming. Real time PCR, sensitive specific and rapid technology, is an effective alternative. We propose a new real-time PCR based diagnsotic tool for *C. pneumoniae* and *M. pneumoniae* diagnosis.
Methods: Nucleic acids were extracted from nasopharyngeal specimens by using easyMag (bioMérieux) or MagNA Pure Compact (Roche) extraction systems. Purified nucleic acids were added to the ready-to-use Chla/Myco pneumo r-gene™ amplification premix. *C. pneumoniae* and *M. pneumoniae* were distinguished in a duplex single reaction tube. Amplification was performed on ABI7500 Fast, Bio-Rad CFX96 or Roche LC480 platforms.
Results: On QCMD European Proficiency Panel 2010, the 12 positive/negative samples were correctly identified with Chla/Myco pneumo r-gene™. All positives were detected, including weak positives (0,049IFU/100 µL for *C. pneumoniae* and 50CCU/100 µL for *M. pneumoniae*). Analytical Sensitivity study on *C. pneumoniae* and *M. pneumoniae* samples was performed in respiratory specimens. Specificity study showed no cross reaction with other respiratory bacteria or viruses.
Conclusion: The high quality associated with its compatibility with the major extraction and real time PCR platforms allows an immediate integration of Chla/Myco pneumo r-gene™ in most routine diagnostic laboratories.

R2434 Preliminary clinical study using a multiplex blood PCR for rapid detection of bacterial and fungal pathogens in ICU patients with presumed sepsis

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Objectives: Sepsis is a serious medical condition that requires rapidly administered, appropriate antibiotic treatment. The rapid detection of pathogens in blood is critical for a favourable outcome of patients with suspected sepsis. Although blood culture (BC) is considered the criterion standard for diagnosis of bloodstream infection, it take three or more days for final pathogen identification and antimicrobial susceptibility testing. In this observational study, the clinical impact of a commercially available multiplex PCR system in ICU patients with suspected sepsis was been analysed.
Methods: Blood samples from patients with presumed sepsis were cultured with the Bactec 9240 system (Becton Dickinson, Heidelberg, Germany) and blood in EDTA from the same patients subjected to analysis with the LightCycler SeptiFast M(grade) Test (LC-SF; Roche Diagnostics, Mannheim, Germany) at two tertiary care centres. LC-SF test is a multiplex, real-time PCR system allowing detection of 16 pathogens at the species level and four groups of pathogens at the genus level (Gram-positive, Gram-negative and fungal microorganisms). For samples with PCR-detected pathogens, the actual impact on clinical management was determined by chart review. Furthermore a comparison between the time to a positive blood culture result and the LC-SF result was made.
Results: From 33 patients, 24 (73%) yielded concordant negative and 7 (21%) concordant positive results. In one patient two more pathogens were detected with molecular method (*E. coli* in blood cultures vs *E. coli*/*S. maltophilia*/*C. albicans* in LC-SF assay) and one patient was LC-SF positive only (negative blood cultures vs *P. aeruginosa* in LC-SF assay). LC-SF results were obtained in 7–15 h, in contrast to the 24–72 h required for blood culture. According to the LC-SF results, initial therapy was inadequate in five patients, and antibiotic treatment was changed.
Conclusion: This rapid, multiplex pathogen detection LC-SF system complemented traditional culture-based methods and offered some added diagnostic value for the timely detection of causative pathogens having a relevant impact on clinical management for a subset of patients with clinically suspected sepsis.

R2435 A seven-year survey of pertussis incidence in the capital city of Sofia, Bulgaria

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Bordetella pertussis, the causative agent of whooping cough is endemic in Bulgaria despite extensive nationwide vaccination since 1950s.
Objectives: Goal of this study was to investigate the incidence of pertussis infection among children and adults in the capital city of Sofia, Bulgaria for a seven years period.
Methods: Since seven years the National reference laboratory in molecular microbiology started molecular diagnosis of pertussis. As target PCR marker we selected pertussis toxin gene. 162 bp amplified fragment was detected on agarose gels. DNA was extracted from nasopharyngeal swab samples by automated robot system. A total of 2227 samples were analyzed. Samples from patients clinically suspected for pertussis were analyzed.
Results: A total of 2227 samples were analyzed for pertussis by PCR. Of these 496 samples were positive and 1731 negative for pertussis. Positive samples represent 22.3%. The capital city of Sofia has about 1.3 million inhabitants. The incidence of pertussis illness is estimated to be 38.1 patients per 100.000 inhabitants.

Year	B.pertussis	
	PCR (+)	PCR (-)
2004	57(30.8%)	128
2005	27(31.8%)	58
2006	36(27.5)	95
2007	140(28.2%)	357
2008	69(24.3%)	215
2009	142(17.9%)	650
2010	25(9.9%)	228

Table 1. Incidence of pertussis in the capital city of Sofia Bulgaria.

Conclusions: Children in Sofia have high immunization coverage. Acellular vaccination is applied since April 2010. Whole cellular vaccine was previously applied. Highest prevalence of pertussis incidence is in group age 0–3 years.

R2436 Evaluation of the GenoType® CDiff for detection of *Clostridium difficile*-DNA and the multiplexed identification of toxins and different ribotypes from stool specimens

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Objectives: The purpose of this study was to evaluate the new PCR based *Clostridium difficile* (CD) assay GenoType® CDiff (Hain Lifescience, Nehren, Germany). This assay is able to identify *Clostridium difficile*, toxins A and B, the binary toxin cdtA/B, and the highly pathogen and virulent ribotypes 078, 126 and 027. The detection is done in a line probe format (DNA-strip).
Methods: DNA isolation from stool was performed with an automated nucleic acid purification instrument (GenoXtract) and the GXT Stool Extraction Kit (Hain Lifescience). The GenoType® CDiff assay was performed according to manufacturer's instructions.
Results: 175 stool samples positive with the Glutamate dehydrogenase (GDH) antigen test (C.diff Check™-60-EIA, Techlab, Blacksburg, VA, U.S.A.) were compared to results from an EIA for the detection of toxin A and B (Premier™ TOXINS, A&B, Meridian, Saco, ME, U.S.A.) performed on cultured CD colonies and directly from stool. EIA based toxin detection directly from stool had a sensitivity of 73% and a NPV of 29%.
In 167 GDH positive and culture positive stool specimens *C. difficile* was confirmed in 161 cases by PCR (sensitivity 96%). Eight GDH positive stool specimens remained negative when cultured.
152 samples were congruent positive by PCR and tox EIA (culture + direct testing), 17 were congruent negative and 4 only positive by the EIA. Two samples were excluded (sensitivity toxin-PCR 97%, specificity 100%, PPV 100%, NPV 81%).
The GenoType® Cdiff assay was able to identify ribotype O27 in 9 specimens and in 2 specimens 078/126.
Conclusions: The GenoType® Cdiff assay for the direct detection of *Clostridium difficile* and major ribotypes from stool shows rapid, sensitive and specific results. The DNA isolation is fully automated. The turnaround time (including hands on time) is approximately 1.5 hours for DNA isolation, 2 hours for amplification and 2 hours for hybridization. The assay provides more information (ribotypes, toxins, binary toxins and Moxifloxacin resistance) as any presently available commercial CD test.

R2437 Molecular confirmation of resistance to first- and second-line antituberculosis drugs using GenoType MTBDRplus and GenoType MTBDRsl assays

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Objective: To detect mutations in genes associated with resistance to first and second line antituberculous drugs in multidrug resistant (MDR) clinical isolates of *M. tuberculosis*.
Methods: GenoType MTBDRplus and GenoType MTBDRsl assays were used according to the instructions of the manufacturer in 22 Cuban clinical isolates of *M. tuberculosis* previously reported as MDR by Proportion Method (PM) and Nitrate Reductase Assay (NRA). These methods were also used to detect resistance to second line drugs.
Results: 13 strains had mutations associated with resistance to isoniazid and only one showed a wild type pattern for katG gen. Mutations in rpoB gen were found in 95.45% of MDR strains. Resistance to ofloxacin was found in one strain showing mutations in the gyrA gen. 4 out 5 strains considered resistant to kanamycin by phenotypic methods, reveal mutation in rrs gen. For capreomycin, 80% of resistant strains had mutations related with resistance to this drug. Five strains had mutations in embB gen but only 40% were phenotypically resistant to ethambutol (EMB). Otherwise one EMB-resistant strain showed a wild type pattern to embB gen.
Conclusion: Genotype MTBDRplus is a very useful tool for MDR resistance detection while GenoType MTBDRsl assay is a promising test for rapid identification of the most common mutations involved in resistance to second line drugs.

R2438 Molecular detection of bacterial bloodstream infections: the SepsiTest™ assay

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Objectives: Implementation of rapid molecular diagnostics in bloodstream infections could significantly improve speed of diagnosis, and thereby outcome. Current molecular tests directly on whole blood samples have suboptimal sensitivity, due to the use of small volumes. Larger volumes show an inhibitory effect of human DNA. The Sepsitest™-assay (Molzym, Germany) incorporates a pre-test enrichment method, which selectively eliminates human cells. This may lead to an increase in input volume and subsequent diagnostic sensitivity. We investigated the use of this assay in a cohort of patient with sepsis on the ICU.
Methods: We analysed 55 septicaemic patients on the ICU in whom blood cultures (BC) were taken. Together with the BC (n = 90), an additional blood sample (EDTA) was taken from the same sample site, for analysis with the Sepsitest™-assay (ST). The assay consists of a pre-test-enrichment on 1 ml EDTA-sample, followed by bacterial lysis and DNA-isolation, and subsequent amplification using 16S-based universal primers for Gram-negative and Gram-positive bacteria. Amplicons are detected by agar-based gel-electrophoresis. In accordance with instructions of the manufacturer, EDTA-samples were analysed in duplicate, and a sample was considered positive if at least one of the duplicates was positive.
Results: Of the 90 BC, 5 (6%) yielded positive results; *S. aureus* (n = 1), *E. faecalis* (n = 3), and CNS with *E. faecalis* (n = 1). Bacteraemia was diagnosed in 3/50 (6%) of the patients. ST was positive in 11/90 (12%) of the samples. Compared to BC, sensitivity was 80% (4/5 positive BC). In total 2/3 patients (66%) with positive BC was positive with ST. Seven patients had positive ST and negative BC. In all of these patients, the clinical suspicion of bacterial infection was high, and 3 of them showed positive BC during septicemia, but at another time point when no EDTA-samples had been taken for this study. These results might therefore represent additional yield of the Sepsitest™-assay. Sequencing of amplicons is currently being performed, to provide species-specific results and control for false-positive results.
Conclusions:
- The Sepsitest™-assay can be used in clinical practice for molecular analysis directly on blood samples.
- Implementation of the assay may provide additional detection of bacteraemic patients, but results have to be evaluated with sequence results.
- Gel-based analysis is applicable, but may be troublesome to implement in the routine workflow.

Molecular virology

R2439 Development of novel multiplexed molecular assays for detection of respiratory viruses using room temperature stable reagents, the AmpliVue™ influenza A/B and AmpliVue™ hMPV/RSV assays

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Background and Objectives: Respiratory Infections cause significant morbidity and mortality in both developed and developing countries. Influenza A and B (Flu A&B), RNA viruses of the family Orthomyxoviridae, spreads in regular epidemics resulting in the deaths of more

than 250,000 people worldwide annually. Human respiratory syncytial virus (RSV) is a negative single-stranded RNA virus of the family Paramyxoviridae. RSV is the major cause of lower respiratory tract infection and hospital visits during infancy and childhood. Human metapneumovirus (hMPV) is a negative single-stranded RNA virus of the family Paramyxoviridae, and may be the second most common cause (after RSV) of lower respiratory infection in young children. Utilizing proprietary chemistries and processes, we have developed novel, room temperature stable reagents for use in our AmpliVue Taqman®-based, multiplexed, rt-PCR assays for the detection of Flu A&B or hMPV/RSV. As an added benefit, the AmpliVue™ assays provide a simplified workflow, significantly reducing the number of end-user manipulations required to perform testing. These attributes allow for more widespread and reliable use of molecular diagnostics in the detection of respiratory viruses. In this report, we describe the initial studies performed with these reagents on both the Applied Biosystems® 7500 FastDx and the Cepheid® SmartCycler.

Methods and Results: Testing was performed on cultured isolates or clinical specimens to establish initial performance of the assays. RNA was extracted on either a NucliSENS® easyMag® or Roche MagNA Pure Compact and 5 ul of each sample was added to reconstituted master mix. Each cultured influenza A and B isolate was detected; 19/19 and 14/14, respectively. Clinical specimens analyzed for the presence of either RSV A, RSV B or hMPV were able to detect 10/10 RSV A, 13/13 RSV B, and 26/26 hMPV. Specificity was 100% for all samples evaluated. Initial analytical sensitivity tests for the various viruses indicated detection limits less than 50 TCID50/ml and/or 10vp/mL for each target. Testing with isolates of other common viruses and bacteria confirmed that these reagents are not cross reactive with other common respiratory pathogens.

Conclusion: Results from these studies indicate that our room temperature stable reagents, coupled with the simplified workflow for our AmpliVue molecular assays, provides end-users with sensitive and specific assays for the detection of Flu A&B and hMPV/RSV.

R2440 Quantitative PCR for detection and monitoring of active CMV infection in routine diagnostics

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Objectives: Infections with CMV are usually asymptomatic or related with harmless symptoms. Severe problems occur if fetuses, transplant patients, or patients under immunosuppressive therapy get infected. The aim of this study was to investigate the impact of quantative PCR for diagnosis and monitoring of an active CMV infection.

Methods: A total of 138 patients were enrolled in this study, 72 males and 66 females aged 30 days to 72 years. Both children (n = 16) and adults (n = 122), immunocompetent (n = 40) and immunosupressed (n = 98), hospitalized with clinical suspicion of CMV infection, in the University Hospital of Ioannina, Greece, were screened for CMV-IgM antibody (AxSYM, Abbott) and CMV-DNA (COBAS AMPLICOR, Roche). Viral load and clinical data was also investigated.

Results: The confirmation of CMV infection by the two methods was obtained in 14 patients. One hundred four patients were positive for CMV-IgM and negative for CMV-DNA. Antibodies for HSV, VZV or EBV were also detected in some of them. By performing PCR, 20 extra cases of active infection were diagnosed, for which no antibodies could be detected. This translates to a 15% rise in the number of diagnoses of active CMV infection as compared with serological approach. The CMV-DNA positive patients were 6 children and 28 adults. The major underlying disease was haematological malignancies (14/34). Pneumonitis was the most common clinical presentation (20/34). Overall, the median viral load was 2255 copies/ml (472–58700), while in them with CMV mononucleosis was 583 copies/ml (472–874). The overall mortality rate was 12% and major cause was respiratory failure. Eighteen of the 34 PCR positive patients received ganciclovir. Treatment led to a marked decrease in CMV DNA copy number. The median time interval necessary to obtain a negative result after implementation of treatment by PCR was 27 days.

Conclusion: Quantative PCR CMV assay is rapid, and linear for quantifying CMV viral load, and it seems to be useful in the diagnosis and management of affected patients for predicting disease and monitoring response to antiviral therapy and can serve as surrogate markers for antiviral resistance and clinical relapse in these patients. Compared with traditional serologic assays that detect antibodies to CMV, Q-PCR offers a significant advance through the direct detection of viral DNA, which is independent of a functioning humoral immune system.

R2441 Detection of HSV1 and HSV2 DNA from external anogenital lesion specimens using the BD Viper™ System in extracted mode

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Objective: To characterize the performance of the new BD ProbeTec™ Herpes Simplex Viruses (HSV1 & 2) Q^x Amplified DNA Assays* using swab specimens collected from external anogenital lesions expressed into BD™ Universal Viral Transport medium (UVT) or equivalent Copan Universal Viral Transport Medium (UTM-RT) System.

Methods: The BD ProbeTec Herpes Simplex Viruses (HSV1 & 2) Q^x Amplified DNA Assays were designed to be compatible with transport via the Swab Diluent for the BD ProbeTec CT/GC Q^x Amplified DNA Assays (Q^x Swab Diluent) and BD UVT or Copan UTM-RT collection devices. This study focused on evaluating performance of the HSV assays using simulated specimen comprising 0.5mL BD UVT medium added to a pre-filled Q^x Swab Diluent Tube. Samples were pre-warmed, and then loaded directly onto the BD Viper System for DNA extraction and amplification.

Results: The analytical limits of detection (95% proportion positive) for the HSV1 Q^x and HSV2 Q^x assays using clean BD UVT medium were estimated to be 24 viral particles (vp)/mL and 88 vp/mL, respectively. The limits of detection for the assays in the presence of external anogenital swab specimen matrix were estimated to be 17 vp/mL and 127 vp/mL for HSV1 Q^x and HSV2 Q^x, respectively. Both assays were shown to be tolerant to common exogenous and endogenous substances that may be present in external anogenital lesion specimens, including but not limited to blood, mucus, semen, leukocytes and various prescription and over-the-counter medications. In addition, both assays were found not to cross-react with a variety of bacteria, viruses and fungi that could be found in external anogenital lesion specimens. Stability of HSV DNA in simulated external anogenital swab specimens was demonstrated at ambient, refrigerated and frozen temperatures in both the original UVT specimen and once diluted in the Q^x Swab Diluent.

Conclusion: The BD ProbeTec Herpes Simplex Viruses (HSV1 & 2) Q^x Amplified DNA Assays* offer excellent analytical sensitivity and robust performance for the detection of HSV1 and HSV2 DNA from external anogenital swabs expressed into BD UVT medium. The BD Viper System software allows the user to test both CT/GC specimens and HSV1/HSV2 specimens within the same Viper run, providing workflow flexibility.

*Product not for sale, for investigational use only in the US.

R2442 Comparative evaluation of two commercial HCV genotype tests: Versant assay (LiPA) 2.0 versus 1.0

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Background: HCV genotyping is used to predict the response to antiviral therapy and also to optimize the duration of treatment. The 5-untranslated region (5-UTR) is the region of choice for routine genotyping of HCV. However, due to its high level of conservation, the 5-UTR is limited in its ability to discriminate genotype 6 from genotype 1 and subtypes within genotypes 1, 2, 3, 4, and 6. The newly developed Versant HCV genotype assay (LiPA) 2.0 (Versant 2.0) uses sequence information from both the 5-UTR and the core region, allowing

distinction between HCV genotype 1 and 6 and between subtypes a and b of genotype 1. Previously, Versant HCV genotype assay (LiPA) 1.0 (Versant 1.0) only used sequence information of 5-UTR. In this study, the results of both genotyping assays were evaluated.
Methods: A total of 556 HCV-positive samples (EDTA plasma) were genotyped using the Versant 2.0 according to the manufacturer's instructions. For the comparison study, Versant 1.0 was used. HCV RNA was extracted by using the NucliSENS® easyMAG™ (BioMèrieux). In each extraction run, positive and negative controls were included. Only interpretable results by both assays were included in the study. Only differences found at the genotype 1 and subtypes 1a and 1b level were taken into account. The Versant 2.0 was considered the reference method.
Results: Table 1 gives an overview of the results of the comparison study after testing by both assays. The correlation rate of both tests was 85.8% (477/556). Results from 79 specimens (16.2%) were discordant between the two assays, being the genotype 1a the most difficult to discriminate by the Versant 1.0 (40 samples).
Discussion: Versant 2.0 showed an improvement in identifying the correct subtype of genotype 1. This improvement can be attributed to the additional information available from the core region of the HCV genome. As the clinical management of patients infected by genotype 1a and 1b is equal, disagreements among both tests may present epidemiological consequences but at least Versant 1.0 errors do not affect treatment dosage and duration.

VERSANT 1.0	VERSANT 2.0			TOTAL
GENOTYPE	**1**	**1a**	**1b**	
1	5	**40**	**9**	54
1a	**1**	209	**3**	213
1b	**3**	**23**	263	289
TOTAL	9	272	275	556

R2443 Prevalence of human papillomavirus in cervical samples from sexually active young women: a cross-sectional age-stratified study

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Objectives: This cross-sectional study aimed at the identification of HPV infection prevalence in sexually active young women aged 14–26 years – target of HPV vaccination programme – residing in Northern Italy.
Methods: 651 cervical swabs were collected from women aged 14–26 years. The population was age-stratified in 4 groups: (1) 14–17 years, (2) 18–20 years, (3) 21–23 years, and (4) 24–26 years.
Samples were centrifuged at 3500xg for 15′ to obtain the cell pellet. DNA extraction was carried out by a commercial kit (NucliSENS®miniMAG®, BioMérieux, France) and the amplification of a L1 gene fragment (450 bp) was performed by degenerated primers pair. Genotyping was carried out through restriction fragment length polymorphism technique using 3 restriction enzymes (RsaI, HaeIII, DdeI, Recombinant Enzyme, BioLabs Inc, New England).
Results: The overall prevalence of HPV infection was 25.8% (168/651). The age-stratified prevalence of HPV infection was: 41.3% in women aged 14–17 years; 18.2% in those aged 18–20 years; 35.5% in women aged 21–23 years, and 37.6% in those aged 24–26 years.
The prevalence of genital high-risk HPV was 78.9% in group 1, 82.8% in group 2, 83.3% in group 3, and 93.1% in the last group. HPV-16 was detected in 32/651 (4.9%) cervical swabs and HPV-18 in 6/651 (0.9%) samples.
Conclusion: The high prevalence observed in women aged 14–17 years is probably due to both the increased susceptibility of this population and an early sexual debut, major risk factor for the acquisition of HPV infection. A characteristic ascending age-specific curve of infection prevalence was observed in the other 3 groups. These data support the existence of an inverse relationship between age and HPV infection prevalence. In addition, vaccination of this whole population would have prevented about 6% of occurred infections.

R2444 HPV prevalence in adolescent population at the beginning of immunisation policy in northern Italy

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Objective: The recent introduction of vaccination against HPV in adolescent girls in Italy has focused the attention on the virological surveillance in this age group. Since HPV is one of the major pathogen sexually transmitted worldwide, it will be important to extend the surveillance also to the adolescent males, who could be the next target of the immunization strategy. This study aimed at evaluating the HPV infection prevalence in adolescents both females and males. On purpose, a molecular assay based on urine samples, easy to collect and acceptable for this young individuals, was applied for the detection and genotyping of HPV-DNA.
Materials and Methods: 545 urine samples were collected from adolescents (14–17 years) attending community clinics, youth centres of the local sanitary units or day clinics in Northern Italy in the period spanning from September 2009 to July 2010. Analysed subjects were 233 females (mean age 15.3 years) and 312 males (mean age 15.4 years). Samples were centrifuged at 3500xg for 20′ to obtain the cell pellet. DNA extraction was carried out using a commercial kit (NucliSENS®miniMAG®, BioMérieux, France) and the amplification of a L1 gene fragment (450bp) was performed by degenerated primers. Genotyping was performed by restriction fragment length polymorphism technique using 3 restriction enzymes (RsaI, HaeIII, DdeI, Recombinant Enzyme, BioLabs Inc, New England).
Results: HPV-DNA was detected in 2% (11/545) of analysed samples. In particular, in 3.9% (9/233) and in 0.6% (2/312) of samples collected from female and male adolescents, respectively. Both high and low-risk genotypes were identified: high-risk HPV-16 (11.1%) and HPV-66 (11.1%), low-risk HPV-70 (33.3%), HPV-6 (22.2%), HPV-11 (22.2%) and HPV-87 (11.1%). Among female adolescents, 8 infections were due to a single genotype and 1 was sustained by HPV-16/HPV-70 co-infection. The two infections found in male subjects were sustained by HPV-70.
Conclusions: These data show that HPV infection is present in 14–17 years old subjects, more frequently in females than in males. These infections were supported both by high- and/or low-risk genotypes and, among these, 45% (5/11) could now be preventable by the available vaccines that include one or two types found (HPV-6 and HPV-16). Molecular testing on urine sample seems to be a good alternative to those conducted on cervical swab, especially for very young women. Finally, this method seems to be applicable also to the male population.

R2445 Molecular diagnosis for human herpesvirus in critical patients affected by pneumonia

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The aim of this study was the evaluation of the often underestimated potential role of herpes viruses in the onset or inauspicious evolution of respiratory pathologies in critical patients. We analyzed 158 bronchoalveolar washes from patients hospitalized with severe acute respiratory pathologies at the intensive care units of some hospitals in Catania, Sicily, Italy.

For the retrospective study we used viral isolation methods and Real-Time PCR, respectively, for viral replication activity and the clinical significance expressed in terms of viral load, correlated with the days the patients were on assisted ventilation until the time of the analysis.
In 57.6% (91/158) of the samples DNA was found of at least one, though often two or more, of the herpes viruses (HSV1, VZV, CMV, EBV, HHV7). In particular: 19% (30/158) were HSV1, 10.7% (17/158) CMV, 16% EBV (26/158) and 46% (68/158) HHV7; there was no positivity for VZV. Based on the data relative to the finding of viral nucleic acid and the duration of mechanical ventilation, a statistically significant association was found only for HSV1 DNA with assisted ventilation of more than 7 days ($p < 0.05$). The increase of the viral load, in some cases, was directly proportional to the days on assisted ventilation reaching a value of 108 gEq/ml for HSV1, compared to 102–104 gEq/ml for CMV and EBV.
The prevalence of herpes viruses, and above all the finding of HSV1, accompanied by a substantial viral load, would confirm the importance that these viruses could have in the onset of respiratory pathologies in immunocompromised subjects. Therefore, the introduction of tests for the detection of the above mentioned viruses in diagnostic protocols would favor early diagnosis and correct therapy that could reduce the rate of mortality in critical long-term patients affected by respiratory pathologies who need assisted ventilation.

R2446 A etiology of bronchiolitis in hospitalised children in south eastern Spain

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Objectives: Bronchiolitis is the most common respiratory disease in children under 2 years-old and a major cause of hospitalization in young children, especially during the winter. Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis. During the epidemic period, over 90% of diagnosed cases are caused by this virus. However, the incorporation of new molecular techniques such as RT-PCR in the last decade has enabled the detection of other additional viral agents. The aim of this study was to investigate the prevalence and etiology of respiratory viruses, as well as ascertain the involvement of mixed viral respiratory infections in children hospitalized during the winter of 2008–2009 in south-east of Spain.

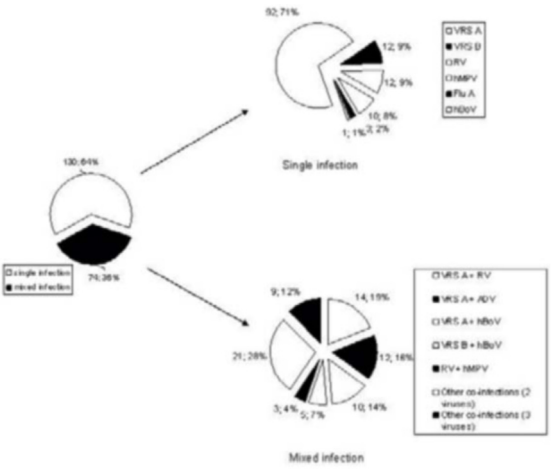

Figure 1: Frequency of single and mixed respiratory infections

Methods: It was a prospective study during the bronchiolitis season (December-April). Children below 18 months-old admitted to the hospital for a first bronchiolitis episode were included. The study excluded all infants with previous episodes of bronchiolitis as well as those bronchiolitis cataloged as acquired in the hospital. Nasopharyngeal aspirate were collected. It was performed a reverse transcription polymerase chain reaction (RT-PCR) (CLART® PneumoVir, Genomica) for the detection of 16 respiratory viruses including RSV A and B; influenza virus (Flu) A, B, and C; coronavirus (229E), adenovirus (ADV), rhinovirus (RV), parainfluenza (PI) 1–4; metapneumovirus (hMPV)A and B; bocavirus (hBoV) and enterovirus (echovirus).
Results: 235 children were included. The mean age was 3.4 months (mean±SD months, 3.4±3.3 months), range 8 days-17 months of which 78.7% were under 5 months-old. A total of 287 viruses were detected in NA from 204 infants (86.8%). Respiratory syncytial virus was the virus detected more frequently (56.4%) followed by rhinovirus. Co-infections were found in the 36% of children. The most common associations were respiratory syncytial virus A with rhinovirus (Figure1).
Conclusions: This study showed that respiratory viruses were detected in most of the children below 18 months-old hospitalized with bronchiolitis and that 36% of them showed a mixed infection. 3/4 of the children had infection by RSV, whereas the frequency of infection with other respiratory viruses was low and mainly associated to mixed infections.

R2447 Epidemiology of viral infections in acute respiratory illnesses in children

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Objectives: Acute children respiratory infections (ACRI) are a common reason for consulting general practitioners. In most cases the aetiology is unknown, yet most result in an antibiotic prescription. The objective was to study the epidemiology of respiratory virus infections and in particular to determine the virus-specific positivity rates and seasonality in pediatric age for respiratory virus infections over a 15 month period in Pordenone (ITALY), using a multiplex real-time PCR assay to detect multiple viruses in the same reaction.
Methods: A total of 195 nasopharyngeal specimens were collected from symptomatic pediatric inpatients (average 3 years) between November 2008 and January 2010. Each specimen was split into two aliquots, one aliquot was processed by using multiplex real-time PCR test and the second was tested for adenovirus using a nested PCR. Total nucleic acid was extracted using the BioMerieux easyMAG. Multiplex real-time PCR test was performed using Dia-ResRNAVir-050 (DIAGENODE) for detection of Influenza A virus (IA) and Influenza B virus (IB), Respiratory Syncytial Virus (RSV), Metapneumovirus (MPV), Rhinovirus (RVs) and Parainfluenza virus (PIV) 1, 2, 3 and 4. Adenovirus was detected by nested PCR (NANOGEN).
Results: Of the 195 specimens tested, 158 (81.02%) were positive for at least one respiratory virus including: 64 (32,8%) RSV (A or B), 34 (21,4%) Rhinovirus, 23 (11,8%) Flu A or B, 15 (7.8%) Parainfluenza (1–4), 11 (5,6%) Adenovirus and 11/185 (5,9%) Metapneumovirus. Most pediatric patients (86,6%) was pre-school age. All patients RSV and MPV (38,5%) positive were less than 4 years of age. We have seen a dual respiratory virus infection in 11/195 (5,6%) patients and only one triple virus infection. In terms of seasonal distribution, RVs were distributed across the majority of months. RSV peaks were between December 2008 and March 2009. IA infections were distributed from January and February 2009, with another "atypical" peak between October and December 2009, including Influenza A/H1N1v. IB showed a peach activity in March and April. PIVs peaked in November 2008 and October 2009. MPV peaked between the end of winter and spring months. Adenovirus infections were distributed in winter and spring months.
Conclusion: The molecular assay has increased our understanding of the epidemiology of respiratory viral infections and should assist us in the diagnosing the etiology of respiratory tract infections in individual and in outbreak situation.

R2448 Presence of rhinovirus and enterovirus in patients with suspected influenza A/H1N1 infection

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Objective: To determine the presence of rhinovirus and enterovirus in patients with suspected influenza infection during the 2009 A/H1N1 influenza pandemic.
Methods: During the peak incidence of H1N1 influenza in our hospital (last week of October 2009), a total of 91 patients were diagnosed with influenza according to strict clinical criteria. Nasal aspirates were collected and stored at −80ºC. DNA extraction was performed with the automated system Maxwell 16MDX (Promega, USA) using 200 µl of sample. All the samples were first tested for influenza A (H1N1 and H3N2) and influenza B using a real-time RT-PCR (Applied Biosystems, USA) with primers published by CDC. Then, all the samples were simultaneously screened for rhinovirus and enterovirus using a real-time RT-PCR (PrimerDesign, UK).
Results: A total of 91 patients (47 men and 44 women), ranging from 0 to 81 years, were prospectively included in the study. Of the 91 nasal aspirates, 43 were positive for influenza A/H1N1 (47,3%), 10 were positive for rhinovirus (11%), 3 were positive for enterovirus (3,3%) and the remaining 37 were negative (40.7%). All the samples were negative for H3N2 and influenza B. Overall, 54 out of 91 samples (59,3%) were positive for any of the viruses tested. In addition, two different patients were coinfected by A flu - rhinovirus and enterovirus-rhinovirus. These coinfections represented 3,7% of the overall positive samples. The average age and range of the infected patients were as follows: for H1N1, 33 years (SD±20.1) and 0–76 years, respectively; for rhinovirus, 28 years (SD±23.7) and 0–69 years respectively; and for enterovirus, 25.7 years (SD±22.4) and 0–42 years, respectively.
Conclusions: Our results showed the involvement of other respiratory viruses in patients with influenza-like illness. Thus, a notable proportion of rhinovirus infection was found in a collection of patients diagnosed with influenza during the peak incidence of H1N1 pandemic. These data would suggest a potential indication for rhinovirus detection in acute respiratory diseases. Regarding age and gender distribution of the infected patients, no significant differences were found for all the viruses tested.

R2449 Comparison of HIV and HCV viraemia counts obtained with Roche Cobas®Amplicor HIV-1 or HCV Monitor® and Cobas® TaqMan® HIV-1 or HCV respectively following a NucliSENS® easyMag™ (bioMérieux) extraction

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Objectives: Our objective was to validate the viraemia results obtained with the new Roche HIV-1 Cobas® TaqMan® and HCV Cobas® TaqMan® Test platforms following an extraction with NucliSENS®easyMag™ instead of the High Pure manual extraction recommended by the manufacturer.
Methods: Ninety-seven plasma samples from HIV patients and 37 plasma samples from HCV patients whose viraemia counts were obtained with Roche Cobas® Amplicor HIV-1 Monitor® Test v1.5 and Cobas® Amplicor HCV Monitor® Test, respectively, were tested with the new Roche Cobas® TaqMan® HIV and Cobas® TaqMan® HCV Tests. Five hundred microlitres of each of the 134 samples were extracted with the specific B protocol on the NucliSENS®easyMag™ (bioMérieux) instrument. The results obtained with the two different platforms were then compared.
Results: Fifty-three out of the 97 HIV plasmas yielded viraemia counts lying within the linearity limits of both methods. The linear regression analysis for these samples generated a R2 (square of correlation coefficient) of 0.95 comparable with the R2 (0.94) given by Roche for Cobas® Amplicor HIV-1 Monitor® and Cobas® TaqMan® HIV with High pure extraction. Regarding the HCV test all 37 samples yielded results within linearity limits with both methods and the R2 was 0.97. Regarding HIV tests we could observe that the two tests were poorly correlated for the detection of viraemia counts below the lower limit of quantification (kappa correlation coefficient: 0.39).
Conclusions: From these results we conclude that an extraction of plasma samples with the NucliSENS® easyMag™ instrument can be used with both the Cobas® TaqMan® HIV and Cobas® TaqMan® HCV platforms instead of the High Pure manual extraction, avoiding cumbersome work and long manual hands-on time without significant changes in final results.

R2450 Human papillomavirus prevalence and types among women in Herzegovina

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Objectives: Human papillomavirus (HPV) is a group of small DNA viruses that cause warts and certain cancers and precancers of the skin lining the lower genital tract and mouth. The genital HPV types can be divided into two broad groups (low-risk and high-risk HPVs) depending upon their association (or lack of association) with cancers of the lower genital tract. Genital HPV is very common. It is the most common viral sexually transmitted infection (STI) and is likely to be the most common STI overall. The aim of this study was to investigate the prevalence of HPV DNA, to determine HPV types distribution among women in 3 Cantons in Herzegovina and prevalence of hr HPV infection in different age groups of women (women over 30 years and under 30 years).
Methods: A total of one hundred sixty (n = 160) women were retrospectively evaluated between March 2009 and December 2010. Abbott RealTime polymerase chain reaction was used to detect the presence of HPV types in cervicovaginal samples obtained from patients during gynecologic examination. Abbott RT hr HPV test was performed on the m 2000 rt PCR instrument and was designed to identify 14 hr HPV genotypes: 16, 18 and other high risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).
Results: Eight (n = 8) of the women were excluded from the study because of the incomplete data and a total of 152 women were used for the final analysis. Forty-five patients (29.6%) were found to be infected with a genital HPV. As expected, viral prevalence was higher among women younger than 30 years of age (15.8%) in comparison to those aged 30 or older (13.8%). The rate of o HPV types were as follows: 16 (37.8%), 18 (8.9%) i other (53.3%). Coinfection with multiple HPV types had one woman. The largest number of positive samples was from Herzegovina-Neretva Canton.
Conclusion: Our results indicated that HPV infections represent a significant public health concern in Herzegovina. Detailed knowledge of HPV type circulating patterns in specific local geographical areas is essential for appropriate implementation of screening, prevention, and surveillance campaigns.

R2451 Contribution of influenza viruses, human metapneumovirus and respiratory syncytial virus to acute respiratory infections in children in northern Greece, 2008–2010

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Objectives: Influenza viruses, respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) are the most common pathogens that cause acute respiratory disease in children. The aim of this study is to present the contribution of the above three pathogens to influenza-like illness (ILI) in children (aged <6 years old) during 2-year (2008–2010) influenza seasons in N. Greece.
Materials and Methods: 430 pharyngeal swabs from children younger than 6 years, presented as ILI infections during the last two influenza seasons (2008–2009 and 2009–2010) were examined for influenza A and B, RSV and hMPV, by one step Real-time RT-PCR.

Results: Influenza viruses were detected in 122 (28,3%) of the 430 spesimens, RSV in 45 (10,4%) samples and hMPV in 28 (6,5%). RSV and influenza viruses' co-infections were observed in eight cases, RSV and hMPV co-infections in four cases and hMPV with influenza viruses was found in one case. The majority of the patients (67,7%) were between 3 and 6 years old.
Conclusion: Our results demonstrate that influenza viruses, RSV and hMPV contribute to ILI presenting infections at a rate of 45,2% in children younger than 6 years old.

Molecular mycology

R2452 A quick and low-cost PCR-based assay for detection of *Candida* DNA from blood culture bottles: a pilot study

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Differences in susceptibility of *Candida* species to antifungal drugs make identification to the species level important for clinical management of candidaemia. Molecular tests are not yet standardized or available in most clinical laboratories, although they can reduce the time required for species identification, as compared to the conventional methods. To cut laboratory costs and improve diagnostic accuracy, different molecular methods have been proposed, including DNA extraction protocols to produce pure DNA free from PCR inhibitors.
Objective: To identify *Candida* species causing candidaemia by analyzing fungal DNA from blood culture bottles positive for yeasts using a low cost-effectiveness assay.
Methods: For DNA extraction an "in house" protocol based on organic solvent extraction was tested. Additional steps of liquid nitrogen incubation followed by mechanical disruption were processed for complete cells lysis. The purity of extracted DNA was evaluated. Fifty blood culture bottles positive for yeasts were processed. PCR assays amplified ITS region of rDNA gene. The amplicons of twenty samples were sequenced and these sequences were submitted for comparison on Genbank database (NCBI) for species identification. Molecular yeast identification was compared to results provided by conventional methods.
Results: The organic solvent extraction protocol showed good reproducibility on the amount of DNA extracted, pure DNA and high PCR sensitivity (10 pg of *C. albicans* DNA and 90% amplification on PCR assay). Therefore, this method was used to analyze all clinical samples available. The molecular species identification showed 100% concordance to the conventional culture. However, the molecular method could identify one sample with mixed infection caused by *C. albicans* and *C. glabrata*, while the conventional culture identified just *C. albicans*. Moreover, 3 samples identified as *C. parapsilosis* by the classical method were molecularly identified as *C. orthopsilosis*, a species belonging to the *C. parapsilosis* complex.
Conclusions: Organic DNA extraction, PCR assay and sequencing could efficiently identify mixed infections, as well as *Candida* species that only can be molecularly identified. This test can be easily implemented in routine laboratories providing earlier and low cost species identification when compared to the traditional methods. The organic DNA extraction was 4-fold less expensive than protocols using commercial kits.
Support: FAPESP 2007/08575–1.

Molecular typing

R2453 Use of modified PCR ribotyping for detection of *Clostridium difficile* ribotypes directly in stool samples

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Objectives: *Clostridium difficile* infections represent significant burden for health care system with small and large outbreaks constantly being present in hospital environment. Some of the PCR ribotypes were lately associated with outbreaks and with increased mortality and morbidity: 027, 078, 017 and 053. It is therefore important to recognize these ribotypes as rapid as possible. Standard typing method in Europe is culturing of *C. difficile* from faecal sample and subsequent PCR ribotyping. Here we describe for the first time the modification of PCR ribotyping that can be used for direct detection in stool samples and its use during emergence of PCR ribotype 027 in a single hospital.
Methods: For direct PCR ribotyping from stool sample we have modified existing primers described by Bidet et al. (1999) to increase specificity for *C. difficile*. A total of 50 *C. difficile* culture positive and 43 negative stool samples from the routine laboratory were then used for validation of the method. DNA was isolated from faecal sample and PCR ribotyping was performed with Bidet and with new primers. Additionally, five stool samples from general hospital, which were detected as "*C. difficile* positive, presumptive 027/NAP1" by Cepheid Xpert *C. difficile* assay were submitted to the reference laboratory for ribotype 027 confirmations.
Results: Direct PCR ribtyping from stool samples was possible in 37 out of 50 *C. difficile* positive samples using new primers. Other 13 samples were negative (n = 9) or the banding pattern was too weak to be analyzed (n = 4). In 36 out of 37 cases PCR ribotype determined directly from the stool was identical as PCR ribotype of the strain isolated from the same stool sample (sensitivity 0.72–36 out of 50 positive samples). All, but one *C. difficile* culture negative samples were negative on direct PCR ribotyping with modified primers. In contrast, 30 out of 43 *C. difficile* negative samples reacted with Bidet primers. All five Cepheid Xpert "*C. difficile* positive presumptive 027/NAP1" samples were confirmed as ribotype 027 by direct ribotyping as well as conventional ribotyping of the cultured isolate.
Conclusion: The direct culture-independent PCR ribotyping of *C. difficile* is a useful and rapid method for detection of emerging strains and outbreak identification.

R2454 Genotyping of atypical *Vibrio cholerae* eltor strains from Siberia and Far Eastern region of Russia

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Objectives: A feature of the seventh cholera pandemic during last decades is a replacement of typical El Tor (ET) biotype strains to an altered ET biotype possessing determinants of cholera toxin (CT) of the classical (CL) biotype. The new strains are common in endemic areas and demonstrate higher pathogenic properties. The purposes of this study included revealing and molecular-epidemiological characteristic of atypical *V. cholerae* strains isolated in Siberian and Far Eastern regions of Russia.
Methods: Sample set included a toxigenic *V. cholerae* strains (n = 32) from patients and environmental samples, isolated during outbreaks and sporadic cases of cholera in 1970s and 1990s. Twenty nine strains belonged to ET biotype, including 19 and 8 strains isolated in Siberia and at the Far East during 1990s and 1970s, respectively. Two ET strains from European part of Russia and three CL strains were used as external group for comparison. *V. cholerae* eltor M-878 and *V. cholerae* cholerae 569B were used as a control. Biovar-specific identification of tcpA, rstC, rstR, hlyA, rtxA, rtxC genes and TLC-element were carried out by PCR as described previously. Allele-specific PCR (AS-PCR) for detection of ctxB genomovars was fulfilled according to Morita et al. (2008). Standard sequencing procedure of ctxB gene was performed for confirmation of AS-PCR results and for detection of numbers of ToxR-binding repeats in the zot-ctxA intergenic region.
Results: Among total ET strains isolated during 1970s CT ET biotype gene (ctxB3) was revealed. ET strains isolated in 1990s harbored CT CL biotype gene (ctxB1). At the same time all these strains had ET biotype-specific genetic determinants. Among nearly all ET strains we observed four ToxR-binding repeats in the zot-ctxA intergenic region. ctxB1 strains demonstrated heterogeneous TCL and rstR pattern: part of TCL+ strains harbored rstREL, part of TCL+ and all TLC− strains had both rstREl and rstRCL.

Conclusion: Three genotypes of atypical ET strains imported from different regions were revealed (ctxB1TLC+rstRET+rstRCL−; ctxB1TLC+rstREL+rstRCL+ and ctxB1TLC−rstREL+rstRCl+). Analysis atypical ET genotypes allowed to conclude a consecutive acquisition of genetic determinants from CL biotype and formation of more pathogenic clones.

R2455 Molecular typing of rotaviruses, isolated on the territory of Ukraine

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Objective: Diarrheal diseases can be caused by viruses that belong to different species, but rotaviruses are most often the cases of severe diarrhea with fatal consequences. The aim of the present study was the investigation of rotavirus circulation among children under 5 years old, hospitalized with severe diarrhea in different regions of Ukraine and rotavirus genotype identification.
Methods: Stool specimens were selected from 600 young children under 5 year old, hospitalized in 6 Ukrainian regions: South, North, West, East, Center and Kyiv from 2006 to 2009. The detection of rotaviruses group A was performed by chromatographic immunoassay (CITO TEST ROTA, CerTest Biotec. S.L., Spain). All specimens positive for rotaviruses were confirmed and identified by RT-PCR (AmpliSens® Rotavirus-290, InterLabService, Russia).
Results: It was shown that proportion of severe diarrhea, caused by rotaviruses in 5 regions of Ukraine in the period of study was: in the East – 10% in the South – 44,5% in the North – 24,8% in the West – 45,4%, in the Center – 21,1%. The winter-spring seasonality was confirmed, and it was found that in the age group of children under 3 years the average frequency of rotavirus identification was the highest and amounted to 70,1±4,0%. As a result among 210 positive samples it was detected G-genotype in 182 cases (86,7%) and P-genotype in 176 cases (83,8%). P-genotype and G-genotype were not identified in 3,3% and 4,3% of samples, respectively. In 5,7% of samples both genotypes were not identified. It was shown that during each epidemic season from 2006 to 2009 in Ukraine G1P[8] was the dominant genotype, which varied from 30% to 80% of all positive samples. The second most distributed genotype was G4P[8] (40%), third – genotype G3P[8] (25%), and the fourth – G2P[8] (11%). During the epidemic period 2006–2009 in Kiev, for the first time genotype G9P[8] was identified in 5% of cases. Thereafter it was found seldom during 2007, then appeared in rare cases. In some clinical samples multiple genotypes were identified: G1P[8] + G3; G1P[8] + G2; G3P[8] + G4. Genetic variant G2P[4] was the cause of rare cases of diarrhea during the studied period.
Conclusions: For the first time the features of rotavirus group A circulation in Ukraine among children under 5 years old were shown. The obtained data of the major rotavirus genotypes has a great importance in deciding the implementation of specific prevention of rotavirus diarrhea in Ukraine.

R2456 Cost-effectiveness of PCR versus culture for MRSA in hospitals: evaluation of the published evidence

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Objectives: The much decreased turnaround time of PCR tests has still controversial impact on MRSA infection rates in hospitals. MRSA rapid diagnosis however does not only concern screening but severe life threatening infection diagnosis as well. The cost effectiveness for a variety of important clinical outcomes of PCR for MRSA in hospitals has not been sufficiently evaluated. We sought to systematically review all studies in the English literature that evaluate the comparative cost effectiveness of MRSA molecular testing versus traditional methods and report the associated findings.
Methods: A literature search was performed in PubMed, Scopus and the NHS Economic Evaluation Database. Quality evaluation of the selected articles was performed using a standardized instrument for the quality of economic analyses.
Results: Eleven studies were identified comparing molecular assays for MRSA over traditional culture and/or chromogenic agars for a total of 22757 patients. 4/6 cost-effectiveness studies found that PCR incurs higher costs but better outcomes while 1/6 found multiplex PCR cost effective among 31 methods when length of ICU stay is <2 days. 1/6 found PCR not cost effective. 3/4 cost-benefit studies found PCR for MRSA cost saving, and 1/4 found PCR not cost saving in an existing search and destroy policy. One cost utility analysis reported that PCR leads to important mortality reduction at a low cost/LYS, due to an under the hour change in empirical antibiotics. Factors that determine cost effectiveness in various settings are the baseline prevalence rate of MRSA, applying screening and pre-emptive isolation to high-risk patients, the availability and cost of isolation and last but not least, the assay characteristics and individual test costs.
Conclusion: The use of PCR instead of culture screening for MRSA is found to be cost effective and/or cost-saving particularly in surgery. The significant benefits are less isolation, less overall hospital stay, more infections avoided, timely and cheaper treatment choices and lower mortality. Although more studies are urgently needed, especially in severe infections diagnosis, infection control teams for MRSA in hospitals can be preliminarily informed about the particulars of the cost effectiveness of molecular assays over traditional techniques according to each hospital's settings.

R2457 Genomic changes in the populations of *Bordetella pertussis* strains isolated in Poland before and after the 1990s

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Objectives: The aim of the study was the evaluation of genomic diversity differences among *B. pertussis* strains collected from patients before and after the 90-ties in respect to vaccine strains used for production of the DTP vaccine.
Material and Methods: In the study, two collections consisting of 29 and 111 *B. pertussis* clinical isolates originated from 1960–1977 and 1995–2005 periods, respectively and six historical (A/63, 21/60, 7/60, 25593/65, 1326/62, 60623/67) and three current (606/67, 186/65, 629/65) vaccine strains were investigated. EUpertstrain Pulsed-Field Gel Electrophoresis (PFGE) procedure with XbaI was applied and compared with PFGE procedure with AflII enzyme. The obtained PFGE profiles were analyzed using GelCompar software. The UPGMA algorithm was used as the clustering method, with 2% band tolerance and 1.5% optimization settings with the Dice coefficient. PFGE profiles obtained for clinical isolates and vaccine strains were compared to *B. pertussis* PFGE reference strains.
Results: Among 111 strains isolated in the period from 1995–2005, 59 PFGE-XbaI and 64 PFGE-AflII profiles were identified. In the period 1960–1977, 18 PFGE-XbaI and 24 PFGE-AflII profiles were found among 29 strains. *B. pertussis* strains from 1960–1977 typed with PFGE-XbaI, were classified into 2 clusters: A and B, and 2 groups: III and IV. The same set of strains typed with PFGE-AflII, were found in 3 clusters: Afl-A, Afl-B and Afl-III. *B. pertussis* strains isolated within 1995–2005, typed with PFGE-XbaI, were found in the groups III, IV and V and in clusters A and C. All currently used vaccine strains were found in the group III. In the period from 1995–2005, compared with the period from 1960–1977, frequency of strains belonging to groups III and IV and cluster A increased, new group V and new cluster C have been found and cluster B has disappeared. According to PFGE-AflII results, new clusters: Afl-C, Afl-D, Afl-E and Afl-IV have been recognized and cluster Afl-B has disappeared. In the period 1995–2005 genetic similarity has not changed significantly.
Conclusions: The study showed that genetic similarities of *B. pertussis* strains coming from 1995–2005 and 1960–1977 do not differ significantly and represent level 80.5–81.8% for PFGE-XbaI and 77–79% for PFGE-AflII. Optimized PFGE-AflII system has been found as potential additional tool for discrimination among closely related *B. pertussis* isolates.

R2458 Clonality of *Escherichia coli* isolates that cause repetitive urinary tract infections in patients with spinal cord injury

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Introduction: Recurrent infections are not low among urinary tract infections (UTI). One of the most important complications for patient with spinal cord injury is urinary tract infections. Recurrence rate of UTI among these patients are very high. One of the main questions is if these recurrent infections are due to the same clone or different clones. The aim of this study was to evaluate clonality of infectious agents isolated from urinary samples of patients with spinal cord injury.
Materials and Methods: A total of 68 *E. coli* isolates from 23 patients were included to present study retrospectively. Of 23 patients 13 were women (56,5%) and 10 men (43,5%). All patients had at least 3 samples. The antimicrobial susceptibilities of recurrent infection agents were tested by disk diffusion method. Clonality was tested by PFGE. Total DNA was restricted with XbaI and DNA fragments were separated using CHER DR III.
Results: Among 23 patients 21 were infected with the same clone at least twice. Among these patients 50% were women and 50% were men. Of 68 *E. coli* isolates from 23 patients, were 9 days minimum and 200 days maximum. Of 22 clones 12 were ESBL positive. A total of 31 isolates were among 12 ESBL clones. Clones isolated from women were relatively lower (56,5%) than from men (43,5%).
Discussion: The present study showed high rates of clonal urinary tract infections among patients with spinal cord injury. Further studies are needed to evaluate risk factors for recurrent urinary tract infections among patients with spinal cord injury.

Diagnostic/laboratory methods (other than molecular)

R2459 *Legionella pneumophila* detection and viability evaluation by flow cytometry

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Objectives: Legionellosis occurs through inhalation of contaminated aerosols expelled by wet cooling systems or hot water distribution systems of healthcare facilities. Despite the prolonged incubation period, culture remains the standard diagnostic method. Alternative methods like antigen detection and molecular techniques are expensive, often unavailable and give no information regarding *Legionella pneumophila* viability, which can be of minor importance in clinical specimens but of major impact in water samples and therefore in public health security. Considering its high mortality rate and the need of specific treatment, the development of a rapid, sensitive and specific method for *L. pneumophila* detection is crucial. Also, distinction between viable and non-viable cells in water is warranted. Therefore, we propose a flow cytometry protocol for *L. pneumophila* detection in clinical and water samples and viability evaluation in the latter.
Methods: *L. pneumophila*, ATCC33155, was used for protocol optimization. Fifty respiratory samples and twenty water samples previously analysed by immunofluorescence were screened by flow cytometry. All samples were stained with a specific antibody MONOFLUO™ Anti-*L. pneumophila* (BioRad, California) (green-530nm), which reacts with an external membrane protein found in all known *L. pneumophila* serogroups, and with Propidium iodide (red-FL3) to evaluate bacteria viability in positive water samples, where the analysis was made before and after killing bacteria by heat. Flow cytometry analysis was performed on FACSCalibur Cytometer (BD Biosciences, Sydney). Specificity was studied by mixing *L. pneumophila* with suspensions of *E. coli*, *S. aureus* and *C. albicans* type ATCC strains, according to the same protocol.
Results: *L. pneumophila* cells displayed an intense green fluorescence, indicating bacteria recognition by specific antibody. Dead cells showed high intensity of fluorescence in both channels, FL1 and FL3. A 100% correlation was achieved between immunofluorescence and flow cytometry assay, in both clinical and water samples. Viability assessment was successful.
Conclusion: Flow cytometry proved to be a sensitive and a specific method to detect *L. pneumophila* and simultaneously to assess its viability. Furthermore, while it has been used in the detection of *Legionella* in environmental specimens and experimental systems, this is the first time that this technique is applied to detect *L. pneumophila* in clinical specimens.

R2460 Antimicrobial suseptibility of *Aspergillus*, *Candida* and *Fusarium* species isolated from keratitis in eastern India

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Purpose: To find out the antifungal drug sensitivity pattern of common fungi responsible for cases of fungal keratitis in Eastern India and to provide better guidance for appropriate choice of antifungal drugs.
Methods: A retrospective, noncomparative study of 25 commonly selected fungal keratitis patients for antifungal susceptibility between August 2008 to July 2010.
Results: Amphotericin B and Natamycin were sensitive to all selected species isolated for susceptibility test. Amphotericin B had a lowest MIC against *Fusarium* sp. Voriconazole was the most effective antifungal with lowest MIC against all *Aspergillus* sp isolated from fungal keratitis except *Candida* sp. *Aspergillus*, *Candida* and *Fusarium* were insensitive to fluconazole and miconazole. Ketoconazole had a best MIC against *Candida*.
Conclusion: Voriconazole is still the first choice in the treatment of mould keratitis. Disk diffusion method might be a practical method for preliminary assessment of in vitro antifungal susceptibility testing.

R2461 Assessment of mycoplasma duo kit culture system for the detection of *Mycoplasma hominis* and *Ureaplasma urealyticum* in cervicovaginal secretions

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Objective: Comparing results of a commercially available selective culture system for isolation of *Mycoplasma hominis* and *Ureaplasma urealyticum* with their detection using PCR amplification.
Materials and Methods: Two endo-Cervical swabs have been collected from each case of a cohort of 40 infertile females attending fertility centers for management of infertility (group A), and from a control group of 40 multiparus females attending contraception clinic (group B). One swab have been subjected to culture for isolation of urogenital mycoplasmas using a commercially available selective culture system (Bio-Rad, USA) and the other sample was eluted in a buffer for PCR amplification using specific primers for *Mycoplasma hominis* and *Ureaplasma urealyticum*.

Agreement of PCR with culture

	Culture				FEp	Significance
	Positive		Negative			
	No.	%	No.	%		
PCR						
Positive	17	42.5	2	5.0	<0.001*	S
Negative	2	5.0	19	47.5		
	Sensitivity	Specificity	PPV	NPV	Accuracy	
	89.47	90.48	89.47	90.48	90.00	

Results: Using PCR, 19 of group A and 9 of group B were positive for *Mycoplasma hominis* while 12 of group A and 8 of group B were positive for *Ureaplasma urealyticum*. According to Duo kit results, 5 cases of

groupA and one of control group B were positive for *Mycoplasma hominis* while 10 and 8 cases were positive to *Ureaplasma urealyticum* in group A and B respectively. The sensitivity of Duo kit culture system in relation to PCR was found to be 89.47% and the specificity was 90.48.
Conclusion: Our study confirm the reliability of *Mycoplasma* Duo kit culture system for diagnosis of genitourinary colonization of the female genital system with *Mycoplasma hominis* or *Ureaplasma urealyticum*. Also we report a higher sensitivity than the PCR.

R2462 Review of the Tuberculosis External Quality Assessment (EQA) programme in Zambia from 2005 to 2008

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Introduction: The NTP has been conducting quality assurance through the reference laboratory for the past four years to determine quality improvement in Tb control. Quality improvement is a process by which the components of smear microscopy diagnostic services are analyzed with the aim of looking for ways to permanently remove obstacles to success.
Direct sputum smear microscopy remains the most cost effective tool for diagnosing patients with infectious tuberculosis and for monitoring their progress on treatment. The case detection rate for Zambia is 58% and falls short of the target of detecting 70% of all infectious cases of TB.
Specific objectives:
1. To evaluate blinded rechecking results
2. To examine proficiency testing outcomes
3. To determine the solutions provided during on site evaluation

Methodology: This was a retrospective descriptive study, using the quarterly reports and the data collected by the Chest Diseases Reference laboratory from nine provincial hospitals between 2005 to 2008.
Results: Copperbelt had (15), Eastern (4), Northern (3) Southern (4),UTH (7) Central (0) and Western (5), Luapula (2) North-Western (2) errors.
The data for 2007: North-western (25) High False Negatives, 15 (6LFN, 4HFP, 2LFN) Southern and Central 28 (12HFP, 12LFP, 4LFP).
The data for 2008: There were (9) Arthur Davison Hospital, UTH and Kasama General hospitals. Seven (7) Kitwe, Central, Mansa General. Kabwe General (6).
On-site evaluation: No laboratory communication network which resulted in inefficiency in results transmission and feedback. Lack of adequate work space and ventilation in 90% of the laboratories.
Conclusion: The laboratory performance was above the expected major errors. Proficiency testing outcomes were below 70% case detection in Zambia.

R2463 Comparison of the performance of IFA, CFA, and ELISA assays for the serodiagnosis of Q fever by quality assessment

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Objectives: Serology is important in diagnosing Q fever. Different tests are available and comparability is not always clear. A quality assessment for various Q fever tests was performed by comparing IFA, CFA and ELISA, using serum samples from patients from a large outbreak in the Netherlands.
Methods: A total of 25 serum samples were included from negative controls, acute Q fever patients, and a serial diluted high positive sample. Thirteen laboratories participated: eight from the Dutch Q fever endemic area, two Dutch national laboratories and three reference laboratories from outside The Netherlands. Six labs performed CFA, 9 performed IFA, of which three were in-house assays, and 5 performed ELISA.
Results: IFA, ELISA and CFA values between laboratories using the same methods were within close range and all three methods correctly identified the Q fever patients. In house and commercial IFAs were well comparable quantitatively. In titres reached some differences could be observed.
All tests were reasonably specific (92–100%), however the sensitivity showed more variation. IFA was the most sensitive test for both phase 1 and 2, with a sensitivity of 100% if IgG and IgM responses were combined. For phase 2 antibodies the CFA performed well (100%) but the sensitivity for phase 1 was only 61%. The phase 1 IgG ELISA was more sensitive (67%) than phase 1 CFA, while phase 2 IgG and IgM ELISAs were less sensitive than CFA (using the manufacturer's instructions).
Discussion: The higher sensitivity of the IFA was supported in the serial dilution, however these observations were based on a limited number of samples and should be refined using a larger set of sera. The IFA appears the method of choice if high sensitivity is required (e.g. early phase of illness). ELISAs can be an alternative or for screening of large sample numbers.

R2464 The utilisation of gluconic acid in certain strains of *Acinetobacter baumannii*

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Objective: A characteristic feature of glucose oxidizing acinetobacters is their ability to produce a brown pigment in blood agar. The aim of this study was to evaluate the nature of brown pigment produced by *Acinetobacter baumannii*.
Methods: Two (A/B) *A. baumannii* strains were isolated from diabetic patient and identified by blaOXA-51-like PCR and restriction analysis of 16S and 23S r-RNA spacer sequences using AluI and NdeII. MICs were estimated by BSAC guidelines. Isosensitest (IST)/M9 glucose broth was used for the growth of strains. Growth of the strains was monitored over 48 hour period using IST broth having gluconic acid concentration range from 0.1 to 4%. The inhibitory activity of the pigment produced by strain A was checked by the ditch plate method.
Results: The MICs for Imipenem, meropenem, ceftazidime and cefepime were 0.5, 0.5, 8 and 4mg/L respectively for both strains. Both the strains were positive for blaoxa-51-like and blaADC but negative for blaoxa-23/40/58 and metallo-β-lactamases. Strain A produced a brown pigment in presence of gluconic acid. It also grew better than strain B over a period of 48 hours in the presence of IST broth containing gluconic acid concentrations ranging from 0.1 to 4%. Neither strain produced pigment in M9 glucose medium. When gluconic acid is added in excess, it increases the pigment in strain A. The brown pigment produced by strain A did not have any inhibitory effect against *S. aureus* NCTC6571, Ps. aeruginosa NCTC10662, *A. baumannii* ATCC19606 and *E. coli* NCTC10418. The glucose dehydrogenase enables both the strains to form 6-phosphogluonate which is free to enter the Entner-Doudouroff pathway hence there is no pigment production seen in both the strains when M9 glucose broth is used. On the other side human blood being enriched with nutrients including gluconic acid helps strain A to survive better by converting gluconic acid to 2,5 diketogluconate which leads to the formation of brown pigmentation.
Conclusion: The survival of *A. baumannii* A in gluconic acid enriched medium helps it to survive better than *A. baumannii* B. Excess gluconic acid in strain A leads to brown pigmentation which may offer protection against antioxidant stress. The results show that strain A has multiple routes of metabolism which offers it better chance for survival than strain B.

R2465 Rapid identification and antimicrobial susceptibility testing of Gram-negative bacilli directly from positive blood cultures using Vitek2

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Objectives: Prompt empirical antimicrobial treatment, after blood culture is recommended for optimal management of sepsis. Rapid

pathogen identification (ID) and antimicrobial susceptibility testing (AST) results permit early streamlining of the empirical antimicrobial treatment to the pathogen-directed treatment with potential healthcare benefits.
We compared, using the Vitek 2 automated system, ID and AST of Gram-negative bacilli (GNBs) from positive blood culture by 'direct' inoculation from blood culture broths with the 'indirect' inoculation from pure subcultures to improve the turnaround time of microbiological analysis.
Methods: Between May-October 2010, 97 consecutive GNBs from 64 monomicrobial positive blood cultures (33 sets in 2/2 and 31 sets in 1/2 bottles) were included in the study. Vitek 2 GN ID and AST N142 (Enterobacteriaceae) cards were 'directly' inoculated with bacterial suspensions, prepared by differential centrifugation of the positive blood culture broth at 160Xg 5min, to separate blood cells and 650Xg 10min to pellet bacteria. Vitek 2 GNID, AST N142 and N 143 (Non-Enterobacteriaceae) were also 'indirectly' inoculated from overnight subcultures, following manufacturer's instructions. The 'indirect' method results were considered the 'gold standard'. API ID system was used if the GNB was not identified by the Vitek 2. Discrepant AST results were interpreted as: a 'very major error' if the result of the direct method was susceptible ("S") and that of the 'Indirect' method was resistant ("R"); a major error was the opposite. All other discrepancies were considered minor errors.
Results: Of the 97 GNBs, 95 (98%) were identified by the indirect method vs. 90 (93%) by the 'direct' method. The two methods concordantly identified 95.4% (83/87) of the Enterobacteriaceae and 100% (6/6) of the *Pseudomonas aeruginosa* isolates. Nine valid isolate-antibiotic comparisons were possible between 'direct' AST N142 and 'indirect' AST N143 for Non-Enterobacteriaceae vs. 19 for Enterobacteriaceae. For 92 GNBs (Enterobacteriaceae [n = 87], *P. aeruginosa* [n = 5]), a total of 1698 valid isolate-antibiotic combinations were compared between the 'Direct' AST N142 and, 'Indirect' AST N 142 and N143 cards. Overall, the rates of the 'very major', 'major', and 'minor' errors were: 0.35% (6/1698), 1% (18/1698) and 1.3% (22/1698) respectively.
Conclusions: Direct inoculation of the Vitek 2 is rapid and reliable for ID and AST of the GNBs from the positive blood cultures.

R2466 Bloodstream infections: is there a seasonal variation?

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Objectives: Seasonal variation in rates of infection with certain microorganisms has already been described with a higher incidence rate during the warmest months than the remainder of the year. The aim of the study was to detect seasonal variation, if any, in the recovery rate of different pathogens from the positive blood cultures obtained from patients of our hospital.
Methods: All the bloodstream infections reported during the last five years were included in the study. After incubation in a continuously monitoring blood culture system (BACTEC 9050, Becton Dickinson, USA), positive blood-cultures were inoculated onto appropriate plates for standard aerobic and anaerobic cultures and incubated at 37^0 C for 24h and 48h, respectively. A Gram-stained smear was examined under microscope to obtain valuable information about the types of microorganisms present. The isolated pathogens were identified using the automated system VITEK 2 (BioMerieux, Marcy l'Etoile, France).
Results: No seasonal variation was observed in the isolation of *Staphylococcus aureus* and the coagulase-negative staphylococci. In contrast, an increase in the isolation of enterococci and *Candida* species during the warm months of the year was noted. Specifically, 52.2% of enterococci and 60.8% of *Candida* species were isolated during June-September or 68.5% and 70.3%, respectively, during May-September. The same was true for *Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella pneumoniae*. For these microorganisms, the 75.0%, 52.2% and 45.3%, respectively, of the isolates were recovered during June-September or 76.6%, 56.7% and 57.2%, respectively, during May-September.
Conclusions: Our data suggest a seasonal variation with an increase of the bloodstream infections caused by enterococci, some Gram-negative rods and *Candida* species during the warm months of the year.

R2467 Evaluation of Copan SL-solution for pre-treatment of lower respiratory samples for bacterial culture inoculation on the automated Walk away specimen processor (WASP)

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Objectives: Respiratory specimens (RS) contains a lot of mucus that must be dissolved before plating. Copan has introduced to the market the SL-Solution (SL), a ready to use tubes with 1.0 ml of mucus dissolving solution. The objectives of our study were to compare: 1) the performance of SL versus Sputasol (SP) (Oxoid) for pre-treatment of RS; 2) manual plating versus automated plating before implementing RS inoculation on the Walk away specimen processor (WASP).
Method: 166 RS were tested: 31 expectorates (ESP), 83 bronchial aspirates (ABR), 44 bronchoalveolar lavages (BRL) and 8 Pharyngo-Nasal Aspirates (AFN). 31 ESP and 8 AFN were tested in duplicate, one sample was prepared using a 1:1 (vol/vol) ratio (RS/SP), vortexed and incubated for 30 minutes at 35ºC, the other sample was prepared by transferring 1 ml of specimen to a tube of SL to obtain a 1:1 ratio (RS/SL). The other 127 samples were tested in duplicate: un-treated and SL treated. Ten microliters of RS treated or untreated samples were manually plated, samples in SL tubes were loaded on the WASP and processed following the WASP protocol (Vortex at 2500 RPM for 30 seconds and plated with 10 ul loop). All samples were incubated as per laboratory SOPs at 24 and 48 hours for semi-quantitative bacteria growth.
Results: Among the 166 samples processed, 100 were positive (30 ESP and AFN, 70 ABR and BRL) and 66 had no significant growth; no qualitative differences were noted between manual and automated streaking on the WASP. In the 30 ESP and AFN positive, pre-treated with SP and SL, there was 100% agreement for bacteria and fungi strains isolation and 93% agreement (28/30) when analyzing the bacterial load, 77% agreement (23/30) for the fungi.
Manual plating of untreated ABR and BRL vs. SL pre-treated (diluted 1:2) had 100% agreement for isolated bacteria and fungi and 91% agreement (64/70) for bacterial load, 89% agreement (62/70) for the fungi. A 99% agreement (76/77) was noted in the semi quantitative evaluation of bacteria (manually vs. Wasp-plated). The loads differences of 1 log in SL that were noted mainly with *Candida*, may due to the 1:2 SL dilution factor uneven sampling and inconsistency evaluation for seeding in quadrants.
Conclusions: Copan SL-Solution demonstrated an excellent performance and results agreement compared to the Sputasol with both manual and WASP plating. It's easy to use, doesn't require a pre incubation step and samples in SL tube can be loaded directly on the WASP.

R2468 Quantitative comparison of new liquid-based transport swabs for aerobic and anaerobic organism recovery

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Background: The development and introduction of automation to inoculate and streak samples in Microbiology laboratories, will lead to a move from gel based transport swabs to liquid based transport systems.
Objectives: To compare and evaluate the survival of fastidious organisms in two liquid based transport swabs (Copan Eswab® and Medical Wire Σ® swabs) against two gel based systems (Copan M40 and Medical Wire Transwab®). The Eswab® has a flocked swab in 1mL of modified liquid Amies, and is designed to elute the entire sample into the medium. The Σ® swab is foam tipped in 1mL of liquid Amies.
Method: The study was based on CLSI procedure M40-A using the quantitative elution method for the liquid based swabs and the roll plate method for the gel swabs, to evaluate performance. Suspensions of *Neisseria gonorrhoeae* ATCC 43069, *Haemophilus influenzae* ATCC 10211, *Streptococcus pneumoniae* ATCC 6305, *Streptococcus pyogenes*

ATCC 6305, *Fusobacterium nucleatum* ATCC 25586, *Prevotella melaninogenica* ATCC 25845 and *Peptostreptococcus anaerobius* ATCC 27337 were prepared by diluting 0.5 McFarlane solutions, made from fresh cultures. The swabs, in triplicate, were inoculated by immersion in 100 μL of the suspensions for 10 seconds. Recovery was measured by quantitative plate counts after 0, 24, and 48 hours storage at room temperature.

Results: After 24 hours *P. anaerobius*, *S. pneumoniae* and *S. pyogenes* were recovered from all swabs. *F. nucleatum*, *P. melaninogenica* and *N. gonorrhoeae* were recovered from the Eswabs® and Copan gel swabs only. *H. influenzae* was recovered from both the Eswab® and the Σ® swabs. At 48 hours only *S. pyogenes* was recovered from all swabs. *P. anaerobius*, *F. nucleatum*, *P. melaninogenica* and *H. influenzae* were recovered from the Eswabs but not the Σ swabs. *N. gonorrhoeae* was not recovered from any swabs after 48 hours.

The highest number of organisms recovered was consistently achieved with the Eswab®.

Conclusions: The best results were obtained by the Eswab®; which showed superior recovery of all the organisms, compared to the others tested. It would be an acceptable transport system for these fastidious organisms. It is also suitable for use with automated inoculation systems.

R2469 Rickettsial infections during 2000–2010 in northern Greece

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Background: Rickettsiae are a group of bacteria that cause disease in both animals and humans. The cause of Mediterranean spotted fever is *Rickettsia conorii*, transmitted to humans by the dog tick *Rhipicephalus sanguineus*. *Rickettsia mooseri* is the cause of mourine or endemic typhus and is transmitted to humans by the rat flea *Xenopsylla cheopis*.

Purpose: The aim of the study was to determine the prevalence of rickettsial infection among patients with clinically suspected rickettsiosis. Also the distribution among several demographic variables, including sex, age, and season during the period 2000–2010 was evaluated.

Methods: Serum samples were collected from 903 patients (537 males, 366 females aged 10 months to 85 years old) and tested by indirect immunofluorescence assay (*Rickettsia conorii* Spot IF, *Rickettsia mooseri* Spot IF, bioMerieux) for the detection of IgM and IgG antibodies. All serum samples were tested in a 1/40 initial dilution. Intense fluorescence of the Rickettsiae situated in or outside the cells was considered positive reaction. End-point titers were obtained by serial dilution on positive specimens. Sera showing a typical pattern of fluorescence at titers of ⩾1:80 for IgG and/or for IgM antibodies were considered positive. In 64 patients a second serum sample was tested for seroconversion or a fourfold titre increase.

Results: Of the total 903 patients, IgM and IgG antibodies to rickettsiae were found in 250 patients. Antibodies to *R. conorii* were detected in 210 patients (84%), 115 males (54.8%) and 95 females (45.2%). In addition antibodies to *R. mooseri* were found in 40 patients (16%), 26 males (65%) and 14 females (35%). Difference in prevalence of antibodies to rickettsiae between age groups in men was not significant. However, the highest prevalence of antibodies to rickettsiae was observed in women aged >40 years old. Rickettsial infections are significantly seasonal, with most cases appearing during summer months. Patients from the prefectures of Kavala and Xanthi demonstrated a high prevalence of antibodies to rickettsiae.

Conclusions: Data show a wide distribution of *R. conorii* in Northern Greece and indicate the low frequency of *R. mooseri*. Men are affected more often than women. Increased incidence of disease occurs during the summer months, especially in Eastern Macedonia and Thrace.

R2470 Evaluation of Liaison® Biotrin Parvovirus B19 kits for IgG and IgM antibody detection

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Objectives: The purpose of this study was to compare the LIAISON® Biotrin Parvovirus B19 chemiluminescent immunoassay (CLIA) kit's performance to the Biotrin Parvovirus B19 Enzyme Immunoassay (EIA).

Methods: 273 serum samples were collected from patients with different antibody status according to Biotrin EIA: 112 IgG positive-IgM negative samples, 51 IgM positive samples and 110 IgG negative samples. Potential cross-reactions were evaluated using 45 serum samples from patients with other infectious diseases (n = 30) or immunological pathologies (n = 15). LIAISON® results were compared with those obtained with the Biotrin Parvovirus B19 EIA. Discordant results between LIAISON® and EIA were confirmed by Biotrin Parvovirus B19 Immunofluorescent Assay (IFA).

Results: Of the IgG positive-IgM negative group we found one sample was IgM positive by LIAISON®, negative by IFA. Of the IgM positive group, two sera were negative by LIAISON®. One was confirmed negative and one was weakly positive by IFA. Of the IgG negative group no discrepancies were found between the two tests. Of the potentially cross-reactive IgM samples, 2 were positive with LIAISON®, one of them was negative with EIA but both were positive with IFA. One showed doubtful results with the three methods. All those three false positive samples came from patients with acute CMV infection. Coefficients of variation for interassay variability for IgG and IgM antibodies were 4.5% and 4%, respectively.

Conclusions: The agreement between LIAISON® and Biotrin EIA was 100% for IgG. Concerning IgM we observed 3 discrepancies, of which 1 was confirmed by IFA. We can conclude that the fully automated LIAISON® Biotrin Parvovirus B19 CLIA kits showed a good agreement with Biotrin EIA for detection of IgG and IgM antibodies.

R2471 Increase of efficiency of antigen p24 HIV-1 detection

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Background: Antigen p24 is detected in blood serum at early stages of HIV-infection as a result of virus replication and at later stages of infection, when anti-p24 concentration decreases, and the antigen becomes detectable again. When antibodies to HIV are revealed, antigen p24 is often no longer detectable as a result of formation of a complex between the antigen and antibodies in blood. All existing commercial kits detect only the free p24 antigen, and their sensitivity level unable to determine low concentrations of p24 antigen that is required for early diagnosis of HIV infection.

The purpose of the work is the study of diagnostic efficiency of detection of free and bound HIV-1 p24 antigen by the modified kit "DS-EIA-HIV-Ag-screen". An increase the sensitivity at antigen HIV-1 p24 detection is achieved by dissociate the immune complex and detecting total antigen p24 HIV-1 in patients' samples.

Materials: Method for the dissociation of immune complex and preserving the free HIV p24 antigen was developed. The samples were treated with glycine hydrochloride to dissociate the immune complexes, followed by neutralization with TRIS-hydrochloric acid. Reagents for immune dissociation designed for use with the kit of the "DS-EIA-HIV-Ag-screen".

The following categories of sera were used in the work: 43 sera positive in EIA and PCR and indeterminate in the immunoblot (IB), 107 well defined and positive in the IB reaction sera from HIV-infected patients.

Results: At testing 43 blood sera samples positive in EIA, PCR and indeterminate in the IB, antigen p24 is detected in 31 samples (72%) using the kit "DS-EIA-HIV-Ag-screen" and in 37 samples (86%) using the modified kit. At testing of 107 the samples from HIV-infected patients, confirmed in the IB reaction, antigen p24 is detected in 8 samples (7.48%) using the kit "DS-EIA-HIV-Ag-screen" and additionally in 11 samples after the dissociation of immune complex. The

total number of samples positive for p24 among samples with confirmed HIV infection by IB is 19 (17, 76%).
Conclusion: The dissociation of the p24 immune complexes is a simple method to increase the proportion of HIV samples with p24 antigen. The greatest percentage of overall detection of antigen p24 HIV-1 is detected in the sera with indeterminate or negative result of the IB and may be valuable in diagnosing HIV infection.

R2472 Using MALDI-TOF Biotyper 2 for identification of clinical isolates of undetermined species by routine methods

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Objectives: Routine identification of clinical isolates is performed in our laboratory using an automated Phoenix identification system (Becton Dickinson) and various API strips (bioMérieux). In the absence of identification, sequencing of 16S rRNA gene is performed. The aim of this study was to include the MALDI-TOF Biotyper 2 identifications in our protocol before sequencing in order to evaluate its efficacity.
Methods: Since May 2010, all strains not identified by the phenotypic methods were tested by MALDI-TOF (Ultraflex III, Bruker Daltonics, Bremen) at the genomic platform of the University Victor Segalen Bordeaux 2 since the laboratory does not currently have a mass spectrometer on site to test all strains routinely.
All strains for which the biologist asked complementary identification 1) from May to July 2010 isolates were placed in an Eppendorf tube containing a water-ethanol mixture, then, an extraction was performed before testing on the Ultraflex III. 2) since August, the extraction was eliminated and the bacteria were subcultured the day before testing directly deposited on the target.
Results: A total of 170 strains were tested on the Ultraflex III: 94 strains (55%) were identified with a score above 2.00, 37 strains (22%) were identified with a score between 1.70 and 1.99 and for 39 strains (23%) a score below 1.70 was obtained. Finally, an identification was obtained for 77% of the bacterial isolates, avoiding sequencing. Moreover, even if for 23% of strains, no valid identification was obtained (low score), in practice the identifications given by the MALDI-TOF mass spectrometer often helped the biologist in orienting the identification and carrying out additional tests before performing the sequencing.
Conclusion: In conclusion, after 6 months of this protocol, more than 75% of bacteria thus treated were identified and all biologists are convinced of the contribution of spectrometry in clinical bacteriology. Valuable time is saved and in some cases the therapeutic management of patients has been modified and adapted quickly to the results given by mass spectrometry.

R2473 Performance of two commercial antigen tests for the diagnosis of respiratory syncytial virus on respiratory paediatric specimens

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Objectives: Rapid antigen detection assays for the respiratory syncytial virus (RSV) antigen are widely available with large differences in performance characteristics, sensitivities varying between 59% and 97% (Henrickson KJ and Hall CB. Pediatr Infect Dis J., 2007 Nov; 26(11 Suppl): S36–40). We evaluated the performance of two commercially available immunochromatographic assays: the BinaxNOW RSV and Clearview RSV (Inverness Medical).
Methods: One hundred paediatric nasopharyngeal aspirates were collected and stored during the winters 2007–2010 in the University Hospital of Antwerp and were used for the evaluation of the BinaxNOW; 75 of these specimens were also tested with the Clearview RSV. The results of these rapid assays were compared to the combination of immunofluorescence (IF) directly on the specimens and/or culture on inoculated Hep2 shell vials followed by IF after two days of incubation (VC), which was considered as the gold standard. True positives were defined as positive for either IF and/or VC.
Results: Of the 100 samples tested, 49 were found positive (49%) for RSV with either IF and/or VC. The sensitivity and specificity of the Clearview and the BinaxNOW assay were 77.1% and 92.5%, 87.8% and 98.0% respectively (table 1). There was no significant difference in sensitivity (P = 0.3) and specificity (P = 0.3) between the two assays.
Conclusion: These easy to perform RSV antigen tests, which have a comparable performance, facilitate urgent testing outside batched runs or outside normal laboratory working hours. In order to increase sensitivity, negative results should be confirmed by IF.

RSV	BinaxNOW			Clearview		
VC and/or IF	-	+	Total	-	+	Total
-	50	1	51	37	3	40
+	6	43	49	8	27	35
Total	56	44	100	45	30	75

Table 1: Performance characteristics BinaxNOW RSV and Clearview RSV.

R2474 Access® HIV combo: clinical evaluation of sensitivity and specificity on serum and plasma samples

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Objectives: The diagnosis of infection with human immunodeficiency virus 1 and 2 (HIV-1, HIV-2) is based on the detection of antibodies (Ab) to HIV-1/-2, and, during the stage of primary infection, the detection of p24 antigen or HIV-1 viral RNA. The objectives were to assess the performance of the Access HIV combo assay (Bio-Rad) on Access 2 system (Beckman Coulter) in terms of specificity and sensitivity on serum and plasma samples. This study was performed in three laboratories: Virology Department from Pitié-Salpêtrière hospital, laboratory "CQFD" from the EFS Nord de France (EFS-1) and laboratory "Non-Thérapeutique" from the EFS Normandie (EFS-2).
Methods: The hospital tested on the one hand, specificity on 510 serum samples from routine, 200 serum samples from pregnant women and 29 serum samples from patients infected by HTLV-I, and, on the other hand, sensitivity on 103 plasma samples (patients followed for HIV-1 infection using viral load) and 8 serum samples from routine, 209 serum samples from patients at the chronic stage of HIV-1 infection (Genotypes: subtypes A, B, C, D, F, G, H, J, K, 14 different CRFs and group O), 86 serum samples from patients recently contaminated by HIV-1 (pre- or per-seroconversion), and 75 serum samples from patients infected by HIV-2. Results were compared with those obtained with ELISA combined (p24 Ag/Ab to HIV-1/-2) or kit of third generation (Ab to HIV-1/2) according to the period of initial testing.
EFS-1 and EFS-2 tested specificity on 2551 and 2561 serum samples from blood donors, respectively. Those samples were negative for HIV testing using the routine method (Prism®/Abbott or Genscreen™ ULTRA HIV Ag-Ab/Bio-Rad).
Results: Specificity at hospital was 99.80% for fresh serum samples, 100% for serum samples from pregnant women and patients infected by HTLV-I. Specificity was 100% for EFS-1 and EFS-2 on blood donor samples. Overall specificity was 99.98% for all blood donor and patient samples from the three sites.
Sensitivity was 100% for serum samples from patients during chronic stage of HIV infection and 97.7% during primary infection.
Conclusion: The clinical evaluation of Access HIV combo on Access 2 system showed an excellent specificity for HIV testing for blood donors and hospital patients. All patients infected by HIV-1 or -2, at the chronic stage of the infection, were also identified as positive. These performance are fully suitable for HIV screening in private or hospital laboratory.

R2475 Detection of antibodies to *Campylobacter jejuni* antigens in children with Guillain-Barré syndrome by ELISA with four different antigen preparations

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Objectives: *Campylobacter jejuni* is the organism that has most frequently been described in association with Guillain-Barré syndrome (GBS). Although infections with *C. jejuni* are recognized mainly by culture, serodiagnosis is often useful tool in diagnosis of neurological complications and reactive arthritis occurred after intestinal campylobacteriosis. Many various bacterial antigens have been used for the detection of *C. jejuni* specific antibodies. In this study we evaluated ELISA with four different antigen preparations for serological diagnosis of *C. jejuni* infections in patients with GBS.
Methods: Sera were obtained from six pediatric patients (age range 4–16 years old; two males and four females) which met the established clinical criteria for GBS. A few weeks before the development of neurological symptoms all the six patients had a diarrhea episodes. Paired serum specimens were obtained from 2 patients. For control of specificity and sensitivity of different ELISA we used sera from culture-positive *C. jejuni* patients and from blood donors. The serological tests for *C. jejuni* were performed using one commercial ELISA (Mikrogen) and three home-made ELISA tests, with whole-cell antigen (Virion/Serion), LPS antigen and whole-cell antigen prepared according to method described by Strid and colleagues.
Results: The cut-off limit of serum antibodies in home-made ELISA was set at mean antibody titre determined in the sera of 100 blood donors exceeded by three standard deviations. IgG antibodies, in diagnostically significant level, were presented in 4 patients by all four ELISA tests. We observed significant decrease of IgG antibodies titre in serum samples obtained from two patients in chronic phase of disease. ELISA with LPS antigen and ELISA with whole-cell antigen (Virion/Serion) diagnosed the IgM antibodies in four patients. On the other hand the presence of IgA was diagnosed only in serum samples obtained in the acute phase from two patients by commercial ELISA and ELISA with LPS antigen.
Conclusion: The results of our study showed that serological investigation may be a useful tool for identification of *C. jejuni* infections in patients with post-infectious neurological complications. Therefore, development of a worldwide available, standardized ELISA assay which can be used in serological diagnosis of *C. jejuni* infections, and its complications such as GBS, is needed.

R2476 Analysis of recombinant *Echinococcus granulosus* antigen B for serological diagnosis of Echinococcosis by enzyme-linked immunosorbent assay

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Objectives: Echinococcosis or hydatid disease is a zoonotic infection caused by larval (metacestode) stages of cestodes belonging to the *Echinococcus*, family Taeniidae. *Echinococcus granulosus* causes human cystic echinococcosis which is an important public health problem in many regions of the world. There are some problems in primary diagnosis such as cross-reaction with sera from patients with other parasitic disease in serological tests. The use of an appropriate source of antigenic material is a very important and crucial point in the improvement of the serodiagnostic features such as enzyme-linked immunosorbent assay (ELISA) method.
Methods: We expressed and purified recombinant AgB of *Echinococcus granulosus* and used as antigen in ELISA method. Serum samples were given from 36 cystic hydatid disease patients that have been confirmed by surgical operation as well as 36 healthy individuals sera were tested by ELISA method using recombinant AgB and compared with commercial kit (Euroimmun) for specificity and sensitivities value. The sensitivity of 91.66% and specificity of 90.16% were determined by homemade kit.
Results: In this study, recombinant EgAgB (24 kDa) was expressed and purified. The purified protein was coated onto ELISA microplates and tested in an anti-IgG ELISA with sera from patients with or without CHD and healthy individuals.sensitivity and specificity of ELISA test in 36 positive samples and 51sero negative samples were estimated respectively in order as 91.66% and 90.16%.
Conclusion: Overally we came to this conclusion that our results have confirmed the previous studies regarding the detection value of the recombinant antigen. Although our findings are in common with other previous studies in case of high Specificity and sensitivity of this antigen for hydatidosis diagnosis with ELISA test as well. Therefore, the purified recombinant AgB in this study is one of the desirable Ag for the fast and accurate diagnosis of hydatidosis. Therefore, performing this method on more samples and doing more wide researches make it possible to reach more valuable and conclusive results.

R2477 Evaluation of spreading bacteria using manual method versus InoqulA®

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Introduction: Traditionally, clinical samples are manually inoculated and spread onto agar plates by lab technicians using plastic or platinum loops. The most common streaking pattern is the four-quadrant pattern. The aim of this procedure is to isolate discrete colonies for further identification and antibiotic susceptibility testing. KIESTRA Lab Automation (Drachten, The Netherlands) has developed an instrument where the spreading of the samples is performed by beads in an electromagnetic field.
The aim of this study was:
– to compare the number of discrete colonies obtained by manual spreading with automatic spreading performed by InoqulA®
– to study the variation and reproducibility of discrete colonies created by InoqulA®
Methods: Bacteria from two different species were selected, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922. Inoculums of each species were prepared by suspending the bacteria in Phosphate Buffered Saline (PBS) to a density of McFarland 0.5 using VITEK® 2 Densicheck (bioMérieux). The suspension of *E. faecalis* was used undiluted and the suspension of *E. coli* was diluted 1/10. The final inoculum spread on each plate were 108 and 107 respectively. Agar plates used was a cromogenic agar called UriSelect 4 (BioRad).
Five skilled lab technicians were asked to participate in the study and each lab technician inoculated two plates of each bacterial suspension. The same suspensions were used to inoculate the plates with the InoqulA®. All plates were inoculated with 10 µL using a pipette.
For the manual spreading, plastic loops and the four-quadrant pattern, was used. The plates were incubated in ambient air at 35ºC for 16 hours before discrete colonies were counted.
Results: InoqulA® produced three times as many discrete colonies of *E. coli* and five times as many discrete colonies of *E. faecalis* than the manual method (Table 1).
For both *E. faecalis* (fig. 1) and *E. coli* (fig. 2) the amount of discrete colonies isolated by manual spreading varied between different lab technicians. There was also a noticeable variation in number of discrete colonies produced by InoqulA®.
Conclusions:
– InoqulA® produced a greater amount of isolated bacterial colonies than manual streaking.
– The number of isolated colonies obtained by manual spreading varies between different lab technicians.
– Reproducibility and evaluation of different InoqulA® streaking pattern needs to be further studied.

R2478 Association of *H. pylori* infection with recurrent abdominal pain based on *H. pylori* Stool Antigen

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Introduction: Chronic Abdominal Pain of childhood and adolescent is a common disturbance of patients and their Families. Considering different etiologies of abdominal pain, the role of *Helicobacter pylori* is unclear.

Method: This study was case-control and prospective from 1387–1388, carried out in Bandar Abbas. 50 patients aged 4–16 years suffering from recurrent abdominal pain (RAP) over 3 months which interfered with their normal life style were selected randomly. 50 healthy preschool and school children with same age were selected as control group. Demographic data were collected in a questionnaire. After physical examination, both groups were checked for *Helicobacter pylori* stool antigen test (HPSA).
Results: 58% (29) patients were female. 14% in case group suffering from RAP and 10% in control group had a positive HPSAg and 1 person (2%) had a borderline result. HPSAg was more positive in males, and not related to age, route of delivery child was born or the family history of peptic ulcer disease. HPSAg result did not differ significantly in case or control group. Initiation of supplemental feeding after 6 months was a strong risk factor for h.pylori infection.
Discussion: Association of *H. pylori* infection with recurrent abdominal pain is not fully confirmed and other causes of recurrent abdominal pain should be completely evaluated.

R2479 Review of the requests for rapid urinary antigen test for diagnosis of pneumococcal community-acquired pneumonia from the emergency unit

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Objectives: The aim of this study is to know how many of the requests for rapid urinary antigen test for diagnosis of pneumococcal community-acquired pneumonia (cap) meet the criteria for cap which had been previously given by the Hospital (fever, presence of 2 symptoms of a lower respiratory tract infection combined with a new infiltrate on chest radiography).
Methods: During one year (2008) data of cap defined criteria were recorded from every adult patient of who the test was requested. Fever was defined as the temperature was above 38.1ºC. Not only the emergence of a new infiltrate on chest radiography but it was made prior to the request of the test was recorded. Clinical symptoms of lower respiratory tract infection recorded were: malaise, cough, dyspnea and chest pain. Data was recorded and entered into a computer database.
Results: During the period of study 1085 test were requested from the emergency unit to the Laboratory. Only for 227 (20.92%) of the patients a temperature above 38.1ºC was detected, 291 (28.82%) had respiratory symptoms and for 392 (36.1%) patients the chest x-ray was made prior to the test although only in 153 (14.1%) of them a new infiltrate could be demonstrated. The number of positive test was 129 (11.86%) and for 398 (36.68%) of the patients who the test was requested it did not meet any of the criteria.
Conclusions: The rapid urinary antigen test for diagnosis of pneumococcal community-acquired pneumonia has been widely validated in adults. However in our Hospital it is being using in many patients who don't meet the request criteria given by the Hospital. Probably it is due to several factors; The test can be demanded from the laboratory request form along other urine test in a very easy way and that an important number of physicians working at the emergency unit are in their training period from many different medical and surgical specialities and probably they do not know exactly what are the criteria for application the test given by the Hospital.
It would be interesting to know the number of patients diagnosed of cap with a positive test for who the empirical treatment for cap was adjusted after receiving the result of the test, although it was not included in the aim of this work.

R2480 Diagnostic vaule of procalcitonin for infection or noninfection in patients with systemic inflammatory response syndrome

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Objective: The aim of this study is evaluation of procalcitonin role in the diagnosis infection and non infection patients with systemic inflammatory response syndrome (SIRS) patients using meta-analysis.
Methods: The Cochrane Library, PubMed, Ovid, Springerlink, CBM, CNKI, VIP, EMBASE, ISI, and Wanfang Chinese Periodical Database were searched systematically for relevant studies from January 1966 to September 2010. The language of the researches wasn't limited. Inclusion criteria were established based on validity criteria for diagnostic research. Subsequently, the characteristics of the included articles including study background, design information and diagnostic parameters were extracted. Statistical analysis was performed by employing Meta-DiSc 1.4 and SPSS 12. 0 software. To check for publication bias, a funnel plot, using Egger's linear regression method, was constructed. Heterogeneity of the included articles was tested for selecting proper effect model (fixed effects or random effects model) to calculate pooled weighted, sensitivity, specificity and 95% confidence intervals (CI). Summary receiver operating characteristic (SROC) curve was made and the area under the curve (AUC) and Q* index was calculated. Finally, sensitivity analysis and comparison of sensitivity among different groups were performed.
Results: 20 articles (6 English articles, 11 Chinese articles and 1other language articles) were included finally, with 1258 total patients. No significant heterogeneity and no publication bias was observed The sensitivity and specificity of PCT for the differentiation of infectious versus non-infectious systemic inflammatory response syndrome were 0.76 (95% CI = 0.73–0.79) and 0.8 (95% CI = 0.77–0.83) respectively. The area under summary receiver operating characteristic (SROC) curve was 0.85.
Conclusions: Serum measurements of PCT may be valuable in differentiating between on-infectious SIRS sepsis and infectious SIRS, the latter including sepesis.

R2481 Comparative study of the ability of two swab transport systems to maintain viability of clinically important organisms at controlled room temperature

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Introduction: Appropriate conservation of specimens is one of the most critical steps in the microbiological study of infectious diseases. Objective. The aim of the present study was to compare the ability of two extendedly-used swab transport devices with Stuart medium: (1) Copan Venturi Transystem® (Copan Italia Spa, Brescia, Italy) and (2) Eurotubo (Deltalab, Rubí, Barcelona, Spain).
Materials and Methods: The NCCLS (Now CLSI) published recommendations were followed (NCCLS. Quality control of microbiological transport systems, M40-A, Vol. 23 No. 34, Wayne, PA, USA, 2003). In a volume of 0.1 ml appropriate concentrations (approximately 10^5 and 10^4 CFU/ml) of each *Streptococcus pyogenes* ATCC 19615, *Haemophilus influenzae* ATCC 10211, *Neisseria gonorrhoeae* ATCC 43069, or *Streptococcus pneumoniae* were absorbed by the two different swabs. Swabs were held in transport tubes for 10 minutes, 24h and 48h at room temperature (25.1–28.0 oC) before streaking chocolate agar plates (Britania, Buenos Aires, Argentina). All tests were performed in triplicate. Colonies were counted in a blinded way by one of us (HL) and averaged.
Results: By reading the CFU/ml at subsequent time points, we observed the decline in viability for each organism in each kind of device as can be seen in the Table.
Conclusions: Significant differences were observed between the two systems, being Copan the most effective device for maintaining the viability of these selected strains.
Conflict of interests: Nothing to declare.

Table. Average change in cfu (log10) comparing with the zero time counts for each specific device

Organism	(UFC/ml)			Eurotubo (UFC/ml)		
	T=0	T=24	T=48	T=0	T=24	T=48
N. influenzae	100%	17.5%	13.5%	100%	0	0
N. gonorrhoeae	100%	2.5%	0.1%	100%	0	0
S. pyogenes	100%	100%	100%	100%	5.0%	1.9%
S. pneumoniae	100%	100%	34.9%	100%	0	0

R2482 Oxoid Brilliance® agar, a new rapid chromogenic medium for the screening of methicillin-resistant *Staphylococcus aureus* from clinical isolates – an evaluation

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Objectives: Meticillin resistant *Staphylococcus aureus* (MRSA) is one of the major contributors to healthcare associated infection. Although PCR techniques are a faster method for MRSA detection with same day results, chromogenic agars are a lot cheaper and more efficient for use in a routine clinical laboratory. This study sought to evaluate the effectiveness of Oxoid Brilliance® Agar, a new rapid chromogenic medium for the screening of MRSA from clinical isolates.
Methods: 448 screening swabs (nasal, groin and wound sites if present) taken on patient admission were analysed for the presence of MRSA. The first 200 swabs were inoculated onto Oxoid Brilliance® agar (Oxoid) and MRSA ID agar (Biomerieux). The remaining 248 swabs were inoculated onto 3 agars; Oxoid Brilliance agar (Oxoid), MRSA ID® agar (Biomerieux) and chromID® (Biomerieux). Presumptive MRSA colonies were tested for latex agglutination using a Pastorex latex kit (Biorad). Colonies positive for latex agglutination were inoculated onto a DNAse agar plate (Oxoid) and were also tested using the cefoxitin 10 microgram disc diffusion sensitivity test according to the British Society for Antimicrobial Chemotherapy guidelines.
Results: From the 448 samples tested, 17 isolates were positive overall for presumptive MRSA. Sensitivity and specificity were calculated for each media at the incubation time points 18, 20, 24 and 48 hours (Table 1). No false positives for Oxoid Brilliance® within 24 hours were noted. Oxoid Brilliance® was shown to be more sensitive than the other the agars at the earlier time point of 18–24 hours.
Conclusion: In this study, Oxoid Brilliance® agar was shown to be superior to the other agars for the detection of MRSA at the earlier time point of 18–24 hours. Oxoid Brilliance® agar has the potential to cut down on turnaround times by detecting MRSA within 24 hours compared to 48 hours as seen with most other chromogenic agars. This has major implications on cost, infection prevention and control measures and treatment strategies within healthcare facilities.

R2483 Miniaturised γ-IFN assay for diagnosis of congenital toxoplasmosis

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Objective: Diagnosis of congenital toxoplasmosis at birth, even with the most sensitive serological test is possibile in only 70–80% of newborn that must be followed until one year of age. Furthermore treated babies could be serologically negative. In our laboratory immunological test like stimulation index and Cd25 markers have been employed for many years in all doubtful cases; but these tests are time consuming and expensive. In this study we employed a commercial test: Quantiferon CMI Assay (Cellestis-Australia) less expensive, more rapid and easier to perform to the diagnosis of congenital toxoplasmosis.
Methods: 180 eparinized samples (1ml) were obtained from 126 patients referred to outpatients of Neonatal and Intensive Care unit of IRCCS Foundation San Matteo Policlinic Pavia Italy as at risk for congenital toxoplasmosis. Serum was used for serology (IgGVIDAS, IgM ISAGA Biomerieux – Marcy l'Etoile France; Toxok IgA Diasorin Saluggia Italy; LDBIO IgG/IgM western Blot – Lyon France) and replaced with the same amount of RPMI (Σ-US). The test Quantiferon CMI was performed according manufacturer's instruction on a smaller amount of blood (900 μl instead of 3000 μl) and the results were expressed in IU according a standard curve. Toxoplasmic antigen was kindly provided by Diasorin.
Results: Eighty patients were recorded as negative at the first sampling and all but one were true negative in the follow-up. Ten newborn scored positive and all of them were congenitally infected. No one switched negative under treatment in further controls. For 36 newborn test results were undetermined (32 because of inadequate response to phytoemoagglutinine, 4 for an aspecific activation).
Conclusion: These preliminary data according with a previous paper by Peyron et al. suggest that also this commercial test could be useful in early diagnosis of congenital toxoplasmosis and in all cases in which serological test cannot solve diagnostic problems.

R2484 The role of infection in morbidity newborns in critical conditions with brain damage

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Objectives: The prenatal hypoxia is the leading of nervous system patology on one side and infectious processes with tendency of generalization on the other side. We use a tandem of markers in intensive care newborns: PCT – a biomarker of a bacterial infection and S100B – a marker of brain damage.
Methods: 31 newborns (3–6 days) in ICU transported from maternity clinic. The gestational age was 28–40 weeks. PCT and S100 serum levels were measured by Elecsys immunoassay.
Results: 13 newborns (42%) were with HIE and 18 (58%) with IVH. Patients with IVH was with pre-natal pneumonia in 10 (77%) cases and in 9 (69%) necrotizing coloenteritis. Whereas at patients with HIE an aspiration pneumonia is prevailed (11. 61%). Necrotizing coloenteritis is diagnosed in 4 (22%) cases. PCT level was1.54 ng/ml after hospitalization (0.377–4.81) and exceeded 2 ng/ml in 15 cases. Level PCT didn't differ in groups. At the same time patients with IVH have positive hemoculture in 8 (61.5%) cases, than at patients of compared group – 4 (22.2%) ($p = 0.05$). At the same sample S100B 0.325 (0.26–0.45) pkg/l. 0.448 (0.253–0.764) pkg/l from newborns with IVH, 0.301 (0.244–0.336) pkg/l with HIE ($p = 0{,}05$). The level of S100B is directly correlate with PCT level ($r = 0.51$).
Subsequent assay was made by 18 patients. PCT was 0.39 (0.192–1.1) ng/ml. In 9 cases PCT have normalized after initially levels more than 2 ng/ml level. In other cases decreased of PCT level before treatment was more than 50%. While S100B remained the same 0.347 (0.192–0.438) pkg/l and has raised at 8 patients from 0.299 (0.170–0.418) pkg/l to 0.488 (0.202–0.843) pkg/l ($p = 0.05$) (5 newborns were with IVH, and 6 has developed the convulsive syndrome).
In eight cases the level of S100B decreased from 0.480 (0.270–0.630) to 0.223 (0.133–0.307). The gestational age of these patients was 36 (36–39) weeks. Only one patients was IVH. Initial level PCT in this group was 3.92 (1.5–14.5) ng/ml. Whereas in group with raised S100B, it was 0.97 (0.28–4.07) ng/ml ($p = 0.05$).
Conclusions: The tandem of biomarkers allows to start an adequate antibacterial therapy rapidly for newborns in ICU. Monitoring this markers allows to carry out differential diagnostics in cases of an aggravation of clinical condition, to prevent the exceeded escalation antibacterial therapy at suspected bacterium etiology while the processes of CNS damage are prevalence.

R2485 The Walk-Away specimen processor (WASP): a first European evaluation

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Objective: Finding trained technicians specialized in Microbiology nowadays is difficult in Belgium. Microbiology has been, up till now, one of the most labour intensive disciplines of the Clinical

Laboratory. However, recent developments as automation of planting and identification with MALDI-TOF technology initiated an unimaginable revolution.
We evaluated the WASP™ instrument (Copan Diagnostics Inc.) for its accuracy of planting liquid samples.
Methods: Accuracy of the loop content (1 μL and 10 μL) of the WASP was compared with a calibrated 50 μL pipet using a ten times dilution series of a 0.5 McFarland suspension of 3 ATCC strains (*E. coli* 25922, *P. aeruginosa* 27853 and *E. faecalis* 29212) according to guidelines of the American Society for Microbiology.
Every inoculation was performed in triple on blood agar plates and the experiment was repeated four times.
For the implementation of the WASP in the routine microbiology laboratory, results of the inoculation of fifty urine samples and fifty MRSA-screening samples by the WASP were compared with results of manual inoculation. For urine samples, 1 μL was planted on blood agar and McConkey agar plates in a four quadrants streaking pattern. Ten μL of the MRSA screening samples (eSwab) was planted on a chromogenic agar and inoculated in TSB salt enrichment medium. After overnight incubation, enrichment broths were planted onto chromogenic agar plates both by WASP and manually.
Results: The quantitative evaluation of the automated streaking of the 1 μL loop showed that plates inoculated by the WASP had 10 times more colonies growing after overnight incubation as compared to the plates inoculated with 50 μL using the calibrated pipet. This was the same for all three ATCC strains and after a correction was made for the cfu/mL of the initial 0.5 McFarland suspension. Although there were numeric differences between colony count of plates inoculated by WASP and manually, no differences in clinical interpretation was observed.
Testing of the clinical samples will be continued in the next weeks.
Conclusion: Although the WASP is a very promising automated instrument for planting and streaking of bulk samples like urines and MRSA-screening samples in microbiology laboratories, a conscientious validation is recommended before the implementation in daily routine.

R2486 Identification of anaerobic bacteria by MALDI-TOF mass spectrometry

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Objectives: Anaerobic bacteria remain an important group of human pathogens. Most of them are fastidious microorganisms, and their identification by conventional, biochemical methods is frequently tedious and inaccurate. MALDI-TOF mass spectrometry is a fast and reliable technology for microorganism identification, which has been shown useful for microorganisms identification both from culture and from some samples. We have compared the correlation between biochemical identification and MALDI-TOF mass spectrometry (ME) for anaerobic bacteria identification.

Table 1. Correlation between biochemical and MALDI-TOF MS identification in anaerobic bacteria

Rapid 32A id.	No. isolates	MALDI-TOF correlation to the species level (%)	MALDI-TOF correlation to the genus level (%)
Bacteroides fragilis	82	77	96.0
B. ovatus	9	44.4	100
B. caccae	2	0	0
B. stercoris	2	0	0
B. uniformis	2	50	50
B. vulgatus	2	50	50
P. asaccharolytica	10	0	0
P. bivia	6	33.3	50
P. intermedia	4	50	50
Prevotella spp	3	33.3	33.3
P. corporis	1	0	0
P. melaninogenica	1	100	100
P. distasonis	2	50	50
C. perfringens	2	50	50
Bifidobacterium spp	1	0	0
Fusobacterium spp	1	0	0
F. varium	1	0	0
E. lenta	1	0	0
Veillonella spp	1	0	0
TOTAL	133	57.9	68.4

Methods: 133 clinical isolates, previously identified by biochemical methods were evaluated: 82 *Bacteroides fragilis*, 9 *B. ovatus*, 2 *B. caccae*, 2 *B. stercoris*, 2 *B. uniformis*, 2 *B. vulgatus*, 10 *Porphyromonas asaccharolytica*, 6 *Prevotella bivia*, 4 *P. intermedia*, 1 *Prevotella corporis*, 1 *P. melaninogenica*, 3 *Prevotella* spp, 2 *Parabacteroides distasonis*, 2 *Clostridium perfringens*, 1 *Bifidobacterium* spp, 1 *Fusobacterium varium*, 1 *Fusobacterium* spp, 1 *Eggerthella lenta* and 1 *Veillonella* spp.
Species were identified using the Rapid 32A® system (bioMerieux, Marcy L'Etoile France) according the manufacturer instructions. MALDI TOF mass spectrometry was performed with an Autoflex III MS MALDI-TOF device (Bruker Daltonics GmbH, Leipzig, Germany).
Results: Correlation between both methods, to the genus and species levels, appear in Table 1.
Conclusions: Both methods show a good correlation in *Bacteroides* species, especially at the genus level, but correlation is quite worse for other genera such as *Prevotella* and *Porphyromonas*.

R2487 Diagnosing bloodstream infection by MALDI-TOF mass spectrometry

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Sepsis is caused by a variety of different groups of infectious etiologies. Early and adequate antimicrobial therapies correlate with positive clinical outcomes. In recent years matrix-assisted laser desorption-ionization time of flight (MALDI-TOF) mass spectrometry fingerprinting has become a powerful tool in the microbiological diagnostic. The direct identification of microorganisms from a positive blood culture can shorten the diagnostic significantly enabling an earlier specific therapy.
Aim of the study was to compare two different methods of sample preparation for the MALDI-TOF directly from a positive blood culture versus the conventional culture, the current gold standard.
In the first part of the study 192 positive blood cultures have been investigated by using tubes with separator gels (BD) to enrich microorganisms in the serum followed by the standard ethanol/formic acid protein extraction for the preparation of the bacterial extracts. In the second part of the study MALDI Sepsityper Kit from Bruker Daltonic has been used for the preparation of the bacterial extracts out from 132 positive blood cultures. 75% of Gram-negative bacteria and 60% of Gram-positive bacteria were correctly identified using the separator tubes compared to the cultures on species level. Using the MALDI Sepsityper Kit from Bruker Daltonic 95% of Gram-negative bacteria and 68% of Gram-positive bacteria were correctly identified. The overall identification Biotyper score in both studies was significantly higher for the Gram-negative (Septityper 2,2 and BD 2,0) compared to the Gram-positive bacteria (Septityper 1,8 and BD 1,7). An alteration of the algorithm for the interpretation of the Biotyper score for Gram-positive bacteria increases the rate of identification of the germ about 12–18%. The hands-on time is higher for the method using the tubes with separator gels.
Thus, both protocols are suitable methods for the analysis of blood stream infection with better results observed with Brukers Daltonic Sepsityper Kit.

R2488 Plasma levels of N terminal pro-brain natriuretic peptide and biomarkers of sepsis (procalcitonin and C-reactive protein) in critically Ill patients of intensive care units

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Objectives: Natriuretic peptides compose a family of peptides that present diuretic and vasoconstrictive properties and are associated with left heart ventricle functions. Especially, the amino ending end of brain natriuretic peptide (NT-proBNP) is secreted by heart ventricles due to their dilatation in heart failure. High levels of NT-proBNP are also observed during bacterial infection and sepsis. Procalcitonin (PCT) is a calcitonin propeptide, which levels increase in serious bacterial infection

and sepsis, and C-reactive protein (CRP) is an acute phase protein. The purpose of our study was to evaluate NT-proBNP, PCT and CRP serum levels in patients with documented infection that were hospitalized in Intensive Care Units (ICUs) and their correlation with the isolated microorganism and the site of the infection.

Methods: We mearured NT-proBNP, PCT and CRP serum levels in 30 hospitalized patients in ICUs of AHEPA University hospital. Patients presented 31 infection episodes, confirmed with cultures. NT-proBNP levels were determined with electroluminescence (Expected Values <100 pg/ml), PCT levels with chemoilluminescense (Expected Values <0.5 ng/ml) and CRP with nephelometry (Expected 0.0–0.8 mg/dl).

Results: Increased levels of NT-proBNP and CRP were observed in all episodes while increased PCT values were found only in 16 (53%) episodes. The highest levels were observed for NT-proBNP in respiratory truck infections and bacteremias, for PCT in respiratory infections and bacteremias and for CRP in bacteremias and venous catheter infections. Statistically, significant correlation was observed between NT-proBNP and CRP ($p < 0.01$), while no correlation was observed between NT-proBNP and PCT or between CRP and PCT. No significant correlation was observed between the isolated bacteria and NT-proBNP, PCT or CRP. In respiratory truck positive cultures it was found statistically significant correlation between NT-proBNP and CRP ($p < 0.01$) and also between PCT and CRP ($p < 0.05$).

Conclusions:

1. NT-proBNP levels are significantly correlated with CRP levels in severe infections.
2. There is no significant correlation between the isolated bacteria from positive cultures and NT-proBNP, PCT or CRP.

R2489 Inconsistent results of amoxicillin/clavulanic acid susceptibility tests with gradient test strips from three different manufacturers in rapid growing anaerobes

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Objectives: Since we repeatedly observed inconsistent results regarding susceptibility of rapid growing anaerobes to amoxicillin/clavulanic acid using different gradient test strips, we set out to systematically compare test strips from three manufacturers in order to identify possible discrepancies.

Methods: Commercially available gradient test strips for amoxicillin/clavulanic acid from three manufacturers were compared: Liofilchem (MIC Test Strip), AB bioMérieux (ETest®) and Oxoid (M.I.C.Evaluator™). The initial experiment was carried out on 30 isolates of *Bacteroides fragilis*, 18 isolates of *Prevotella bivia* and two isolates of *Parabacteroides distasonis* (total n = 50). The experiment was replicated with 9 isolates of *B. fragilis*, 4 isolates of *P. bivia* and one isolate of *P. distasonis* (total n = 14) using new batches of test strips. Since divergent results in the first experiment were observed mainly with isolates that expressed at least one resistance gene (as tested by PCR), only such isolates were used in the replication experiment. Plates (*Brucella* agar, Becton Dickinson) were prepared with Mc Farland turbidity standard 1. Following application of the test strips, agar plates were incubated at 37ºC in an anaerobic system for 48 hours. After recording the MIC value, susceptibility was determined using the breakpoints established by EUCAST.

PCR for resistance genes and additional species verification of each isolate using 16s rDNA sequencing was performed by the Austrian Agency for Health and Food Safety (AGES). For quality control the ATCC strain 25285 of *B. fragilis* was used.

Results: In 12 out of 64 tested isolates inconsistent results regarding susceptibiliy to amoxicillin/clavulanic acid were documented (see table). These isolates also expressed at least one resistance gene. Strips from Liofilchem consistently produced the highest MIC values, while strips from AB bioMérieux yielded the lowest.

Conclusion: Our results demonstrate, that gradient test strips from different manufacturers may yield contradictory results regarding susceptibility. Possible explanations for this finding include different diffusion times of the diagnostic reagents, different materials of the test strips (Liofilchem uses paper strips, the two other companies use synthetic strips) or instability of the diagnostic reagents (possibly clavulanic acid). These findings need further attention in order to make appropriate clinical decisions.

Table. 12 clinical isolates (total number tested = 64) of rapid growing anaerobes that exhibited inconsistent results regarding their susceptibility to amoxicillin/clavulanic acid with gradient test strips from three different manufacturers. Susceptibility according to EUCAST: S=susceptible, I=intermediate, R=resistant. Asterisk denotes isolates from replication experiment.

Species	Isolate ID	MIC per Manufacturer and susceptibility according to EUCAST					
		AB bioMérieux		Oxoid		Liofilchem	
B. fragilis	RS 150	1,5	S	8	I	> 256	R
B. fragilis	RS 156	0,5	S	2	S	> 256	R
P. distasonis	RS 161	0,75	S	2	S	> 256	R
B. vulgatus	RS 205	0,5	S	2	S	> 256	R
B. vulgatus	RS 214	0,75	S	4	S	> 256	R
P. bivia	RS 226	0,38	S	2	S	> 256	R
B. ovatus	RS 249	3	S	16	R	> 256	R
B. fragilis	RS 139*	3	S	16	R	> 256	R
B. fragilis	RS 150*	2	S	16	R	> 256	R
B. vulgatus	RS 205*	1	S	4	S	> 256	R
B. vulgatus	RS 214*	1	S	8	I	> 256	R
P. bivia	RS 253*	0,75	S	3	S	64	R

R2490 The diagnostic and prognostic value of procalcitonin in patients with systemic inflammatory response syndrome and septic shock symptoms

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Objective: We evaluated the diagnostic and prognostic value of procalcitonin (PCT) in patients with sepsis.

Methods: The study was conducted in Zonguldak Karaelmas University Hospital, Turkey between February, 2009 and February, 2010. A total of 94 patients (ages ranging between 22 and 75) with systemic inflammatory response syndrome (SIRS) due to noninfectious etiology (n = 17), sepsis (n = 51), severe sepsis (n = 15), septic shock (n = 11) were included. Patients were followed for 14 days. PCT levels were recorded on days 1 (T1), 3 (T3), 7 (T7) and 10 (T10). The value of PCT in terms of differential diagnosis between SIRS due to other causes and sepsis, prognostication, identifying the severity of illness and patients at risk of deterioration was evaluated.

Serum PCT levels were measured with the VIDAS®B.H.R.A.M.S PCT test (Biomerieux, Marcyl Etoile, France) on the Minividas Analyzer (Biomerieux, Ponte A Ema, Italy) using Enzyme-linked Fluorescent Assay.

Results: On T1 PCT was higher in patients with sepsis, than patients with SIRS due to noninfectious etiology ($p < 0.001$). PCT levels increased significantly with the severity of illness. The highest levels were observed in patients with septic shock ($P < 0.001$). Patients who died during the follow up period were grouped as "nonsurvivors". Patients who were alive at the end of 14 days (n = 57) or discharged with cure (n = 8) in this period were grouped as "survivors". A total of 29 patients died in the first 10 days, so PCT levels on T10 for these patients were not available. PCT levels were higher in nonsurvivors on T0, T3 and T7 ($p < 0.001$, $p = 0.001$, $p = p < 0.019$ respectively). The change in PCT levels in time was analyzed. A significant decrease was observed in survivors in whom PCT values on T1, T3, T7, T10 were available (n = 57) ($p < 0.001$).

At a cut-off value of 0.07ng/ml on T0, the negative predictive value (NPV) of PCT for differentiating sepsis from SIRS due to non infectious etiology was 100.0% with 95.7% accuracy. When 17 patients with SIRS were excluded, for 77 patients with sepsis NPV of PCT for mortality were as follows:

On T3, at a cut-off value of 0.7ng/ml, NPV: 91.0% accuracy: 70.6%

On T7, at a cut-off value of 3ng/ml, NPV: 97.5%, accuracy: 84.3%

Conclusion: We think that PCT is an important marker guiding the clinician in the differential diagnosis of sepsis and prognostication.

R2491 Differentiating between mastitis through Acute phase proteins in different dairy animals in sub-tropical agro-climate

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Objectives: Mastitis continues to be a costly disease in modern dairy farming and detection at subclinical stage is of immense worth both in terms of animal health and economy. The present study was undertaken to evaluate potential value of Acute Phase Proteins (Serum Amyloid A and Haptoglobin) in detecting clinical and subclinical mastitis and their correlation with pH and electro-conductivity in Zebu (Sahiwal), Holstein-Friesian Cross and Murrah buffaloes.
Methods: In the present study plasma Acute Phase Proteins (Serum Amyloid A-SAA and Haptoglobin-Hp) were measured (Life Diagnostics Inc., USA) and their correlation with pH and electro-conductivity of milk in 200 cows of each breed of Sahiwal, HF Crossbred and Murrah buffaloes. The subclinical and clinical mastitis cases were confirmed by Californian Mastitis Test.
Results: The haptoglobin levels in the subclinical, clinical and healthy cases were 2.4±0.77, 1.69±0.94 and 0.17±0.02 μg/l. The SAA levels in subclinical, clinical and healthy cases were 1.427±2.04, 2.58±1.09 and 0.03±0.04 μg/l. The milk electro-conductivity of healthy, subclinical and clinical mastitis in Sahiwal cows was 5.25±0.19, 7.13±0.53 and 7.94±0.91 mS/cm in crossbred cows was 5.88± 0.23, 6.96±0.76 and 7.67±1.13 mS/cm and in Murrah was 5.44±0.35, 6.42±0.72 and 7.54±1.02 mS/cm, respectively. The milk pH of healthy animals was 6.55±0.19, 6.68±0.23 and 6.64±0.35, subclinical mastitis 6.63±0.27, 6.79±0.34 and 6.78±0.41 and mastitis was 6.93±0.37, 7.17±0.43 and 7.09±0.29, in Sahiwal, Crossbred and Murrah respectively. The correlation coefficient between Hp and Electroconductivity was 0.87 in subclinical and 0.94 in clinical cases and SAA electro-conductivity was 0.73 and 0.91.
Conclusion: In our experiment, haptoglobin proved to be a better indicator of mastitis. The higher level of Hp in subclinical mastitis than clinical may be due to the chronic nature of the subclinical cases. Both the APP's showed a very correlation with the electrical conductivity. Hence as APP's are biomarkers for mastitis, it can be concluded that the electroconductivity can be a good predictor of mastitis.

R2492 Performance evaluation of the Access® HIV combo assay on the UniCel® DxI 800

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Objectives: A new automated HIV combo assay has been developed by Bio-Rad for the qualitative detection of HIV-1 p24 antigen and antibodies to HIV-1 (groups M-N-O) and HIV-2 using the UniCel DxI 800 Immunoassay system (Beckman Coulter). The purpose of this study was to evaluate the performance of this new assay in terms of sensitivity, specificity and precision.
Methods: All studies were performed on UniCel DxI 800 immunoassay system. The analytical sensitivity was estimated by dilution study of the AFSSAPS panel and the NIBSC Panel 90/636 (WHO standard). The clinical sensitivity was evaluated by testing 62 subtype and variant samples, 289 commercial positive samples, 199 hospital patient samples and 61 seroconversion panels (including 131 early seroconversion samples). The clinical specificity was studied with 2,552 samples from blood donors, 1,969 selected negative hospital patient samples and 617 non selected hospital patient samples. The precision study has been studied following CLSI EP5A2 guidance by the analysis of 13 samples: a negative sample, 2 low positive samples, a medium positive sample for HIV-1, HIV-2, HIV-1-O and HIV-1 antigen.
Results: The specificity was found to be 100% on blood donor samples, 99.85% on selected negative hospitalized patient samples and 100% on non selected hospitalized patient samples.
The analytical sensitivity obtained with the NIBSC 90/636 was equal to 1.1 IU/mL. It was estimated at 20.60 pg/mL with the AFSSAPS panel.
The clinical sensitivity for all positive samples including HIV-1-M, HIV-1-O, HIV-1-N, HIV-2 antibodies and HIV-1 antigen was 100%. The seroconversion sensitivity gave performance in accordance with the state of the art as 131 early seroconversion samples were all detected.
Intra-assay and inter-assay precisions were found below 10% with positive samples.
Conclusion: The evaluation of the Access HIV combo on the highest throughput UniCel DxI immunoassay system showed excellent performance in terms of global specificity, analytical sensitivity, clinical sensitivity and precision. This new Access HIV combo is fully suited for the screening of HIV, in hospitals or private laboratories.

R2493 Prognostic, economic and epidemiological significance of p24 antigen for early detection of HIV infection

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Background: In Russia for screening purposes assays for simultaneously detection of anti-HIV and antigen p24 HIV-1 are used. The confirmation of screening positive results carried out by immunoblot assays (IB) only. IB are not intended for detection of presence p24 antigen. Therefore there is problem with confirmation screening result at early stage of seroconversion. The aim of this study was to develop optimal algorithm of diagnostics of HIV infection.
Materials: The work was done on the basis of the Regional Centre of AIDS Prevention from 01.01.2007 to 01.01.2010. All sera (n = 4271) with IB indeterminate and negative results were additionally tested in high sensitive EIA for p24 antigen detection. The majority of these samples (57%) were also evaluated for viral load. Cost-effectiveness of analysis included the calculation of the cost of identifying one case of HIV infection. The reliability of differences was determined by Mann-Whitney's criterion.
Results: Out of 4271 analyzed sera with IB indeterminate and negative results, antigen p24 was detected in 4,5% and 2,5% respectively. In p24 positive samples, viral load was detected in 98%. More than in 25% of cases, the viral load exceeds the sensitivity level of the used assay (>750000 copies/ml). A median was more than 100,000 copies/ml in samples with indeterminate IB results and more than 300000 copies/ml in samples with negative IB results. HIV-status was confirmed in 58% of tested patients. The median time until confirmation by IB was 107 days. 40% of patients with indeterminate and negative IB results did not come for the repeat testing. A few of false-positive results (2%) were due to the errors of pre-analytic phase.
The cost of identifying one case of HIV infection is $420 when used currently available algorithm of diagnostics of HIV infection. The additional testing for p24 allows to save about $10 due to a larger number of identified cases.
Conclusion: The additional analysis of samples with indeterminate or negative IB result for p24 antigen, can detect HIV infection in average on 107 days earlier. Each detected case of early seroconversion can prevent 1–3 cases of a HIV of an infection. Thus, additional testing on p24 allows not only lowering total cost of revealing of each case of HIV infection, but also will allow slowing spread of epidemic.

R2494 Pathogens isolated from central vascular catheters in patients with positive blood cultures

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Objectives: The use of central vascular catheters (CVC) was introduced in practice more than 60 years ago and become essential to modern medical practice, especially in the intensive care units (ICU). The aim of this study was to evaluate the etiology of CVC-related infections in patients with positive blood cultures.

Methods: Catheter tips from patients hospitalized during January 2008 to October 2010 were processed using the semiquantitative method and blood cultures were incubated in the automated BACTEC 9050 System (Becton Dickinson, USA). The positive blood cultures and the CVC were cultured under standard conditions. The identification of the isolated microorganisms and their susceptibility to different antimicrobial agents was performed using the automated system VITEK 2 (BioMerieux, Marcy l'Etoile, France).
Results: During the study period, samples from 127 CVC obtained from different patients were evaluated (42 in 2008, 58 in 2009 and 27 in 2010). Positive cultures were found in 115 (90.6%) of cases [39 (92.9%) in 2008, 50 (86.2%) in 2009 and 26 (96.3%) in 2010]. Blood cultures were available from all patients studied. Out of the 115 positive CVC cultures, 96 (83.5%) yielded the same microorganism as from the blood culture, while in 19 (16.5%) cases a different pathogen was isolated in the blood culture. Out of the 96 cultures yielding the same pathogen with the blood cultures, Gram-negative bacteria as well as Gram-positive cocci were isolated in the same percentage, 48%, while the remaining 4% was represented by fungi. Resistance to glycopeptides among staphylococci and enterococci was not detected, whereas 60% of Gram-negative bacteria were resistant to β-lactams.
Conclusions: In the majority of cases, the same microorganisms were isolated from both CVC and blood cultures.

R2495 Acute phase DMSA renal cortical scintigraphy for identifying dilating vesicoureteral reflux in children with a first febrile urinary tract infection: a diagnostic test accuracy meta-analysis

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Objective: Controversy exists regarding the type and/or sequence of imaging studies needed during the first febrile urinary tract infection (UTI) in young children. Several investigators claim that since acute phase Tc-99m dimercaptosuccinic acid (DMSA) renal scan results are abnormal when there is dilating vesicoureteral reflux (VUR), a normal DMSA scan makes voiding cystourethrography (VCUG) unnecessary in the primary examination of infants with UTI. We aimed to evaluate the accuracy of acute phase DMSA scan in identifying dilating (grades III-V) VUR documented by VCUG in children with a first febrile UTI.
Methods: We performed a diagnostic test accuracy meta-analysis of relevant studies identified through the PubMed and Scopus databases. Two separate analyses, patient-based and renal units-based, were performed.
Results: Overall, 13 cohort studies were identified. Nine studies involved patients <2 years old, 3 children ⩽16 years, and 1 involved exclusively neonates. Females constituted 22% to 85% of the involved children. Pooled (95% confidence intervals) sensitivity and specificity of DMSA scan was 79% and 53%, respectively for the patient-based analyses (8 studies) and 60% and 65% for the renal units-based analysis (5 studies). The respective areas under the hierarchical summary receiver operating curves were 0.67 and 0.66. Marked statistical heterogeneity was observed in both analyses, as indicated by an I2 index value of 91% and 87%, respectively.
Conclusion: Acute phase DMSA renal scan cannot be recommended as replacement for VCUG in the evaluation of young children with a first febrile UTI.

R2496 Cytomegalovirus immunity and infection in first trimester pregnant women in Greece

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Aim: The aim of this study was to assess immunity to Cytomegalovirus (CMV) and infection in pregnant women in Greece.
Methods and Subjects: In this study, we present data of pregnant women from years 2008 & 2009 in the 1st semester of pregnancy based on a total of 4440 samples. CMV infection in pregnant women was identified in our laboratory by a diagnostic algorithm utilizing CMV IgG, IgM, CMV IgG avidity (Abbott Axsym and VIDAS BioMérieux) and home-made PCR.
Results: For 2008, 60.2% of the women tested were CMV IgG seropositive, 1.0% CMV IgM positive and only 19% of the latter were tested with CMV IgG avidity and 3.8% with PCR. For 2009 63.5% seropositive for CMV IgG, 1.1% positive for CMV IgM and 4.3% tested with CMV IgG avidity and none with PCR. In addition, 35% of women without antibody titers for both IgG & IgM were re-tested in 2008 and 23.2% in 2009 respectively.
Conclusions: The follow-up of pregnant women was found to be inadequate. It seems that financial issues could be the reason for this. Comparing the two years, we observed that despite the homogeneous distribution of the number of samples which were tested per trimester, a significant decrease in the number of the IgM positive samples in the 3rd trimester of the year, (4.7% for 2008 & 8.8% for 2009) was possibly due to the increase of outbreaks in this period of the year.

Methods for antibacterial susceptibility testing

R2497 Direct detection of *Mycobacterium tuberculosis* drug resistance by nitrate reductase assay

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Objective: The aim of this research was to evaluate the performance of the direct nitrate reductase assay (D-NRA) for detection of resistance to the first line antituberculous drugs.
Methods: The D-NRA was performed in 174 smear positive sputum. We used Löwenstein-Jensen (LJ) medium with 1 mg/mL of KNO3 and with or without isoniazid (INH), streptomycin (STR), ethambutol (EMB), rifampicin (RMP) and nicotinamide (NIC) at critical concentration of 0.2, 4, 2, 40 and 1000 μg/mL, respectively. Previous to the D-NRA sputum samples were decontaminated and part of the decontaminated suspension was diluted 1:10. For each sample, 0.2 ml of the undiluted suspension was inoculated into LJ medium containing KNO3 and the antituberculous drugs, and 0.2 ml of the 1:10 dilution was inoculated into three drug-free LJ medium tubes containing KNO3. The tubes were incubated at 37ºC. After 14 days of incubation, 0.5 ml of freshly prepared Griess reagent was added to one drug-free tube. If any color appeared, the drug containing tubes were developed. Else, the other tubes were reincubated, and the procedure was repeated at day 21 or 28. An isolate was considered resistant if the drug-containing tube produced a color identical or greater than growth control.
PM was performed in 100 isolated strains with the recommended critical concentration for INH, STR, EMB and RMP. PZase activity was assayed in 100 isolated strains by the Wayne assay using Dubos agar. The pink color indicates the enzymatic hydrolysis of the PZA into free POA, so that a strain was considered susceptible to PZA.
The MedCalc software program was used to calculate the sensitivity and the specificity using the results obtained by PM and Wayne Assay as a reference.
Results: Of the 174 samples evaluated by D-NRA, only 100 of them were evaluated by the indirect PM and Wayne Assay. D-NRA results were obtained at day 14 for 33 specimens, for 40 specimens at day 21 and the remaining 27 specimens at day 28. The sensitivity of the D-NRA was 73.33%, 93.91%, 85.71% and 54.55% for INH, STR, RMP and NIC, respectively; for EMB the D-NRA don't identified resistant results. Specificity was 97.65%, 97.40%, 97.94%, 100% and 98.88% for INH, STR; EMB, RMP and NIC, respectively.
Conclusion: The D-NRA is simple to perform, rapid and cost-effective tool for the first line antituberculous drugs resistance detection. Nevertheless, additional studies are requires to improving its sensitivity almost for INH and RMP.

R2498 Microarray-based assay for the rapid detection of genes encoding extended-spectrum β-lactamases and carbapenemases of A, B and D classes

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Objectives: The production of β-lactamases is the predominant cause of resistance to β-lactam antibiotics in Gram-negative bacteria. To date, more than 700 β-lactamases have been described. Among them extended-spectrum β-lactamases (ESBLs) are the most widespread and clinically significant enzymes which are active against practically all penicillins and cephalosporins. During last years the clinical value of carbapenemases of molecular classes A, B and D increased noticeably. So far as many clinical isolates produce more than one β-lactamase and due to the high diversity of these enzymes their determination at the molecular level ensures adequate information for the identification of antimicrobial resistance. In the present research we have developed the technology of oligonucleotide microarray with horseradish peroxidase (HRP)-based detection for the identification of genes encoding ESBLs and carbapenemases of A, B and D classes.
Methods: Specific oligonucleotide probes were designed to determine β-lactamase type and important mutations responsible for the broadening of substrate specificity or resistance to inhibitors. The method of DNA microarray consisted of several steps involving DNA extraction, amplification and labeling of target DNA with biotin by two multiplex PCRs and the subsequent hybridization of a PCR product with specific oligonucleotide probes immobilized on porous membrane support. After hybridization biotin in DNA duplexes was developed with the streptavidin–HRP conjugate followed by colorimetric detection of the enzyme.
Results: Careful optimization of oligonucleotide probes and hybridisation conditions ensured specific identification of all control isolates producing ESBLs and carbapenemases in a single array.
The method developed has been applied successfully for the detection of ESBL and carbapenemase genes in a series of clinical isolates of Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp.
Conclusion: DNA microarray technique offers the identification of a pathogen antibiotic resistance at the molecular level and is proposed as a useful tool for epidemiological investigation of ESBL- and carbapenemase-producing microorganisms.
The work was supported by the Russian Federal Programme "National Technological Resources 2007–2011" (Contract GP/07/442/NTB/K).

R2499 The *Brucella* blood agar for disk diffusion antimicrobial susceptibility testing of the *Bacteroides fragilis* group?

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Objectives: The EUCAST disk diffusion antimicrobial susceptibility testing method for fastidious organisms is based on the Mueller-Hinton fastidious agar (MH-F). In a pilot study, most anaerobic bacteria did not grow well enough on MH-F to permit antimicrobial susceptibility testing. We decided to investigate whether or not the *Brucella* Blood Agar supplemented with hemin and vitamin K (BBA), recommended for antimicrobial susceptibility testing of anaerobic bacteria with gradient strips, might also be suited for disk diffusion.
Methods: *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741 were tested with E-test gradient strip (piperacillin/tazobactam, meropenem, metronidazole and clindamycin) on BBA according to the manufacturer's instructions. The corresponding disk (EUCAST disk strength) was included on each plate. Twelve plates were incubated at 37ºC in an anaerobe environment (10% CO_2, 10% H and 80% N_2) for 24 hours with each antimicrobial agent on two different days for intra and inter day variability. E-test results were compared with the acceptable ranges for the two strains (reference agar dilution testing, CLSI guideline M11-A7). Twelve plates with disks only were also incubated at 35ºC.
Results: All E-test results were within acceptable ranges and the intra and inter day variability was ⩽1 dilution step. Zone diameter mean and range are shown in Table 1 (n = 12).
The maximum difference between two means was 2.9 mm and the maximum range was 5.5 mm. Overall, growth was better and zones smaller at 37ºC than at 35ºC.
Conclusion: Only a small intra and inter day variability was observed with disk diffusion on BBA with the tested antimicrobial agents. Whether this small variability can be reproduced with clinical isolates and whether resistant isolates can be separated from wild type zone diameter distributions of the *Bacteroides fragilis* group remains to be investigated. Also the impact of different temperatures needs to be evaluated further.

Antimicrobial agent	*Bacteroides fragilis* Zone diameter (mm)		*Bacteroides thetaiotaomicron* Zone diameter (mm)	
	37°C	35°C	37°C	35°C
Piperacillin/tazobactam (30/6 mikrog)	32.4 (30.6-33.5) 32.2 (31.3-33.7)	37.7 (36.5-38.9)	20.5 (19.6-21.0) 19.9 (19.2-20.6)	22.2 (21.0-22.8)
Meropenem (10 mikrog)	37.6 (35.7-39.0) 36.7 (35.9-38.1)	41.2 (39.3-42.7)	34.4 (31.9-37.4) 32.3 (30.9-33.7)	35.0 (33.9-35.7)
Metronidazole (5 mikrog)	30.2 (29.3-31.7) 30.3 (28.5-32.9)	35.5 (34.2-37.1)	29.1 (26.6-30.8) 27.3 (25.4-29.3)	30.9 (30.4-31.6)
Clindamycin (2 mikrog)	18.7 (17.9-20.3) 21.6 (20.9-22.8)	30.9 (29.7-32.4)	8.4 (7.8-9.1) 8.5 (7.8-9.0)	10.4 (10.0-10.8)

Table 1.

R2500 Verification of the 2009–10 EUCAST Cephalosporin Breakpoints for Enterobacteriaceae Using Sensititre® MIC Susceptibility Microdilution Panels

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Objectives: In 2009 EUCAST published revised breakpoints for the interpretation of antimicrobial susceptibility tests; some of which were further modified in 2010. The new breakpoints described for Enterobacteriaceae lowered the susceptible criteria for multiple drug classes, including the cephalosporins. Using disk diffusion (DD) and frozen microdilution panels as reference methods, we evaluated the accuracy of the Sensititre® GN4F standard MIC panel (Trek Diagnostic Systems, Cleveland, OH, USA) using current EUCAST interpretative breakpoints.
Methods: Susceptibility testing was performed on 55 enteric Gram negative enteric bacilli. Bacteria from five different genera, comprised of wild-type (n = 30) and ESBL positive strains of *E. coli* and *K. pneumoniae* (n = 25) were included. MIC and DD testing were performed simultaneously using the same inoculum, followed by 18–24 hours of incubation in ambient air or within the Automated Reader and Incubation System (ARIS). The MIC distributions for ceftriaxone, ceftazidime, and cefepime for ESBL positive isolates were also examined.
Results: The MIC dilutions spanned a larger range on the frozen MIC tray compared to the GN4F standard panel; however, the S, I, R, interpretations of the MIC values and DD results were the same for all isolates tested. The distribution of the MICs for ESBL positive isolates varied, and ranged from 0.5 to >64 μgmL for ceftriaxone (AXO), 1 to >32 μgmL for ceftazidime (TAZ), and 0.12 to >32 μgmL for cefepime (FEP).
Conclusions: A Sensititre standard microdilution panel was evaluated against a reference frozen microdilution tray and DD. Accurate determination of MICs and subsequent S, I, R reporting using the new EUCAST interpretative breakpoints were achieved with the Sensititre GN4F panel. Using the current breakpoints, all ESBL isolates tested resistant to ceftriaxone or ceftazidime; one ESBL strain tested susceptible to AXO, while three were susceptible to TAZ, and seven strains yielded FEP MICs of <1 and would be considered susceptible.

R2501 Performance of a new MicroScan WalkAway panel for detection of oxacillin resistance in a French nationwide set of staphylococci isolated from bacteraemia

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Objectives: Detection of Oxacillin resistance (OR) in staphylococci is a daily challenge for clinical laboratories. Erroneous in vitro susceptibility results could lead to therapeutic failure or inadequate antimicrobial selection pressure. Cefoxitin (FOX) testing is now currently used for this purpose. We evaluated the performance of a new MicroScan WalkAway panel (Siemens, Sacramento) including FOX, and the disk diffusion method (DDM) for the detection of OR.
Methods: A set of 217 non-duplicates isolates of *Staphylococcus aureus* (n = 167) and Coagulase Negative staphylococci (CNS, n = 50) was collected in 2007 from a nationwide study through the French College de Bacteriologie Virologie Hygiene network interesting in clinically significant bacteraemia. Identification was performed using gyrB PCR, mass spectrometry (Axima Assurance, Shimadzu) or 16S RNA sequencing. The detection of mecA gene was considered as the gold standard for OR detection. Phenotypic detection of OR was performed according to EUCAST guidelines excepted for moxalactam (MOX) (French official criteria: susceptible to oxacillin if diameter >24 and resistant if <23 mm) using (i) the FOX DDM, (ii) the MOX DDM, (iii) the MicroScan WalkAway PC30 panel which contained oxacillin MIC determination and an additional FOX TEST (4 μgmL); each strain was categorized as OR if the oxacillin MIC was superior to the EUCAST breakpoint or if the FOX TEST was positive (i.e. yielded a growth). The panels was automatically run according to the manufacturer's instructions. ATCC25923 and 43300 were used as quality control strains.
Results: OR was detected by PCR in 31% of *S. aureus* (52/167) and 66% of CNS (33/50), this last group consisted of *S. epidermidis* (34/50), *S. haemolyticus* (8/50), *S. hominis* (5/50), *S. capitis* (2/50) and *S. schleiferi* (1/50). Considering the mecA PCR as the gold standard, the sensibility, specificity, Positive Predictive and Negative Predictive Value were respectively for *S. aureus* 88.5%, 100%, 100% and 95% for the FOXdm, 98.1%, 100%, 100% and 99.1% for the MOXdm, and 98.1%, 100%, 100% and 99.1% for the PC30 panel including the FOX Test. The corresponding results obtained for CNS were 87.9%, 100%, 100% and 81% for the FOXdm, 100%, 100%, 100% and 100% for the MOXdm and 100%, 88.2%, 94.2% and 100% for the PC30.
Conclusions: The new Microscan PC30 panel used with the Walk Away system is a highly accurate method for OR detection in clinical relevant staphylococci.

R2502 Comparison of phenotypic and genotypic tests used for determining methicillin resistance in *Staphylococcus aureus* isolates

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Objectives: The heterogenous expression of methicillin resistance in *S. aureus* make the phenotypic testing difficult and slow. The aim of this study was to compare the results of phenotypic and genotypic methods used for methicillin resistance, determine susceptibilities to antibiotics used for skin and soft tissue infections.
Methods: 92 outpatient and 150 inpatient *S. aureus* strains isolated from skin and soft tissue infection included in the study. The patients were classified as community acquired and hospital acquired by CDC criteria. Methicillin resistance was determined by oxacillin and cefoxitin disk diffusion (DD), then confirmed with oxacillin salt screen agar test, and mecA PCR. Susceptibility test to antimicrobials including clindamycin (CLI), erythromycin (ERY), gentamicin (GEN), penicillin (PEN), mupirocin (MUP), rifampin (RIF), tetracycline (TET), trimethoprim-sulphametoxasole (SXT), teicoplanin (TEC) were determined. Data were compared by the chi-square or Fisher's exact test, using SPSS 15.0.
Results: mecA was detected in 11 outpatient, 66 inpatient. HA strains showed higher positivity than CA-strains (44%, 12%) (5.79; 2.85–11.74, p = 0.001). Oxacillin, cefoxitin DD test, oxacillin screen agar test exhibited sensitivities of 98.7%, 98.7%, 100% and specificity 96.9%, 97.5% and 96.9%, respectively. Based on mecA gene and patient data 242 *S. aureus* were classified as; 81 CA-MSSA, 84 HA-MSSA, 11 CA-MRSA, 66 HA-MRSA. Five isolates were true CA-MRSA. All isolates were susceptible to TEC. 3 CA, 2 HA were high level resistant to MUP. MRSA isolates were more likely to be resistant to antibiotics except MUP, FA compared with MSSA. mecA positive strains were more resistant to ERY (10.3;4.09–27.5), CLI (10.6;3.63–24.35), TET (28.3;12.1–66.4), GEN (35.5;16.7–75.5), and RIF (98.7;35.7–272.6) than mecA negative strains (p = 0.001).
Conclusion: This study showed that with the highest sensitivity and specificity oxacillin salt screen agar test and cefoxitin DD test could be used for methicillin resistance detection as an alternative for mecA PCR. Mupirocin and fusidic acid may be still therapeutic choices fort he treatment of skin and soft tissue infections.

R2503 Comparison of E-test, Vitek2, and Wider for detection of VIM-producing Enterobacteriaceae with the updated CLSI and EUCAST breakpoint systems

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Objective: The aim of this study was to compare the performance of Etest, Vitek2 and Wider in detecting metallo-β-lactamase (VIM)-producing Enterobacteriaceae using the updated CLSI and EUCAST clinical breakpoints as well as EUCAST epidemiological cut-off (ECOFF) values.
Methods: A total 23 VIM-producing Enterobacteriaceae (12 *K. pneumoniae*, 8 *K. oxytoca*, 2 *S. marcescens* and 1 *E. cloacae*) were isolated in our hospital from January 2009 to November 2010. Etest (bioMérieux) with imipenem (IPM) and meropenem (MEM) was performed. Further, all strains were tested with the VITEK2-system (bioMérieux) with the AST N-114 card containing IPM and MEM, and microdilution Wider system (Panel C095–31/W/REV2. Fco. Soria Melguizo, Madrid, Spain).

Method / Antibiotic	Nº of isolates inhibited at each concentration (mg/L)									Range	MIC_{50}	MIC_{90}
	≤0.25	0.5	1	2	4	8	16	32	>32			
Etest												
Imipenem				1	6	3	3		10	2->32	16	≥32
Meropenem	1	2	5	6	2	3	1		3	≤0.25-16	2	16
Vitek												
Imipenem		1		1	13	3	5			0.5-≥16	4	≥16
Meropenem	1		18	3			1			≤0.25-≥16	1	2
Wider												
Imipenem				5	7	5	6			2->8	8	>8
Meropenem			8		3	5	7			1->8	8	>8

CLSI ≤1 2 ≥4 EUCAST ≤2 4-8 >8 ECOFF≤1 Imipenem y ≤0.12 Meropenem

Results: Susceptibility testing results obtained by each method are shown in the Table. With Etest and CLSI criteria, 8 strains were interpreted as susceptible to MEM and applying EUCAST criteria 1 and 14 strains were interpreted as IPM and MEM susceptible respectively. With Vitek2 and CLSI, 1 and 19 strains were IPM and MEM susceptible whereas with EUCAST 2 and 22 respectively. The Wider system failed in detecting 8 carbapenemase producers when using MEM with CLSI and EUCAST criteria. Five additional failures were observed with IPM and EUCAST. Only one strain had MICs below the IMP and MEM

ECOFFs using the VITEK-2 system. With the other methods all strains were detected using these epidemiological cut-offs.
Conclusions: The new CLSI breakpoints performed better that the EUCAST clinical ones in detecting VIM-producing Enterobacteriaceae. However the current breakpoints still do not capture all carbapenemase-producing isolates. This is important because standard doses of carbapenems for treatment of isolates with these MICs could be one reason for the selection of highly resistant strains.

R2504 Anaerobic infections and susceptibility to antimicrobial agents in a Portuguese hospital

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Objectives: Anaerobic bacteria have been established as causing a number of serious human infections. Due to their fastidious nature, they are difficult to isolate from infectious sites and are often overlooked. Increasing resistance to several antibiotics has been reported in the last decades. The aims of the study were to analyze the prevalence of anaerobes and to evaluate the antimicrobial susceptibility in various clinical specimens in our hospital.
Methods: During the year of 2009, 136 anaerobic strains were identified from 2113 clinical specimens. After elimination of duplicates 100 strains were studied (CLSI M-39). The samples were inoculated in Schaedler broth (enrichement media) and cultured on brucella anaerobic agar. Initial identification was made by Gram staining, colony morphology, hemolysis, pigment formation, special-potency antimicrobial agent disks (Becton, Dickinson and Company, USA) and by using the API 20A system (BioMerieux, France). The susceptibility (MIC) for amoxicillin/clavulanic acid (AMC), imipenem (IMP), metronidazole (MET) and clindamycin (CLIN) was determinated with E-test (Biomerieux, France) and interpreted according to clinical breakpoints from the CLSI M11– A7, 2007.
Results: Of 100 anaerobic strains studied, 60% were *Bacteroides fragilis* group; 18% were other Gram-negative (*Prevotella*, *Porphyromonas*, *Veillonella*) and 22% Gram-positive (*Peptostreptococcus* spp., *Propionibacterium*, *Bifidobacterium*, *Clostridium*, *Eubacterium*, *Actinomyces*) anaerobes. Table 1 presents the antimicrobial susceptibility. The infection sources of the samples were: head and neck (2%), lung and thorax (7%), abdomen (38%), soft tissue and extremities (40%), obstetric-gynecological (9%) and surgical wound (4%).
Conclusion: As expected, the most abundant anaerobic species isolated were *Bacteroides fragilis* group (60 strains), which comprised 60% of the total anaerobes isolates. Gram-positive occurred in lower number (22%). More than a half of anaerobes were isolated from intraabdominal and soft tissue infections. All strains of *Bacteroides fragilis* group were susceptible to MET, the IMP was the second choice with 98,30% of susceptibility. Antimicrobial susceptibility testing suggests MET and IMP as a useful spectrum of activity against all isolates and are good alternatives for empiric therapy in our hospital.

Table 1: Anaerobic antimicrobial susceptibility

Antimicrobial	MIC (microg/mL)	*Bacteroides fragilis* group	Other Gram-negative anaerobes	Gram-positive anaerobes
AMC	<=4	68,30%	83,30%	100%
	8	10%	0%	0%
	>=16	21,70%	16,70%	0%
IMP	<=4	98,30%	100%	100%
	>=16	1,70%	0%	0%
METRO	<=8	100%	94,40%	72,70%
	>=32	0%	5,60%	27,30%
CLIN	<=2	51,70%	66,70%	90,90%
	4	11,70%	0%	4,55%
	>=8	36,60%	33,30%	4,55%

MIC= minimal inhibitory concentration

R2505 Antibiotic sensitivity testing with InoqulA® according to EUCAST new recommendations

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Introduction: EUCAST new disc diffusion test for routine antimicrobial susceptibility testing (AST) on Mueller Hinton (MH) agar is a standardized method to be used in microbiological laboratories. The EUCAST method requires that the inoculum is confluent. We combined the EUCAST AST guidelines using InoqulA® (Kiestra Lab Automation, Drachten, The Netherlands). InoqulA® is an instrument for automatic plate inoculation where the spreading is performed by magnetic beads and the spreading patterns can be customized for different agars and purposes.
The aim of this study was to optimize the spreading performed by InoqulA® and to find the ideal pattern for susceptibility testing on MH agar according to EUCAST guidelines, by varying the volume of the inoculum and the length and location of the primary streak. We also wanted to compare the results of InoqulA® spreading with manual spreading.
Method: 160 routine isolates from the following species were tested; 60 *E. coli*, 10 *P. mirabilis*, 10 *K. pneumoniae*, 10 *Enterobacter* spp, 10 *P. aeruginosa*, 40 *S. aureus*, 15 *E. faecalis* and 5 *E. faecium*. The bacteria were cultivated on blood agar incubated in 35ºC ambient air for 18–22 hours. Suspensions of McF 0,5 and McF <0,1 density were prepared from each isolate. All suspensions were also manually spread on MH agar with cotton swabs. After incubation inhibition zones were then measured.
Results: The most ideal spreading pattern to create evenly spread growth and uniform circular inhibition zones was pattern 5 with 1 mm distance between the laps and a full length primary streak. 50 µL of the McF 0,5 suspensions were sufficient for all species except *Enterococcus* spp which required an inoculum volume of 75 µL. Less inoculum than 50 µL increased the diameter of the inhibition zones. Inoculums with a density of McF < 0,1 yielded larger inhibition zones than inoculums of McF 0,5 density. This had an effect on the SIR-interpretation for some antibiotic-bacteria combinations.
Conclusions:
1. It is possible to use InoqulA® for inoculation and spreading of agar plates for susceptibility testing according to the new EUCAST guidelines.
2. There is no significant difference in SIR-interpretation of the included species when comparing inhibition zones after manual spreading and after spreading by InoqulA®
3. Using inoculums of much lower density than recommended increases the size of the inhibition zone, which has an effect on the SIR-interpretation for some antibiotic-bacteria combinations.

R2506 Rapid susceptibility of Enterobacteriaceae to ciprofloxacin using flow cytometry

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Objectives: Increasing resistance to quinolones on Enterobacteriaceae, the most important causes of nosocomial and community acquired infections, have been documented. They constitute the main therapeutic choice, often empirically made to overcome the delayed susceptibility test. A quick test based on flow cytometry was developed to analyze the susceptibility to ciprofloxacin, an antimicrobial agent which inhibits DNA gyrase and blocks DNA replication.
Methods: A total of 30 susceptible and resistant strains of *Escherichia coli*, *Klebsiella pneumoniae* were screened for ciprofloxacin by VITEK 2 automate system (BioMérieux, Paris) and additionally by Etest (BioMérieux, Paris). *E. coli* ATCC 25922 was used as control. Bacterial cells were incubated in filtered Muller-Hinton broth until exponential phase and then exposed to 1, 2 and 4 micrograms per milliliter of ciprofloxacin (Σ-Aldrich) for 0.5, 1 and 2 hours. Several fluorescent dyes were tested in order to obtain the best approach to the subject: Propidium

Iodide (Σ-Aldrich) (FL3- 650 nm) a nucleic acid staining, which only penetrates on cells with severe lesion of the membrane i.e. dead cells; Bis-(1,3-dibutylbarbituric acid) trimethine oxonol (DiBAC4(3)) (Σ-Aldrich) (FL1- 530 nm), a lipophilic anion able to diffuse across depolarized membranes; and SYBR Green I (Molecular Probes) (FL1- 530 nm) fluorescent dye that binds to double stranded DNA. Flow cytometric analysis was performed after staining with those probes in the dark for 30 minutes. Conventional colony-forming units (CFU) assay was performed from the suspensions analyzed by Flow Cytometry.
Results: Propidium Iodide and DiBAC4(3) were not able to discriminate Susceptible and Resistant strains after the tested incubation time. SYBR Green I was able to clearly discriminate between susceptible strains, presenting a decrease in fluorescence intensity in a drug concentration and time dependent manner only on susceptible strains when compared to the viable non-treated bacterial cells even after 0.5 hour treatment. Correlation between conventional CFU assay and Flow Cytometry was successfully achieved.
Conclusion: One hour versus 24–48 hours was enough to characterize the susceptibility to ciprofloxacin staining with SYBR Green I. Flow cytometry proved to be an excellent and accurate method representing an alternative approach to evaluate the susceptibility of bacteria to ciprofloxacin.

R2507 Use of the MicroScan Walkaway system for the detection of extended-spectrum β-lactamase production by Enterobacteriaceae

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Objectives: The detection of extended spectrum β-lactamases (ESBLs) in the laboratory has led to a rise in the use of carbapenems worldwide. Many laboratories now rely on automated systems to perform this task, and the reliability of their results is of crucial importance. Here, we assessed the performance of one such system – the MicroScan WalkAway system (Siemens) and panel NM36 as a screening tool for the detection of ESBL production by a range of enterobacteriaceae.
Methods: We collected 105 consecutive enterobacteriaceae isolates which were reported as either 'ESBL positive' or 'possible ESBL' on the NM36 panel. Isolates were further identified by MALDI-tof (Bruker) mass spectrometry analysis. Combined disc diffusion tests (cefpodoxime and cefepime +/− clavulanate) were used to confirm ESBL production and all isolates were then further characterised using multiplex PCR methods to detect genes encoding TEM, SHV, OXA, CTX-M, PER, VEB and plasmidic ampC enzymes.
Results: Of the 105 isolates, 84 were reported as ESBL producers, and 21 flagged by MicroScan as 'possible ESBL' producers, 72/84 were identified as *Escherichia coli*, 7/84 *Klebsiella* spp, 3/84 *Enterobacter* spp, 1/84 *Citrobacter* spp, and 1/84 *Proteus* spp. 90% (76/84) of those called positive by MicroScan were also positive by combined disc testing. Of the 8 negative by disc diffusion, 6 were also negative for all genes detected by PCR. Of the 'possible ESBL producers', 90% (19/21) were negative in the disc assay, and 12 of these were also negative by PCR for all β-lactamase genes tested. Of note the identification of organisms flagged as 'possible ESBL' producers differed from those confirmed as positive – 7/21 *Enterobacter* spp, 6/21 *Escherichia coli* 2/21 *Klebsiella* spp, 2/21 *Proteus* spp, 2/21 *Morganella* spp, 1/21 *Citrobacter* spp, and 1/21 *Serratia* spp.
Conclusion: The MicroScan NM36 panel was effective at identifying common ESBL phenotypes in *E. coli* and *Klebsiella* species. Isolates identified as 'possible' ESBL producers should be further investigated using phenotypic disc tests or molecular methods, particularly if they are not members of the genus *Klebsiella* or *E. coli*.

R2508 Comparison of genotypic and phenotypic susceptibility testing in multidrug-resistant Gram-negative bacteria

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Objectives: Multidrug-resistant gramnegative bacteria (MDR) became an emerging problem throughout Europe within the last years. To prevent transmission of these bacteria especially in the hospital setting and to start earlier with an appropriate antibiotic therapy new and rapid molecular-based diagnostic tools are needed.
Here we compared results from conventional phenotypic antibiotic susceptibility testing with newly developed PCR based genotypic methods to estimate the benefit of such new tools in future.
Methods: A set of 237 clinical isolates was selected (196 Enterobacteriaceae, 41 non-fermenter). 193 of these clinical isolates exhibited a MDR phenotype. MDR was defined as resistance to at least 3 antibiotic classes or an ESBL phenotype. Genotypic testing was carried out by a multiplex PCR assay system targeting 3 classical β-lactamases (ambler class A) and 2 families of plasmid coded ampC genes (ambler class C). Moreover a surrogate marker for multidrug resistance was included in the assay. The complete assay comprises genes coding for 17 different pathogens and 22 resistance markers.
Results: 237 clinical isolates were investigated with the new multiplex PCR assay system. No false positive results of non-MDR phenotypes were observed. In 151 of the 170 (89%) Enterobacteriaceae and 20 of the 23 (87%) non-fermenter included in this study the MDR phenotype was confirmed. Occurrence of genes coding for β-lactamases in Enterobacteriaceae are species specific. Interestingly, the presence of more than one β-lactamase gene is very common and the combinations are also species specific. Furthermore, detection of the MDR surrogate marker shows a strong correlation with an ESBL phenotype in Enterobacteriaceae. In the *Acinetobacter baumannii* isolates included in this study this marker was suitable to predict a resistance to aminoglycosides, whereas the correlation in Enterobacteriaceae was poor.
Conclusion: Genotypic antibiotic susceptibility testing demonstrates a rapid and therefore valuable tool to quickly detect MDR. Faster detection should help to accelerate the implementation of hygienic measures and to select the appropriate antibiotic therapy in time.

R2509 Implementation of EUCAST in a clinical laboratory: a piece of cake?

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Objective: EUCAST aims to set European breakpoints for antimicrobial resistance and to streamline methods of antimicrobial susceptibility testing (AST) to increase the comparability of results from different laboratories.
In our laboratory AST was performed by micro-broth dilution on Phoenix™ and disk diffusion (DD) according to CLSI guidelines. At the moment BD Diagnostics discontinued production of certain non-EUCAST panels, we decided to implement EUCAST-guidelines and breakpoints for DD and automatic testing at the same time.
This study describes the draw-backs encountered during the implementation of these guidelines in a routine microbiology laboratory.
Methods: The EUCAST DD manual (EUCAST DD-Manual v. 1.0 18–12–2009) was used for the validation of the AST with EUCAST breakpoints.
The checklist to facilitate implementation of AST with EUCAST breakpoints (v. 1.0 25–08–2010) was used to guide the implementation.
Results: Although in 2008 a National Antimicrobial Committee was founded for a nationwide validation of the implementation of EUCAST methodology, no Belgian validation data for DD and Phoenix were available at the moment of our implementation.

Besides, breakpoints for several antibiotics tested in our lab are lacking in EUCAST-guidelines e.g. temocillin for Enterobacteriaceae, ceftazidime for *Acinetobacter* and erythromycin for Enterococci.
Practical issues encountered included the unavailability of some of the required disks and plates from some companies at the moment of implementation. Also, Phoenix EUCAST-panels do not all fit completely into EUCAST-strategy as some antibiotic ranges do not include the new breakpoints recommended e.g. erythromycin for enterococci and rifampicin for staphylococci.
Due to these issues, a complete quality control testing on 20 consecutive days had to be performed using ATCC strains 25922, 27853, 29213, 29212, 49619 and NCTC strain 8468. Zone diameters were measure daily by 2 trained microbiologists and compared to ranges provide by EUCAST (Tables of QC targets and ranges v. 1.3 21–12–2010).
The implementation of the technicians (read a correct antibiogram 3 days in a row) required more than 4 days and is still ongoing (increasing). Most problems are seen with PSAE and meropenem, STAU and penicillin, ENFA and ampicillin, SRPN and penicillin and oxacillin and HAIN.
Conclusion: The implementation of EUCAST guidelines for performance and interpretation of AST in a routine microbiology laboratory is no piece of cake!

R2510 New immunochromatographic assays for detection of methicillin resistance and identification of *S. aureus* directly on primary culture or blood culture bottles in a few minutes

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Objective: Evaluation of:
- Clearview Exact PBP2a™ (CPBP2a) (Alere) for detection of PBP2a directly on primary cultures in 5 minutes.
- BinaxNOW *Staphylococcus aureus*™ (BNSA) and BinaxNOW PBP2a™ (BNPBP2a) (Alere) for SA identification and methicillin résistance (MR) detection directly on blood culture bottles in 30 minutes.

Methods: Prospective study of CPBP2a: 333 consecutive staphylococcal strains were tested from primary plates.
Evaluation and prospective study of BNSA and BNPBP2a: *Evaluation*: 17 strains representative of MRSA clones circulating through the world were inoculated into artificial blood cultures (BacT/Alert FA and FN with 10 ml fresh human blood, 100 CFU/vial) and incubated in the BacT/Alert automat. *Prospective study*: 58 clinical blood culture bottles positive for Gram-positive cocci were included.
All immunochromatographic results were compared with phenotypic results (Vitek or Phoenix®). Any discrepant result was checked by PCR.
Results: Test CPBP2a: 329 tests were interpretable. For SA (MSSA, n = 216; MRSA, n = 30), Se, Sp, PPV and NPV were 90%, 100%, 100% and 98.6%, respectively. For coagulase negative staphylococci (CNS) (MSCNS, n = 23; MRCNS, n = 60), Se, Sp, PPV NPV were 70%, 100%, 100%, 56%. No false positive was observed for SA and for CNS. For MRSA and MRCNS isolates not detected on primary culture, the test was positive for all MRSA and for 14 of 18 MRCNS after subculture. Two out of the 4 remaining MRCNS were detected after induction (around cefoxitin disc) and 2 could not be retested.
Tests BNSA and BNPBP2a: *Evaluation*: for BNSA, 1 out of the 34 blood culture bottles (17 pairs aero/ana) remained negative. For BNPBP2a, all strains were positive for PBP2a. *Prospective study*: for BNSA, Se, Sp, PPV and NPV were 100%, 93.1%, 87.8%, 100%, respectively. All SA were accurately detected while 4 CNS yielded false positive results. For BNPBP2a, Se, Sp, PPV and NPV were 100% for SA (MSSA, n = 27; MRSA, n = 2) while for CNS Se, Sp, PPV NPV were 75%, 90%, 93.8%, 64.3%, respectively.
Conclusion: For SA, CPBP2a presents relevant PPV and NPV, enabling early consideration of the MR directly on primary culture. For CNS, the test has an acceptable PPV but a low NPV and improvement would probably require an optimization of the protocol.
For BNSA and BNPBP2a, evaluation study as well as preliminary results from the prospective study appears promising.

Public health and community-acquired infections

R2511 Clinical and laboratory characteristics of patients with measles for the period February-June 2010

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Introduction: Measles as a highly infectious disease that can result in serious complications returned in Bulgaria. Launched in 2009 a major outbreak covered the majority of the population in the Roma districts of Plovdiv and the region. The reasons for this vary from high-risk populations unimmunized for cultural or religious beliefs to low knowledge of the means of transmission and severity of measles. Objectivity required accepting the fact that infectious diseases and responsibility of them challenge the health system to increase the immunization coverage up to 95%.
Material and Methods: During February and July, incl. 2121 cases of measles were registered and 1969 of them were hospitalized in the University Clinic of Infectious Diseases, town of Plovdiv. Laboratory tests were conducted on the standard methodology; virus and serological parameters were investigated in Regional Public Health Institute. The data were statistically processed with SPSS 14 analysis system, using parametric methods in Gaussian distribution and nonparametric when needed. As a significant difference interval was accepted $p < 0.05$, guaranteeing 95% confidence.
Results and Discussion: The highest incidence of measles was repotted during April and May, 34.3% of hospitalized patients. Mainly medium–heavy forms of disease were observed. 65% of treated were children between 1 to 18 years. Measles complicated with pneumonia was found in 504 patients – 25.6%. Pronounced respiratory failure and need of oxygen therapy had 59 fellows. Antibiotics received all complicated cases. X-Ray control was achieved in 74.3% of lung-affected. We observed complications of the nervous system in 7 patients, aged 8 months to 52 years. Measles, complicated with meningitis – two cases, viral encephalitis – 4 and one 8 years old boy with meningomyelitis.
Conclusion: Outbreak of measles in Plovdiv and the region in 2010 once again put reasonable challenges of organizational, financial, legal and social–legal aspect to epidemiologists and infectious diseases in particular and the healthcare system in the country as a whole. The neurological complications were rare – in analyzing study 0.36% with benign ending.

R2512 *Helicobacter pylori* in inflammatory bowel disease and colorectal cancer patients

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Objectives: Recently, *Helicobacter pylori* proved to be capable to colonise colonic tissue and, due to this, it may have a protective effect against the development of inflammatory bowel disease (IBD). The study aimed at evaluation of cagA-positive and cagA-negative *H. pylori* strain manifestation in active IBD and colorectal cancer (CRC) patients.
Methods: The study was conducted in 44 hospitalized adult patients. Employing standard clinical, radiological, endoscopic and histopathological criteria, 3 study groups were distinguished. Group 1 included 19 patients with active ulcerative colitis (UC). Group 2 included 10 patients with active Crohn's disease (CD). Group 3 included 15 patients with CRC. The standard surgical treatment was applied in all the patients. The study material involved homogenized colonic tissue. DNA was isolated using QIAamp DNA Mini Kit (Qiagen). *Helicobacter pylori* and cagA gene of *H. pylori* were identified using PCR-*Helicobacter pylori* diagnostic kits (DNA Gdansk). PCR was conducted using Mastercycler gradient (Eppendorf). Positive and negative controls were used. Positive

result of *Helicobacter pylori* identification involved presence of 262 bp PCR product, positive identification of cagA gene involved presence of 445 bp PCR product.
Results: In Group 1 *H. pylori* infection was disclosed in 6 patients (31.6%), but in only a single case cagA-positive *H. pylori* was identified. In Group 2 only a single patient (10%) carried cagA-negative *H. pylori* infection. In turn, in Group 3 infection with *H. pylori* was detected in 8 patients (53.3%) and in five of them cagA-positive *H. pylori* was identified. In parallel, cagA-positive *H. pylori* infection was shown to significantly more frequent in CRC patients than in patients with IBD ($p = 0.0317$).
Conclusion: In patients with IBD infections with cagA-negative *H. pylori* strains prevail, which may carry no pathogenic significance. On the other hand, in CRC infections with cagA-positive *H. pylori* strains prevail, which seems to be involved in development of colorectal cancer.

R2513 Spread of blood-borne viruses among conscripts of the Bulgarian army

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Objectives: Contact with blood of battlefield casualties, injection drug use, tattoo, piercing and sharing of personal hygiene items play an important role in the transmission of blood-borne infections among military personnel. The purpose of this study was to determine the prevalence of the main four blood-borne infections and to examine the risk factors among conscripts of the Bulgarian army.
Methods: Anonymous cross-sectional data were collected from May 2009 to November 2010 from recruits who voluntary agreed to participate in the study and who completed a self administrated questionnaire. A blood sample was taken from each subject and tested in order to detect anti-HBc, HBsAg, anti-HCV, anti-HDV and anti-HIV by enzyme-linked immunosorbent assay (ELISA).
Results: In this study, 504 army conscripts at the age of 18–28 (20.1 ± 6.8) were enrolled. 71 (14.1%) (95% confidence interval 8.6% to 18.8%) had antibody to hepatitis B core antigen, 21 (4.2%) (95% confidence interval 2.8% to 6.4%) were positive to hepatitis B surface antigen, 12 (2.4%) (95% confidence interval 1.8% to 2.6%) were anti-hepatitis C positive, 9 (1.8%) (95% confidence interval 0.9% to 2.8%) had antibody to hepatitis D and 0 (0%) were positive to antibody to HIV.
Conclusions: Although this population theoretically had a low risk for HBV, HCV and HDV infection, these results are higher than expected in accordance with the age range. It has not been found any recruits positive to HIV. Based on the prevalence of serological markers we recommend vaccination against HBV infection after prevaccination screening.

R2514 Prevalence and correlates of hepatitis C virus infection among inmates of Bulgarian prisons

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Objectives: To determine the prevalence and predictors of hepatitis C virus (HCV) infection and associated risk factors for this infection among inmates of Bulgarian prisons.
Methods: This study was carried out in 2009, among inmates of the fifth biggest Bulgarian prisons (men $n = 367$ and women $n = 131$) and a juvenile correctional institution ($n = 86$). Anonymous cross-sectional data was collected from prisoners who agreed to participate in the study and who were interviewed using a standard questionnaire including demographic, imprisonment history and HCV related risk behaviors items. Thereafter, the blood drawn from the participants were tested for anti-HCV antibodies and HCV-RNA. Discarded serum samples were tested also for a presence of hepatitis B core antibodies (anti-HBc), hepatitis B surface antigen (HBsAg), hepatitis D antibodies (anti-HDV) and antibodies to human immunodeficiency virus (HIV).
Results: A total number of 498 (74% male and 26% female) inmates participated in our study. The overall rate of antibody positivity to HCV among inmates was 25.6%. Among male inmates 32.6%, and among female inmates 20.2% were anti-HCV positive. The most important risk factor for HCV infection was injection drug use. Total number of i.v. drug abusers (IDA) and non-i.v. drug abusers (NIDA) was 142 (28.5%) and 109 (21.9%), respectively. Of all IDAs, 61.4% and of NIDAs, 26.5% had serological evidence of HCV infection. In addition, anti-HBc rates were 59.2%, HBsAg 32.4%, anti-HDV 9.4% and anti-HIV 0.6%.
Conclusion: The seroprevalence of HCV infection among prisoners comparing with the general population in Bulgaria, is very high (25.6% vs. 1.1%). These data indicates that this infection is common among both men and women entering prisons in Bulgaria. However, male inmates are much more likely to be infected with HCV. Our results emphasize the importance of policies to prevent transmission of viral hepatitis during and following incarceration.

R2515 A bacteriologic study of diabetic foot ulcers

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Objectives: Diabetic foot ulcers are frequently complicated by infection which represents an important cause for hospitalization, enhancing the risk for subsequent amputation. Usually these infections are polymicrobial in nature so correct and early isolation and identification, as well as prompt initiation of appropriate antibiotic therapy are important steps toward a successful outcome. This study was undertaken to identify the pathogens associated with diabetic foot infections in our hospital.
Methods: A total of 283 consecutive patients were included in the study during the period January 2007 to October 2010. Only diabetic patients presenting with foot infection and who did not receive antibiotics for the past 30 days were included in the study. Clinical specimens collected from patients were inoculated onto appropriate plates for standard aerobic and anaerobic cultures and incubated at 37^0 C for 24h and 48h, respectively. A Gram-stained smear from the specimen was examined under microscope to obtain valuable information about the types of microorganisms present. The isolated pathogens were identified using the automated system VITEK 2 (BioMerieux, Marcy l'Etoile, France).
Results: The mean age of the patients was 60.2 years (range 33–78) with 165 (58.3%) of them being males and 118 (41.7%) females. A total of 501 pathogens were isolated: 191 aerobic Gram-positive cocci representing 38.1% of all pathogens, 221 (44.1%) aerobic Gram-negative rods, 82 (16.4%) anaerobic bacteria and 7 *Candida* isolates (1.4%). *Staphylococcus aureus* was more frequently isolated among the Gram-positive cocci (60.9%), *Proteus mirabilis* was more frequently isolated among the Gram-negative rods (31.4%) and *Bacteroides* species represented the 86.4% of all anaerobic bacteria isolated. One hundred and thirty-eight (48.8%) patients had one microorganism, 65 (23.0%) had 2 pathogens, 32 (11.3%) had 3, 25 (8.8%) patients had 4 pathogens and 7 (2.5%) patients had 5 pathogens isolated from their foot ulcers.
Conclusion: In our study group diabetic foot infections were mostly monomicrobial. The most frequently isolated microorganisms from the ulcers were *S. aureus*, *P. mirabilis* and *Bacteroides* species. Constant awareness of isolated pathogens in these infections is essential for the optimal management and a successful outcome of diabetic foot ulcers.

R2516 Influenza A/H1N1 in Libya: implementation during pandemic influenza

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Objective: To analyse the epidemiology and clinical characteristics of novel influenza A (H1N1). In March 2009 H1N1 influenza virus began its inexorable spread around the world. The National Centre for Diseases Prevention and control (NCDC) in Libya performs on-going influenza surveillance and tracking of patients in Libya. We also evaluated the performance of our diagnostic tools for the detection of H1N1 2009.

The aim is to describe the NCDC experience and lessoned learned from H1N1 pandemic.
Methods: Surveillance and investigation procedures were modified in accordance with NCDC policy. From the outset of the outbreak and until March 15, 2010, all suspected cases of influenza A/H1N1 were investigated and laboratory verified. Starting December 1, 2009, lab confirmation was reserved for severely ill patients or those at high risk of complications. All hospitalized cases were monitored and tracked daily. We also evaluated a new rapid influenza diagnostic test (the Remel Xpect Flu A&B test) for the pandemic (H1N1) 2009 virus, using real-time RT-PCR for 684 samples which randomly selected and were negative by rapid test.
Results: The first A/H1N1 2009 cases were identified by the surveillance system in the first week of July 2009. The timeline showed epidemic peak occurred in November 2009. In the six months from July to March 2010, there were 1192 confirmed cases of H1N1 in Libya. During the peak of the pandemic period, 35% of samples collected from patients with influenza like illness (ILI) were reported to be positive for influenza; 95% of those positive for influenza were H1N1. At this time there was an increase in the rates of patient visits to outpatient clinics for ILI, especially in the age group 0–18 years old (54%) and in residents of Tripoli, The capital of Libya. However, 99% of patients are less than 65 years old.
The Sensitivity of Remel Xpect Flu A&B test to the pandemic influenza (H1N1) 2009 were 41%.
Discussion: Data indicate spread particularly to younger populations, expressing non-specific respiratory symptoms. NCDC surveillance system provided a valid picture which facilitated how to deal with the influenza epidemic.
The low sensitivity value of the rapid test during these outbreaks highlights the limitations of using this test alone to establish diagnosis and aid clinical management.

R2517 Outbreak of Shigatoxin producing *Escherichia coli* O174:H2 infections, Austria, 2010

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Objectives: Shigatoxin producing *Escherichia coli* (STEC) are an important cause of foodborne disease. It is transmitted to humans primarily through consumption of contaminated food, such as raw or undercooked ground meat products and raw milk. We report on the first occurrence of STEC O174:H2 in Austria.
Methods: Biochemical identification, serotyping and virulence testing were done at the National Reference Centre for *Escherichia coli*. A descriptive investigation was performed in order to describe the outbreak and to identify the outbreak genesis.
Results: The outbreak involved 7 persons (6 male). The index isolate was from a 46 year old, previously healthy male, who had a fecal specimen taken under intensive care for haemolytic uraemic syndrome (HUS) on April 13 in Graz. The second isolate was from a 46 year old Tyrolean patient (specimen received on April 28); this patient suffered from discharge of slime and bowel irregularities, following watery diarrhea. The patient attended a book fair in Graz 5 days before onset of illness in Innsbruck. He and two working colleagues consumed appetizers (bred with various handmade spreads) at some of the exhibition booths. The two colleagues fell ill with diarrhea (one in Innsbruck, one in Munich, Germany) but had not stool specimens tested. Another stool isolate (June 10) was from a 66 year old male treated for HUS in Vienna. A fourth isolate was from this latter patient's wife (age 63), who developed bloody diarrhea three days after her husbands discharge from hospital. A fifth stool isolate was from a 92 year old patient with diarrhea from Graz (specimen received May 12). All five isolates were sorbitol-fermenting, positive for stx1, stx2 and hlyA, but negative for eae. Analyses by PFGE using XbaI as restriction enzyme yielded patterns indistinguishable from each other, but clearly different from five other STEC O174:H2 provided by the German National Consulting Laboratory on HUS and 48 disease associated Austrian STEC isolates from 2010.
Conclusion: Our report emphasizes that stool samples from patients with HUS must be tested by other techniques than sole use of Sorbitol-MacConkey media. Direct person-to-person transmission underlines the importance of instructing family members about the need for post-defecatory handwashing. For five of the six remaining patients, consumption of an unidentified contaminated handmade spread produced and distributed in the Graz area is the likely source of infection.

R2518 Expression of virulence hallmarks in *Escherichia coli* aquatic strains submitted to different stress conditions

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Purpose: To investigate the expression of 11 virulence factors (VF) of 100 *E. coli* aquatic strains isolated from Black Sea in different conditions.
Material and Methods: Cell-associated (i.e. adhesion to HeLa cells) and soluble (i.e. lecithinase, lipase, gelatinase, caseinase, amylase, aesculin hydrolysis, DN-ase, lisindecarboxilase, hemolysin, and CAMP test performed in the presence of *Staphylococcus aureus* ATCC 25923 and *Rhodococcus equi* ATCC 6939) VF were investigated at different temperature (22ºC, 37ºC, 44ºC), salinity, pH and glucose concentration, in aerobic and respectively, anaerobic conditions. The genes encoding for VF which remained negative, were detected by multiplex PCR for Ecp/dA – lipase and EchelD – DN-ase and by simple PCR for Ech/yB – α-hemolysin.
Results: The adherence ability, siderophores, amylase and caseinase were better expressed at 37ºC. At 0% NaCl only amylase and siderophores, at 2% and 3% NaCl the amylase was better expressed and at 6% NaCl, siderophores and caseinase showed the best expression, thereafter, the VF expression gradually decreasing till 10% NaCl. The adherence to HeLa cells was decreased by higher salinities. The tested strains proved high resistance to a broad range of pH from 5 to 9.6. The amylase and caseinase were better expressed at pH 9.6 and siderophores at pH 7. The higher glucose concentrations (3%) inhibited the expression of amylase and caseinase. The incubation conditions exhibited no influence on the VF expression. The molecular detection of VF genes evidenced the Ecp/dA in 82% and EchelD in 85% of the tested strains. The densitometric analysis of the electrophoretic bands obtained in multiplex PCR showed different intensities, suggesting that, in a number of cases, the lipase or DN-ase genes could be lost in a great percent in the bacterial population.
Conclusion: Our results proved the high adaptation ability of aquatic enterobacterial strains to different stress conditions and the expression of virulence determinants even in limiting environmental conditions, demonstrating the role of these parameters in the preservation of the virulence gene pool in water.

R2519 Evaluation of one-sample testing of self-obtained vaginal swabs and first-catch urine samples separately and in combination for the detection of *Chlamydia trachomatis* by two amplified DNA assays in women visiting a sexually transmitted disease clinic

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Objective: *Chlamydia trachomatis* (CT) detection in both self obtained vaginal swabs (SVS) and first-catch urine (FCU) in two separate assays, results in the highest sensitivity. In most laboratories however, one-sample testing is performed for reasons of cost efficiency. To further improve one-sample testing, we assessed the laboratory performance of three different testing approaches to find the most sensitive one-sample test procedure: SVS versus FCU versus a combined specimen of FCU/SVS.
Methods: All women visiting a STD clinic above the age of 16 were asked to participate in the study. Each client was asked to take a FCU

and a SVS with a dual swab. The FCU, SVS and FCU/SVS combination were tested for CT by Strand Displacement Amplification assay (SDA) of Becton Dickinson (ProbeTec ET system, Maryland, USA) or Polymerase Chain Reaction (PCR) by Roche Diagnostics Inc. (Cobas Amplicor system, California, USA). Clients with at least one out of three sample types (SVS, FCU, SVS/FCU combination) tested positive for CT by NAAT, were regarded as CT positive (comparison standard).
Results: In total 791 females were included and CT prevalence was 12% (96/791). The CT detection rate for SVS, FCU and SVS/FCU combination were 94%, 90% and 94%, respectively, if results of NAAT by SDA and by PCR were analyzed together. The detection rate was not significantly different between any of the sample types, when tested solely. Discordance in NAAT results within the different sample types was found in 16 out of 96 CT positive results.
Conclusion: Our results show that the detection rate of SVS/FCU combination is equal to that of FCU or SVS alone. SVS is an acceptable and feasible specimen for females. Moreover, SVS is the most cost-effective sample type for a STD clinic population We can therefore conclude that SVS is the specimen of choice to detect CT in females.

R2520 Characteristics of an outbreak of pandemic H1N1 2009 among healthcare personnel

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Objectives: During an outbreak of pandemic H1N1 2009, healthcare personnel (HCP) were thought to have substantial risk for acquirng influenza for their frequent and close interaction with infected patients. This study was performed to investigate the characteristics of an outbreak of pandemic H1N1 2009 among HCP.
Methods: A multicenter survey of HCP was conducted in 4 general hospitals of the Republic of Korea between July and August 2010. All were assigned to local influenza centers by the Korean government during the outbreak.
Results: The questionnaires were distributed to 4555 HCP and those of 3365 (73.9%) were responded. Of the study HCP, 578 (17.2% of 3365) received diagnostic tests for pandemic H1N1 2009 and 141 had positive results (4.2% of 3365; range, 3.8–4.6%). More than a quarter of HCP (27.6%) had influenza-like illness (ILI) during the outbreak. Independent risk factors for ILI were female gender, <40 years of age, presence of chronic diseases associated with influenza complications, and working in influenza outpatient, non-influenza outpatient and emergency departments. The preventive effect of isolation precaution was not shown prominently. HCP with pandemic H1N1 2009 more frequently had the infection among their household members than those without. Whereas pandemic H1N1 2009 tended to be transmitted from the hospital to the household through HCP who were directly involved in patient care, it did from the houshold to the hospital through HCP who had a infected child or adolescent within their households.
Conclusion: ILI and pandemic H1N1 2009 was frequently observed among HCP and the type of facility where HCP had worked principally was one of the most important determinants for ILI. Influenza outpatient and emergency departments were the highest risk for ILI of HCP. During an influenza pandemic, infection control measures should be educated and implemented for HCP who are actively involved in influenza patient care, to prevent themselves and their households from the infection.

R2521 Predisposing factors for *Streptococcus mutans* septicaemia

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Objectives: *Streptococcus mutans*, a viridans group streptococcus and the primary etiological agent of dental caries, is rarely identified as the cause of streptococcal sepsis or infective endocarditis (IE) by conventional methods. The use of molecular methods allows species level analyses of the etiology of systemic infection complications of oral origin. This study describes ten patient cases of *S. mutans* septicaemia identified by sequence-based methods.
Methods: This retrospective, population-based cohort study comprises patients with *S. mutans* identified in blood cultures during 2002–2007 at our hospital in Helsinki. Characteristics of hospital stay recorded were diagnostic methods, supportive treatment, C-reactive protein levels, white blood cell (WBC) counts, body temperature, length of stay (LOS), need for intensive care, antimicrobial therapy and outcome. Dental records were obtained from public and private dentists. Medical records were verified by telephone interview of the patient during years 2006–2007.
Results: Ten patients (8 men, 2 women) were included with matched controls. The mean age was 49.8 (24–77) years. IE (9/10) was the most common distant site infection, one patient developed spondylitis. All patients had some predisposing condition for distant site infection: mitral valve insufficiency (5/10), bicuspid aortic valve (3/10), atrioventricular septal defect (1/10) and discus protrusion (1/10). Five patients had had a preceding dental procedure, which in most cases was removal of dental plaque and calculus (4/10). None of the patients received antibiotic prophylaxis preoperatively. All ten patients had active oral infection foci, most often untreated periodontitis (5/10), likely to cause spontaneous bacteraemia. Patients reported more childhood infections than the controls ($p = 0.01$). WBC counts (10(3)/μL) on admission were median 8.0 (4.5–10.6) and at maximum 9.9 (5.5–33.6). LOS was 27 days in median (9–75).
Conclusion: Patients with predisposing medical conditions seem to be more susceptible for distant site infections caused by *S. mutans*. The major predisposing factor in our study material was cardiac structural abnormality. All these patients subsequently developed IE as a distant site infection. The results highlight the importance of oral health in patients with cardiac abnormalities and support the use of antibiotic prophylaxis during dental care of oral infection foci in these patients, especially if colonised with *S. mutans*.

R2522 "The body gets used to them": patients' interpretations of antibiotic resistance and the implications for containment strategies

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Background: Antibiotic resistance is a term that is frequently used by clinicians in their discussions with patients. However, patients and clinicians may not share the same assumptions about the meaning of this term.
Objective: We aimed to explore patients' interpretations of the term 'antibiotic resistance' and to consider the implications for strategies to reduce the overuse of antibiotics.
Design: Qualitative interview study.
Participants: 121 adult patients from nine European cities who had recently consulted a primary care clinician with symptoms of Lower Respiratory Tract Infection (LRTI).
Approach: Semi-structured interviews were conducted with participants following their consultation. Data were subject to Framework Analysis.
Results: The dominant theme in all networks was that antibiotic resistance arose from having or developing a 'resistant body', where the barrier to antibiotic effectiveness was individual loss of responsiveness. Less commonly, patients correctly conceptualised antibiotic resistance as a property of bacteria. Nevertheless, the over-use of antibiotics was a strong central concept in almost all patients' explanations, whether they viewed resistance as located in either the body or in bacteria.
Conclusions: Most patients were aware of the link between antibiotic use and antibiotic resistance. The identification of the misinterpretation of antibiotic resistance as being located in the body could lead to clinician-patient discussions and public health interventions which are much clearer about the location and mechanism of antibiotic resistance, explaining the transferability and societal relevance rather than focusing on individualised risk, thereby emphasising the public health argument for the prudent use of antibiotics.

R2523 Enteroaggregative *Escherichia coli* as a possible cause of sporadic diarrhoea in children from Romania

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Objectives: Diarrhoea remains one of the main sources of morbidity and mortality in the world and enteroaggregative *Escherichia coli* (EAEC) have been increasingly recognized as an emerging pathogen, causing diarrhoea in both developing and industrialized countries. In Romania, the real contribution of EAEC to diarrhoea disease burden is not known because of the lack of routine EAEC detection protocols in clinical microbiological laboratories. The aim of the present study was to determine the prevalence of faecal carriage of EAEC in Romanian children with sporadic acute diarrhoea.
Methods: *E. coli* isolates originating in stool samples, collected during diarrheal episodes from 477 children, under five years of age, previously found negative for enteric pathogens such as *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Campylobacter* spp. and other diarrheagenic *E. coli* pathotypes, were included in the study. From each specimen, one biochemically confirmed *E. coli* isolate was screened by PCR for the presence of EAEC-associated genes aat, aggR, aap and astA. EAEC isolates were evaluated, using a PCR-based protocol, for their phylogenetic background and tested for antimicrobial susceptibility by the disk diffusion methods.
Results: The molecular approach revealed that 41 (8.6%) *E. coli* isolates carried at least two of EAEC-associated genes targeted. The most frequently detected genes were aat (40 isolates) and aap (38 isolates). Twenty-nine isolates harboured concurrently aat, aggR, and aap genes. Almost half of these carried also astA gene. Most of the EAEC isolates derived from phylogenetic group A (29 isolates), while the rest belonged to groups B2 (6 isolates), D (4 isolates) and B1 (2 isolates). Twenty-four EAEC isolates expressed resistance, of which five were extended-spectrum β-lactamase producers. All EAEC isolates were fluoroquinolone susceptible.
Conclusion: This study revealed that EAEC pathotype might be a significant cause of sporadic diarrhoea among children in Romania. Our results provide additional support for the reconsideration of the local diagnostic and surveillance strategies.

R2524 The occurrence of selected intestinal helminthoses in marginalised group of Roma living in settlements

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Material dimension of poverty in Roma national minority is particularly noticeable in the sphere of living. Especially, in the segregated settlements with illegal huts (mostly built of wood, iron waste, flat metal stock and other materials obtained from waste dumps or surrounding countryside), devastated environment and with no access to basic infrastructure such as electricity, tap water (they mostly use water wells or streams), sewerage system and waste disposal what in many cases has influence on bad health status and troubles with hygiene. According to the Office of Government Plenipotentiary for Roma issue in Slovakia there is approximately 680 Roma settlements at present. These are often located in rural communities without the necessary basic infrastructure. According to several studies in many settlements, which are often built on loose soils are lacking in their drinking water, sewage, waste pits and landfills, sanitary facilities and lack of garbage disposal.
The settlements are concentrated in a small area with a large number of people (about 500 000 people) whose health status is unsatisfactory.
The aim of our work was to study the occurrence of helminthoses in Roma children population in selected areas of Eastern Slovakia, especially from Roma settlements (Michalovce, Prešov, Vranov nad Toplou, Secovce) and Lunik IX (Košice).
In total, 246 stool samples from children aged 0–14 years were examined.
For detection of parasites was used coprological method Paraprep L (DiaMondial, France).
The helminth eggs were detected in 19.9% of examined children, at which *Ascaris* sp. (18.3%) and *Trichuris* sp. (5.3%) eggs dominated. The highest positivity was detected in children in the age group 6–14 years (27.1%).
Low standard of housing, communal and personal hygiene, lack of drinking water and sanitation in Roma settlements increases the risk of oro-faecal infections.
Indirectly to faecal contaminated environment shows the high prevalence of enteronematodes. The occurrence of epidemiologically low-risky parasitoses in Roma population in Slovakia suggests low hygiene standard in Roma settlements.
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R2525 Neural involvement in brucellosis: clinical classification, treatment and outcomes

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Aim: The aim of this study was to describe and to categorize different clinical pictures of patients with neurobrucellosis in our clinic, and to present demographical and laboratory data about the patients.
Material and Methods: Hospital records, between 2003 and 2009, of about 430 patients with brucellosis were followed and retrospectively reviewed in our clinic.
Results: Out of 430 patients, 19 (4.4%) had neurobrucellosis. These patients were classified into four groups: Meningitis group (n = 14; 13 cases of subacute-chronic meningitis and one case of acute meningitis), Encephalomyelitis group (n = 3; one case of meningoencephalomyelitis, one case of cerebellar abscess, and one case of transverse myelitis), Polyradicular group (n = 1; one case of Miller Fisher Syndrome), and others (n = 1; one case of intradural abscess).
Ten patients (52.6%) were female, and nine patients (47.4%) were male and the mean age of the patients was 48.8 years. About 47.4% of the patients had fever, 26% of the patients had neck stiffness, and 5% of the patients were in an unconscious state. Out of 19 patients, 18 underwent lumbar puncture (LP). According to cerebrospinal fluid (CSF) analyses, the mean leukocyte count was 113/mm3, the mean glucose level was 45 mg/dl, and the mean protein level was 123 mg/dl. Standard tube agglutination test showed brucellosis in all patients who underwent LP. Microorganisms were detected in four patient's blood culture and one patient's CSF culture. There were cranial nerve involvements in five cases. The most frequent was the sixth cranial nerve involvement. Out of 19 patients, 3 recovered with sequels (paraparesis, hearing loss, dementia, and sphincter dysfunction) and 16 patients recovered completely.
Conclusion: Although neurobrucellosis is most frequently accompanied by subacute-chronic meningitis, it may have many different clinical pictures. The classical triad of meningitis (fever, neck stiffness, and unconscious state) is rarely seen in brucellosis-related meningitis. Brucellosis should be kept in mind in patients with unexplained neurological findings in regions where brucellosis is endemic. In addition, a classification of brucellosis, which reflects locations of nervous system involvement, clinical picture, and pathogenesis, is needed.

R2526 Oral candidal colonisation and oral candidiasis in the institutionalised and non-institutionalised elderly

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Objectives: Oral candidiasis is an opportunistic infection of the oral cavity and it is common among the elderly. This study was carried out in

order to evaluate colonization of the oral cavity with *Candida* species and oral candidiasis in the institutionalized and non-institutionalized elderly.
Methods: A total of 341 elderly were included, 280 institutionalized elderly in nursing home (134 males, 146 females, mean age 72.68±8.43) and 61 non-institutionalized elderly (23 males, 38 females, mean age 70.39±6.16). Specimens were isolated from oral cavity with sterile swabs (Copan, Zagreb, Croatia) from the surface of oral mucosa and were inoculated on a isolation medium, Sabouraud's dextrose agar. Cultures were incubated at 37ºC for 48 hours. In cases of no growth, the plates were considered negative and discarded. The count of colony-forming units was recorded. Oral cavity is considered colonized with *Candida* spp. in the case of positive microbiological analysis and normal oral mucosal appearance. The diagnosis of oral candidiasis was established by taking account of the clinical appearance (pseudomembranous candidiasis, erythematous candidiasis, denture-related stomatitis or candida-associated lesions) and positive microbiological analysis. The diagnosis was made according to the number of the colonies described by Budtz-Jorgensen.
Results: Altogether, 68.92% of institutionalized elderly in our study were colonized with *Candida* spp. This result was statistically different compared with the non-institutionalized elderly (50.81%; $p < 0.001$). The results of our investigation also showed an increased number of the institutionalized elderly with oral candidiasis compare with the non-institutionalized ones (17.14% for institutionalized elderly; 9.83% for non-institutionalized elderly; $p = 0.035$).
Conclusion: Results of this study showed a higher prevalence of colonization of the oral cavity with *Candida* spp., as well as oral candidiasis in the institutionalized elderly compared with the non-institutionalized elderly.

R2527 Study of the incidence of different *Corynebacterium* spp. strains among the healthy population in Romania

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Diphtheria is controlled in Romania by the National Program for Communicable Diseases Surveillance. Since 1989 no diphtheria case was recorded in our country. As a consequence, since 2002 the disappearance of toxigenic *Corynebacterium diphtheriae* is declared. At the same time, annually reports showed also a decrease of the number of non-toxigenic *C. diphtheriae* strains isolated in different districts. As members of DIPNET Diphtheria Surveillance Network, the surveillance of *Corynebacterium diphtheriae* as well as *C. ulcerans* and *C. pseudotuberculosis* strains represent an important part of our activity.
Objectives: The aim of this study was the screening of healthy target population to identify the *C. diphtheriae* and other toxigenic species.
Materials and Methods: 696 pharyngeal and nasal samples were tested in our laboratory. The target population was represented by 1–19 years old children from care centers in 8 different geographical regions of country. The analyses were performed according to WHO Laboratory Diagnosis of Diphtheria protocols.
Results: No *C. diphtheriae* or *C. ulcerans* strains were isolated. A number of 106 strains were isolated and identified as upper respiratory tract saprophytic species belonging to the genus *Corynebacterium*, some of them with pathogen potential especially in immunocompromised hosts as follows: 56% *C. pseudodiphtheriticum*, 29% *C. propinquum*, 6% *C. striatum*/*amycolatum*, 5% C. group C, 2% *C. macginleyi* and 2% *C. glucuronolyticum*.
Conclusions: We can conclude that strains involved in diphtheria disease are not circulating in Romania in the surveillance period, and that the frequency of other species of *Corynebacterium* spp. isolated is low, only 16% of subjects being carriers.

R2528 Evaluation of geriatric patients hospitalised in infectious diseases department

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Introduction: Infection is a common problem in elderly patients. In geriatric practice, infection is most frequently seen in combination with many other problems. During the evaluation of geriatric patients, atypical presentations are the main cause of missed diagnosis, mostly in infectious diseases.
Method: This retrospective, cohort study identified elderly patients (age $\geqslant$65 years) hospitalized in Infectious Diseases Clinic of a tertiery hospital between January 2008 and July 2009. The initial major symptom, co-morbidities, definite diagnosis, clinical course and prognosis of these elderly patients were evaluated.
Results: 103 of 601 hospitalized patients in Infectious Diseases Department were aged 65 or older.
Co-morbidities were detected in 91 of the patients as; hypertension and coronary artery diseases (n: 60, 58.2%), neurologic disorders (n:29, 28.1%), diabetes mellitus (n:22, 21.3%), chronic obstructive lung disease (n:17, 16.5%), malignancy (n:9, 8.7%) and other as history of recent surgical operation, hypothyroidism, renal insufficiency and rheumatological disorders (n:18, 17.4%). The common symptom of the patients were fever (62.1%) followed by unconsciousness (22.3%) and cough (16.5%). The common infections were urinary tract infection (n:37, 40%), pneumoniae (n:18, 17.4%), and sepsis (n:16, 15.5%). Four patients had the diagnosis other than infectious diseases. The duration for the definite diagnosis beginning from the initial symptoms was varied from longer than 4 weeks (n:16) to less than 7 days (n:57) The mean hospitalization day was 9.5 days (5–26 days) Following the consultations, 15 patients were transferred to other departments for the need of other disciplines support. Nine of the patients (5 were in other departments) died.
Conclusion: As the atypical presentations of infectious diseases are very common in elderly patients, the most important problems are delayed or missed diagnosis and long hospitalization periods. Data reveals that neither the lack of fever directs the diagnosis to non-infectious disease nor the unconsciousness is the constant symptom of CNS infection in elderly patients. Detailed investigation of differential diagnosis is very important in geriatric patients.

R2529 Risk factors for surgical site infections in hip and knee arthroprosthesis: role of microbial air contamination and adherence to guidelines for antimicrobial prophylaxis

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Objectives: The main aim of the study is to evaluate the role of microbial air contamination on the risk of surgical site infection (SSI) in hip and knee arthroprosthesis controlling for adherence to guidelines for antimicrobial prophylaxis. The project has been funded by the Italian CCM (Centro Controllo Malattie, Ministry of Health).
Methods: Hospitals were invited to join the project by GISIO members. SSI surveillance was conducted according to the HELICS protocol (version 9.1, 2004). Microbial air contamination was evaluated at the patient area, once at rest and during each operation, by passive sampling to determine the Index of microbial air contamination (IMA) (Pasquarella et al., 2000) and in some cases also by active sampling to determine the colony forming units (cfu)/m3. Surgical antimicrobial prophylaxis refers to a very brief course of an antimicrobial agent initiated just before an operation begins. A web-based data collection procedure was adopted using three electronic data forms. The two years project started in July 2010, preceded by a three-month pilot study to assess the overall feasibility of the programme.

Results: The project has included so far 8 Hospitals and 20 operating rooms (OR). According to the OR ventilation system in place, 76.5% of OR were with unidirectional airflow, 6.0% with turbulent air ventilation, and 17.6% mixed. A total of 289 surgical procedures, 67.0% hip and 33.0% knee arthroprosthesis, were included until December 2010. Mean duration of operations was 93 minutes for hip and 117 minutes for knee arthroprosthesis. IMA values in OR at rest were as follows: range = 0–8, mean±SD = 0.8±2.1, median = 0; during surgical procedures the following values were registered: range = 0–156, mean±SD = 7.2±14.7, median = 3. A total of 99.6% of patients received antibiotic prophylaxis: 30–60 minutes (36.7%), 1–2 hours (18.5%) and >2 hours (44.8%), before incision. The most frequently administered antimicrobial agents were cefazolin (44.7%), tobramycin (29.1%) and teicoplanin (19.6%).
Conclusion: Although one year of follow-up after implant is requested for SSIs surveillance, the study has depicted the epidemiological scenario in which a complex network of risk factors for SSIs is embedded, already highlighting potential areas for improvement both for air quality and antibiotic prophylaxis.

R2530 Brucellosis: a five-years experience from southeastern Anatolia

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Brucellosis is a chronic granulomatous infection caused by intracellular bacteria. It is an endemic disease in Mediterranean countries and Turkey. Brucellosis shows the involvement of many systems and it seems to be responsible for the high incidence of relapse. The aim of this study is to analyze the clinical, laboratory findings and therapeutic features in patients with brucellosis.
Patient and Methods: We conducted a retrospective study of patients who developed brucellosis between 2005 to 2009 admitted in the Department of Infectious Diseases of Dicle University Hospital, Diyarbakir. The diagnosis was based on clinical findings compatible with brucellosis, serological tests positive, and/or isolation of *Brucella* species from blood, or other tissues.
Results: 91 cases were included. The mean age was 33 years (16–67 years). Sixty-threes of patients (69.2%) were male. An occupational history relevant for *Brucella* exposure was present in 44% of the cases and consumed contaminated animal product was noted in 93% of cases. The mean diagnostic delay was 15 days, much longer in focal brucellosis. Acute brucellosis was predominant, in 85% of cases. The focal brucellosis complications were seen in 42.8%: osteoarticular involvement (82%), epididimo-orchitis (10%), and nervous system central (8%). Chronic brucellos occurs in 3.3% of cases. Clinical manifestations include non-specific symptoms such as fever (95%), sweats (90%), arthralgia and lower back pain (63%). 92% of the patient had serological titre = 1/160. Overall, 31% of blood cultures were positive. All of the patients were cured by antibiotherapy included Doxycycline and rifampicin/Doxycycline, streptomycine and rifampicin/Doxycycline, ceftiraxone and rifampicin. Relapse in follow-up period was observed in five patients.
Conclusion: Brucellosis is an infection with multiple presentations. Its early diagnosis was mandatory to avoid severe complications.

R2531 Boric acid for recurrent vulvovaginal candidiasis: the clinical evidence

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Objectives: Recurrent vulvovaginal candidiasis remains a challenge to manage in clinical practice. Recent epidemiological studies indicate that non-*albicans Candida* spp. are more resistant to conventional antifungal treatment with azoles and are considered as causative pathogens of vulvovaginal candidiasis.
Methods: We searched PubMed and Scopus, for studies that reported clinical evidence on the intravaginal use of boric acid for vulvovaginal candidiasis.
Results: We identified 14 studies (2 RCTs, 8 case series and 4 case reports) as eligible for inclusion in this review. In 7 studies, boric acid was compared either with nystatin, or azoles (terconazole, flucytosine, itraconazole, clotrimazole, ketoconazole, fluconazole, buconazole and miconazole), while as monotherapy boric acid was studied in 7 studies. The mycological cure rates varied from 40% to 100% in patients treated with boric acid. Four of the 9 included case series reported statistically significant outcomes regarding cure (both mycological and clinical) rates. None of the included studies reported statistical significant difference in recurrence rates. Regarding the adverse effects caused by boric acid use, vaginal burning sensation (<10% of the cases), water discharge during treatment and vaginal erythema were identified in 7 studies.
Conclusion: Our findings suggest that boric acid is a safe, alternative, economic option for women with recurrent and chronic symptoms of vaginitis when conventional treatment fails due to the involvement of non-*albicans Candida* species or azole-resistant strains.

R2532 *Yersinia enterocolitica* detection in drinking and recreational waters

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Objectives: The objective of the present survey was the study of *Yersinia enterocolitica* infestation in drinking and surface waters in Northwestern Greece.
Methods: Over a period of 12 months (November 2008–October 2009) a total number of 180 water samples were examined for the presence of *Y. enterocolitica*. The water samples were collected from the area of Epirus (Northwestern Greece) and included: 60 samples of drinking water, 60 samples of lake water and 60 samples of marine water. The drinking water samples were tap-water collected from houses from three different municipalities of the area, the lake water samples were collected from two lakes (Pamvotis and Voulkaria) representing two different ecosystems (polluted/urban and rural/unpolluted) both used for recreational activities and the marine water samples were collected from five different point sources of Ionian Sea, including areas of recreational activities. The APHA/AWWA, Standard Methods for the examination of water and waste water were employed for the detection of *Y. enterocolitica* and other bacterial indicators (total microbial flora, coliforms, fecal coliforms, enterococci).
Results: *Y. enterocolitica* was isolated from two samples of marine water and two samples of lake water. The positive samples were isolated from the most polluted aquatic environments (the urban lake and the Amvrakikos Golf). The isolation of *Y. enterocolitica* coincided with increased numbers of total coliforms (⩾2500 cfu/ml) and enterococci (⩾2000 cfu/ml). No *Y. enterocolitica* strains were isolated from the drinking water samples.
Conclusion: The findings of the present survey underline the presence of *Y. enterocolitica* in marine and lake water as an indicator of microbiological pollution and a potential threat for public health if the contaminated aquatic environment is used for recreational activities. Also, the absence of *Y. enterocolitica* in the drinking water samples of the examined area proves the efficacy of the chlorination practices.

R2533 Tick invasion in the blood transfusion unit of a general hospital, following unusual weather conditions

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Objectives: The aim of the present study is to report the event of tick invasion in the premises of the blood transfusion unit (BTU) of a General Hospital following the unusual climatic conditions of spring and summer 2010 in North-western Greece.
Methods: During the first week of July 2010, large numbers of ticks appeared in the external and internal walls and inside the premises of the BTU of the "Hatzikosta" General Hospital of Ioannina, causing uneasiness to the unit's medical and paramedical personnel. The BTU

is located next to the Microbiology and the Clinical Biochemistry Laboratories and above and below the BTU there are various Clinics. None of the surrounding laboratories and clinics reported any appearance of any ticks, which was also confirmed by in situ Inspection. Inspection of external walls of the BTU building revealed large numbers of ticks, the presence of a number of bird nests and many cracks on the walls.
Results: The tick specimen collected by the Microbiology Department of the Medical School of Ioannina were identified to belong to *Ixodes* spp. The Greek Ornithological society was called and there were identified 80 nests of swallows belonging mostly to the species *Delichon urbica*, on the BTU building and surrounding hospital buildings. The swallow species *Delichon urbica* is host of haematophagous ticks belonging to the family of Ixodidae and apparently the nests were the source of the ticks, which astonishingly enough, were moving only towards and into the BTU premises and nowhere else in the Hospital buildings.
Conclusions: The swallow nests have been on the hospital buildings for years and were re-visited every spring by their bird habitats. However, the 2010 appearance of large numbers of ticks is correlated with the earlier spring with high temperatures and heavy rainfalls continued through middle of July. The favorable weather conditions worked well with the cracks on the old building walls resulting in the multiplication and establishment of the ticks on the hospital walls of the BTU.

R2534 Clinical appearance of brucellosis in adults: are there any changes? Response based on 14 years of experience

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Purpose: This study has been planned to indicate the clinical course and termination of brucellosis in our region during the recent years, and to compare it to the literature.
Methods: The study has been based on a review of the medical records of adult patients older than 14 years, followed with the diagnosis of brucellosis from March 1997 to October 2010. The demographical data, disease diagnosis, course, treatment and termination data of the patients were recorded.
Results: 317 patients, including 136 males (43%) with average age 40±17 were included in the analysis. There was a statistically significant relationship between advanced age and development of spondylitis and arthritis (respectively $p = 0.000$ and $p = 0.028$). Besides, the frequency of splenomegaly and neurobrucellosis among the young population was found high, which was statistically significant (respectively $p = 0.005$ and $p = 0.001$). In cases of spondylitis, the most common (~85%) involvement was of the lumbar vertebrae. Also there was a statistically significant relationship between high ESR and spondylitis, sacroilliitis and visceral abscess ($p = 0{,}001$, 0,013 and 0,049 respectively).
Conclusion: The study is sad to see that there haven't been any significant changes in the frequency of the disease and its complications in time. Osteoarticular involvement, and particularly the presence of spondylitis should be searched, and the advanced aged patients should be meticulously scanned for complications. Laboratory parameters, patient's age and duration of symptoms may help to identify complicated cases.

R2535 Infectious diseases among EUFOR military personnel

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Objectives: Infectious diseases and non-battle injuries has been recognized for its impact on the combat effectiveness of military units during operations in Bosnia and Herzegovina.
Methods: An epidemiological analysis was conducted of the impact of infectious diseases on the European Union Force (EUFOR) troops for the period from 02.12.2004 to 31.12.2010. Standar-dized collection of medical data from all EUFOR medical treatment facilities (MTF) using 14 diagnostic categories based on the International Classification of Diseases, 9th Revision were conducted.
Results: The average infectious diseases rate for this 6-years period was 8.1 per 100 soldiers per week (range, 5.8–11.1/100/week). Most frequently causes for soldier visits to MTF were respira-tory (36%), gastrointestinal (31%), arthropod-borne (12%) and "other" infectious diseases (21%).
Respiratory infections were the leading causes of infectious disease morbidity among EUFOR troops showed a distinctive seasonal pattern. Adenovirus, influenza virus, *S. pyogenes* and *B. pertussis* were particularly problematic. It has been reported 12 outbreaks of influenza, chicken pox, Q-fever and pertussis.
Diarrheal diseases were the most common cause of acute infections among EUFOR troops. The primary enteropathogens identified were norovirus, enterotoxigenic *E. coli*, Sh. sonnei and *S. enteritidis*. Five outbreaks of norovirus and shigella were reported among EUFOR soldiers.
From arthropod-borne infections it has been confirmed cases of Lyme disease, Mediterranean spotted fever and Q-fever. Thirty two EUFOR soldiers were tested positive to brucellosis and four confirmed cases of hemorrhagic fever with renal syndrome have been reported. There were a few cases of sexually transmitted diseases and viral hepatitis C but they were not a significant problem during this deployment.
Conclusions: The incidence rate of infectious diseases, during this conflict was relatively low because of combination of factors: the presence of a comprehensive infrastructure of medical care, extensive preventive medicine efforts and several fortuitous circumstances. Nevertheless EUFOR military personnel, because of crowding and unique stressors, were subject to respiratory and diarrheal disease outbreaks. A few diseases (brucellosis, hemorrhagic fever with renal syndrome and Q-fever) caused by potential biological weapon agents were prevalent.

R2536 Study of *Campylobacter* strains isolated in the decade 2001–2010

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Background: *Campylobacter* has been recognised as a causative agent of bacterial infectious diarrhoea and remains one of the most prevalent bacterial enteropathogens in the industrial countries. *Campylobacter* infection is generally foodborne disease.
Objectives: The aim of this report is the study of *Campylobacter* cases recorded during the decade 2001–2010, as well as the susceptibility of *Campylobacter* isolates to antibiotics. Also the frequency of the disease according to age, sex and season was estimated.
Methods: A total of 898 *Campylobacter* strains were isolated from patients with acute gastroenteritis hospitalized in the Infectious Diseases Hospital of Thessaloniki. The selective Skirrow medium was used. Skirrow medium is blood agar infused with antibiotics: vancomycin, polymixin-B and trimethoprim. The incubation was under microaerophilic conditions at 37ºC and 42ºC. Following isolation on selective media, identification was carried out using the biochemical profile of each *Campylobacter* strain. Furthermore, *Campylobacter* strains were tested for susceptibility to antibiotics (ampicillin, gentamicin, tobramycin, cephalothin, ceftriaxone, ciprofloxacin, nalidixic acid, co-trimoxazole, erythromycin) using the Kirby-Bauer method.
Results: Of the 898 strains, 846 (94.2%) were *C. jejuni* and 52 (5,8%) *C. coli*. From the total strains, 521 (58%) were isolated from males and 377 (42%) from females. Furthermore, 704 strains (78,4%) were isolated from children (47.4% under the age of 2 years) and 194 (21.6%) from adults. In addition, leucocytes were found in 34.4% of the stool specimens. The peak of *Campylobacter* infection in the study was in the spring months (31.4%) and in the summer months (28.5%). An increase in fluoroquinolones and co-trimoxazole resistance (nalidixic acid 23.9%, ciprofloxacin 12% and cotrimoxazole 47.7% respectively) among *Campylobacter* isolates has been observed. Erythromycin (macrolides) is considered the drug of choice with low resistance.
Conclusions: *Campylobacter* is a major cause of bacterial enteritis, following *Salmonella* in Northern Greece. Most frequently isolated was *C. jejuni*. Age specific infection rate was highest in children less than

two years old. Campylobacteriosis occurs much more frequently in the spring and summer months. Erythromycin is still an effective antibiotic for the therapy of *Campylobacter* infection.

R2537 *Chlamydia pneumoniae* seropositivity and its association with carotid intima media thickness, c-reactive protein and oxidant stress in subjects with only underlying hypercholesterolaemia

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Background: The association between *Chlamydia pneumoniae* and atherosclerosis in hypercholesterolaemia subjects in the absence of other conventional risk factors is unclear. This study was done to determine whether *C. pneumoniae* seropositivity is associated with increase in intima media thickness (IMT) of the carotid artery, hs-C reactive protein (CRP) and oxidant stress in subjects with hypercholesterolaemia.
Methods: Fifty-two hypercholesterolaemic subjects were recruited during health screening programmes organized by our institution. Subject inclusion criteria include baseline LDL-c of $\geqslant$3.4 mmol/l. Subjects with diabetes, hypertension, severe obesity, smoking, primary hypercholesterolemia and presence of acute inflammation (C-reactive protein $\geqslant$10 units) were excluded. IgG and IgA to *C. pneumoniae* were measured by microimmunofluoresence test. IgG titre $\geqslant$64 and IgA $\geqslant$16 were considered to be seropositive. IMT of the far wall of carotid artery was measured by B-mode ultrasound. hs-CRP was measured by immunoturbidimetric method using an automated analyser. Isoprostanes (an oxidative stress marker) was quantified by liquid chromatography mass spectrometry.
Results: Seropositivity for *C. pneumoniae* was detected in 40.4% of the hypercholesterolaemic subjects. There was no significant difference in the IMT in the hypercholesterolaemic subjects who were *C. pneumoniae* seropositive compared to the *C. pneumoniae* seronegative subjects (Mean IMT$\pm$SD mm: 0.6$\pm$0.7mm vs. 0.6$\pm$0.6 mm, $p > 0.05$). hs-CRP did not show a statistically significant difference between *C. pneumoniae* seropositives and seronegatives individuals (Mean hs-CRP + SD mg/dl; 1.56$\pm$0.29 vs 1.85$\pm$0.35; $p > 0.05$). By contrast, a significant difference was observed when comparing isoprostane, a marker for oxidative stress by serostatus to *C. pneumoniae* (Mean + SD pg/ml; 46.2$\pm$30.6 vs 37.7$\pm$20.4; $p < 0.05$).
Conclusion: Our data demonstrates that seropositivity to *C. pneumoniae* increases the oxidative stress but it is not associated with increase in carotid IMT or in hs-CRP in subjects with hypercholesterolaemia in the absence of other conventional cardiovascular risk factors.

R2538 Role of matrix assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry as a tool for rapid diagnosis of potentially toxigenic *Corynebacterium* species in the laboratory management of diphtheria-associated bacteria

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Objectives: The rapid and reliable identification of the three potentially toxigenic *Corynebacterium* species *Corynebacterium diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis* is usually essential for diagnosis and treatment of diphtheria and diphtheria-like diseases. Classical differentiation of suspected isolates is done by biochemical tests, which are time consuming and may often give unclear results. Recently, matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (MS) was shown to allow identification of isolated microorganisms within 15 minutes and is therefore a fast alternative for species differentiation.
Methods: We used matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in comparison with classical microbiological (API Coryne) and molecular methods (rpoB sequencing) on 116 *Corynebacterium* strains.
Results: All 90 potentially toxigenic *Corynebacterium* spp. strains collected by the German National Consiliary Laboratory on Diphtheria in a period of more than ten years were correctly identified by MALDI-TOF MS. In 103/116 strains (88.8%) biochemical identification by API Coryne yielded identical results as rpoB gene sequencing. Twelve strains showing unreliable or ambiguous API results were concordantly identified by rpoB sequencing and MALDI-TOF MS except for one isolate of *C. tuberculostearicum*. In conclusion, 99.1% of the tested Corynebacteria were correctly identified by MALDI-TOF MS when compared to rpoB gene sequencing. Moreover, both the positive and negative predictive values for identification of potentially toxigenic *Corynebacterium* species were 100% with MALDI-TOF, respectively.
Conclusion: In conclusion, species identification of potentially toxigenic *Corynebacterium* spp. can be accomplished by MALDI-TOF MS within 15 min. when applied to suspicious colonies. In this scenario, MALDI-TOF MS technology might be used as a rapid screening method helping to decide whether suspicious colonies should be analyzed for the presence of tox by real time PCR. We propose an algorithm for fast and reliable diagnosis of diphtheria incorporating MALDI-TOF MS, real-time tox PCR and Elek testing.

Emerging infectious diseases

R2539 A case of fulminant myocarditis complicating scrub typhus in Korea

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Scrub typhus, which is caused by *Orientia tsutsugamushi*, is an acute febrile illness characterized by fever, rash, and myalgia. Severe complications are very rare. Recently, cases of scrub typhus with severe complications, such as acute respiratory distress syndrome, septic shock, acute renal failure, myocarditis and meningitis have been increasingly reported. Fulminant myocarditis is characterized by critical illness at presentation. However, if affected patients recover with pharmacologic therapy and mechanical circulatory support, they may have a better long-term prognosis than patients with other forms of myocarditis. An adult patient with scrub typhus presented with normal EKG on admission and no hypotensive period, subsequently developed chest pain on 6 th hospital day. T inversion of EKG and diffuse ventricular dyskinesia of echocardiography appeared on 6th hospital day. We report a cases of acute fulminant myocarditis in adult with scrub typhus. This complication led to severe cardiogenic shock and death.

R2540 Septic arthritis and bacteraemia caused by *Streptococcus suis*: phenotypic and molecular confirmation

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Streptococcus suis (*S. suis*) is a swine pathogen that is responsible for meningitis, septicemia, pneumonia and endocarditis. Since the first case report of human infection in Denmark in 1968, human infection with *S. suis* has been reported in many countries and especially Southeast Asia because of its high density of pigs. We report here on a patient with septic arthritis and bacteremia caused by *S. suis*. To the best of our knowledge, a case in which *S. suis* is isolated from joint fluid is very rare and this is first case report of *S. suis* infection in Korea. This organism was confirmed by phenotypic characterization and 16S rRNA sequence analysis. An 81-year-old Korean female who presented with fever, arthralgia and headache was seen in a secondary referral center in Korea. The aspirated joint fluid and the blood culture revealed growth of *S. suis* biotype 2, which was identified by the Vitek 2 GPI and API 20 Strep systems (bioMérieux, USA) and this organism was susceptible to penicillin-G and vancomycin. The 16s rRNA sequences of the blood culture isolates showed 99% homology with those of the *S. suis* subsp. *suis* reported in GenBank. Although her fever subsided and the subsequent blood and joint cultures were negative after antibiotic therapy, the swelling and pain in the left knee joint persisted. She is planning to receive total knee replacement.

R2541 Tick-borne encephalitis: variability presentation in children

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Objectives: Tick-Borne Encephalitis (TBE) is an infectious zoonotic disease present in Central Europe but rare in Italy. Over the last 30 years a continuous increase in the disease morbidity was observed in the endemic countries, where about 13.000 cases occur each year. We report the first paediatric cases described in Italy.
Case reports: First: A 7 year old boy came for a remarkable involvement of the Central Nervous System, 2 week after a flu-like syndrome. Non tick-bites were reported, daily consumption of non-pasteurized milk was referred. Lumbar puncture revealed clear cerebrospinal fluid (CSF) with polymorphonuclear pleocytosis, glucose and protein content were normal. The boy received treatment with dexamethasone, ceftriaxone, ampicillin and acyclovir, with regression of symptoms within 24 hours. TBE specific serology in blood and CSF was positive while serology for *Borrelia burgdorferi* revealed past infection. The diagnosis was a meningeal form of TBE. The boy had a normal neurological outcome. Second: A 12 year old boy came for the onset of walking difficulties, myalgia, leg heaviness, fatigue and nausea, after 4 days of fever and headache. To note the removal of two ticks on the day before the start of symptoms. Clinically he presented only a slight instability in the march on his heels. The blood examination revealed: leuconeutropenia, thrombocytopenia, elevated aminotransferase levels and significant creatine kinase increase. Serology for common viruses and bacteria, also *Borrelia burgdorferi*, were negative. A first TBE serology was positive only in IgM, after 15 days it was positive in IgM and IgG. There was no need for any treatment because of spontaneous regression of symptoms.
Conclusion: We described the first pediatric TBE cases documented in Italy. Generally the disease was transmitted by a tick of the genus *Ixodes*, even if there have already been described sporadic cases from non-pasteurized cow's milk, and it manifested by the typical diphasic febrile course (a flu-like syndrome in the first phase, involvement of the central nervous system in the second phase).
In our first case no tick bites were known, while the boy often fed non-pasteurized milk and dairy products. The peculiarities of the second case was the short incubation period and the absence of the second phase of infection. These show that in children it is impossible to define a single clinical picture that characterizes the TBE, because of its several clinical forms.

R2542 A *Clostridium sordellii* fatal toxic shock syndrome post-medical-abortion in Portugal

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Background: *Clostridium sordellii* (*C. sordellii*) is a Gram-positive anaerobic bacillus that has been reported as a rare cause of fatal toxic syndrome after medical abortion. Portugal's legal therapeutic abortion, before 10 weeks of gestation, was approved in 2007. We report a case of a young patient who underwent a medical induced abortion and died of a *C. sordellii* toxic shock syndrome.
Case summary: A 16-year-old women who underwent a medically induced abortion by means of 200 mg of oral mifepristone followed by 800 μg of vaginal misoprostol, presented to the maternity hospital's emergency five days after receiving mifepristone, complaining of lipothimia in the night before and abdominal cramping. On admission, she was conscient, afebrile and hypotense (76/35mmHg). A few hours later she developed a rapid onset-sepsis with marked leukocitosis (83,400 white-cells/μL with 88% of neutrophils), hemoconcentration (hematocrit of 63.4%; hemoglobin of 21.2) and severe metabolic acidosis. The patient underwent a hysterectomy and uterus biopsy cultures and anatomopathological analysis were requested. Patient was transferred to intensive care unit and died 18h after presenting to emergency. At the microbiology laboratory a direct examination by Gram-stain smear of the uterine biopsy showed large, Gram positive rods. The biopsy was inoculated in appropriated agar plates mediums and incubated aerobically and anaerobically. 48 h later only the anaerobic culture was positive and colonies were smeared by Gram stain (showing Gram positive rods) and a *C. sordellii* was identified by the semi-automated system, Vitek 2 (bioMerieux®) with an ANC card (Anaerobes and *Corynebacterium* card).
Discussion: This was a fatal case of pos-abortion *C. sordellii* sepsis. The distinctive clinical features developed are the same reported in other studies. Gram staining of a uterine biopsy is a good and rapid mean of having a presumptive result and help to diagnose. *C. sordellii* was identified through uterine biopsy cultures with a semi-automated system which is different from other studies that used anti-clostridium species immunochemical assay and PCR assays performed on formalin fixed uterine tissue pos-autopsy.
Conclusion: To improve diagnosis Gram staining and cultures of an endometrial biopsy specimen are a good approach to an earlier recognition of the disease's etiology.

R2543 Tularaemia (cases from Turkey)

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Objectives: The oropharyngeal form of tularemia is known to be common particularly in Eastern European countries including Turkey. The aim of the study was to evaluate the patients with tularemia and to make a point for the disease.
Methods: Between February and August 2010, five patients with unilateral cervical mass were admitted to our outpatient clinic with the preliminary diagnosis of tularemia. The diagnosis of tularemia was confirmed by micro-agglutination test and PCR.
Results: All of the patients had oropharyngeal tularemia with the suspicion of contaminated water ingestion. Age of the patients ranged from 19–59, three of them were male (60%) and two were female (40%). In initial stage of the disease, all the patients had general symptoms such as fever, headache, malaise and sore throat which lasted about two weeks, after that the general symptoms disappeared but the cervical lymph nodes started swelling in all patients. β-lactam antibiotics were administered to three of them in different centers before admission to our clinic. However there wasn't any response to these treatments.
At admission unilateral cervical or submandibular lymphadenopathy and malaise were detected in all patients. One patient (20%) had suppurative lymphadenitis with spontaneous drainage. Leukocytosis was found in one patient (20%), elevated ESR in three (60%) and elevated CRP in three (60%) of patients. Lymph node aspiration was performed when fluctuation was detected but *F. tularensis* could not be grown in the cultures. However *F. tularensis* subsp. *holarctica* DNA was detected in four lymph nodes aspirates by conventional PCR. Micro-agglutination test was positive in four patients (80%) with titres of 1/640 in three patients (60%) and 1/160 in one patient (20%).
The patients were treated with a streptomycin and doxycycline or a ciprofloxacin and doxycycline combination for a 2 weeks period due to a delay in initiation of treatment. One patient was received a second coures of antibiotics because of insufficient response to first therapy and continuing suppurative lymphadenitis. No severe complication was observed.
Conclusion: Tularemia should be kept in mind in the differential diagnosis of patients with fever, pharyngitis or tonsillitis and cervical lymphadenopathy, especially unresponsive to β-lactam antibiotics in the endemic regions and early treatment with proper antibiotics should be started.

R2544 Imported malaria as a threat to Egypt

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This work evaluated the clinical and parasitic status of malaria as a cause of fever among patients admitted to the Military fever hospitals. Thirty six patients were included twenty already diagnosed as malarial

patients, who were recruited from Peace Keeping Mission Forces in Africa and sixteen cases presented with prolonged fever coming from different locations in Egypt.
The results showed that El-Gabal El-Ahmar area (Cairo) was the most extensively infested region (37.4%). This might be due to change of its ecolo-gical pattern since the year 2003 and the environmental conditions favoured by breeding and flaring mosquitoes. El-Sharkia and El-Fayoum Governorates (G.) were next in order (18.7%) and (12.5%) and this might be due to increased rural areas and agricultural projects and re-establishment.
Plasmodium vivax was the main species among locally acquired patients (81.25%), while the imported patients coming back to Egypt from Africa especially (Sudan) had *P. falciparum* (100%). However, *P. falciparum* was also present in 6.2% of cases from El Fayoum Governorate while *P. ovale* and *P. malariae* were not encountered. Of interest, was a case recruited from Ard-El-Golf, Heliopolis, an area with high social and hygienic standard, and the same condition applied to that from El-Nozha El-Gidida. Such cases included the "runway" or "airport" malaria, in which local transmission of disease has been attributed to an infected mosquito that was transported on a long haul flight. The two locally acquired cases were malaria positive by bone marrow smears and negative by peripheral blood examination. However, the thick blood film was the most sensitive (97.2%). The patients (75%) were clinically and parasit-ologically cured, but one patient died. The best therapeutic response for locally acquired malaria infection was the monotherapy-based one such as Chloro-quine or Mefloquine.

R2545 Abdominal lymphadenopathy and multiple splenic micro-abscesses in an immunocompetent child with cat-scratch disease

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Background: Cat-scratch disease (CSD),caused by *Bartonella henselae*, is a benign, self-limiting disease in immunocompetent children with history of contact with cats. The organism is commonly found in the blood of cats and other felids. Classic presentation is tender and swollen regional lymph nodes with or without a papule at the site of initial infection. In immunocompromised patients, however, more severe forms of presentation can occur. Diagnosis is usually possible by serology and or histopathology.
Case summary: An 14-year-old Kuwaiti girl, previously healthy, was admitted with fever, abdominal pain, nausea and generalized weakness. She had arrived from a trip to Egypt five days earlier. There was no history of diarrhea, vomiting, urinary symptoms or rash. On Examination the patient looked ill & had an oral temperature of 40 ºC with abdominal tenderness and guarding. Initial blood investigations showed a WBC of 14.1×10^9, ESR 44 mm/h, C-Reactive Protein (CRP) 150 mg/l, urea 3.2 mmol/l and creatinine 39 umol/l. Urinalysis showed presence of RBC & WBC but the culture was negative. A diagnosis of appendicitis was made and appendicectomy was performed. The patient, however, continued to run high grade fever (>40ºC). Therapy with piperacillin-tazobactam (TAZ) and metronidazole was initiated but no clinical response was observed. CT of the abdomen showed mesenteric and para-aortic lymphadenopathy and multiple splenic lesions. A suspicion of enteric fever prompted changing TAZ to ceftriaxone. Blood culture, brucella agglutination, T spot test, & Widal test were all negative. several antibiotic courses including anti-TB & anti-fungal were tried but none helped to improve her condition. Finally, splenectomy and lymph node biopsy was done and the pathology report concluded multiple splenic granulomas with a picture suggestive of CSD. Serology, however, proved negative (IFA titre: IgG: <1:64; IgM: <1:20). History of contact with cats in Egypt was elicited from the patient after pathology report was available. There was a dramatic improvement in her condition following splenectomy.
Conclusion: To the best of our knowledge this is the first case of CSD being reported from Kuwait. We recommend to consider CSD in the differential diagnosis in children presenting with PUO, lymphadenopathy and splenic involvement. Despite several antibiotic courses, which included those recommended in CSD our patient showed defervescence only after splenectomy was performed.

R2546 *Salmonella* monophasic serotype 4,[5],12:i:- in Greece

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Objectives: *Salmonella* is one of the most common causes of bacterial foodborne diseases worldwide. Recently, *S. enterica* serovar 4,[5],12:i:- emerged and is now among the most common serovars isolated from humans in many countries. This serovar is considered a monophasic variant of serovar Typhimurium (4,[5],12:i:1,2). In Greece, this monophasic serovar was firstly recorded in human isolates in 2007 (0.3% of total isolates), increased sharply the next two years and in 2010, it was the 3rd most frequent serotype (9.5% of total isolates).
In the present study, *S. enterica* 4,[5],12:i:- strains isolated from humans, pigs and foods during 2007–2010 in Greece were examined using phenotypic and molecular methods.
Methods: A total of 50 *S. enterica* 4,[5],12:i:- strains of human (29), pig (16) and food (5) (chicken and salami) origin were included in this study. Serotyping of isolates was performed by slide agglutination according to White–Kauffmann–Le-Minor Scheme. Phage typing of the strains was performed in Health Protection Agency, UK (standard HPA protocols). Antibiotic resistance test (against 16 antibiotics) was performed by the disk diffusion method according to NCCLS. PFGE was performed after digestion of genomic DNA with XbaI according to the Pulse-Net protocol.
Results: As shown in Table 1: a. Phage typing using the Typhimurium typing phages identified 5 different PTs. The most commonly identified PTs were DT120 (62%) and DT193 (24%), b. Eighty-six percent of the isolates expressed resistance to ampicillin, sulphonamides, streptomycine and tetracycline (R-type ASSuT) with or without additional resistance(s), c. PFGE analysis identified 8 unique profiles (A-H). Eighty-eight percent of strains were represented by three profiles (A,B,C) that shared more than 95% similarity.
Conclusion: Combining phenotypic and genotypic characteristics, the most frequent pattern (54%) appeared the one with phage type DT120, R-type ASSuT (plus additional resistances) and the closely related PFGE profiles (A,B,C), followed by the pattern (18%) with phage type DT193, R-type ASSuT (with or without additional resistances) and closely related PFGE profiles (A,B,C). These results are consistent with the possible presence of two different clones of *S. enterica* serovar 4,[5],12:i:-, that prevail in both human and pig isolates in Greece. Similar data have been recorded in several European countries.

Table 1: Phage type (PT), R-type, PFGE profile of Salmonella enterica serovar 4,[5],12:i:- isolates.

	ASSuT				ASSuT plus other resistances				Other resistances			
PT	PFGE profiles				PFGE profiles				PFGE profiles			
	A	B	C	Other	A	B	C	Other	A	B	C	Other
120					23	1	3	1	2		1	
193	2	3	1	2	2		1			1		
NT*						1	2					
195												1
7												2
97					1							

*NT: Not Typable

R2547 First case report of pacemaker *Brucella suis* endocarditis via zoonotic transmission from Canadian caribou (*Rangifer tarandus*)

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Background: The use of implantable cardiac devices such as permanent pacemakers (PPMs) continues to increase, with infection complicating up to 10% of inserted devices. Management of pacemaker endocarditis generally requires surgical removal of the entire system. *Brucella suis* is an uncommon cause of endocarditis, not previously reported in association with a PPM.

Case: An 81 year old gentleman from Inuvik (Nunavut, Canada) with a PPM for 4 years was evaluated for syncope at the University of Alberta Hospital (Edmonton, Canada). Investigation revealed evidence of cardiac lead vegetations on a transesophageal echocardiogram. Pre-operative blood cultures and intraoperative cultures taken during laser lead extraction became positive for *Brucella suis* (with MICs of 0.25 mg/L to each of ciprofloxacin, doxycycline, and rifampin). Upon further questioning, the patient a subacute history of constitutional symptoms, and reported hunting, skinning, and butchering Canadian caribou (*Rangifer tarandus*). The patient's brother, a hunting partner, had been treated for *B. suis* infection 2 years previously. Our patient was treated with laser lead extraction and combination oral antimicrobial therapy with doxycycline and rifampin for 3 months. Follow up blood cultures were negative.
Discussion: *Brucella suis* type 4 is a known pathogen of Canadian caribou herds. It is hypothesised that cutaneous exposures when while skinning and butchering or ingestion of undercooked meat resulted in zoonotic transmission of the organism to our patient causing bacteraemia and secondary seeding of his pacemaker wires. From our literature search, this is the first reported case of pacemaker endocarditis due to *B. suis*. It is important that clinicians be aware of the possible zoonotic transmission of *B. suis* to patients exposed to caribou in the Canadian north and the risk of secondary infection of underlying foreign bodies (such as PPMs). Clinicians are also reminded to alert microbiology labs if *Brucella* is a suspect pathogen, to allow appropriate biosafety precautions.

R2548 The first case of *Neisseria meningitidis* serogroup X-caused meningitis in Turkey

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Neisseria meningitidis, an agent of bacterial meningitis is classified into 13 different serogroups based on immunologic reactivity of its capsular polysaccarides. Serogroups A, B, C, W135 and Y are among the predominant serogroups.
In Turkey, A, B, and C serogroups of *N. meningitidis* are encountered as the most frequent cause of menigococcal meningitis. A meningococcal vaccine containing serogroups A and C has been used to prevent recruits from this fatal disease till May, 2009. From then on, administration of the quadrivalent vaccine consisting of "A+C+W135+Y" serogroups has been implemented because of the emergence of menigococcal meningitis cases caused by serogroup W135. We present the first known menigococcal meningitis case caused by *N. meningitidis* serogroup X since the start of the quadrivalent meningococcal vaccine administration to recruits.
Serogroup X has emerged as a cause of meningococcal disease in a susceptible population because of suppression of other serogroups not included in the quadrivalent vaccine.
The results of mass vaccination campaigns have implied that the mass vaccination programs solely cannot prevent the disease totally and the improvement in environmental conditions especially in risky populations is also essential in prevention of the disease.

R2549 A most unusual case of superinfection with *Arcanobacterium haemolyticum* in a primary treated *Staphylococcus aureus* mitral valve endocarditis in an intravenous drug-user

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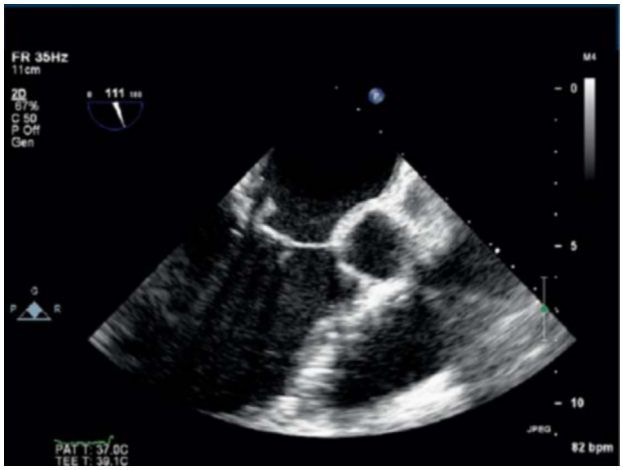
Background: *Arcanobacterium haemolyticum* (AH) is an extremely rare cause of infective endocarditis. We present here an unusual case of super-infection with AH in a primary *Staphylococcus aureus* (SA) mitral valve endocarditis in a Hepatitis B/C positive intravenous drug user[IVDU]. After a protracted course (with multi-organ impairment) of 3-months the patient was successfully treated.
Case study: A 29-year-old IVDU male was transferred from emergency unit of neighbouring hospital to intensive care of this hospital on February 4, with signs of severe sepsis and seizures. CT head revealed small parietal bleed; lumbar puncture was non-conclusive and CTPA was negative for pulmonary embolism, but showed extensive non occlusive thrombus in superior vena cava. Systolic murmur prompted trans-esophageal echo revealing mitral regurgitation with vegetation. Following isolation of meticillin-sensitive SA grown from two sets of blood culture, the patients was treated with Flucloxacillin IV 1g q6h for 4-weeks. After 3 weeks of satisfactory response, the patient started getting intermittent pyrexia, elevation of CRP and WCC; mild renal and hepatic impairment. Blood culture with AH from single bottle was not considered significant and dose of Fluclox was increased to 2g q6h. Further pyrexia, raised inflammatory markers and impairment of renal functions prompted biopsy revealing focal necrotizing glomerulonephritis. Two sets of blood cultures grew AH [penicillin resistant – MIC 0.5mg/L (breakpoint 0.125mg/L); Teicoplanin MIC 0.064 mg/L and Vancomycin MIC 1.0 mg/L]. Reference laboratory confirmed AH and susceptibilities. The patient was started on Teicoplanin 10mg/Kg q24h (following loading doses).
The patient responded successfully to 4-weeks of Teicoplanin with normalization of his organ functions. Details and ECHO pictures to be presented.
Discussion: AH infrequently causes pharyngitis and cutaneous infections. Penicillin resistant AH super-infection of a primary MSSA native mitral valve endocarditis is extremely rare. The multi-organ impairment in this high-risk Hep B and C positive IVDU patient complicated the course of management. Regular clinical input from clinical microbiologist helped optimize treatment successfully. Details to be presented.



R2550 Emerging *Vibrio vulnificus* septicaemia associated with environmental change

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Vibrio vulnificus is a human pathogen, frequently transmitted to humans via raw oysters and is isolated from the majority of raw oyster and shellfish harvested from subtropical areas during summer season. A 73-year-old woman with diabetes mellitus and liver cirrhosis presented to the emergency department with generalized edema for one month and mental change on a day. She was diagnosed *V. vulnificus* septicemia and treated ceftazidime and doxicycline. We also isolated *V. vulnificus* from sea water of outbreak sea and same season (summer). *V. vulnificus* septicemia was emerged in Jeju island associated with climate change in island. This is the first report about relationship between clinical and environmental isolates according to the climate or environmental change in Jeju island, Korea.

R2551 CT findings in novel 2009 influenza A (H1N1) virus infection in Isfahan, Iran

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Objectives: Pandemic 2009 influenza A (H1N1) virus causes a broad spectrum of clinical syndromes, ranging from mild to severe. The role of radiologic imaging in detection of severity and response to therapy is evolving. We want to find whether CT scan of the lungs in patients with influenza can help triage of the patients and predicts clinical outcome in similar outbreaks.
Methods: We retrospectively reviewed the archive of all patients with a diagnosis of H1N1 influenza A, in Saint Alzahra hospital, Isfahan, Iran, between September23, 2009 to February 20, 2010. From 216 patients with confirmed, probable, or suspected 2009 influenza A (H1N1), 26 cases with abnormal CT were enrolled the study. Radiologic findings were characterized by type and pattern of opacities and zonal distribution.
Results: Patchy infiltration (34.6%), lobar consolidation (30.8%), and interstitial infiltration (26.9%) with airbronchogram (38.5%) were the predominant findings. Bilateral distribution was seen in 80.8% of patients. None of our patients showed a nodular pattern on the CT. Only one patient (3.8%) showed ground-glass opacity, the predominant radiographic finding in the previous reports and SARS. Consolidation was detected in 30.8% of our patients that may represent either a severe viral infection, or a secondary bacterial infection. In patients with a more severe clinical course of 2009 H1N1 virus infection, we observed a radiographic pattern of areas of consolidation often associated with patchy or interstitial infiltration. In fatal cases we observed consolidation with airbronchogram and diffuse alveolar infiltration. Extensive involvement of both lungs, presence of bilateral opacities, interstitial infiltration, lobar consolidation, and patchy infiltration were associated with poor prognosis. Patients with abnormal CT findings had greater risk of intubation, ventilation, ICU admission and death than those with normal CT, but some patients with similar CT findings had various outcomes from improvement and discharge to death.
Conclusion: Chest CT findings may be significant in prediction of clinical outcome and management of the disease (e.g., initiation of antibiotics) in patients with influenza infection but CT findings alone does not exactly predict the outcome and other risk factors should be considered.

R2552 Evaluation of epidemiological, clinical and laboratory characteristics of pandemic influenza A (H1N1) cases in a tertiary care hospital in Turkey

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Objectives: In April 2009, H1N1/09 virus was identified in Mexico, spread throughout the world and caused an influenza pandemic. Worldwide more than 214 countries and communities have reported pandemic influenza H1N1 2009 cases, including over 18449 deaths. Our country has also influenced from this pandemic with 656 deaths. In this report we present the epidemiological, clinical and laboratory data of hospitalized patients in our clinic.
Methods: Totally 117 patients were hospitalized due to the probable pandemic influenza H1N1 between 25 October 2009 and 31 January 2010. Nasal and/or nasopharingeal samples were tested for Influenza A (H1N1) with real-time RT-PCR in National Influenza Reference Laboratory. Patients' data were evaluated retrospectively from the records.
Results: Of the patients, 77 were female (65.8%), mean age was 44±19.4 years and the mean hospital stay was 5.8±4.6 days. Five patients (4.3%) were vaccinated with seasonal and three (2.6%) with pandemic flu vaccine. Twenty-one patients (17.9%) had a history of close contact with persons presenting symptoms of a respiratory infection. 38 patients (32.5%) had not have any underlying disease while 79 patients (67.5%) had one or more. Of these 25 patients were pregnant. On admission, most common symptoms were cough, myalgia, fever and headache (92.3%, 82.9%, 82.1% and 64.1%, respectively). Forty-six (39.3%) had dyspnea on the admission and three of them required mechanical ventilation. On the physical examination, 79 patients (67.5%) were febrile. Fifty-two patients (44.4%) had crepitation on the lung. Radiological investigations revealed bilateral extensive consolidation and ground glass opacities. Mean SGOT, GGT, LDH, CK, creatinine and ferritine levels were higher while mean zinc level was lower than normal limits. Remaining routine biochemical and blood count tests were within normal limits. Of patients, 68 (58.1%) were found to be PCR positive for pandemic Influenza. 110 patients (94%) received oseltamivir therapy,83 (70.9%) received additional antibiotics. All pregnant patients recovered without complications. 4 patients died, all of them had underlying conditions and three had required mechanical ventilation.
Conclusion: During influenza pandemic, four patients died in our hospital. Lower zinc levels may correlate with the severity of the disease, though controlled studies are needed. We also suggest that those patients with underlying conditions must receive a close follow-up.

R2553 High GGT levels: as a prognostic factor for surviving from CCHF

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Objectives: Γ glutamyl transferase (GGT) is an enzyme that is present in the cell membranes of many tissues. High GGT level is used as a prognostic factor for alcoholic liver disease, metabolic syndrome, cardiovascular diseases, liver cancer and liver metastasis of different cancers. Crimean Congo Hemorrhagic Fever has been emerging in Turkey for the last 8 years. Elevation of alanine amino transferase (ALT) and aspartate amino trasnsferase (AST) levels are used both severity criteria and prognostic factor for fatality in the management of CCHF. However, we observed an association between GGT levels and the course of the disease. In this study we aimed to figure out the relation between serum GGT levels and CCHF and its effect on survival, if there is any.
Methods: Patients with laboratory-confirmed diagnosis of CCHF were included in to the study. Serum ALA, AST, gama glutamyl trasnferase (GGT) were retrospectively investigated from the records. Two parameters were used for evaluation of relation between CCHF and GGT; serum GGT levels and serum AST-GGT ratio. Because of AST elevation is a prominent feature of CCHF than ALT elevation, AST/GGT ratio was chosen as a marker.
Results: Totally 126 patients were included. Of total, 71 (56.3%) were female. The mean age was 50.2 years (15–83 years). The mean hospital stay of the patients was 7.5 days (1–28 days). Remarkable laboratory findings were as follows (mean, min-max); WBC: 2472/mm3 (500–8900). Platelets: 59000/mm3 (7000–361000), AST: 291U/L (17–3060), ALT:133U/L (9–879) and GGT: 91 U/L (6–759). Comparing the serum levels of GGT between fatal and non fatal cases, mean GGT levels were statistically significantly higher in fatal cases on the 2nd and 3rd days of admission. Serum AST levels were also found higher in those days in the fatal group. The mean GGT level continued to increase during the course of the disease (Figure 1). When AST/GGT ratio was investigated, the ratio was >1 in the first 7 days of admission but <1 after the 7th day. Higher serum GGT level was the most prominent laboratory finding in all non fatal cases (Figure 2). A positive correlation was detected between higher AST/GGT ratio and mortality.

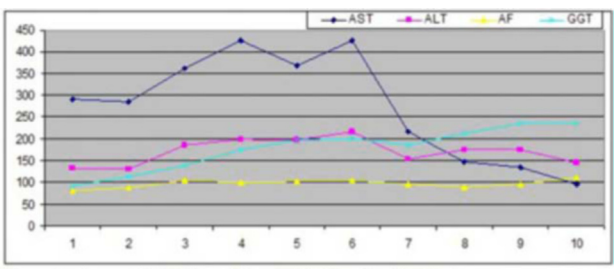

Figure 1. Serum AST.ALT, AF and GGT levels of patients

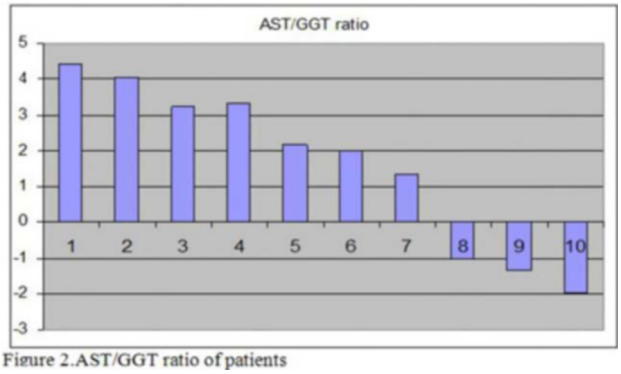

Figure 2.AST/GGT ratio of patients

Conclusion: Serum GGT levels can be used as a prognostic factor for CCHF. However an increase of GGT level during the recovery period of CCHF has not yet been fully explained. Therefore more studies are needed in this area of research.

R2554 A retrospective study of Dengue virus infection in northwestern Italy in travellers from endemic areas

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Background and Aim: Dengue fever is increasingly recognized to be the most frequent agent of febrile illness in tourists returning from dengue-endemic areas. Domestic outbreaks of dengue (DENV) fever from imported cases have to be considered a possible risk in countries where, even if the virus is not endemic, Dengue vectors are, such as Italy.
Objective of this study was to review imported DENV infections in a one-year survey in Piemonte area, North-West Italy.
Methods: From January to November 2010 we investigated 68 patients traveling from endemic areas studied for DENV infection due to symptoms such as fever, skin rushes and leukopenia. IgG and IgM DENV specific serology and DENV-RNA with molecular test were performed. Confirmation of DENV IgG/IgM required a second blood test within 15 days from the first one.
Results: Active DENV infection was confirmed in 20/68 (29%) patients (3 IgM+/IgG–, 16 IgM+/IgG+, 1 IgM–/IgG– and DENV-RNA+) while DENV exposure was identified in 4/68 (6%) patients.
Conclusion: Our findings outline the high rate of imported Dengue infection and emphasize the need for a continued dengue surveillance in non-endemic countries and a careful evaluation and follow-up of febrile patients returning from countries in which dengue is endemic.

R2555 Vascular endothelial growth factor levels in patients with Crimean-Congo haemorrhagic fever

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Background: Crimean Congo hemorrhagic fever is a serious viral hemorrhagic fever. Immunopathogenesis of the disease is not clear enough yet. Vasculitis due to endothelial damage is a major pathologic component of the disease. Vasculitis may be caused by a virus and/or cytokine and other important proteins which were released during infflamatory process. Vascular endothelial growth factor (VEGF) is an important protein on endothel functions, and it significantly influence vascular permeability and VEGF induces the synthesis of von Willebrand factor in endothelial cells. Additionally, it is also a potent chemoattractant for monocytes and thus has procoagulatory activities. In microvascular endothelial cells VEGF induces the synthesis of plasminogen activator and plasminogen activator inhibitor type 1.
Objective: We aimed to investigate the role of VEGF in pathogenesis of CCHF.
Methods: Sixty CCHF patients observed between 2009–2010 years were included in the study. Diagnosis was made by anti-CCHF IgM and/or PCR positivity. Patients' sera samples were obtained during hospitaliaztion and stored at −80ºC. VEGF levels were detected by commercial ELISA kit; and mean values were compared in severe, mild patients and 18 healthy control.
Results: Sixty patients included in this study, and 34 of them were male (56.7%), 26 were female (43.3%). All of the patients have been living in rural areas, and their job was farming. Patients were grouped according to the severity criteria, and 42 (70%) patients were severe, 18 (30%) of them were mild cases. Mean VEGF levels were 487+422 pg/mL (range = 2–2258 pg/mL) in patients and 496+410 pg/mL (range = 0.2–1337 pg/mL) in control group ($p = 0{,}768$). It was lower (381+467pg/mL, range:8–1839pg/mL) in severe case than that of mild ones (534+398pg/mL, range = 69–2258 pg/mL) ($p = 0.016$).
Conclusion: Low free VEGF levels may be related to vasculer permeability, and serum VEGF levels might be a prognostic factor in CCHF.

R2556 Interleukin-12 levels in patients with Crimean-Congo haemorrhagic fever

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Background: Crimean Congo hemorrhagic fever is a serious viral hemorrhagic fever. Immunopathogenesis of the disease is not clear enough yet. Interleukin (IL)-12 is a pivotal cytokine that strongly stimulates Th1-associated cellular immunity, and activates humoral immunity to both T-dependent and T-independent antigens. IL-12 has an immunoregulatory functions which may play a role in promoting endogenous protective responses during infections and/or contribute to pathology resulting from unregulated cytokine expression. Pathogen induction of IL-12 elicits interferon-γ production by natural killer cells, which contribute to early defense during certain bacterial, parasitic, and viral infections.
Objective: We aimed to investigate the role of IL-12 in immunopathogenesis of CCHF.
Methods: Sixty CCHF patients observed between 2009–2010 years were included in the study. Diagnosis was made by anti-CCHF IgM and/or PCR positivity. Patients' sera samples were obtained during hospitaliaztion and stored at −80ºC. IL-12 levels were detected by commercial ELISA kit; and mean values were compared in severe, mild patients and 18 healthy control.
Results: Sixty patients were included in this study, and 34 of them were male (56.7%), 26 were female (43.3%). All of the patients have been living in rural areas, and their job was farming. Patients were grouped according to the severity criteria, and 42 (70%) patients were severe, 18 (30%) of them were mild cases. Mean IL-12 levels were higher in patients (200+89pg/mL range:56–459 pg/mL) than control group (151+68pg/mL, range: 42–282pg/mL) ($p = 0.009$). It was 181+82pg/mL (range: 69–336pg/mL) in severe 208+91pg/mL (range: 57–459pg/mL) in mild cases ($p = 0.206$).
Conclusion: IL-12 levels have been increased during CCHF infection, but this response is lack in severe cases compared to mild ones. It is indicated that, low IL-12 levels may lead inadequate immune stimulation and poor outcome in the disease.

R2557 Emergence of *Clostridium difficile* PCR ribotype 176, highly related to type 027 in Poland

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Objective: The aim of this study was to establish etiological agents of nosocomial diarrhea caused two outbreaks in two hospitals in region Mazovia, Poland in 2008–2009.

Methods: Subjects were 10 patients who suffered from *C. difficile* infection (CDI). Patients were diagnosed in the period between July 2008 and September 2009 (hospital H1) and between November 2008 and April 2009 (hospital H2); 5 patients from Infant Jesus Hospital in Warsaw and 5 patients from Province Hospital in Plock. Faecal samples from patients with antibiotic diarrhoea were submitted to toxigenic culture to and toxigenicity status of *C. difficile* strains were confirmed by using PCR for detection of tcdA, tcdB and binary toxin genes. Susceptibility to antimicrobial agents was investigated on *Brucella* blood agar using E-test method. Isolates of *C. difficile* were typed by the PCR-ribotyping. MLSB resistance to CM and EM carried by ermB gene, was confirmed by PCR. To investigate the relatedness of Type 176 with Type 027 isolates, MLVA was applied.
Results: Mean age of patients was 68,9 (age range 49 to 91 years) and 5 were womens. Five patients were admitted to Internal and Cardiology Medicine Department (H1), four patients to the ward of infections diseases (H2), and one outpatient (hospitalized before in hospital localized in Warsaw). In the retrospective survey, PCR ribotyping in Polish laboratory of 10 *C. difficile* isolates shown similarity with ribotype 027 with one band in gel electrophoresis difference. All isolates were analized in Reference Laboratory in Leiden and showed that the strains belonged PCR ribotype 176 and shared many similarities with PCR ribotype 027, including the presence of the binary toxin gene, an 18 bp deletion in TcdC and a point mutation on pt 117bp. To investigate the relatedness of Type 176 with Type 027 isolates, MLVA was applied on 59 type 027 strains collect in 14 different European countries, 10 Type 176 isolates from Poland. Type 176 isolates clearly differed from 027 isolates in 3 of the 7 loci tested with a sum tandem repeat difference of 14, whereas tested Type 176 correlated with one another. The strains had high level resistance to fluoroquinolones and to erythromycin (EM) and clindamycin (CM) and carried of ermB gene. Resistance to metronidazole and vancomycin not observed.
Conclusion: A highly to 027 related new type (ribotype 176) has emerged in Poland, underscoring the need for a local and regional surveillance to detect and control CDI.

R2558 *Mycoplasma hominis* cultured from cerebrospinal fluid after subarachnoid haemorrhage

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Introduction: Urogenital colonization with *Mycoplasma hominis* is common in sexually active adolescent females. Extra-genitourinary infections caused by this species are described in the literature. However, it is still a rare pathogen cultured from cerebrospinal fluid.
Methods: A 48 years old woman was admitted because of a subarachnoid hemorrhage. Fever (39ºC) was noted six days after craniotomy. On the ninth hospital day, blood cultures became positive with *Staphylococcus aureus*. Flucloxacillin intravenous and a combination of intravenous and intrathecal vancomycine were administered. This infection responded, however, 3 days later, she again developed high fever in spite of the antibiotics. After 6 days of incubation, small colonies were detected on blood agar from CSF taken on day 9, 16 and 17. There were no bacteria present on the Gram stain. No identification could be obtained by using MALDI-TOF MS. No growth was detected in seven CSF cultures before day 9 and 18 cultures after day 17.
Result: *M. hominis* was detected from CSF by using 16S rDNA gene amplification. Moxifloxacin (400 mg daily) was given intravenous for two weeks. Clinical conditions improved with negative repeat CSF cultures.
Conclusion: Amplification of 16S rDNA for *M. hominis* in CSF should be included in diagnostic workup of patients after subarachnoid hemorrhage. Clinicians should consider this rarely recognised pathogen in the differential by those not responding to standard therapy with negative results in routine bacterial cultures.

R2559 H1N1 influenza mimicking cardiac diseases

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Objectives: In early April 2009, cases of human infection with 2009 pandemic influenza A (H1N1) virus were first identified and then H1N1 virus spread rapidly around the world. H1N1 influenza pneumonia presents with dry cough, fever and myalgia. In this study we aimed to call attention to H1N1 pneumonia mimicking cardiac diseases.
Methods: We report 7 cases presented with cardiac symptoms during the H1N1 pandemic and admitted to Cardiology department of a tertiary care superspecialty hospital. The clinical and laboratory features of these cases were investigated through chart review.
Results: During the H1N1 pandemic, 4 patients with initial diagnosis of pulmonary edema and 3 patients with initial diagnosis of pulmonary emboli were admitted to cardiology department. All of these patients had previously diagnosed cardiac diseases. After hospitalization, throat samples were sent for H1N1 test. Polymerase-chain-reaction assay confirmed the diagnosis of H1N1. Five of them were females and mean age was 50.4±15.8 (range 35–72). Mean duration of symptoms were 4 days before hospital admission. All of them had dyspnea and cough, four of them had fever, only one had myalgia and one had somnolence. None of these patients had sore throat and rhinitis. Two patients had monocytosis. Six patients had elevated LDH levels (956±393 IU/L). Six patients had bilateral ground glass opacities and one patient had bilateral patchy consolidations at chest x-ray. Four patients were directly admitted to cardiology ICU, required mechanical ventilation and had a fatal outcome. Oseltamivir were administered to all patients after diagnosis.
Conclusion: H1N1 influenza pneumonia can mimic cardiac diseases especially pulmonary edema and pulmonary emboli. High level of suspicion is required in outbreak periods.

R2560 Tick-borne infections as a cause of heart transplantation

T. Chmielewski, I. Maczka, E. Walczak, S. Tylewska-Wierzbanowska (Warsaw, PL)*

Objectives: Infective endocarditis is a heart disease which could be caused by many uncultured (blood-culture negative) bacteria. The aim of presented studies was to establish if any tick-borne infection can contribute to serious heart disorders which lead to heart transplantation.
Material and Methods: Samples of myocardium, aortic and tricuspid valve fixed in formalin of twenty three hearts removed from patients undergoing heart transplantation between 2006 and 2009 were tested.
DNA was extracted with the QIAamp Tissue kit (QIAGEN Gmbh, Hilden, Germany) according to the manufacturer's recommendations. Extracted DNA from above mentioned tissues were examined by PCR with primers targeting following genes: OspA and 16S rRNA for *Borrelia burgdorferi* sensu lato, htpAB fragment for *Coxiella burnetii* and citrate synthase (gltA) for *Bartonella* spp. and *Rickettsia* spp. Specificity of all positive results was confirmed by sequencing the amplicons with the ABI 377 DNA Analyzer (Applied Biosystem, USA) according to the manufacturer's recommendations. All sequences were edited using Auto assembler software (Applied Biosystem, USA) and identified using the BLAST software by comparison with sequences available in GenBank.
Results: DNA of *Borrelia afzelii* has been found in aortic valves from two patients. Mixed infection has been found in two patients. DNA characteristic for *Bartonella* spp. were detected in valves and DNA of *C. burnetii* in myocardium. DNA of *Rickettsia* spp. was not found.
Conclusions: Detected pathogens occurring in natural environment in ticks have a clinical importance in heart transplantology. Obtained results indicate that among patients with cardiac diseases, infections caused by *B. burgdorferi*, *C. burnetii* and *Bartonella* spp., should be tested routinely.

R2561 The Q fever outbreaks in Poland

T. Chmielewski, B. Fiecek, I. Maczka, S. Tylewska-Wierzbanowska (Warsaw, PL)*

Objectives: Q fever is a health problem affecting humans and animals worldwide. In the last decade five outbreaks occurred occurred in cattle farms in Poland. They occurred in southern Poland: first one on the border of Silesian and Malopolskie voivodships in May of 2005, the second one in Podkarpackie voivodship in August 2008, the third one in Opolskie voivodship in 2009 and two outbreaks in Silesian voivodship in 2010. The aim of this study was to characterize detected foci, to determine the number of infected animals and humans recognized with serological and PCR methods, and describe clinical symptoms.
Methods: Serum samples were collected from: farm workers, veterinarians and their family members. Blood samples were obtained from 302 persons. Levels of human serum IgM and IgG antibodies to *C. burnetii* antigens phase I and II were determined. Serum titers $\geqslant$20 of IgM and IgA and $\geqslant$64 of IgG were interpreted as positive. Blood human and animal samples were tested for presence of *C. burnetii* DNA with PCR method.
Results: Specific *C. burnetii* antibodies were found in 82 from 302 persons in titers ranged from 20 to 80 in phase II of IgM and from 64 to 4096 in phase II of IgG. Among all blood samples tested, 7 were positive with PCR. Three samples were positive in both methods. The highest attack rate was revealed in groups of veterinary stuff (63%) and farm workers (33.0%) consisting of cattlemen, milkmaids, safeguards, mechanics and electricians. According to information on possible exposure in the group of family members and all those occasionally spending time on the farms an attack rate of 11.0% was determined. Clinical symptoms presented 26 persons. There was no correlation between level of serum antibodies and positive results in PCR.
Conclusions: In Poland infected cattle is the main source of infections for humans. Veterinary stuff is at the highest occupational risk to acquire Q fever.

Outbreak	No of tested	No of seropositive	Group of workers	Family members	Group of veterinarians
Malopolskie	148	37	22 (29%)	3 (6%)	12 (60%)
Podkarpackie	130	38	16 (37%)	12 (15%)	10 (100%)
Opolskie	8	2	2 (25%)	0	0
Silesian I	9	3	3 (43%)	0	0
Silesian II	7	2	2 (66%)	0	0
Total:	302	82	45 (33%)	15 (11%)	22 (63%)

Table 1. Humans infected (seropositive) with *C. burnetii* in outbreaks in Poland.

R2562 *Actinobaculum schaalii* – invasive pathogen or innocent bystander?

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Objectives: *Actinobaculum schaalii* is a Gram-positive, facultatively anaerobic coccoid rod, classified as a new species in 1997, formerly belonging to the species of *Actinomyces suis*. It grows slowly and therefore is easily overgrown by other bacteria, which are often found concomitantly. Since 1999, *Actinobaculum schaalii* is routinely investigated at our hospital, whenever its presence is suspected due to the detection of minute grey colonies on blood agar plates and negative reactions for catalsae. The objective of this study was to determine the clinical significance of *Actinobaculum schaalii*, identified in our microbiology laboratory over the last 11 years.
Methods: All consecutive isolates with *Actinobaculum schaalii* were obtained from the computerized database of the clinical microbiology laboratory and patients whose cultures from any body site yielded this pathogen were analyzed. Observation of tiny colonies of Gram-positive, catalase-negative rods triggered molecular identification based on partial 16S rRNA gene sequencing. An infectious diseases specialist reviewed the medical charts and collected data regarding underlying diseases, clinical manifestations, antibiotic therapy, and clinical outcome.
Results: 27 patients with positive isolates were identified in the last 11 years. The patient's median age was 81 (19–101) years, 25 (92.6%) had underlying diseases and 12 (44.4%) had a genitourinary tract pathology. *Actinobaculum schaalii* was isolated in 12 urine cultures, 21 blood cultures, and 7 deep tissue biopsies (n = 40). Twenty-five (62.5%) specimens were monobacterial, the remaining 15 (37.5%) were polybacterial (all deep tissue samples, three bloodcultures and five urine cultures). Recovery from urine was interpreted as colonization in 5 (18.5%) cases (41.6% of all urine samples). Thirteen patients (48.1%) suffered from genitourinary tract infections, four (14.8%) from abscesses (skin, intraabdominal, and surgical site infections) and 5 (18.5%) from bacteremia (associated with spondylodiscitis, intraabdominal infection or pneumonia).
Conclusion: *Actinobaculum schaalii* caused an infection in 81.5% of our patients. It is often detected together with other pathogens (37.5%), especially in urine cultures and abscesses. Knowledge of its existence is crucial as it can lead to serious invasive infections, as bacteremias associated mostly with urinary tract or intra abdominal infections, especially in patients with underlying genitourinary tract pathologies.

R2563 Implications of the detection of human influenza B virus haemagglutination-inhibiting antibodies in unvaccinated pigs in Ibadan, Nigeria

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Objectives: Unlike Influenza A viruses which cause natural infection in many species including aquatic birds, Influenza B viruses cause influenza almost exclusively in humans. However, considering the role of pigs in genetic reassortment of influenza viruses, using serological survey, we investigated the possibility of human-to-swine transmission of two influenza B virus strains, one belonging to B/Victoria/2/87 lineage and the other belonging to B/Yamagata/16/88 lineage, which circulated among humans in Ibadan, south-western Nigeria, in 2008 and 2009.
Methods: Serum specimens obtained from ninety-one out of one hundred and ninety-nine (91/199) apparently healthy, unvaccinated, Landrace pigs at three locations within Ibadan were tested for influenza B virus Haemagglutination-inhibiting antibodies. Virus strains used for Haemagglutination Inhibition (HI) Assay were influenza B/Shanghai/361/2002-like (B/Yamagata/16/88 lineage) and B/Malaysia/2506/2004-like (B/Victoria/2/87 lineage) CDC reference antigens. HI Assay was performed according to W.H.O Protocol for Animal Influenza Diagnoses and Surveillance Manual (2002). Results obtained were analyzed by two-way ANOVA and Student's t-test, using GraphPad Prism (GraphPad Software Inc., San Diego, USA). Values of $P < 0.05$ were considered significant.
Results: Prevalence of antibodies to influenza B/Shanghai/361/2002-like virus among pigs was 3.3% (n = 91). None of the pigs tested had HI antibodies against influenza B/Malaysia/2506/2004-like virus. Titres of antibodies to influenza B/Shanghai/361/2002-like virus in each of the three pigs that were seropositive were 160, 160, and 320 HIU/25 μl respectively (mean = 213.3 HIU/25 μl).
Conclusion: Considering the role of the pig as an important 'mixing vessel' for influenza A viruses in which novel reassortants, which could be highly virulent in the human population, could be generated, detection of HI-antibodies to a human strain of influenza B virus among pigs in Ibadan, should prompt more detailed studies to establish the susceptibility of pigs to influenza B viruses and determine the roles of pigs in the evolution of influenza B viruses. It should also serve as an urgent call for effective surveillance among live pig handlers and swine herds in south-western Nigeria, not only for influenza A viruses, but also for influenza B viruses.

R2564 Evaluation of predictor factors of Crimean Congo haemorrhagic fever: new suggestions

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Objectives: Crimean-Congo hemorrhagic fever (CCHF) is one of the viral hemorrhagic fevers caused by tick bites. Common symptoms of the infection are fatigue, high fever, headache, and myalgia. In some patients hemorrhage may accompany these symptoms and is a sign of bad prognosis. The typical laboratory changes are thrombocytopenia, leucopenia, elevation of AST, ALT, CPK, and LDH, prolongation of PT and aPTT. Mortality rates vary between 3–30%. The aim of this study was to determine the factors affecting the prognosis of CCHF.
Methods: A total of 70 patients who were followed with the diagnosis of CCHF in our clinic between 2005 and 2008 were included in this study. Besides the epidemiological history of the patients, biochemical parameters that were tested during the first five days and prognosis were evaluated. Non-parametric statistical tests were used for statistical analysis.
Results: When the laboratory parameters of patients who died and recovered were compared, PT, aPTT, INR, AST, LDH, fibrinogen, CRP, hs-CRP, D-dimer, IgM, IgG, C3 and C4 levels and thrombocyte count were found to be positively correlated with fatality. On the other hand, there was no significant difference between groups regarding ALT, CPK, prealbumin, ceruloplasmin, protein C, protein S and antithrombin III levels and WBC counts.
Conclusions: CCHF is an important infectious disease that may cause loss of labour force and deaths and still an epidemic disease in our country. Estimating the predictor factors for fatality is essential to take possible precautions. Thus, it is important to re-evaluate the existing parameters that were shown to be related with fatality and to suggest new predictor factors for fatality.

	NON FATAL (n = 61), Mean (sd)	*FATAL* (n = 9), Mean (sd)	p value
AST (I/U)	287 (387.48)	*1727 (3513.39)*	**0.009**
ALT (I/U)	137.2 (145.35)	*428.33 (741.34)*	0.107
LDH (IU/L)	821 (402.53)	*2011.22 (1626.18)*	**0.006**
CPK (I/U)	588.8 (695.31)	*1307.56 (1269.74)*	0.061
PT (second)	13.6 (3)	*18.37 (6.61)*	**0.003**
INR	1.7 (4.39)	*1.97 (1.41)*	**0.005**
aPTT (second)	46.8 (9.8)	*81.98 (24.98)*	<**0.001**
WBC/mm^3	2849.5 (1703.73)	*4988 (4817.79)*	0.289
Thrombocyte/mm^3	37393.4 (25086.70)	*11444.44 (4693.37)*	<**0.001**
Fibrinogen (mg/dl)	395.3 (159.9)	*300.3 (171.9)*	**0.027**
CRP (mg/L)	14.00 (17.1)	*59.27 (60.98)*	**0.004**
D Dimer (mg/dl)	4425.83 (3479.87)	*9356.00 (1555.89)*	**0.002**
Prealbumin (mg/dl)	0.14 (0.06)	*0.10 (0.03)*	0.071
HsCRP (mg/L)	16.87 (20.23)	*42.32 (50.12)*	**0.049**
Ceruloplasmin (mg/dl)	0.3 (0.08)	*0.23 (0.1)*	0.307
Prot C (%)	87.9 (30.9)	*67.4 (28.9)*	0.355
Prot S (%)	55.8 (19.1)	*57.0 (4.3)*	0.817
Antithrombin 3 (%)	105.4 (22.9)	*83.7 (30.0)*	0.176
IgM (mg/dl)	2.11 (1.05)	*1.25 (0.47)*	**0.003**
IgA (mg/dl)	2.89 (1.34)	*2.98 (1.17)*	0.235
IgG (mg/dl)	15.51 (3.53)	*13.13 (4.72)*	**0.040**
C3 (mg/dl)	1.43 (0.45)	*1.08 (0.42)*	**0.048**
C4 (mg/dl)	0.46 (0.43)	*0.22 (0.14)*	**0.025**

R2565 BNP, PCT and CRP as diagnostic and prognostic markers in patients with sepsis

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Objectives: BNP, PCT and CRP are well known indexes in critically ill patients such as those in sepsis and septic shock. The Purpose of our study was to determine which of these markers's serum levels increase more in sepsis and if they can predict the outcome or be a valuable prognostic marker.
Methods: We retrospectively examined 114 patients with febrile bacterial infections admitted to our department over a period of 2 (two) years. No one had a history of established cardiac failure. The diagnostic of the sepsis was based on clinical and laboratory data. We measured BNP, PCT and CRP in blood samples, taken the first 2 (two) days of hospitalization. The outcome was determined as survivors and non-survivors.
Results: We had 66 female and 48 male patients. Among those patients, 68 were survivors (59,6%) and 46 (40,4%) non-survivors. There was a significant increase of BNP levels in non-survivors patients (1027) vs. BNP levels in survivors patients (432,55). Also the PCT mean levels were higher in the non-survivors's group (20,65) vs. the survivors's group (3,6). Finally, the mean levels of CRP in survivors was 167,21 and in the non-survivors was 166,4.
Conclusions: In our study BNP and PCT levels measured soon after admission were increased significantly in sepsis and they seem to be a valuable prognostic marker in the outcome of these patients. The difference of the CRP mean levels in the 2 (two) groups was not significant.

R2566 H1N1 pandemic influenza during pregnancy and their newborns: risks of infection and antiviral medication

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Introduction: H1N1 influenza pandemic caused higher morbidity and mortality among pregnant women than in the general population underscores the medical community's urgent need for data regarding the safe and effective use of medications during pregnancy. Although the benefits of treatment appears to outweigh the theoretical risks of antiviral use there is few data about the newborns who borned from mothers who took medications for H1N1 during their pregnancy. In this study we aimed to investigate the clinical characteristics of H1N1 influenza in pregnant women and follow up the health status of newborns of these mothers after delivery.
Methods: Twenty pregnant women with clinical symptoms and diagnosis of influenza A (H1N1) were included in this study from October 2009 to January 2010. All the study population were followed up until delivery and newborns were evaluated for any complication associated with infection or medication.
Results: Twenty of 276 hospitalized patients with pandemic H1N1 influenza were pregnant (7.2%). The mean age was 24±3 (17–30years). Thirteen (65%) pregnant women were in 2nd trimestre, 5 (25%) were in 3rd trimestre and 2 (10%) were in 1st trimestre when they admitted to hospital. Although 80% of patients had pathologic respiratory auscultation findings, only 4 pregnant patients accepted chest X-ray test and all of them had bilateral patchy alveolar opacities. Two of them required mechanical ventilation. One of the mechanically ventilated pregnant woman died at the second day of hospitalization. Six of the patients didn't accept to get antiviral medication. Only one pregnant women who got antiviral medication (oseltamivir) had premature birth at 36th week. All other deliveries were in term and no complications were detected in newborns up to 4th week of their life.
Conclusion: Although early evidence suggests that pregnant women may be at higher risk for severe complications (including stillbirth) from novel H1N1, the benefits of treatment appears to outweigh the theoretical risks of antiviral use. Early anti-viral therapy, may bring clinical benefits to pregnant patients.

R2567 Will Lombardia and North Italy soon begin as a new leishmaniasis endemic area? A worrisome case report

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Background: Canine leishmaniasis (CanL) due to *Leishmania infantum*(LI) is a veterinary disease with an heavy burden on public health.

In humans, LI cause visceral (VL) and cutaneous (CL) leishmaniasis and their distribution overlap that of CanL. The spreading of autochtonous foci of CanL toward Lombardia (LO) and North Italy is well evidenced: above all, during a 2003–2005 prospective survey were detected 5 new foci of CanL in LO; we report a CL clinical case from a patient living nearby the focus called PV-1.

Case report: A.C., 60 years old, male, was admitted for an evaluation of a cutaneous lesion on the upper right wrist whit no trend to healing. The patient was suffering of a Chronic Lymphatic Leukemia (CCL), actually in recovering. He worked as forest warden for a natural reserve placed nearby PV-1 area and he's living in that area, too. The man didn't report travels abroad in the last years; nevertheless, because the patient lived and worked nearby a documented new focus of CanL, was considered CL for differential diagnosis. A biopsy of the outer rim lesion was performed: a part of the sample was stained with Hematoxylin and Eosin (HE) stain, the other was cultured in NNN terrain at TA for growing of *Leishmania* promastigotes. A serum sample was collected for specific antibodies assays (anti rK39 IgG antibodies Leishmaniasis Rapydtest© DID and *Leishmania* Western Blot IgG WB LDBIO Diagnostic).

Results: HE stain resulted positive for intracitoplasmatic Donovan bodies, diagnostics for CL. NNN culture failed because was contaminated by cutaneous microbic flora. Serological assays were negative for rK39 antibodies immunochromatographic test but a strongly positive pattern for specific IgG was pointed out by WB. Patient's lesion was treated as CL by 1 ml of Meglumine Antimoniate (Glucantim©) vials by local intradermic injection in three times (1/month) with a complete lesion recovering.

Conclusions: The reported case suggest us two important considerations: this clinical report could be the first case of zoonotic transmission of CL in human being by one of the new foci of CanL identified in LO and since now could be better for LO medical practitioners arguing that ought to be suggested differential diagnosis for CL every time in clinical diagnostic routine for cutaneous lesions.

R2568 Identification and study of a cowpo virus isolated from llama (*Lama glama*) farmed in central Italy

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Objective: In July 2009, 5 llamas, farmed in Central Italy, exhibited skin lesions at different sites evolving from nodules to crusts; some of them had a crater morphology typical for pox lesions. On the farm, there are present many species of birds (local and exotic) and mammals (goats, cattle, pigs, donkeys, horses) none of which showed any of the above mentioned symptoms.

Methods: From one moribund female which was euthanised samples were collected for laboratory analysis. Skin lesions were processed for electron microscopy techniques. Two mammal cell lines (Vero and BHK21) were used for virus isolation attempts. Total viral DNA was extracted from both homogenized crusts and Vero cells supernatant. Real-time PCR, targeting the crmB gene, sequencing and phylogenetic analysis were performed to identify the virus. Other viral and bacterial diseases were considered as differential diagnosis.

Results: Transmission electron microscopy revealed brick particles typical of orthopoxviruses. CPXV-antibodies were detected from llama and human sera (farmers). A Real-Time PCR gave positive results for both crust and cells supernatant. Phylogenetic analysis of two poxvirus genes (HA and crmB) confirmed a Cowpoxvirus infection, homologous to the CPXV-GuWi strain isolated in Germany in 2007.

Conclusion: After this outbreak, no other symptoms have been described among animals and human beings in the farm and no other news about CPXV have been reported in Italy. About the origin of the infection, all the llamas were born in the farm and had had no contact with exotic mammals, as declared by the owner. we can suppose that the virus has been introduced in the farm through bred mice, used as food for birds of prey. Such mice came from a German farm, which had sold infected rats to a zoo were mongooses have recently resulted affected by CPXV.

R2569 Orthopoxviruses seroprevalence among veterinarians and cats in northeastern Italy

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Objectives: Two cases of orthopoxviruses disease occurring in veterinary personnel scratched by sick domestic cats from 2006 have been reported in Friuli Venezia Giulia (FVG), Northeastern Italy. There are few studies on seroprevalence of orthopoxviruses infection in cats from Europe (rates from 2% to 10.1%), no one in Italy.

A surveillance program has been launched among local veterinary clinics to identify the seroprevalence of orthopoxviruses in veterinarians and cats to evaluate the extent of the infection in FVG.

Methods: In order to investigate the seroprevalence of orthopoxvirus infections in veterinarians, wild and domestic cats >1 year old from 11 selected veterinary clinics of FVG, both human and animal blood samples were collected to test signs of previous exposure to orthopoxviruses. Microneutralization assays were utilized. A standardized questionnaire was also used to record the past history, as well as to evaluate all possible risk factors.

Results: A total of 36 veterinarians and 85 cats were included in the study, from February 2010 to May 2010. The median age of the veterinarians was 41.5 years (range 25–57 years) and 17 of them (47.2%) reported a previous smallpox vaccination. The median clinic work experience was 15.5 years (range, 1–31 years). Twenty-four subjects (66.7%) reported a weekly exposure to more than 10 cats. Ten veterinarians (28.6%) reported a previous exposure to more than 10 cats affected by ulcerative dermatitis. Only 9 (25%) of the subjects considered the orthopoxvirus infection a professional risk.

Antibodies against orthopoxviruses were detected in 12 veterinarians (33.3%). In particular, the seroprevalance decreased gradually for younger age groups (Table 1).

The 85 cats analyzed have a median age of 5 years (range 1–17 years); 65 cats (77.4%) are domestics and 40 cats (48.2%) live in rural area. The seroprevalence rate among cats was 35% (37.5% in rural area vs 38% urban area).

Conclusion: Circulation of orthopoxviruses in wild and domestic animals, together with decreased immunity in humans, may lead to the increased occurrence of human cowpox.

The high prevalence reported in the cats studied should alert veterinarians and physicians to consider this infection as possible in different diagnosis, especially with regards to potential fatal consequences for immunosuppressed subjects as consequence of the ceasing of the smallpox vaccination (1976 in Italy).

Table 1: orthopoxvirus antibodies in veterinarians, Friuli Venezia Giulia

Veterinarians (y)	n	Positive	Prevalence (%)
≤ 30	5	0	0
31-40	13	2	15.4
41-50	10	5	50
> 50	8	5	62.5

Infection control

R2570 Transrectal ultrasound-guided prostate biopsy. Antibiotic prophylaxis of post-biopsy bacteraemia

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Introduction and Objectives: Transrectal ultrasound guided prostate biopsy (TRUSPB) is the technique of choice in the diagnosis of prostate cancer. There is no consensus on antibiotic, dose and duration of the prophylaxis.

The main objectives of the study were: to calculate the incidence of postbiopsy bacteremia (PB), to know the microorganisms and their antibiotic susceptibility and to evaluate the effectiveness of different antibiotic prophylaxis used in our hospital.

Patients and Methods: The study is a retrospective revision of patients subjected to TRUSPB between January 2005 and March 2010 in Parc Taulí Hospital, Spain. The information was obtained from clinical registers and from the databases of Radiology and Microbiology Departments. We have used the Pearson's χ^2 test for statistical analysis with significance set at $p < 0.05$.
Results: The study included 2,427 TRUSPB. 1,954 procedures (80.5%) were carried out with local anaesthesia and 1,924 (79.3%) implied the extraction of 8 to 14 biopsic pieces. The antibiotic prophylaxis regimens were: cotrimoxazol 1,166 (48%), ciprofloxacin 575 (23.7%), tobramycin 361 (14.9%), other antibiotics 68 (2.8%) and not registered 257 (10.6%). The global incidence of PB was 2.06% (50/2427).
Escherichia coli was isolated in 98% of episodes (49/50) and *Klebsiella oxytoca* in 2% (1/50). *E. coli* susceptibility to antibiotics used for prophylaxis was: 81.6% for tobramycin, 73.5% for ciprofloxacin and 22.5% for cotrimoxazol. Susceptibility to ceftriaxone – usually proposed in literature as prophylaxis – was of 98%. Only one strain of *E. coli* was Extended-Spectrum-β-Lactamase (ESBL) producing.
The PB incidence based on the prophylaxis regimen was: ciprofloxacin – 1.04% (6/575); cotrimoxazol – 2.49% (29/1166) and tobramycin – 3.60% (13/361). The lower incidence of PB in ciprofloxacin's group is statistically significant ($p < 0.05$ versus cotrimoxazol; $p < 0.01$ versus tobramycin).
Conclusions: The global incidence of PB was 2.06%. *Escherichia coli* was isolated in 98% of episodes.
Ciprofloxacin prophylaxis was the most effective regimen with lower PB, however susceptibility of *E. coli* isolates wasn't optimal (73.5%). Taking into account that *E. coli* susceptibility to ceftriaxone was of 98%, the use of this antibiotic as a new prophylaxis regimen could reduce PB incidence.

R2571 Compliance, knowledge and attitudes towards hand hygiene guidelines among healthcare workers in a tertiary hospital in Greece

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Objectives: The objectives of the study were to evaluate healthcare workers' (HCWs') compliance to the hand hygiene guidelines issued by the World Health Organization in 2009, and to assess their knowledge regarding and perceptions toward hand hygiene. We also searched for associations between observed compliance and various factors.
Methods: The study was conducted in "Evaggelismos" Hospital, a 950-bed, tertiary care general hospital in Athens, Greece, between March and June 2010. Compliance data collection was based on direct and overt HCW observation during patient care, while anonymous, self-administered questionnaires were used to collect information on knowledge and perceptions. We used the data collection forms and questionnaires provided by WHO.
Results: During 41 observation sessions with average duration of 18.6 min, we recorded 587 hand hygiene opportunities. Total average compliance was 13.6%. Compliance was significantly higher among physicians than among nurses [21.6% vs. 9.5% ($p < 0.001$)]. Professional category (doctor vs. nurse), type of indication (before contact vs. after contact), day of observation (after "on take" day vs. other days) and activity index were found to correlate strongly with compliance.
142/228 knowledge questionnaires were completed. The average number of correct answers to the 25 knowledge questions was 12.38 (SD = 3.40) and differed significantly among age groups, professional categories, departments and wards. Only 4.2% of HCWs answered correctly to more than 19 (75%) questions.
139/228 perception questionnaires were completed. Behavioral beliefs in favor of hand hygiene seemed to be strong and produced a positive attitude toward the behavior. Normative beliefs were also relatively favorable with respect to hand hygiene. As for control beliefs, most HCWs felt that a big effort is required to perform good hand hygiene. According to HCWs' views, the most effective action to improve hand hygiene in the hospital is to make alcohol-based handrub always available at each point of care. Patient empowerment is regarded as the least effective measure.
Conclusions: Compliance rates in "Evaggelismos" Hospital are very low compared to those of other studies carried out in Greece or in other countries. Doctors' and nurses' level of knowledge is not satisfactory, while low perceived behavioral control seems to exert strong influence on HCWs' behavior. Action needs to be taken, involving a combination of system change, education and motivation.

R2572 Does oral care with chlorhexidine influence bacterial epidemiology or incidence of nosocomial pneumonia in the intensive care unit?

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Objective: To investigate the effect of oral hygiene with chlorhexidine influences bacterial epidemiology or incidence of nosocomial pneumonia in intensive care unit.
Methods: Patients admitted and stayed for more than 48h to our 18-bed polyvalent ICU of a Chinese tertiary care teaching hospital were enrolled in our study. From May 2008 to April 2009, during pre-intervention period, protocol of oral hygiene consisted of metronidazole in the morning and boiled water in the evening and has been changed to 0.2% chlorhexidine gluconate twice daily since May 2009. Bacterial epidemiology of nosocomial pneumonia (NP) and ventilator associated pneumonia (VAP) was studied. Statistical analysis was performed by SAS 8.0.
Results: Five hundred and forty-two patients admitted in our ICU from May 2008 to April 2010, of which four hundred and eighty-seven patients were screened, 234 patients, 103 episodes of NP, of which 44 VAP (21/33 for patients intubated) for 1070 device-day, occurred during the pre-intervention period; while 253 patients, 93 NP, of which 42 VAP (28/43 for patients intubated) for 1205 device-day during the intervention period. There was no significant difference between the intubated patients for sex (60.6% vs. 78.7%, $p = 0.078$), age (62.6 ± 21.3 vs. 62.2 ± 17.7, $p = 0.968$), median ICU stay (37d vs. 29d, $p = 0.259$) and ventilator-day (1070d vs. 1205d, $p = 0.496$). Overall rate of catheter related pneumonia (VAP) and rate of NP was 37.8 (episodes per 1000 ventilator-day) and 20.0 (episode per 1000 patient-day), 41.1 and 34.9; 24.3 and 16.7 for pre-intervention and intervention period, respectively. Among 143 bacteria isolated for the first year, 32 (22.4%) were G+, of which were18 MRSA, 111 (77.6%) were G-, of which 63.1% were non-fermentive bacteria. During the intervention period, among 113 bacteria, 28 (24.8%) were G+ of which were 19 MRSA, 85 (75.2%) were G-, of which 72.9% were non-fermentive bacteria. For MRSA and non fermentive bacteria distribution, there was no significant difference between 2 studied periods for VAP.
Conclusions: Oral hygiene with 0.2% chlorhexidine twice daily changed little bacterial distribution of NP in ICU compared with pre-intervention period. However, the slightly decreased 15% (41.1 to 34.9) incidence of VAP by episodes per 1000 catheter-day and 31% (24.3 to 16.7) incidence of NP by 1000 patient-day were observed during the intervention period suggests a possible benefit of oral hygiene for ventilated patients in ICU.

R2573 Twenty-three years of countermeasures against all postoperative MRSA infections

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Introduction: In a previous paper, we reported that the number of cases of MRSA isolated from the site of postoperative infection after surgery decreased 4.1% (34/833) to 0.3% (3/2073) of all cases of digestive surgeries, and maintained this low rate of MRSA isolation from March 1990 to August 1997 (Kusachi S et al. Surg Today, 1999).
Patients and Methods: 8,991 cases of digestive organ surgery were investigated for 23 years. Sparked by a increase in MRSA infections both

Surgical Site Infections (SSI) and Remote Infections (RI), we classified our countermeasures to class into period A (1987.9–1990.2), period B (1990.3–1997.8), period C (1997.9–1999.2), period D (1999.3–2004.10), and period E (2004.11–2010–8), and compared the periods. In period B, Cefazolin and cefotiam were administered as prophylaxis. The dosing continued for 4 days, including the day of surgery. Moreover, non-screening pre-emptive isolation and cohorting (NSPEI&C) were applied to the patients undergoing endotracheal intubation or tracheotomy who were admitted to single rooms (isolation) and multiple patients with similar conditions in one room (cohorting), regardless of whether or not MRSA was isolated. Since August 1997, however, the idea of evidence-based medicine (EBM) has spread over Japan so that we had no other choice than stopping NSPEI&C because NSPEI&C had no confirmed evidence. Since then, MRSA become isolated from postoperative patients even more often. Then, NSPEI&C was strictly instituted again, and the dosing period of prophylactic antibiotics for postoperative infection being shortened or extended, the isolation rate of MRSA after digestive surgeries could be significantly reduced. We report herein the method to prevent the isolation of MRSA for two decades. However, NSPEI&C was stopped in period C, but was implemented again, and prophylactic antibiotics were administered only on the day of surgery in period D. In period E, prophylactic antibiotics were administered for 3 days.

Results: In period A, MRSA was isolated from 4.1% (34/833). In period B, the MRSA isolation rate decreased to 0.3% (8/2,722). In period C, the MRSA isolation rate increased to 3.4% (23/681). In period D, the MRSA isolate rate decreased to 2.2% (40/1807). In period E, MRSA isolation cases significantly decreased to 0.4% (196/4948; $p \leqslant 0.002$, vs. period D).

Conclusion: The comprehensive management, the 3 days administration of prophylactic antibiotics and NSPEI&C were effective.

R2574 In vitro efficacy of antibiotic supplementation in PMMA-bone cements against staphylococci

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Objectives: Infections of prostheses and surrounding tissue are one of the most serious complications in orthopedic surgery. Most, these infections caused by staphylococci, especially *Staphylococcus aureus*. Nowadays *S. aureus* often has a complete resistance against β-lactams (methicillin-resistant *S. aureus* (MRSA)), mostly connected with a resistance against other antibiotics, e.g. lincosamides, quinolones. Exchange of the implant and temporary implantation of antibiotic-containing spacers is the mode of choice in treatment of these biofilm-associated infections. Our in vitro-study was aimed to determine the antimicrobial activity of antibiotic-containing specimens over a time period of up to three months.

Methods: Specimens were prepared from commercially available bone cements (without antibiotics, with 12.5 mg gentamicin/g, with 25 mg gentamicin and 25 mg clindamycin/g cement). Additionally the cement containing 12.5 mg gentamycin was supplemented with 25 mg and 100 mg/g vancomycin. Specimens were placed in nutrient broth with five test strains (2 methicillin-susceptible *S. aureus* (MSSA), 2 MRSA, 1 *S. epidermidis*). If bacterial growth was visible in the broth specimens were removed and bacteria attached to the surface of the specimens were counted. Further, specimens were soaked into nutrient broth and phosphate buffered saline; eluates were taken for determination of antimicrobial activity by means of agar diffusion test.

Results: Cements containing gentamicin and clindamycin showed a higher antimicrobial activity against MSSA and *S. epidermidis* in comparison to cement containing only gentamicin. All specimens were infected 15 d after starting the experiments. Specimens with gentamicin and clindamycin inhibited biofilm formation and acted antimicrobial against MSSA strains and *S. epidermidis* strain up to 84 days. Cements with gentamicin also combined with clindamycin did not have any effect on MRSA strains. Only supplementation of vancomycin was able to inhibit growth of MRSA strains; concentration of 100 mg was more active up to 25 days.

Conclusions: Microbiological diagnosis should exclude as fast as possible an infection by MRSA. If infection is caused by an MSSA, combination of gentamicin and clindamycin can be recommended. For treatment of MRSA, application of an vancomycin-containing cement seems to be necessary.

R2575 Seroprevalence of antibodies against *Treponema pallidum* in pregnant women

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Objectives: To know the prevalence of antibodies against *Treponema pallidum* in pregnant women in our health area; to compare Spanish-born women with those born abroad.

Methods: The information source used to select patients was the Microbiology Laboratory database (Hospital de Albacete, Spain). It included all women who applied for syphilis serology at the Obstetrics Service (January-December 2009). Serological screening consisted in determining antibodies against *T. pallidum* by chemiluminescence enzyme immunoassay (Syphilis TP Abbott® Laboratories). Confirmation of the positive sera was done by passive hemaglutination (Kit TPHA 500, Newmarket Laboratories Ltd.) and by determining nontreponemal antibodies (Macro-Vue RPR, Beckton Dickinson®). Seropositive patients' medical histories were consulted to know the country they were born and if they had been previously diagnosed and treated. The Agresti-Coull method was used to calculate 95% confidence intervals (95CI) for prevalence. Relative risk (RR) and its (95CI) were calculated by the Epi Info 3.4.1 program, to compare proportions.

Results: 4379 women participated, with a mean age of 30.4 years (standard deviation [SD] = 5.7 years). Of these, 3305 (75.0%) were born in Spain, 880 (20.1%) were born abroad, and country born was not established for 194 (4.4%). The mean age of seropositive patients was 29.5 years (SD = 4.6 years). Treponemal antibodies overall prevalence was 0.73% (95CI: 0.52%-1.04%). Of the 32 seropositive patients, 30 were foreign (94.0%). Seroprevalence among Spanish pregnant subjects was 0.06% (2/3305; 95CI: 0.002%-0.24%), and 3.4% among foreign subjects (30/880; 95CI: 2.4%-4.9%). Of the 30 seropositive immigrants, 19 came from Latin America, 9 from East Europe and 1 from sub-Saharan Africa; country born was not determined in 1 case. When compared with Spanish pregnant subjects, seroprevalence was higher in women born in Latin America (RR = 92; 95CI: 22–395) or East Europe (RR = 48; 95CI: 10–220). Of the 32 seropositive pregnant women, 11 had been diagnosed and treated before pregnancy. In the remaining 21 cases, no former diagnosis was reported and women received specific treatment. Prevalence of previously undiagnosed syphilis was 0.48% (95CI: 0.31%-0.74%).

Conclusion: Syphilis seroprevalence is very low among Spanish pregnant women, but high in pregnant women from Latin American and East Europe. The strategy of prevention of congenital syphilis should pay special attention to immigrant women.

R2576 The comparison of the sterilisation effect against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* using the supersonic wave and plasma irradiation

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Objectives: The nosocomial infection caused by *Acinetobacter baumannii* and *Pseudomonas aeruginosa* has been problem worldwide. It is known that these bacteria contaminate the water supply environments such as faucet, sink and bath of the hospital for a long time. And it is extremely difficult to sanitize strains on these environments. The aim of the present study is to evaluate the bactericidal effect against biofilm-formed these strains using the supersonic wave or plasma irradiation.

Methods: The clinical isolate of *A. baumannii* and the standard strain of *P. aeruginosa* PAO1were used in this study. To form biofilm, the 5cm square stainless steel plate was added in TSB of each bacterial

suspension of McFarland No.0.5 and incubated at 37ºC for 24hr under anaerobic conditions.
These stainless plates were added in water tank and irradiated a supersonic wave of 38 kHz for 5, 10 or 30 minutes. Whereas, a plasma flow generated by a dielectric barrier discharge in air at 12 kVpp with 2 k Hz was irradiated to another plates with each biofilm-formed bacteria for 1, 10 or 30 minutes. After irradiation, each plate was cultured on the MH agar plate at 37 oC for 24hr.
Bactericidal effect was observed using the scanning electron microscope (SEM) and the above-mentioned cultural method.
Results: Both strains of *A. baumannii* and *P. aeruginosa* except biofilm-formed strains were sterilized by the supersonic wave for 5 minutes. However, biofilm-formed both strains were not sterilized by that of 30 minutes. On the other hand, all strains were sterilized within 10 minutes by the plasma irradiation. With or without biofilm, the bursting of bacteria by the plasma irradiation was confirmed using the SEM.
Conclusion: This study results showed that the plasma irradiation is effective for sterilization of the hospital environmental contamination by *P. aeruginosa* and *A. baumannii*.

R2577 Evaluation of hand-hygiene compliance rate among healthcare workers in Isfahan hospitals, 2010

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Objectives: Nosocomial infections are those which are created during hospitalization or as its result. The health care workers' hands are the main route of microorganism transmission. Hand washing is the most important measure to prevent spread of this infection. Compliance of hand hygiene (HH) among healthcare workers is generally low (less than 50%). This study aimed to determine the acceptance level of hand hygiene in Isfahan hospitals, 2010 to design interventions to increase awareness, motivation and performance among Healthcare workers.
Methods: This cross sectional study was conducted in three large hospitals in Isfahan (a public training medical center, a public medical center, and a private medical center) and a total of seven trained observers for different working shifts at three hospitals were selected. 7 working days during two different weeks and every day, eight 20-minute sessions was considered. During these sessions, observers monitored healthcare workers during their routine activities through a check list. Five indications (before touching a patient, before a procedure, after a procedure or body fluid exposure risk, after touching a patient, after touching a patient surroundings) were observed based on the World Health Organization guideline. Four different professional groups (physician, nursing, student, others) were considered for this study. HH compliance was calculated as "(number of positive hand hygiene actions/number of total hand hygiene indications) $\times$ 100" based on each indication and professional group.
Results: From the total 3097 observed indications, in general, the HH compliance rate was 5.6% and statistically significant between three different hospitals (7.4%, 4.1%, and 1.4% respectively). The most rates were observed among the nursing group (7.3%), and then the student group (6.5%), (p-value less than 0.001). Among the various indications, the highest rate of compliance was observed after a procedure or body fluid exposure risk (9.4%), (p-value less than 0.001).
Conclusion: In this study, the hand hygiene compliance rate in hospitals studied was low and needs to plan for improve.

R2578 Phenotypic characterisation of gonococci isolates in an Irish university teaching hospital

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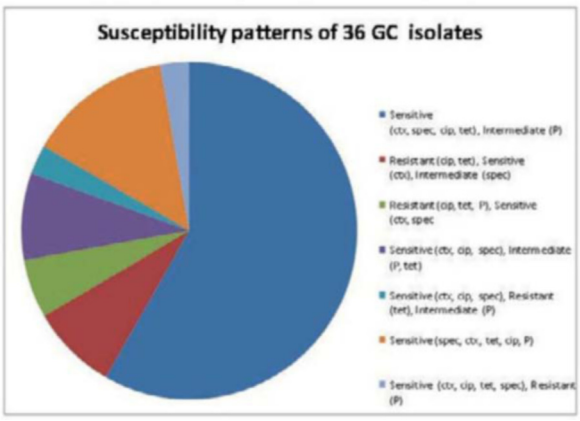
Objectives: Gonorrhoea remains an important clinical and public health problem worldwide. *Neisseria gonorrhoeae* infection have been shown to increase the risk of acquiring and transmitting human immunodeficiency virus (HIV) infection thereby making gonorrhoea control an important part of HIV prevention (Barry and Klausner, 2009). This study examined prevalent phenotypes and resistance pattern of gonococci isolates in an Irish university teaching hospital.
Method: Thirty six *Neisseria gonorrhoeae* strains isolated from urogenital specimens adults using New York City agar (NYC, Oxoid, UK) at Microbiology Laboratory, Waterford Regional Hospital, Waterford, Republic of Ireland between January 2007 and June 2010 susceptibility pattern were studied using E-test strips (Biomerieux SA, France) and adopting the clinical laboratory standards institute (CLSI) breakpoints. Phadebact monoclonal antibody typing (Boule Diagnostic AB, Hudding, Sweden) was performed on these isolates. Penicillinase production was performed using nitrocephin method.
Results: All 36 isolates were WII/WIII (IB serovars), yielded 100% in-vitro susceptibility to ceftriaxone (MIC $\geqslant$0.5mg/l) and spectinomycin (MIC $\geqslant$128mg/l). Three isolates (8.3%) were resistance to penicillin (MIC $\geqslant$2mg/l) and were penicillinase producing strains. Twenty eight isolates (77.8%) yielded reduced susceptibility to penicillin (MIC 0.12–1mg/l). Thirty one isolates (86%) were resistance to penicillin or yielded reduced susceptibility to penicillin. Five isolates (13.9%) were resistant to ciprofloxacin (QRNG) (MIC $\geqslant$1mg/l) of these five isolates three yielded penicillin reduced susceptibility pattern while two were resistant to penicillin. Six (16.7%) were resistant to tetracycline (MIC $\geqslant$2mg/l) (TRNG). All the ciprofloxacin resistant isolates were from male subjects. Seven distinct antimicrobial susceptibility patterns were observed. A 0.74% isolation rate was obtained in this study population.
Conclusion: Ciprofloxacin and penicillin resistance is increasing. Cephalosporin resistance was not observed but, local continuous antimicrobial surveillance is paramount and should be ongoing. There was no distinct link between the phadebact monoclonal typing of these isolates and their antimicrobial susceptibility pattern.

Table 1. Susceptibility pattern of 36 GC isolates

Sensitive (ctx, spec, cip, tet), Intermediate (P)	58.30%
Resistant (cip, tet), Sensitive (ctx), Intermediate (spec)	8.30%
Resistant (cip, tet, P), Sensitive (ctx, spec	5.60%
Sensitive (ctx, cip, spec), Intermediate (P, tet)	8.30%
Sensitive (ctx, cip, spec), Resistant (tet), Intermediate (P)	2.80%
Sensitive (spec, ctx, tet, cip, P)	13.90%
Sensitive (ctx, cip, tet, spec), Resistant (P)	2.80%

Ctx- ceftriazone, Spec- spectinomycin, Cip- ciprofloxacin, Tet- tetracycline, P – penicillin.



R2579 The incidence of infections and MDRO prevalence in long-term-care facility in Krakow, Poland

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Nosocomial infections have been a very well-know public health problem with many consequences like medical, economical, ethical etc. Information about epidemiology and microbiology is used as a basis for legislation of special programs for infection prevention and control in hospitals and long term facilities (LTCF). Unfortunately, is not known situation in polish LTCF.

The aim of this work was to analyze the epidemiology of infections and multidrug resistance organisms among 108 residents in one LTCF.
Results: 108 residents and 33 968 resident days (pds) were included in the studied period. 61,6% of studied residents had wheelchair disability or were bedridden, 10,6% were disoriented in time and/or space, the average age was: 76,3. Prospective epidemiological surveillance was carried out, with trained professionals using McGeer definitions. The incidence density per 1000 pds was 3,6. The most common infection was lower respiratory tract infection including pneumonia: 38 cases (31,1% all) and skin infections – WI (lower leg ulcerations, decubitus ulcers): 26 cases (21,3%). 22 cases (18,0%) of symptomatic urinary tract infections and 36 cases (11,5%) of other infections were reported.
The most common aetiological factor for all types of infections were Gram negative bacteria: 75,6% with Enterobacteriaceae (46,3%). *Pseudomonas aeruginosa* was resistant in 70% for fluoroquinolones, in 40% for amikacin and imipenem/meropenem. 75% of isolated strains of *Klebsiella pneumoniae* were ESBL+ and 80% of isolated *Staphylococcus aureus* (12,2% of all bacteria) strains were MRSA.
The empirical therapy was used in 76,2% of all infections, 29 cases of infections (23,8%) were microbiologically tested.
Conclusion: This study supports the need for screening and control of infections and multidrug resistance organisms. Observed infections are rarely subject to routine microbiological diagnostics. The incidence rate and resistance of microorganisms showed importance of cooperation between the microbiology lab and medical personnel for both: competent diagnosis and treatment of illness as well as nosocomial infection surveillance and control activities. Further study should examine more risk factors in detail to identify opportunities for prevention of infections and MDRO.
This study was supported by the grant from the Polish State Committee for Scientific Research (No N N404 047236).

R2580 The role of silver ion coated external fixators in preventing bacterial colonisation: in vitro microbiological study

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Objectives: Foreign body (external fixator, prosthesis) infections cause a significant morbidity and mortality in orthopedic surgery. Silver ion coating of metal external fixators can reduce bacterial colonization consequently infections.
Methods: In the study, 66 metal external fixators were used. Titanium (22 TiA14V) external fixator was coated with silver ion containing calcium phosphate based ceramic powder by using electrospray method. 22 implants were coated with hydroxyapatite in the same way, and the remaining 22 external fixators were without any coating. To induce experimental infection, *Staphylococcus epidermidis* clinical isolate which have slime factor, was used. In the study, 1×10^4 CFU/mL suspension of the clinical isolate in tryptic soy broth was prepared. Following 24 h incubation, quantitative culture of bacteria and determination of silver ion by atomic absorption were performed on the broth. Quantitative culture of bacteria on the external fixators was performed in addition to microscopic examination and the possible antibacterial efficacy of implants due to silver ion coating was investigated for 8 weeks (day 2, week 2, 4, 6 and 8). Accordingly, the implants in glass tubes (diameter 0.4 cm) were placed in shaking incubator at 35 ºC/80 rpm. Implants were rinsed with distilled water in aseptic conditions once a week.
Results: The bacterial growth was statistically higher in broth containing titanium external fixators compared to broth media containing silver ion-coated and hydroxyapatite-coated external fixators at 24 hours ($p = 0.036$ and $p = 0.009$, respectively). The release of bacteria from silver ion-coated fixators was statistically less compared to hydroxyapatite- and titanium-coated fixators ($p = 0.039$ and $p = 0.002$, respectively). MIC levels for 5% silver ion powder was 8 μg/mL for CNS. No free silver ions were detected in broth media using this method which has a detection threshold as low as 0.02 ppm. In electron microscopy, it was observed that less bacteria adhered to silver ion-coated external fixators.
Conclusion: We observed that there was no bacteria release from silver ion-coated external fixator to surrounding liquid. As a result of silver ion coating of metal external fixators, bacterial colonization reduces significantly. Such effect may help preventing post-operative infections caused by external fixators commonly used in orthopedic operations. In-vivo evaluation of this effect is warranted.

R2581 An evaluation of nebulised hydrogen peroxide post routine cleaning for environmental surface disinfection

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Objectives: Environmental contamination with methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) establishes reservoirs of infection and facilitates transmission. Routine cleaning methods may be inadequate for environmental disinfection and hypochlorite disinfection is time-consuming, potentially toxic and therefore no longer widely used. This study evaluated the efficacy of nebulised hydrogen peroxide (6% H2O2; Nocospray, OXY′PHARM) following routine cleaning, for surface disinfection.
Methods: Environmental surfaces (bed, bedside rail, bedside table, blood pressure cuff, intravenous pump, call button, dresser, door handle, toilet seat, toilet rail and curtain rail) in a closed room measuring $80m^3$ artificially contaminated with MRSA and/or VRE were sampled with sterile swabs (Copan) prior to and after routine cleaning (using neutral detergent and quaternary ammonium compound [Viraclean, Whiteley]); and after further disinfection with Nocospray (delivered from a portable aerosoliser over 18 minutes). Swabs were plated onto MRSA (Oxoid) and VRE (bioMérieux) chromogenic agars and placed into enrichment broth. After 48 hours incubation at 37^{oC}, the presence of MRSA and/or VRE was noted; if absent, enrichment broth fluid was plated onto chromogenic agar and read after 48 hours incubation. RODAC plates (BD Diagnostics) were applied to five surfaces (bed, bedside rail, blood pressure cuff, toilet seat and toilet rail) prior to and after Viraclean and Nocospray, and bacterial colonies counted.
Results: MRSA was detected in 22 (100%), 9 (40%), 11 (50%), 7 (32%) and 7 (32%) and VRE in 22 (100%), 13 (59%), 17 (77%), 4 (18%) and 5 (23%) of 22 surfaces tested: pre-cleaning, post Viraclean with direct plating, post Viraclean with enrichment, post Nocospray with direct plating and post Nocospray with enrichment, respectively. Blood pressure cuffs were least likely to be adequately cleaned; MRSA and VRE were recovered after both Viraclean and Nocospray. The table below shows the number of bacterial colonies detected on RODAC plates at each stage.
Conclusion: Nocospray further reduced environmental contamination with MRSA and VRE following Viraclean. The process is quick, convenient, safe and allows rapid turnaround of rooms after disinfection. Reducing environmental reservoirs should reduce transmission of MRSA and VRE.

	Pre-clean (n=15)	Post Viraclean® (n=15)	Post Nocospray® (n=15)
No growth	0	1 (7%)	5 (33%)
<10 colonies	0	1 (7%)	4 (27%)
10-50 colonies	0	7 (47%)	4 (27%)
50-100 colonies	1 (7%)	4 (27%)	2 (13%)
>100 colonies	14 (93%)	2 (13%)	0

R2582 Towards European core competencies for training infection control/hospital hygiene professionals

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Objective: European Centre for Disease Prevention and Control (ECDC) commissioned the project "TRaining in Infection Control in Europe" (TRICE) to produce a guidance document to develop and harmonize Infection Control Professionals' Training Programmes across Europe.
Methods: The project utilised the information in the report of Improving Patient Safety in Europe (IPSE) project Working Pary 1 defining European Core Curriculum for training for Infection Control Practitioners and the EU council recommendations on patient safety of June 2009. The proposed competencies resulted from the following steps:
– the IPSE Core Curriculum for Infection Control Practitioner was submitted to the national representatives, who were asked to score(?or grade?) each item of the curriculum:
– in a meeting held in Udine, results, graduation and future endorsement strategies were discussed;
– after the meeting a coordination group elaborated the final document;
– this document was sent again to all NCPICT for their final approval.
All 33 NCPICT participated in the process and approved the document.
Results: The proposed European Core Competencies aim to be helpful in:
– standardizing Infection Control/Hospital Hygiene Professionals (IC/HHPs) competencies in Europe;
– designing and implementing training courses according to different national contexts and facilitating the mutual recognition of competence across Europe;
– providing an opportunity for IC/HHPs to review their own performance and to plan their professional development;
– providing an opportunity for health care institutions and organizations to evaluate their needs in terms of professional human resources.
The document is organized into three main areas: Programme Management, Quality Improvement and Infection Control. Each consists of different professional tasks common to infection control doctors and nurses (except one: related to antibiotic prescribing). For each professional task, competencies were classified into foundation practice (this applies to newly appointed IC/HHP staff with little or no previous experience and expert practice (for a IC/HHP confident and experienced in all competencies).
Conclusions: We have achieved a consensus document on European Infection Control/Hospital Hygiene Core Competencies. However, to ensure that they will make ae a positive impact it will be necessary to spread and endorse them with all the relevant stakeholders.

R2583 De-escalation strategy reduces Gram-negative pathogen resistance in infectious complications of thermal burns

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Objectives: To assess the influence of a new carbapenem-based de-escalating strategy of antimicrobial therapy in treatment of infectious complications of severe burn injury by resistance of nosocomial Gram-negative bacteria.
Methods: A retrospective analysis of antibiograms from burn patients admitted to our burn care unit (BCU) of the sensitivities to common Gram-negative pathogens before (2007) and after de-escalation strategy (2010) was applied. The results of microbiological analyses of wound discharge of 100% BCU patients from January to December 2007 and from January to November 2010 were compared. De-escalation strategy (meropenem (MEM) in combination with vancomycin) was introduced in January 2008. Data were collated, and statistical significance was assessed with the chi-square test.
Results: 302 patients in 2007 and 217 patients in 2010 with confirmed burn wound infection were analyzed. The most common Gram negative pathogens were *P. aeruginosa* (20.3% of all samples) and *A. baumannii* (7.1%). There was a significant decrease of resistance to antibiotics in 2010 as compared with resistance in 2007 for *P. aeruginosa* to amikacin (AMI) (21.0% vs. 44.9%; $p = 0.001$), ceftazidime (CEF) (62% vs. 78%; $p = 0.022$), cefoperazone/sulbactam (CFP/SUL) (67.2% vs. 85.3%; $p = 0.01$). There is a tendency of resistance decreasing to ciprofloxacin (CIP) (65.2% vs. 75.7%; $p = 0.13$), MEM (52.8% vs. 61.2%; $p = 0.29$). The level of susceptibility of the pathogen to imipenem (IMP) remains the same (61.4% vs. 69.3%; $p = 0.47$).
The amount of resistant strains of *A. baumannii* significantly decreased to AMI (23.6% vs. 62.2%; $p < 0.001$), CIP (36.8% vs. 69.0%; $p = 0.013$), CFP/SUL (2.7% vs. 17.4%; $p = 0.039$). Also there is a trend to decreasing resistance to CEF (59.0% vs. 75.8%; $p = 0.083$). None of the strains of *A. baumannii* from the samples collected in 2010 were resistant to MEM (0% vs. 9.7%; $p = 0.093$).
No difference was seen in mortality between2007 and 2010 (5.3% vs. 7.7%; $p = 0.079$). The rate of mean inpatient-day decreased from 19.7 in 2007 to 15.1 in 2010.
Conclusions: The implementation of a de-escalation strategy of treating infectious complications of thermal burns in our burn care unit increased the susceptibility of multidrug-resistance to Gram-negative pathogens. There is a potential benefit in reducing the overall costs of treatment, due to direct costs of antibiotic treatment and shortening the length of stay in the hospital without increasing the mortality rate.

R2584 Laboratory personnel is a high-risk group for latent tuberculosis infection: evaluation by interferon-γ release assay and tuberculin skin test in an intermediate incidence setting

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Background: This is the first large-scale study that focused on the prevalence of LTBI in the laboratory personnel, especially in the intermediate incidence setting.
Methods: We recruited 173 laboratory personnel and performed QuantiFERON-TB Gold In-Tube test (QFT-G) and tuberculin skin test (TST). Agreement between QFT-G and TST was analyzed, and risk factors for the positivity of each test were assessed.
Results: QFT-G was positive in 21.4% of the enrolled laboratory personnel, and TST was positive in 33.3% with a cut-off of 10 mm. The agreement between the two tests was fair (kappa = 0.234; 95% confidence interval = 0.054–0.414). Age and duration of health care profession were significantly associated with positive QFT-G and TST results by univariate analyses. With multivariate logistic regression analyses, household contact to TB patients ($P = 0.013$), the laboratory sections of microbiology ($P = 0.045$) and chemistry/immunology ($P = 0.014$) were significantly associated with positive QFT-G results.
Conclusions: Our data shows the high prevalence of LTBI in the laboratory personnel, and emphasizes the importance of LTBI screening for the health care workers. QFT-G seems to be superior to TST for the LTBI screening.

R2585 Incidences of *C. difficile* infections in cardiothoracic transplant recipients

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Background: *C. difficile* infections (CDI) are emerging over the past ten years. Only few data is available about incidences of CDI in hospitalized cardiothoracic transplant recipients (CTx) in comparison to non-transplanted hospitalized patients. Therefore, we investigate CDI incidences in hospitalised CTx recipients in comparison to non-CTx recipients.
Methods: In a university hospital a prospective surveillance of all CDI patients was performed from 2007 to 2009. Psychiatric patients were excluded. CDI was defined as diarrhea with a positive toxigenic *C. difficile* result, or the detection of pseudomembraneous colitis

(diagnosed by endoscopy or positive histopathology). Incidences and incidence densities in CTx and non-CTx-patients were determined. All *C. difficile* isolates were typed by PFGE.
Results: CDI incidences in Ctx and non CTx recipients were depicted in table 1. 39 (2007), 68 (2008), or 38 (2009) patients were already admitted with CDI (all non CTx). CDI incidences and incidence densities declined over the time in both groups (table 1). Post transplantation CDI incidences declined significantly in CTx patients over the observed period ($p < 0.05$). CDI incidences in CTx patients were higher in 2007 than in 2009 and in comparison to the hospital-wide incidences, but incidence densities of CDI in CTx declined remarkably over the three years. 5 out of 366 CTx patients (1.4%) developed CDI pre-Tx. Only one patients required a colectomy (non CTx). No NAP I strain was detected.
Conclusion: CDI occurred more frequently in patients post CTx compared to other patients admitted to a university hospital. CDI densities in CTx declined over a three years period. It is not clear whether the decrease of CDI cases in CTx patients was due to more rigorous infection control measures, a strictly targeted antibiotic therapy, or both.

Table 1: CDI incidence and incidence densities

	2007	2008	2009
CDI cases hospital	188	222	148
CTx patients	137	99	130
CDI post CTx	10	6	5
CDI per 100 patients	0.39	0.43	0.28
CDI per 1000 patient days	0.51	0.58	0.39
CDI per 100 CTx patients	7.3	6.1	3.85
CDI per 1000 CTx patient days	1.07	1.0	0.65

CDI = *C. difficile* infection; CTx = cardiothoracic transplant recipients

R2586 Evaluation of the antimicrobial properties of copper against clinical isolates of carbapenamase-producing Enterobacteriaceae

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Objectives: Copper has been shown to have antimicrobial properties and when used on hospital surfaces it minimized environmental contamination by pathogenic bacteria. We investigated the effects of two copper alloys on the survival of VIM and/or KPC-producing multi-drug resistant (MDR) Enterobacteriaceae.
Methods: MDR clinical isolates were screened for the production of VIM or KPC carbapenemase using the EDTA-imipenem or the boronic acid-meropenem synergy test, respectively. The genes were confirmed by PCR with specific primers. To assess the antimicrobial properties of copper, coupons (1 cm × 1 cm) of copper alloys (A and B containing Cu99% and Cu63%Zn37%, respectively) were sterilized, inoculated with 20 µl of bacterial suspension (10^8 cfu/ml) and incubated at room temperature for 0, 1, 3, 5, 6 and 24 hours. Then coupons were placed in sterile neutralizing broth (Difco) and vortexed. The broth was serially diluted and quantitatively cultured for recovery of viable bacteria. The lower limit of detection was 2.6 log10 cfu/ml. Stainless steel (C) and polyvinylchloride (D) coupons were used as controls. Mean viable counts (log10 cfu/ml) after incubation at each time interval with each coupon were compared for statistical analysis. Reduction of viable counts by $\geqslant 3$ log10 from starting inoculum was characterized as bactericidal activity.
Results: Thirteen isolates including *K. pneumoniae* (7), *E. coli* (4) and *Enterobacter* spp. (2) producing either VIM (2), KPC (10) or both (1) were tested. At 3 and 5 hours, mean viable bacterial counts on coupon A decreased by 1.2 and 3.4 log10, respectively while the reduction produced by coupon B at 5 and 6 hours was 2.1 and 3.2 log10, respectively. Controls C and D reduced bacterial counts by 2.1 log10 at 6 and by 2.3 log10 at 24 hours, respectively. A bactericidal effect was detected by coupons A and B at 5 and 6 hours, respectively. Viable bacteria could not be recovered from 10/13 tested strains incubated on coupon A after 5 hours and from 8/13 strains after 6 hours of incubation on coupon B.
Conclusions: Copper alloys reduced the number of viable carbapenemase-producing bacteria significantly at 3 hours and produced a bactericidal effect at 5 hours. Cu99% was more effective than CuZn. These data suggest that the use of copper as surface material in the hospital could aid in diminishing the environmental reservoir of MDR Gram-negative pathogens.

R2587 Educational approach to reduce catheter-related bloodstream infections and catheter-related urinary tract infection rates in intensive care unit

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Objectives: Central venous catheter (CVC) usage is becoming more and more frequent in intensive care units (ICU). Besides its mechanical complications, catheter related bloodstream infections (CRBSI) are frequent, preventable and costly with high mortality and morbidity. Although it is essential for an ICU patient, urinary catheterisation also brings the risk of catheter related urinary tract infection (CRUTI).
We aimed to decrease our CRBSI and CRUTI rates with observation, education, feedback of infection rates, and reward.
Methods: We performed an observational intervention study in a 550-bed education and training hospital in Ankara, Turkey with twelve neurology-neurosurgery, ten coronary, six cardiovascular surgery and nine general ICU beds. The study was carried out in three phases, as preintervention, intervention, and postintervention. After six months of preintervention period, we performed education meetings to all ICU doctors and nurses. Education program highlighted prevention bundle of urinary catheter (UC) and CVC related infections. We observed CVC and UC with checklists and follow-up charts. CRBSI and CRUTI diagnosis were performed according to CDC definitions.
Six months after the education, all staffs of the departments which decreased their infection rates were promised to be awarded with 10% pay raise.
Results: During the study period 462 CVCs and 1258 UCs were inserted to 1075 patients and catheter days were 4313 and 6918, respectively. Most observed indications of CVC are peripheral intravenous access problem (41%) and haemodialysis (29%). Insertion sites were 41% jugular and 41% subclavian. 20% of CVCs were inserted in emergency conditions. Mean lenght of catheterisation was 11 days. Compliance to maximum barrier precautions was 41%, but this rate was correlated with doctors' education years. Most common displacement reasons for CVC were exitus (50%) and discontinuity of indication (32%). Compliance to prevention bundle was 13%. Mean lenght of catheterisation was 13.25 days. There was a relationship between the clinic which inserted the catheter and compliance to maximum barrier precautions for CVCs and UCs. We decreased our CRBSI rates from 8.2 to 3.19 (61%) and CRUTI rates from 3.7 to 2.19 (40.8%).
Conclusion: Our study showed that education, feedback of infection rates, and reward are effective for reducing CRBSI and CRUTI rates but continuity of education is essential for maintaining low infection rates.

R2588 Outbreak of carbapenem-resistant enterobacteria in a Brazilian university hospital

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Objectives: Report an outbreak of carbapenem-resistant enterobacteria in a university hospital, control measures and mortality associated with these pathogens.
Methods: Retrospective observational study about the experience of controlling an outbreak of carbapenem-resistant enterobacteria in a Brazilian University Hospital during the period of February 2009 to November 2010. Pathogens were isolated from vigilance cultures or cultures from sites suspected of infections.
Results: During study period 360 patients were identified as having positive cultures for carbapenem-resistant enterobacteria, mainly by carbapenemase production as mechanism of resistance (KPC-2).

Microorganisms more frequently isolated were *K. pneumoniae* (94.4%) and *Enterobacter* spp (5%). The outbreak began on February 2009 with nineteen cases, decreased to one case in June 2009 after control measures that included isolation, cohort, contact precautions and decreasing number of circulating people inside the hospital and closing of most affected units. On July 2009 there were new cases, despite control measures, and nowadays the number of monthly identified patients is around 21 new cases, becoming endemic in our hospital. Most identified cases were hospitalized (97.8%) in the following units: Intensive Care Unit (49.0%); Wards (33.1%); Emergency Department (12.3%) and Burn Unit (3.4%). Approximately half the patients were considered colonization (55.2%). Infected patients were treated with polymyxin with or without associated antibiotics. Among infected patients, more frequently identified sites of infections were: lungs (38.0%), urinary tract (27.7%), blood (9.0%) and catheter (5.1%). There was more than one of site infection in 14.8% of infected patients. Hospital mortality was 47.8% for all patients. Infected patients had higher mortality rate (58.8%) compared with colonized patients (38.3%; $p < 0.001$). Patients with positive blood cultures for these pathogens had higher mortality (70%) compared to other patients (46.4%; $p = 0.03$).

Conclusions: Carbapenem-resistant enterobacteria are a great challenge concerning control and treatment. These outbreaks take place frequently in the Intensive Care Units, nearly half the patients manifest infections and mortality among infected patients is high.

R2589 Incidence of nosocomial methicillin-resistant *Staphylococcus aureus* after a hospital prevention and control programme implementation

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important multidrug-resistant bacteria. Its surveillance and control must be a priority in a hospital setting. The aim of this study is to describe the incidence of MRSA in our hospital after the implementation of a prevention and control programme.

Methods: The programme of prevention and control was implanted in 2003 after a nosocomial MRSA outbreak, with an incidence of 0.24% in 2002. It is based in a bundle of recommendations: identification, isolation and control of the incidental cases, early detection of the condition of carrier and record of incidental cases in a database. This computing record is linked to the hospital information system (HIS) that allows us to detect the hospital readmissions of colonized/infected patients. These patients are isolated immediately.

Results: After the programme implementation, a gradual increase of the incidence was observed in the last six years at expense of community-associated MRSA (0.08% in 2003, 0.09% in 2004, 0.13% in 2005, 0.15% in 2006, 0.16% in 2007, 0.16% in 2008 and 0.25% in 2009) while the incidence of nosocomial MRSA has kept around 0.1% (0.15% in 2003, 0.10% in 2004, 0.11% in 2005, 0.07% in 2006, 0.14% in 2007, 0.12% in 2008 and 0.12% in 2009). The most frequent location of infection for nosocomial MRSA during these years has been always the low respiratory tract infection with or without pulmonary condensation (25.0%) followed primary bacteraemia (11,8%), and for community-associated MRSA the cutaneous infections (72,7%). All patients were isolated after detecting the MRSA: the daily average was 5 isolated patients which represented 2.5% of hospital bed occupancy.

Conclusions: We have not detected any nosocomial MRSA outbreak in our hospital from 2003. The incidence of nosocomial MRSA is kept in rates around 0.1%. It is important to emphasize the decrease of the proportion of nosocomial MRSA isolations, whereas community-associated MRSA have increased. A dynamic record connected to the hospital information system allows us to identify and control the colonized/infected patients before any entry to the hospital.

R2590 Evaluation of the use of a commercial PCR to detect methicillin-resistant *Staphylococcus aureus* in screening swabs during one year

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Objectives: Retrospective evaluation of the results in the detection of MRSA of a commercial PCR (Genohm®, Becton Dickinson) compared to culture (chromID™ MRSA, Biomérieux) after one year use (2009) in two geriatric wards (60 beds) and an intensive care unit (8 beds) of a 415 bed community hospital. Any impact on MRSA epidemiology is also evaluated.

Methods: All patients were screened on admission in the geriatric wards and in the intensive care unit. Swabs taken from nose and perineum were pooled, tested by PCR and inoculated on chromogenic agar to detect MRSA. No enrichment in broth culture was made. Samples arriving on Friday evening and in the weekend were not examined by PCR. Patients were isolated as soon as MRSA positive results of PCR or culture were known.

Results: 1136 samples were examined by PCR. Results of PCR are evaluated as false positive if culture was negative in primary and all subsequent samples in the same period with a minimum of two. A PCR negative result in combination with positive culture is regarded as false negative. A total of 35 samples were evaluated as MRSA positive. The results for PCR are shown in the table. The sensitivity of the PCR is 71.4%, specificity 99%, PPV 67.6% and NPV 99%.

Four patients from the involved wards screened positive in culture while PCR was not performed because of admission in the weekend.

From 2007 until 2010 there was a decline in MRSA incidence: from 11/1000 admissions in 2007 to 8/1000 in 2009. A proportionate reduction was seen in screening on admission (also in the geriatric wards and ICU), in infection on admission and infection >48h after admission. Remarkable is the decline in MRSA positivity on admission in patients residing in a nursing home: from 61 MRSA+ patients in 2007 to 39 in 2009.

Conclusion: In our hands the Genohm® PCR test had a rather low sensitivity and positive predictive value compared to culture even if culture was not ideal (only two sites, no enrichment). Introduction of the PCR was meant to reduce transmission of MRSA and hence reduction of infection by faster isolation because of the speed of the test. We can not deduce from our data that this policy has had any impact on the decrease of nosocomial MRSA infection.

	MRSA+	MRSA-
PCR+	25	12
PCR-	10	1089

R2591 Turning foe into friend: an innovative multidisciplinary *Clostridium difficile* performance management meeting to spearhead trust CDI programme

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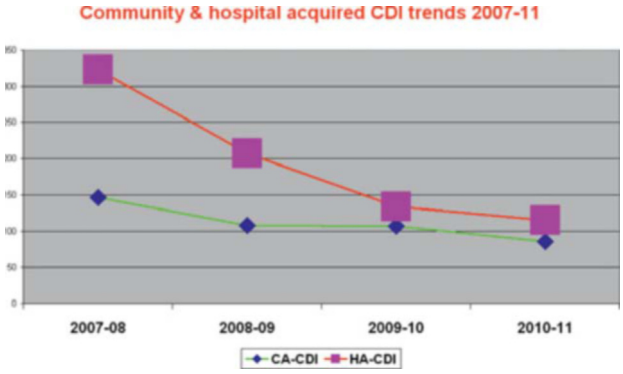
Background: Blackpool Victoria, a large district teaching foundation trust hospital in NW England, operates a very successful comprehensive CDI containment programme. There is special emphasis on antibiotic stewardship and real time programme monitoring initiatives like root cause analysis of all CDIs in community/acute trust, multidisciplinary audit and surveillance. We present findings from 1st 6-months of an innovative pilot project "multidisciplinary CDI performance management meetings [MCPM]" aimed to spearhead the trust CDI programme in future. The aims of MCPM include team working, problem solving, real time "shop-floor" troubleshooting, raising profile and awareness in a friendly interactive session. The results have been used to inform a regularly monitored strategic action plan.

Methods: MCPM are scheduled with 7days of an avoidable CDI. A rapid turnaround [within 24h] rootcause analysis is carried out jointly by infection control and microbiologist to identify avoidable/unavoidable

CDI. Following which MCPM is organised by DIPC/dep medical director or Director of Nursing to which are invited divisional head nurse, matron, staff nurse, antibiotic/ward pharmacist, consultant/s from clinical areas during patient transfer, consultant microbiologist. Initial RCA is now discussed in details dissecting out sequential aspects of patient care and clinical management leading upto episode of CDI. Database review.
Results: CDI rates have consistently delined since launch of CDI programme. An overall 58%[469/2007–08 to est 200/2010–11] reduction; with community and acute trust CDI reductions of 41%[146/2007–08 to est 85/2010–11] and 64% [323/2007–08 to est 115/2010–11] respectively. Key results of rootcause analysis [2009 till date] and MDT audit/surveillance to be presented. Key outcomes from MCPM include:inappropriate [choice, duration, dose, review/stop, guideline non-compliance] antibiotic use; poor/missing documentation; low staffing; fall of communication between nurse/medical staff;absence of risk based prescribing, etc. Details to be presented.
Conclusion: Trust comprehensive CDI programme is aimed at reducing avoidable CDIs, associated morbidity, mortality & costs and increase quality of care and patient safety. The key results of the RCAs and MDT audit/surveillance to be presented. The MCPM have been found to be most useful for all attendees. The results have been used to inform regularly monitored CDI strategic action plan.



R2592 Epidemiologic survey of nosocomial infections with molecular typing methods in haematologic patients

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Objectives: Epidemiologic survey of infections in cancer patients is an important tool to understand the nosocomial pattern and emerging antibiotic resistance; in addition regular monitoring is essential to identify outbreaks and possible transmission route. To evaluate this risk we conducted a prospective molecular epidemiologic investigation in hematologic patients.
Methods: From 2006 to 2009, 540 hematologic patients were monitored employing the Vigi@ct epidemiological information system and the f-AFLP (fluorescent-amplified length fragment polymorphism) microbial fingerprinting method.
Results: Bloodstream infections (BSIs) was documented in 32% of total study population (175/540). BSIs were present in 69% of acute leukemia, in 16% of non-Hodgkin lymphoma, in 5% of myeloma and in 10% of other hematologic diseases. Among the pathogens responsible for BSIs, 117 (67%) were Gram-positive: CoNS 63%, enterococci 28%, *S. aureus* 3%, other 6%; 53 (30%) were Gram-negative: *E. coli* 43%, *P. aeruginosa* 28%, *S. maltophilia* 13%, KES group 16%; 5 (3%) were fungi.
Of Gram-positive, 100% of CoNS and 59% of *S. aureus* were meticillin resistant, whereas 18% and 50% of enterococci were VRE (VanA-type) and HLAR (High-level Aminoglycoside-Resistance), respectively.
Of Gram-negative, resistance to ceftazidime occurred in 16% of *E. coli*, 46% of KES and 75% of *P. aeruginosa*; resistance to quinolones occurred in 75% of *E. coli*, 53% of KES and 52% of *P. aeruginosa*; resistance to β-lactamase inhibitors occurred in 20% of *E. coli*, 46% of KES and 33% of *P. aeruginosa*; resistance to carbapenems (meropenem) occurred in 8% of KES, 14% of *P. aeruginosa*, while no *E. coli* strain was resistant. In 2008 we recorded one *P. aeruginosa* PAN-R strain, sensible only to colistin. In May-September 2008, an unexpected high incidence of VRE was reported. AFLP analysis of these VRE strains documented a genetic relationship between two different groups of isolates: 2 isolates show similar AFLP patterns but different from those of the other 4 genetically related isolates. These data suggest a possible person to person transmission. After corrective measures no other case of VRE was documented. No Gram-negative outbreak was recorded in the same period.
Conclusion: Our results showed a new increase of Gram-negative infections and a progressive antimicrobial resistance. The high discriminatory AFLP method was fast enough to allow a 'real-time' monitoring of the outbreak, permitting additional preventive measures.

R2593 *Clostridium difficile* detection: identification of colonisation, subclinical and overt disease

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Background: Detection of C difficile or its toxins has been observed to be problematic. False negative results have meant that patients were tested repeatedly when symptomatic. Among the reasons for poor negative predictive value are the labile nature of the toxins and the limitations of available technologies. Newer and more sensitive technologies were introduced such as PCR. These technologies have reduced the rates of false negatives and eliminated the need for multiple samples. In recent years, and through a combination of infection control measures, CDAD rates were reduced dramatically. However, these successes brought to the front the significance of high false positive rates especially with the ultra-sensitive new technologies. It is well-established that toxin-producing *C. difficile* can colonise young children and older patients with history of repeated and prolonged hospitalisation. There is a need for a new approach to determine the clinical value of a positive result.
Objectives: This study was designed to assess the concordance between different toxin assays and clinical presentation in Antigen positive/Toxin negative patients.
Methods: Twenty six Antigen positive/toxin negative stool samples (Techlab EIA for both GDH Ag and toxin) were included in the study. Patients with multiple samples were identified. Samples were then tested using VIDAS EIA, culture and ribotyping, CEPHEID GeneXPert PCR and cytotoxicity assay. Clinical presentation and follow up data were then analysed to assess clinical significance of each result.
Results: Patients who had alternative explanations for their transient diarrhoea (e.g. laxatives), were found to be toxin-negative by EIA. However, 86% of these samples were positive by PCR and all produced cytotoxic effects following culture. Isolates were from a number of ribotypes (001, 002, 015 and 054).
Conclusions: These results suggest that combined clinical/laboratory interpretation is of more value at the current epidemiological setting. Highly sensitive technologies, such as PCR, may detect the presence of toxin genes in cases of colonisation or subclinical presence of *C. difficile*. Our data suggest that samples found to be Antigen positive but toxin negative by EIA, in the presence of alternative clinical causes for diarrhoea, should be treated as colonisation. However, these patients should be monitored closely and should be isolated, while having diarrhoea, to limit environmental contamination.

R2594 Patients with chronic foot osteomyelitis: 10-year evaluation (1999–2009) in a Greek reference clinic of a tertiary university hospital

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Objectives: Chronic foot osteomyelitis (CFO) is a diagnostic and therapeutic challenge for appropriate regimen and length of antimicrobial therapy. Most CFO occurred on the ground of diabetic foot (DF). This study aims to evaluate factors influencing successful outcome in patients (pts) with CFO with or without DF.
Patients and Methods: Pts with CFO were retrieved from a data-base of 700 pts with bone and joint infections followed in our reference clinic from 1999 to 2009. Demographics, DF, foot vascular and neurologic complications, charcot joint and gangrene were assessed following standard definitions. Diagnosis of CFO was based upon clinical, imaging and laboratory/microbiological evaluation. A completely restoration of all the above parameters was considered as cure. Chi-square and Mann Whitney tests were applied for categorical and continuous variables respectively. A logistic regression analysis of factors predictive of outcome was performed.
Results: DFO pts (n = 137), were male (59%) with median age + IQR(25–75) = 60 (52–70) and comorbidities (n = 76) including diabetes mellitus-DM (n = 64, insulin dependent, n = 60), rheumatic arthritis (n = 3), non DM vascular disease (n = 6), vascular (n = 44), neurological (n = 27) DM complications, charcot joint (n = 22) and gangrene (n = 24) with amputations (n = 25) and orthopedic device (n = 2). HbA1C was above 7 in32 pts. Median IQR(25–75) time from infection onset to treatment was 5 (2–12) months. Sinus tract and ulcer but no fever were found in 71 and 49 pts respectively. Microbiological documentation of DFO was based on intra-operative cultures (n = 83), prone to the bone or aspiration samples (n = 21). Gram positive bacteria (86%) included MRSA (32.5%), MRSE (17%) while Gram negative bacteria accounted for 49%. Among 84 polymicrobial cases, 16.6% were *S. aureus* plus *P. aeruginosa*. ESR/CRP baseline were abnormal in 90% of pts. Combined antimicrobial therapy was given to 117/137 pts including fluoroquinolones (n = 92,67%). Only 16 fully reversible adverse events were noted. Median IQR (25–75) treatment duration was 6 (6–8) months. However, 34% were treated for up to 3 months, 42% up to 6 months and 24% more than 6 months. Median follow up was 12 months. Cure Analysis demonstrated that only HbA1C more than 7 was independently related to adverse outcome (p = 0.0001).
Conclusions: Only normal HBA1c and n were predictive of the pts' outcome. Strict glycemic control is mandatory in the management of pts with DFO.

R2595 Comparison of two chromogenic media, Oxoid Brilliance™MRSA II Agar and ChromID™MRSA, for detection of methicillin-resistant *Staphylococcus aureus* from surveillance specimens

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Objectives: Rapid routine screening of patients for methicillin-resistant *Staphylococcus aureus* – MRSA is very important. Improved chromogenic media can detect MRSA within 18–24 hours. The purpuse of this study was to evaluate the abillity of two chromogenic media Oxoid Brilliance™MRSA II Agar (Oxoid, Ltd, Basingstoke, UK) and ChromID™MRSA (bioMérieux, Marcy-l'Etoile, France), for detection of MRSA from surveillance specimens.
Methods: Brilliance and ChromID were tested using six strains of MRSA (ATCC 43300), hVISA (ATCC 700698) and hGISA (ATCC 700699), two strains of MSSA (ATCC 29213) and a strain of *E. faecalis* and *E. coli*. The rest were strains isolated from patients (laboratory strain collection). One to two colonies of the test strain from the third subcultivation were inoculated on Brilliance and ChromID. 55 patient samples were processed. Skin, nose or throat swabs were sent in an Amies transport medium. Each sample was inoculated into Todd-Hewitt enrichment broth (THBS), blood agar (BA), Brilliance and ChromID. The first 28 samples were inoculated in the following order: 1. THBS 2. BA, 3. Brilliance, 4. ChromID. The next 27 samples were inoculated in a different order: 1. THBS 2. BA, 3. ChromID, 4. Brilliance. The plates were inspected after 18–21h of incubation at 35ºC. The plates were inspected after 18–21h of incubation at 35ºC. All denim-blue colonies on Brilliance and green colonies on ChromID were processed according to CLSI guidelines. Antimicrobial susceptibility testing was performed for all DNAse and coagulase positive strains with positive result of methicillin-resistance screening (MRSA strains) according to CLSI guidelines. Denim-blue and green colonies that were DNAse and coagulase negative (regardless of the result of methicillin-resistance screening) were considered false positive (CNS); DNAse and coagulase positive and oxascreen negative colonies were considered false positive (MSSA).
Results: All MRSA strains produced denim-blue or green colonies following 18–24 hours of incubation (Table 1). One discrepant result was obtained from a patient sample, the colonies denim-blue were on Brilliance, and yellow on ChromID. MRSA was confirmed in both cases. Strains of MSSA, *E. faecalis* and *E. coli* failed to grow on both chromogenic media.
Conclusion: Both agars are fast, easy to use and reliable. They require no additional costs and are accessible to every microbiological laboratory in comparison with rapid PCR methods.

Table 1. Results for Brilliance and chromID media after 20 h of incubation.

Isolate	No. of strains with positive results on Oxoid Brilliance™MRSA II Agar/total number of strains (%)	No. of strains with positive results on chromID™MRSA /total number of strains (%)
MRSA	4/4 (100)	4/4 (100)
hVISA	1/1 (100)	1/1 (100)
hGISA	1/1 (100)	1/1 (100)
MSSA	0/2 (0)	0/2 (0)
E.faecalis	0/1 (0)	0/1 (0)
E.coli	0/1 (0)	0/1 (0)
CNS	1/1 (100)*	1/1 (100)*
MRSA isolates from laboratory strain collection and routine patient samples	62/62 (100)	61/62 (98)

*different color of colonies

R2596 Cytokines in the ascitic fluid of cirrhotic patients with and without spontaneous bacterial peritonitis

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Aim: The compartmental immune response of ascitic fluid seems to be important, especially in spontaneous bacterial peritonitis (SBP). The aim of our study was to determine the pattern of cytokine synthesis in the ascitic fluid (AF) of cirrhotic patients, with or without SBP.
Patients and Methods: We prospectively studied 13 cirrhotic patients with ascitis, who were admitted at the University Hospital of Patras from May 2006 to December 2007. Patients were separated into two groups: (a) group 1: patients with SBP (b) group 2: patients without SBP. Cirrhosis was diagnosed on the basis of typical clinical, laboratory, ultrasonographic findings and/or liver biopsy. Upon admission a paracentesis of ascetic fluid was performed. Ascitic levels of IL-1b, IL-1ra, IL-6, IL-10, TNFa, sTNFRI and sTNFRII were measured by using an ELISA method. Data are presented as mean±standard deviation and were compared using t test. A P value <0.05 was considered significant.
Results: Characteristics of patients with SBP VS those without SBP are: age 69±12 vs 62±15, male/female ratio 5/2 vs 5/1, ethanol use 5 vs 4, viral hepatitis 1 vs 2, cryptogenic 1 vs noone. The ascitic concentrations

of individual cytokines in group 1 vs group 2 are the following: IL-10: 160±142 vs 80±74, IL-6: 2413±3183 vs 2257±2478, TGF-a: 11±20 vs 0.5±1, sTNFRI: 8191±3077 vs 5618±2384, sTNFRII: 5259±2354 vs 2883±932, IL1-ra: 946±703 vs 131±84, TGF-1b: 279±681 vs 378±671.
Multivariate analysis showed significant ($P < 0,05$) differences in the levels of sTNFRII and IL-1ra between the two groups. Ascitic levels of IL-10, IL-6, IL-1ra, TNF-a, STNFRII and STNFRI were higher, while TGF-b1 levels were lower in the ascitic fluid of patients with SBP (differences not significant). It is remarkable that IL-1b was not expressed in patients either with or without spontaneous bacterial peritonitis.
Conclusions: We demonstrated an increased cytokine production in ascitic fluid of cirrhotic patients, while the levels of anti-inflammatory cytokines sTNFRII and IL-1ra are significantly increased in patients with SBP. It seems, therefore, that an ascitic fluid anti-inflammatory response is characteristic in SBP,and this might compromise the final outocome.

R2597 Screening for extended-spectrum β-lactamase-producing Enterobacteriaceae: which sites?

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Introduction: Extended-spectrum β-lactamases (ESBL)-producing organisms belong to the most important multiresistant pathogens in hospitals. More recently, ESBLs are also spreading in the community worldwide. Carriers of ESBL-producing Enterobacteriaceae remain colonized for prolonged periods, possibly representing an important source of spread. Knowledge on the body sites most commonly colonized with these pathogens is therefore of great importance in order to develop appropriate screening schemes. The aim of this study therefore, was to determine the frequency of colonization for each body site.
Methods: From January 2008 to December 2010 all patients with detection of an ESBL-producing Enterobacteriaceae in any specimen were screened for colonization by examination of urine samples, rectal swabs, inguinal swabs, throat swabs, as well as wound samples. ESBL production was identified as recently described (Buehlmann M. et al., ICHE 2010;31: 227–8;none of these pusblised data are included in our study sample. The frequency of detection of ESBLs was calculated for each site.
Results: ESBL-producing Enterobacteriaceae were detected in 749 samples from 303 patients. 369 (38.6%) of all urinary samples, 219 (22.9%) of all rectal swabs, 92 (9.6%) of inguinal skin swabs, 35 (3.7%) of all throat swabs and 34 (3.6%) of all wound samples revealed ESBL-producing Enterobacteriaceae.
Conclusions: Urine and the rectum are the sites most commonly colonized with ESBL-producing Enterobacteriaceae (61.5%) followed by the inguinal fold. These three sites should therefore be considered when screening for ESBL carriage is performed. Throat swabs and wound samples were positive in less than 5% of all specimens, suggesting that these sites are not useful for screening, however should be considered in the individual patient if a decolonization regimen is performed.

R2598 Paediatric and neonatal intensive care healthcare workers: knowledge of evidence-based guidelines for preventing central venous catheter-related infection

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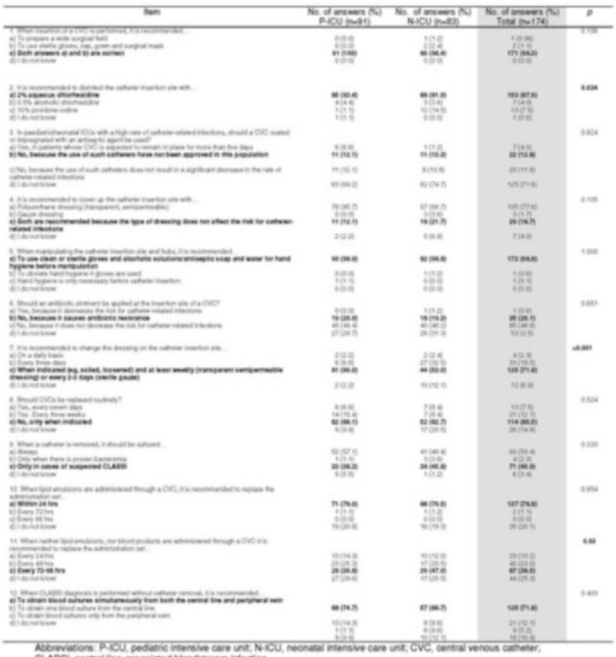
Assessment of knowledge of CDC guidelines for the prevention of central venous catheter (CVC)-related infection is recommended for healthcare workers (HCWs). Information on this knowledge in pediatric and neonatal intensive care units (P-ICU, N-ICU) is very scarce, and there are no comparative data on knowledge between physicians, nurses, and medical/nursing students.
Objectives: We compared different professional groups in order to assess HCWs knowledge of guidelines for preventing CVC-related infection in the P-ICU and N-ICU of a large teaching institution.
Methods: We distributed a 12-question multiple-choice questionnaire to HCWs in both units between November 2009 and May 2010. We also recorded participants' demographic data. Each correct answer was scored as 1 and incorrect answers as 0. We created individual and grouped scores of adequate responses ranging from 0 to 12.
Results: We collected 174 questionnaires from both the P-ICU and the N-ICU (91 and 83, respectively). Of these, 6 (3.4%) were returned by medical staff, 2 (1.1%) by medical residents, 99 (56.9%) by nurse staff, 18 (10.3%) by replacement nurses, 8 (4.6%) by nursing students, and 41 (23.6%) by nursing assistants. The overall mean score was 7.05. As for professional category, there were statistically significant differences between mean scores for nursing assistants (5.9) and medical/nursing staff (8.7 and 7.6). Moreover, years of experience were associated with better scores (>10y, 8.1 vs. <1y, 6.9 and 1–5 y, 6.7) and those participants who received training sessions in the last year had better scores than those who did not (7.7 vs. 6.6).
Conclusions: Our results show that there is room for improvement in HCWs knowledge of prevention of CVC infection. Simple, easy-to-obtain scores can help to evaluate the impact of educational and interventional bundles.

Table 1. Answers on multiple-choice questions regarding prevention of central venous catheter-related infection



Abbreviations: P-ICU, pediatric intensive care unit; N-ICU, neonatal intensive care unit; CVC, central venous catheter; CLABSI, central line-associated bloodstream infection.
The correct answer according to CDC guidelines is indicated in bold type.

Clinical epidemiology of nosocomial infections (POWI, VAP, UTI, BSI, . . .)

R2599 Antibiotic usage and risk factors in increasing antimicrobial resistance rates

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Background: Aim of this study was to investigate the risk factors associated with high antibiotic resistance rates belong to Gram positive and negative organisms isolated in 2010 using epidemiological analysis.
Methods: A case–control study of 72 patients with Extended Spectrum β Lactamase producing organisms and 33 patients with Amp C producing organisms, 222 patients with non-ESBL organisms and 75 patients with Gram positive organisms was conducted in a subsidial care hospital of Çanakkale province. Demographics, co-morbidities, antibiotics usage were analysed for all patients.

Results: ESBL and AmpC (+) producing microorganisms were more isolated from patients that belong to over 65-year age and 0–1 age groups (p:0,005). The risk factors including hospitalisation (OR:3,1; 1,19–8,1; p:0,018), co-morbidity (OR:2,1; 1,12–7,3, p:0,024), were found higher in ESBL (+) group compared to ESBL(–) group. Hospitalisation at last three months was found as a risk factor for resistance to any 3rd generation cephalosporins (OR:2,4; 0,9–6,3; p:0,04). There was a correlation between ESBL (+) production and resistant to many antibiotics that are generally used for ampiric treatment ($p < 0,05$, Pearson correlation r: 0,78), except for piperacillin-tazobactam ($p > 0,05$). ESBL production was significantly higher in *K. pneumoniae* (p: 0,0005), *E. cloacae* (p:0,001), *E. sakazakii* (p:0,001) and *A. baumannii* (p:0,005) strains more than other Gram negative strains revealed in Table-1. Hospitalisation was single significant risk factor for patients with MRSA (OR:1,8, 0,7–1,2, p:0,005; Table-1). Vancomycin resistant *Enterococcus* was not isolated.

Discussion: Hospitalisation and co-morbidity causing to colonisation and impairment on immunity are the most important risk factors for ESBL producing microorganisms related infections as our study revealed. Although antibiotic usage including 2nd, 3rd generation cephalosporins and quinolones was cited as a risk factor for ESBL related infections, it was not found significant statistically in our study. Piperacillin-tazobactam could be thought as an option to treat the resistant Gram negative infections associated with *K. pneumoniae*, *E. cloacae*, *E. sakazakii* and *A. baumannii* whose ESBL rates are frequently higher in our local settings but carbapenems, should be opted for serious patients with co-morbidites. Consequently, immunity and infection control programs are important factors against increasing antibiotic resistance rates.

Table 1. Characteristics of patients and isolated microorganisms

	ESBL(+) Group, n: 72	AmpC(+) group, n: 33	ESBL and AmpC (–) Gram negative Group, n: 222	Gram positive group, n: 48	Total, n: 375
Female/Male	42/30	18/15	150/72	33/42	243/159
AGE					
Median	1	6	1	3	1
Range	0–78	0–69	0–77	0–78	0–78
0–1 age	24	9	93	33	159
1–5 age	18	6	30	9	63
5–15	6	3	42	6	57
15–65	9	6	39	24	78
>65	18	9	12	9	48
SAMPLES					
Urine	51	21	68	54	194
Sputum	6	3	12	–	21
Blood	3	3	–	6	12
Wound	3	3	3	6	15
Conjunctiva	–	–	–	15	15
Catheter	6	3	–	–	9
Abscess	–	–	3	–	3
CO-MORBIDITY					
Urinary system abnormality	9	6	45	9	69
Chronic Obstructive Lung Disease	6	6	12	9	33
Diabetes mellitus	–	–	12	9	21
Chronic Renal Failure	–	–	6	6	12
Catheter	–	–	6	6	12
MICROORGANISMS					
E.coli	18	9	126		153
K.pneumoniae	12	3	30		45
Enterobacter cloacae	15	9	15		39
Enterobacter sakazakii	6	6	–		12
Pseudomonas aeruginosa	–	–	9		9
Enterobacter aerogenes	3	–	3		6
Citrobacter freundii	6	6	6		18
Morganella morganii	3	–	3		6
Procidencia stuartii	–	–	3		3
Acinetobacter baumannii	3	–	–		3
Enterobacter gergoviae	–	–	6		6
Klebsiella oxytoca	–	–	24		24
Enterococcus faecalis	–	–	–	33	33
Enterococcus faecium	–	–	–	3	3
Methicillin resistant *S.aureus*	–	–	–	3	3
Methicillin susceptible *S.aureus*	–	–	–	9	9
Methicillin resistant coagulase negative staphylococci	–	–	–	15	15
Methicillin susceptible coagulase negative staphylococci	–	–	–	18	18
Antibiotic usage (at last 3 months)					
Cefixime	45	18	123	42	228
Ceftriaxone	33	15	78	18	144
Amoxicillin-clavulanate	57	30	150	54	291
Amoxicillin	63	33	156	48	300
Cefuroxime	15	9	51	15	90
Quinolones	12	9	15	6	42
Hospitalisation	51	15	84	51	186
Resistance to any 3rd-generation cephalosporin	19	16	15		50

R2600 Infections in patients with traumatic brain injury: a 7-year retrospective study

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Objective: Infections in patients with traumatic brain injury (TBI) are associated with prolonged hospitalisation and adverse outcomes. The acknowledgement of the pre-disposing risk factors may help decrease the morbidity and mortality. We conducted a retrospective cohort study to determine the incidence, bacteriology and risk factors for development of infection after TBI.

Methods: The records of patients >18 years old, admitted with head injury in Crete Univ Med Ctr between 1999 and 2005 were abstracted. Data were analysed with SPSS.

Results: 760 patients (75% men, median age 41) were analyzed. Two hundred fifty eight patients (33% of the admissions) underwent 342 surgical procedures. Burr-hole was the most common procedure (29.2%). The median duration of surgery was 2 hours. In 23% of the patients there was some kind of drain inserted.

Two hundred fourteen infections were observed. The majority were infections of the lower respiratory tract (47%), mainly ventilator associated pneumonia (VAP) (33%), followed by surgical site infections (SSI) (17%). Fifty three of the admissions (6.3%) were complicated with at least one SSI, superficial or deep. The most common SSI was wound infection (2.2% of the cohort).

The patients who underwent neurosurgery had a lower Glasgow Coma scale (GCS) on admission, they were more prone to be admitted to the Intensive Care Unit and bear drains. They were also more prone to develop meningitis, SSI and had increased mortality.

Multivariate analysis showed that SSI development was independently associated with performance of >2 procedures ($p < 0.001$), presence of concomitant infections, namely VAP ($p = 0.004$) and urinary tract infections ($p = 0.001$), insertion of lumbar ($p = 0.002$) and ventricular drains ($p = 0.050$) and cerebrospinal fluid (CSF) leak ($p = 0.050$). Meningitis was independently associated with prolonged hospitalisation ($p = 0.001$) and insertion of lumbar ($p < 0.001$) and ventricular drains ($p = 0.0017$).

There was a predominance of *Acinetobacter* spp as a VAP pathogen (38%) whereas Gram(+) organisms (especially coagulase-negative staphylococci) remained the most prevalent in SSI (53%).

Conclusions: Respiratory tract infections were the most common among patients with head injury. Device-related communication of the CSF with the environment and prolonged hospitalisation were independent risk factors for SSI and meningitis. The prevalence of the pathogens must be determined upon institutional basis for the establishment of proper treatment of these life threatening infections.

R2601 Prevalence of hospital-acquired infections at Duzce University Hospital, Turkey

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Objectives: Rates of hospital-acquired infections have varied between 5 and 10 episodes per 1000 hospital admissions and continue to be a major problem, causing high morbidity, mortality and significantly increasing the length of hospitalization and cost of treatment. The purpose of this study was to evaluate the hospital-acquired infections at Duzce University Hospital, in Turkey.

Methods: The study was carried out at the Duzce University Hospital (350-bed). 2009 prevalence of hospital-acquired infections in our hospital was prospectively evaluated. A total of 11333 inpatients were searched.

Results: During one year follow up period, 384-hospital-acquired infection episodes were detected in hospitalized patients ($n = 11333$). The prevalence rates of patients with hospital-acquired infections were 3.4%. The incidence density was 5.2/1000 patient days. The most common hospital-acquired infections by primary site were pneumonia (47.1%), followed by urinary tract infection (19.5%), surgical site infections

(16.9%), bacteremia (14.8%), and other infections (1.6%). Hospital-acquired infections were seen frequently in the department of neurology (7.7%), internal medicine (3.3%), neurosurgery (2.9%), general surgery (2.5%), urology (2.4%), orthopedics (1.9%), infectious disease (0.5%), pediatrics (0.5%). Hospital-acquired infection rate in intensive care units was 26.2%. The most prevalent microorganisms were *Pseudomonas* spp. (33.5%), *Escherichia coli* (14.5%), *Staphylococcus aureus* (12.6%), *Acinetobacter* spp. (9.5%), coagulase-negative staphylococci (8.1%), *Enterobacter* spp (7.2%), *Klebsiella* spp. (6%), *Enterococcus* spp. (3.1%), *Stenotrophomonas* spp. (2.3%), and other microorganisms (3.1%).

Conclusion: Hospital-acquired infections in inpatients should be screened by proper nosocomial infection control programs. The clinical and microbiological monitoring of hospital-acquired infections for nosocomially infected patients should be necessary. The routine application of standard infection control practices may reduce the incidence of hospital-acquired infections.

R2602 Twelve-year survey of severe *Candida* infections; results from the Greek participation in the ARTEMIS global antifungal surveillance study

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Objectives: Greece has been participating in the ARTEMIS global surveillance programme since 1997 through October 2009, in order to study the epidemiology of *Candida* strains in our hospital and monitor their susceptibility to fluconazole and voriconazole using a low cost, reproducible and accurate standard in vitro test.

Methods: Strains isolated from patients with severe *Candida* infections from all body sites and all hospital locations were collected, identified and analyzed. Susceptibility was investigated with disk diffusion testing according to CLSI M44 method. Test results were read, interpreted and recorded with the Biomic plate reader system.

Results: A total of 1461 *Candida* strains were recorded. Most prominent species was *C. albicans* (63.6%), with a year-frequency ranging from 52 to 75%, followed by *C. glabrata* (14.5%, range 6.9–23.5%) and *C. parapsilosis* (7.2%, range 0.9–9.6%, except for the years 2007 and 2009 in which a 24.1% frequency was observed). All other species together had a frequency of 5–24%.

The sensitivity of *C. albicans* to fluconazole equalled that to voriconazole (92.5% versus 92.6% of strains respectively). For the *C. glabrata*, the respective percentages were 72.4 and 75 and for the *C. parapsilosis* 85.7 and 92.8. Most of the resistant strains of all species were isolated from miscellaneous body fluids, the upper respiratory tract and blood and derived from the ICU and the surgical wards. During the years 2006–2009, the *C. albicans* strains resistant to fluconazole were 13.1% and to voriconazole 14.3%. In the previous years only 5% and 4.4% were resistant, respectively. A comparable increase in resistant strains was noted also for *C. glabrata* (47.8% versus 33.4% in previous years to fluconazole and 43.9% versus 16.9% to voriconazole) and *C. parapsilosis* (11.2% versus 6.6% and 4.6% versus 2.3% respectively). The remaining species did not show increasing rates.

Conclusions: Of the *Candida* species isolated in our hospital, the most prominent was *C. albicans*, followed by *C. glabrata* and *C. parapsilosis*. The frequency of isolation of these strains varied from year to year without a clear trend. However, an increase in strains resistant to fluconazole and voriconazole was observed in the last four years.

These results confirm the need for monitoring the local epidemiology and antifungal susceptibility of *Candida* strains in order to optimize the empirical or targeted therapy, especially in critically ill patients.

Travel medicine, tropical and parasitic diseases

R2603 Ruptured hydatid cyst following minor head trauma and few signs on presentation

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Introduction and Aim: Hydatid cyst is an infection of Echinococci and still represents a serious problem in endemic regions, especially in the Middle East, Mediterranean countries and Australia. Rupture of a hydatid cyst commonly gives rise to allergic phenomena, including anaphylactic shock. Rupture of cyst can occur spontaneously or during surgery as well as due to trauma. We describe a patient who after trauma presented to the emergency department with scant physical signs.

Case report: A 17-year-old girl was admitted to our emergency department after falling down of motorcycle, 15 to 20 minutes previously. She was only complained of pain of her head and she had two minor traumas on her face. On presentation, her Glasgow Coma Scale was 15, blood pressure 120/80 mmHg, pulse rate 110 beats per minute, temperature was 36.8ºC and her physical examination was normal except for mild abdominal tenderness. There was no evidence of any other injury and the secondary survey was otherwise normal. White blood cell count was 16,400/mm^3, eosinophilia was absent and the other laboratory tests were within normal limits. She underwent head and neck computed tomography without sciagraphic fluid and during examination general urticaria developed and it was progressive. The examination stopped and fluid resuscitation, methyloprednisolone and diphenylhydramine were administered intravenously. Once her condition had stabilized she underwent abdomen computed tomography to determine the cause of the abdomen pain. There was no free fluid which usually excludes intra-peritoneal bleeding. There was one ruptured cyst on the right lobe of the liver and another cyst measuring 104 mm with thin wall on the left lobe of the liver. The patient turned to have a rupture of a hydatid cyst and she was immediately taken to the operation room by the general surgeons. Drainage and capitonage of the cysts were performed. The patient was treated with albendazole for three months and at six months' follow-up she remained well without any complications.

Conclusion: Rupture of hydatid cyst is rare and can occur spontaneously following serious injuries or even minor trauma. Anaphylaxis is the most frequent cause of death in cases of hydatid cyst rupture. *Echinococcus* liver cysts should be suspected in cases of anaphylaxis of uncertain etiology. Acute vascular collapse, generalized cutaneous erythema, urticaria and edema are suggestive of anaphylaxis from hydatidosis, especially in endemic areas.

R2604 Travel: health risk perception, knowledge and prophylaxis practices among immigrants who travel to visit friends and relatives

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Introduction: Travelers visiting friends or relatives (VFRs) typically demonstrate travel and behavioural patterns which render them at high risk for acquisition of preventable infection. Pre-travel services are rarely sought by VFRs, whereas misconceptions that they possess life-long immunity against malaria make them less likely to receive or adhere to antimalarial chemoprophylaxis recommendations.

Objective: We analyzed the adherence of this group against these measures of prophylaxis and health problems resulting from the travel.

Methods: All VFRs travellers attended in a tropical medicine referral unit between 2007–2010 were interviewed with a questionnaire investigating use of malaria prophylaxis, vaccination, and knowledge of dietary and hygiene measures before and during the travel. VFR was defined as all immigrants permanently established in Spain who temporarily travelling to their place of origin.

Results: We analyzed 61 immigrants (mean time of travel: 91 days). They travelled to Senegal (33%) Equatorial Guinea (25%), Ecuador (18%), Bolivia (8%), Venezuela (5%), Brazil, Cameroon and Colombia (3% each) and Ethiopia (2%). Only 23% patients received health's advice before travel, 72% of travellers didn't received any vaccine and 78% did not carry out antimalaria chemoprophylaxis. Only 25% used safe water. No one use repellents. Twenty-eight patients (46%) were to be illness to return home. Twenty-six patients had diarrhea during the trip and 20% fever, being the most frequent reasons for consultation. In 32% of cases the diarrhea was due to parasitic infections. Eight patients had malaria, in 6 cases due to *P. falciparum* and two to *P. vivax*.
Conclusions: VFRs are a group of high risk for imported disease due to poor adherence to vaccination and prophylaxis measures.
At present, immigrants visiting friends and relatives (VFRs) constitute the most significant group of travellers for malaria importation in developed countries, with sub-Saharan Africa destinations carrying the highest risk. There is an urgent need to increase health's travel advice in this group of travellers.

R2605 Trichinellosis in Serbia – influence of early diagnosis and treatment on outcome and complications

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Objectives: Trichinellosis represents acute infective disease caused by tissue nematodes *Trichinella* species. Despite the reduction of the incidence of the disease resulted from obligatory meat check, this disease still represents significant medical and social–epidemiological phenomena. The goal of the study was to show epidemiological and clinical features of patients suffering from trichinellosis, and to investigate possible influence of early diagnostic and begining of the treatment on the outcome of the disease.
Methods: Descriptive-analytic retrospective study included 44 patients with trichinellosis, treated at the Clinic for Infectious and Tropical Diseases, Clinical Center of Serbia, Belgrade, from 2006 to 2010. Statistical data analysis used descriptive and and analytic methods – Mann–Whitney U test, Wilcoxon signed-rank test and Spearman's rank correlation coefficient. The value of $p < 0.05$ was considered statistically significant.
Results: Within 5 years 44 patients were treated with trihinellosis. Around 2/3 (68.18%) of them were men, average age being 41.18±14.51, mostly from urban region (65.9%). Source of infection was known in 95% of the cases, out of which in 90% the cause was found to be with domestic animals. Duration of the disease before making diagnosis was on the average 11.5±6.55 days, and duration the hospitalisation lasted 16.5±8.1 days. There were no lethal outcomes, the disease have ended well with 39 patients, while 5 patients (11.36%) had a disease complications-two of them hepatitis, one neurotrichinellosis with neurological consequences, while two patients had myocarditis (one of them developed deep venous thrombosis). Comparing groups of patients with or without complications, the statistically significant difference was shown in time between the intake of food and the appearance of symptoms ($p = 0{,}461$). Duration of the disease before the diagnostic and treatment had no effect on the frequency of complications ($p = 0{,}643$), while there was statistically significant correlation between the duration of the sickness before the diagnosis was made and duration of the hospitalisation ($p < 0.05$).
Conclusion: Although trihinellosis is a rare disease, it still represents a significant medical problem which we need to be reminded about. Timely diagnosis and beginning of treatment affects the shortening of the hospitalisation and time of recovery, but has no influence on the frequency of complications.

R2606 Clinical and epidemiological characteristics of imported infectious diseases in immigrants

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Spain could be a potential area in Europe for the development and spread of emerging diseases from the tropics due to its characteristics, and their important migratory flows We analyzed clinical-epidemiological characteristics of infectious diseases imported by immigrants from developing countries in the north of Spain.
Methods: A retrospective descriptive study of 341 immigrants from developing countries who live in Spain was conducted. Demographic data, details of country of origin, time on Spain, clinical syndromes, and diagnoses were analyzed.
Results: The countries of origin were Ecuatorial Guinea (28%), Senegal (21%), Ecuador (10%), Bolivia (7%), Nigeria (6%), Guinea-Conakry (4%), Marruecos (4%), Brazil (3%). The mean time of Spain was 910 days. Most common syndromes were abdominal pain (25%), diarrhea (21%), cutaneous syndrome (10%) and fever (7%). Most frequent diagnoses were intestinal parasites (48%), filariasis (17%), Chagas disease (5,5%), malaria (4,5%). and schistosomiasis (3.5%). The most frequent intestinal parasites were: *Entamoeba histolytica* (22%), *Strongyloides* spp (12.6%), *Trichuris trichiura* (10%) and *Uncinaria* spp (5%). 70% of patients had one parasite, 17% two parasites, 9% three parasites and 4% 4 or more parasites. 69,8% of patients were from Subsaharian Africa. All patients diagnosed of filariasis were from Ecuatorial Guinea. The most frequent were *M. perstans* (70%), filariasis *Loa loa* (20%) and *Onchocerca volvulus* (10%).
Conclusions: Increased migratory flows is a key factor for the development and spread of emerging pathogens. Information on these diseases is essential to early diagnostic and treatment. Intestinal parasites were the most frequent disease following to filariasis, malaria and Chagas disease.

R2607 Determination of visceral leishmaniasis reservoir dogs epidemiology with real-time PCR method

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Objectives: World Health Organization (WHO) has determined that Leishmaniasis is considered one of the most important zoonotic diseases. Leishmaniasis consists of four main clinical syndromes: cutaneous leishmaniasis; muco-cutaneous leishmaniasis; visceral leishmaniasis (VL) and post-kala-azar dermal leishmaniasis. Each year, 500.000 new cases of visceral leishmaniasis (VL) are reported worldwide. VL is a systemic disease that is fatal if left untreated and is caused by *Leishmania infantum* in Europe. VL is endemic along the tropical and subtropical regions in the world while in Turkey VL is endemic along the Aegean and Mediterranean coasts, it occurs sporadically in other regions. In epidemic Mediterranean countries, prevalence is changing between 1–37% percentages. According to data from the Turkish Ministry of Health annually 40 new cases of VL have been reported.
A relationship between canine leishmaniasis (CanL) and human visceral leishmaniasis has been detected. Transmission of CanL is from animal to vector to human. Humans are occasional hosts and dogs, are the reservoir of *L. infantum*. In this research, dogs in two rehabilitation and treatment centers of Istanbul Metropolitan Municipality examined with Real Time PCR assay.
Methods: 4–5 ml of venous blood samples were collected into EDTA tubes from 93 dogs and taken to the Microbiology and Clinical Microbiology Department. Qiagen™ (Germany) Dneasy Blood&Tissue Kit was used for DNA extraction. Parasite DNA was examined by using PrimerDesign™ (UK) *Leishmania infantum* Real Time PCR pathogen detection kit.
Results: Bloods from 93 dogs in two rehabilitation and treatment centers of Istanbul Metropolitan Municipality examined that whether they carry parasites or not with the Real Time PCR assay. Two dogs (2.15%) had a positive *L. infantum* DNA.

Conclusion: Leishmaniasis is the most important zoonotic diseases in Turkey. Transmission of *L. infantum* is from dog to vector to human. The percentage of positive dogs of our study is in accordance with previous studies performed in Turkey. Since a high proportion of infected dogs are asymtomatic, diagnosis of *Leishmania* infections is an serious problem. The parasitological methods present complex interferences, such as NNN culture has a low sensitivity and seeing the parasites fixed in glass slides after Giemsa staining is difficult. Therefore Real Time PCR is effective for especially differential diagnosis of sub-clinical and asymptomatic Dogs.

R2608 Clinical characteristics and aetiology of traveller's diarrhoea among Korean travellers visiting South-East Asia

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The morbidity of travelers' diarrhea (TD) is still high. This study examined the incidence of common pathogens and characteristics of TD among Korean travelers who visited South-East Asian countries. We performed a prospective study involving 479 Korean travelers with diarrheal disease from February 2009 to April 2009 and stool samples were examined and questionnaires were done after arrival. Enterotoxigenic *Escherichia coli* (ETEC) was found in 36.0% of TD cases, as were the following: Enteroaggregative *Escherichia coli* (EAEC) in 27.0%, *Vibrio parahaemolyticus* in 13.1%, and Norovirus in 11.5%. The detected rate of classic TD was higher in men, in patients who had a shorter duration trip and in patients who drank more than 1 liter of water per day ($P = 0.007$, $P = 0.023$, and $P = 0.037$, respectively). Positive stool culture rates were higher in men, in hospitalized patients, and in those who consumed impure water or raw foods ($P = 0.005$, $P = 0.013$, and $P = 0.033$, respectively). A higher severity of disease corresponded to a significantly higher culture positivity rate ($P = 0.029$). We should consider the possibility of other pathogens in addition to ETEC in patients with TD who visit South-East Asia and travelers need to educate about risk factors associated with TD.

R2609 The aetiology of diarrhoea among primary refugee arrivals in Timis County, Romania

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Objectives: Diarrhea is an important cause of morbidity and mortality worldwide, although its etiology remains unknown in many cases. The present study aimed at establishing the etiological spectrum of pathogens causing diarrhea in a group of refugee arrivals to Center for Immigrants from Timisoara, Romania.
Methods: There were retrospectively analyzed the medical records of 68 immigrants hospitalized with diarrhea at Clinic of Infectious Diseases in Timisoara during 2008–2009. Diagnosis was established based on the epidemiological aspects (the acute onset following collective or individual consumption of food with inadequate physical and organoleptic properties, stored in poor conditions or even expired), physical examination (fever, repeated shivers, nausea, vomiting, headache, multiple watery diarrhea, fetid stools, tenesmus, abdominal colic, pyrosis, dizziness, paresthesias of lower limbs), laboratory tests (erythrocyte sedimentation rate, blood counts, fibrinogen, C reactive protein, glycemia, blood cultures, lingual swabs, throat swabs, stool cultures, stool parasitological examinations, natremia, kalemia, calcaemia) and other medical explorations (abdominal ultrasound, electrocardiography). Data of the epidemiological survey were collected from the Institute of Public Health from Timisoara. The data was statistically processed using Epi Info 3 software.
Results: Of the total number of patients, 28 (44.1%) were diagnosed with acute enterocolitis, 5 (7.4%) had dysentery, 16 (23.5%) had foodborne, 14 (20.6%) were diagnosed with giardiasis and 3 (4.4%) had intestinal amoebiasis. The etiologic agent was established in 40 (58.8%) cases as follows: *Staphylococcus* spp. (5 cases), *Salmonella* spp. (8 cases), *Shigella* spp. (5 cases), enterotoxigenic *Escherichia coli* (5 cases), *Entamoeba histolytica* (3 cases), and *Giardia lamblia* (14 cases). Most of the patients (86.8%, $p < 0.0001$) required endovenous hydroelectrolytic replacement with glucose, normal saline and lactated Ringer solutions, along with symptomatic medication (analgesics, antipyretics, antispasmodics, antacids). Twelve cases received antibiotics therapy prior to hospital admission, thus complicating the detection of the etiologic agent. All patients had favorable outcomes.
Conclusion: Knowledge of the etiology of diarrhea among immigrants patients allows the proper implementation of targeted therapy and prophylaxis measures in order to limit diarrheal morbidity in persons at risk.

R2610 Clinical and epidemiological characteristics of scabies in hospitalised patients with infectious diseases

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Objectives: Scabies is a highly contagious parasitic dermatosis with a worldwide distribution that may cause significant morbidity and large nosocomial outbreaks. The present study aimed at analyzing the clinical and epidemiological characteristics of scabies in hospitalized patients with infectious diseases.
Methods: Data were retrospectively collected from the medical charts of 62 patients found with scabies lesions at Clinic of Infectious Diseases, Timisoara during 2009. The positive diagnosis was established based on the epidemiological aspects (recent travels, family or collective outbreaks, sex with multiple partners), physical examination (lesions such as papules, pustules, burrows, nodules, and occasionally urticarial papules and plaques with patognomonic distribution: interdigital web spaces of the hands, perimalleolar and periumbilical region, ribs, horizontal gluteal crease, radiocarpal joint) and intense generalized skin itching at night. The diagnosis was confirmed either by microscopic identification of mites in skin scrapings or by dermoscopy. Patients followed treatment with precipitated sulfur apply topically to entire trunk and extremities every 24 hours for 3 days. Disinfection was carried out at all linen and personal items.
Results: Of the study group, 40 (64.5%) patients were rural inhabitants and 22 (35.5%, $p = 0.002$) lived in urban areas. Six familial outbreaks occured in rural regions. Scabies was mainly associated with respiratory diseases (22.6%), liver diaseases (19.4%), intestinal diseases (12.9%), psychiatric disorders (16.1%), urinary infections (9.7%). Scabies lesions had the following patterns: edematous and urticariform papules (26 cases), papulo-vesicular lesions (22 cases), escoriations due to scratching (48 cases), and scratching related superinfected lesions with streptococcal or staphylococcal bacteria (35 cases). Common form of scabies was evidenced in 24 patients (38.7%) whereas 38 cases (61.3%) had atypical course of the disease ($p = 0.02$). All patients had favourable outcomes following therapy with precipitated sulfur associated with symptomatic medication (calcium, antihistamincs, antibiotics in bacterial superinfections), individual hygiene and disinfection.
Conclusion: The study of clinical and epidemiological characteristics of scabies in hospitalized patients with infectious diseases allows early diagnosis of this parasitosis as well as timely implementation of effective prophylactic measures.

R2611 *Cryptosporidium*: the role of speciation in understanding epidemiological trends

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Background: *Cryptosporidium* sp taxonomy has gone through a number of changes in the last three decades. Initially a single species, C parvum – with a number of clonal genotypes loosely corresponding to specific vertebrate hosts, had been proposed. The current nomenclature suggests, for all cryptosporidial organisms with an oocyst size of 4–6 um, a number of cryptic species that are identified based on

molecular genotyping. This is believed to facilitate better understanding of transmission and other epidemiological factors.
Objectives: This study was designed to assess the value, to the clinician and the epidemiologist, of the new cryptic species and whether this information would improve practice.
Methods: Isolates that were positive for *Cryptosporidium* oocysts by auramine staining were typed by PCR. The resulting molcular speciation was then analysed with clinical data (age, travel history, contact with animals, sporadic or part of an outbreak).
Results: In this study 27 isolates were typed, 13 *C. parum*, 12 *C. hominis*, 1 *C. meleagridis* and 1 *C. felis*. There were some trends. Two isolates from Egypt and 1 from Greece were identified as *C. hominis*, while isolates from Pakistan and St Lucia were identified as *C. parvum*. This may represent epidemiological differences between the various travel desinations. One patient has both *C. parvum* and *Shigella sonnei* isolated, this would be strongly suggestive of a contaminated water source of infection. A second patient had both *C. felis* and *Campylobacter* sp isolated. This patient had recently acquired a kitten. It is possible that both pathogens contributed to the diarrhoea, or that the *Campylobacter* was the cause of the diarrhoea while the presence *C. felis* oocysts (a primary pathogen of feline hosts) may represent oocysts passing through the patient's gut rather than tissue invasion.
Conclusions: Identification of cryptosporidial isolates either by genotype or as one of the cryptic species allows better understanding of potential source. Further studies are needed to confirm the trends seen in this study.

Resistance and mechanisms of action of antifungals

R2612 Evaluation of the Neo-Sensitabs diffusion method for determining the antifungal susceptibilities of *Candida* species

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Objectives: The purpose of this study was to compare the Neo-Sensitabs method with the CLSI reference broth microdilution (document M27-A3, S3) method for testing the susceptibility of 98 *Candida* spp. isolates to five antifungal agents (amphotericin B, fluconazole, itraconazole, ketoconazole, and voriconazole).
Methods: In this study, the broth microdilution method was performed according to the CLSI recommendations. The Neo-Sensitabs method was performed according to the manufacturer's instructions (Neo-Sensitabs; A/S Rosco Diagnostica, Taastrup, Denmark). *C. parapsilosis* ATCC 22019 was used as quality control strain.
Results: A total of 98 isolates of *Candida* spp.[*C. albicans* (n = 56), *C. tropicalis* (n = 22), *C. parapsilosis* (n = 6), *C. glabrata* (n = 4), *C. dubliniensis* (n = 3), *C. krusei* (n = 3), *C. famata* (n = 2), *C. lusitaniae* (n = 1), *C. pelliculosa* (n = 1)] were evaluated by both of the methods. MIC ranges were found as 0.03–8.0 μg/ml, 0.05–64 μg/ml, 0.03–0.5 μg/ml, 0.03–1.0 μg/ml, and 0.03–0.12 μg/ml for amphotericin B, fluconazole, itraconazole, ketoconazole, and voriconazole for the microdilution method respectively. All isolates were susceptible to voriconazole by both of the methods. Ninety one (93%) of all isolates were determined as susceptible, two (2%) (*C. albicans*) were found susceptible dose dependent (SDD), and five (5%) [*C. krusei* (n = 3), *C. glabrata* (n = 1), *C. albicans* (n = 1)] were found resistant to fluconazole by both of the methods. Ninety two (94%) of all isolates were determined as susceptible, six (6%) [*C. krusei* (n = 3), *C. albicans* (n = 3)] were found SDD to itraconazole by both of the methods. There was 100% agreement between two of the methods for these three antifungal agents in this study. Two (2%; *C. albicans*, *C. glabrata*) and eight [8%; *C. albicans*(n = 3), *C. krusei*(n = 3), *C. glabrata*, *C. tropicalis*] isolates were determined as resistant to amphotericin B and ketoconazole by both of the methods respectively. One (*C. albicans*) and three [*C. albicans* (n = 2), *C. glabrata*] isolates were intermediate to amphotericin B and ketoconazole by Neo-Sensitabs method, but the same strains were susceptible to these antifungals by standard method. In this situation minor errors were calculated 0.01% for amphotericin B, and 0.03% for ketoconazole by Neo-Sensitabs method.
Conclusion: The Neo-Sensitabs method may be an alternative and easy method for the clinical laboratories to determine the susceptibility of yeast isolates.

R2613 Fungistatic and fungicidal activity of antifungal agents against *Candida* spp. strains isolated from patients with invasive candidiasis

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Objectives: To study the activity of antifungal agents against *Candida* spp strains isolated from invasive candidiasis and to assess the fungicidal activity of amphotericin B, caspofungin and anidulafungin. Fungicidal activity of azoles was not studied because they are considered fungistatic.
Methods: All the strains of *Candida* spp isolated from invasive candidiasis in Pisa Hospital in the 2009–2010 period were studied. MICs of all strains were performed with the Sensititre method under manufacturer instructions. All the wells without growth of yeast were sub-cultured in order to measure the fungicidal activity of amphotericin B, caspofungin and anidulafungin. Minimal fungicidal concentration (MFC) of these drugs was defined by a 99,9% reduction of the inoculum.
Results: Thirty-three *Candida* spp isolates were included in this study. Thirty strains were isolated from blood, 1 from liver abscess, 1 from vertebral osteomyelitis, 1 from peritonitis. Among these strains, *C. albicans* were 17 strains and *Candida* non-*albicans* 16 strains (7 *C. parapsilosis*, 4 *C. glabrata*, 3 *C. tropicalis* and 2 *C. krusei*). MIC_{50} and MIC_{90} for all strains are shown in table 1. All antifungal agents, except amphotericin B have an increase of MIC_{90} for *Candida* non-*albicans* strains, with an increase of several dilutions with respect to those of *C. albicans* strains. MFC50 and MFC90 are shown in table 1. MFC50 and MFC90 of amphotericin B were 2 mg/L and between 4 and 8 mg/L, instead values for echinocandins were superior especially for *Candida* non-*albicans*.
Conclusion: *Candida* non-*albicans* strains have elevated MICs for all antifungal agents. Amphotericin B fungicidal activity is superior to that of echinocandins, especially among *Candida* non-*albicans* strains. If this activity might be used in the clinical ground has to be studied.

Table 1. Minimum inhibitory and fungicidal concentrations (mg/L) of antifungals (MICs and MFCs) vs *Candida albicans* and non *albicans*. U.O. Malattie Infettive, Pisa, Italy, 2009-2010

	Candida albicans				Candida non albicans			
	MIC_{50}	MIC_{90}	MFC_{50}	MFC_{90}	MIC_{50}	MIC_{90}	MFC_{50}	MFC_{90}
Anidulafungin	0.003	0.06	2	16	0.06	1	8	32
Caspofungin	0.016	0.094	4	32	0.125	0.5	16	32
Fluconazole	0.5	1			2	128		
Itraconazole	0.06	0.25			0.25	0.5		
Voriconazole	0.003	0.008			0.12	1		
Posaconazole	0.008	0.06			0.06	1		
Amphotericin B	0.5	0.5	2	4	0.25	1	2	8

R2614 Influence of anidulafungin or voriconazole on biofilms of various *Candida* species in a small tube system of continuous flow cultures

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Objectives: We investigated the antifungal effect of anidulafungin (AND) or voriconazole (VRC) on biofilms and MIC values of different *Candida* species in a small tube system of continuous flow culture.
Methods: We carried out 21 experiments with 23 strains. Strains of *C. albicans* (Ca), *C. glabrata* (Cgl), *C. tropicalis* (Ct), *C. krusei* (Ck), *C. parapsilosis* (Cp), *C. orthopsilosis* (Co), *C. metapsilosis* (Cm), *C. lusitaniae* (Cl) and *C. guilliermondii* (Cgui) from blood culture isolates were grown in small tubes (V = 0.48 ml). Peptone-yeast extract (0.5%/0.2%) + 50 mM glucose±AND (8 mg/l) or VRC (16 mg/l) was supplied at very low flow rates (1.3 ml/h). The flow in the tube was

63-times within 24 h. Biomass production, glucose concentration as metabolic activity, pH and planktonic CFUs were measured. MIC testing was performed by Etest® on RPMI. Morphology of adherent fungal cells was assessed microscopically after staining with lactophenol cotton blue.
Results: In the tube system the development of biofilm varied from species and strains and took place after 2 or 3 days. After continuous input of AND the production stopped in case of sensible species like Ca or Ct, but the metabolic activity measured by glucose concentration did seldom reach the beginning concentration. This could partly be shown after continuous input of VRC. Different strains of Cp showed different results against AND, but there was no increase of resistance in these long term trials up to 10 days. Biofilm of strains with a low susceptibility against AND like some Cp strains or candidaemia strains of Cl or Cgui stopped growth after input of VRC as also metabolic activity did so.
Conclusion: The small tube system is a model to get some information from biofilms of different *Candida* strains under conditions of long term cultivation with supply of low nutrition more similar to real conditions than batch culture.

R2615 Nosocomial candidaemia: epidemiology and antifungal susceptibility patterns at the University Hospital of Modena

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Objective: Aim of the present study was to evaluate the incidence of candidemia in an Italian tertiary care hospital, and the antifungal susceptibility patterns of isolates of *Candida* spp. The antifungal agents tested were fluconazole, itraconazole, voriconazole, posaonazole, amphotericin B, caspofungin, anidulafungin and micafungin.
Materials and Methods: A retrospective, observational study was carried out at the University Hospital of Modena. Data were collected from January, 2007 to July, 2010. Candidemia was defined as the detection of at least two consecutive positive blood cultures yielding *Candida* spp. during the same hospital admission. MICs were determined according to the CLSI guidelines (M27A-3 protocol).
Results: During the study period a total of 142 episodes of candidemia occurred in 129 patients, accounting for a global incidence of 12.1 episodes/10,000 in-patients. Median age of the patients was 56 years and 58% of them were males. The most common species isolated was *C. albicans* (79 episodes, 55.6%), followed by *C. parapsilosis* (26 episodes, 18.3%), *C. glabrata* (18 episodes,12.7%), *C. tropicalis* (10 episodes, 7.0%), *C. guilliermondii* (4 episodes, 2.8%), *C. krusei* (3 episodes, 2.1%), *C. dubliniensis* and *C. norvegensis* (1 episode, 0.8%). During the study period the incidence of candidemia increased from 7.1 to 18.5 episodes/10,000 in-patients ($p < 0{,}01$). *C. glabrata* and *C. krusei* fungemia occurred more frequently in patients admitted to intensive care units ($p = 0.04$). All the isolates resulted susceptible to voriconazole, posaonazole, amphotericin B and to the three echinocandins The susceptibility rate of fluconazole and itraconazole was 99.3 and 72.5%, respectively. The antifungal agents tested had the following MICs90: fluconazole 8 μg/dl, itraconazole 2 μg/dl, μg/dl voriconazole 0.125, μg/dl posaconazole 0.5, amphotericin B 1 μg/dl, caspofungin 0.5 μg/dl, anidulafungin 2 μg/dl, and micafungin 2 μg/dl.
Conclusions: Candidemia is still a frequent complication among hospitalized patients. We observed a progressive increase during the study period; this trend could be due to the increase of immunocompromised patients admitted to our institution, in particular hematological patients, solid organ transplant recipients and surgical patients admitted to intensive care units. Nevertheless, *C. albicans* remained the prevalent species and the resistance rates to antifungal agents were very low.

Fungal infections

R2616 Candidaemia in adult non-neutropenic intensive care unit patients in 2 Turkish university hospitals: factors associated with non-*albicans Candida* spp. and antifungal susceptibility patterns

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Objectives: The objective of this prospective, observational, multicenter study was to describe factors associated with bloodstream infections (BSIs) with *Candida* non-*albicans* (NAC) species, compared with *Candida albicans* BSIs, and antifungal susceptibility patterns in intensive care unit (ICU) patients.
Methods: The study was conducted in adult medical/surgical ICUs at two university hospitals. All adult patients with ICU-acquired BSI due to *Candida*, excluding patients with neutropenia, malignancy or AIDS, between 2007–2009 were included. Potential factors occurring up to 30 days before candidemia, including demographic characteristics, comorbidities, exposure to antibiotics and antifungals, ICU-related factors (i.e TPN, invasive procedures) and outcome were determined. Antifungal susceptibility testing was performed using broth microdilution assay method described by the Clinical Laboratory Standards Institute.
Results: Clinical characteristics of the patients were presented in the Table. Seventy-six cases (59,4%) of candidemia were due to *C. albicans* and 52 (40,6%) to NAC spp. Distribution of the first three NAC spp. was *C. tropicalis* (22/52, 42,3%), *C. glabrata* (12/52, 23,1%) and *C. parapsilosis* (9/52, 17,3%). Multivariate logistic regression analysis of the factors showed that presence of glucocorticosteroid treatment and a central venous catheter were variables independently associated with BSI due to NAC, compared with BSI due to *C. albicans* (P:0,001, OR: 7,18, 95% CI: 2,22–23,22; and P0,001, OR: 20,13, 95% CI: 3,64–111,18, respectively). A total of 65 patients died within 30 days of the diagnosis of candidemia. The mortality rate was higher in those with NAC than *C. albicans* BSI (61,5% vs 43.4%) and candidemia due to NAC spp. was independently associated with death (P: 0.04). Except for one *C. lusitaniae* strain, which was resistant to amphotericin B and four *C. glabrata* strains, which were fluconazole susceptible dose dependent, all *Candida* species were susceptible to fluconazole, caspofungin, voriconazole and amphotericin B.
Conclusion: Presence of a central venous catheter and glucocorticosteroid treatment were significantly associated with BSI due to NAC. BSI due to NAC was significantly associated with death, compared with BSI due to *C. albicans*. The in-vitro activity of fluconazole is encouraging, and this agent, an efficacious, inexpensive and safe drug, can continue to play an important role in the management of invasive candidiasis.

Table . Risk factors for blood stream infection with *C. non-albicans* spp. in intensive care units by univariate and multivariate analyses, *n* (%)

Univariate analyses	*C. albicans* (n= 76)	*C. non-albicans* (n=52)	Unadjusted OR (95% CI)	*P*
Male Sex	37 (48,7)	29 (55,8)	1,33 (0,65-2,70)	*0,43*
Age (years)[1] mean ±SD	58,95 ±18,50	57,37 ±18,05	1,58 (-4,94-8,11)	*0,40*
Hospital length of stay (mean)[a]	42,37 ±32,01	40,73 ±29,69	1,64 (-9,44-12,71)	*0,77*
30 days mortality	33 (43,4)	32 (61,5)	0,48 (0,23-0,98)	*0,04*
APECHE II score[b]	23,93 ±4,16	25,02 ±5,58	1,09 (-2,79-0,62)	*0,21*
Type of patient, medical	44 (57,9)	32 (61,5)	0,86 (0,42-1,77)	*0,68*
			0,86 (0,42-1,77)	*0,68*
Underlying diseases and conditions				
Diabetes mellitus	20 (26,3)	15 (28,8)	0,89 (0,40-1,94)	*0,75*
Pulmonary disease	17 (22,4)	10 (19,2)	1,21 (0,50-2,90)	*0,67*
Liver disease	9 (11,8)	8 (15,4)	0,74 (0,26-2,10)	*0,56*
Chronic renal failure	45 (59,2)	35 (67,3)	0,70 (0,34-1,47)	*0,35*
Cardiac disease	18 (23,7)	11 (21,2)	1,16 (0,49-2,71)	*0,74*
Inpatient procedure				
Surgery within prior 4 weeks	29 (38,2)	18 (34,6)	1,16 (0,56-2,43)	*0,68*
CVC in place at time of diagnosis	52 (68,4)	49 (94,2)	0,13 (0,04-0,47)	***<0.001***
Total parenteral nutrition	51 (67,1)	32 (61,5)	1,27 (0,61-2,66)	*0,52*
Glucocorticosteroids	10 (13,2)	19 (36,5)	0,26 (0,11-0,63)	***0,002***
Assisted ventilation	42 (55,3)	28 (53,8)	1,12 (0,55-2,27)	*0,76*
Antifungal therapy within prior 4 Wee ks	9 (11,8)	8 (15,4)	0,74 (0,26-2,06)	*0,56*
Fluconazol	8 (10,5)	8 (15,4)	0,65 (0,23-1,85)	*0,41*
Other	1 (1,3)	-	1,69 (1,46-1,96)	*0,41*
Antibiotic therapy within prior 4 weeks	74 (97,4)	52 (100)	0,59 (0,51-0,68)	*0,24*
Broad-spectrum cephalosporin[c]	34 (44,7)	24 (46,2)	0,94 (0,46-1,92)	*0,87*
Carbapenem[d]	47 (61,8)	32 (61,5)	1,01 (0,49-2,09)	*0,97*
Piperacillin-tazobactam	26 (34,2)	15 (28,8)	1,28 (0,60-2,75)	*0,52*
Quinolone[e]	16 (21,1)	13 (25)	0,80 (0,35-1,84)	*0,60*
Aminoglycoside[f]	13 (17,1)	10 (19,2)	0,87 (0,35-2,16)	*0,76*
Glycopeptid[g]	56 (73,7)	41 (78,8)	0,75 (0,32-1,74)	*0,50*
			Adjusted OR (95% CI)	*P*
Multivariate analyses				
Glucocorticosteroids			**7,18 (2,22-23,22)**	***0,001***
CVC in place at time of diagnosis			**20,13 (3,64-111,18)**	***0,001***

[a] The number of days of hospitalization before the onset of candidemia
[b] APACHE: acute physiology and chronic health evaluation
[c] Third and fourth generation cephalosporins including ceftriaxone, cefoperazone-sulbactam, ceftazidime, cefepime
[d] Imipenem, meropenem
[e] Ofloxacin, moxifloxacin, levofloxacin, ciprofloxacin
[f] Amikacin, gentamicin, netilmicin
[g] Vancomycin, teicoplanin

R2617 Evaluation of candiduria in patients with neoplasia

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Objectives: Candiduria is encountered in cancer patients (pts) but its significance is not defined. However, it may be indicator of urinary tract infection (UTI) or invasive infection. The study aimed to determine the significance of candiduria in neoplastic pts.
Patients and Methods: Adult pts with neoplasia and positive urine cultures for *Candida*, admitted to the Oncology or Haematology department of the University Hospital of Heraklion, Greece from 2005 to 2010 were retrospectively reviewed.
Results: Seventy one pts with candiduria were identified. Of them 48 (68%) were males. The median age was 71 (range 40–88). The underlying disease was solid tumor in 51 (72%) and hematologic malignancy in 20 (28%). The most frequent risk factor was use of urinary catheter or nephrostomy (69%), followed by antimicrobial treatment (60%), corticosteroids (42%), anatomic malformations of the urinary tract (29%), neutropenia (24%) and diabetes mellitus (21%). Fever was present on admission in 59 (83%). Nineteen pts (27%) had dysuria. Flank pain and vomiting were prominent clinical features in 7 (10%) and 5 (7%) pts respectively. UTI by established criteria was present in 51 (72%). Among the 71 positive urine cultures, *C. albicans* was isolated in 44 (62%), and non-*albicans* in 27 (38%) [*C. tropicalis* 9 out of 27 (33%), *C. parapsilosis* 7 (26%) *C. glabrata* 6 (22%), *C. famata* 3 (11%), *C. guilliermondii* 1 (4%) and *C. lusitaniae* 1 (4%)]. Blood cultures were obtained from 59 (83%) and were positive in 15 (21%). However, only 4 pts (6%) had *Candida* in their blood. Death occurred in 26 (37%) pts with candiduria. Factors associated with death included disease progression in 7 (27%) and sepsis syndrome in 19 (73%). *Candida* spp. has been present in the blood of 2 pts who died. All pts who died had advanced neoplasia.
Conclusions: Factors predisposing to candiduria, included urinary catheter or nephrostomy, prior use of antimicrobial agents, corticosteroids, anatomic malformations of the urinary tract and diabetes mellitus. Candiduria was rarely associated with candidemia, however, has been associated with high mortality, probably because it occurred in pts with advanced neoplasia.

R2618 Epidemiology of dermatophytoses in western Greece, 2003–2010

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Objectives: Dermatophytoses are considered as one of the major public health problems in many parts of the world which distribution varies in different countries and geographical areas depending on several factors. This study was conducted to investigate the aetiological agents and the clinical variants of dermatophytoses, in Western Greece during a 8-year period (2003–2010).
Methods: A total of 3210 samples (skin scales, nail and hair fragments) obtained from 2616 patients with suspected dermatomycoses referred to the laboratory of Clinical Microbiology at the University Hospital of Ioannina, Greece. The causative agents were identified by direct microscopy and culture.
Results: Dermatophytes were isolated from 524 patients (20.03%), mainly adults. The most common type of infection was onychomycosis (34.6%) followed by tinea pedis (28.5%), tinea corporis (18.5%), tinea cruris (8.5%), tinea capitis (4.6%), tinea manum (4.0%) and tinea facei (1.3%). In forty seven patients the same dermatophyte was isolated from two different sites of infection. The frequency for all types of tineas was higher in males than in females, while males exceeded females in cases of tinea corporis and tinea facei. *Trichophyton rubrum* was the most prevalent dermatophyte species isolated from 51.5% of the 607 positive samples, followed by *T. mentagrophytes* (20.3%), *Microsporum canis* (8.2%), *Epidermophyton floccosum* (5.4%), *T. violaceum* (3.0%), *Trichophyton* spp (1.8%) and *M. gypseum* (1.7%). Other species as *T. tonsurans*, *T. verrucosum* and *T. schoenleinii* were occasionally isolated.
Conclusion: In our region, *Trichophyton rubrum* is the most common aetiological agent in all types of dermatophytoses except of tinea capitis and tinea facei. The epidemiological data collected would serve as reference for future research while many factors that contribute to the change of prevalence of dermatophytoses alter to the pass of time.

R2619 In vitro combination of anidulafungin and voriconazole against azole susceptible or resistant *Aspergillus* spp.

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Background: Voriconazole (VRZ) remains the first line therapy for invasive aspergillosis. Because some *Aspergillus* species are resistant to azoles and because the prognosis or aspergillosis remains poor; antifungal combinations could be of interest. We tested here the in vitro combination of Anidulafungin (ANI) and VRZ against azole susceptible or resistant *Aspergillus* spp by two different methods.
Methods: Thirty clinical isolates of *Aspergillus* spp from five various *Aspergillus* species identified by β-tubulin sequencing were tested. The ANI-VRZ combination was evaluated using checkerboard (CK) based on the CLSI broth microdilution method M38-A3 and using an agar diffusion technique (Etest). For CK, final inoculums were 10^4/mL. After 48h at 35ºC, both MIC and MEC were recorded visually. The fractional inhibitory concentration index (FICI) was interpreted as synergic if FICI $\leqslant 0.5$, no interaction if $0.5 < \text{FICI} < 4$ and antagonistic if FICI $\geqslant 4$. Tests were run in duplicates. For Etest, MIC of VRZ and ANI were determined alone as well as in combination after 48h at 35ºC. In combination, ANI strip was placed on agar for 1h, removed and then VRZ strip was applied over demarcation left from previous strip. Synergy and antagonism were defined respectively as a decrease or an increase of $\geqslant 3$ dilutions of the resultant MIC.
Results: Using CK method, there was no interaction between VRZ and ANI with MEC as endpoint (table) except for 1 *A. nidulans* isolate for which an antagonism was shown. Using Etest, no interaction was observed in 27 isolates. According to the endpoint used, an antagonism or a synergism was observed for 2 and 1 *A. calidoustus* isolates, respectively.
Conclusions: Overall no in vitro interaction was shown between VRZ and ANI against most of the azole susceptible or resistant *Aspergillus* isolates by using different techniques. Confirmation of these results in vivo is warranted.

	MIC VRZ µg/mL	MEC ANI µg/mL	FICI MEC
A. fumigatus (n=11)	0,25-0,5	0,001-0,03	0,53-1,59
A. flavus (n=5)	0,5-1	0,004-0,06	0,75-2,92
A. terreus (n=5)	0,25-0,5	0,002-0,03	1,06-1,48
A. calidoustus (n=5)	2-4	0,008-0,06	0,51-1,43
Other *Aspergillus* spp. (n=4)	0,06-0,25	0,002-0,06	0,68-8,35

R2620 Diagnostic issues, clinical characteristics and outcome for patients with fungaemia

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Objectives: This study investigated diagnostic issues, underlying host factors, management and outcome factors for Danish fungaemia patients.
Methods: Isolates and clinical information were collected at six centres. 335 isolates from 319 episodes in 306 patients were included corresponding to 2/3 of the national episodes.
Results: Species distribution varied by age, prior antifungal treatment (more isolates with intrinsic reduced fluconazole susceptibility $P = 0.007$)

and clinical specialty (61% *C. glabrata* and *C. krusei* at haematology wards versus 29%, P = 0.002). Colonisation samples were not predictive for the invasive species in 11/100 cases with species identification.
Blood culture positivity varied by system, species and procedure. Thus, cultures drawn via arterial lines or with *C. glabrata* needed longer incubation and cases with concomitant bacteraemia were less common using BacT/ALERT compared to the BACTEC system (9% (11/124) versus 28% (53/192); P < 0.0001).
56% of the patients had undergone surgery, 51% were ICU patients and 33% had malignant disease. Mortality varied by age (increasing by age, P = 0.009), species (numerically highest for *C. krusei* 36%, lowest for *C. parapsilosis* 25% and other *Candida* species 14%), severity of underlying disease (47% in ICU patients versus 24%; P = 0.0001), and choice but not timing of initial therapy (12% versus 48% in patients with *C. glabrata* receiving caspofungin versus fluconazole, P = 0.023). Initial antifungal choice was deemed suboptimal upon species identification in 15% of the cases, which would have been 6.5% had current guidelines been followed.
Conclusion: A high proportion of Danish fungaemia patients were severely ill and received sub-optimal initial antifungal treatment. Optimisation of diagnosis and therapy is possible.

R2621 Impact of voriconazole in fungal keratitis in eastern India

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Aim: To describe the role of prepared voriconazole (2%) eye drop in the management of fungal keratitis.
Methods: It is a retrospective observational case series involving patients of culture proven fungal keratitis from April 2008 to March 2010 attending to the cornea clinic of a tertiary care eye hospital in eastern India. Among the 144 fungal keratitis cases 66 (45.83%) cases were identified for analysis. The ulcer size, organism, treatment modalities and MIC value were analyzed. Voriconazole was used either topically (2%), or in AC wash (50 μg/0.1 ml),) and Intrastomally (50 μg/0.1 ml).
Results: Among 66 cases, 39 showed healing with corneal scar formation while 27 underwent therapeutic keratoplasty. 25 cases (64.10%) required topical use, 9 cases (23.07%) required intracameral use and 5 (12.82%) cases required intrastomal administration. Among filamentous fungi *Aspergillus* sp responded well (25/66;37.87%) followed by *Fusarium* sp (5/66;7.57%),unidentified sp (3/66;4.54%), and equal no of *Penicillium* sp, *Scedosporium* sp, and *Cladosporium* sp (2/66;3.03%). Among the cases undergoing therapeutic keratoplasty *Candida* keratitis was maximum with 11 cases followed by 7 cases of *Fusarium* sp, 5 cases of *Aspergillus* sp and 2 case each of *Alternaria* sp and *Penicillium* sp. In relation with ulcer size response to voriconazole therapy obtained in (20/39;51.28%) where ulcer size is <6 mm and remaining (19/39;48.71%) cases where ulcer size is >6 mm. The minimal inhibitory concentration of voriconazole was 0.125 μg/ml against *Aspergillus* sp and 0.5 μg/ml against *Fusarium* sp. *Candida* sp showed resistance activity upto 2% of voriconazole.
Conclusion: Voriconazole has promising activity against filamentous fungi and may prove useful in the management of fungal keratitis but it shows no response in *Candida* keratitis from our centre.

R2622 Cerebral human pythiosis: the first case report of *Pythium insidiosum* infection presented with brain abscess

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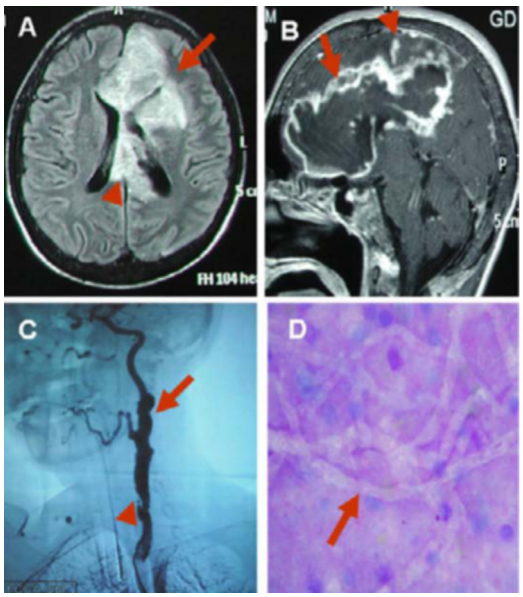
We here reported a thalassemic patient suffering from cerebral pythiosis (left common carotid pythiosis arterial aneurysms, septic embolisms evolving to brain abscess over the left anterior cerebral artery territory). This was the first case in the world of *Pythium insidiosum* involving left common carotid artery and producing septic emboli, causing pythiosis brain abscess.
A 27-year-old man, developed nasal congestion with whitish discharge from the left nostril. Three weeks before admission, he developed high grade fever. One week later, he complained of occipital headache, followed by a focal seizure starting at right hand, and then he developed secondarily generalized tonic-conic seizures. Computed tomography scan of the brain was performed. A 5x6 cm hypodensity lesion was noted over the left frontoparietal region. Intravenous ceftriaxone 4 gm/day and metronidazole 1,500 mg/day were prescribed. One week later, fever was still persisted and right-sided weakness was worsened.
Personal and past medical history: This patient was diagnosed with β-thalassemia haemoglobin E disease. The pertinent physical findings were high grade fever, significant left carotid bruit, right facial palsy and spastic right hemiparesis.
MRI brain showed a large rim-enhancing brain lesion over the left hemisphere. MRA showed multiple aneurysms at left common and internal carotid arteries. His serum was tested for antibody against pythiosis using 3 different techniques, which were ELISA, immunodiffusion and Western blot. The results of all the tests were positive. We also prescribed itraconazole, terbinafine and *P. insidiosum* antigen immunotherapy, but the patient eventually died from brain herniation. Autopsy revealed gross pus at left frontal area. The left common and internal carotid arteries also found 2 cm in diameter of aneurysm. Pus Wright's stains showed hyphae with infrequently septate and branching. Tissue culture from his brain was confirmed the diagnosis of pythiosis by isolation the organism and proof by Polymerase chain reaction (PCR) with specific primers in the COX II region.
Discussion: We couldn't eradicate infected brain tissue as well as infected carotid arteries because of the area of brain and carotid artery involvements were harmful to resection. Cerebral pythiosis should be the differential diagnoses of brain abscess in thalassemic patients. Early recognition and prompt surgical treatment should be considered to reduce mortality or morbidity.



R2623 Bronchoscopy sampling, culture technique and real-time PCR for *Aspergillus* affect diagnostic yield

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Background: Fungal culture methods for respiratory specimens have never been formally compared. Real time PCR for *Aspergillus* spp. has recently been introduced in Europe, Africa and Canada. We compared 2 culture methods and PCR on multiple sputum and bronchoscopy samples from 3 patients with aspergillosis.

Method: We bronchoscoped 3 patients: ABPA, prior IA and COPD and *Aspergillus* bronchitis. We compared (1) the UK standard method for processing respiratory cultures (BSOP57) (modified to plate 10uL instead of 1uL) with (2) high volume culture (range 20 μL – 2.3mL, avg 0.5mL) (100% plated) on Sabouraud dextrose agar (37ºC) and 3) real time PCR (MycAssay *Aspergillus*) preceded by DNA extraction using the MycXtra kit. The sensitivity of this assay is <1 genome, following a >10% extraction efficiency. Sputum samples were obtained before and after the bronchoscopy procedure. Material obtained was split into 'highly mucoid' material and more liquid material, if possible. Approximately equal volumes of material (33% each) were used for the PCR and 2 culture methods.
Results: 21 samples were cultured. All (100%) were *Aspergillus* negative by routine culture and 14 of 21 (67%) negative by high volume culture. Only 2 (10%) were negative by PCR, 3 were below clinical cutoff (Ct <36) and 16 (76%) positive (Ct values 28.9–35.7). Of the 6 sputum samples (2 split), all were positive by PCR and 5 of 8 (63%) were positive by high volume culture (1–6 CFU). BAL samples were all *Aspergillus* culture negative, and 8 of 10 samples (80%) were PCR positive. In 2 patients the highest PCR yield was the initial bronchoscopy trap aspiration (often discarded as contains lignocaine), but not in one patient; hyphae and Charcot-Leyden crystals were visible in this sample from patient with ABPA and 19 CFU were grown in high volume culture.
Conclusion: The UK standard method for culture is grossly sub-optimal for *Aspergillus* spp. and needs revision. Improved culture methods may be of value for sputum but are inferior to real time PCR using the MycXtra DNA extraction system and MycAssay *Aspergillus* assay. Bronchoscopy sampling has considerable variability in diagnostic yield; sputum may be superior.

Sample	n	Positive samples (%)		
		Aspergillus culture		MycAssay Aspergillus real time PCR
		Routine	High volume	
Pre-bronch sputum	4	0	4 (100)	4 (100)
Post-bronch sputum	4	0	1 (25)	4 (100)
First trap aspiration	3	0	2 (67)	3 (100)
First BAL (10-20mL)	5	0	0	4 (80)
Second BAL (10-50mL	5	0	0	4 (80)

R2624 Detection of *Trichophyton rubrum* by multiplex PCR and all other dermatophytes from nails

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Objectives: To evaluate the performance of a multiplex PCR (m-PCR) method for detection of dermatophytes, in general, and specifically *T. rubrum* as compared to conventional methods (microscopy and culture) for the diagnosis of tinea unguium.
Methods: A total of 65 nail samples from clinically suspected tinea unguium were examined by direct microscopic examination and culture, as well as, by an m-PCR method (Dermatophyte PCR Kit – SSI Diagnostica – Denmark). DNA from a clinical *T. rubrum* isolate was used as a positive control. m-PCR includes a two step extraction, a primer mix containing two primer pairs directed toward genes encoding chitin synthetase 1 (chs1) for detection of dermatophytes in general (PCR product = 366bp) and internal transcribed spacer 2 (its2) for detection of *T. rubrum* (PCR product = 203 bp), an internal control (PCR product = 660bp), and two positive controls (Dermatophyte and *T. rubrum* genomic DNA). After 45 thermal cycles, PCR products are electrophoresed in a 2% agarose gel and stained with ethidium bromide. Results are obtained within 5 hours.
Results: Cultures of 65 nail samples, yielded growth of 2 *T. rubrum*, 1 of *T. rubrum* and *Candida* non-*albicans*, 3 of *Candida* non-*albicans*, 1 of *C. albicans*, 1 of *Aspergillus niger*, 1 of *A. fumigatus* and 1 of *Alternaria*. Overall 5/65 (7.7%) of samples were positive for dermatophytes by microscopy (2) and culture (3). By m-PCR 13/65 (20%) of samples were positive for dermatophytes, 12 for *T. rubrum* and 1 for another dermatophyte. PCR results of 8 nail specimens with positive culture for non dermatophytes showed: 1 *A. niger* and PCR (+) for dermatophytes, 1 *A. fumigatus* and PCR (+) for *T. rubrum*, 3 *Candida* non-*albicans* and PCR (+) for *T. rubrum*. The other 3 cases, positive for *C. albicans*, C. non-*albicans* and *Alternaria*, gave negative PCR results. Fifty nail samples (77%) were negative for dermatophytes by either conventional or PCR methods.
Conclusions: m-PCR is a rapid method with high specificity which increases sensitivity of laboratory diagnosis of nail dermatophytosis.

R2625 Disseminated sporotrichosis in a patient with hairy cell leukaemia treated with amphotericin B and posaconazole

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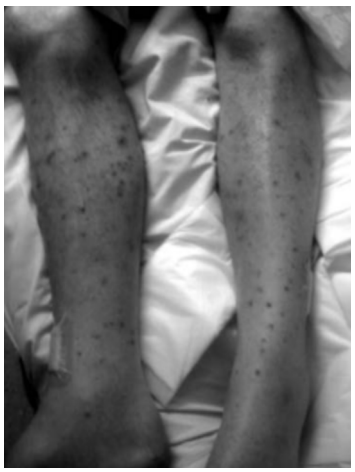
We describe a case of disseminated *Sporothrix schenckii* infection in a man with underlying hairy cell leukemia.
A 44-year-old man, who was initially treated with immune suppression for a presumed autoimmune inflammatory disorder, developed disseminated sporotrichosis with involvement of his skin, lungs and eyes. His immmunity was further impaired by a previously unrecognised hematological malignancy, hairy cell leukemia.
The degree of dissemination was so severe that the organism, *S. schenckii*, was isolated from blood cultures while using standard automated culture techniques.
His infection was initially refractory to antifungal therapy with traditional amphotericin B, in part due to drug toxicity. Treatment with liposomal amphotericin B and oral posaconazole, along with treatment of his underlying leukemia, resulted in dramatic clinical improvement with no residual evidence of infection.
The immunological defects associated with this malignancy, as well as the management of refractory sporotrichosis are reviewed.



R2626 22 months of *Saccharomyces cerevisiae* in Princesa University Hospital of Madrid, Spain

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Introduction: *Saccharomyces cerevisiae* is a yeast used as adjuvant treatment and prophylaxis of diarrhea. It produces two possible infections: superficial and invasive. It can colonize the respiratory tract, gastrointestinal tract and genitourinary tract of host. Can lead to invasive infection: gut translocation and nosocomial. There is a possibly environmental transmission and from person to person. Administration of probiotic mainly in neutropenic and critical patients is the main risk factor. Infection also may emerge without previous risk factors.
Objective: Study the prevalence of *S. cerevisiae* in different group of patients: in hospital and in community, and establish a relation between it use as treatment and infection and see another possible origin.

Method: 4652 samples were collected from March of 2009 to December of 2010. All of them were grown in Sabouraud dextrose agar (SDA) for 24 and 48 hours at 30ºC. We looked for macroscopic morphology in SDA and microscopic morphology in Corn Meal agar. We differenced saccharomyces cerevisiae from other yeast by chromogenic medium (CHROMagar™ *Candida*) and by test of assimilation of sugars (auxacolor™, BIO-RAD). Risk factors in this study: previous use of antibiotic/antifungal, probiotics based in *S. cerevisiae*, hospitalization and immunosuppression. We followed clinical and microbiological criterias to prove if we were in an infection or colonization.
Results: Risks factor weren't brake down into their different headings due to the difficulty of ensuring that patients received *Saccharomyces cerevisiae* (Ultralevura®). Only we found that only patients admitted with chronic diarrhea and elderly in hospital received probiotic. In community was impossible to know. Vaginitis and cervicitis, due to *S. cerevisiae*, were the main infection after using of azoles or antibiotics to treat other infections. We had not isolated in blood or sterile samples.
Conclusions: We have not had any invasive infection. We should unify criteria to decide which patients can be given or not Ultralevura® to prevent infections. We should study phenotypic and genotypic factors of yeast related to its ability to invade the host and antifungal test to establish epidemiological criteria for treatment. It is complicated to know the origin of *S. cerevisiae*.

SAMPLE	N (AS)	PLACE	N./ ORIGIN	RISK FACTOR	CLINICAL CRITERIA TO GIVE PROBIOTICS	INFECTION/COLONIZATIO N
BRONCHOASPIRATE	4	ICU	3	SURE	VERY RARELY ARE GIVEN	COLONIZATION
		PNEUMOLOGY	1	SURE	NO PROBIOTICS ARE GIVEN	COLONIZATION
ESPUTUM	3	PNEUMOLOGY	2	SURE	NO PROBIOTICS ARE GIVEN	COLONIZATION
		INFECIOUS UNIT	1	SURE	CHRONIC DIARRHEA AND ELDERLY	COLONIZATION
ORAL LESION	2	INFECIOUS UNIT	1	SURE	CHRONIC DIARRHEA AND ELDERLY	SUPERFITIAL INFECTION
		EMERGENCY	1	PROBABLY	NONE	SUPERFITIAL INFECTION
PHARYNGEAL EXUDATE	4	OTOLARYNGOLOGY	3	PROBABLY	NONE	ESOPHAGITIS
		ONCOLOGY	1	SURE	VERY RARELY ARE GIVEN	ESOPHAGITIS
LINGUAL EXUDATE	2	OTOLARYNGOLOGY	2	PROBABLY	NONE	SUPERFITIAL INFECTION
STOOL SAMPLE	13	DIGESTIVE UNIT	11	SURE	NO PROBIOTICS ARE GIVEN	COLONIZATION
		INFECIOUS UNIT	1	SURE	CHRONIC DIARRHEA AND ELDERLY	COLONIZATION
		ENDOCRINOLOGY UNIT	1	PROBABLY	NONE	COLONIZATION
VAGINAL EXUDATE	13	HEALTH CARE PRIMARY	13	PROBABLY	NONE	VAGINITIS
CERVICAL EXUDATE	2	HEALTH CARE PRIMARY	2	PROBABLY	NONE	CERVICITIS

R2627 A study of fungal keratitis cases in Sirte Province, Libya

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Objective: Fungal keratitis is one of the major causes of the ulcerative and sight threatening infection of the cornea but its incidence is usually underestimated. Such a study has never been carried in this part of the world. The objectives of this study was to determine its incidence and associated etiological risk factors in this region.
Materials and Methods: A prospective case study of patients presenting with clinically suspected keratitis was conducted at Al-Rehma Hospital, Sirte, Libya between January 2008 and November 2010. Patients presenting with clinical features of keratitis were included in this study. Detailed history and Ophthalmic findings were documented. Cornea was scrapped from the edge of the base of the ulcer and smears were examined with Gram stain, Giemsa stain and KOH and inoculated on blood agar, chocolate agar and sabourds dextrose agar.
Results: A total of 85 patients with clinically suspected keratitis were examined of which fungal infection was found in 28 (32.9%). Out of them 24 (85.7%) had fungal infection alone while 4 (14.2%) had fungus mixed with bacteria. Out of all the fungus isolated *Aspergillus* was cultured in 8 (28.5%) while *Fusarium* was cultured in12 (42.8%). Trauma was the major cause (n = 22,78.5%) of which vegetative injury was found in 17 (60.7%). Associated history of diabetes mellitus was noted in 5 (17.8%) while of contact lens in 6 (21.4%) patients. Of the total 85 patients included in the study 62 were (72.9%) males while of the total 28 patients diagnosed with fungal keratitis 17 (60.7%) were males.
Conclusion: Contrary to popular perception of low incidence of fungal keratitis in this region, high incidence has been found in this study. It can be attributed to increasing trend towards farming activity in this region which predisposes the people to vegetative injury. Seasonal variations throughout the year in this region leads to altered tear film which again could be a major predisposing factor. Increasing use of the contact lenses also predisposes the users to contact lens related infection.
Diabetes mellitus was found in some patients which can be a predisposing factor.

Prevalence of individual fungi

Type of fungus	No. of cases
Aspergillus spp.	14
fumigatus	8
niger	6
Fusarium	11
Penicillium	2
Sporotrichos	2
Cephalosporium	1

R2628 *Candida* spp. bloodstream infections: trends in epidemiology, susceptibility and antifungal use during three years in a French university hospital

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Candidaemia are an important cause of morbidity and mortality in hospitals, especially among severe patients. Over the past decade, the epidemiology and antifungal therapies have evolved. Our university hospital is committed since 2005 in a multidisciplinary approach to help diagnosis and treatment of invasive fungal infections (IFI) initially focused on costly treatments. Our objectif was to study trends in epidemiology, susceptibility, and antifungal use in our hospital.
Methods: All patients with a blood culture yielding yeast between January 2007 and December 2009 were included. Patient data, epidemiology, susceptibility, treatments and interventions of our antifungal team were collected from computerized patient records, the Mycology Laboratory and the Pharmacy.
Results: 74 patients were included. During this period a diversification of yeast species was observed, with a significant reduction of *Candida albicans* (25% in 2009 vs 52% in 2007). The rate of resistance to fluconazole has not exceeded 6% among strains of *Candida* and the first strain of *Candida parapsilosis* resistant to caspofungin was isolated in 2009. First line antifungal treatments have been established according to local recommendations (96%). The antifungal team has intervened for nearly 80% of patients in 2009, to continue the initial antifungal treatment or to propose a de-escalation therapy (20%). In 2009, 45% of patients (11/24) died, underlyng the severity of those IFI.
Conclusion: This work has underlined our local specificity both in terms of epidemiology (most important decrease in *Candida albicans* compared the litterature), and in terms of multidisciplinary management of IFI. Among the proposals from this study include the systematic monitoring of Candidaemia during our antifungal team meetings wathever the drug and the search for a possible nosocomial cause of these severe infections.

R2629 Imported histoplasmosis in a region of Spain

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Objective: The increased presence of immigrants from Latin America and increased travel to that continent has increased the incidence

of Histoplasmosis in Spain. Our aim was to review the cases of histoplasmosis diagnosed in our hospital.
Methods: Review the diagnostic methods employed and clinical characteristics of cases of histoplasmosis diagnosed during the last six years.
Case reports: We diagnosed 4 cases, three of whom were HIV positive (male, 28–43 years) and one patient (female 50 years) treated with immunosuppressive drugs. All of them were from South America. The spectrum of disease in HIV positive patients was one liver disease, one pulmonary and one gastrointestinal. Two had a good outcome but the other, in spite of treatment with amphotericin B, had a rapidly progress to a multiorganic failure and died. The female had a liver histoplasmosis and she also had a good clinical response. The treatment in all cases was amphotericin B 5 mg/kg/day (two weeks) followed by itraconazole 200 mg/12h.
The laboratory diagnosis was carried out by histological (PAS and PAS-Diastase Stain) and microbiological study (culture and PCR directly): in three cases, we isolated *H. capsulatum* var. capsulatum and in the other, the microbiological diagnosis was thanks a direct PCR in the tissue.
Conclusion: Most infected people by histoplasmosis remain asymptomatic, but they can develop serious clinical forms depending on the level of exposure and the patient's immune status. Usually, severe forms are seen in HIV positive patients, but as has occurred in our series of cases it's also possible in patients with immunosuppressive therapy.
In our country, histoplasmosis is an imported infection and because of this it is necessary to have a high index of suspicion and perform a detailed history to get a diagnosis. It is an infection to be considered in the differential diagnosis in immunosuppressed patients, both HIV positive and immunosuppressive therapy, which originate from endemic areas or who have a history of stay in them.

R2630 Invasive zygomycosis in Saint Petersburg, Russia

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Objectives: To determine risk factors, clinical features, aetiologic agents and outcomes in patients with zygomycosis.
Methods: The diagnosis of zygomycosis was made according to EORTC/MSG criteria (2008). Clinical materials from patients were examined by direct microscopy, culture and histopathology.
Results: During 2002–2010 y.y. twenty one cases of zygomycosis have been diagnosed. The mean age of patients was 37 years (range 11–60), female – 57%, male – 43%. Risk factors were as follows: surgery – 46%, haematological malignancies – 44%, diabetes mellitus – 5%, tuberculosis – 5%.
Lungs and paranasal sinuses were the most common sites of infection (38% each). Disseminated form of zygomycoses was diagnosed in 10% of patients, as well as the disease of soft tissues (10%). Rhinocerebral form of zygomycosis was revealed in 4% of cases.
The spectrum of aetiologic agents included: *Rhizopus oryzae*, *Rhizopus microsporus*, *Rhizomucor pusillus*, *Rhizomucor variabilis*, *Absidia corymbifera*, *Syncephalastrum racemosum*.
Seventeen patients were treated with antifungals: amphotericin B deoxycholate (59%), amphotericin B lipid complex or liposomal amphotericin B (10%), posaconazole (41%). Combined antifungal therapy was conducted in 18% of cases. Surgical treatment was performed in 11 patients. The six-month overall survival in all patients was 62%, in haematological patients – 30%.
Conclusions: Early diagnosis, antifungal and surgical treatment, elimination of immunosuppression are needed for successful treatment of invasive zygomycosis.

R2631 Evaluation of zygomycosis patients: a retrospective multicentre study

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Objectives: The incidence of Zygomycosis has increased due to immunosuppression in recent years. We aimed to evaluate 16 cases of Zygomycosis from eight centers retrospectively.
Methods: Zygomycosis patients were collected between 2004 and 2010. Zygomycosis was diagnosed by culture positivity and/or histopathological findings in addition to clinical findings. The clinical forms of zygomycosis have been described on the basis of organ involvement: rhino-orbito-cerebral, cutaneous, pulmonary, gastrointestinal and disseminated. Disseminated infection was defined as infection at $\geqslant 2$ non-contiguous sites.

Table: Underlying conditions, clinical forms and treatments of 16 patients, 10 of whom died

Underlying conditions*	n (%)	Mortality n (%)
Diabetes mellitus	10 (62.5)	8 (80)
only	2 (20)**	1 (50)
and Corticosteroid use	5 (50)**	4 (80)
and Chronic renal failure	3 (30)**	3 (100)
and Broad spectrum antibiotic use	2 (20)**	2 (100)
and Surgery	2 (20)**	1 (50)
and Cirrhosis	1 (10)**	1 (100)
Haematological disorders	4 (25)***	3 (75)
and Neutropenia	3 (75)***	2 (66.7)
and Broad spectrum antibiotic use	3 (75)***	2 (66.7)
and Corticosteroid use	2 (50)***	2 (100)
Solid tumour	1 (6.3)	0 (0)
SOT*, Corticosteroid use, cirrhosis	1 (6.3)	0 (0)
Clinical Forms		
Rhino-orbito-cerebral	7 (43.75)	5 (71.4)
Rhino-orbital	3 (18.8)	2 (66.7)
Disseminated	3 (18.8)	3 (100)
Pulmonary	1 (6.3)	0 (0)
Cutaneous	1 (6.3)	0 (0)
Gastrointestinal	1 (6.3)	0 (0)
Treatment		
Antifungal therapy alone	3 (18.8)	1 (33.3)
Antifungal therapy + surgery	8 (50)	4 (50)
Antifungal therapy + surgery + irrigation with AmB	4 (25)	4 (100)
No therapy (Postmortem diagnosis)	1 (6.3)	1 (100)

*: There are more than two conditions in some patients.
**: Calculated for 10 patients
***: Calculated for 4 patients
SOT: Solid organ transplant, AmB: Amphotericin B deoxycholate

Results: There were 11 female and five male subjects. The mean age was 52.50±14.55 (22–68) years. The majority of cases (15 cases, 94%) were immunocompromised patients, mainly diabetes mellitus (10 cases, 62.5%). Seven patients had received corticosteroid treatment. The most common symptoms and clinical signs in patients was fever (n = 9), retroorbital pain (n = 7). The most common form was rhino-orbito-cerebral. The mycological cultures were performed in 14 patients and half of them had a positive culture. The isolated pathogens: *Rhizopus* spp. (n = 4), *Mucor* spp. (n = 2) and *Rhizomucor* spp. (n = 1). In 2 (12.5%) of patients the diagnosis was based on only culture positivity, in 9 (56.25% of patients) on histopathological findings, in 5 (31.25% of patients) on both culture positivity and histopathological findings. *Aspergillus flavus* was found as a second agent in two patients. The average time elapsed for diagnosis was 14.26±13.96 (2–52) days. Antifungal therapy was administered to 15 patients (94%). Twelve patients underwent one to four times surgical interventions with antifungal therapy. The average duration of antifungal therapy was 61.4±58.02 (1–180) days. On the other hand, the median duration of treatment in survivors was 62.5 (42–

180) days. Characteristics of underlying conditions, clinical forms and management were given in table.
Conclusion: Diabetes mellitus and corticosteroid use are common underlying conditions in Zygomycosis. Zygomycosis is an infectious disease with high mortality despite antifungal therapy and surgery interventions.

R2632 Fungemia in the intensive care unit: a five-year study in two centres

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Objectives: Candidemia is frequently encountered in the nosocomial setting, particularly in the Intensive Care Unit (ICU) causing considerable morbidity and mortality. The increasing incidence of non-*albicans Candida* species could also be important. The aim of the present study was to record the epidemiology, risk factors, mortality, strains susceptibility to antifungal drugs. This is a clinical and microbiological retrospective study of all fungemia episodes registered in two medical–surgical ICUs between 1/1/2005 and 31/12/2009. The records of the research laboratory of the 4th department of Internal Medicine, the microbiology department of Attikon university hospital and the microbiology department of Thriassion Hospital were used in order to identify patients. Medical records were then retrieved. Special forms were completed for each patient including demographic information, concomitant conditions, Apache II and Sofa severity scores the day of ICU admission, the risk factors within the preceding 10 days, data of colonization and candidemia related information.
Results: Attikon hospital is a 640-bed teaching tertiary care hospital with a 25-bed medical and surgical ICU and Thriassion hospital is a 433-bed tertiary care hospital with an ICU with 8 beds. During the study period a total of 1663 pts were hospitalized in both ICUs. Among them 67 patients developed fungemia. Median patients' age was 56 years. Median ICU length of stay was 37 days. Medical cause of admission was present in 31 cases. Species isolated were *C. albicans* (38.8%), *C. parapsilosis*, *C. tropicalis* (8,9%), C.spp, *C. glabrata*, and non-*albicans* spp. Median time elapsed between ICU admission and candidemia was 19 days. Mean Apache II score was 19 on the day of admission and overall mortality was 50.7%. Attributable mortality was 55,9%. Ostrosky prediction rule was positive in 41 patients. Urine or lung colonization was present in 20.5%,and multiple site colonization in 29%. Fifteen pts were submitted to an intraabdominal operation. Fifteen pts received TPN prior to candidemia episode. Caspofungin was the most commonly introduced treatment.
Conclusions: Compared to other blood infections fungemias are not common among our patients but they are often lethal. A high Apache II score at admission, multiple site colonization in combination with abdominal surgery should raise a high suspicion index and a prophylactic therapy should start. Non-*albicans* species are on the rise.

R2633 20 months of dermatophytes onychomycosis in Princesa University Hospital, Madrid, Spain

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Introduction: Onychomycosis is the mainly cause of nails disease in developed countries. The prevalence is increases with age and in certain conditions, such as diabetes and immunosuppression. The dermatophytes are responsible primary of infections. The majority of cases are presented as distal and lateral subungual onychomycosis, which has the typical appearance in most dermatophyte infections.
Objective: Show the prevalence of onychomycosis dermatophytes in the area of our Hospital in a period of time of twenty months.
Methods: Of 2005 samples, firstly, we looked for fungal structures by optical microscopy using KOH 10%. We used Sabouraud agar with and without actidione, PDA and Lactrimel agar. They were incubated at 30ºC until thirty days. We looked for typical macroscopy morphology of dermatophytos twice a week and microscopy morphology by lactofenol-blue from PDA or Lactrimel.
Results: Of the 2005 samples, we obtained a total of 680 samples with growth of molds. We reported 357 (52.5%) *Trichophyton rubrum* and 29 (4.26%) *Trichophyton mentagrophytes* and 294 (43.23%) corresponded to other molds.
In 2009 the most isolations were in May (8.53%) and August (5.00%) and in 2010 were in July (5.44%) and in October (8.23%) as cause of onychomycosis. No dermatophytes were isolated more than these.
Conclusions: Confirmation of the diagnosis should be considered as essential. A bad treatment predispose to advanced invasion with dermatophytes and/or other molds. We should assay sensibility to antifungals in our area.

R2634 Typing of the dermatophytes and yeasts detected as cause of superficial mycotic infections in diabetes patients and antifungal sensitivity of yeasts

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Objective: The present study was undertaken to determine the causative agents of superficial mycotic infections and their prevalence in diabetes mellitus patients.
Materials and Methods: In this study, a total of 130 skin and finger nail scratches were taken from 80 diabetes patients who had been being followed up by Department of Internal Medicine-Endocrinology Branch polyclinics and/or clinics between 2009–2010 and the samples were submitted to Mycology Laboratory of Department of Medical Microbiology, Faculty of Medicine, Firat University for mycological analysis.
The samples were subjected to mycological direct microscopic examination with 15% KOH, and cultured in mycobiotic agar (Oxoid) medium. In addition to conventional mycological identification methods, API ID32C kits (Mini API, Biomerieux, France) were used For identification of yeast and dermatophytes. Furthermore, *Candida* spp. Isolates were subjected to disk diffusion antifungal sensitivity test using vorikonazol and flukonazole disks and using CLSI criteria.
Results: In microscopic examination, 89 (68%) of clinical specimens were found positive while 41 (32%) was negative. In the mycological cultures, growth was detected in 78 (60%). No growth was seen 46 (35%) while contamination was detected in 6 (5%) of the samples. Of those 78 samples that were growth positive, 28 (36%) were *Trichophyton rubrum*, 3 (4%) were *Trichophyton mentagrophytes*, 11 (14%) were *Trichophyton rubrum* + *Candida* spp., 2 (3%) were *Trichophyton mentagrophytes+Candida* spp., 5 (6%) were *Trichophyton rubrum* + *Trichophyton mentagrophytes* and 29 (37%) were *Candida* spp. Identification of those 29 *Candida* spp. revealed that 16 (55%) were *Candida albicans*, 7 (24%) were *Candida sake*, 4 (14%) were *Candida parapsilosis*, 2 (7%) were *Candida krusei*. Results of antifungal sensitivity tests showed that 13 (45%) of *Candida* spp. were resistant to flokonazole where as 4 (14%) were resistant to vorikonazole.

R2635 A pooled analysis of 100 cases of mucormycosis from Turkey

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Objectives: In this study, it was aimed to review systematically the published mucormycosis cases from Turkey.
Method: Published mucormycosis cases from Turkey in national and international medical literature in the last fifteen years were retrieved from three national (Ulakbim, Turkish Medical Literature and Medline Plexus) and two international databases [Pubmed and Science Citation Index Expended (SCI)]. As keywords "mukor", "mukormikoz" were used in national databases and "mucor", "mucormycosis" adding "Turkey" in international databases. Data related to age, gender, comorbidities, signs, diagnostic tools, therapeutic modalities, and mortality were analysed from the retrieved articles.

Results: Data for a total of 100 mucormycosis patients (47 female, 53 male, aged 46.1) with definitive diagnosis of invasive fungal infections according to criteria of European Organization for Research and Treatment of Cancer (EORTC) were obtained from 48 reports (22 international, 26 national). We could not achieve the full texts of two reports published in international databases and three reports in national databases. In terms of common clinical findings, the most common symptom was swelling of eye and face (64%) followed by headache (56%), fever (54%), opthtalmoplegia (38%), loss of vision (31%) and other neurological signs (%27). The most common comorbidity was diabetes (53%) followed by, hematological malignancies and corticosteroid usage (33%). A total 40 of patients had mycological culture and in 21 it was positive. In radiologic imaging 76% of patients had findings in favor of fungal infection. Diagnosis was made by the help of histopathological investigation in 83 cases. Three patients had been diagnosed on autopsy. Both surgical debridement and antifungal therapy were administered in 85 patients. Five patients had received only surgical debridement and seven only antifungal therapy. Three patients died before treatment. Total mortality rate was %57. Although all patients treated by only surgical debridement were alive, seven patients who had only antifungal therapy died. Patients with diabetes had tendency of higher mortality according to others (Chi square test, p = 0.052).
Conclusion: Despite new diagnostic tools and therapeutic agents mucormycosis has still very high mortality. Suspicion of mucormycosis is crucial for early diagnosis. In the management, biopsy should be made immediately.

R2636 Clinical characteristics and risk factor analysis of patients with persistent candidaemia

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Objectives: Candidemia can persist even with antifungal therapy. However the risk factors and clinical characteristics of patients with persistent candidemia are not well known yet.
Methods: The medical records for all patients with candidemia at the Ajou University Hospital from August 2006 to July 2008 were reviewed. Persistent candidemia was defined as the isolation of same species of *Candida* from repeated blood culture at least 72 hours after administration of systemic antifungal agent. Cases of persistent and non-persistent candidemia were compared and risk factor analysis was performed. Antifungal susceptibility tests were also performed and the relationship between laboratory susceptibility data and microbiological failure was evaluated.
Results: Fifty-three episodes of persistent candidemia and 63 episodes of non-persistent candidemia were identified. Male gender, high Charlson's comorbidity score, mechanical ventilation, antifungal treatment with azoles, central venous catheter tip culture positivity and catheter reinsertion the same day as catheter removal dwere identified as risk factors by univariate analysis. Using multivariate analysis, male gender (OR 4.72, 95% CI 1.47–15.19), antifungal treatment with azoles (OR 5.75, 95% CI 1.63–20.27) and catheter tip culture positivity (OR 4.06, 95% CI 1.03–15.99) were identified as independent risk factors. Ninety-eight percent of *Candida* isolates (114 of 116) were susceptible to the administered antifungal agents. Only one pair of isolates displayed altered minimal inhibitory concentration value during treatment.

Table. Multivariate analysis of independent risk factors for persistent candidemia

Variables	Odds ratio (95% CI)	*P* value
Male gender	4.72 (1.47-15.19)	0.009
Mechanical ventilation	1.13 (0.37-3.42)	0.828
Charlson's comorbidity score ≥3	1.69 (0.57-4.99)	0.346
Azoles antifungal treatment	5.75 (1.63-20.27)	0.007
Catheter tip culture positivity	4.06 (1.03-15.99)	0.045
CVC reinsertion at removal day	3.00 (0.78-11.55)	0.110

Conclusion: Microbiological treatment failure of patients with *Candida* blood stream infections is influenced by various related factors including host factor, efficacy of antimicrobial agents and management of central venous catheter. Antimicrobial resistance is not a major determinant to the persistence of candidemia.

R2637 Fungicidal effect on yeasts of photodynamic therapy with 1,9-dimethylmethylene blue

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Objective: To evaluate the in-vitro fungicidal effect of PDT using DMMB against various yeast strains.
Methods: *Candida parapsilosis* (ATCC 22019) *Candida krusei* (ATCC 6258) *Candida albicans* (ATCC 10231) *Saccharomyces cerevisiae* (ATCC 9763) *Candida albicans* (CETC 1001) *Saccharomyces cerevisiae* (CETC 1170) and *Saccharomyces pastorianus* (CETC 1970) were included in study. Starting with 24 hour-old yeast cultures at 0.5 (± 0.05) and 4 or 5 (+0.05) McFarland standard solutions were obtained. 90 µL of these inocula were diluted to 100 µL with different concentrations of DMMB and incubated for 0,15,30 and 60 minutes at 35 ºC. Yeasts were irradiated with a light-emitting diode lamp emitting at 630 nm (±10nm) with an emission power of 1,22 mW, and using a fluence of 18 J/cm^2. Irradiated samples cultured on Sabouraud-Chloramphenicol agar plates were incubated 48 h at 35ºC. Fungicidal effect was studied combining varied incubation times with different concentrations of DMMB. Microtiter inocculated without light exposure, only with DMMB or none of them were used as controls.
Results: A fungicidal effect of three logarithmic units was observed irradiating immediately after adding the photosensitizer for *C. parapsilosis* and *S. cerevisiae* with DMMB concentrations of 1.25 mcM; *C. albicans* (CETC 1001) *S. cerevisiae* (CETC 1170) and *S. pastorianus* of 0.625 mcM; and *C. albicans* (ATCC 10231) and *C. krusei* of 2.5 mcM.
A fungicidal effect of six logarithmic units with 0 minutes of incubation with DMMB previous to irradiation was observed for *S. pastorianus* with a concentration of 5 mcM; *C. krusei*, *C. albicans* (ATCC 10231) and *C. albicans* (CETC 1001) of 10 mcM; *S. cerevisiae* (CETC 1170) and *C. parapsilosis* of 20 mcM; and *S. cerevisiae* of 40 mcM. Incubating the photosensitizer for 15, 30 o 60 minutes before illumination halved the minimal fungicidal concentration of DMMB for all of the strains studied either to obtain 3 or 6 logarithmic units reduction.
Conclusions: PDT with DMMB has fungicidal effects in-vitro (for three and six logarithmic reduction) on *C. parapsilosis*, *C. albicans*, *S. cerevisiae*, *C. krusei* and *S. pastorianus*. 15 minutes was the optimal time of incubation with DMMB to obtain maximum fungicidal effect (for three and 6 logs reduction) with the minimal photosensitizer's concentration in all the strains. Future evaluations in vivo in animal models, should provide a better assessment of the utility of this therapy for yeast infections.

R2638 Posaconazole failure in a renal transplant patient with invasive aspergillosis due to *Aspergillus fumigatus* with attenuated susceptibility

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Objective: To describe the first case of an azole-naïve patient with progressive aspergillosis around and in her renal transplant due to an *Aspergillus fumigatus* isolate resistant to voriconazole and with reduced susceptibility to posaconazole, in whom posaconazole treatment failed, even though the posaconazole regimen had been maximized to 6 times 200 mg daily with intake after high-fat-containing food. The patient succumbed to septic emboli complicating the aspergillosis.
Methods: Antifungal susceptibility testing of *A. fumigatus* strains using EUCAST methodology. Molecular identification and sequence-analysis of the fungal isolates. Monitoring of posaconazole concentrations by HPLC-fluorescence method.

Results: Fungal isolates were borderline to intermediate susceptible to posaconazole (0.25 to 0.5 mg/L) according to proposed guidelines (Verweij et al, Drug Resist Updat 2009; 12:141–7). Sequence-analysis of the infecting fungal strains showed two mutations in the Cyp51A-gene and a tandem repeat in the gene promoter. During treatment, posaconazole trough levels were 0.6 microg/ml. Blood and postmortem tissue posaconazole concentrations indicated AUC/MIC ratios of 30 to 60. Table 1. Culture results and posaconazole drug concentrations in blood and tissue samples obtained at autopsy.
Conclusion: The efficacious AUC/MIC for posaconazole is probably above 200, but this could not be achieved in our patient. Plasma levels >4 microg/ml would have been required to achieve the pharmacodynamic target for these *A. fumigatus* strains with posaconazole MIC of 0.25 and 0.5, which was impossible to achieve with the current posaconazole formulation. Posaconazole should be used caution in invasive aspergillosis caused by strains with attenuated posaconazole susceptibility, as drug exposure may be inadequate resulting in therapeutic failure.

Site	Fungal Culture	MIC μg/ml, (classification)[1]			POS level (microg/g)
		ITZ	VCZ	POS	
Abscess	*A fumigatus*	2 (I)	>16 (R)	0.5 (I)	5.1
Kidney – swab	*A fumigatus*	1 (S)	>16 (R)	0.5 (I)	5.9
Kidney – tissue	*A fumigatus*	1 (S)	>16 (R)	0.5 (I)	6.5
Renal fat tissue	*A fumigatus*	1 (S)	>16 (R)	0.25 (S)	7.1
Liver	Negative				18.4
Spleen	Negative				5.8
Lung	Negative				4.1
Blood	Negative				1.1 (microg/mL whole blood)

[1]ITZ, itraconazole; VCZ, voriconazole; POS, posaconazole

R2639 Identification and antifungal susceptibility of *Candida haemulonii* isolates in a national reference laboratory

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Background: The list of uncommon fungal species causing human infections is growing. Conventional methods of classification based on morphological, biochemical and physiological features have proven ineffective in accurately identifying such species. *Candida haemulonii* has been reported among uncommon yeasts with decreased susceptibility to antifungal agents causing human disease. We have reviewed the identification and antifungal susceptibility results of a collection of clinical isolates of *C. haemulonii*.
Methods: A total of 10 isolates, received in our institution over 10-year period (2001–2010), were evaluated. One of them was isolated from blood and the other nine strains were recovered from superficial sites. They were identified by morphology and biochemical tests. In addition, molecular identification was done by sequencing of ribosomal DNA (ITS domain). Susceptibility testing followed the recommendations proposed by the EUCAST.
Results: A total of 90% (9/10) isolates were not discriminated by phenotyping and strains were classified as unidentifiable according to their biochemical profile. One strain (10%) was misidentified as *Candida sake*. Sequencing-based methods were able to identify all strains analyzed. ITS sequencing of *C. haemulonii* isolates resulted in identity ⩾97% related to the respective type/validated control strain. Most of the isolates (90%) exhibited amphotericin B MIC ⩾1 mg/L. In addition, azole compounds showed poor activity since all isolates exhibited a fluconazole MIC >4 mg/L and eight out of 10 strains showed itraconazole, voriconazole and posaconazole MICs >0.125 mg/L. On the other hand, echinocandins demonstrated good activity in vitro against most of the isolates.
Conclusions: (i) Phenotyping-based methods are not useful for *C. haemulonii* identification. (ii) Molecular identification based on ITS sequencing shows a good performance for classifying of that species. (iii) *C. haemulonii* seems to be resistant to amphotericin B and azoles, thus its accurate identification can be compulsory for appropriate clinical management and treatment of infections caused by it.

R2640 Clinical and epidemiological changes in candidaemia – a comparison of periods 1985–1990 and 2001–2005

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Objectives: *Candida* spp. is the fourth most common pathogen causing nosocomial blood stream infections and invasive infections in immunocompromised and seriously ill patients.
Methods: Two retrospective data sets each with 100 patients from the time periods 1985–1990 (period 1) and 2001–2005 (period 2) respectively were evaluated. All patients whose data were included into this study have been diagnosed with candidemia at the University Hospital of Vienna, a 2200-bed referral centre. The aim of the study was to identify changes in the epidemiology, the clinical manifestation and the outcome in these two groups. The demographic dissemination in terms of age, gender etc. was analysed as well as differences in underlying diseases in both periods. The duration of hospitalisation and time until *Candida albicans* isolation from blood culture were determined. The progress of candidemia, the success of therapy and the mortality rate in these two periods were evaluated.
Results: During period 1 significantly more patients with proven candidemia were found at Intensive Care Units, in period 2 significantly more at medical wards. The main hospitalisation duration until isolation of candida was 23 days in period 1 and 27,9 days in period 2. In both periods two third each of the patients were diagnosed as immunocompromised.
In period 1 60 patients died: 20 in consequence of candidemia, 10 later but from the persistent fungal infection, 16 patients from their underlying disease and 12 from bacterial sepsis. Thirty-seven patients received antifungal therapy, 23 remained untreated. Fifty-four percent of the treated patients died, as opposed 74 percent of the patients without therapy.
In period 2 51 patients died: 23 in consequence of candidemia, 2 later but from the persistent fungal infection, 20 from their underlying disease and 6 patients from bacterial sepsis. Forty-five of the 51 deceased patients received antifungal therapy, 6 remained untreated. Fifty-four percent died in the treated group, 50 percent in the group without treatment.
Conclusion: Although much progress has been made in the treatment of *Candida albicans*, mortality of patients is still high. For a further decrease in candida infections better tools for diagnosis, guidelines for management and more awareness of this pathogen are absolutely essential.

R2641 Trends in candidaemia in one Italian region, Lombardia

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Objectives: To perform a 1-year survey on candidaemia in one Italian region, Lombardia, to verify the epidemiological changes in comparison with the results of a previous survey conducted ten years ago (Tortorano et al. J. Hosp. Infect. 2002; 51: 297).
Methods: The survey was carried out prospectively during 2009 involving 30 Microbiology Laboratories of Lombardia that notified candidaemia episodes defined by at least one blood culture positive for *Candida*.

Results: A total of 354 episodes of candidaemia occurred in 344 patients accounting for an incidence of 1.19 per 1000 admissions (range 0.19–2.3) and 1.20 per 10000 patient days (range 0.2–2.2). These rates were higher compared to those obtained in the previous '90s survey (0.38 per 1000 admissions, range 0.03–1.45, and 0.44 per 10000 patient days, range 0.04–1.64). In the present survey, candidaemia occurred more frequently in patients aged ⩾80 years (15.4 vs 8.1% of the cases) and remained associated mainly, even if at a reduced rate, to surgery (45% of the cases vs 56% in the previous study) and intensive care treatments (35% vs 45%). A shift of the species causing fungaemia was demonstrated by the comparison of the two periods: while the proportion due to *C. albicans* decreased from 58 to 52%, an increase of *C. glabrata* (from 13 to 20%) and *C. tropicalis* (from 6 to 8%) was noted. The proportion of *C. parapsilosis* remained unchanged (14.5%). A decreased of the crude mortality at day 30 from 35 to 32% was observed. The highest mortality rate was detected in patients with *C. tropicalis* (41%) and *C. albicans* (33%) bloodstream infections.
Conclusion: The present study revealed an increasing incidence of candidaemia, mainly in aged subjects, and an increasing proportion of isolates with decreased susceptibility to fluconazole.

R2642 Comparative epidemiology of *Candida* spp. isolated from deep and superficial sites in intensive care, haematology and renal transplant patients during a 2-months period in 8 French hospitals

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Objectives: A prospective, multicentre observational study was conducted in France to compare the epidemiology of *Candida* spp. isolated in high risk patients from intensive care units (ICU), haematology units (HU) and renal transplantation units (RTU).
Methods: From January to February 2010, all *Candida* spp. isolated from patients hospitalized in medical and surgical ICU, adults and paediatrics HU and RTU from 8 French university hospitals were collected. *C. albicans* was identified using chromogenic agar media, and screened with BichroDubli® (Sofibel) to identify *C. dubliniensis*. *C. krusei* and *C. glabrata* were identified with KruseiColor® (Sofibel) and Glabrata RTT® (Sofibel), respectively. All other *Candida* spp. were identified using ID32C® (BioMérieux). Unequivocal identification using rDNA sequencing was done for all *C. glabrata*, *C. parapsilosis*, *C. kefyr*, *C. inconspicua*/norvegensis, and those with ID32C identification score <95%. In vitro antifungal susceptibility was determined by E-test® (BioMérieux). First isolate of each *Candida* sp. from each different body site was tested in each patient.

Candida species	Adults haematology N=444	Paediatrics haematology N=24	Medical intensive care N=495	Surgical intensive care N=381	Kidney transplant unit N=79
C. albicans	58.8%	37.5%	63.2%	62.5%	51.9%
C. dubliniensis	1.1%	8.3%	2.0%	4.5%	0
C. glabrata	16.9%	8.3%	6.7%	13.6%	17.7%
C. tropicalis	4.5%	0	6.5%	6.3%	11.4%
C. parapsilosis	2.0%	8.3%	10.5%	4.2%	5.1%
C. krusei	4.5%	8.3%	3.2%	1.3%	5.1%
C. kefyr	6.5%	0	4.6%	1.6%	5.1%
C. lusitaniae	1.8%	0	1.4%	1.3%	1.3%
C. guilliermondi	0.9%	20.8%	0	0	0
C. inconspicua	1.4%	4.2%	1.0%	0.3%	0
Other species	1.6%	4.2%	0.8%	4.5%	2.5%

Results: Among the 1425 *Candida* spp. isolated from 537 patients, 1346 were from a superficial body site and 78 from a deep one. *C. albicans* was the dominant species with 60.5% of the isolates, followed by *C. glabrata* (12.4%), *C. tropicalis* (6%), *C. parapsilosis* (5.8%), *C. kefyr* (4.4%), *C. krusei* (3.3%), *C. dubliniensis* (2.5%) and others (5.1%). Species distribution differed according to the clinical unit (table) and the body site sampled. Conventional identification methods failed to identify recently described species or uncommon species. ID32C frequently misidentified *C. inconspicua* as *C. norvegensis*. Antifungal susceptibility was determined for 905 *Candida* isolates. None was resistant to amphotericin B. The global rates of resistance were 8.6%, 5.2%, 2% and 0.1% for flucytosin, fluconazole, voriconazole and caspofungin, respectively. Except *C. krusei*, 69% of fluconazole resistant isolates were *C. glabrata*. Among voriconazole resistant isolates, 89% were *C. glabrata*. Isolates with non-susceptibility to caspofungin were *C. guilliermondii* (n = 2) and *C. parapsilosis* (n = 1).
Conclusion: This epidemiological study conducted on a short period of time allowed us to point out differences in *Candida* spp. isolated from different clinical units and body sites. Local epidemiology must be considered for an appropriate empirical antifungal therapy.

R2643 The incidence of *Candida dubliniensis* in clinical material in Czech Republic

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The most commonly isolated yeats *Candida albicans* as well as other yeasts can colonize the gastrointestinal tract of humans and be a reservoir for the development of infections at other body sites. In 1995, a new species of the *Candida* genus was identified and classified as *Candida dubliniensis*. This species shares many phenotypic similarities with *C. albicans*, which leads to its misidentification and underestimation. It was primarily isolated and identified from the samples of HIV-positive individuals and AIDS patients suffering from oral candidiasis, and many other publications reported its prevalence in the oropharynx. The occurrence of *C. dubliniensis* in the human feces samples has been tested only rarely.
C. dubliniensis is not routinely identified in most of clinical laboratories in the Czech Republic. The aim of this study was to evaluate the incidence of *C. dubliniensis* in human clinical material, especially in stool samples. Three common phenotypic methods were used for discriminating *C. dubliniensis* from *C. albicans* (the color of colonies on CHROMagar *Candida*, the inability to grow at 42 ºC and the colony morphology on Staib medium). The identification of *C. dubliniensis* was verified by polymerase chain reaction with the species-specific primer pair and universal primer pair.
The experiments took place from December 2007 to December 2010. We have collected almost 500 samples from the gastrointestinal tract and about 1500 isolates from the airways and oropharynx, all of which were originally identified in the laboratories as *C. albicans*. Two to three % of these isolates were identified as *C. dubliniensis*. The incidence in the oropharynx and airways (3%) was significantly higher than in the stool samples (2%).
The significance of the finding lies primarily in the fact that only a small number of publications deals with this particular clinical specimen and that not many publications about the incidence of *C. dubliniensis* in any other clinical materials have been published in Czech republic.
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R2644 Prophylaxis of invasive fungal diseases with posaconazole in acute myeloid leukaemia: a real-life experience

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Background: Acute Myeloid Leukemia (AML) patients are at high risk of Invasive Fungal Diseases (IFDs). We report our real-life experience with POS prophylaxis in AML. We also compare the performance of POS prophylaxis with an historical, well matched, control group of AML pts who received prophylaxis with Fluconazole (FLUCO) or Itraconazole (ITRA).
Patients and Results: Fifty-five unselected and consecutive AML pts received POS prophylaxis (600 mg daily) between Jan 2009 and Oct 2010. Median age of this population was 47 yrs (range 18–69). All cases were given chemotherapy with anthracyclines and cytarabine. The

POS was started when neutrophil (PMN) count was less than 1000 mL and was stopped at PMN recovery. The median duration of severe neutropenia (PMN lower than 500 mL) was 15 days (range 7–41); 10/55 (18%) of cases had an oral mucositis grade II-III CTC (common toxicity criteria) and 73% (40/55) of these pts received a proton pump inhibitor. An active diagnostic work up was made in all cases with Galactomannan assay, standard chest X-ray and thoracic CT scan in case of fever (FUO) lasting over 48 hours. The median duration of POS prophylaxis was 15 days (range 7 to 41). Only 4/55 (7%) of pts required parenteral empiric or pre-emptive antimycotic therapy and only 2/55 (4%) experienced a proven IFDs (*Fusarium solani* fungemia and *Aspergillus* sp pneumonia). Mortality IFDs related was 0%. POS was well tolerated and only 9% (5/55) of pts experienced mild drug related side effects. No cases of POS discontinuation, due to the side effects or intolerance, were reported. When we compare the 55 pts who received POS with an historical control group of 55 AML pts who received FLUCO (45/55) or ITRA (10/55)prophylaxis, between Jan 2008 and Jun 2009, no significant differences were observed for underling disease status, age, IFDs risk factors, days of severe neutropenia and days of prophylaxis. Instead, there were significant differences in breakthrough IFDs (4% in POS group vs 16% in control group; P = 0,02), and in days of parenteral antymicotic therapyn (37 vs 163).

Conclusions: This real-life experience confirms that POS prophylaxis is feasible, safe, well tolerated and effective (prevention of IFDs) in unselected AML patients. Only 7% of these high risk pts required parenteral antimycotic therapy and only 4% experienced breakthrough IFDs. We also confirm that POS is more effective than FLUCO or ITRA as antifungal prophylaxis in AML pts.

R2645 Elevated serum IgG responses against *Aspergillus fumigatus* proteins prior to haematopoietic stem cell transplant identify patients at risk for invasive aspergillosis

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Background: There is a need for screening tests that identify patients who are at particular risk for invasive aspergillosis (IA). Patients (pts) with elevated serum antibody responses against *A. fumigatus* proteins prior to haematopoietic stem cell transplant (HSCT) may be at risk to develop IA.

Methods: We screened *A. fumigatus* expression libraries with serum from a pt who survived IA. We collected serum prior to HSCT (baseline) from 19 pts who developed IA and 54 controls, all of whom received fluconazole prophylaxis. We also collected serum at the time of diagnosis (acute), and, for 13 pts, 4 weeks after diagnosis. We measured serum IgG concentrations against 6 purified recombinant proteins by ELISA.

Results: We identified 20 *A. fumigatus* proteins, and purified Eno1, Ahp1, Hsp90, Pep2, Crf1 and Cdc3. Among pts with IA, 68% (13/19) were diagnosed within 30 days of HSCT (early) and 32% after 60 days (late). 47% (9/19) of pts with IA died. Serum IgG concentrations were highest against Eno1 and Ahp1. Baseline IgG concentrations against Eno1, Hsp90, Pep2, Crf1 and Cdc37 were significantly higher among pts with IA than controls (all $p \leqslant 0.05$). Sensitivities and specificities of baseline IgG in identifying pts with IA ranged from 67–84% and 52–65%, respectively. Baseline responses against Eno1, Ahp1, Hsp90, Crf1 and Cdc37 were associated with development of IA (all $p \leqslant 0.05$), with best results for Eno1 ($p = 0.003$). Assuming 15% prevalence of IA, PPV and NPV of baseline IgG against Eno1 would be 25% and 95%, respectively. For the combination Eno1/Cdc37, sensitivity and specificity of baseline IgG were both 72% ($p = 0.001$), and PPV and NPV would be 31% and 94%, respectively. Positive IgG responses against Hsp90, Pep2, Crf1 and Cdc37 were specifically associated with early IA (all $p \leqslant 0.02$). Overall, there were no significant differences in IgG concentrations against any of the proteins across three time points. Pts who survived IA were more likely to demonstrate increased IgG concentrations against Hsp90, Pep2 and Crf1 in week 4 compared to baseline (all $p \leqslant 0.05$).

Conclusions: Baseline IgG concentrations against Eno1 and other proteins prior to HSCT may identify pts likely to develop IA. Our results suggest that some pts are infected or colonized with *A. fumigatus* at the time of HSCT, and IA may result from progression of infection/colonization. At the same time, elevated IgG responses may reflect defects in innate immunity that predispose to fungal infection.

R2646 Nosocomial bloodstream yeast infections in children and adolescents in Baskent University Adana Research and Practice Center: a 2-year retrospective study (2008–2010)

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Objective: As nosocomial candidemia is one of the most important problem of hospitalized patient, we investigated the epidemiologic features and antimicrobial susceptibility patterns of bloodstream *Candida* isolates from paediatric and adolescent patients in our centre during 2008–2010, retrospectively.

Material and Methods: Blood cultures were performed using BACTEC 9240. *Candida* species were identified with standard tests and API 20C AUX (bioMerieux) kit. Fluconazole, voriconazole, itraconazole susceptibilities were investigated by ATB FUNGUS 3 (bioMerieux).

Results: During the 2 years of period total 159 *Candida* and 11 other yeast species were isolated from bloodstream infections which 103 (60%) of them from pediatric and 67 (40%) of them from adolescents patients. Clinical distributions of adolescent patients were intensive care units (31%), oncology-haematology clinics (22%) and burn unit (16%) and pediatric patients were from paediatric intensive care units (40%), paediatric oncology-haematology clinics (28%) and burn unit (25%). Species distributions were given in table.

The values of MIC_{50} values of *Candida* isolates from pediatric patients were 1mg/L, 0.06 mg/L, 0.125 mg/L and MIC_{90} values were 16 mg/L, 0.5 mg/L, 0.5 mg/L for fluconazole, voriconazole, and itraconazol, respectively. For adolescent patients, MIC_{50} values were same as pediatric group but MIC_{90} values were 1 mg/L, 0.125 mg/L, 0.5 mg/L for fluconazole, voriconazole, and itraconazol, respectively. For pediatric patients, one of the fluconazole, voriconazole and itraconazole resistance were obtained from of *C. albicans* strains and nonalbicans strains were 6.9%, 11.3%, respectively.

Conclusion: Our data showed that candidemia was aserious problem espacially in intensive care units. *C. albicans* was still the predominat species. *C. parapsilosis* is the second most frequently reported nosocomial bloodstream infection for candidemia agents. MIC_{50} values of azoles were same in both groups, whereas MIC_{90} values were higher in pediatric patients for fluconazole and voriconazole.

Table: *Candida* species distribution according to patient groups.

Candida species	Pediatrics patients	Adolescents patients
C.albicans	48	26
C.parapsilosis	32	20
C.tropicalis	7	7
C.famata	4	5
C.krusei	2	1
C.pelliculosa	2	-
C.glabrata	1	4
Other*	7	4
Total	**108**	**67**

* *Rhodotorula, Saccharomyces, Trichosporon.*

R2647 *Aspergillus*-specific immunohistochemistry and in situ hybridisation facilitate diagnosis of aspergillosis

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Background: The diagnosis of invasive asperigllosis (IA) is limited by poor sensitivity and specificity of microbiologic cultures, and the inability of conventional histopathologic tests to distinguish IA from invasive fungal infections (IFI) caused by non-*Aspergillus* moulds. *Aspergillus*-specific immunohistochemistry (IHC) and in situ hybridization (ISH) could facilitate direct diagnosis of IA within tissue.
Methods: IHC and ISH were performed on deparaffinized, formalin-fixed sections from previously diagnosed cases of proven IFI, and slides interpreted by pathologist blinded to diagnosis. IHC: Primary *Aspergillus* antibody (Abcam, MA) and secondary anti-rabbit antibody were detected with Avidin Biotin Complex and visualized using liquid DAB. ISH: Commercially synthesized Locked Nucleic Acid probe for *Aspergillus* was detected using antiflourescein AP. Poly T stained tissue section was used to determine the area of tissue to be studied.
Results: 8 tissue sections were available from patients with IA (*A. fumigatus* = 5; *A. niger* = 1; non-speciated = 2, and 12 sections were available from patients with IFI due to other fungi (Zygomyces = 6; *Candida* = 5; *Dactylaria gallopava* = 1). IA samples were obtained from 6 tissue biopsies (3 lungs, 2 upper airways, 1 vocal cord), and 2 autopsy samples. 2 serial sections from one patient with IA due to *A. fumigatus* were excluded from the study because positive control stains were negative. The sensitivity of IHC and ISH for IA was 83% (5/6) and 100% (6/6), respectively. IHC was falsely negative in 1 pt with IA due to *A. fumigatus*. The specificity of IHC and ISH was 100% (12/12), as the tests were each negative in all sections associated with IFI due to non-*Aspergillus* fungi. IHC was associated with significant levels of non-specific background staining in 33% of the sections that could be analyzed, compared to 0% for ISH.
Conclusion: Data from this pilot study suggest that *Aspergillus*-specific IHC and ISH will facilitate diagnosis of IA and exclude IFI due to non-*Aspergillus* fungi. ISH was superior to IHC by limiting false negative results and non-specific background staining. We are currently assessing IHC and ISH prospectively on non-fixed tissues.

R2648 First Romanian surveillance study of fungaemia: species distribution and antifungal susceptibility

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Objectives: This paper presents the preliminary results of a multicentre study on fungaemia performed in four tertiary hospitals from Romania between 2007 and 2009. The aim of this study was to evaluate the species distribution and the antifungal susceptibility of fungal strains isolated from bloodstream cultures.
Methods: The study has included a total number of 12215 blood cultures from patients hospitalized in departments of cardiovascular surgery, general surgery, intensive care, oncohaematology, and infectious diseases. The range of age varied between 12 days and 86 years. The major clinical sign that required the bloodstream cultures has been the persistent fever. In order to detect the fungal strains we used the Hemoline Performance Duo bottles, the BacT/ALERT FA bottles, and the BacT/ALERT Microbial Detection System. The isolated strains have been identified using specific tests. In vitro susceptibility was assessed by following the guidelines of AFST-EUCAST E. Def. 7.1. Two azoles (fluconazole and voriconazole) and three echinocandins (caspofungin, micafungin, and anidulafungin) were tested.
Results: The overall percentage of fungaemia was 1.49% (182 positive blood cultures). Out of a total number of 182 yeast strains, 56 (30.76%) were *Candida albicans*, 112 (61.54%) were other *Candida* species, and the rest – 14 strains (7.70%) belonged to different genera (i.e. *Cryptococcus*, *Geotrichum*, *Rhodotorula*, *Saccharomyces*, and *Trichosporon*). The distribution of minimum inhibitory concentrations (MICs) was as follows: fluconazole (MIC_{50}: 0.5 mg/L; MIC_{90}: 32 mg/L), voriconazole ((MIC_{50}: 0.0156 mg/L; MIC_{90}: 0.25 mg/L), caspofungin (MIC_{50}: 0.125 mg/L; MIC_{90}: 1 mg/L), micafungin (MIC_{50}: 0.25 mg/L; MIC_{90}: 2 mg/L), and anidulafungin (MIC_{50}: 0.0312 mg/L; MIC_{90}: 0.125 mg/L).
Conclusions: The rate of positive blood cultures emphasizing fungaemia is still low comparatively with those exhibiting bacterial infections. The study underlines the diversity of fungal strains isolated from bloodstream cultures. It can be noticed a relatively low frequency of *Candida albicans* strains and the emergence of fungaemia due to non-*albicans* species accordingly to worldwide trend. The rates of resistance to fluconazole and voriconazole were 16.49% and 12.09% respectively. The MIC_{90} value for all echinocandins indicates in vitro susceptibility for more than 90% of the total strains, with no significant differences between caspofungin, micafungin, and anidulafungin.

R2649 Antifungal use at a tertiary care centre using only electronic data

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Objective: There are limited data on the clinical use of antifungals, especially the echinocandins. The objective of the study was to describe the use of antifungals among patients admitted to a tertiary care centre using only electronic medical data.
Methods: Patients admitted to Barnes-Jewish Hospital (BJH), a 1250-bed tertiary care centre in the US, from 10/1/2004 to 12/31/2009, who received systemic antifungal treatment, were included in this study. Electronic data was collected from the Washington University and BJC Healthcare Medical Informatics Database.
Results: There were 3229 patients in the analysis. Mean age was 56±16 years. 55% (1782/3229) were males; 74% (2401/3229) were Caucasian, 21% (668/3229) were African-Americans, 1% (25/3229) were Asians, and 0.3% (9/3229) were of Hispanic ethnicity. Median length of stay was 27±24 days. Median APACHE II score was 11 and median Charlson Index of Co-Morbidity was 3. 38% (1226/3229) received anidulafungin, 36% (1152/3229) received fluconazole, 22% (729/3229) received caspofungin, and 4% (121/3229) received amphotericin. Crude in-hospital mortality was 27% (818/3229). 56% (1797/3229) were admitted to the ICU during their admission. 27% (861/3229) had neutropenia (ANC < 500). Co-morbid illnesses included malignancy (37%; 1199/3229), heart disease (26%; 851/3229), chronic lung disease (19%; 626/3229), diabetes (17%; 542/3229), and chronic kidney disease (12%; 392/3229), and hepatic dysfunction (10%; 329/3229). 19% (604/3229) had a positive blood culture for *Candida* species. 54% (325/604) of the isolates collected from blood were *C. albicans*, 20% (118/604) were *C. glabrata*, 16% (95/604) were *C. parapsilosis*, 6% (37/604) were *C. tropicalis*, 6% (37/604) were *C. lusitaniae*, and 3% (19/604) were *C. krusei*. An additional 16% (500/3229) were treated for *Candida* species isolated from a normally sterile site. 1% (44/3229) of patients received treatment for non-candida invasive fungal infections with 75% (33/44) being probable or definitive aspergillus infections.
Conclusions: During the study period, the majority of patients requiring systemic antifungal treatment were treated with echinocandins, primarily anidulafungin and caspofungin. Less than 40% of antifungal therapy was used to treat a probable or definitive fungal infection.

R2650 Invasive pulmonary aspergillosis in patients admitted with acute exacerbation of chronic obstructive pulmonary disease

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Objectives: Invasive pulmonary aspergillosis (IPA) among patients with chronic obstructive pulmonary disease (COPD) is increasing in frequency and associated with mortality exceeding 70% in some series. We conducted this study to find out the approximate incidence of IPA in

patients hospitalized with acute exacerbation of COPD (AECOPD), its true mortality and to identify potential factors associated with mortality.
Methods: We retrospectively included all patients admitted with AECOPD and isolation of *Aspergillus* over the last 3 years. IPA was defined according to Bulpa criteria. Charts were retrospectively reviewed and patients were classified into probable IPA and colonization.
Results: We identified 68 patients admitted in the last 3 years with AECOPD and aspergillus isolation. Thirty-seven (54%) had COPD stage III-IV. Forty-six (68%) were male and median age was 69 (55–90). Fifty-five patients (81%) had increased dyspnoea and 52 (76%) had radiological alterations, mostly new infiltrates in chest x-ray. Thirty-nine (57%) received >700 mg of steroids during admittance and 16 (24%) had received >20 mg/day during the past 3 months. Twenty patients (28%) had more than one aspergillus isolation. Twenty four patients (35%) received voriconazole for a median of 19 days (1–50 days). Crude mortality was 21% (14/68).
Patients with COPD stage III-IV were older (71 vs 63 years, p = 0,02) and had higher mortality, 13/37 (35%) vs 3/32 (9%) (p = 0,01, OR: 3,9 CI95: 1,8–15). Similarly, patients with COPD stage III-IV received more frequently high dose steroids 28/37 (76%) vs 11/32 (34%), p < 0,001, and had more frequently increased dyspnoea during admittance, 36/37 (97%) vs 20/32 (62%), p < 0,001. Neither daily dose of steroids nor voiconazole therapy were associated with increased mortality. In multivariate analysis, only increase dyspnoea was significantly associated with mortality.
Conclusion: COPD patients stage III-IV had increased mortality because of IPA although it is lower than described by other authors. Neither previous nor high steroid doses during admittance were associated with mortality.

R2651 Accuracy of the β-D-glucan assay for the diagnosis of *Pneumocystis jirovecii* pneumonia: a meta-analysis

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Objectives: The diagnosis of *Pneumocystis jirovecii* pneumonia (PCP), which affects various types of immunocompromised patients, can be at times problematic. We sought to evaluate the diagnostic accuracy of (1?3)-β-D-glucan (BDG) for the diagnosis of PCP.
Methods: We did a meta-analysis of relevant studies, identified through PubMed and Scopus. Eligible studies were those that reported BDG diagnostic data in cases with documented PCP and controls with other conditions. We excluded cases of invasive fungal infections or healthy controls. We performed a bivariate meta-analysis of sensitivity and specificity and constructed a hierarchical summary receiver operating characteristics (HSROC) curve.
Results: Twelve studies were included in the meta-analysis. BDG data were provided for 334 PCP cases and 1663 controls. The pooled (95% confidence interval) sensitivity and specificity of BDG were 94.7% (90.2–97.2%) and 86.6% (81.5–90.5%), respectively. The positive and negative likelihood ratios were 7.1 (5.1–9.9) and 0.06 (0.03–0.12), respectively. The area under the HSROC curve was 0.964 (0.944–0.977). We did not identify the presence of a threshold effect or of substantial statistical heterogeneity.
Conclusion: Serum BDG can be a useful test in the diagnosis of PCP. It shows excellent sensitivity and very good specificity. Still, in clinical practice the test results should be interpreted in the context of the underlying clinical characteristics of the individual patient. Further studies could determine the optimal cut-off level of the test and the appropriate diagnostic strategy for suspected PCP.

R2652 Antifungal management strategy for high-risk neutropenic patients based on itraconazole levels

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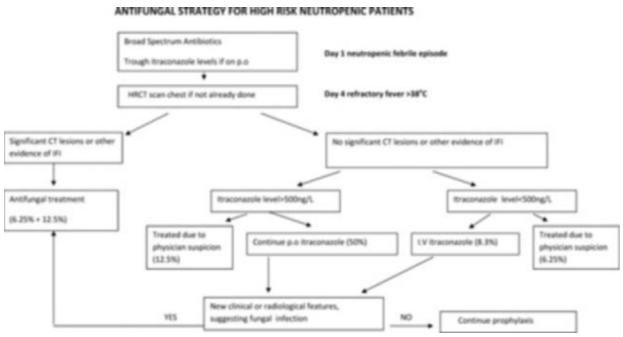
Objectives: This study assessed a pilot antifungal prevention strategy based on itraconazole levels in consecutive high risk patients with the aim of reducing empirical treatment. The patients underwent chemotherapy or allogeneic stem cell transplantation for haematological malignancies and received itraconazole suspension prophylaxis. The study is ongoing.
Methods: 48 neutropenic febrile episodes, 25 of which were refractory at 4 days to broad spectrum antibiotics initiated on day one, were studied. All patients had serum galactomannan assays (EIA) twice a week and trough itraconazole levels measured on day one of fever. If they were still febrile after 4 days, HRCT scan chest was performed. Antifungal treatment was required to be given only when well defined clinical, microbiological and radiological criteria were present. Invasive Fungal Disease (IFD) is defined according to EORTC/MSG criteria 2008.
Results: Patients with normal intraconazole levels were included in the following groups: a. NO ACTION TAKEN (no evidence IFD) 24 patients all alive (50%) b. TREATED DUE TO PHYSICIAN SUSPICION (no evidence IFD) 7 alive (12.5%) c. TARGETED TREATMENT (HRCT:IFD and or gal:pos) 5 possible alive,1 probable alive, 1 possible dead (totally 12.5%). Patients with subtherapeutic levels were included in the following groups: d. SWITCHED TO IV ITRACONAZOLE (no evidence of IFD) 4 patients alive (8.3%) e. TREATED DUE TO PHYSICIAN SUSPICION (no evidence IFD) 2 patients alive,1 dead (totally 6.25%) f. TARGETED TREATMENT (HRCT:IFD and or gal:pos) 1 possible alive, 1 possible dead (totally 6.25%) g. NO ACTION TAKEN (no evidence IFD) 1 alive (2%).
Conclusions: The incidence of probable or proven IFD was 2%. Three patients with possible IFD died (all had received iv antifungal therapy). 20% of the patients overall had subtherapeutic itraconazole levels. Our strategy reduced the rate of antifungal use by 50%.



R2653 Efficacy and safety of voriconazole and posaconazole as second line therapy in ABPA and SAFS

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Background and Objectives: Allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitisation (SAFS) are progressive allergic fungal lung diseases. Current treatment with itraconazole (itra) is associated with a 40% failure rate and adverse events (AEs). Very little is known about the response rates or appropriateness of treatment with voriconazole (vori) or posaconazole (posa). This study assessed the effect of vori or posa as second and third line therapy.
Methods: We conducted a retrospective audit of 27 adult asthmatic patients who fulfilled diagnostic criteria for either ABPA or SAFS. All patients had previously received itraconazole. Vori (300–600 mg/day) or posa (800 mg/day) (adjusted by TDM) was given for at least 6 months if tolerated. Clinical, radiological and immunological evaluation was used to assess response. We defined response as improvement in symptoms and either fungal serology or radiological abnormalities. The rates of clinical response or failure and their adverse effects (AEs) after 3, 6, and 12 mos of treatment were analyzed. Co-existing diagnoses, *Aspergillus* antibody titre, vori and posa levels and lung function were used as co-variates.
Results: There were 27 patients, ABPA (n = 22) or SAFS (n = 5), 11 males, median age = 59 yrs. All patients had failed itra (n = 11) or developed AEs (n = 12), had low serum concs (n = 2) or itra resistance

(n = 2). There were 34 courses of therapy analysed, 25 with vori and 9 with posa; only 2 posa courses were not preceded by vori (resistance). Clinical response to voriconazole was observed in 17/25 (68%) at 3 mos, 15/20 (75%) at 6 mos and 12/16 (75%) at 12 mos, compared to 7/9 (78%) at 3, 6 and 12 mos for posa. 6/25 (24%) vori pts had AEs requiring discontinuation before 6 mos compared to 0/9 posa patients. Vori AEs included GI upset (7), skin photosensitivity (11), blistering (4), visual light flashes (10), insomnia (2), visual hallucinations (2), depression (1), adrenal suppression (2), peripheral neuropathy (4), eye irritation (2), vivid dreams (1), dizziness (1) and headache (1) but most of them were transient and mild. Posa AEs included insomnia (2), GI upset and mild liver impairment. Among those who discontinued, 4 relapsed (one at 3 mo, 3 at 12 months).

Conclusion: Both voriconazole and posaconazole are safe and effective treatment options as second line antifungal therapy for SAFS and ABPA. Larger prospective studies are required.

		Outcome of courses of therapy (%)		
		3 mo	6 mo	12 mo
ABPA				
Vori	Response	13/20 (65)	11/15 (73)	9/13 (69)
	Stable	2/20 (10)	2/15 (13)	2/13 (15)
	Failure	1/20 (5)	0/15	2/13 (15)
	Discontinued (AEs)	4/20 (20)	2/15 (13)	0/13
Posa	Response	7/9 (78)	7/9 (78)	7/9 (78)
	Stable	2/9 (22)	2/9 (22)	0/9
	Failure	0/9	0/9	2/9 (22)
	Discontinued (AEs)	0/9	0/9	0/9
SAFS				
Vori	Response	4/5 (80)	4/5 (80)	3/4 (75)
	Stable	1/5 (20)	1/5 (20)	1/5 (20)
	Failure	0/5	0/5	0/5
	Discontinued (AEs)	0/5	0/5	0/5

AIDS and HIV infection

R2654 Levels of HIV-1 RNA suppression in two groups of salvage patients: relation to therapy and viral dynamics

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Background: The new methods of measuring HIV-1 RNA have lowered the levels of detection, and not all those patients whose viremia was classified as undetectable have levels below the new cut-offs. In this study we tried to assess some of the factors related to deeper HIV-1 RNA suppression among subjects on rescue therapy.

Methods: The VERSANT HIV-1 RNA 1.0 Assay kit (kPCR, Siemens) is a procedure of kinetic polymerase chain reaction (kPCR) with reverse transcription (RT) for the direct quantification of HIV-1 RNA, with a range of detectability between 37 and 11.000.000 copies/mL. Below 37 copies, the system can still see some signal (SS) or no signal at all (NS), suggesting that the virus concentration in the sample in this case is extremely low. We selected our adherent patients on salvage therapy with darunavir/raltegravir-based regimens (the deep salvage patients, A) and matched each with patients on late lopinavir-based regimens (the experienced patients, B).

Results: The two populations (A, n = 33, and B, n = 32) were homogeneous by age (48, A, vs 45.1 years, B, P = 0,13), baseline CD4+ T-cells (292 vs 338/mmc, P = 0,4) HIV-1 RNA log10 copies/mL (4,6 vs 4,8, P = 0,6), and time on viral suppression (112,6 vs 115,1 weeks, P = 0,77). However, group A was on average in 11th treatment line with a mean GSS = 1,88, while group B was scattered around the 5th line, having a GSS = 2,83, P < 0,001. 21/33 subjects in group A and 16/32 in group B reached NS viremia, p = 0.32. Reaching NS viremia does not seem to be correlated with lower baseline viral load (4,6 in NS vs 2,9 log copies/mL in SS, P = 0,69), nor even with baseline GSS (2,3 vs 2,4, P = 0,81). Also the CD4-T cell nadir (158 vs 149, P = 0,77) and zenith HIV-1 RNA log log10 copies/mL (5,2 vs. 5,6, P = 0,21) were not predictive of NS response.

Conclusions: Overall, a fair proportion of subjects in rescue therapy had NS viral loads (57%), and late treatment lines were not associated to worse virologic outcome. The relationship between extreme viral load suppression (NS) and the latently infected T-cell pool will be further investigated.

R2655 Cost of antiretroviral treatment in senior naïve and experienced HIV-infected patients

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Introduction: Since HAART introduction, the decreased mortality rate, the longer survival and the availability of novel drugs is accompanied to an increase in senior HIV infected patients.

In this cross sectional study is evaluated antiretroviral (ARV) cost according to treatment line in senior (sHIV) compared to junior (jHIV) HIV infected pts.

Methods: ARV regimens in sHIV infected patients older than 50 years are compared to those used in jHIV patients followed up in the II Div Inf Dis, L Sacco Hospital, Milan – Italy.

Clinical, immunovirological and current treatment data were collected on divisional data base on 30 November 2010. Cost of antiretroviral treatments are based on the prices reserved to L Sacco Hospital and are presented as mean cost/patient/day (CPD).

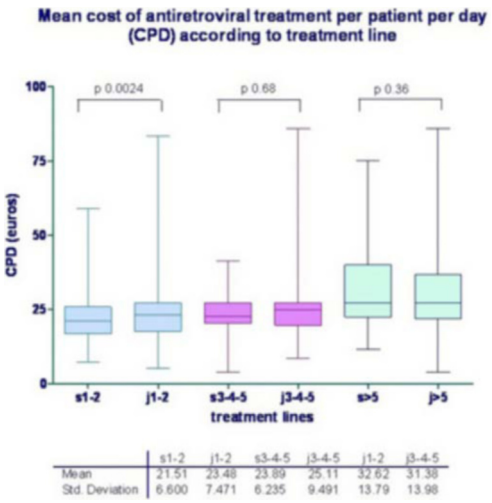
Results: Among 1251 HAART treated pts (404 sHIV and 847 jHIV), 534 (42.68%) were on their first or second line treatment, and 712 pts had experienced ⩾3 antiretroviral regimens.

Viral load suppression <50 cp/ml was achieved in 908 (85.66%) out of 1060 available viral load assessed within 1 month from the enrollment with no difference between sHIV and jHIV (88.3% and 84.4% respectively; p = 0.092); no difference in rate of suppression by treatment line (χ^2 p 0.74) was assessed. The two groups were comparable for class of antiretrovirals to be included in the treatment regimen (χ^2 p 0.4) even if a NNRTI based regimen in sHIV and a PI based regimen in jHIV was preferred.

Increasing proportion of rescue molecules (which include enfuvirtide, maraviroc, raltegravir and darunavir) are included in therapeutic regimen in multi-experienced patients associated to a proportional reduction in NNRTI containing regimens.

Both in sHIV and jHIV patients the mean CPD of each treatment line progressively increases from €22.02 and 23.71 in first line treatment to €4083 and 38.85 CPD in patients with >10 treatment lines previously experienced respectively.

When CPD were compared in sHIV and jHIV pts according to treatment lines a significantly lower cost was observed in sHIV in the first-second line treatment while comparable costs are registered in multi experienced patients (figure).



Discussion: Preliminary results of this cross sectional study show a difference in the early treatment choices in sHIV versus jHIV pts with NNRTI based regimens preferred in the older aged group with a comparable efficacy rate. In experienced patients PI based or rescue molecules based regimens are equally represented in the two groups.

R2656 Comparisons of three methods to estimate HIV incidence rates among persons seeking voluntary, anonymous counselling and testing services in Taiwan

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Objective: The annual case number of persons who are newly diagnosed with HIV infection continues to increase in Taiwan after successful control of HIV outbreak among injecting drug users that took place between 2003 and 2007. Whether the increasing case number is related to increased awareness and HIV testing activities or increasing incidence in high-risk populations, such as men who have sex with men (MSM), remains to be investigated. In this study, we aimed to compare 3 methods to estimate the incidence rate of HIV infection among persons seeking voluntary, anonymous counseling and testing services (VCT) at a university hospital.
Methods: Between 1 May, 2006 and 30 September, 2010, 9410 persons were tested anonymously for HIV antibodies at the National Taiwan University Hospital. Demographics and behavioral data were obtained at the time of the counseling. Three methods were used to estimate HIV incidence: first, based on self-reported dates of prior tests; second, based on linking prior records with an unique testing code; and third, based on the Serologic Testing Algorithm for Recent HIV Seroconversion (STARHS).
Results: During the 4-year study period, 311 individuals (3.50%) were diagnosed as HIV-positive. Comparable overall seroconversion rate was found by the three methods, 5.19, 4.64, and 3.88 per 100 person-years [PY], respectively. The incidence rate of HIV infection in MSM by the three methods was 7.44, 6.91, and 6.22 per 100 PY, respectively, which was significantly higher than that in heterosexuals (0.70, 0, and 0.89 per 100 PY, respectively). Greatest variability was observed among the 3 methods in the estimation of the HIV incidence rate in non-MSM injecting drug users (31.58, 0, and 25.95 per 100 PY, respectively). An increasing trend of overall HIV incidence rate was observed from 2007 (3.99, 4.11, and 2.34 per 100 PY) to 2009 (7.60, 5.51, and 3.79 per 100 PY) by the three respective methods.
Conclusions: The overall incidence rate of HIV infection was increasing among VCT clients, and MSM had a significantly higher annual rate of HIV infection than heterosexuals. The findings suggest that, in addition to increased testing activities that contribute of to the increasing case number of HIV infections in Taiwan, increased transmission of HIV continues to occur among MSM.

R2657 Prevalence of HIV infection and the correlates among homeless in Tehran, Iran

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Objectives: This study was conducted to determine the prevalence of HIV rate infection among homeless population and also to find out HIV related risk behaviors in Tehran, Iran.
Methods: Tehran municipality stacks up homeless people for assessment HIV and began collaboration with Iranian Research Center for HIV/AIDS (IRCHA) departments during one year, to carry out prevalence of HIV infection among 10657 homeless people. The results were analyzed for associations between demographic information, family support, status of drug abuse, relation with family and friends and HIV infection prevalence.
Results: Overall HIV prevalence was 1.7% (95% confidence interval 1.4–1.9). Factors independently associated with HIV infection included history of using drugs [AOR 8.15 (4.86–13.67)], older age [AOR 1.80 (1.08–2.99) for 40–55 yr], occupation [AOR 1.64 (1.19–2.24) for unemployed], and no relation with family [AOR 1.82 (1.30–2.54)].
Conclusion: This study supports that using injection drug is contributing to the increased spread of HIV. Harm reduction programs should be urgently expanded, particularly among IDU's homeless.

R2658 A new trend of rapid disease progression in Japanese patients recently infected with HIV-1

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Objective: The natural course of HIV-1 infection includes 10 years of asymptomatic period before the development of AIDS. However, in Japan, the disease progression process seems faster in recent years. This study is to document the natural disease progression of recently HIV-1-infected patients in Japan.
Methods: The study subjects were all 108 new patients with primary HIV-1 infection during the period from 1997 through 2007. We evaluated their clinical symptoms and laboratory data, and then analyzed disease progression in 82 eligible patients. Disease progression was defined as fall in CD4 count below 350/microliter and/or initiation of antiretroviral therapy.
Results: Ninety percent of the patients were infected through homosexual intercourse. All patients had at least one clinical symptom (median; 5, range; 1–11) related to primary HIV-1 infection, with a median duration of 20 days (4–66) and 53% of them had to be hospitalized due to severe symptoms. The median CD4 count and viral load at first visit were 320/microliter (48–980) and 4.81 log10/ml (3.66–5.71), respectively. None developed AIDS during the study period. Estimates of risk of disease progression were 61.0% at 48 weeks and 82.2% at 144 weeks. In patients who required antiretroviral therapy, the median CD4 count was 215/microliter (range, 52–858) at initiation of such therapy. Among patients with CD4 count <350/microliter at first visit, 53% never showed recovery of CD4 count (>350/microliter) without antiretroviral therapy.
Conclusion: Despite possible bias in patient population, disease progression seemed faster in symptomatic Japanese patients with recently acquired primary HIV-1 infection than the previously defined natural course of the disease.

R2659 Anti-HIV low-avidity persistence in recently infected patients, treated and not treated with early antiretroviral therapy (HAART)

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Objective: To evaluate the maturation of HIV-1 specific antibody avidity in patients with recent HIV-1 infection and to study the early highly active antiretroviral therapy (HAART)impact.
Methods: From May to November 2010 we collected sequential samples (23) from 6 patients with recent HIV-1 infection (seroconversion); three of them were treated with HAART, three were not treated. Samples were tested with HIV Ab/Ag Combo assay (ARCHITECT, Abbott Diagnostics), HIV 1–2 Western blot (ALFA-Biotech) and with HIV-1 RNA (RT-PCR,Siemens Healthcare). The anti-HIV avidity index (AI) was calculated by diluting two aliquots of each sample in 1M guanidine chlorydrate or in PBS, by using ARCHITECT HIV Ag/Ab. The S/CO ratio values between the two aliquots was calculated according to literature: AI <0.80 indicates a recent infection (<6 months). We assayed AI also in three patients not recently infected (16 samples) and 42 long-term HIV-1 infections (22 with CD4+ <200, 10 HIV-1 B and 10 HIV-1 non-B genotypes (6 C, 2 F1, 1 D, 1 CRF02_AG).
Results: AI remained low (<0.80) for the first 6 months from seroconversion in one patient treated very early after diagnosis (average: 0.31+0.05), while in the two patients treated later the AI reached levels >0.80 within 4–5 months from seroconversion. Fourteen out of 16 patients not recently infected had high AI (average: 0.96+0.12). The average AI in HIV positive subjects with low CD4+ was 0.86+0.16, and

in three cases a low AI was detected. All other sera from long-standing HIV infection had a high AI (average: 1.01+0.08), with no differences between B and non-B genotypes.
Conclusions: These results may have relevant implication in understanding the complex mechanism of maturation of the immune response to HIV. We will expand our study to other patients with recent HIV-1 infection and to treated patients in order to confirm this preliminary data.

R2660 H1N1 A influenza in HIV-positive patients: a case-control study

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Objective: The aim of this study is to compare the outcome and the presence of risk factors between HIV patients and general population diagnosed with H1N1 influenza.
Materials and Methods: Case-control study of 12 HIV adult patients diagnosed with H1N1 A Influenza. ⩾2 controls were recruited for each case, choosing in random order. Diagnosis was confirmed in all patients by identifying antigenic influenza virus A and B RNA immunochromatography and Influenza A Virus H1N1 by Real Time PCR in nasopharyngeal swab. The study period was July 29 to December 31, 2009 performed in a 592 beds Universitary Spanish hospital serving an urban population around 400,000.
Statistical analysis: Descriptive statistics of quantitative variables was calculated with the SPSS program v 15.01. The odds ratio (OR) and confidence interval (CI) were analyzed by an exact test (Mid-p).
Results: During the pandemic phase, 662 samples of symptomatic patients resulted positive for H1N1.12 were HIV+, 2 women,10 men. 6/12 (50%) were hepatitis C virus (HCV) coinfected. Median CD4 + count was 443/mm3 (167–1297). 6/12 had <20 copies HIV/ml. Only 2 patients required hospitalization because of bacterial pneumonia and exacerbation of COPD (Chronic Obstructive Pulmonary Disease). There was no mortality in any case. 30 controls were selected: 12 men,18 women. Median age was 32.5 years (18–72). 3 controls required hospitalization due to decompensation of underlying diseases. Outcome was favorable in all cases. Smoking was more common in HIV patients than in controls (OR 2.44). 1/3 patients and controls suffered from COPD. 40% of controls had some underlying condition in contrast to none of the HIV patients, excluded chronic liver disease and COPD. 23.3% of controls were on chronic steroid therapy for various reasons, mainly COPD. Two of the controls were obese.
Conclusions: Although the HIV associated immunosuppression may initially predict a more severe clinical course of influenza, our results suggest that evolution does not differ from not HIV-infected people. We found a higher frequency of smoking among HIV patients, with an odds ratio of 2.4. It is striking also the high occurrence of COPD in both groups, without differences between them. If we exclude HCV co-infected patients with liver disease, none of the HIV patients had other chronic diseases, unlike controls.

R2661 Mal/TIRAP S180L variant polymorphism is associated with decreased infection risk in patients with advanced HIV-1 infection

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Objectives: MyD88 adaptor-like (Mal/TIRAP) is an adaptor protein bridging activation of Toll-like receptors 2 and 4 after stimulation by exogenous and endogenous ligands. We investigated the association between the presence of the S180L SNP of Mal and the risk of severe infection in individuals with human immunodeficiency virus (HIV)-1 infection.
Methods: The SNP S180L was determined in a cohort of 179 HIV-1 infected Greek patients. Analysis of the prevalence of this SNP in relation to the infectious complications was evaluated.
Results: One hundred and thirty two (73.3%) patients were bearing the wild type (WT) haplotype, 43 (24%) were heterozygous (HT) for the SNP, and four (2.2%) were homozygous (HO) for the variant allele. 39 patients carrying the WT haplotype experienced an infection (29.5%) compared to 12 HT patients (27.3%) and one HT patient (25%) (p ns). The individuals with a nadir CD4 count <200 cells/mm^3 who carried the S180L variant demonstrated a 4-fold decrease in the odds ratio (OR) for any serious infection compared with those who carried the wild-type 180S genotype (OR 0.58 vs OR 2.6, p = 0.016). Six patients developed B-cell non-Hodgkin lymphomas: two among patients bearing WT haplotypes (1.5%); and four among patients bearing the HT haplotype (9.3%, p = 0.040).
Conclusions: This study suggests a protection effect of the Mal S180L SNP against serious infections in HIV-1 infected individuals with low CD4 cell counts.

R2662 Never ever give up until you succeed: a case report of repeated unclear febrile episodes in a 55-year-old HIV-infected man

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In February 2010, a 55 year-old man with human immunodeficiency virus (HIV) infection was referred to our hospital with fever, weakness and diarrhea. The past three months he has been suffering from several febrile episodes. Extensive work-up during previous hospitalisations revealed no cause. HIV was diagnosed in 2007. Despite an excellent virological response to antiretroviral treatment with an undetectable plasma viral load the patient remained severely immunosuppressed with 107 CD4 cells/μl (5%).

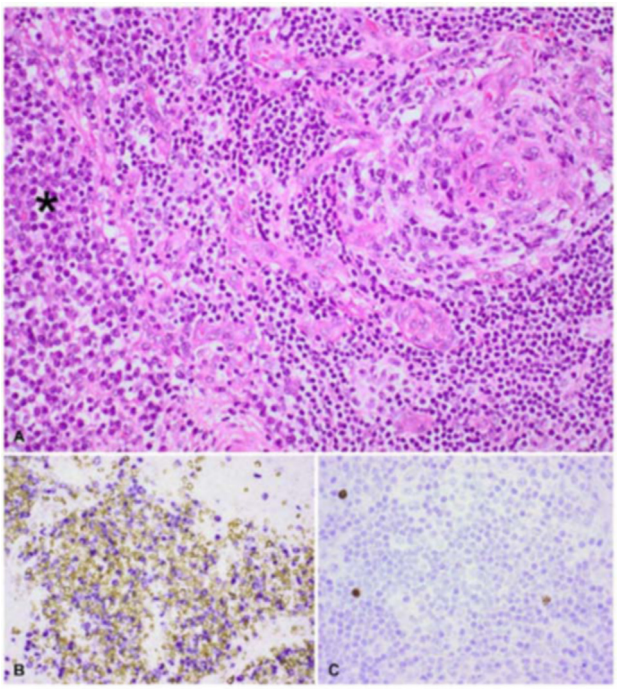

Figure: Lymph node histology showing **A)** a lymph follicle with a regressed germinal center and radially penetrating blood vessels (right) accompanied by an increased interfollicular plasma cell content (asterix). **B)** Double staining for the light chains kappa (brown) and lambda (blue) highlights the massive increase of polytypic plasma cells. **C)** Nuclear staining for the latency-associated nuclear antigen (LANA-1) in a few plasmablasts within the follicular mantle zone.

On admission he was in reduced general condition, febrile (39.5ºC), had a normal heart rate, a low blood pressure, multiple marginally enlarged lymph nodes and an enlarged spleen. Laboratory tests showed pancytopenia, elevated C-reactive protein and acute renal failure. Vast

examinations for bacteria, mycobacteria, helminths, protozoas remained all negative. Upper and lower endoscopic bowel examination with tissue biopsies showed no evidence of disease. Fluorodeoxyglucose (FDG)-positron emission tomography scan revealed a pathological FDG-uptake in lymphatic tissue. Immunohistochemical examination was positive for human herpes virus type 8 (HHV-8) latency-associated nuclear antigen (Figure), which allowed the diagnosis of multicentric Castleman's disease (MCD). A treatment with rituximab, etoposide and valganciclovir for six cycles was started. Ten months later, the patient was free from new febrile episodes, was working full-time and CT scan revealed a decreased size and number of lymph nodes.

MCD is a HHV-8 associated lymphoproliferative disease and should be considered as a possible cause of episodic fever. Disease presentation varies widely and the optimal treatment-regime is not yet established. Improvement of the immune system and treatment of the underlying disease is crucial. Antiviral agents have been successfully investigated. A promising approach for treatment in selected cases is rituximab. Antineoplastic agents such as etoposide or vinblastine are highly active in preventing the evolution of MCD towards lymphoma. Because of the unspecific findings, MCD can be difficult to diagnose in the setting of episodic fever and malaise.

We present a case of MCD for which it was extremely challenging to establish the diagnosis and assume that MCD is still underestimated even though it is more important than ever to have MCD diagnosed timely as new very promising therapeutical approaches exist.

R2663 Hyperhomocysteinaemia in HIV-infected patients – An underestimated problem?

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Objective: HIV-infected individuals are prone to several metabolic disturbances and are at higher risk for premature arteriosclerosis compared to HIV-negative persons. Homocysteine is an intermediate product of methionine metabolism; hyperhomocysteinemia may indicate functional deficiency of either folate, vitamin B12 and vitamin B6. Additionally, large population-based series have documented hyperhomocysteinemia as an independent risk factor for atherosclerosis and subsequent cardiovascular incidents.

Little is known about the role of hyperhomocysteinemia in individuals with HIV infection.

Goal of this study was to describe the extent of hyperhomocysteinemia in HIV-infected patients with or without antiretroviral treatment.

Patients and Methods: In 139 HIV-infected individuals from a single outpatient clinic homocysteine was determined. Results were correlated with clinical, laboratory, immunological and virological parameters. In 22 of those (15,8%) assays for folate, vitamin B12 and vitamin B6 were done and compared to the results of homocysteine serum levels.

Results: In 98 (70.5%) patients homocysteine was elevated (median serum levels 13.8 μmol/l, range 7.6–27.2). Individuals with HIV-1 viral load below detection limit (20 copies/ml) had no difference in homocysteine levels compared to those with detectable viral loads (median homocysteine 13.95 vs 13.6, $p > 0.9$). There was a trend towards an inverse correlation of homocysteine with the extent of immunodeficiency ($p = 0.148$) with the highest homocysteine values in those with CD4+ T lymphocyte counts below 0.2/nl. Also, no difference was found in patients on antretrovirals vs. naïve patients. In neither examined case ($n = 22$) an association of homocysteine levels with cobalamine, pyridoxine or folate deficiency was found.

Conclusions: Hyperhomocysteinemia is a common feature in HIV-infected individuals. Elevations are not significantly correlated to either immunological or virological parameters although there was a trend towards higher levels in severely immunocompromised patients neither are there inverse correlations with folate, cobalamine or pyridoxine plasma concentrations. If this reflects the significance of homocysteine as an independent biomarker for premature arteriosclerosis remains to be determined.

R2664 Late diagnosis of HIV infection in Iasi County, Romania – frequency, associated factors, therapeutical options

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Late diagnosis of HIV infection has unfavorable repercussions on both the patients themselves – due to higher morbidity and mortality rates – and the society as a whole, given the higher infection transmission risks and increased therapy and recovery costs.

Material and Method: Retrospective study of naïve patients that have entered the records of the Regional HIV/AIDS Center of Iasi over the last 10 years (2001–2010), based on the initial clinical, paraclinical and therapeutic data found in the follow-up charts of these patients. Late diagnosis was established when the patient presented for care with a CD4 count below 350 cells/mL or having an AIDS-defining event.

Results: 73.4% of the 192 patients included in the study were considered late presenters (58.5% with advanced HIV infection), according to the latest consensus definition. The average age of late presenters was 23.7 years, 53.9% of them being born between 1998 and 1991; M/F ratio – 1.3, U/R ratio – 1.14, average CD4 number – 130.8/mm3. The factors usually associated with late diagnosis in literature (patients over 30 years of age, males, co-infection with hepatitis viruses) had no statistical significance in our study group. Mortality rates were considerably higher in late presenters (31.9 vs. 7.8%, $p = 0.002$) and were correlated with a low number of CD4 ($p < 0.001$), younger age ($p = 0.004$); 57.8% of the deaths occurred within 6 months from diagnosis. ARV therapy was started by combining 2 INRT and IP in 52.7% of the patients, 2 INRT and one INNRT in 32.1% of them.

Conclusion: Late diagnosis rates were higher in Iasi County than in literature, while the risk factors detected by other authors had no statistical significance, possibly due to the regional epidemiological specificity.

R2665 Positive impact of a nurse intervention on hepatitis B immunity in a HIV clinic

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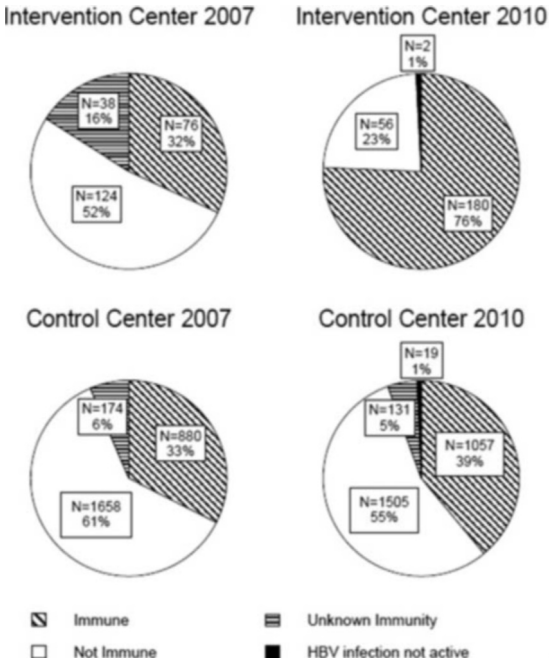
Objectives: Hepatitis B virus (HBV) infection is more frequent and severe in HIV-infected patients than in the general population. We assessed the impact of a 3.5-year nurse intervention to improve immunity against HBV in patients from the Swiss HIV Cohort Study (SHCS). The intervention was conducted in one center (intervention center) and 6 other centers were used as a comparator (control centers).

Methods: SHCS participants who were seronegative for HbsAg and anti-HBc in January 2007 were included in the study groups and followed up until June 2010. Non immune patients (anti-HBs <10 IU/L) and patients with unknown immunity were eligible for nurse intervention, consisting of (1) documenting HBV serostatus in patients with previously missing information, (2) providing vaccination (3 doses with a minimal interval of 1 and 6 months) to non-immune patients, (3) measuring vaccination effectiveness $\geqslant$1 month after the 3rd dose and (4) providing a second course of vaccination to non-responders.

Results: A total of 238 and 2712 patients seronegative for HbsAg and anti-HBc were included in the intervention and control centers, respectively (Figure 1). Between 2007 and 2010, the number of patients with absent immunity decreased from 124 (52%) to 56 (24%) in the intervention center, and from 1658 (61%) to 1505 (55%) in the control centers ($P < 0.001$). The number of patients with undocumented immunity decreased from 76 (32%) to 0 (0%) in the intervention center, and from 174 (6%) to 131 (5%) in the control centers ($P < 0.001$).

Conclusions: HBV immunity in the HIV population is insufficient in Switzerland and can be significantly improved by nurse intervention.



R2666 HIV infection is associated with reduced aortic distensibility

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Objective: Atherosclerotic cardiovascular disease is an increasing menace for patients with HIV infection. Aortic distensibility (AD) is an elasticity index of the aorta, and reflects aortic stiffness. Reduced AD is considered as an early marker of atherosclerosis. The aim of this study was to test the hypothesis that AD is lower in HIV-infected patients compared to healthy controls.
Methods: One hundred and five HIV-infected patients (86 males, mean age 41±0.92 years), including 60 patients with AIDS, and 124 age and sex matched HIV-uninfected controls (104 males, mean age 39.2±1.03 years) were evaluated by high-resolution ultrasonography to determine AD. Measurement of carotid intima media thickness (c-IMT) was also performed. For all patients and controls clinical and laboratory factors associated with atherosclerosis were recorded. For HIV-infected patients, clinical and laboratory parameters such as the CD4(+) cell count, HIV viral load, disease stage and exposure to HAART were recorded, as well.
Results: HIV infected patients had significantly reduced AD in comparison with the control subjects: 2.2±0.01 vs 2.62±0.01 10^{-6} cm^2 dyn^{-1}, respectively ($p < 0.001$). No statistically significant difference was found in c-IMT between the two groups. In univariate analysis obesity, hypertriglyceridemia, hypercholesterolemia, systolic and diastolic hypertension, smoking, family history of cardiovascular disease and HIV infection were predictors for AD. In multiadjusted analysis, though, the most influential predictor of decreased distensibility was HIV infection (β −0.43, $p < 0.001$). Moreover, in multiadjusted analysis among HIV-infected patients, only increasing age was significant contributing factor for decreased AD.
Conclusion: This study underscores the significant role of HIV-infection in the pathogenesis of early atherosclerosis, as depicted from decreased AD. Among HIV-infected patients, increasing age is a further contributing parameter to decreased AD.

R2667 Drug resistance mutations in HIV-1 CRF06_cpx viruses from anti-retroviral-treated patients in Estonia

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Introduction: Development of drug resistance mutations (DRMs) is one of the greatest challenges of the successful HIV-1 ARV therapy. There are some studies but not all suggesting that character of DRMs might be HIV-1 subtype specific. The Estonian concentrated HIV epidemic is predominantly caused by the CRF06_cpx and CRF06/A viruses that are rich of naturally occurring polymorphisms in the protease (PR) region.
Objectives: We aimed to evaluate the distribution of DRMs in HIV-1 CRF06_cpx in treatment experienced patients who have failed ARV therapy and to compare it with subtype B viruses.
Methods: We included 97 patients sent to HIV drug resistance testing due to failure of ARV therapy between January 2008 and November 2010. Genomic viral DNA was sequenced in PR and reverse transcriptase (RT) region and DRMs were detected using Stanford University HIV-1 Drug Resistance Database. The data on ARV consumption in 2007 were collected by the State Agency of Medicines.
Results: A total of 29%, 57% and 14% of patients, respectively were treated with regimens containing 1 PI + 2 NRTI, 1 NNRTI + 2 NRTI or both. Most patients (78/97) had failed first HAART regimen. Median viral load was 50,586 (QR 9,514; 261,418) and median CD4+ count 170 (QR 94; 260) cells/mm3. The most frequently used ARV agents were 3TC (0.57 DDD) followed by ddI (0.31 DDD), AZT (0.22 DDD), EFV (DDD 0.20) and LPVr (0.15 DDD). Altogether 83 viruses were successfully sequenced; majority (79%) belonging to the HIV-1 CRF06_cpx. There were 29 wild type viruses, 31 possessing resistance against two and 5 against three ARV classes. At least one primary DRM was detected in 54 cases (65%) including 42/54 of NRTI, 47/54 of NNRTI and 7/54 of PI resistance. In NRTI treated population the most common primary DRMs were M184I/V (36/42) and L74I/V (14/42), followed by K70E/R/Q and K219E (both 4/42) and T215Y/F (3/42). In NNRTI treated population the DRMs were as follows: K103N (38/47), V179E (19/47), V108I (6/47), K101E/N and G190A/S (both 5/47), others were seen in low frequency. In PI treated population the following primary DRMs were represented in decreasing order – L90M (3/7), M46I, I54V, V82S/A (all three 2/7), I47V and F53L in single case only.
Conclusions: The distribution of DRMs HIV-1 CRF06_cpx viruses corresponds to the pattern seen in subtype B viruses with similar ARV treatment regimen in previous studies suggesting that resistance development rather depends on the ARV agent and not on the viral subtype.

R2668 HIV testing in patients with hepatitis B and C infection in the United Kingdom

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Objectives: Universal HIV testing is recommended for those diagnosed with hepatitis B and hepatitis C virus (HBV and HCV) infection under the UK National Guidelines for HIV testing 2008. Offering and uptake of such testing should be reviewed annually by the Hepatology/Gastroenterology/Infectious Diseases (ID) team. The audit was done to ascertain the practice of universal HIV testing in this setting and to identify measures to improve things if necessary.
Methods: This audit was done in a UK district general hospital and included all patients who tested positive for Hepatitis B surface antigen (HBsAg) and/or hepatitis C antibody or PCR between Oct 2008 and September 2009. The pathology IT system was used to establish whether these patients had an HIV test. In addition we looked at testing for a second hepatitis virus (HBV/HCV) as well as other parameters in relation to their hepatitis infection (viral load, AST, genotype).
Results: We identified 102 patients who tested positive for hepatitis C (98 antibody positive, 4 PCR positive). The tests were requested by specialist teams (gastroenterology/genitourinary medicine/ID) in 39 (38%) patients. 51 patients (50%) had HIV serology checked of whom 6

(12%) were HIV antibody positive. Sixty six patients (65%) had HBsAg checked but only one was positive. Fifty nine (58%) patients had a viral load measured and 31 (30%) had the virus genotype checked. The AST was measured in 75 (74%) patients.
99 patients were HBsAg positive. These tests were requested by above specialist teams in 43 (43%) patients. HIV serology was checked in 63 (64%) patients and this was positive in 6 (10%). 52 (53%) had HCV antibody checked. 49 (49%) patients had a HBV viral load checked and AST was measured in 77 (78%) patients. One patient was positive for HBV, HCV and HIV.
Conclusions: HIV testing in hepatitis B and C patients is far from universal, however the same applies to testing for a second blood borne hepatitis virus in these patients. The laboratory currently recommends referral to gastroenterology services in cases of positive viral hepatitis B & C markers on the reports. This should be extended to include considering testing for other blood borne viruses including HIV. The results suggest that a substantial number of requests did not come from the specialist teams in the hospital suggesting they may not have been involved with all patients. Further investigation is warranted to establish the targets for further education.

R2669 A new approach for CD4 targeting and HIV-1 inhibition by antibody therapy

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The fusion process of the Human Immunodeficiency Virus type 1 (HIV-1), is mediated by the gp120 surface protein and the gp41 transmembrane protein. To facilitate viral entry, the gp120 glycoprotein must bind to cell-surface CD4, alter its conformation to reveal a site for co-receptor attachment, and trigger conformational rearrangements in the gp41 glycoprotein to mediate fusion of viral and host cell membranes. Therefore the gp120-CD4 interaction is critical for virus-cell fusion. The gp120 region that binds CD4 is the target of the broadly neutralizing antibody B12. The B12 antibody targets gp120 and recognizes a highly conserved epitope overlapping the CD4-binding region of gp120. This antibody is one of the four known human monoclonal antibodies identified that can efficiently neutralize a broad array of primary isolates of HIV-1 in vitro and can protect against viral challenge in vivo.
The goal of this project is to evaluate the potential of CD4 targeting and virus-cell fusion inhibition of HIV-1 by a single domain antibody grafted with the gp120 highly conserved epitope recognized by the B12 antibody.
This will be done using the 23 amino acids loop of gp120, grafted at the CDR1 of a highly stable rabbit single domain VL antibody, and designated VL-B12. The potential of this VL-B12 construct has been tested for CD4 binding by ELISA assay against soluble CD4 and by FACS analysis using HeLa, HeLa-P4, 293T and Jurkat cell lines. VL-B12 is highly specific and exhibits a high binding to CD4 in the conditions tested.
Preliminary inhibition assays performed in Jurkat cells, a Human T lymphocyte cell line, in the presence of the VL-B12 construct show no apparent HIV-1NL4–3 inhibition, indicating that VL-B12–CD4 binding alone is not sufficient to block virus-cell fusion and that a steric effect due to the presence of a larger molecule may be necessary for HIV-1 fusion inhibition. The VL-B12 is being tested in Cell-to-cell inhibition assays and standard Jurkat cell line inhibition assays in interaction with different molecules to further evaluate its HIV-1 inhibition potential.
In conclusion, this VL-B12 appears to be very promising for CD4 targeting and a valuable mediator of biopharmaceuticals.

Hepatitis

R2670 Incidence of and associated factor with recent hepatitis C virus infection in patients with HIV infection in Taiwan

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Objective: Incidence of hepatitis C virus (HCV) infection has been increasing in HIV-infected patients in several western countries, where clustering of HCV infection was recently detected among men who have sex with men (MSM). The objective of this study was to investigate the incidence of and associated factor with recent HCV infection in HIV-infected patients in Taiwan.
Methods: From June 1994 to November 2010, HIV-positive MSM or heterosexuals with negative anti-HCV antibody at baseline and tests for anti-HCV at least twice within one year were included in the present study. Serial CD4, plasma HIV RNA load (PVL), and Venereal Disease Research laboratory (VDRL) titers were obtained at baseline and within 6 months of the last anti-HCV test. Recent syphilis was defined as new seroconversion or a 4-fold increase in VDRL titers within 6 months of the last anti-HCV test. The HCV NS5B fragment was amplified with the use of PCR and sequenced and the phylogenetic trees were constructed.
Results: During the 16-year study period, 854 patients (39.8% [854/2143]), 699 MSM and 155 heterosexuals, were enrolled. After follow-up for 4223 person-years [PY], 22 patients (2.6%) had HCV seroconversion during follow-up, with an incidence rate of 5.210 per 1000 PY. All cases occurred during the last 5-year study period. The median duration from the last negative to the first positive anti-HCV was 183 days (range, 14–350 days). 4 HCV strains clustered in the phylogenetic analysis, and no genotype 4 or genetic relatedness with the reported strains in the western countries was observed. Compared with the patients without HCV seroconversion, patients with seroconversion were more likely to have syphilis at baseline (54.5% vs. 20.8%) and recent syphilis (40.0% [4/10] vs. 9.0% [57/631]) within 6 months of the last anti-HCV test (both $P < 0.05$), while no differences were observed between the two groups regarding age, CD4 count, or PVL. Syphilis at baseline (odds ratio [OR] 4.660; 95% CI, 1.917–11.330, $P = 0.001$) and older age (OR 1.043; 95% CI, 1.001–1.086, $P = 0.001$) were independent factors associated with HCV seroconversion after adjustment for HIV transmission routes. With age, HIV transmission route, and recent syphilis included in the model, only recent syphilis (OR 6.236; 95% CI 1.679–23.165, $P = 0.006$) was independently associated with HCV seroconversion.
Conclusion: Recent HCV infection was increasing in HIV-infected MSM in Taiwan, which was associated with acquisition of syphilis.

Virology (non-HIV/non-hepatitis)

R2671 Viral shedding duration of 2009 influenza A (H1N1) in young adults

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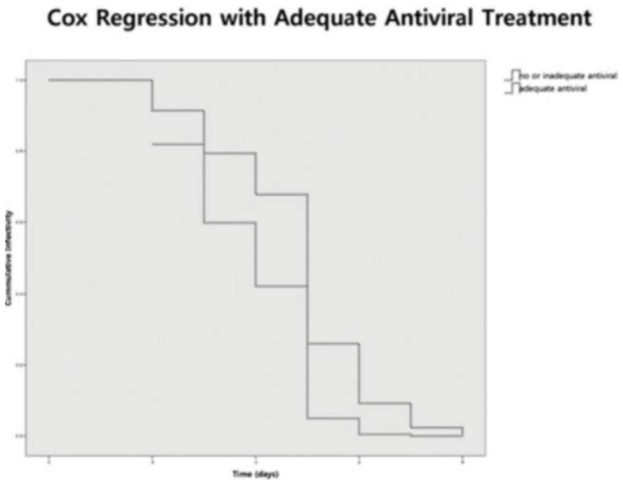
The auxiliary police of Korea are a group of early 20s healthy men, who are conscripted police as an alternative military service. They live in a same space like military barrack, but have frequent contact with general population. So in case of contagious disease, rapid and easy spread, and need of isolation care can be predicted. During 2009 influenza A (H1N1) pandemic, patients with influenza like illness (ILI) among them should be admitted and isolated for adequate infection control and treatment. We investigated the viral shedding duration and predictive factors of 2009 influenza A (H1N1) infection in the young adults with these data. From 3rd November 2009 to 2nd February 2010 (prior to vaccination), we performed daily real time reverse transcriptase polymerase chain reaction (rRT-PCR) test (Bioneer, AccuPower® New Inf.A Real-time PCR kit, Korea) to the all enrolled influenza patients till confirming negative result and checked the symptoms, physical findings and vital signs.

Nasopharyngeal-swab samples for rRT-PCR were collected by a same doctor.
During the study period, there are 62 patients were confirmed 2009 influenza A (H1N1) by rRT-PCR out of 104 ILI, and negative conversions in the serial tests were confirmed in 45 patients. These 45 patients were finally analyzed. The data were analyzed nonparametrically with Mann-Whitney U test by Windows SPSS 13. The median age of enrolled patients was 21 years old (19–22). The median duration of fever (interval from the onset of fever (body temperature $\geqslant$37.5ºC) to the time at which temperature <37.5ºC was attained and maintained for >24 hours) was 42 hours (9–132). Short febrile group included patients with shorter duration of fever than 24.5 hours (1st quartile of distribution). Adequate antiviral treatment was defined as administering antiviral agents within 48 hours after the onset of symptoms. Symptoms included fever (presented in 100% of patients), cough (95.6%), sputum (73.3%), sore throat (68.9%), rhinorrhea (73.3%) and gastrointestinal symptoms (26.7%). Viral shedding duration was defined as the time interval between the onset of symptoms and the last day of positive result of serial rRT-PCR test.
In 2009 influenza A (H1N1) among healthy young adults, the median viral shedding duration was 5 days (2–8). Adequately treated patients ($p = 0.002$) and patients with short febrile group ($p = 0.001$) had significantly shorter infective period.



R2672 **Crimean Congo haemorrhagic fever: modern approaches to diagnostics, epidemiology, prevention, therapy and preparedness**

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Over the last years, large outbreaks of Crimean Congo Hemorrhagic fever virus (CCHFV) in several European countries and neighbouring areas are on the rise. This disease poses a great threat to public health due to its high mortality rate, modes of transmission and geographical distribution. Climate changes and observation of the CCHFV vector in central Europe alarm the European Community as we cannot exclude that future outbreaks will take place in non-endemic area of Europe. To date, there is no vaccine available and no selective antiviral drug for the management of the disease. The general knowledge of migration, epidemiology, re-assortment and recombination of the virus is very limited. To fill these gaps, the CCH Fever project proposes to create a multidisciplinary collaborative research environment by bringing together selected competitive advantages such as: operative capacity with appropriate high security research facilities, reference centers and clinical samples from endemic areas and an international network of e xperienced researchers. This multidisciplinary research consortium will facilitate the progress in several key research areas of the field. This program will mainly focus on (i) developing sensitive and biosafe state-of-art diagnostic tools for CCHFV, (ii) gathering the forces and resources in Europe to build a Biobank of clinical samples, (iii) building a comprehensive database consisting in clinical, laboratory and surveillance data, (iv) taking advantage of unique and state-of-art tools to progress towards vaccine candidates and specific antivirals against this bio-treat and (v) disseminating the appropriate knowledge to the health care workers in endemic regions and contributing to capacity building. These achievements will provide tools for local and European public health authorities to prevent or counter future outbreaks and monitor the spread of the disease thanks to the established novel and unique tools and resources.

R2673 **Herpes genitalis in women under the guise of recurrent lower urinary tract infection**

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Aim: To study the prevalence of herpes genitalis in women with symptoms of recurrent lower urinary tract infection (LUTI).
Materials and Methods: Of 60 female patients aged 18–45 years with symptoms of recurrent LUTI were investigated during 2006–2010 years. All patients were examined by gynecologist. There was drawn the biopsy material from cervical canal of uterus, the blood serum in each patient. At the time of relapse a PCR method and a fluorescence immunoassay were used for detection of HSV2 in biopsy material and immunoglobulin G to HSV2 in serum respectively. Also urinalysis and urine culture were done. All patients were treated by acyclovir.
Results: All patients had a history of more than 4–8 episodes of LUTI per year. The most common complaints were frequent and painful urination, itch and burning. There was observed the relationship of LUTI relapses with menstrual cycle in each woman. The gynecological examination was displayed no abnormal changes in each patient. In the urinalysis there was revealed the increased amount of epitheliocytes only. All urine cultures were negative. All biopsy materials from cervical canal of uterus were negative for HSV2, but about 75% (45/60) patients with LUTI symptoms were found high level of immunoglobulin G to HSV2 (1:1600–1:3200) in blood serum. Three weeks later the antibody G titer increased twice. The patients were treated by acyclovir 400 mg three times a day for five days. Then the suppressive therapy with acyclovir 400 mg twice a day was given for 3–5 months. After the full course of treatment no one patient complained of LUTI.
Conclusions: In the patients with frequent episodes (more than 4 per year) of LUTI symptoms and normal urinalysis herpes genitalis should be suspected. The fluorescence immunoassay may be used in case of receiving negative PCR results.

Mycobacterial infections (including diagnosis)

R2674 **Clinical manifestations of *Mycobacterium marinum* infections in Taiwan**

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Objectives: To our knowledge, there are no standard regimens to treat *M. marinum* infections to date. The role of surgery is still a controversial issue. In Taiwan, some unsatisfactory results were observed. In this study, we would like to unravel the optimal anti-mycobacterial regimens, benefits and risk factors influencing the outcome.
Methods: From January 1, 1999 to May 31, 2009, 27 patients were enrolled for this study at Chang Gung Memorial Hospital – Linkou Medical Center. Medical charts were retrospectively reviewed for demographic data, contact history, infection sites, comorbidity, histological results, anti-mycobacterial regimens, surgery history and outcomes. All of them received anti-mycobacterial therapy. Ten received surgery. The clinical outcomes are classified as: successful, remission of lesions without any sequelae; not-successful, persistent symptoms and signs of infection or lost to follow-up.

Results: In our study, 27 patients were enrolled due to *M. marinum* infections. Among thirty isolates, three were repetitively isolated from two patients. According to the criteria of clinical outcomes mentioned in methods, 18 patient's outcomes were successful, eight were failed, and one was lost to follow-up. Statistically, 8 failed and 1 lost to follow-up were pooled into "not successful" group. In "successful" group, the mean (±SD) age is 50.1 (±21.6). In "not successful" group, the mean (±SD) age is 45.8 (±19.3). Twelve patients had either fish tank contact or fishing hobby. Two had shrimp contact, and one played in a swimming pool. One received intra-articular steroid injection. Three patients got minor or superficial trauma on their extremities. Eight had no documents about their contact history.
In successful group, 9 patients had ever received surgical debridement; in not-successful group, 1 patient had received surgical debridement (2-sided Fisher's exact p = 0.0912).
Conclusion: In Taiwan, this is the first study to unravel the relationship between clinical outcome and susceptibility testing of bacterial strain. Optimal anti-mycobacterial regimens have not well been established, all drugs had been reported as successful agents or failed agents. In our study, we cannot identify the risk factors of treatment failure, but the duration of prescribing clarithromycin, rifampicin, ethambutol, and doxycycline were different between "successful" group and "not-successful" group.

Table 1. Demographic data of *Mycobacterium marinum* infection patients.

	Successful (n=18)	Not successful (n=9)	*p*-value
Age	50.1±21.6	45.8±19.3	0.6431
Gender (female: male)	6:12	5:4	0.4105
Contact history			
Fish	9	3	
Shrimp	1	1	
Swimming pool	1	0	
Steroid injection	1	0	
Trauma	1	2	
ND	5	3	
Histologic findings			
granulomatous inflammation	5	2	
suppurative granulomatous inflammation	4	4	
caseating granulomatous inflammation	2	0	
necrotizing granulomatous inflammation	2	0	
chronic inflammation	1	0	
rheumatoid nodule	1	0	
focal alveolar damage	0	1	
cornea ulcer	0	1	
ND	3	1	
Antimicrobial agents			
INH	75.4±112.5	10.1±30.3	0.1063
RIF	156±121.8	49.7±70.4	0.0352
EMB	163.6±121.6	10.1±30.3	0.0016
DOX	5.9±18.6	8.8±9.4	0.0492
SXT	5.8±18.9	3.6±7.1	0.5603
CIP	7.8±28.2	19.9±46.8	0.197
CLR	114.8±116.1	6.2±12.8	0.0122
E	0	14.9±44.7	0.1573
Surgical excisions	9	1	0.0912

Footnote: ND: not documented, INH: isoniazid, RIF: rifampin, EMB: ethambutol, DOX: doxycycline, SXT: sulfamethoxazole-trimethoprim, CIP: ciprofloxacin, CLR: clarithromycin, E: erythromycin.

R2675 Tuberculosis: bacteriological and epidemiological aspects in the central region of Tunisia

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Objective: To present the situation of TB in the region of Sousse-TN.
Materials and Methods: A retrospective study conducted during 4 years (2006–2009) concerning all TB cases in the region of Sousse diagnosed according to bacteriological or histo-pathological and/or clinical criteria.
Results: The total of TB cases is 494. The incidences noted for the period study were respectively 20.7, 20.6, 24.7 and 22.4. The mean age was 39.7 years, the sex ratio was 1.4. Close contact with patient was found in 29.7%. Twenty patients were prisoners. The mean delay for TB diagnosis was 45 days. Symptoms were predominated by cough and fever in pulmonary TB. HIV infection was noted in only 2 patients with extrapulmonary tuberculosis. TB was 233 times pulmonary and 261 times extra pulmonary (31% lymph nodes). Bacteriology confirmed the diagnosis in 207 cases; microscopy was positive in 67.6% of pulmonary TB cases and 15.9% of extra pulmonary TB cases. Multidrug resistance (MDR) was observed for 8 strains/patients. Mortality attributed directly to TB was 0.2%.
Conclusion: TB remains endemic in our region. TB location is primarily lung and lymph nodes. TB is not related to HIV infection. MDR and mortality attributed to TB are rare in our region.

R2676 Agreement between clinical scoring methods for the diagnosis of childhood tuberculosis in a Venezuelan indigenous population

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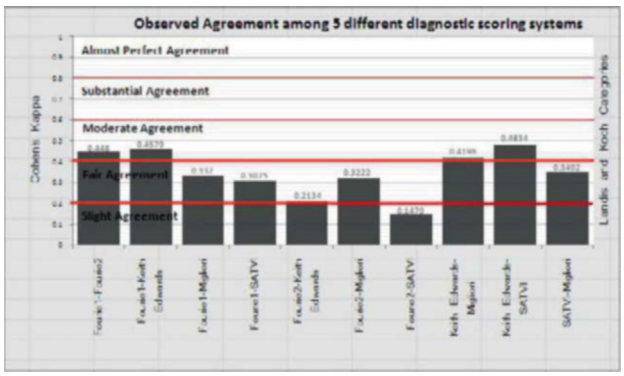
Objective: Compare different scoring methods for the diagnosis of childhood tuberculosis (TB), in Warao Amerindian children.
Methods: We selected 61 children aged <16 years, with a history of close contact with culture-confirmed TB patients. Clinical, radiological and microbiological data (smear microscopy and culture of sputa or gastric aspirates) were collected prospectively, previous a signed informed consent. We calculated the number of children diagnosed with TB according to the Fourie, Keith-Edwards, Migliori and SATVI (The South African Trial Vaccine Initiative) diagnostic scoring systems. Differences between case frequencies were calculated using the McNemar test with Yates correction. The simple percent agreement and the concordance (Cohen's Kappa coefficient) for the binary results (TB/Not TB) were calculated. Strength of agreement was classified using Landis and Koch categories.
Results: Childhood TB case frequency varied from 19.67% using the Fourie scoring system to 85.25% using the SATVI scoring system. Significant difference in case frequency ($P < 0.05$) occurred in 8 of 10 pair-wise comparisons between diagnostic scoring systems. The simple percent agreement varied from 6.93% to 78.68% (median: 68.81%). Cohen's kappa ranged from 0.15 to 0.48 (median: 0.34). There was a high variability in the case frequency between the different scoring systems. The strongest concordance (moderate) found was between Keith-Edwards and SATVI scoring systems (Kappa: 0.48); with a concordance median value in the fair agreement category (Kappa: 0.2–0.4).



Conclusion: Although this study doesn't support the systematic use of the investigated diagnostic scoring systems for the diagnosis of childhood TB in Warao children, the criteria that showed high frequency and moderate concordance (SATVI and Keith-Edwards) could be used as important tools in the screening of childhood contacts of TB patients.

R2677 Tuberculosis revisited: a 10-year experience from a tertiary hospital outpatient infectious diseases clinic

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Objectives: To evaluate definite cases of mycobacterium tuberculosis (MTB) infections, according to ATS criteria.
Methods: Retrospective chart review of 10year outpatient visits in the infectious diseases department of a tertiary hospital.
Results: Among 110 referred patients (pts) with suspected TB, 85 definite cases were documented and analyzed [male 39 (45.9%), age: median 59ys, range 21–93ys, caucasian 71pts (91.2%)]. In 49 pts (57.6%), no risk factor (RF) for TB was recognized. Among the rest, the most prominent RF was underlying malignancy [11pts (30.6%)], followed by diabetes mellitus [8 pts (22.2%)] and steroid use [7pts (19.4%)]. Pulmonary TB was present in12 pts (14.1%), pleural in 4 (4.7%), extrathoracic lymphadenopathy 22 (25.9%), bone and joint infection 9 (10.6%), spinal TB 15 (17.6%), 9 CNS (10.6%), urinary tract 6 (7.1%), abdominal 5 pts (5.6%). Fever was present in 36.5%, appetite and/or weight loss in 14.1%, cough in 16.5%, bone-joint pain in 23.4% and neurological signs in 11.8% (the last two directly related to the site of infection).
Plain chest radiography (PR) was positive in 36.5% and CT scan in 46% of pts adding significant diagnostic clues. Direct sputum examination (DE) was positive in 36.5%; in 55% of them plain radiograph was insignificant. Cultures were positive in 50pts (58.8%); in 50% of them DE was negative. PCR was positive in 27/28 cases, contributing to the diagnosis of 17 additional cases; the same was true for 11 biopsies. Among 48 sensitivity tests 89.6% revealed fully sensitive MTB, whereas only two were multidrug-resistant.
Median duration of treatment was 9 months (mo) [range 2–24]; for pulmonary TB 6mo. Drug toxicity experienced 21/85 pts (24.7%), mostly as liver toxicity attributed to rifampin. Successful treatment was confirmed in 73/78 (93.6%); 7 pts (8.2%) were lost to follow-up. Among 4 pts who failed treatment, 3 died (mortality 3.8%).
Conclusions: Traditional diagnostic methods (DE, cultures, PR) retain a singificant diagnostic value, which can be significantly augmented by the addition of newer diagnostic techniques as CT and PCR. Molecular methods added importantly in the diagnosis of extrapulmonary TB. Particularities of the population served by our reference center could probably explain the epidemiological features observed regarding risk factors and clinical picture.

R2678 Highly individualised treatment for XDR-/MDR-tuberculosis: case series from a single centre

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Objective: Treatment options for patients with extensively resistant (XDR) or multi-drug resistant (MDR) tuberculosis (TB) are highly limited. Individualized treatment using WHO group II, IV or V tuberculostatic substances may improve outcomes.
Goal: To determine the clinical and microbiological outcomes of patients with confirmed MDR- or XDR-TB.
Patients and Methods: Retrospective analysis of all MDR- and XDR-TB cases in a single tertiary referral centre. Outcomes were correlated with underlying conditions, treatment schedules, duration of treatment and microbiological treatment response.
Results: From 2006 to 2009 eleven patients with either XDR (n = 5) or MDR-TB (n = 6) were recorded. Pulmonary manifestations were present in 10/11, one patient had only extrapulmonary tuberculosis (pleural, mammary and lymph node involvement). Disseminated disease involving lung, liver, spleen, lymph nodes or central nervous system was found in three patients. Eight out of eleven patients were from Eastern Europe (Russia, Ukraine, Georgia) two from Asia (Iran, Afghanistan), and one from Africa (Cameroon). Underlying condition were found in four (HIV infection, n = 1; HCV infection, n = 2, HIV/HCV co-infection, n = 1). Nearly all (10/11) had at least one incomplete tuberculostatic pre-treatment (duration 3 months to five years, in all cases non-compliance was documented). Isolated strains were resistant to a median of five antituberculous drugs (range 3–8). Treatment regimens provided by DOT in every patient included mainly WHO group IV and V drugs with at least three drugs selected by susceptibility testing (median 3, range 3–5). Two patients underwent surgery (lung surgery, n = 1; pleurectomy, n = 1). Microbiological, radiological and clinical cure was documented in 10/11 (72.7%). One patient died after upper lobe resection because of pulmonary thromboembolism.
Conclusions: Prognosis in patients having at least three effective substances left is favourable. Complete cure can be achieved by implementing DOT strategies for the long-term therapy. Drug compliance history is crucial for estimating prognosis and directs the management of patients especially with XDR-TB.

R2679 Rapid detection of *Mycobacterium tuberculosis* complex in spinal aspirate from cases of Pott's spine at tertiary care hospital, North India

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Objectives: Tuberculosis of spine causing paraplegia is common and increasing its prevalence in developing country like India. Spinal tuberculosis (Pott's disease) covers 50% of the cases of skeletal tuberculosis. 15% of the cases extra pulmonary tuberculosis and 2% of all cases of tuberculosis. It has poor prognosis due to late diagnosis. Therefore, rapid diagnosis is essential to decrease the morbidity and mortality of this disease. Conventional methods are time consuming and of low sensitivity. The polymerase chain reaction (PCR) based diagnosis provide fast diagnostic tool and hope for early diagnosis of this disease. The aim of the study was to compare assess the applicability of rapid methods i.e. PCR, BACTEC culture and Microscopy for detection of mycobacterium tuberculosis complex (MTBC) in spinal specimens from cases of suspected Pott's spine admitted at tertiary care hospital in North India.
Method: Sixty two non-repeated clinical samples of suspected cases of Pott's disease were included. All the samples were processed for Ziehl-Neelsen staining for acid fast bacilli (AFB) and BACTEC culture for *M. tuberculosis*. All the samples were also processed for PCR amplification with primers targeting 123 bp fragment of insertion element IS6110 of *M. tuberculosis* complex. The Sensitivity, specificity and positive-negative predictive value were assessed for ZN stain and PCR. Kappa coefficient calculated for agreement culture between ZN stain and PCR.
Result: Out of 62 suspected Pott's cases 32 (51.6%) cases were male and 28 (49.4%) cases were female. ZN staining detected AFB in 19 (30.6%); BACTEC culture was positive for MTBC in 27 (43.5%) and PCR detected MTBC in 33 (53.2%) cases. BACTEC culture was considered as the gold standard. The Sensitivity, specificity, positive and negative predictive value (PPV&NPV) were 96%, 80%, 79%, 97% correspondingly. The sensitivity, specificity, PPV and NPV of ZN staining were 52%, 91%, 82% and 71% respectively. Kappa agreement between Culture with ZN (kappa 0.51), Culture with PCR kappa agreement (kappa 0.75) respectively.
Conclusion: PCR disclosed good agreement with culture, hence PCR can be used for early diagnosis of tuberculosis in spinal samples (Pott's disease) that can help to initiate timely anti-tubercular treatment and prevent progression to irreversible changes.

R2680 Pulmonary infections with non-tuberculous Mycobacteria: management under guidance of Infectious Diseases Board

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Objective: In recent years pulmonary infections with nontuberculous mycobacteria (NTM) appear to be increasing worldwide in HIV negative

patients, although accurate data are lacking. Aim of the study was to evaluate clinical and treatment aspects in patients with pulmonary NTM-infections.
Methods: In our center we included all patients with pulmonary NTM-infection without concomitant HIV-infection in the period from 2007 to 2010. All patients fulfilling the ATS (American Thoracic Society) criteria for NTM pulmonary infection were evaluated, and structured treatment recommendation was discussed in the interdisciplinary infectious diseases board (ID-Board).
Results: Seven patients were diagnosed with pulmonary NTM-infection meeting the criterias given by the ATS. Five were female, two male; mean age was 55, 7 years of age (range 31–77 years).
Microbiological specimens included *Mycobacterium kansasii* (3), *M. xenopi* (2), *M. avium* complex (MAC) (2).
Radiological findings included disseminated small nodules (*M. xenopi*, MAC), infiltrates/nodules (*M. kansasii*, *M. xenopi*) and one cavitary lesion (MAC).
Three women had malignancies: one received chemotherapy, another the monoclonal antibody Alemtuzumab, both had a complete course of antimycobacterial therapy for 12 or 24 month, respectively; the third with MAC had a stable clinical course, even if treatment has been stopped after 3 months due to side effects.
From two patients with chronic pulmonary disease, one with *M. kansasii* is cured after 22 months of therapy, the other one with MAC-associated cavitary lesion is clinically stable while still under treatment (for 6 months). In two patients (*M. kansasii*, *M. xenopi*) we could not identify underlying risk factors.
Conclusion: Pulmonary NTM infection appears not to be a uniform disease. The clinical and radiological presentation varies, but seems independent of mycobacteria species involved. None of our patients progressed or deteriorated due to NTM-infection, four patients are cured. In our opinion this result was achieved because of thorough interdisciplinary discussions in the ID-board considering the clinical presentations, co-morbidities and expected drug-toxicities which led finally to a favourable outcome of our patients.

R2681 The role of interferon gama-release assays in the diagnosis of latent tuberculosis infection in contacts of smear-positive pulmonary tuberculosis patients: Kuwait experience

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Introduction: Knowledge of the prevelance of latent *Mycobacterium tuberculosis* (MTB) infection is crusial for effective TB control. In contacts of smear-positive pulmonary TB cases, tracing is usually done by tuberculin skin test (TST), chest radiography and clinical signs and symptoms of which are either nonspecific or difficult to interpret.
Objectives: This prospective survey was done to compare the new interferon gama-release assay, T.Spot TB, (Oxford Immunotech, UK) with the conventional tests and to evaluate its value in the decision on administration of isoniazid (INH) chemoprophylaxis.
Methods: All contacts of smear-positive pulmonary TB cases from November, 2008 till July, 2010 were included in the survy. They were screened by TST, T.Spot TB and chest radiography. Their nationality, BCG status together with the administration of INH prophylaxis were recorded.
Results: Out of 743 contacts, 381 (51%) were Kuwaiti nationals and 691 (93%) were BCG-vaccinated. TST was above the cut off value (10mm) in 704 (95%) of the total. Only 55 (7%) of those contacts had abnormal chest X-ray findings. In BCG-vaccinated contacts, 660 (89%) showed TST above 10mm while only 355 (48%) of them had reactive T.Spot TB. Forty-four percent of the contacts with TST above 10mm had non-reactive T.Spot TB. Furthermore, out of 68 contacts with TST above 20mm, 26 still had non-reactive T.Spot TB. In contacts with reactive T.Spot TB, 347 (89%) had normal chest X-ray while only 42 (11%) had abnormal chest X-ray findings. Out of the 704 contacts with TST above 10mm, 363 contacts with reactive T.Spot TB correctly received INH prophylaxis, while 194 contacts with non-reactive T.Spot TB correctly did not receive INH prophylaxis. Follow up of those 194 contacts is being carried out for any possible active disease development. Till date non developed active disease.
Conclusion: T.Spot TB, a type of interferon gama-release assay, shows promising results in the diagnosis of latent TB infection.

R2682 Performance evaluation of an in-house interferon-γ release assay in detecting tubercular infection in HIV patients

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Objectives: To evaluate the performance of interferon-γ release assays (IGRA) in relation to CD4 count in a group of patients with HIV infection with a risk of tubercular infection.
Methods: 132 patients (pts) [M = 100, F = 32] with HIV infection, have been tested with IGRA: 113 pts because suspected of active tuberculosis, 19 pts for screening. 91 pts were also tested with tuberculin skin test (TST). The home-made IGRA detects the number of specific interferon-γ producing T cells by means of an enzyme-linked immunospot assay, using a restricted pool of synthetic highly immunogenic peptides derived from ESAT-6 and CFP-10 proteins (IGRA ESAT6-CFP10). The interferon γ response to purified protein derivative (PPD) was also measured (IGRA PPD). IGRAs were considered positive for values greater than 20 spot forming cells per million lymphocytes (SFCML). CD4 cells count, collected within 3 months of IGRA, was performed and classified using four classes: CD4 <50 cells/ml, between 50 and 200 cells/ml, between 200 and 500 cells/ml and >500 cells/ml. Chi-square, Kruskal-Wallis, Mc Nemar test and k statistics were calculated.
Results: The four classes considered do not differ for gender and place of birth distributions and for the median age (respectively chi square: $p = 0.7852$, $p = 0.5958$; Kruskal Wallis test: $p = 0.4638$). Among different CD4 cell count classes, no differences in the distribution of the IGRA ESAT6-CFP10 qualitative results and in the means of quantitative measures were found (chi square: $p = 0.4412$; Kruskal Wallis test: $p = 0.4458$).
Considering the results of the TST and the IGRA PPD, no differences in distribution were found among the CD4 classes (chi square: $p = 0.2221$ and $p = 0.823$).
Fair agreement was found between TST and IGRA PPD in the whole sample ($k = 0.378$, McNemar $p \leqslant 0.0001$). In particular poor agreement was found in classes of CD4 <50 cells/ml ($k = 0.111$, McNemar $p = 0.0133$), fair agreement between 50–200 cells/ml ($k = 0.364$, Mc-Nemar $p = 0.364$), fair agreement between 200–500 cells/ml ($k = 0.371$, McNemar $p = 0.0233$) and good agreement in pts with CD4 >500 cells/ml ($k = 0.831$, McNemar $p = 1$).
Conclusions: Our results show that IGRAs performance are not influenced by a different CD4 cells count levels.
Comparison between test in vivo (TST) and in-vitro (IGRA PPD) demostrated that IGRA performes better than TST when CD4 cells are below 500 cells/ml; TST is not useful in detecting immunological response to tubercular infection in case of immunodeficiency.

R2683 Diagnosis of tuberculosis and susceptibility testing by conventional and molecular methods in Southwestern Greece

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Objective: An increase in the number of tuberculosis cases in Southwestern Greece is observed. The re-emergence of disease combined with isolation of multidrug-resistant strains has intensified the need for rapid diagnostic methods for mycobacteria. The rate of mycobacteria isolation with the Bactec/9000MB system and the Löwenstein-Jensen (LJ) medium in 2949 clinical specimens obtained from 2087 patients during 19 months (1/1/2009–31/7/2010) was compared.
Methods: Ziehl-Neelsen (ZN) staining was performed in 2796/2949 various samples. Inoculation of 2438 samples onto Löwenstein-Jensen slants (LJ, bioMérieux) and 2189 into Bactec/9000MB culture vials (Becton Dickinson) was also carried out. Among 1858 samples, real-time PCR for *Mycobacterium tuberculosis* complex was applied (MTB,

COBAS TaqMan MTB, Roche). Isolates were identified at species level by PCR and hybridization (GenoType MycobacteriumCM, Hain). Susceptibility testing was performed by phenotypic and genotypic methods (MGIT, Becton Dickinson and GenoType MTBDRplus, Hain, respectively).
Results: ZN-positive were found 30 samples (1%). Twelve out of 24 tested (50%) were MTB-positive by RT-PCR. Nine out of 30 were LJ-positive (30%), whereas 13/25 (52%) were Bactec-positive. RT-PCR was performed in 1819/2766 ZN-negative samples. Forty-five (2.5%) were MTB-positive and 26/2368 (1.1%) LJ-positive. Among Bactec vials, 27/2049 (1.3%) were positive. Forty-seven patients had positive culture by either method: 41 *M. tuberculosis*, three *M. kansasii*, and one of each *M. avium*, *M. simiae*, *M. fortuitum*. Susceptibility testing of 31 MTB showed that all were rifampicin-susceptible, while 28 were sensitive to isoniazid, 26 to ethambutol and 20 to streptomycin. The molecular method of 39 MTB showed 18 isoniazid-susceptible and one resistant, while 17 more carried one mutation (14/17 phenotypically susceptible). Furthermore, 14 were rifampicin-susceptible and 4 resistant, while 18 carried one mutation (16/18 phenotypically susceptible).
Conclusions: Sensitivity of Bactec system is higher in ZN-positive samples (100%) compared to LJ medium (66.7%). Among ZN-negative samples the sensitivity of Bactec system and LJ medium is the same (62.9%). RT-PCR shows high sensitivity (100%) in ZN-positive and lower (68.4%) in ZN-negative samples. It is important to apply a molecular method for susceptibility testing, in order to discover mutated subpopulation, avoiding the predominance of completely resistant strains.

R2684 Evaluation of the genotype microbacterium CM-Hain assay for identification of non-tuberculous Mycobacteria Species from clinical specimens

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Although *Mycobacterium tuberculosis* complex is by far the most important mycobacterial species from a public health perspective, several non tuberculous mycobacteria (NTM) species are associated with pulmonary disease.
NTM are ubiquitous in environmental reservoirs and sometimes clinically insignificant colonization or contamination can be difficult to distinguish from true disease.
Detection of acid-fast bacilli (AFB) in stained smears is the first bacteriologic evidence of the presence of mycobacteria in a clinical specimen, but acid-fast bacteria seen on smear may represent either *M. tuberculosis* or NTM.
A positive nucleic amplification technique (NAT) on an AFB-positive specimen is usually taken as evidence of infection with *M. tuberculosis* complex, while a negative NAT in such specimens means NTM infection. Unfortunately, NTM species identification still requires growth in culture media which often requires a prolonged incubation.
We decided to test smear-positive, PCR negative respiratory specimens with a molecular assay based on the DNA strip technology (Genotype *Mycobacterium* CM-Hain®) (GMCM) aimed to identify several NTM to a species level.
After decontamination and concentration according to standard procedures, 23 AFB-positive respiratory samples which tested negative with a commercial PCR test (Xpert MTB_RIF, Cepheid®) were submitted to GMCM-testing. The results obtained were compared with culture results after 56 days.
Fifteen NTM (3 *M. intracellulare*, 4 *M. avium*, 4 *M. kansasii*, 3 *M. xenopi*, 1 *M. chelonae*) were detected by GMCM by this procedure. Four specimens were reported to contain *M. abscessus* by GMCM but no growth was detected by culture. Three specimens grew NTM (1 each of *M. abscessus*, *M. intracellulare* and *M. avium*) but were negative by GMCM. One AFB-positive specimen was negative by both GMCM and culture.
For the 15 concordant specimens the mean time to positivity in culture was 11.5 days (range 5.0 to 26.0). Direct identification of NMT from AFB-positive specimens may be useful to reduce time to diagnosis.

R2685 Extrapulmonary tuberculosis in fever of unknown origin

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Objectives: The aims of this study were to determine prevalence of extrapulmonary tuberculosis in patient with fever of unknown origin (FOU) who was HIV negative, to show prevalence of, and types of extrapulmonary tuberculosis (TB) and to determine trends of prevalence among patients with FOU.
Methods and Results: During period 1995–2010, 2642 with FUO were evaluated and treated in the Clinic for infectious and tropical diseases, Belgrade. Extrapulmonary TB were diagnosed in 128 (4,8%) patients. Genitourinary TB in 67 patients (renal – 57, orhiepididymitis – 3, salpingo-oophoritis – 7); TB lymphadenitis in 11, meningitis in 15, TB pericarditis in 8, spondylodiscitis in 6, liver TB in 4, in 2 patients cerebral tuberculoma and in one patient small intestine TB. In 14 patients we did not confirm tuberculosis and after pulmonary TB was excluded, they were treated empirically with antituberculous drags, and had a good response. As a diagnostic methods we used: PPD skin test, cerebrospinal fluid (CSF), sputum and urine cultivation, computed tomography, echocardiography, intravenous pyelography, patohistological examination of lymph nodes, intestine and liver, and gynecological laparoscopy. For urine and CSF specimens PCR test was used after year 2001. The sensitivity of conformation test were: CSF culture 100% (PCR 100%), urine culture 45% (PCR 68%), for histopathology lymph nodes 78%, small intestine 100% (single patient) and liver 85%. Cerebral tuberculomas was confirmed by magnetic resonance spectroscopy and reduction of lesion dimensions during antituberculous therapy. As a diagnostic criteria, clinical course of the illness, radiological examination, laparoscopy and other endoscope examinations and response to empiric therapy, were also used. Incidence of extrapulmonary TB was in slight increasing after year 2001. Isoniazid, rifampin, pyrazinamide, ethambutol and streptomycin were used for treatment. Multi drug resistant TB were confirmed in 4 patients and in 7 patients (drug sensitive TB) had relapses of the illness after treatment.
Conclusions: Extrapulmonary TB is increasing cause factor in patients with FOU and should be always considered during evaluations of this patients.

R2686 CD4+ T-lymphopenia in HIV-negative tuberculous patients at the King Khalid University Hospital, Riyadh, Saudi Arabia

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Objectives:
1. Compare the baseline and post treatment values of CD4 and CD8 of patients with tuberculosis and controls.
2. To analyze the sequential CD4 counts during and after treatment.

Subjects and Methods: Twenty eight (28) consecutive adult patients diagnosed with different clinical forms of tuberculosis were recruited. Eligible patients were enrolled based on compatible symptoms of TB and positive *Mycobacterium tuberculosis* based on Ziel-Nielsen smear and/or culture of relevant specimens as determined by the Bactec system and/or Lowenstein-Jensen culture methods. All controls were selected relying on the absence of history suggestive of tuberculosis and negative tuberculin tests. Both subjects and controls were screened for HIV and ensured negative using Enzyme-linked immunosorbent assay (ELISA) and Recombinant immunoblot assay (RIBA). Flow cytometry study was done as per protocol.
Patients and controls were excluded if they have the following conditions: any form of immunodeficiency syndromes, diabetes mellitus, chronic kidney disease and concurrent use of immunosuppressant medications. Informed consent was sought from both subjects and controls before enrollment.

Results: Twenty eight consecutive (28) patients with varied forms of tuberculosis were enrolled. The baseline CD4 counts of patients (mean ±SD of 556.8±297.8) were significantly reduced as compared with matched controls (1132.3±259.9) at a p value of 0.000. Further the pretreatment and post treatment values of patients were significantly different as recorded as follows: 556.8±297.8 versus 954.3±210.9 with a p value 0.000.
The baseline CD8 counts were not significantly reduced (p value 0.013) as the values were 1136.0±512.1 and 1461.9±367.0 for patients and controls respectively. However, there were improvement of the CD8 counts after treatment; baseline counts of 1136.0±512.1 versus 1316.5±286.2 after treatment (P value, 0.002).
Conclusion: The study showed significantly lower baseline CD4 counts among patients with tuberculosis as compared with the controls. Further, the counts significantly rose towards normalization at the end of treatment. We therefore conclude that tuberculosis is associated with CD4 lymphoenia independent of other notable causes. The counts are reversible and may indicate a success of treatment.

R2687 Diagnosis of tuberculosis infection in health care workers. Comparison of tuberculin skin test with QuantiFERON® TB Gold In-Tube

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Objectives: To compare the use of tuberculin skin test (TST) and Interferon-γ (IFN-g) Release Assay using three specific antigens (ESAT-6, CFP-10 and TB7.7) (QuantiFERON®-TB Gold in tube) for the diagnosis of tuberculosis infection (TBI) and indication of treatment in health care workers.
Methods: We conducted a prospective transversal study of health personnel who came for routine health care study (may 2007 to june 2010). All were screened with TST and QFT (Cellestis, Australia) and risk factors were registered in a questionnaire. Patients with a positive result (TST or QFT) were screened also with chest X-ray. TST was performed by Mantoux method and a positive test was defined as an induration ⩾5mm in non-vaccinated and ⩾15 mm in vaccinated people. QTF was made according to the manufacturer specifications. We considered as vaccinated persons those presenting with a suggestive scar. CDC recommendations were followed for the interpretation of the QFT. Agreement between TST and QFT was assessed by the Cohen kappa coefficient.
Results: We studied 316 health care workers (72.2% women) from the General Hospital of Jerez. Average age was 43.4 years (SD: 8.8), 76.4% had been vaccinated with BCG. TST was not done in 104 (32.9%) persons because of a previous positive TST. TST was positive in 50 (68.5%) and QFT in 30 (41.1%) non-vaccinated people. Whereas, for vaccinated people TST was positive in 93 (39.1%) and QFT was positive in 53 (22.2%). Agreement between the TST and QFT was 64.4% (Kappa 0.33, CI (0.15–0.51)) among the non vaccinated group; when we defined positive test for TST as an 10 mm induration, agreement was 71.2% (Kappa 0.43, CI (0.23–0.63). Agreement was 64% (Kappa 0.18, CI (0.06–0.30)) for the vaccinated group. Two indetermined results were detected by QFT. The indication of TBI treatment made by TST and risk situation was modified in 70% of cases according to QFT test. We prescribed treatment of TBI by QFT in 13% of the patients that did not have this indication according to the TST.
Conclusions:
1. Agreement between TST and QFT was low in vaccinated and non-vaccinated people.
2. QFT was better than TST for recent tuberculosis infection diagnosis in health care workers because of its high specificity and no interference of booster
3. QFT was a better indicator for treatment of tuberculosis infection.

Further studies addressing IFN-g sero-conversion and -reversion in health care workers for the follow-up of health care personnel are needed.

R2688 Comparison of QuantiFERON®-TB Gold In-Tube with tuberculin skin test for the diagnosis of tuberculosis infection among drug users

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Objectives: To evaluate the agreement of tuberculin skin test (TST)test and the QuantiFERON® TB Gold In Tube (QFT) for the diagnosis of tuberculosis infection (TBI) in patients attended in Drug addicts Rehabilitation Centers, and to establish the utility of QFT as a tool for the indication of TBI treatment.
Methods: We studied 347 immunocompetent intravenous drug users (92.5% men) since june 2007 to april 2010; 66% had been vaccinated with BCG. All patients were older than 17 years (mean age 39.6 years (SD: 10.2) and came for screening of tuberculosis infection. All were screened with chest X-ray, TST, QFT (Cellestis, Australia) and risk factors were registered in a questionnaire. TST was performed by Mantoux method and a positive test was defined as an induration ⩾5mm in non-vaccinated and ⩾15 mm in vaccinated people. QFT was made according to the manufacturer specifications. We considered as vaccinated persons those presenting with a suggestive scar. CDC recommendations were followed for the interpretation of the QFT and the treatment of the TBI. Agreement between TST and QFT was assessed by the Cohen kappa coefficient.
Results: Agreement between TST and the QFT among non-vaccinated patients was 84.2% (Kappa 0.69, CI (0.56–0.82)). When we redefined positive test for TST as an induration ⩾10 mm, agreement rose to 86.8% (Kappa 0.74, CI 0.61–0.86)). Agreement for vaccinated people was 70.7% (Kappa 0.38, CI (0.26–0.50)). QFT (−)/TST (+) results was the most frequent disagreement in non-vaccinated people. QFT was indeterminated for 5 patients, 4 of these were negative for TST. We prescribed treatment of TBI by QFT in 14% of the patients that did not have indication according to the TST. The indication of TBI treatment made by TST and risk situation was modified in 46% of cases according to QFT test.
Conclusions:
1. Agreement between TST and QFT was good in non vaccinated intravenous drug users.
2. Agreement between TST and QFT was low in vaccinated intravenous drug users even if TST was considered positive with the ⩾15 mm criterion.
3. QFT test is a better tool to identify infected individuals and to reduce the number of unnecessary TBI treatment.

R2689 Rapid molecular detection of tuberculosis and rifampin resistance using Xpert-MTB/RIF

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Background: Global control of tuberculosis is hampered by slow, insensitive diagnostic methods, particularly for the detection of drug-resistant forms and in immunocompramised patient's Early detection of tuberculosis is essential to reduce the overall mortality and morbidity as well as elimination of disease transmission, but the complexity and infrastructure needs of sensitive methods limit their accessibility and effect.
Methods: We assessed the performance of Xpert MTB/RIF, an automated molecular test for *Mycobacterium tuberculosis* (MTB) and resistance to rifampin (RIF), with fully integrated sample processing in 40 patients with suspected pulmonary tuberculosis. Eligible patients in from our hospital provided three sputum specimens each. Twenty Two specimens were processed with N-acetyl-L-cysteine and sodium hydroxide before microscopy, solid and liquid culture, and the MTB/RIF test, and current culture confirmation ProbTec PCR, the rest of 18 samples underwent direct MTB/RIF test and direct ProbTec PCR kit.
Results: Among culture-positive patients, a single, direct MTB/RIF test identified 25 of 26 patients (96.15%). In smear-positive patient the test

identified 15 of 16 smear positive cases (93.75%) and 6 of 24 with smear-negative tuberculosis (25.00%). The test was specific in 38 of 40 patients without tuberculosis (95.00%). Among patients with smear-negative, culture-positive tuberculosis, the addition of a second MTB/RIF test increased sensitivity to a total of 92.2%. As compared with phenotypic drug-susceptibility testing, MTB/RIF testing correctly identified 1 of 1 patients (100%) with rifampin-resistant bacteria and 21 of 23 (91.3%) with rifampin-sensitive bacteria.
Additional comparison between ProbTec Strand displacement amplification (our current MTB PCR) and Xpert MTB/RIF showed a total of match of 39 of the 40 samples (97.5%) and one sample that was smear negative were positive with ProbTec and negative with Xpert system, subsequently culture confirm it's MTB positive.
Conclusions: The MTB/RIF test provided Simultaneous detection of both MTB and rifampicin resistance, as a marker for MDR strains directly from untreated sputum in less than 2 hours with minimal hands-on time. The test has high sensitivity for detecting MTB – even in smear negative, culture positive specimens. The test can be performed On-demand basis enable physicians to treat rapidly and effectively.

R2690 Risk factors for long-term treatment in lymph node tuberculosis

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Introduction: Lymph node tuberculosis (LNTB) is the most frequent extrapumonary tuberculosis (TB), but some authors reported huge differences between duration of treatment instead of WHO 2003 recommendations for 6 to 9 months of antiTB therapy. We conducted a retrospective multiple centre study in the aim to describe the clinical factors for prolongation of antiTB drugs.
Material and Method: We included retrospectively all patients who presented with at least LNTB during 1998–2010 periods from seven hospitals in North of France. TB was diagnosed either by culture or histology or clinical suspicion improving with antitubercular drugs. Five universities hospitals participated. We excluded from treatment duration analysis patients with bone and neurological involvement.
Results: 148 patients were included, 57 of them were men (38.5%), and 58 (41.1%) were Africa born. Median age was 43.2 years (extremes 13.2–91.3). There were 21 (16.2%) HIV infected patients, 13 of them (54.2%) were diagnosed HIV positive at the same time at the TB diagnosis. Mean CD4 cell was 193.7/mm3±151. Forty two patients (28.8%) had another TB localisation (of whom 4 had bone or neurological involvement), 91 (61.9%) had superficial lymph nodes, 73 (49.6%) had cervical localisation only. Four (2.7%) patients died, 13 (8.8%) were lost of follow up.
Duration treatment analysis was undertaken on 126 patients: median duration was 9 months (extremes 2–30). Treatment was significantly longer in HIV positive patients ($p < 0.01$), in patients with other TB localisation than lymph node ($p < 0.01$), in patients with loss of weight ($p = 0.02$), and if patient had presented new lymph node during treatment ($p = 0.023$). The treatment was significantly shorter when no complication occurred, and the main cause for treatment prolongation was non favourable evolution. Seven patients presented relapse, 2 have been treated for 9 months, 2 for 6 months, 2 for 4 months, one for 30 months before relapse.
Conclusion: LNTB is treated much longer than WHO recommendations. Only 58% of treatment length is in accordance with 2003 recommendations, and 23% with 2009 recommendations which suggest that 6 months are sufficient for all extrapulmonary TB except for bone and neurological TB. Factors associated with treatment duration are HIV infection, loss of weight and other tuberculosis localisation.

Infection in the immunocompromised host and transplant recipients

R2691 Prevalence and aetiological pathogens of asymptomatic bacteriuria in type 2 diabetic patients with and without microalbuminuria

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Objectives: The prevalence of asymptomatic bacteriuria (ASB) in diabetic patients is high, especially in women. The aim of this study was to evaluate the prevalence and to identify the aetiological pathogens of ASB in patients with type 2 diabetes mellitus (T2D) with and without microalbuminuria (MA).
Methods: A total of 200 patients with T2D (100 with MA and 100 without MA) were recruited in the study. Patients with overt diabetic nephropathy or nephropathy from other causes, with symptoms of urinary track infection or use of antimicrobial drugs in the last 14 days were excluded by the study. Microalbuminuria was diagnosed measuring the albumin excretion rate (AER) by radioimmunoassay (RIA) method (Pharmacia, Pharmacia and Upjon Diagnostics AB, Uppsala, Sweden). Midstream clean voiding urinary specimens were collected for urinalysis, examined by Gram stain and cultured on blood and MacConkey agar for detection of uropathogens. Any isolated pathogen (after 24–48hours incubation) was identified using BBL™ Enterotube™ II (BD Diagnostic Systems, Germany), Api System and Vitek 2 Compact (Biomerieux, France).
Results: The prevalence of ASB was increased in diabetic patients with MA compared to diabetic patients without MA (21% versus 8%, $P < 0.001$, respectively). *Escherichia coli* was the most prevalent pathogen isolated in diabetic patients with and without MA (12% versus 3.0%, $P = 0.01$, respectively) followed by *Proteus mirabilis* (6% versus 5%, $P = 0.75$, respectively) and *Klebsiella* spp (5% versus 1%, $P = 0.09$, respectively).
Conclusion: ASB was more prevalent among T2D patients with MA and the main causative agent was *E. coli*. Screening for ASB is warranted in diabetic patients especially if pyuria is detected in urine analysis since ASB has been found to be a risk factor for developing symptomatic urinary tract infection.

R2692 Pandemic 2009 influenza A (H1N1) virus infection coinciding with invasive pulmonary aspergillosis

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Introduction: Prior reports have suggested a possible association between invasive pulmonary aspergillosis (IPA) and influenza infection in otherwise healthy patients. In patients receiving anti-neoplastic chemotherapy, however, the impact of influenza on the incidence of IPA remains unknown.
Methods: We used data from the Cologne Cohort of Neutropenic Patients to analyse the impact of the influenza A (H1N1) 2009 pandemic on the incidence of invasive pulmonary aspergillosis among cancer patients. We then compared our findings to historical data.
Results: During the pandemic we diagnosed influenza A (H1N1) by PCR in five patients with malignancies and febrile neutropenia refractory to antibiotic therapy. Probable invasive pulmonary aspergillosis was diagnosed in three of these patients on grounds of typical CT morphology and microbiologic results. During the pandemic, three of five patients receiving AML induction chemotherapy developed aspergillosis although receiving posaconazole prophylaxis. In the three years before the influenza pandemic, only 2/77 patients of this group developed IPA (RR 23.1; 95% CI: 4.93–108.16).
Discussion: Our findings indicate that infection with influenza A (H1N1) may increase the risk for invasive aspergillosis in neutropenic patients. During pandemic and seasonal influenza, persistently febrile neutropenic

patients should continue to receive standard diagnostic work-up, even if diagnosis of influenza has been made. Pulmonary aspergillosis is an important additional differential diagnosis in neutropenic influenza patients with pneumonia.

R2693 Common pathogens isolated from diabetic foot infections in a university hospital, Hungary

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Objectives: Foot infections are the most common problems in persons with diabetes. These individuals are predisposed to foot infections because of a compromised vascular supply secondary to diabetes. Local trauma and/or pressure (often in association with lack of sensation because of neuropathy), in addition to microvascular disease, may result in various diabetic foot infections. Infectious agents are associated with the worst outcomes, which may ultimately lead to amputation of the infected foot, unless prompt treatment strategies are ensued. The present study sought to reveal bacterial etiology of diabetic foot ulcers in diabetic patients of our region.
Methods: A retrospective review of clinical and microbiological data of 139 diabetic patients suffered from moderate-to-severe diabetic foot infections (DFIs), including out- and inpatients in the special wards of our University was carried out over a 5-year period. After debridement, investigators collected wound specimens, mostly by curettage or biopsy, and sent them to the Hungarian Anaerobic Reference Laboratory for aerobic and anaerobic culture.
Results: All of the samples were culture positive, only anaerobic bacteria were present in 34 samples (24.5%). Among the cultures, 97% were polymicrobial, 2 grew only one microorganism: *C. perfringens* and *C. septicum* in pure culture, 43.7% had both aerobes and anaerobes. A total of 832 bacterial strains were isolated, resulting in an average of 5.98 organisms (range 1 to 21) per lesion: 619 anaerobic-, and 209 aerobic- or facultative bacteria and 4 yeasts were isolated. The predominant aerobic organisms were oxacillin-susceptible *S. aureus* (31.7%), β-haemolytic (mostly group B) *Streptococcus* species (12.2%), *Enterococcus* species (16.7%), members of the family Enterobacteriaceae: *E. coli*, *Proteus*, *Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter* (52.3%), and *P. aeruginosa* (15.8%). The predominant anaerobes were Gram-positive cocci: 103 isolates, *Prevotella* species: 198 isolates, *Clostridium* species: 50 isolates, and the members of the *Bacteroides fragilis* group: 41 isolates.
Conclusion: Clinical grading and bacteriological study of 139 patients with diabetic foot lesions revealed polymicrobial aetiology. Our findings showed that the density of growth of anaerobes and the number of the isolated anaerobic species were significantly higher than that in previous studies, due to good anaerobe laboratory practice and the adequate sampling.

R2694 Clinical and laboratory investigation of KPC-2 *Klebsiella pneumoniae* bacteraemia in patients of a haematology ward

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Objectives: The present the emergence and the dissemination of KPC-2 producers *K. pneumoniae* (Kp) strains from blood cultures of hematological patients, in a tertiary hospital in Greece.
Methods: During the period October 2008-May 2010, Kp strains, that exhibited resistance to carbapenemes, were isolated from 12 patients of the hematology ward. Susceptibility to carbapenemes was perfomed by the use of Kirby-Bauer method and MICs were determined by VITEK II and E-test method, according to CLSI. KPC production was initially investigated by Hodge test and double disk synergy test (meropenem+boronic acid) and was confirmed by molecular techniques. Molecular typing was performed by pulse field gel electrophoresis (PFGE). A retrospective review of the patients' history took place to estimate underlying diseases, previous antibiotic usage and clinical outcome.
Results: Kp strains revealed various susceptibility to imipenem and meropenem. All strains were resistant to ertapenem, but most of them were susceptible to gentamicin, tetracycline, tigecycline and colistin. All strains were KPC-2 producers and belonged to the same clone. Patients' mean age was 70 years (range 25–81). Clinical diagnosis were: nine acute leukemia, one chronic lemphogenic leukemia, one aplastic anemia and one non Hodgkin lymphoma. The duration of patients' hospitalization was 0–24 days, before the isolation of Kp strains. In three patients, a simultaneous isolation of KPC strains from urine cultures was observed. Two patients with bacteremia were found to be colonized in the intestine with with KPC strains, while four patients developed first the bacteremia and intestine colonization followed. Neutropenia was observed in eight patients, with an absolute number of neutrophils 0–400/μl. Before the isolation of the KPC strains, all patients were treated with a combination of a β-lactam antibiotic and an aminoglycoside, while after the isolation the treatment changed to colistin, tigecycline and/or gentamycin. The response was poor and ten patients out of twelve (83%) died because of the KPC infection.
Conclusions: Bacteremia from KPC-2 producers Kp, in hematological patients, has high mortality and consists a serious medical-nursery problem, in addition with the already existing high bacteria resistance.

R2695 Risk factors contributing to treatment success in severe diabetic foot infections

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Objectives: To identify risk factors contributing treatment responses and prognoses in severe diabetic foot infections.
Material and Method: Between April 2008 and October 2010, a prospective study was conducted by Diabetic Foot Study Group on severe diabetic foot infections.
Results: A total of 62 patients were included in this study, the mean age was 64.09 (range 37 to 87) years and forty three (69.4%) patients were male. Eighty patients were diabetic for more than ten years. 56.5% of the patient had previous hospital stay and 83.9% had previous antibiotic use within the last three months. The duration of wound infections were more than four weeks in 84% of the patients and 56.5% of them had purulent discharge. Leucocytosis was found in 26 (46.8%) patients and 41 (66.1%) patients had elevated CRP levels more than five fold. 14.5% of patients were classified as Wagner stage 4 and 8% were Wagner stage 5. Thirty three (53.2%) of the patients were presented with osteomyelitis. Etiology was identified in 41 (66.1%) patients. Gram negative bacilli were isolated in 19 (46.3%), Gram positive cocci were isolated in 10 (24.4%) and 12 (29.2%) grew mixed bacteria. Ten of 41 microorganisms isolated were ESBL producing Gram negative bacilli, three were MRSA, nine were MRSE, seven were multidrug resistant (MDR) *P. aeruginosa* and three were MDR *A. baumannii*.
The mean duration of hospitalization was 38.4 days; 37 (59.7%) patients underwent debridement and amputation was performed in 30 (48.4%) patients. Major amputation was reqüired in 9% of cases.
Conclusion: Complete clinical improvement was observed in 26 (41.9%) patients. Duration of diabetes over ten years ($p = 0.017$), higher fever ($\geqslant 38.5$ºC) ($p = 0.004$), existance of purulent discharge ($p = 0.020$), higher Wagner stage of the wound ($p = 0.000$), elevated CRP levels (>5 fold) ($p = 0.01$), infection with MDR bacteria ($p = 0.066$), requirement of amputation ($p = 0.000$) and prolonged hospital stay ($p = 0.01$) were negative predictors of treatment success and prognosis.

R2696 The efficacy of low-dose valganciclovir prophylaxis for cytomegalovirus infection in renal transplant recipients

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Objectives: Cytomegalovirus (CMV) infection is one of the most important viral infections causing graft loss in renal transplant recipients (RTr). The present study aims to determine the efficacy of low-dose valganciclovir (VGC) prophylaxis in CMV infection.

Methods: 103 CMV seropositive patients over the age of 18 – and whose donors were also seropositive – who had undergone renal transplantation between January 2007 and June 2010 were included in the study. The data on these patients were recorded retrospectively using follow-up forms. The form included the following information: age, gender, primary disease, cold ischemia time, type of transplantation, immunosuppressive treatment scheme, duration of valganciclovir use, adverse effects, CMV antigenemia level and whether CMV infection/disease developed after transplantation or not. In the prophylaxis group VGC (n = 29) was administered 450mg/day. For the other group (n = 26) VGC was not administered only short time IV gansiklovir was administered. If the patient received antithymocyte globulin (ATG) treatment, IV ganciclovir was administered only during the treatment. The patients were followed using CMV antigenemia tests (1–3 times/month). All patients were followed for 6–30 months after transplantation. The data were evaluated using chi-square test and t test for independent groups on SPSS 15 software.
Results: The demographic characteristics of the RTr who did or did not receive VGC prophylaxis were similar. Total ATG dose was significantly higher in the prophylaxis group than in the other group. It was determined that, although not significant, the rate of CMV infection development was lower in the patients to whom prophylaxis was administered (Table 1). All of the eight patiens were determined CMV infection delayed-onset in the prophylaxis group VGC. The mean time between transplantation and CMV infection were found 242,62±141,36 (95–524) days. During the use of VGC, drug administration was interrupted in 8.3–34.5% of the patients due to leucopenia/thrombocytopenia.
Conclusion: The use of high-dose ATG is a risk factor for CMV infection. Although the use of low-dose VGC decreases the rate of CMV infection/disease, the decrease is not significant and does not bring out a decrease in the rate of adverse effects.

Table 1: Characteristics of renal transplant recipients

	CMV Prophylaxis (+) ATG (+) n=29 (%)	CMV Prophylaxis (-) ATG (+) n=26	CMV Prophylaxis (-) ATG (-) n=18	Statistics (p)
Age (Year)	40,97 ± 12,8	43,50 ± 11,23	36,33 ± 10,92	0,441
Sex (Male/Female)	18 / 11	17/9	27 / 21	-
Primary diseases	Hypertension (7) Chronic GN* (8) Idiopathic (5) Others (9)	Hypertension (7) Idiopathic (7) Chronic GN (5) Others (7)	Hypertension (11) Chronic GN (11) Idiopathic (8) Chronic PN** (5) Others (13)	-
Living / Cadaveric	13 / 16	4 / 22	28 / 20	
Cold ischemia time (min)	638,43±613,70 (45-2160)	828,08±517,58 (45-1560)	595,02±492,32 (40-1620)	0,139
Total steroid dosage (mg)	2039±696,03 (750-4000)	1868,75±662,14 (500-2500)	1714,29±659,05 (500-3000)	0,320
Total ATG dosage (mg)	1694,20±1065,44 (180-4550)	1191,67±536,07 (200-2375)	-	**0,034**
Duration of VCG use (day)	90,00±22,31 (27-105)	63,80±31,36 (12-107)	60,87±34,98 (12-122)	-
Advers effects (n)	10 (34,5)	3 (12,5)	4 (8,3)	
CMV infection (n)	8 (27,5)	12 (46,1)	15 (31,25)	0,152

R2697 Ribavirin treatment for human metapneumovirus and methicillin-resistant *Staphylococcus aureus* co-infection in adult haematological malignancy

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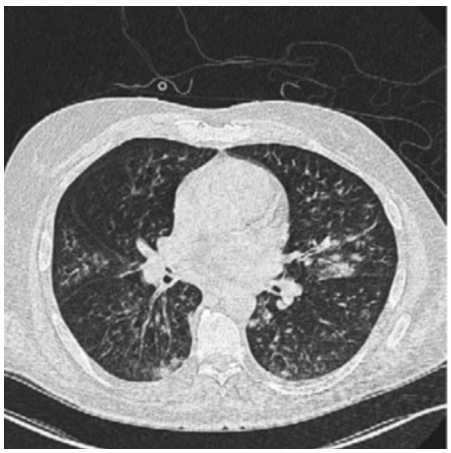
Objective: To report the use of intravenous ribavirin in two adult cases of severe pneumonia demonstrating co-infection with human metapneumovirus (HMPV) and methicillin resistant *Staphylococcus aureus* (MRSA) in the setting of lymphopenia following chemotherapy for haematological malignancy.
Method: Case report of clinical, radiological and microbiology findings with literature review of the role of ribavirin in HMPV infection in adults with haematological malignancy.
Results: 2 cases of HMPV pneumonia were identified in an Australian haematology unit in spring, 2010. The cases were in separate but adjacent rooms raising the possibility of nosocomial transmission. Lymphopenia was present in both as a complication of chemotherapy for anaplastic large cell lymphoma (case one) and after autologous peripheral stem cell transplant for multiple myeloma (case two). MRSA and HMPV were identified in bronchoalveolar lavage (BAL) fluid. Respiratory intubation, ventilation and intensive care management was required for progressive respiratory failure whilst on maximal therapy for MRSA infection. Bilateral ground glass infiltrates progressed to nodular, confluent parenchymal changes. Salvage treatment with ribavirin was started more than a week after symptom onset. Ribavirin 25mg/kg/day (intravenous) was followed by 15mg/kg/day (intravenous) for a total of 7 days in both cases. One patient cleared virus from respiratory tract, but progress was complicated by a cerebrovascular accident. The second patient developed extensive air space consolidation and cavitation. HMPV was detected by PCR at 6 weeks after treatment with ribavirin.
Conclusion: HMPV infection is associated with significant morbidity and poor outcomes. Lymphopenia is a risk factor for infection with HMPV in adult patients receiving treatment for hematological malignancy. Co-infection with MRSA occurs. Neutrophil recovery and hypogammaglobulinaemia may contribute to severity of infection. Ribavirin was well tolerated. Late treatment with intravenous ribavirin for seven days did not eradicate viral shedding in one patient, and may have contributed to clinical and virological cure in the other. HMPV may be detected in respiratory secretions for greater than 6 weeks in adult patients with haemopoeitic malignancy, with implications for infection control measures.



R2698 Polymicrobial bloodstream infections in patients with cancer

D. Kofteridis, D. Dimopoulou, A. Valachis, S. Maraki, A. Christidou, A. Thanasia, V. Theodorakopoulou, I. Aristeidou, N. Spernovasilis, G. Samonis (Heraklion Crete, GR)*

Objectives: Polymicrobial bloodstream infections (PBSIs) occur frequently in patients with cancer and are associated with high mortality. However, there are only a few reports specifically addressing PBSIs. The aim of the present study was to compare epidemiological features and mortality attributable to PBSIs with those attributable to monomicrobial bloodstream infections (MBSIs) in patients with malignancy.
Methods: A retrospective matched case-control study, with 1:1 ratio was performed. All patients with malignancy, hospitalized with bloodstream infection in the departments of Haematology and Medical Oncology, from 2005 to 2010, were reviewed. Each patient with PBSI was matched to another with MBSI by age, sex and type of malignancy. Univariate and multivariate analyses were performed.
Results: A total of 114 episodes of BSI among 110 patients were identified. Of those, 34 patients (31%) had a PBSI. Gram-negative organisms were predominant in 85% of the episodes, while Gram-positive in 53% of the PBSIs. Grade 4 neutropenia was present in 6 patients with PBSI and 5 with MBSI. The presence of chronic renal

disease (odds ratio [OR] 12.2; 95% confidence interval [CI], 1.3–113.1) was an independent risk factor for PBSI, while prior blood transfusion was revealed as protective factor (OR 3,9; 95% CI, 1.4–11,5). Empirical inappropriate antimicrobial treatment has been given to 20 patients (59%) with PBSIs, and 7 (21%) with MBSIs ($p = 0.003$). The infection-related mortality was 29% in patients with PBSI and 12% in those with MBSI ($p = 0.072$). No differences in duration of hospitalization or overall mortality were observed.

Conclusion: PBSIs represent a significant percentage of BSIs among patients with malignancy. Although inappropriate empirical antimicrobial treatment has been given in higher percentage of patients with PBSI than those with MBSIs, no differences in duration of hospitalization or overall mortality have been observed. Recognition of the risk factors for PBSIs and knowledge of their microbiology are important for the selection of appropriate empirical antimicrobial treatment that may result in improved outcome.

R2699 Risk factors for urinary tract infections caused by ESBL-positive *E. coli* in renal transplant recipients

H. Arslan, O. Özalp, Ö. Azap, S. Aktas, A. Tekindal, M. Haberal (Ankara, TR)*

Objectives: Urinary tract infections (UTI) are the most common infections in renal transplant recipients. The aim of this study is to determine the risk factors for UTI caused by extended-spectrum β-lactamase (ESBL) producing *Escherichia coli*.

Methods: Renal transplant recipients with the diagnosis of UTI during the two year period from January 1st, 2009 to December 1st, 2010 were included in this study. Fifty-one adult patients. Demographic charactersitics, clinical and laboratory values, antibacterial susceptibility results were evaluated from patient records retrospectively. Risk factors for UTI caused ESBL positive *E. coli* strains were determined.

Results: Sixty-one UTI episodes were diagnosed in 51 patients. Sixty-nine percent of the patients were female. The range of the ages were 18–60 years. More than one UTI episodes were seen in 8 patients. The three leading uropathogens were *E. coli*, *Klebsiella* spp and enterococci. *E. coli* was detected in 41 (67%) episodes, *Klebsiella* spp. was detected in 11 (18%) episodes and enterococci was detected in 5 (8%) episodes. Twenty-one of the *E. coli* isolates (51%) were ESBL positive. The antimicrobial resistance rates of ESBL negative and ESBL positive *E. coli* isolates were shown in the Table.

The risk factors determined are hospitalization at the time of diagnosis or hospitalization within 1 month, recent (within 1 month) antibiotic use, urinary catheterization.

Conclusion: Risk factors for urinary tract infections caused by ESBL producing bacteria among renal transplant recipients are similar with the other patient groups but nearly half of the uropathogen *E. coli* strains are ESBL positive in renal transplant recipients. This high percentage of resistant pathogens causes difficulties in the management of these patients.

	AMP	AMC	NIT	FOS	GN	AN	CIP	NOR	CRO	CZ	TZP	SXT	IPM/MEM
ESBL positive	21/21	21/21	3/21	1/21	10/21	3/21	15/21	15/21	21/21	21/21	7/21	21/21	0/21
ESBL negative	19/20	7/21	1/20	0/20	4/20	0/20	8/20	8/20	0/20	3/20	1/20	16/20	0/20
Total	40/41	28/41	4/41	1/41	14/41	3/41	23/41	23/41	21/41	24/41	8/41	37/41	0/41

Table. Antimicrobial resistance rates of uropathogen *E. coli*.

R2700 Bacterial infections in the early period after liver transplantation

H. Arslan, F. Timurkaynak, Ö. Azap, S. Aktas, M. Haberal (Ankara, TR)*

Multidrug resistant bacteria are increasingly isolated from transplant recipients and cause high morbidity, mortality. The aim of this study is to determine the disribution and etiologic agents of bacterial infections seen in the early period after liver transplantation.

A retrospective study was conducted on 90 patients who underwent orthotopic liver transplantation consecutively from January 2008 to October 2010 at Baskent University Hospital. Microbiologically documented bacterial infections seen during the first month after liver transplantation were included in this study.

A total of ninety liver transplantations were performed during the study period. Twenty eight bacterial infection episodes were diagnosed in 20 patients within 30 days after transplantation operation. Twelve (13%) of the infections were intraabdominal (9 Gram negative and 3 Gram positive), 8 (9%) were catheter-related (4 Gram negative, 4 Gram positive), 6 (6%) urinary tract infection (5 Gram negative, 1 Gram positive), 2 ventilator associated pneumonia (1 Gram negative, 1 Gram positive). A total of 19 episodes were caused by Gram negative bacteria and the remaining 9 were caused by Gram positive bacteria. Strains susceptible only to colistin and tigecycline were defined as extensively drug resistant (XDR). Seven of the isolated Gram negative strains were *Klebsiella pneumoniae* (2 ESBL positive, 2 XDR strains), 6 were *Escherichia coli* (4 ESBL positive strains), 4 *Acinetobacter baumannii* (3 XDR strains), 1 *Pseudomonas aeruginosa*. The distribution of the Gram positive pathogens were 4 *Enterococcus faecium* (2 were vancomycin resistant), 3 methicillin resistant coagulase negative staphylococci, 1 methicillin sensitive *Staphylococcus aureus* and 1 methicillin resistant *Staphylococcus aureus*. A total of 20 bacteremia episodes were detected of which 12 (60%) were secondary and 8 (40%) were primary.

The incidence of colonization and infection with multi-drug resistant bacteria particularly Gram negative strains has been increasing throughout the world. Data regarding the transplantation patients are limited but common usage of extended spectrum antibiotics among these patients both during the preoperative and postoperative phases undoubtedly predispose to difficult-to treat infections. The infection rates seen in the early postoperative period obtained in this study are comparable with the previous ones but the high percentages of multi-drug resistant strain is the alarming issue.

Community-acquired infections including CAP, sepsis, STD, ...

R2701 Urethritis epidemiology in men in central Madrid, Spain

M.A. Orellana, M.L. Gomez-Lus (Madrid, ES)*

Objective: To study the epidemiology and risk factors of urethritis in men and compare these with the microorganisms isolated.

Methods: We conducted an epidemiological survey to 270 men with urethral exudate petition during the years 2006–7. The parameters obtained were: age, nationality, symptoms and risk factors (previous STD, HSH, >1 sexual partner). To urethral exudates we performed: GRAM stain, culture in habitual plates, study of *C. trachomatis* by PCR in the COBAS AMPLICOR System (ROCHE), *U. urealyticum* and *M. hominis* by *Mycoplasma* IST (Biomerieux), *T. vaginalis* by microscopic examination and HSV by cellular culture.

Results: The age of patients were 33.1±10 years, the 52.6% were Spanish, the 29.3% were HSH, the 61.8% had >1 sexual partner and the 31.8% had a previous STD. The symptoms were: pain 17%, discharge 40%, urethral itching 21.5% and dysuria 39.3%. In GRAM stain it was observed >5 LPMN/C in 18.9%. The percentage of positive samples was: 39.3% The isolated microorganisms were: *C. trachomatis* 13%, *N. gonorrhoeae* 11.1%, *U. urealyticum* 10%, *Haemophilus* sp 4.8%, *S. agalactiae* 1.5%, HSV 1.5%, *M. hominis* 0.7%, *Candida* sp 0.7% and *S. aureus* 0.4%. The patients with urethritis compared with non-urethritis had significant difference for: age (31.4±8 vs 34.2±10), HSH (49.4% vs 35.1%), >1 sexual partner (45.5% vs 29.1%), discharge (52.8% vs 31.1%) and leukocytes in GRAM stain (76.5% vs 23.5%). Depending on isolated microorganism, we found that:

N. gonorrhoeae was significantly most frequent isolated in HSH (27.8% vs 4.2%), >1 sexual partner (16.2% vs 2.9%), pain (23.9% vs 8.5%), discharge (27.8% vs 0%) and leukocytes in GRAM stain (49.0% vs 1.1%).

C. trachomatis was significantly most frequent isolated in: >1 sexual partner (17.4% vs 5.8%), discharge (18.5% vs 9.3%), dysuria (18.9% vs 9.1%) and leukocytes in GRAM stain (21.6% vs 10.1%).
U. urealyticum had significant difference for age (28.6± 7 vs 33.6±10) and we did not find significant difference for the rest of issues studied.
We did not find significant differences for the rest of isolated microorganisms.
Conclusions: Urethritis was most frequent in young men, with >1 sexual partner, with discharge and leukocytes in GRAM stain. *N. gonorrhoeae* was most frequently isolated in HSH, with >1 sexual partner, with pain and discharge like symptoms and leukocytes in GRAM stain. *C. trachomatis* was isolated in men with >1 sexual partner, dysuria and leukocytes in GRAM stain and *U. urealyticum* was most frequent in youngest men.

R2702 Peritoneal dialysis-related peritonitis in both a paediatric and an adult population: a four-year retrospective analysis

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Microbial etiology of peritoneal dialysis (PD) related peritonitis seems to vary widely in children and data for adults are scarce. The objective of this study was to determine the microbial etiology, correlation between leucocyte (wbc) count and culture results as well as the concordance between culture and the Gram stain of PD related cases of peritonitis in both children and adults.
We reviewed the records of all patients whose peritoneal dialysate (n = 1285) was sent to the lab for microbiology of the Antwerp University Hospital between January 2005 and August 2009. Microbial etiology, wbc count, Gram stain and antibiotic treatment were analyzed per episode. 142 adults and 22 children were included.
From each episode, microbial etiology was based on culture results of the first sample. Gram stain was performed when the leucocyte count exceeded 200 per mm3. Logistical and linear regression were used in addition to a mixed effects model with repeated patient effect to determine the degree of correlation between the wbc count and positive cultures.
In 158/39 episodes of adult/pediatric peritonitis, 70.7/70.0% of samples were culture positive (+): 72.1/52.4% of isolates were Gram positives (G+), 21.2/38.1% Gram negatives (G–), 1.9/0.0% anaerobes and 4.8/9.5% yeasts. In 29.3/30.0% no etiology was found: 32.6/22.0% of the culture – were taken during antimicrobial treatment.
Gram stain showed predominant morphotypes concordant with culture results in 30.5/16.7%.
In adults, the G+ were mainly coagulase negative staphylococci (CNS) (70.7%), *S. aureus* (8.0%) and viridans streptococci (8%); in children, predominant pathogens were *E. faecalis* (27.3%), CNS (27.3%), Coryneforms (18.2%) and *S. aureus* (9.1%).
The most prevalent G– pathogens in adults were *E. coli* (22.7%), *E. cloacae* (18.2%), *K. pneumoniae* (18.2%), *P. aeruginosa* (13.6%) and other non-fermenters (13.6%). In children, the most frequent G– were *E. cloacae* (25.0%), *P. aeruginosa* (12.5%) and other non-fermenters (25%).
A significant difference in wbc count was found between culture+ and culture– samples in adults (p = 0.001).
In conclusion, a microbial etiology was found in the majority of cases. Gram positive related cases of peritonitis are more frequent in adults, Gram negatives in children and the distribution of pathogens also differs. The concordance between Gram stain and culture appears low. Finally, there are significantly less leucocytes in culture negative dialysates compared to culture positive dialysates.

R2703 International PMEN clones equal or exceed the fitness of other strains despite the accumulation of antibiotic resistance

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Objectives: A small number of global pneumococcal clones defined by the Pneumococcal Molecular Epidemiology Network (PMEN) dominate the population of antibiotic-resistant pneumococci. It remains unclear why PMEN clones spread so successfully despite the scientific paradigm that a loss in biological fitness is the price for acquisition of resistance. The aim of this study was to detect PMEN clone related clinical isolates from adult patients with community-acquired pneumonia (CAP) collected by the German CAPNETZ surveillance study during 2002–2006 and to compare them to unrelated clones in terms of antibiotic-resistance, biological fitness and clinical parameters.
Methods: Multi-locus sequence typing (MLST) data were used to define relatedness between PMEN clones and clinical pneumococcal isolates. Relatedness was defined by the sharing of alleles at ⩾5 of 7 loci. Fitness was determined by the measurement of growth curves. The bacterial growth was measured by a microplate reader at optical density of 600 nm at intervals of five minutes over a period of 16 hours. To compare bacterial growth we analysed the maximum slope of each curve and the experiment was repeated nine times. Statistical analysis were performed by the chi-square test or Fisher's exact test for categorial variables and the t-test or analysis of variances (ANOVA) for continuous variables.
Results: We analysed 154 pneumococcal isolates and 46 (30%) isolates showed a close relationship to the global PMEN clones. These isolates were equal or exceeded the fitness of isolates without relationship to PMEN clones (1.48±0.73 vs. 1.18±0.54; P = 0.015) and constituted 80% of antibiotic-resistant isolates. The survey of clinical parameters showed no prominent significant difference between both groups.
Conclusion: The success of international PMEN clones is based on the combination of resistance and fitness and may result in the endurance of these strains despite a reduction of antibiotic usage. New vaccines can interrupt the transmission of resistant strains, but continued attention to the replacement of nonvaccine serotypes and development of vaccines with a broader coverage will be necessary.

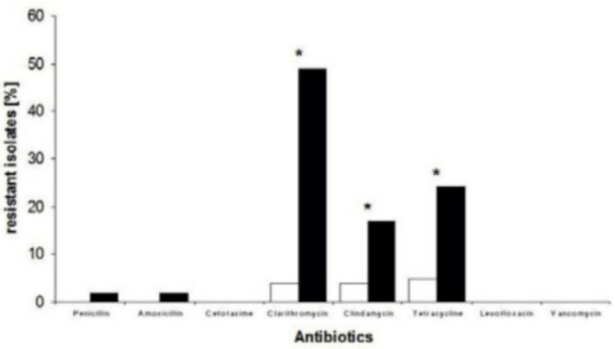

Figure 1. Antimicrobial resistance profile of pneumococcal isolates (n=154).

■ PMEN clone related
□ Non PMEN clone related

* P-Value ≤ 0.01

R2704 Microorganisms isolated in urethral samples in women

M.A. Orellana, M.L. Gomez-Lus (Madrid, ES)*

Objective: To study the isolated microorganisms in urethral samples in women and to analyse the epidemiology and risk factors depending on isolated microorganisms.
Methods: We studied 377 urethral exudates between 2003–2007. The samples were cultured in habitual plates; *C. trachomatis* was performed between January 2003 and May 2007 by ICT CHLAMY-CHECK-1

(GRIFOLS) and between June-December 2007 by RCP by COBAS AMPLICOR System (Roche); *U. urealyticum* and *M. hominis* by *Mycoplasma* IST (Biomerieux) and HSV by cellular culture.
We performed an epidemiological survey to 90 women during the years 2006–7. The obtained parameters were: age, nationality, symptoms and risk factors.
Results: The percentage of positive samples was 54.4%. The isolated microorganisms were: *U. urealyticum* (UU) 41.1%, *Candida* sp 10.3%, *C. trachomatis* (CT) 2.9%, *M. hominis* (MH) 6.4%, Enterobacteries 4.8%, *S. agalactiae* 2.4%, *N. gonorrhoeae* 0.8%, *Haemophilus* sp 0.5%, *G. vaginalis* 0.5% and SHV 1.3%. It was isolated >o= 2 microorganisms/sample in 15.1% and the most frequent associations UU+MH 42.1%, UU+*Candida* sp 28.1%, UU+*E. coli* 8.8%, UU+CT 7% and UU+*S. agalactiae* 5.3%.
In the epidemiological survey we found: age 36.4±15, Spanish 52.2%, previous STD 15.5%, >1 sexual partner 25.5%. The symptoms were: pain 20%, discharge 37.7%, urethral itching 34.4%, dysuria 25.5% and recurrent urinay tract infection 33.3%. The isolated microorganisms in this group were: UU 44.4%, CT 6.7%, Enterobacteries 7.7%, *Candida* sp 8.8%, GV 6.6%, *S. agalactiae* 2.2% and HSV 1.1%.
Among women with positive samples there were significant differences for discharge (79.4% vs 48.2%) and urethral itching (77.4% vs 50.8%).
Depending on isolated microorganisms, we found that:
U.U. was significantly most frequent isolated in patients with discharge (58.8% vs 35.7%) and >1 sexual partner (65.2% vs 37.3%).
was most frequent isolated in patients with discharge (14.7% vs 1.8%), urethral itching (16.1% vs 1.7%), dysuria (17.4% vs 3.0%) and >1 sexual partner (17.4% vs 3.0%).
Enterobacteries were most frequently isolated when dysuria existed (21.8% vs 3.0%).
Candida sp was not significantly associated with none of the parameters studied.
Conclusions: UU, *Candida* sp and MH were the most frequent isolated microorganisms. Discharge and uretrhal itching were the most frequent symptoms in women with positive culture. Discharge was present in patiens with isolation of UU and CT and dysuria in patients with isolation of CT and enterobacteries.

R2705 *Chlamydia trachomatis*, *Mycoplasma hominis*, *Mycoplasma genitalium* and *Ureaplasma urealyticum* infections and seminal quality in infertile men

N. Al-Sweih, A. Hadad, V. Rotimi, A. Omu (Kuwait, KW)*

Objective: The aim of this study was to compare the occurrence of genital *Chlamydia trachomatis*, genital mycoplasmas and ureaplasmas in semen samples of fertile and infertile men in Kuwait.
Materials and Methods: A total of non-duplicated 315 semen samples collected from 127 infertile and 188 control men seen at the infertility clinics in Maternity hospital were studied after informed consent. Semen analysis was performed according to the guidelines of World Health Organization. The specimens were examined for the presence of *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Mycoplasma genitalium* and *Chlamydia trachomatis* by PCR using published specific primers. Biodata, such as age, nationalities, and marital status were carefully recorded.
Results: The frequency of genital *U. urealyticum*, *M. hominis*, *M. genitalium* and *C. trachomatis* in all semen samples was respectively 26% (82/315), 27% (86/315), 5.4% (17/315) and 8.3% (26/315). Mixed infections were detected in 14% (44/315). The infertile participants positive for *U. urealyticum* and *M. hominis*, respectively had semen samples that showed statistically significant difference in the mean of sperm concentration, vitality percentage, total progressive and rapid progressive motility in comparison to control fertile participants ($P < 0.001$). Similar statistical significance difference was noted for those infertile and fertile men positive for *M. genitalium* and *C. trachomatis* ($P < 0.001$). Infections in infertile men who had been married for less than 5 years were significantly higher than in infected fertile men of the same length of marriage.
Conclusion: Infections caused by these urogenital pathogens were more common among infertile men than fertile men and may possibly play a role, in part, in the etiology of male infertility in this part of the world. Genital mycoplasmas and chlamydial infections may negatively influence semen quality.
Acknowledgement: This study was supported by Kuwait University Research Administration Grant No. YM 03/09.

R2706 Validity of hospital diagnosis of pleural empyema in 224 patients: a clinical validation study

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Objective: Pleural empyema is a serious infection characterized by accumulation of pus in the pleural space. In the US, 65,000 patients suffer from empyema each year, with 30-day mortality up to 15%. Many studies addressing risk factors and outcome of empyema rely on hospital discharge diagnosis codes recorded in health care databases. We validated the ICD-10 diagnosis of pleural empyema in the Danish National Registry of Patients (DNRP).
Methods: We randomly selected hospitalized inpatients in the North Denmark Region with a first-time discharge diagnosis of pleural empyema between 1995 and 2009. We retrieved and reviewed medical records and estimated the positive predictive value (PPV) of the empyema diagnosis. Definite empyema was defined by frank pus aspirated from the pleural space, a positive Gram stain/subculture for pathogenic micro-organisms in the pleural fluid, and/or an autopsy diagnosis of empyema. Patients with clinical symptoms suggestive of empyema in association with compatible radiographic features (e.g. pleural thickening, loculated and/or septated pleural effusions) who did not meet the criteria for definite empyema were classified as having probable empyema.
Results: We could retrieve the medical records of 224/225 sampled patients with empyema (99.6%). Of those, we classified 182 (81.3%) as being definite empyema cases. Another 21 patients were classified as probable empyema cases. In 21 patients whose empyema diagnosis was rejected, eight had pneumonia, one had a pulmonary abscess, one pulmonary tuberculosis, one had sarcoidosis, two had emphysema.
The overall PPV of the empyema diagnosis was 90.1% (95% CI 86.0–94.1). The PPV was 81.3% (95% CI 81.3–86.1) when including definite cases only. PPVs of empyema diagnosed in 1995–1999, 2000–2004, and 2005–2009 were 90.7% (95% CI 81.7–96.2), 94.6% (95% CI 86.7–98.5), and 86.7% (95% CI 76.8–93.4), respectively, indicating that no major changes in coding validity occurred over the 15-year study period. The PPV decreased slightly from 95.7% in patients aged 15–39 years to 87.5% in patients aged 80 years and over but was uniformly high regardless of study period, hospital or department type, or cause of empyema.
Conclusion: The high overall PPV indicated good agreement between ICD-10 codes for pleural empyema and medical records. Registry-based discharge codes may be a suitable source of data on pleural empyema for epidemiological research.

R2707 Pulmonary nocardiosis – clinical analysis of 21 patients

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Introduction: *Nocardia* is a rare pathogen causing predominantly pulmonary and/or skin and soft tissue infections. Since immunosuppression is one main predisposing factor, nociardial infections may increase because of the rising number of patients on immunosuppressive therapy (e.g. solid organ or bone marrow transplantation). Data on futher risk factors, clinical course and treatment of nocardial infections are limited to case reports and small case series. We aim to establish a register for nocardia infections in Germany, Switzerland and the Netherlands.
Methods: Retrospective analysis of all microbiologically proven pulmonary *Nocardia* spp. infections in 4 hospitals in Germany and the

Netherlands between 1999 and 2009, defined as detection of *Nocardia* spp. in respiratory samples + radiolagical changes and signs and symptoms of pulmonary infection.
Results: Twenty-one cases of pulmonary nocardiosis could be identified (18 male, 3 female; mean age 54.7±18.1 years). In addition to pulmonary involvement, disseminated disease was detected in 3 patients (all with cerebral abscess formation). Eleven of 21 patients had pulmonary comorbidities (n = 4 COPD; n = 3 bronchiektasis, n = 2 cystic fibrosis, n = 1 asthma, n = 1 post-TB). Twelve of 18 patients had identifiable causes of immunosuppression (hematological dieseases, malignancies, drug induced immunosuppression, diabetes mellitus). All patients tested (16/21) were HIV negative. Time from sampling to availability of microbiological results was 9.1 days. All cases were proven by culture and in 15 patients additional PCR-sequencing was performed (*N. farcinica* n = 4; *N. abscessus* n = 3; *N. asteroides* n = 3; *N. nova*, *N. carnea*, *N. cyriacigeorgica*, *N. otitidiscaviarum* and *N. paucivorans* each n = 1) The pathogen was isolated from BAL (n = 9), sputum (n = 7), biopsy (n = 3), tracheobronchial aspirate (n = 1), and abscess aspirate (n = 1). Most patients received cotrimoxazol treatment (15/21), mean duration of treatment was 12.2 weeks. One patient died.
Conclusion: Pulmonary nocardiosis remains a rare disease. Although immunosuppression (e.g. drug induced immunosuppression or malignancies) is its major risk factor, it can also occur in patients without obviously impaired immunocompetence. These patients most frequently suffer from chronic pulmonary diseases such as COPD. The delayed in vitro growth of *Nocardia* spp. may lead to misdiagnosis or underestimation of nocardiosis in patients without typical risk factors.

R2708 A prospective study on predictors for early death from bacteraemia

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Objective: Inactive empiric antibiotic occurred in 33% of patients with bacteraemia, with mortality of 27% at discharge in 2006. A blood culture service started in April 2007 to ensure active antibiotic within 2 days of blood culture collection. A prospective bacteraemia study from February to June 2009 noted mortality at discharge decreased to 9%. Death occurred in 2% before positive blood culture was notified. We aim to study patients with early death for clinical predictors.
Methods: Patients who died before (early death) and after (late death) positive blood culture was notified were compared, so was early death with survivors. Univariate and multivariate analysis were performed for independent predictors of early death.
Results: Of 452 patients with 467 cases of bacteraemia, 42 patients died, and 9 were early death. Male comprised 60%, median age was 78 years and Charlson's score 5. *Staphylococcus aureus* occurred in 10 (7 was methicillin-resistant [MRSA]), *Escherichia coli* and *Klebsiella pneumoniae* 5 each (6 carried extended-spectrum β-lactamase), *Proteus mirabilis* 3 and *Candida* species 2.
On univariate analysis of early death vs. late death, age ⩾75 years (odds ratio [OR] 9.14, confidence interval [CI] 1.01–82.44), renal disease (OR 0.122, CI 0.02–0.71), pneumonia (OR 28, CI 3.81–205.79) and acute renal impairment (OR 0.17, CI 0.03–0.94) were significant. Only pneumonia was independent risk factor (adjusted OR [AOR] 41.2, CI 3.68–463.74, P = 0.003).
Comparison of early death vs. survivors noted significant univariate risk factors: age ⩾75 years (OR 14.68, CI 1.82–118.45), intensive care unit (OR 5.64, CI 1.35–23.42), solid tumour (OR 4.36, CI 1.05–18.06), pneumonia (OR 26.53, CI 6.32–111.40), *Acinetobacter baumannii* (OR 53.38, CI 3.06–930.79), MRSA (OR 6.51, CI 1.26–33.58), inactive empiric antibiotic (OR 4.74, CI 1.17–19.25), hypotension (OR 13.02, CI 3.17–53.52), and ventilatory support (OR 30.29, CI 4.74–193.47). On multivariate analysis, the independent predictors of early death vs. survivors were: pneumonia (AOR 18.61, CI 2.68–129.53), inactive empiric antibiotic (AOR 24.40, CI 1.57–378–23), hypotension (AOR 17.01, CI 2.29–127.78), and ventilatory support (AOR 59.59, CI 1.27–2786.41).
Conclusion: Our study showed that hypotensive patients needing ventilatory support for pneumonia with inactive empiric antibiotic were more likely to die before blood culture could be notified. It is crucial to ensure adequate empiric antibiotic in this cohort.

R2709 Evaluation of Abbott® real-time m2000 CT/GC assay, Roche Cobas®Amplicor™ CT/GC test and SiemensVersant® CT/GC DNA 1.0 assay (kPCR) for the detection of *Chlamydia trachomatis*/*Neisseria gonorrhoeae*

H. Tuokko (Oulu, FI)*

Objectives: Abbott RealTime m2000 (AR) method was compared to Roche Cobas Amplicor (RCA) for detection of *Chlamydia trachomatis* (Ct) and *Neisseria gonorrhoeae* (Gc) in urine samples. Siemens Versant (SV) was the third test for the minor series of the urine samples. AR and SV are automated systems with short hands-on-time. Tecan miniprep was used for DNA extraction for RCA.
Methods: Ct was tested in 297 urine samples and Gc in 200 urine samples in RCA and AR. The samples were taken in the years 2008–10, tested with RCA within 1–2 days after the collection and frozen. The minor selection included 75 frozen urine samples for AR. Also these were tested within 1–2 days after the collection with RCA- and SV-methods.
Results: AR detected 170 Ct-positive while RCA 176 among 297 samples with the correlation of 0,966 for Ct-positive and of 1,05 for negative results. In Gc-testing, RCA found 13 (confirmed) positive samples while AR 12. AR detected as Ct-positive 45 out of 75 samples while RCA 47 with the correlation of 0,957 and SV 49 with 1,042, respectively. All the tests gave the same 20 samples as Ct-negative and 40 as Ct-positive. RCA and SV detected the same samples negative and SV 2 positive more than RCA, while RCA compared to AR, AR had 5 negatives and 1 positive more than RCA. SV and AR tested the same samples Ct-negative but SV 4 Ct-positive samples more. And, RCA detected the other 3 Ct-negative samples more than AR- and RCA. All 3 methods detected the same 4 Gc-samples as positive.
Conclusion: RCA used the DNA extractor more sensitive for contamination than the other two. The manual pipetting may have been the risk for false-positivity, too. There is also possibility of false-negative samples, because of inability to detect the new variant of Ct (nvCt) unlike AR and SV. Sample storing as frozen may have some effect on results. AR needed sample volume less than SV. AR offers separated units for DNA extraction and PCR itself. Gc-positive samples detected by AR (target the opaA gene) need no confirmation because of high specificity. These make AR a suitable system for clinical microbiology laboratories. The prevalence of nvCt needs to be tested.

R2710 Clinical manifestations of actinomycosis in a university hospital

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Objectives: Actinomycosis is a chronic infection caused by anaerobic or microaerophilic, Gram-positive bacteria, *Actinomyces* spp. classically it involves cervicofacial (55%), abdominopelvic (20%), thoracic (15%), and mixed organs (10%), including skin, brain, pericardium, and extremities. But recent studies reveal change about types of actinomycosis. This retrospective study describes the clinical manifestations of patients with actinomycosis.
Methods: We retrospectively studied clinical manifestation of patients who had diagnosed actinomycosis by histopathological methods, from January 2001 through January 2009. The medical records were reviewed for clinical data of the patients, including presenting symptoms, predisposing factor, lab findings, whether surgery was performed, and the duration of IV or oral antibiotic treatment.
Results: There were 16 cases of actinomycosis diagnosed at Chung-Ang university Hospital in Seoul, South Korea from January 2001 to January 2009. Three types of actinomycosis were found in this study: thoracic (44%), abdominopelvic (31%), cervicofacial organs (25%). In

this study, the duration of IV antibiotic treatment ranged from 10 to 30 days. 2 patients received oral antibiotic therapy without IV antibiotics. The duration of oral antibiotic treatment ranged from 67 to 150 days. One patient received surgical treatment only without antibiotics.
Conclusion: Over the last three decades, the incidence of actinomycosis has declined markedly because of better oral hygiene and more extensive use of antibiotics and clinical pictures have changed. The incidence of cervicofacial type is declined and thoracic type is increased. The treatments of actinomycosis are also changed, short-course chemotherapy has recently been reported to be successful.

R2711 Comparison of the severity of invasive pneumococcal infections between adults and children

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Background: *Streptococcus pneumoniae* is a common cause of community acquired invasive bacterial infections both in children and adults and infects both previously healthy and diseased subjects. Incidence of invasive pneumococcal diseases in different age groups is well known but the effect of age on the clinical outcome of invasive pneumococcal diseases has not been documented. We wanted to analyze whether our clinical impression that children recover more quickly from invasive pneumococcal diseases than adults is true.
Methods: We reviewed the medical records of all blood culture positive community acquired cases of pneumococcal diseases admitted to University Hospital of Oulu in years 2000–2007. Data on clinical symptoms, laboratory values, imaging studies, medications and outcome were collected and compared between children and adults. During the study period vaccination against pneumococcal diseases was recommended only to specific risk groups (patients with immune deficiencies and people older than 65 years).
Results: There were 56 paediatric (<18 years) and 229 adult cases. One hundred and seven of the patients (26% of adults and 84% of children) were previously healthy. None of the children died while there was a 15% (35/229) mortality among the adults (difference 15%, 95% confidence interval, CI, 8–21%). The median length of stay at the hospital (LOS) was 2 days in children and 9 days in adults (difference of the medians 6 days, CI 5–7). When taking account only the patients with underlying conditions the median LOS was 2 days in children and 8 days in adults ($P < 0.001$).
Conclusion: The course and outcome of invasive pneumococcal infections are far more severe in adult patients compared to children. This may explain the differences in the attitudes towards pneumococcal vaccination among physicians taking care of either children or adults.

R2712 Influence of sexual intercourse on vaginal lactoflora

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Under physiological conditions, the vaginal microflora (VMF) contains high numbers of lactic acid bacteria which provide the colonization resistance. At the same time VMF is an open ecosystem that can be significantly affected by sexual intercourse.
Our aim was to clarify the influence of sexual intercourse on partner's vaginal lactoflora.
Methods: Study group included 17 women with mean age 29.9 (21–39) years. Two self-collected vaginal samples were taken in the follicular phase, before intercourse and 8–12 hours after intercourse. VMF was assessed by Nugent method. Lactobacilli were cultured on MRS agar, typed by AP-PCR method and identified by sequencing.
Results: According to the Nugent scores, normal vaginal flora (score 0–3) was found in 9 women in both specimens. Intermediate score (4–6) was found in 3 women and bacterial vaginosis (score $\geqslant 7$) in 1 woman. In 4 women normal microflora was found in the first sample but intermediate microflora in post-intercourse sample. In addition, the score increased in two more women, and in total, the mean score was higher after intercourse (1.94 vs 2.71).
Culturable lactobacilli were found from 15 out of 17 women. The mean proportion of lactobacilli in total microflora was somewhat lower in after-intercourse sample ($49.7 \pm 33.8\%$ vs $34.6 \pm 26.2\%$). All isolated strains were identified as *Lactobacillus jensenii* (in 67% of women), *L. crispatus* (58%) and *L. gasseri* (25%). In one third of women more than 1 species was isolated. AP-PCR allowed us to confirm the persistence of the same strains in all cases though in a quarter of women strains of the same species but different fingerprints were revealed.
Conclusions: *Lactobacillus* species composition in Estonian women coincides with that of described earlier. Sexual intercourse causes shifts in vaginal lactoflora as revealed by increased Nugent score and decreased lactobacillus proportion in VMF.

R2713 AQP1 and AQP4 in the CSF of bacterial meningitis patients

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Objectives: Aquaporins (AQP) are proteins that facilitate water transport through cell membranes. Due to the main localization of AQP1 in the plexus choroideus and of AQP4 in the perivascular endfeet of astrocytes in the brain, these channels are supposed to play a pivotal role in oedema formation. Cerebral oedema formation is one of the main contributors of neuronal damage in bacterial meningitis (BM).
Our study aimed to determine whether AQP1 and AQP4 are present in cerebrospinal fluid (CSF) and if BM induced an increase of these proteins in the CSF, and whether these concentrations correlated with routine CSF parameters of inflammation.
Methods: CSF AQP1 and AQP4 concentrations of 35 consecutive patients with BM treated at the Department of Neurology of the University Medical Center and 27 healthy control persons (C) were measured. CSF aquaporins were quantified using ELISA technique.
Results: The mean concentration of AQP1 was significantly elevated in patients with BM (3.8 ± 3.4ng/ml[BM] vs. 0.8 ± 0.5ng/ml[C]; $p < 0.001$). AQP4 was also increased, however not significantly (1.8 ± 3.1ng/ml[BM], 0.1 ± 0.2ng/ml[C]; $p = 0.092$). AQP1 and AQP4 concentrations in the CSF of BM patients were inversely inter-correlated ($R = -0.47$, $p = 0.004$) but we could not find any other correlation between the concentrations of AQP1 or AQP4 with routine CSF parameters (leukocytes, lactate, protein, Qalbumin), age, a prediction-score, outcome-score or GCS at admission.
Conclusion: Bacterial meningitis causes an increased release of AQP1 and AQP4 into the CSF. Whether the significant elevation of AQP1 is due to a higher expression on intact choroidal cells or due to the destruction of glial cells needs to be determined.

R2714 Experience of an infectious disease ward with prosthetic joint infections: clinical outcome and economical impact

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Objectives: The aims of our study were: 1) to evaluate the outcome of prosthetic joint infections (PJIs) due to different microorganisms and treated with different options 2) to analyze the economical impact of PJIs.
Methods: We consider retrospectively patients with PJIs from 2004 to 2009. The isolation of microorganisms was obtained from blood culture and/or deep samples and/or intraoperatory samples. We defined as cured patients without signs of local infection and negative inflammatory index >6 months after antimicrobial therapy interruption. In order to quantify the costs of the treatment we consider the average weekly costs of antimicrobial therapy.
Results: 50 PJIs were included in the analysis. Only in 27 (54%) we obtained the isolation of the microrganism (9 Coagulase Negative Staphylococci, 8 Methicillin Resistant *Staphylococcus aureus*, 7 Methicillin Sensitive *Staphylococcus aureus*, 3 Gram-negative). PJIs were cured in the 71,4% of patients; if we consider Methicillin Resistant *Staphylococcus aureus* (MRSA)/Methicillin Resistant *Staphylococcus epidermidis* (MRSE) infection, we achieved a positive outcome only

in 61% of cases. Furthermore, we evaluated the importance of the choice of the therapeutical option: with prosthetic removal and consequent substitution we cured 89% vs 69% of patients who underwent debridement and retention and 64% of patients treated only with long-term suppressive antimicrobial treatment. The mean weekly cost of PJIs in our analysis was 278€ but when the microrganism involved were MRSA/MRSE was increased to 404€.
Conclusion: In our analysis. there is a significant proportion of PJIs where the pathogen remains unidentified. As previously described, the two stages substitution of the prosthesis resulted to be the most successful option. Taken as a whole, our data suggest the importance of a multidisciplinary approach to the management of prosthetic joint infections at all stages, allowing a timely identification of the microorganism whenever is possible and a medical and surgical approach that allows more favourable outcome. MRSA/MRSE continue to be a serious challenge in patients with prosthetic infections, being associated to have a worse outcome and greater costs when compared to infections caused by other microorganisms.

R2715 Clinical significance of delta neutrophil index in patients with sepsis

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Objectives: To evaluate the clinical significance of delta neutrophil index (DNI), which corresponds to the immature granulocytes in the peripheral blood, in patients with sepsis.
Methods: We reviewed medical and laboratory records of the consecutive 116 hospitalized adult patients with sepsis from May 2007 to June 2010 in one tertiary university hospital. DNI was measured by blood cell analyzer (ADVIA 120, Siemens, Inc.).
Results: Of a total 116 patients with sepsis, mean age was 69.3±12.0 years and mean DNI value was 5.5±7.4% (range 0–34.6%) and mean APACHE II score was 17.8±6.9 (range 6–42). DNI values of sepsis (n = 43), severe sepsis (n = 25) and septic shock (n = 48) were 4.2±4.3%, 6.5±8.9% and 6.0±8.6% respectively (p = 0.34). DNI value of bacteremic patients (n = 45) was higher than non-bacteremic patients (n = 71) (7.9±8.6% vs 4.0±6.0%, p = 0.01). DNI values were no significant difference in survivors (n = 98) and non-survivors (n = 18) (5.0±6.8% vs 7.9±9.8%, p = 0.24). But, DNI values in bacteremic patients were significantly higher in non-survivor (n = 7) than survivor (n = 38) (14.2±12.1% vs 6.7±7.5%, p = 0.03).
Conclusion: The present findings indicate that DNI may be useful prognostic marker of bacteremic sepsis.

R2716 Epidemiological characteristics of community-acquired pneumonia among hospitalised children in the Republic of Belarus

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Objective: To evaluate epidemiology of community-acquired pneumonia (CAP) in hospitalized Belarusian children.
Methods: All consecutive immunocompetent children hospitalized in Main city children's infectious diseases hospital with radiographically confirmed CAP were evaluated retrospectively from January 2009 through December 2009. We analyzed age, past history, clinical manifestations, length of hospital stay, the severity of the disease and the influence of repeated cases of CAP in the same child.
Results: 743 patients were studied during 12 months. Ages ranged from 1 month to 17 years old (55% were boys). The majority of pneumonia cases (34%) were registered in two-years-old children. The second place was occupied by one-year-old children (19%), and the third place – by three-years-old children (13%). Children younger than 1 year of age had pneumonia only in 9% of cases.
The majority of patients were hospitalized during the first week of their illness (mostly on 2–4 days). In only 32.23% of cases children were admitted with initial diagnosis of pneumonia.
Pneumonia had moderate severity level in 90% of hospitalized children. While 10% of patients had severe CAP. The mean length of hospitalization was 10.5±0.34 days (range 1–38 days).
686 and 183 children had the history of the acute upper respiratory tract infection and bronchitis, respectively. 68 patients experienced one or more CAP episodes in their past history. Influenza was registered in the anamnesis of 53 patients. 60% of studied patients attended pre-school and school organizations.
The analysis of the repeated cases of pneumonia among studied patients hasn't revealed the statistically significant interrelation between the severity of the disease and the presence of the pneumonia episode in the patient's history (odds ratio: 1.3; 95% confidence interval 0.6–2.8, p = 0.695).
CAP cases were registered round the year. The morbidity increased in September then slightly decreased in December. Another increase was recorded in January. The maximum levels were noted in November (101 cases) and in April (100 cases).
Conclusion: Children's CAP is the significant problem for Belarusian Health System. The main group associated with CAP was children aged from 1 to 3 years old. In the future, immunization with the use of licensed pneumococcal and influenza vaccines may reduce the frequency of pneumonia.

Lyme borreliosis, toxoplasmosis

R2717 Seroprevalence of *Toxoplasma gondii* among pregnant women living in a rural northern Greek province

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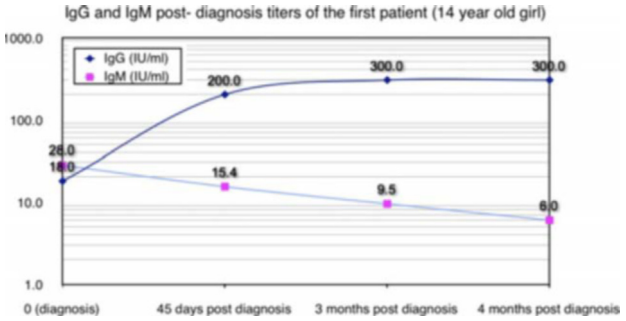
Objectives: Purpose of this study is to evaluate toxoplasmosis seroprevalence among pregnant women who live in a rural Province of Northern Greece, where eating undercooked meat is quite unusual. Also, 3 toxplasmosis cases were evaluated, concerning the source of infection.
Methods: Laboratory of Hygiene in collaboration with a private Microbiology–Virology Laboratory of Drama Province, collected blood samples from 128 pregnant women (age range: 20–38 years old) during the period 17/1/2007– 5/5/2010. VIDAS (BIOMERIEUX) was used to determine IgG & IgM *T. gondii* levels. Statistical analysis was done with SPSS 16.0 package. Moreover, clinical and laboratory data for 3 individuals suffering from toxoplasmosis were evaluated, and patients were interviewed concerning the source of infection.
Results: Among 128 women, 26 (20.31%) had IgG(+) – past infection, and 102 (79.69%) had IgG(–) – no previous contact with *T. gondii*. None had recent *T. gondii* infection [IgM(+)].
Toxoplasmosis cases:
14 year old girl, clinical signs: fever and lemphadenopathy, IgG= 18IU/ml, IgM= 28IU/ml, her IgG and IgM titers through time is shown on the diagram attached.
47 year old man, father of the first patient, IgG= 140IU/ml, IgM= 1.08IU/ml. They hosted stray cats at their garden and had close and long-term contact with them.
40 year old woman, IgG= 300IU/ml, IgM= 6.60IU/ml, infection is attributed to eating raw meat.
Conclusions: 20.31% of women tested proved to be seropositive, a percentage which comes in total compliance with other studies conducted in Northern Greece (20% among women of reproductive age). Comparing our results to previous studies we can clearly observe a decrease in seropositivity rates during the last decades (1984: 35.6%, 1994: 25.6%). 79.69% of women tested had no previous contact with *T. gondii*, and therfore are susceptible. Possible sources of infection for susceptible individuals, taking into account people's habits in Drama Province:
Eating habits: low consumption of undercooked meat, high consumption of lamp, goat, sheep, smoked pork sausages, and salads consisting of raw and wild vegetables and fruits (well documented sources of *T. gondii*)
Great number of stray cats

Individual's habits: close contact with cats, special attention to the cat's litter container.

Apart from serological testing of pregnant women, there is no further official recommendation concerning toxoplasmosis management during pregnancy, in order to prevent congenital disorders to the growing fetus.



R2718 Course and outcome of Erythema migrans in patients with underlying rheumatological disease

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Objectives: To evaluate the course and outcome of erythema migrans (EM) in adult patients with rheumatologic disease (RD).

Methods: Information was obtained from database on patients with EM, examined at the University Medical Center, Department of Infectious Diseases, Ljubljana, Slovenia, from 1992 to 2009. The data were acquired prospectively using a structured questionnaire. EM was defined according to modified CDC criteria. During 18-year period 59 patients, 50 females and nine males, aged 58 (30–81) years, with typical EM and pre-existent RD (arthritis and related disorders – 51 patients, collagen vascular disorders – 8 patients), were examined. 33/59 patients (55.9%) were receiving immunosuppressive therapy (methrotrexate, corticosteroid, gold, leflonamide). Their pre-treatment characteristics and outcome after treatment were assessed and compared with 118 previously healthy age-, sex- and antibiotic treatment-matched persons, diagnosed with EM in the same year.

Results: Comparison revealed analogous findings for the frequency of tick bite, incubation, duration of EM prior to diagnosis and its largest diameter, proportion of patients with multiple EM, seropositivity, as well as for *Borrelia* skin and blood culture results. Systemic symptoms were more common in patients with RD (51% versus 25%, $p = 0.0014$); the most prominent difference was found for headache (36% versus 8%, $p < 0.0001$). In 2/59 (3.4%) patients with RD but in none of the control group ($p = 0.1098$) objective extracutaneous manifestations of Lyme borreliosis (monoarthritis, meningoradiculitis) were found at presentation. Duration of EM after the beginning of antibiotic treatment was comparable. Four (6.8%) patients with RD, and one (0.8%) in the control group ($p = 0.0429$) were re-treated within 3 months due to pronounced subjective symptoms (two patients) or persistence of EM (two patients with and one patient without underlying RD). Nonetheless, the clinical course was smooth and comparable in both groups.

Conclusions: Higher proportion of systemic symptoms and a tendency for more common presence of objective extracutaneous manifestations of Lyme borreliosis before antibiotic treatment as well as more often need for re-treatment indicate that early Lyme borreliosis has a more severe course in patients with RD than in immunocompetent persons. However, the outcome one year after treatment with antibiotics as used for immunocompetent individuals is excellent.

R2719 Neuroborreliosis in Bulgaria – Clinical manifestation and serological findings

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Objectives: Lyme borreliosis is a multisystemic disease called neuroborreliosis when neurological symptoms are pre-eminent. Bulgaria is an endemic region for Lyme disease with increasing number of cases reported annually. The aim of this study is to reveal clinical forms and evaluate serological findings in patients with diagnosed neuroborreliosis in Bulgaria.

Methods: Medical records of patients with neuroborreliosis during 2006–2010 were studied. A total of 254 paired serum/CSF samples were collected from patients with neurological symptoms compatible with neuroborreliosis and tested by ELISA. For detection of specific intrathecal antibody production, antibody index towards total IgG was calculated.

Results: Presence of IgG and IgM antibodies was detected in 39,76% (101/254) of the patients. In 8,66% (22/254) of the patients, anti-*Borrelia* IgG antibodies were found in serum samples. Anti-*Borrelia* IgM antibodies were detected in 32/254 (12,5%) serum samples and both IgM and IgG antibodies – in 9/254 (3,54%) patients. Thirty one (12,2%) patients had intrathecal synthesis of anti-*Borrelia* antibodies. Antibodies against *B. burgdorferi* s.l. in both serum and CSF samples were found in only 6,69% (17/254) of the patients. IgM antibodies were detected in 3/31 (9,68%) of the patients with intrathecal antibody synthesis, while IgG antibodies were found in 90,32% (28/31) patients. Various clinical manifestations of neuroborreliosis were observed – mostly affection of the peripheral nervous system-cranial neuritis, polyneuropathy and paresis. Encephalopathy was observed in 10 patients. Four were misdiagnosed as multiple sclerosis.

Conclusions: Neuroborreliosis is a frequent manifestation of Lyme borreliosis in Bulgaria. This study showed that common serological findings included IgM anti-*Borrelia* antibodies in serum and IgG antibodies in CSF samples. Polyneuropathy was the most common clinical manifestation of neuroborreliosis.

R2720 Seroprevalence of Toxoplasmosis in HIV/AIDS Iranian patients

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Objectives: *Toxoplasma gondii* has arisen as an important opportunistic agent particularly in the central nervous system and in advanced HIV disease; it can cause significant morbidity and mortality. This study was performed to determine the seroprevalence of toxoplasmosis among HIV-positive patients in Iran.

Methods: Blood samples were collected from 201 HIV positive patients and anti-toxoplasma antibodies were detected by using conventional ELISA. An antibody titer of more than 3 Iu/ml was considered positive.

Results: The majority of studied patients were male (male to female ratio: 5 to 1) with the mean age of 36 ± 0.65 yrs. The seroprevalence of toxoplasmosis in HIV positive patients was 49.75%. The mean CD4 count in HIV patients with positive toxoplasma serology was $332.46 \pm 22.45/\mu l$. Only 1% of the patients had IgM anti-toxoplasma antibodies and 10% of the patients had clinical toxoplasma encephalitis. The mean CD4 count in this group was $66.4 \pm 15.5/\mu l$ and there was a significant statistical association between CD4 count and rate of toxoplasma encephalitis ($P < 0.001$).

Conclusion: In view of the relatively high prevalence of toxoplasma infection has found among the HIV infected patients in our study moreover a significant proportion of HIV infected with toxoplasmosis will be presented in future with toxoplasma encephalitis that could be otherwise prevented by appropriate chemoprophylaxis, it can be logical that screening for toxoplasma should be suggested for all HIV infected patients in Iran.

Antimicrobial clinical trials

R2721 Biapenem versus meropenem: a multicentre, randomised, single-blind, controlled clinical trial

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Objective: This study aimed to compare the efficacy and safety of injectable biapenem and meropenem in the treatment of bacterial lower respiratory and urinary tract infections.
Method: A randomized, single-blind, controlled clinical trial was performed in 9 medical centers from January 2009 to March 2010 in mainland of China. In this phase 2 study, patients were randomly (1:1) assigned to receive IV biapenem or meropenem for $\leqslant$2 weeks. The primary efficacy endpoints were clinical response at the test-of-cure visit (7 days after therapy) for the intent-to-treat (ITT). Differences in clinical efficacy, bacteriology and safety between the two groups were subjected to statistical analysis, including ITT analysis.
Results: A total of 272 cases received $\geqslant$1 dose of study drug were enrolled and comprised the ITT population. with 241 per-protocol set (PPS) cases (121 biapenem, 120 meropenem). A total of 272 cases (136 biapenem, 136 meropenem) were included in the safety set (SS) analysis. Overall efficacy rates of biapenem and meropenme were 94.70% and 93.94%, respectively. Overall bacterial eradication rates of the two groups were 96.39% and 93.83%, respectively. Among the biapenem group, 16 patients (11.76%) had probable drug-related adverse events. Among the meropenem group, 21 patients (15.44%) had probable drug-related adverse events. The major ones were gastro-intestinal symptoms and rash, the elevations of AST or/and ALT and decrease of white blood cells. All differences between the two groups were insignificant.
From the above-mentioned results of clinical efficacy, bacteriological efficacy, and safety, injectable biapenem was confirmed to be useful in the treatment of moderate, severe and/or refractory infections in lower respiratory and urinary tract infections caused by β-lactamase-producing (including ESBL) bacterial isolates.

R2722 Microbiological analysis of a prospective, randomised, double-blind trial comparing moxifloxacin and clindamycin in the treatment of infiltrates and abscesses

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Objectives: Aim of the study was the analysis of oral pathogens in odontogenic abscesses and gingival infiltrates and their susceptibility to moxifloxacin (MXF), levofloxacin (LVX), penicillin (PEN), amoxicillin/clavulanic acid (AMX/CLA), doxycycline (DOX), and clindamycin (CLI).
Patients and Methods: A prospective, randomized, double-blind, multicenter, phase II trial compared the efficacy and tolerability of MXF and CLI in the treatment of odontogenic abscesses and inflammatory infiltrates. The analysis of microbial parameters presented here were part of the secondary endpoints. 71 patients with either infiltrates or abscesses were enrolled into this study.
Clinical results: MXF was significantly more effective at mean pain reducing in patients with inflammatory infiltrates on day 2–3, compared to CLI (61.0% vs. 23.4%, $p = 0.006$). Global efficacy assessment at days 2–3 and 5–7 showed faster clinical responses with moxifloxacin in both abscess and infiltrate patients.
Microbiological results: 205 bacteria were isolated from 71 patients (viridans streptococci 77x, *Prevotella* spp. 56x, *Neisseria* spp. 19x, *Streptococcus* (*S.*) *anginosus* group and hemolytic streptococci 17x, other anaerobes 15x, and other bacteria 21x). 98% of pathogens were susceptible to MXF followed by AMX/CLA (96%), LVX (85%), PEN (67%), CLI (60%), and DOX (50%). *S. anginosus* group and hemolytic streptococci were detected significantly more frequent ($p = 0.04$) in patients with abscesses (12/95) compared to patients with infiltrates (5/110).
Only eight patients with odontogenic infections did not recover following surgical intervention and primary antibiotic therapy. Bacteria isolated from patients with therapy failure and resistant against the antibiotic given are analysed. From the abscess group a wide range of bacteria were isolated including various kind of streptococci, *E. faecalis*, *Neisseria* spp., and anaerobes. In contrast, from the infiltrate group viridans streptococci (3x) and *Neisseria* spp. (3x) were isolated only.
Conclusions: In this study MXF showed a promising in vitro and in vivo activity against odontogenic bacteria compared to CLI that justify its use for treatment of odontogenic abscesses and infiltrates. Our analyses clearly indicate that *S. anginosus* group and hemolytic streptococci are associated with odontogenic abscesses and that viridans streptococci and *Neisseria* spp. are involved in the pathogenesis of odontogenic infiltrates.

R2723 Comparative study of the antimicrobial activity of chlorinated and non chlorinated antiseptics against *C. albicans*

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Objectives: The efficacy of chlorinated and non-chlorinated antiseptics on *Candida albicans* is still not completely elucidated. Hence the general objective of this study is to determine the anti-candidal efficacy of six commonly available chlorinated antiseptics with and without cetrimide and three without chlorine and cetrimide.
Methods: The organism was challenged with diluted (according to the manufacturers instruction) of each of the antiseptics for a period between 30 seconds and 180 seconds and the microbial cell reduction rates were determined at every 30seconds contact by Time kill Test.
Result: The undiluted chlorinated antiseptic containing cetrimide revealed 100% reduction in *C. albicans* cell count at 60secs contact time while at 90secs undiluted chlorinated antiseptics without cetrimide produced the same 100% lethal effect. Non chlorinated antiseptics did not produce significant cell reduction even at 180secs just like the control. Chlorinated antiseptic containing cetrimide diluted according to the manufacturer's recommendations produced 100% cell reduction at 120 and 150secs. Diluted chlorinated antiseptics without cetrimide were able to produce 93.8% and 96.1% cell reduction at 180secs. Also, the pH of the antiseptics had significant association with their efficacy on *Candida albicans* ($\chi^2 = 3.54$, $P < 0.05$).
Conclusion: Chlorination and pH of antiseptics has significant effect on the efficacy of antiseptics against *C. albicans*.

R2724 A prospective, open-label, non-comparative study of anidulafungin for treatment of documented candidaemia/invasive candidiasis in selected intensive care unit patients in Europe and Canada (ICE Study) – Focus on microbiologic efficacy

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Objectives: Evaluate anidulafungin (ANI) for therapy of candidemia/invasive candidiasis (C/IC) in selected populations of intensive care unit (ICU) patients (pts) from Europe and Canada. Here we present detailed results on microbiologic efficacy.
Methods: Prospective, open label, multicenter, phase 3b study in adult ICU pts (APACHE II score <25) with post-abdominal surgery, age $\geqslant$65 years, renal/hepatic insufficiency, solid organ transplant, neutropenia and/or solid tumour. C/IC had to be confirmed within 96 h before to 48 h after therapy initiation. Planned total treatment duration was 14 to 56 days: $\geqslant$10 days of ANI (200 mg day 1, 100 mg/d thereafter), optionally followed by oral voriconazole (VOR)/fluconazole (FLU). Global response at end of all therapy (EOT) in the evaluable modified intent-to-treat (MITT) population, i.e. excluding pts with missing/

unknown responses, was the primary efficacy endpoint. Microbiologic response was defined as eradication or presumed eradication. Secondary endpoints were global response at end of IV therapy (EOIVT) and 2 and 6 weeks after EOT, time to negative blood culture, survival at day 90 and adverse events (AEs).

Results: 216 pts received study drug and 170 were included in the MITT population. Most had candidemia (71%) and C albicans only was the most common causative pathogen (56%). Common C/IC risk factors included broad-spectrum antibiotics (90% of pts), central venous catheter (87%), prior surgery (67%), and total parenteral nutrition (58%). Most tested baseline isolates (153/167) were fully susceptible to all of ANI/FLU/VOR, with the exception of: 2/96 C albicans to both FLU and VOR; 4/27C glabrata to FLU; 1/21 C parapsilosis to ANI and 5/21 to FLU; and 2/2 C krusei to FLU. Overall MIC_{90} for ANI, FLU and VOR was 0.5 μgmL, 8 μgmL and 0.5 μgmL, respectively. Microbiologic and global response at different time points, across the selected subpopulations/baseline pathogens and by infection site are shown in the table. Mean time to first negative blood culture was 4 days and 90-day survival was 54%. 33/216 (15%) of patients had treatment-related AEs, which led to study discontinuation in 5 pts. Infusion-related AEs occurred in 6 pts.

	Microbiologic response (95% CI)	Global response (95% CI)
Time point		
EOIVT	73.9% (66.4%, 80.5%)	70.7% (62.9%, 77.7%)
EOT	72.8% (65.1%, 79.6%)	69.5% (61.6%, 76.6%)
2 weeks post-EOT	60.2% (51.1%, 68.7%)	60.2% (51.1%, 68.7%)
6 weeks post-EOT	50.5% (40.7%, 60.2%)	50.5% (40.7%, 60.2%)
ICU population*		
Post-abdominal surgery	68.8% (57.4%, 78.7%)	68.4% (56.9%, 78.4%)
Elderly	72.0% (60.4%, 81.8%)	68.1% (56.0%. 78.6%)
Renal insufficiency	78.7% (66.3%, 88.1%)	75.9% (62.8%, 86.1%)
Solid tumour	76.2% (60.5%. 87.9%)	75.6% (59.7%, 87.6%)
Hepatic insufficiency	84.0% (63.9%, 95.5%)	72.0% (50.6%, 87.9%)
Neutropenic	58.3% (27.7%, 84.8%)	50.0% (21.1%, 78.9%)
Organ transplant	50.0% (15.7%, 84.3%)	37.5% (8.5%, 75.5%)
Baseline pathogen*		
C albicans	77.5% (67.4%, 85.7%)	74.4% (63.9%, 83.2%)
C glabrata	68.2% (45.1%, 86.1%)	68.2% (45.1%, 86.1%)
C parapsilosis	73.3% (44.9%, 92.2%)	66.7% (38.4%, 88.2%)
C tropicalis	50.0% (21.1%, 78.9%)	36.4% (10.9%, 69.2%)
Type of infection*		
Candidemia	72.3% (63.1%, 80.4%)	67.6% (57.9%, 76.3%)
Invasive candidiasis only	73.9% (58.9%, 85.7%)	73.9% (58.9%, 85.7%)

* Response assessed at EOT; percentages based on evaluable MITT pts.

Conclusion: In these selected ICU populations, ANI was effective, safe and well tolerated as first-line therapy of C/IC. EOT outcomes, incl. microbiologic responses, were similar across baseline pathogens, infection sites and patient populations.

R2725 Efficacy and safety of probiotic in *Helicobacter pylori*-positive patients with duodenal ulcer

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Background: The *Helicobacter pylori* (*H. pylori*) eradication rate following standard triple therapies is decreasing worldwide. The lowest incidence of side-effects to standard therapy has been observed when probiotics were combined with antibiotics. Because of the low eradication rates and lack of treatment regimens alternative approaches for *H. pylori* have been explored to assess the efficacy and safety of synbiotic compared with prebiotic for *H. pylori* eradication in duodenal ulcer (DU) patients.

Methods: Randomized, double-blind, placebo-controlled trial lasting 8 weeks. Each of pills contained either specific synbiotic (*E. faecium* L-3 10^{6-7} CFU/g, pectin and soy protein hydrolyzate, *Laminaria saccharina*) or prebiotic (pectin and soy protein hydrolyzate, *Laminaria saccharina*) or placebo. Pills were manufactured to conduct the trial.

81 *H. pylori*-positive patients in remission of DU were randomly assigned to three groups: Group A received synbiotic, group B received prebiotic, group C received placebo. All patients took three pills three times daily. Gastroscopy was performed before and 6 weeks after the end of treatment for DU activity evaluation. *H. pylori* detection was carried out by rapid urease test and polymerase chain reaction (PCR) with the primers vacA, ureaB and ureaC in gastric biopsies. Differences between eradication frequency were evaluated by the chi-square test. Both intention-to-treat (ITT) and per-protocol (PP) analyses were used for the assessment of the eradication rates of *H. pylori*.

Results: *H. pylori* eradication rates for the groups A, B, and C were 38% (11 of 29 patients), 8.7% (2 of 23 patients) and 10% (3 of 25 patients), respectively according to PP and ITT. Synbiotic showed a higher eradication rate than prebiotic ($p = 0.0156$) or placebo ($p = 0.0317$). Clinical or endoscopic relapses of DU or chronic gastritis were observed in 14 patients in group A, 6 patients in group B and 8 patients in group C. Though *H. pylori* was eradicated, relapses still occurred in some cases. Attributive relapse risk was 0.48, 0.26 and 0.28, respectively. Relapse risk ratio was 2.45 (synbiotic vs. placebo). No cases of enterococcus colonization of gastric mucosa were revealed by PCR with *E. faecium* specific primers.

Conclusion: Monotherapy with synbiotic based on *E. faecium* is more effective then prebiotic or placebo for *H. pylori* eradication. Monotherapy with synbiotic increases the risk of DU relapse, indicating *H. pylori* is the major cause of DU but not the only one.

R2726 Phase 2 prospective randomised study investigating daptomycin 6 and 8 mg/kg versus standard antibiotic therapy in the treatment of staphylococcal prosthetic joint infections

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Objectives: Evolving resistance of Gram-positive organisms support the investigation of higher doses of daptomycin in the management of prosthetic joint infections (PJI).

Methods: Prospective, open-label study of 75 subjects randomised to daptomycin (DAP) 6 or 8 mg/kg, or comparator (vancomycin, teicoplanin, or semi-synthetic penicillin) undergoing 2-stage replacement for a hip or knee PJI. After removal of the prosthesis, subjects received 6 weeks treatment and a 2–6 week antibiotic-free period before implanting a new prosthesis. Test of cure (TOC) was 1–2 weeks after re-implantation. The primary objective was evaluation

of Creatine Phosphokinase (CPK) levels. Secondary objectives were efficacy assessments by the Investigator and Sponsor.
Results: Of 73 CPK Safety Population subjects, a CPK elevation of >500 U/L occurred in 16% (4/25) DAP 6 mg/kg, 22% (5/23) DAP 8mg/kg, and 8% (2/25) comparator subjects (difference and 90% CI for DAP 8 vs. 6 mg/kg was 5.7% [-17.6%, 29.4%]). Elevations were sustained (⩾2 consecutive values of >500 U/L) in 2 DAP 6 mg/kg and 3 DAP 8 mg/kg subjects. Four subjects withdrew due to increases in CPK, 1 DAP 6 mg/kg and 3 DAP 8 mg/kg subjects.
In the 68 modified intent-to-treat subjects, DAP had a higher blinded Sponsor-defined success rate (clinical and microbiologic response) at TOC [Table 1]. Clinical success was also similar when assessed by Investigators at TOC and follow-up. Microbiological failure (⩾1 sample positive with original organism at surgery #2) or discontinuation due to an AE, accounted for most cases categorised as failures; only 2 subjects (both DAP 6 mg/kg) failed for an unsatisfactory clinical response.
Eradication rates at TOC for MRSA were 3/5 DAP 6 mg/kg, 3/7 DAP 8 mg/kg, and 2/5 comparator; MSSA 8/11, 5/9, and 5/10, respectively; and coagulase-negative staphylococci 7/12, 7/11, and 13/15, respectively. AE rates were similar between groups.
Conclusion: DAP at 6 and 8 mg/kg, given for up to 6 weeks, was well tolerated and appeared effective for treatment in subjects with PJI.

Table 1: Overall Success, N (%)

	DAP 6 mg/kg (n = 24)	DAP 8 mg/kg (n = 23)	Comparator (n = 21)
TOC Visit			
By Investigator	14 (58)	14 (61)	8 (38)
By Sponsor	13 (54)	13 (57)	8 (38)
3-4 Month Followup Visit			
By Investigator	12 (50)	11 (48)	8 (38)

R2727 Treatment of chronic wounds with maggot excretion/secretion: early clinical experience

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Objective: Clinical efficacy and safety of hydrogels with maggot excretion/secretion (ES) in chronic wounds was investigated.
Patients and Methods: Consecutive patients with chronic wounds were included in the study. *Lucilia sericata* maggot ES was sterilized by filtration and used to prepare 60% formulation in 6% alginate hydrogel base. Hydrogel was applied to wounds three times daily for three days. Patients were examined at 2 to 4 days, then at 9 to 11 days and finally 3 to 4 weeks after the end of treatment Wound swabs for bacterial isolation were taken at every patient visit. The improvement in the size of the wound, secretion, necrosis, granulation tissue formation, epitelisation, pain and odour was assessed by two independent investigators. A scale from 0 to 5 was used to estimate the extent of improvement.
Results: 10 patients (6 females and 4 males) with 14 wounds in total (8 patients with one wound, two patients with three wounds each) were included in the study. Patients were 67,3 years old on average (range 49 to 79). Six patients suffered from diabetes mellitus, three patients had peripheral vascular disease of other etiology and one patient had limb trauma. Wounds were open for 20 months on average before being inclued into the study. Seven patients finished the study per protocol, 3 patients did not attend the last visit. Improvement was observed in all but one wound. The most prominent was the improvement in necrosis (2,65 points), followed by epitelisation (2,1 points), granulation tissue formation? (1,95 points), and odour (1,5 points). Seven patients experienced 10 adverse events. Pain after application of the hydrogel was reported most often. No patients discontinued the treatment. Maceration of surrounding skin was observed in the patient with no improvement.
Conclusion: Maggot ES has a potential in chronic wound treatment. Further investigations of the maggot ES pharmacodynamics, pharmaceutical form optimisation, and the appropriate ES dosing in chronic wound treatment are warranted.

R2728 Tigecycline use in surgical wound infections

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Objective: Tigecycline (TGC) has demonstrated clinical efficacy and safety in both complicated intra-abdominal infection (cIAI) and complicated skin and skin structure infections (cSSSI). Because cSSSI due to prior surgery is common, TGC is a potential option for surgeons and infectious diseases physicians who treat these infections. A pooled analysis of phase 3–4 clinical trial subjects with cSSSI due to a surgical infection was conducted.
Methods: Pooled data from subjects with cSSSI and a surgical etiology from 3 double-blinded and 1 open-label study of tigecycline versus various comparators (COM) were analyzed. The primary efficacy endpoint was the clinical cure rate at the test-of-cure assessment.
Results: A total of 200 subjects were identified (112 TGC subjects and 88 COM subjects). TGC subjects were older (mean age 57.6 vs. 53.7) and more had diabetes (25.9% vs. 18.2%) and peripheral vascular disease (20.5% vs. 13.6%). The abdomen and lower extremity were the most common sites for surgical wounds and the median therapy duration was 9 days in both treatment groups. Clinical cure rates in the clinically evaluable population were 81.2% and 79.4% for TGC and COM respectively (treatment difference 95% CI −11.5, 15.7). *Staphylococcus aureus* was the most common pathogen with similar cure rates for TGC and COM respectively for both MSSA (95.0% vs. 91.7%) and MRSA (82.1% vs. 76.5%). Clinical response by clinical diagnosis, diabetes, and baseline bacteremia demonstrated no difference between groups. Discontinuations of test article were greater in TGC versus COM treated subjects (16.1% vs. 10.2%; $p = 0.298$). The number of subjects reporting treatment-emergent adverse events were comparable between treatment groups ($p = 1.0$) with significantly more TGC subjects experiencing nausea (43.8% vs. 21.6%) and vomiting (30.4% vs. 6.8%). Three TGC subjects and no COM subjects had an adverse event with an outcome of death.
Conclusion: Tigecycline appeared effective and safe in the treatment of cSSSI due to surgical infections.

R2729 Changes in faecal flora observed during a clinical trial of linezolid or IV vancomycin for the treatment of patients with nosocomial pneumonia caused by methicillin-resistant *Staphylococcus aureus*

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Objective: We investigated the impact of linezolid (LZD) or vancomycin (VAN) administration on the fecal flora of subjects enrolled in a double-blind randomized controlled clinical trial comparing the safety and efficacy of LZD and VAN for the treatment of nosocomial pneumonia (NP) caused by methicillin-resistant *Staphylococcus aureus* (MRSA). All patients received concomitant antibiotics with coverage for Gram negative bacilli (GNB).
Methods: Rectal swabs were obtained at baseline, at Day 6 (± 2 days) and at End of Treatment (EOT). Quantitative cultures and susceptibility testing of aerobic GNB were performed to detect the emergence of resistance of Enterobacteriaceae to ceftriaxone, ceftazidime, cefepime, or piperacillin/tazobactam and for R of non-nonfermentative GNB (NFs) to imipenem, aztreonam or ciprofloxacin. Changes in log10 number colony forming units (cfu) for Day 6 and EOT were compared to baseline.
Results: 130 swabs were received from 69 subjects. Baseline, Day 6, and EOT samples were available for 25 subjects, 9 in the LZD and 16 in the VAN treatment arm. From baseline to Day 6, the median log cfu/g of Enterobacteriaceae decreased from 10^6 to $<10^2$ cfu/g in LZD-treated subjects. In VAN-treated subjects, a 2 log reduction occurred between baseline and Day 6. At baseline, the proportion of aerobic GNB resistant to at least 1 of the specified antibiotics for each organism group was 18% and 14% in the LZD and VAN treatment groups, respectively. At Day 6 and EOT, the proportion of resistant GNB increased to 50% and 41% in the LZD group compared to 17% and 22% in the VAN group. In the

LZD arm, the average number days of GN therapy (DOT) per subject during the pre-study period was 5.8 compared to 3.5 for subjects in the VAN arm. The average number of DOT per subject for concomitant antibiotics during the study period was 5 and 5.2 for LZD and VAN treatment arms, respectively.
Conclusion: In this small study, the emergence of resistance among GNB and changes in cfu/g during therapy was greater in the LZD arm compared to VAN. The administration of GN active agents prior to the study period, which was more common in LZD subjects, was likely to have contributed to the noted changes in fecal flora.

R2730 Linezolid and vancomycin in the treatment of patients with nosocomial pneumonia proven due to methicillin-resistant *Staphylococcus aureus* by body weight

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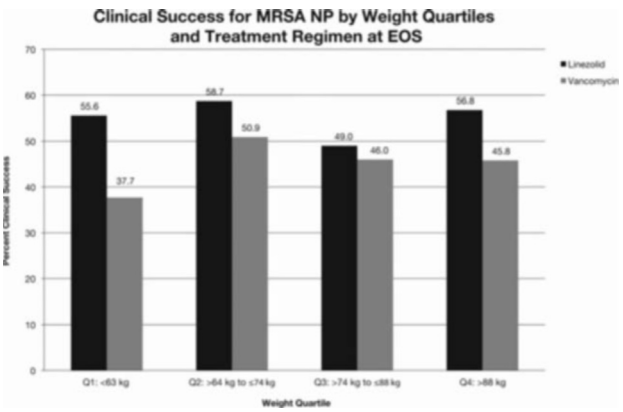
Objectives: The prevalence of adult obesity exceeds 30% and 20% in adults in the USA and several European countries, respectively. Patient body weight may need to be taken into consideration for the optimization of drug therapy. The objective of this study was to determine the effect of body weight on outcomes by treatment regimen for nosocomial pneumonia (NP) due to culture proven methicillin-resistant *Staphylococcus aureus* (MRSA).
Methods: Data from a prospective, double-blind, randomized clinical trial were analyzed to examine body weight-related differences in the efficacy and safety of linezolid (LZD) 600 mg IV Q12h and vancomycin (VAN) 15 mg/kg IV Q12h (adjusted for renal function) for up to 14 days. We stratified the study population into 4 weight quartiles (Q1-Q4) (Figure). Clinical success was evaluated by weight quartiles and treatment group at end of study (EOS = 7–30 days after last treatment dose). We also analyzed rates of adverse events (AEs) by weight quartiles and treatment.
Results: There were 447 patients treated with at least one dose of study drug for MRSA NP (224 treated with LZD and 223 with VAN). The treatment duration for all quartiles by treatment was approximately 10 days. The average maximum VAN trough was lowest in Q1 (16.2 μgml), compared to Q2 (18.3 μgml), Q3 (18.4 μgml) and Q4 (18.9 μgml). The number of patients with AEs were similar regardless of treatment regimen and were numerically higher in Q1 (92%) and Q2 (92%) compared to Q3 (88%) and Q4 (85%). The rates of anemia were highest in Q1 (16%; LNZ = 14% vs. VAN = 17%) compared to Q2 (6%; LNZ = 6% vs. VAN = 5%), Q3 (11%; LNZ = 12% vs. VAN = 9%) and Q4 (9%; LNZ = 7% vs. VAN = 11%). There were no differences in the rates of thrombocytopenia among the quartiles; Q1 (6%; LNZ = 5% vs. VAN = 7%), Q2 (4%; LNZ = 4% vs. VAN = 4%), Q3 (1%; LNZ = 0% vs. VAN = 2%), Q4 (0).
Conclusions: The efficacy of LNZ was maintained at 600 mg IV every 12 h regardless of body weight and consistently numerically higher than weight-based VAN dosing (15 mg/kg IV Q12h) for the treatment of MRSA NP by weight quartiles. Except for higher rates of anemia in Q1, there were no significant differences in safety between the weight quartiles or treatment groups.



R2731 Susceptibility patterns of baseline methicillin-resistant *Staphylococcus aureus* isolates from a global clinical trial of nosocomial pneumonia

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Objective: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of nosocomial pneumonia (NP) and is associated with substantial morbidity and mortality. ZEPHYR is a randomized, double-blind, Phase 4 study that compared linezolid (LZD) to vancomycin (VAN) for the treatment of NP due to culture-proven MRSA. This analysis characterizes resistance patterns in baseline MRSA isolates collected during this recently completed trial.
Methods: 1225 patients were enrolled from Oct. 2004 to Jan. 2010. NP was defined by onset of clinical signs/symptoms and chest radiograph consistent with pneumonia ⩾48 hours after hospitalization. Baseline respiratory specimens were obtained bronchoscopically, by endotracheal aspirate or from sputum with <10 epithelial cells and >25 leukocytes/LPF. Susceptibility testing to confirm MRSA presence was performed by broth microdilution at a central laboratory according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Interim data were previously presented at the 2009 meeting of the American Thoracic Society.
Results: 427 baseline isolates recovered from respiratory specimens obtained from patients with NP were confirmed to be MRSA from US (61%), Asia (15%), EU (13%), Latin America (10%) and South Africa (1%). The in vitro activity of study drugs and other comparator antibiotics is presented in the following table. LZD MICs ranged from 1–4 μgml; 72.1% of isolates had LZD MIC = 2 μgml. Mean and modal VAN MIC = 1 μgml. For 6% of isolates, VAN MIC = 2 μgml.
Conclusions: 100% of MRSA isolates from patients with NP were susceptible to linezolid and all but 1 were susceptible to VAN. Resistance rates were high for erythromycin, clindamycin and gatifloxacin.

Antibiotic	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Range (μg/mL)	%S	%R
Linezolid	2	4	1- 4	100	–
Vancomycin	1	1	0.5- 4	99.7	–
Teicoplanin	0.5	2	0.12- >64	99.7	2.0
Erythromycin	>64	>64	0.25- >64	7	90.4
Clindamycin	>64	>64	0.25- >64	36.2	78[a]
Gatifloxacin	8	>16	0.06- >16	11.7	86.7
Tetracycline	0.5	64	0.25- >64	79.2	20.6
Trimetho/sulfa	0.12	16	0.03- >32	87.1	13.1

[a]constitutive and inducible clindamycin resistance

Paediatric infections

R2732 Assessing the association between *H. pylori* infection and the prevalence of iron deficiency anaemia in children, Iran, 2009–2010

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Objectives: Iron deficiency, defined as decreased total body iron content, is among the most common nutritional deficiencies in the world. IDA is a condition with important health consequences regarding reproduction, immunity, work performance, and possibly cognitive development, Emerging evidence seems to place *H. pylori* infection next to helminthiasis as a communicable cause of anemia. We investigated whether there was any correlation between *H. pylori* infection and iron deficiency and IDA in children in Iran.
Methods: In a case-control survey, 64 patients with IDA between 3 and 8 years (median 4.5 years) were enrolled in the study and 70 matched children as control group. Serum hemoglobin, total iron-binding capacity, ferritin, erythrocyte mean corpuscular volume (MCV), fecal occult blood testing, and stool for ova and immunoglobulin G antibody to *H. pylori*

were measured to compare the prevalence of IDA and *H. pylori* infection in the groups.
Results: 52.5% of cases were girls (53.1% of cases and 51.4% of control group) and 47.8% were boys (46.9% of cases group and 48.6% of controls). *H. pylori* serology was positive in 46.3% of cases and 19% of control group were infected. There were a meaningful difference (p-value >0001).
Conclusion: Our results support the proposal that *H. pylori* infection is associated with IDA in children.

R2733 Influence of mother HIV and/or syphilis infection on the outcome of newborns

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Background: Sexually transmitted diseases (STDs) during pregnancy pose a major risk to the fetus due to vertical transmission. Congenital disease still represents a significant public health problem worldwide, particularly in developing countries.
Methods and Materials: A retrospective investigation was performed comprising all pregnant women with HIV and/or syphilis infections admitted at the central hospital cof two Western Cities of Venezuela, during January 2007-September 2010; pregnant women without STDs served as control group. Epidemiological characteristics were reported, anthropometrical variables in newborns were considered. Statistical significance was defined as $p < 0.05$. Statistical analyses were performed on SPSS v.17®.
Results: 76 pregnant HIV patients and 77 patients with syphilis infection were identified, three of them being coinfected. 87 pregnant women without STD served as controls. Mean age of infected mothers (HIV/syphilis) was 26 years (range 14–42 yrs) with a mean of 3 pregnancies (range 1–12). In the control group, pregnancies of 38±1 weeks were observed; newborns had a mean birth weight of 3,220±524 and a mean height of 51±3cm. In HIV infected patients, mean gestation was 36 weeks (range 26–41 weeks). Mean birth weight of newborns was 2,829±686 g (range 510–3,900 g); 22,6% were low birth weight newborns; mean birth height was 49±4 cm (range 30–56). In syphilis infected patients, mean gestation was 37 weeks (range 22–41 weeks). Mean birth weight of newborns was 3,159±649 g (range 700–4,240 g); 11,5% were low birth weight newborns, mean birth height was 51±4 cm (range 33–59 cm).
Maternal VDRL titers were strongly associated with birth weight; higher mother VDRL titers correlated with lower birth weight. Cephalic, thoracic and abdominal circumference did not show considerable differences between groups.
Conclusion: STDs cause considerable morbidity in women during the gestational period. Congenital and perinatal infection of the newborn, miscarriage and low birth weight have been described. In this study, both HIV and syphilis infections resulted in lower birth weight, particularly in newborns from HIV infected patients. Treatment of the etiologic agent is considered effective for prevention of vertical transmission and is recommended for STDs.

R2734 Parvo B 19 infection in children: an emerging disease

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Parvo B19 is a DNA virus responsible for erythema infectiosum or fifth disease, an infectious benign rash, common in childhood. Pregnant women may also develop Parvo B19 infection with severe conjenital malformation.
Objectives: To study the epidemiologic profile of ParvoB19 infection in hospitalized paediatric population of Athens.
Methods: During two years period (2007–2008) IgM and IgG antibodies against Parvo B19 virus were detected in 287 blood samples from hospitalized children, aged 2 months till 15 years, with fever and suspicion of parvoviral infection. Antibody titre was detected with immunoassay and reconfirmed with indirect immunofluorescence (Bios, Germany).
Results: From 287 clinical samples from equal number of patients, ParvoB19 acute infection was diagnosed in 94 patients (32,7%). IgG antibodies were detected in 27.2% of children. A slight difference was observed in the incidence of antibody detection between sexes. The incidence of IgG antibody detection in children aged less than 4 years (27,3%) was inferior in comparison with incidence in children aged above 10 years (34,7%). Increased incidence of infection was observed during winter and spring months.
Conclusions: Early and accurate diagnosis of Parvo B19 infection based on IgM antibody detection or an increase of IgG antibody titre is crucial in hospitalized children with infectious rash and leukopenia or chronic haemolytic anaemia.

R2735 Incidence of *Toxoplasma gondii* infection in the paediatric population of Athens

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Toxoplasmosis is a worldwide public health disease, nowdays. Incidence varies among different countries, depending on living standards, dietary habits and socioeconomic status of population.
Objectives: To determine the incidence of toxoplasmosis in children with cervical or generalized lymphadenopathy, by detection of IgM and IgG antibodies, during five years (2004–2009).
Methods: Determination of IgM and IgG antibody titre against *Toxoplasma gondii* was performed with immunoassay in 1254 sera from children with lymphadenopathy, aged 1 till 14 years, who were hospitalized or admitted at the emergency department. In every child two blood samples were drawn with a distance period of 15 days and examined for TORCH virulent agents. Reconfirmation of acute infection was performed by indirect immunofluorescence as well as by observation of fourth time rise of IgG antibody titre.
Results: From 1254 children examined 85 (6.8%) were positive for IgG and 25 (1.9%) for IgM antibodies against *Toxoplasma gondii* 19 (1.5%) children were positive for both IgG and IgM antibodies. Rise of seropositivity was observed according the increase of age. Higher incidence of infection was observed in the age group between 8 and 14yrs. There was no difference observed in the incidence of antibody detection between sexes.
Conclusions: *Toxoplasma gondii* is an important cause of lymphadenopathy during childhood in Greece, with low incidence, probably due to the habit of eating well-cooked meat and the good socioeconomic status of the study population.

R2736 *Candida albicans* isolated from cerebrospinal fluid in two premature neonates hospitalised in neonatal unit in a tertiary Greek hospital

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Objectives: Isolation of *Candida* spp. in cerebrospinal fluid (CSF) in preterm neonates is rare. We describe the clinical characteristics and diagnosis of two cases of hospitalized premature neonates in whose *Candida albicans* isolated from CSF.
Methods: The first case was about a set of female dizygotic twins, who were born at 32 w gestation. Twin α died within few hours, while twin β was transferred to neonatal intensive care unit (NICU). Their mother was treated for cervical incompetence and vaginal candidiasis during the 2nd trimester of her pregnancy. Neonate β was started on empiric antibiotic therapy, because of elevated CRP, and low CSF glucose level. Blood and CSF cultures were negative. On day 8, the neonate looked slightly unwell and lumbar puncture (LP) was repeated. The second case was about a male neonate, who was born at 28 w gestation. The neonate presented respiratory distress syndrome and neonatal jaundice, and transferred to NICU. Empiric antibiotic therapy was started, although

mechanical ventilation and phototherapy were used. On day 8, the neonate had bradycardia and episodes of apnea with increased leycocytes and CRP. Blood culture and CSF examination were performed.
Results: In first case, CSF glucose remained very low, protein level was high with 478 cells/mm3, and lymphocyte predominance, indicating chronic meningitis. Blood and urine cultures were sterile. On day 40, LP showed similar findings with the above results, but this time CSF culture was positive for *Candida albicans*, which was sensitive in all antifungal agents, according to RPMI method. Antifungal therapy was given for four weeks. At the end of therapy LP were normal. The neonate remained clinically well throughout therapy. In second case, the first LP and CSF culture were negative. On day 35, LP repeated cause the neonate was still unwell. CSF glucose was normal, protein level was high and leycocytes was 30/mm3 (47% PMNs). Blood and CSF culture were positive for *Candida albicans*, which were resistant in fluconazole. Appropriate antifungal therapy was given and the neonate recovered.
Conclusion: *Candida albicans* isolates in CSF are very uncommon, especially in premature neonates. As in described cases, the low fungal load has been previously associated with false-negative results during the first days of *Candida albicans* infections. The clinical and microbiological findings should help in diagnosis and in antifungal treatment selection.

R2737 Newborn skin infection associated with *Staphylococcus aureus* enterotoxin A production

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Objective: Staphylococcal enterotoxin A (SEA) is one of the known enterotoxins produced by *Staphylococcus aureus* (SA). We report about a newborn with a persisting inflammatory skin infection associated with a SAE producing methicillin sensitive SA (MSSA).
Methods: Swabs were cultured on standard media. The MALDI Tof Biotyper System (Bruker Daltonics) was used for identification. Resistance testing was performed on the Microsan (Siemens). Spa-typing, mec-A gene and the panton-valentine-leukocidine (PVL) gene were investigated by PCR. The superantigen tests for SEA-SED and TSST were done by agglutination (National Reference Centre for Staphylococci, Germany).
Results: The investigation of swaps of the newborn skin at different times showed a MSSA. The results for mecA and PVL genes were negative as well as the results for TSST and SEB-SED. The spa-type was t1381 and the test for SEA was positive for all investigated swabs.
Conclusions: Exotoxins especially SEB are known to play a role in atopic dermatitis and psoriasis. The associated immune response is a wide field for research. To the best of our knowledge this is the first report of a newborn skin disease related to a MSSA positive for SEA. After antibiotic treatment the isolated strain is still detectible. The investigation for filaggrin defects is going on but a hyperimmunglobulin E syndrome is excluded. In this case the colonisation or infection with a SEA positive MSSA strain seams to play an important role in the aetiopathology of the disease.

R2738 Detection of specific IgA antibodies in the serum of children with *Mycoplasma pneumoniae* infection

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Objectives: *M. pneumoniae* is a significant cause of community acquired pneumonia and is responsible for about 40% of atypical pneumonias. Serology remains the most useful diagnostic tool, though its interpretation is difficult. In children detection of IgM antibodies in serum with the absence of IgG strongly indicated acute *M. pneumoniae* infection and the presence of IgM and IgG antibodies simultaneously is associated with past infection. The aim of the present study is to investigate the usefulness of IgA antibodies detection in serum for the diagnosis of acute or past *M. pneumoniae* infection in children.
Methods: Serum samples from 184 outpatient or hospitalized children (90 boys and 94 girls) aged 1,5–14 years old, suspected for *M. pneumoniae* infection, from January 2007 to September 2010, were tested for the determination of specific IgM and IgG antibodies to *M. pneumoniae* using Platelia EIA (Biorad). IgG antibodies were discriminated as non significant, low, moderate and high, according to the absorbance reading. Sera were stored at −70ºC before the determination of IgA antibodies using ELISA (Virion/Serion).
Results: IgM antibodies were found in 167 out of 184 serum samples. The detection rate of specific IgA antibodies in the group of IgM positive and no significant IgG titer was 38% (31/81), in the group of IgM positive and low IgG titer was 48% (13/27), in the group of IgM positive and moderate IgG titer was 45% (10/22) and in the group of IgM positive and high IgG titer was 78% (29/37). In the group of children with IgM negative and IgG positive, IgA antibodies were detected in 41% (7/17) of samples, mainly with significant IgG titer (5/7).
Conclusion: In adults, the determination of IgA antibodies is useful for early diagnosis of acute *M. pneumoniae* infection considering that IgA response is more regular than IgM response in these patients. In contrast our results indicate that IgA specific antibodies determination is incapable of differentiating acute or past *M. pneumoniae* infection in children.

R2739 Polymicrobial otitis in childhood

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Objectives: To evaluate the incidence of common otopathogens in paediatric population with acute (AOM) and chronic otitis (COM).
Materials and Methods: Four hundred and eigthy five paediatric patients were examined in our hospital with otitis symptoms during the years 2007–2009. The samples were cultured according to conventional methods. The identification was performed using the API20E system. VITEK2 automated system (BioMerieux) was used for identification and susceptibility testing of pathogens.
Results: Totally, 71,3% of all samples tested (346/485) were found positive for bacteria. The most prevalent ones were: *H. influenzae* 27%, *S. pyogenes* 20%, *P. aeruginosa* 20,8%, *S. pneumoniae* 18,5%, *S. aureus* 9,7%, *M. catarrhalis* 3% and *V. alginolyticus* 1%. Out of 346 positive cultures, 19 developed fungi (5.5%). *Candida* spp (88%) predominated and followed by *Aspergillus* spp (12%). Anaerobes were isolated mainly in cases of chronic otitis, usually in mixed cultures with other bacteria (Peptostreptococci and Clostridia spp). As a whole, one hundred and four mixed polymicrobial cultures were found that developed $\geqslant 2$ otopathogens (30%). In 4.8% of cases $\geqslant 3$ otopathogens were recovered. *H. influenzae* proved to be the most prevalent otopathogenic agent in childhood. Nontypable *H. influenzae*, *S. pyogenes*, *P. aeruginosa* and *S. pneumoniae* were found in culture with other otopathogens in 35%, 26%, 18% and 13% respectively. The most frequent combinations of mixed bacterial populations were *S. pyogenes+S. aureus* 11,6%, *S. pyogenes+H. influenzae* 8,65%, *S. pneumoniae+H. influenzae* 7,7% and *P. aeruginosa+S. aureus* 6,8%. A seasonal variation has been also detected in the incidence of otitis media with peaks in the fall and winter.
Conclusions: A significant proportion of mixed microbial populations (30%) was observed in paediatric patients suffering from otitis. *H. influenzae* was the leading virulent factor in otitis media especially with effusion, while *P. aeruginosa* was the dominant pathogen in external otitis. It is worthy that *S. pyogenes* was encountered in high proportion (21%) of cases and coexisted with *S. aureus*. An accurate differential bacterial diagnosis is essential for ears disease ensuring appropriate treatment.

R2740 Retrospective study of prevalence and resistance profile of *S. enteritidis* and *S. typhimurium* in a paediatric hospital

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Objectives: To investigate the prevalence of *S. enterica* serovars and to determine the antimicrobial susceptibility patterns of different *S. enterica* serotypes isolated from hospitalized children with acute gastroenteritis to current antimicrobial agents.
Materials and Methods: Out of 6800 feaces samples 550 strains of *S. enterica* were isolated during a seven year period (2004–2010). Identification and antimicrobial susceptibility testing were performed with automated system VITEK 2. The strains were serotyped using specific antisera; as well serotyping was confirmed in reference laboratory. The age of patients ranged from 1 month to 14 years.
Results: Out of all samples examined 1265 were found positive for enteropathogens (18,6%). Five hundred fifty strains were identified as *S. enterica* (43,5%) as follows: *S. enteritidis* 391 (71%), *S. typhimurium* 86 (15,6%), and the rest *Salmonella* serovars 73 (13,4%). Among 19 various *Salmonella* serotypes *S. bovismorbificans* (12,3%), *S. oranienburg* (9,6%), *S. hadar* (8,2%), *S. blockley* (5,5%) and *S. abony* (5,5%) were the most prevalent ones. *S. enteritidis* strains were sensitive to aminoglycosides, chloramphenicol (C), fluoroquinolones (FQ), cephalosporines 3rd generation, spectinomycin (SP), streptomycin (STR) while cotrimoxazole (SXT) showed only intermediate resistance 12,7%. *S. enteritidis* presented resistance to: nalidixic acid (NA) 16,5%, ampicillin (AM) 1%, tetracycline (TE) 1% and amoxicillin/clavulanate (AMC) 0,5%. However, higher levels of multidrug resistance was observed in *S. typhimurium* strains to: TE 32,8%, AM 28%, SXT 23,7%, STR 14,6%, C 9%, SP 9%, AMC 5,5%, NA 5.5%, cefotaxime (CTX) 3,7%, ceftazidime (CAZ) 1,9% and tobramycin (TB) 1,9%. All CTX-resistant *S. typhimurium* strains were resistant to b-lactams, aztreonam, but susceptible to CAZ and ciprofloxacin (CIP). Finally, the others *S. enterica* serovars exhibited the following resistance profiles: NA 15%, AM 10%, SXT 4%, TB 2.8%, AMC 1.5% and CIP 0.7%.
Conclusions: 1. The serovars of *S. enteritidis* and *S. typhimurium* were susceptible to FQ. 2. *S. typhimurium* exhibited higher levels of resistance to AM and SXT in relation to *S. enteritidis*. In this regard, treatment of gastroenteritis in childhood might be complicated. 3. The presence of ESBLs and cefotaximases producing *S. enterica* serovars emphasized the need for strict antibiotic regimens.

R2741 Bacteraemia and fungaemia in Vilnius University Children's Hospital, 2007–2009

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Objectives: To determine the most common organisms isolated from blood cultures in children admitted to the different hospital departments.
Methods: All positive blood cultures from the children 0–18 years old isolated at Vilnius University Children's Hospital which currently possesses 469 beds, from 2007 to 2009 were included in the study. The blood cultures were categorized according to likelihood of infection or contamination.
Results: During the study period 6538 blood cultures were obtained from the hospitalised children. The majority (43%) of the blood culture samples were obtained from the oncohaematological patients and only few samples (1.6%) were obtained from the surgical patients. In total, there were 695 (10.6%) positive blood cultures. They were most common from the oncohaematological patients, and least common for the children admitted to the general paediatric wards. Different bacteria were identified in 94.5% of the cases and 5.5% were identified as *Candida* sp. Gram-positive bacteria constituted 57.6% from all positive cultures. Of them, coagulase-negative staphylococci were found in 33.7% of the cases and *Staphylococcus aureus* in 8.1%. Enterobacteria were isolated from 26% of the children, most commonly from the oncohaematological patients and neonates. *Neisseria meningitidis* isolates were identified in 24 (3.5%) children admitted to the general paediatric wards. Out of all 695 cultures, over 40% of them were considered to be either definite or probable contamination.
Conclusion: There were approximately 10.6% positive blood cultures obtained every year. The most common isolates were Gram positive bacteria. Coagulase-negative staphylococci were the most common organisms isolated in our study. The vast majority of bacterial and *Candida* isolates were identified from the oncohaematological patients.

R2742 Clinical significance and antimicrobial susceptibility of *Staphylococcus epidermidis* and *Staphylococcus lugdunensis* in severe infections in children

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Objectives: 1. To assess the prevalence and the distribution of *S. epidermidis* (S.e) and *S. lugdunensis* (S.l) in severe diseases such as bloodstream infections (BSI) or skin and soft tissue infections, in a pediatric hospital. 2. To investigate S.e and S.l antimicrobial resistance patterns.
Material and Methods: A total of 268 coagulase negative Staphylococci (CoNS) isolated from 356 positive blood and 805 wound cultures from hospitalized children, median age 5 years during a four year period (2005–2008) were tested. Identification at the species level and susceptibility profiles was performed by using the Vitek II system and E-test. All CoNS strains were investigated for the presence of biofilm formation (slime test tube adherence).
Results: Out of 19,2% (69/356) bloodstream related CoNS, the species distribution was: S.e (39.13%), *S. hominis* (20.29%), *S. haemolyticus* (17.40%), *S. warneri* (17.39%) and finally *S. capitis* and *S. auricularis* were found in 2.90% each one. The predominant species in 25% CoNS-wound associated isolates was S.e (61%), followed by *S. warneri* 7,53%, *S. hominis* 7.22%, *S. capitis* 6,8%, S. l 6.2% and the remaining 10.89% CoNS strains included a variety of species. The observed prevalence of methicillin resistance (MR) among S.e strains in BSI was 68% versus 28% in wound infections (WI). A significant proportion of MR was found among *S. hominis* (60%) and *S. warneri* (50%) in BSI. Notable rate of resistance (5% and 16%) was detected to teicoplanin ($\geqslant$32mg/l) in S.e and *S. hominis* sepsis related strains isolates respectively. MR CoNS also showed multidrug resistance to antibiotics: erythromycin 65%, clindamycin 60%, tobramycin 20%, fucidic acid 35%, tetracycline 20% and sulfamethoxazole-trimethoprim 15%. In contradiction, all S.l as well as *S. haemolyticus*, *S. capitis* and *S. intermedius* wound-associated isolates were found methicillin susceptible. Biofilm formation in 25 S.e orthopedic wound-strains was also associated with multidrug resistance.
Conclusions: 1. S.e was the leading cause of BSI and WI and was related with multiple resistance patterns and indwelling medical devices. 2. S. l, a virulent pathogen agent, was associated with severe WI. 3. Our results strongly suggest the need for the establishment of control program measures in order to prevent and reduce the level of resistance of S.e to methicillin. A recent concern is the upwards shift in glycopeptides MIC for S.e strains.

R2743 Perinatal risk factors for the development of neonatal septicaemia

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Objectives: Perinatal determinants plays crucial role in the increased incidence of bacterial sepsis. This study was undertaken to determine whether neonatal-maternal risk factors and bacterial pathogens affect the risk of early or late onset sepsis.
Methods: Three hundred neonates at NICU of two hospitals in Tehran, were studied. Blood culture from neonates with suspected sepsis was performed on BHI broth followed by identification of isolates and testing for their susceptibility to antimicrobial agents. Collectively, neonatal and maternal risk factors such as birth weight, gestation age, PROM, Apgar score, ... were studied in the cultures proven cases of neonatal sepsis.

In univariate binary logistic regression models, the impact of neonatal and maternal factors on sepsis risk was estimated in terms of odd ratio (OR) with 95% confidence interval (CI).
Results: This study revealed the impact of bacterial pathogens, neonatal and maternal predisposing factors on sepsis as follows. Maternal factors: PROM affect the sepsis risk to more than 3-fold (OR = 3.8; 95% CI:1.37–10.56; P < 0.05). Neonatal factors: the mean age of neonate ±SD of whom with early-onset sepsis (1.56±0.88) was lower than that of with late-onset sepsis (10.40±5.50) and this difference was statistically significant (P < 0.05). Low birth weight (LBW) <2500 g increase the risk of sepsis to more than 2-fold (OR = 2.9, 95% CI:1.17–9.86; P < 0.01). Gestation age (GA) <29 weeks was significantly associated with sepsis (P < 0.01). The septicemia in turn, increase the risk of death up to more than 5-fold (OR = 5.5; 95% CI:1.98–15.3; P < 0.01). More than half of septic neonates had positive result for CRP whereas only 1.9% of neonates with sepsis were CRP negative and this difference was statistically significant (P < 0.001). Bacterial pathogens:14/300 (4.7%) of neonates developed septicemia. Among infected neonates 64.3% and 35.7% were considered with early onset and late onset sepsis respectively. The most isolated Gram negative organism was *Stenotrophomonas maltophilia* (42.8%) followed by *Klebsiella pneumoniae* (28.6%), *Escherichia coli* (21.4%) and *Serratia liquefaciens* (7.2%).
Conclusion: This study reveals that specific neonatal and maternal factors are associated with increased risk of sepsis. Among the studied factors, prematurity of neonates explained as GA and LBW are the most important contributors to morbidity in neonates suffered from sepsis. Furthermore, PROM as maternal risk factor predisposes a child to neonatal sepsis.

Immunology, host defences, immunotherapy

R2744 Oral administration of heat-killed *Lactobacillus pentosus* strain b240 augments protection against *Streptococcus pneumoniae* infections in mice

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Objectives: The fatalities caused by *Streptococcus pneumoniae* (*S. pneumoniae*) infection are high in the young children and the elderly. Host-defense mechanisms against these pathogenic infections involve both innate and acquired immune systems. In particular secretory immunoglobulin A (IgA) and innate immunities in the airway mucosa play a principal role in preventing these infections. *Lactobacillus pentosus* ONRICb0240 (b240) has been screened as the excellent IgA-inducing bacterium in vitro. The present study aimed to investigate whether nonviable b240 can prevent *S. pneumoniae* infection in mice.
Methods: CBA/J mice were administered with b240 for 3 weeks prior to lethal *S. pneumoniae* infection. The survival and body weight of the mice were monitored daily for 2 weeks after infection. Then, isolated lungs were evaluated the severity of pneumonia. The number of *S. pneumoniae*, concentration of inflammatory cytokines, and expression of toll-like receptor 2 in lung homogenate on 2days after infection were determined.
Results: In the pneumococcal pneumonia model, oral intake of b240 led to the prolongation of survival time and significantly inhibited of body weight loss, as well as reduced bronchitis. There were a significant decrease in the secretion level of inflammatory cytokines and toll-like receptor 2 expression was promoted in b240-treated mice. These results were exhibited in accordance with the alleviated pathological severity of pneumonia.
Conclusion: These results suggest that *Lactobacillus pentosus* ONRICb0240 intake can facilitate protection against *S. pneumoniae* infection via the modulation of innate immunities.

R2745 Oral administration of heat-killed *Lactobacillus pentosus* strain b240 protects mice against *Salmonella enterica* serovar typhimurium

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Objectives: One of the beneficial effects of probiotic bacteria is protection of the host against infection by various enteric pathogens. Viable lactobacilli are used in most probiotic studies, while few studies using heat-killed probiotic bacteria have been reported. The purpose of this study was to investigate the effects of heat-killed *Lactobacillus pentosus* ONRICb0240 (b240) on systemic infection by *Salmonella enterica* serovar Typhimurium (S.Typhimurium) and to determine the mechanism by which b240 protects against infection.
Methods: To determine whether oral administration of heat-killed b240 would affect the survival of mice after S. Typhimurium infection, mice were orally administered b240 or saline daily for 3 weeks. S. Typhimurium was orally inoculated and survival of the mice was monitored until 20 days after inoculation. To study the effect of b240 on bacterial translocation of S. Typhimurium, mice were treated orally with b240 or saline daily for 3 weeks. Various organs were removed 6 days after S. Typhimurium inoculation and viable numbers of bacteria were evaluated. We also examined the effects of b240 on adhesion and invasion of S. Typhimurium into HeLa cells by in vitro assay.
Results: The mice treated with b240 were significantly protected against S.Typhimurium as compared to those fed saline. Moreover, viable numbers of S.Typhimurium in each organ in b240-treated mice tended to be less than in the control mice. In vitro study demonstrated the inhibitory effect of b240 on binding and invasion of S.Typhimurium into cells.
Conclusion: The oral administration of *Lactobacillus pentosus* ONRICb0240 protected mice from S.Typhimurium infection through the inhibition of adhesion and invasion of S.Typhimurium to intestinal epithelial cells in association with a reduction of bacterial systemic infection. Our results suggest that nonviable lactic acid bacteria also play an important role in preventing infection by enteric pathogens.

R2746 Immunogenicity of the pneumococcal conjugate 7-valent vaccine and impact on pneumococcal carriage in infants from Venezuela with sickle cell disease or HIV infection

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Objectives: To evaluate immunogenicity of the pneumococcal 7-valent conjugate vaccine (PCV7) and the impact on pneumococcal carriage in infants with sickle cell disease (SCD) or HIV infection.
Methods: Children with SCD (n = 25) and HIV (n = 12) listed at the Children Hospital in Caracas, Venezuela were enrolled in this study and vaccinated according to their age with the 7-valent conjugate vaccine followed by a booster dose of a 23-valent pneumococcal polysaccharide vaccine (PS-23). Blood samples and nasopharyngeal swabs for the determination of antibody concentrations for vaccine serotypes and for the isolation of *S. pneumoniae* respectively were obtained immediately before and 1 month after the PS-23 booster.
Results: Of the 37 infants enrolled in this study, pneumococcal carriage prior the first immunization was found in 32% (n = 12) of the children with 67% (n = 8) carrying non-vaccine serotypes. After boosting 100% of the subjects had antibody titers >0.35 μg/mL for all the 7 vaccine serotypes, ranging from 0.42 μg/mL (serotype 6B) to 62.21 μg/mL (serotype 14). One month after completion of the vaccination scheme pneumococcal carriage was found in 27% (n = 10) of the children with 80% (n = 8) carrying non vaccine serotypes.
Conclusions: High antibody titers for the serotypes of the 7-valent conjugate vaccine were obtained with this vaccination scheme in our children. However, no significant effect on carriage rate in general or carriage rates for vaccine serotypes was observed.

R2747 Clinical efficacy of interferon-γ (Γ Immunox®) in chronic granulomatous disease

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Objectives: To evaluate clinical efficacy of interferon-γ (IFN-γ) (Γ Immunox®, Exir Co., Iran) in patients with chronic granulomatous disease (CGD).
Methods: Twenty-eight patients with a mean (SD) age of 9.5 (8) years with the established diagnosis of CGD (according to nitroblue-tetrazolium (NBT) and dihydrorhodamine (DHR) tests) were included. The patients received IFN-γ for 6 months. The method of administration consisted of a dosage of 50 micrograms/m^2 of body surface area via subcutaneous injection in deltoid muscle three times a week. The variables documented before enrollment into the study and after 6 months were fungal infections, respiratory infections, admission to hospital, and primary site of infection.
Results: Mean (SD) number of fungal infections among the patients decreased from 1.81 (0.605) times at baseline to 0.38 (0.498) after 6 months. Similarly, Mean (SD) number of respiratory infections at baseline was 2.1 (0.68) times which decreased to 0.68 (0.86) after receiving IFN-γ. Mean (SD) number of hospital admissions decreased from 2.95 (1.071) times at baseline to 0.512 (0.19) after 6 months. During the study period, hepatic infections (6 cases) was the most common primary site of infection followed by subcutaneous (3 cases), bone and/or joint (2 cases), brain (2 cases), lymph nodes (2 cases), and respiratory system (2 cases).
Conclusion: Γ Immunox® showed acceptable clinical efficacy in patients with CGD.

R2748 Phagocytic functions in patients with active brucellosis

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Objective: To evaluate phagocytic functions in Culture-proven Brucellosis.
Method: Eighty (80) Patients with compatible symptoms and signs and positive blood culture for Brucellosis and forty-three normal controls were prospectively studied at the King Khalid University Hospital, Riyadh, Saudi Arabia. Determination of phagocytic function were done by chemiluminesence assay of the oxidative burst, opsonized yeast uptake and turbidimetric measurement of antibody dependent cytotoxicity (ADCC) for whole blood, polymorphonuclear neutrophils (PMNs)and isolated monocytes. Patients with brucellosis were divided into three categories namely: non-complicated, complicated and post treated patients.
Results: Luminol-enhanced chemiluminesence assay of the oxidative burst for PMNs was significantly increase in all patients infected with *Brucella melitensis* as compared to the control group with time to peak in minutes of non-complicated versus control of 5.82±0.03 vs 6.88 ±0.27 ($p < 0.05$). Specifically, the PMNs of patients in the non-complicated group were more active than those with complication. It has been suggested that PMNs depression may contribute to the chronicity of *Brucella* in some individuals. The pattern of whole blood chemiluminesence was found to closely parallel that of the PMNs. The monocyte response of infected patients, however did not always show a significant increase in their function as the time to peak in minutes for control, non-complicated, complicated and post treatment were as follows: 9±0.34, 8±0.45, 7±0.50 and 8±0.50 respectively. The oxidative burst of monocytes of patients was significantly reduced perhaps due to long term survival of *Brucella* within the monocyte. The result demonstrated recovery of activity after treatment indicating temporary and infection-related process. Yeast uptake was significantly reduced in all groups of patients. Measurement of ADCC showed a significant decreased in cellular cytotoxicity as measured by a decrease in target cell elimination for all patients; non-complicated vs control 47.18±3.9 vs 55.77±2.55 ($p < 0.01$); complicated vs control 45.32±3.6 vs 55.77±2.55 ($p < 0.05$) and post treatment vs control 40.95±2.5 vs 55.77±2.55 ($p < 0.05$) compared to the control.
Conclusion: The PMNs and monocyte oxidation burst reaction is dependent on the patients' status. Target cell elimination and yeast uptake however was significantly reduced and did not depend on the patients' status.

R2749 Oral intake of heat-killed *Lactobacillus pentosus* strain b240 accelerates salivary immunoglobulin a secretion in the elderly: a randomised, placebo-controlled, double-blind trial

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Objectives: Secretory immunoglobulin A (IgA) in saliva has been widely used as an indicator of mucosal immunity. A lack of non-specific secretory IgA at the mucosal surfaces can lead to an increased risk of infection. Salivary IgA (SIgA) secretion decreases with age, and may lead to an increased risk of respiratory infections because the salivary glands are the most important source of secretory IgA in the upper respiratory tract. The objective of this study was to demonstrate the acceleration of SIgA secretion by oral intake of *Lactobacillus pentosus* ONRICb0240 (b240) in the elderly.
Methods: A total of 80 healthy elderly individuals were randomly allocated to either an intervention or control group. The elderly individuals in the intervention group were given a water beverage containing heat-killed b240 (4×10^9 cells), while those in the control group were given only a water beverage; both groups received their respective beverages once daily for 12 weeks. Saliva was collected before initiation of the study and every 2 weeks thereafter. Saliva flow rate and SIgA concentration were determined, and the SIgA secretion rate was calculated.
Results: Changes in SIgA secretion rate over the intervention period were significantly greater in the intervention group than the control group and the mean SIgA secretion rate in the intervention group steadily increased until week 4 (exhibiting a 20% elevation relative to that at week 0), and then remained stable until week 12.
Conclusion: Oral intake of *Lactobacillus pentosus* ONRICb0240 for 12 weeks significantly accelerated SIgA secretion, thereby indicating its potential in the improvement of mucosal immunity and resistance against respiratory tract infections in the elderly.

R2750 Cytokine response in patients treated with *S. aureus* autovaccine

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Objectives: Chronic infection with *S. aureus* creates a serious therapeutic and epidemiological problem. In treatment of chronic staphylococcal infections autovaccines are used, prepared from killed *S. aureus* strains, isolated from the patient. The study aimed at evaluation of serum levels of IL-1β, TNF-α and IFN-γ in patients with chronic *S. aureus* infections, treated with autovaccine.
Methods: The studies were conducted on two groups of patients. Group 1 consisted of 20 healthy individuals of 18 to 38 years of age, asymptomatic carriers, in whom nasal smears disclosed presence of *S. aureus*. Group 2 included 16 patients, 17 to 39 years of age, with chronic upper respiratory tract infections induced by *S. aureus*. Strains of *S. aureus* were isolated from throat smears and identified using ID 32 STAPH tests (bioMerieux). In all patients of group 2 treatment with autovaccine (containing 1.5×10^8 killed bacteria/ml) was applied, prepared from *S. aureus* strains isolated from the patients. Serum IL-1b and TNF-a cytokine levels were estimated using high sensitivity ELISA tests (R&D), while levels of IFN-g were quantitated using high sensitivity ELISA test (eBioscience). Estimations of serum cytokine levels were conducted twice: before application of the autovaccine and 24–72 hours following a series of 20 subcutaneous injections (thrice a week) of the autovaccine.

Results: In group 1, consisting of healthy *S. aureus* carriers, mean levels of serum cytokines amounted as follows: IL-1b 0.31±0.3 pg/ml, TNF-a 0.56±0.22 pg/ml, IFN-g 0.21±0.095 pg/ml. In group 2 at the first sampling levels of the cytokines were as follows: IL-1b 0.28±0.16 pg/ml, TNF-a 0.54±0.09 pg/ml, IFN-g 2.37±1.38 pg/ml, and only the level of IFN-g was significantly higher ($p < 0.0001$) than mean level in group 1. In turn, on the second sampling mean level of IFN-g was significantly higher ($p = 0.0152$) than the level noted upon the first sampling and amounted to 4.41±2.6 pg/ml. Serum levels of IL-1b and TNF-a increased in 10 patients (2.77±2.45 pg/ml, $p = 0.0005$ and 1.67±0.69 pg/ml, $p < 0.0001$, respectively) and remained unaltered in the 6 remaining patients. At the same time in 10 patients presence of *S. aureus* could not be detected in throat smears obtained at the time of the second sampling.
Conclusion: The effective immune response against *S. aureus* seems to depend on levels of circulating cytokines IL-1β and TNF-α, the production of which may be stimulated by *S. aureus* autovaccine.

R2751 Anti-cytomegalovirus Avidity Index in women with and without recurrent pregnancy loss

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Background: Some evidence has shown a relationship between human cytomegalovirus (CMV) infection and pregnancy loss. However, whether recurrent or latent CMV infection or altered immune response to CMV may relate to recurrent pregnancy loss (RPL) is unclear and few data are available in this regard. We evaluated CMV infection and humoral immunological response to CMV in women with RPL and compared it to women without any history of abortion.
Methods and Materials: This cross-sectional, observational study was conducted in Clinical Immunology outpatient clinic, Alzahra University Hospital, Isfahan, Iran, between 2008 and 2010. Cases were 43 women with RPL referred by Obstetric and Gynecology outpatient clinic and controls were randomly selected from healthy age match multiparous women without history of abortion. Inclusion criteria were at least 3 recurrent spontaneous abortions, and no history of any diagnosed underlying diseases as etiology of RP L. Blood samples were obtained from patients and controls to evaluate CMV IgG and IgM antibodies and IgG avidity index by the Enzyme Linked Immunosorbant Assay Method (ELISA). Data were analyzed with SPSS version 16.0, using Student's t-test, chi-squared test, and multivariate analyses.
Results: One case (2.3%) of positive IgM in each group of RPL's and controls was detected. Also, there were 39 (90.6%) and 30 (69.8%) cases of positive IgG in RPL's and controls, $P < 0.05$. Patients and controls were similar regarding serum IgM titer ($P > 0.05$). But, IgG titer was significantly higher in RPL group, $P < 0.05$. No differences were found between the two groups in IgG avidity index ($P > 0.05$). In multivariate analyses, only IgG positivity was related to RPL ($P < 0.05$) and avidity was not related to RPL ($P = 0.277$). Also, IgG positivity ($P = 0.159$), IgM positivity ($P = 0.856$), or avidity index ($P = 0.440$) were not related to the number of abortions in RPL patients.
Conclusions: Previous exposure to CMV detected by a positive IgG antibody is significantly related to RPL in the present study. However, we found no relationship between IgG avidity index and RPL. Whether Latent CMV infection starting an indirect process of autoimmune etiology in RPL's or women with RPL had recurrent or reactivation of CMV infection but avidity index is not the best index to detect it, mostly because of altered immune function to CMV in RPL patients need further investigations.

R2752 Specific antibody functional activity in recipients of one-dose vaccine contained Leningrad-3 mumps virus strain: a 3-year follow-up

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Mumps virus-neutralizing antibodies are believed to be the most predictable surrogate marker of protective immunity. The remote history of vaccination also suggests that waning immunity could have contributed to the vaccinees' susceptibility. Mumps outbreaks in vaccinated populations are usually caused by the phylogenetically distinct from the vaccine strains mumps viruses (MuV).
To examine these issues, the kinetics of titer, avidity and neutralizing activity level and spectrum of the specific IgG were studied in 3-year follow-up serum samples from the healthy volunteers ($n = 60$) with no vaccination and mumps in the past, after the one vaccine dose contained "Leningrad-3" (L-3) MuV strain.
Full seroconversion, confirmed by ELISA and PRN against the vaccine MuV strain, was registered in all volunteers 3 months after vaccination. Antibodies induced by the vaccine strain effectively neutralized five heterologous MuV strains (genotype A, B, D, and those earlier isolated during the local mumps outbreaks, C and H) starting from month 6 after vaccination at all time points tested thereafter, albeit at levels lower than those seen against the vaccine strain (>2-fold difference). The specific IgG titers measured in ELISA demonstrated an "early" decrease after 9 months post vaccination. At the same time, the specific IgG functional activity characterized by their avidity, level and spectrum of neutralizing activity reached its maximum within month 18 after vaccination. Antibodies capable of neutralizing the heterologous MuV genotypes in the absence of antigenic boosting demonstrated a decrease to the critical levels (⩽1:8) in a part of vaccinees after the third year post vaccination, while the specific IgG neutralizing activity against the vaccine strain still demonstrated a titer previously associated with protection against MuV infection (⩾1:8).
The current study possessing several limitations demonstrated for the first time a broad-spectrum neutralizing activity of the specific IgG in recipients of one dose vaccine containing the L-3 MuV strain within 3 years after vaccination.

Geometric mean titers of neutralizing antibody against the "Leningrad-3" vaccine strain and five heterologous mumps viruses in one-dose recipients' sera.

MuV (genotype)	0 month	1 month after vaccination	3 month after vaccination	6 month after vaccination	9 month after vaccination	12 month after vaccination	18 month after vaccination	24 month after vaccination	36 month after vaccination
"L-3"	<2.00	2.30±0.01	6.49±4.30	10.56±9.46	16.0±9.66	27.0±13.99	28.22±11.94	20.16±8.18	15.63±8.16
Enders (A)	<2.00	2.44±0.76	3.49±1.69	6.35±3.97	9.05±4.89	13.30±7.06	13.30±6.09*	11.05±4.38	8.77±4.19
Urabe (B)	<2.00	<2.00	2.83±1.02	6.06±3.66	9.19±4.42	12.40±7.40	12.4±6.45*	9.05±4.69	7.64±4.17
Dragl4 (C)	<2.00	<2.00	2.52±0.96	5.16±1.96	5.92±1.98	7.82±3.75	7.82±3.39*	6.65±2.50	5.28±1.99
Lon1/Uk/9 6 (D)	<2.00	<2.00	2.46±0.93	6.06±4.05	7.82±4.66	10.8±6.92	11.31±6.86*	8.57±4.53	7.13±3.71
Patro/Kov (H)	<2.00	<2.00	2.46±0.93	5.03±1.91	6.79±2.03	7.13±3.31	7.46±3.15*	6.35±1.91	5.31±2.00

R2753 Screening of *Lactobacillus pentosus* strain b240 promoting IgA production

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Objective: The foreign substances, such as pathogenic microorganisms, usually invade internal body via mucosal surface with ingestion or breathing. Thus, activation of mucosal immune function is essential for maintaining and better health. Secretory immunoglobulin A (SIgA) play a pivotal role in mucosal immune system by the multiple protective roles. This study objective was to find out lactic acid bacteria (LAB) for promoting IgA.
Methods: First, we tested 150 LAB strains for IgA production capability in vitro using a murine Peyer's patch cell culture system and selected *Lactobacillus pentosus* ONRICb0240 (b240). In addition, comparison of cytokine profile between b240 and low IgA inducible LAB was examined. Next, we administrated heat-killed b240 to the mice for 3 weeks and investigated the intestinal IgA enhancement. Moreover, we attempted an ex vivo study to assess the reactivity of murine Peyer's patch cells against b240.
Results: We found out b240 to be exhibiting excellent IgA producing activities in 150 strains of LAB. Interestingly, plant derived LAB including b240 tended to show higher activity than dairy LAB. After 3weeks of b240 administration, intestinal IgA significantly increased

comparing that of saline treated mice and the IgA production of murine Peyer's patch cells against stimulation of b240 was facilitated.
Conclusion: *Lactobacillus pentosus* ONRICb0240 would be an useful LAB strain for promoting IgA production. Efficacy of b240 ingestion to the other mucosal immune system is expected.

Vaccines

R2754 The side effects of H1N1 pandemic vaccine in pregnant women and comparison to other healthcare workers

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Objectives: To investigate the side effects of H1N1 vaccine in a cohort of pregnant women and compare with in healthcare workers in 2009–2010 pandemic season.
Methods: We recruited 26 pregnant women less than 38 gestational weeks and 85 healthy healthcare workers who applied to our clinic for influenza vaccination. Pregnant patients received Panenza® (unadjuvanted H1N1 pandemic vaccine), whereas healthcare workers received Focetria® (adjuvanted H1N1 pandemic vaccine). Pregnant women were followed up for the entire pregnancy and the newborns were assessed for APGAR score, while healthcare workers were monitored 14 days for the respective symptoms.
Results: The mean age of the enrolled pregnant patients and healthcare workers were 31.7±5.5 and 33.6±6.3 years, respectively ($P > 0.05$). None of the enrolled cases received seasonal vaccine. Of the enrolled pregnant patients 26.9% were in the 1st trimester, 23,1% were in the 2nd trimester and 50% was in the 3rd trimester. The most frequent symptom was pain in the vaccinated arm with 58.6% of the patients being reported. When vaccine associated symptoms were assessed; redness (34.6% vs. 3.5%; $p = 0.00$), sore throat (7.7% vs. 0.0%; $p = 0.05$), fatigue (50.0% vs. 16.5%; $p = 0.01$), myalgia (50.0% vs. 9.4%; $p = 0.00$), hypotension (11.5% vs. 1.2%; $p = 0.03$), emesis (42.3% vs. 2.4%; $p = 0.00$), and dizziness (19.2% vs. 0.0%; $p = 0.01$) were more common in pregnant patients compared to healthcare workers, respectively. All of the pregnancies ended up with healthy term deliveries, but one newborn died after premature labor in the 27th gestational week (the reason was independent from the vaccine).
Conclusion: The frequency of H1N1 vaccine associated symptoms may be different between pregnant patients and healthy subjects. Whether this difference was caused by administering adjuvanted or unadjuvanted vaccines or the presence of pregnancy – per se – confounded the results merits further evaluation.

R2755 Evaluation of gene polymorphisms in saCOL2291 and saCOL2581, candidate genes for a *S. aureus* vaccine

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Objective: *Staphylococcus aureus* is a salient nosocomial infectious agent. The prevalence of antibiotic resistance complicates the treatment of staphylococcal infections. Therefore, the development of an effective vaccine against *S. aureus* is important.
Materials: saCOL2291 and saCOL2581 are potential candidate genes for vaccine development and their sequences are available in GenBank. In this study, gene polymorphisms in saCOL2291 and saCOL2581 were evaluated. Genomic DNA was extracted from thirty clinical *S. aureus* isolates and the genes were PCR-amplified. The amplicons were sequenced, aligned with Puls4 software and compared with saCOL2291 and saCOL2581.
Results: Nucleotide polymorphisms were detected that resulted in amino acid sequence changes, but these polymorphisms were located in the N-terminal domains while the C-terminal domains of these genes were conserved.
Conclusion: Despite the identification of polymorphisms in these genes, both encode candidate proteins for potential use in a universal staphylococcal vaccine. This is the first study for assessing variation in the saCOL2291 and saCOL2581 genes.

R2756 Projecting of rotavirus vaccine effectiveness in Ukraine, based on the distribution of rotavirus genotypes in different regions of the country

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Objective: In recent years, thanks to the emprovement of laboratory diagnosis, and especially the introduction of molecular biology study methods, including polymerase chain reaction (PCR), it has been shown, that rotaviruses as the etiologic agents of rotavirus infection (RVI), dominate in the etiologic structure of acute intestinal infections of viral etiology in infants and children under five years old worldwide. Rotavirus is non-enveloped virus with genome, represented by 11 segments of RNA, which makes these viruses extremely volatile, especially due to reassortment of genes during co-infection. Detection of specific sequences of genes, encoding protective proteins VP4 and VP7 of outer capsid of rotaviruses (G/P-genotyping), using PCR, has become the modern tool in the study of rotaviruses. Nowadays it is particularly relevant in the context of the implementation of vaccination programs aginst rotavirus infection in children. For prediction of the possible genotype-specific effectiveness of vaccination against RVI in conditions of limited molecular epidemiological studies, mathematical modeling of vaccination strategies can become particularly informative, because the distribution of pathogenic strains in children in different geographic regions can vary significantly.
Methods: Using PCR with reverse transcription, we demonstrated the circulation in Ukrainian children four genotypes of group A rotaviruses: G1P[8], G4P[8], G3P[8], G2P[4] and the dominance of G1P[8]. The mathematical modeling method can adequately evaluate the overall effectiveness of the vaccine, depending on the region of its implementation. Using data of genotype-specific effectiveness of monovalent rotavirus vaccine based on strain RIX4114 genotype G1P[8], we estimated its expected genotype-specific effectiveness in the whole country and, additionally, in Kiev and Odessa regions, as the most densely populated regions with intense migration.
Results: It was shown that the expected overall effectiveness of rotavirus vaccine, based on strain RIX4114, was 0.812 for the whole country, 0.841 for Kiev city and 0.804 for the Odessa region. These results were comparable with the results of clinical studies of the effectiveness of this vaccine (0.847) in Latin America and Finland. We should therefore expect that vaccines, including attenuated or reassortant strains of rotaviruses with genotype G1P[8], as a tool of specific prevention, will be most effective in the territory of Ukraine.

R2757 Effect of age at immunization and quality of immune response following multi-antigen MAP vaccination

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Mycobacterium avium subsp. *paratuberculosis* (MAP), the agent of Johne's disease, causes systemic infection and chronic intestinal inflammation in many animal species. Neonates are more susceptible to the infection due to high degree of exposure from their dams and possibly less developed immune system. The only vaccines available against MAP comprised attenuated or killed organisms. However, none of these vaccines are able to completely prevent infection or shedding of bacteria. Considering the fact that MAP infection occurs at a young age and the animal remains in the subclinical phase for 2–3 years, an effective vaccine should not only elicit strong immune response in young animals, but also a quality of the T-cell response that correlates with long term protection. Here we report the effect of age at immunization and quality of immune response following immunization of calves with recombinant MAP proteins formulated with DDA/TDB (CAF01) adjuvant. Vaccine comprised of one heat shock protein, two ESAT-6 family members and two secreted mycobacterial components fused with CAF01 adjuvant. A total of 27 male jersey calves were divided into 3 groups of 9 calves each

with first vaccination at 2, 8 and 16 weeks of age, respectively. Vaccine induced immune response, mainly the Th1 type cytokine secretion, was evaluated in different age groups following booster doses at equal time intervals. Preliminary results show higher antigen specific IFN-γ levels in response to heat shock protein and ESAT-6 family member protein antigens. It was observed that there was no effect of age on the IFN-γ producing capacity of the animals in the different age groups after stimulation of whole blood with SEB. However, animals in the older age group responded well to the MAP multi-antigens and might need only one booster compared to the younger animals. Findings from this work could be interesting to determine the appropriate age of vaccination so as to generate the memory T cell pool and for MAP vaccine challenge experiments.

R2758 Characterisation of new vaccine candidates against smallpox, attenuated and replicative-competent

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Smallpox, caused by Variola virus, is considered as a dreadful scourge for human. Smallpox vaccination, using strains of Vaccinia virus (VACV) has leaded to it eradication in 1980. However, adverse reaction could result, so it was abandoned. Current bioterrorism attack threat are feared the disease re-emergence, then new smallpox vaccines more safe but less effective in animals have been developed.

Attenuation of our candidates have been measured in Swiss Nude or SCID mice. Mice have been vaccinated with 10^5 pfu of each viruses by tail scarification. Signs of disease and mortality were followed over an 8 week period. Protection of BALB/c mice against a lethal challenge have been performed, 28 days after vaccination, with 2.10^6 DICT50 of cowpoxvirus by intranasal route (i.n). VACV neutralizing antibodies (Abs) in serum samples were titrated by plaque assay. Serum samples were first incubated at 56ºC for 30 minutes then submitted to serial 2-fold dilutions. The samples were mixed with an equal volume of VACV containing 35 PFU in 0.1ml for one hour at 37ºC and added to Vero cell monolayers. Two days later virus plaques were counted. To measure T lymphocyte responses the percentage of CD4+ and CD8+ lymphocytes expressing intracellular IFN-g was measured by flow cytometry. Mature bone marrow dendritic cells from uninfected BALB/c mice were infected with VACV then incubated with spleen cell suspensions from infected animals for six hours. Brefeldin A (5 µg/ml) was added for the last four hours to block cytokine secretion. Cells were stained with FITC-coupled anti-CD8b2 and APC-coupled anti-CD4 monoclonalAbs, fixed and permeabilised. IFN-g was stained with a PE-coupled anti-IFN-g mAb and cells were fixed in 2% formaldehyde.

These vaccine candidates have been shown as protective as the traditional vaccine (1G) against an i.n cowpox challenge in mice, and they have been closely as safe as MVA strain in NUDE mice. The neutralizing antibodies titration showed that every deleted viruses induced a humoral response as strong as 1G. Finally intracellular cytokine staining showed a similar specific CD4+ response to that of 1G and a slightly lower specific CD8+ response than that of 1G.

Our results show that several deleted vaccines could advantageously complete the range of the third generation vaccines already developed. The utilisation of one of these candidates as a viral vector for a vaccine against Ebola virus is under investigation in our laboratory.

R2759 Predictive models of adherence to seasonal and pandemic H1N1 influenza vaccine between health care professionals

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Objectives: Several strategies have been designed to increase adherence to vaccination programs aimed at health-care professionals, though the results were not always satisfactory.

Methods: The differences between adherence to seasonal and pandemic influenza vaccination after the implementation of a vaccination program between health-care workers were assessed and compared by age, gender, professional category and workplace. Two different prognostic models for predicting adherence to seasonal and pandemic influenza were developed. In statistical analysis, univariate logistic regression was performed to identify risk factors associated with adherence to vaccinations. Multiple regression analysis backwards, stepwise variable selection was used to identify independent risk factors for adherence. The internal validation of the predictive models was evaluated by measures of calibration and discrimination power.

Results: 7.6% of professionals were vaccinated against pandemic influenza, and 33.7% against seasonal influenza. Statistically significant differences were observed for both vaccines when comparing vaccinated to unvaccinated professionals as for age, professional category and workplace, while sex differences were only related to pandemic influenza. The predictive model of adherence to pandemic H1N1 vaccine, which showed a good discriminatory power (Area under ROC curve: 0.843), included the variables age, professional category, workplace and previous vaccination against seasonal influenza as independent predicting factors. On the contrary, the predictive model of adherence to seasonal vaccine, which showed a poor discriminatory power (Area under ROC curve: 0.567), included the variables age, gender and workplace. Both models showed a good calibration (table 1).

Conclusions: Adherence to pandemic H1N1 and seasonal influenza vaccination program was very low, which suggests the need to implement new strategies into vaccination programs aimed at health-care professionals. A good model for predicting adherence to pandemic vaccine was developed when compared with the poor discriminating power of the predictive model for seasonal influenza vaccination.

MODEL FOR PREDICTING ADHERENCE TO PANDEMIC H1N1 VACCINE		
Variable	**OR (CI 95%)***	**p**
Age	1.02 (1.01-1.04)	0.002
Professional category (ref. non-medical categories**)		
Doctor/Clinical pharmacist	9.46 (5.24-17.10)	<0.001
Medical Residency Training/Fellowship	10.44 (5.05-21.58)	<0.001
Nurses	2.06 (1.11-3.82)	0.022
Medical assistants	1.41 (0.73-2.72)	0.306
Workplace (ref: other centres***)		
Acute care hospital	12.46 (5.05-30.74)	<0.001
Investigation center	6.36 (1.82-22.23)	0.004
Gender (ref: female)	1.23 (0.89-1.70)	0.215
Previous seasonal vaccination	5.71 (4.25-7.68)	<0.001
MODEL FOR PREDICTING ADHERENCE TO SEASONAL VACCINE		
Age	1.01 (1.00-1.02)	0.007
Gender (ref: female)	0.8 (0.67-0.96)	0.016
Professional category (ref. non-medical categories**)		
Doctor/Clinical pharmacist	1.24 (0.96-1.61)	0.102
Medical Residency Training/Fellowship	0.95 (0.65-1.38)	0.774
Nurses	1.06 (0.84-1.34)	0.632
Medical assistants	0.92 (0.73-1.16)	0.467
Workplace (ref: other centres***)		
Acute care hospital	1.72 (1.41-2.08)	<0.001
Investigation center	0.41 (0.22-0.77)	0.005

* OR = Odds ratio, CI95% = confidence interval 95%,
** Includes members of the board, other non-medical categories, administrative officers and workers.
*** Includes a health-care center, a psychiatric centre, two medical assistant schools and primary care.

R2760 In silico analysis of Adenovirus penton protein and proposed vaccine using bioinformatics

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Objective: Adenovirus are responsible for various diseases to humans and many species of animals & birds. There are no antiviral drugs to treat adenoviral infections. The use of immunoinformatics has greatly revolutionized the field of vaccine research, discovery and development. Hence approach has been made to identify the relevant B-cell & T-cell epitopes to design vaccine against adenovirus using bioinformatics.

Methods: Conserved regions of Penton protein of Adenovirus were analyzed in order to assess their antigenic potential. B-cell epitope prediction of conserved sequences was done by using BepiPred method. The binding capacity of this conserved sequence was also assessed for its ability to bind with MHC-I and MHC-II using the ProPred-I server. During last step of study, this penton sequence was also analyzed for its binding affinity with T-cell receptors using the EpiJen server. Structure prediction of this sequence was done by PSIPRED.
Results: The whole sequence of amino acid of penton protein was found to be conserved. There are 16 B-cell epitopes, one epitope at each conserved region. As per the results of ProPred, 32 sequences were found to bind with MHC-I alleles. During the analysis of MHC-II binding, total 51 alleles were analyzed for their binding efficiency with conserved region of penton. The multiepitope peptide showed T-cell binding region at a number of allele positions. Structure prediction of this sequence by PSIPRED revealed that 16 helix are present in this sequence.
Conclusion: The present study finds that penton proteins of Adenovirus can also be an effective candidate for the development of preventive measures against the drastic diseases caused by this virus by blocking its initial infection. In fact in silico approach for target prediction is definitely reducing manpower, time and cost in relation to search for a lead antigenic molecule against penton protein.

R2761 Immunogenicity and protective immunity of a recombinant BCG expressing human GM-CSF and CFP10–ESAT6 fusion proteins from *M. tuberculosis*

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Objectives: Tuberculosis remains a major health threat to the people of the world. Bacille Calmette–Guerin (BCG) is the only using vaccine and provides dissatisfactory protection against *Mycobacterium tuberculosis*. Here we constructed a trivalent recombinant BCG which encodes human GM-CSF and immunodominant antigens CFP10 and ESAT6 fusion protein from *M. tuberculosis* and detected its immune responses in mice. Our objective is to develop a novel BCG vaccine which provides a better immunogenicity and protective efficacy against tuberculosis.
Methods: The sequence of GM-CSF and ESAT6/CFP 10 gene was amplified by PCR respectively. GMCSF–CFP10–ESAT6 (GCE) chimeric gene was amplified by SOE PCR and then transferred into shuttle plasmid pMV 361. The recombinant vectors were electroporated into *M. bovis* BCG. BALB/c mice were divided into seven groups and were injected subcutaneously with BCG or rBCGs. The samples were collected at 6, 8, 10, and 12 weeks after immunization, ELISA was used to determine IgG antibody specificity. The proliferative response of lymphocytes was detected by XTT assay and the lymphocyte subpopulations were analyzed by the flow cytometry. The production of IFN-γ and IL-4 in response to TB-antigen was measured.
Results: The expression of GM-CSF, CFP10, ESAT6 and GCE complex were identified by western blotting from recombinant BCG. Stimulation index values of rBCGs were significantly higher than control groups. The SI values with rBCG:GCE group was the highest in all groups. The rBCG with GEC chimeric gene could induce more antigen specific CD4+ and CD8+ T cells of splenocyte than BCG and single gene r BCGs at different time points. The rBCGs were able to induce higher levels of IFN-γ and IL-4 than control groups. IFN-γ production from group of rBCG:GCE was the highest among groups. Moreover, mice immunized with rBCG:GCE generated the strongest antigen specific antibody responses compared to other groups.
Conclusion: The rBCG:GCE can induce intensive immune response and could enhance the Protective efficacy to *M. tuberculosis*. The results indicate that this novel trivalent recombinant BCG might be a good vaccine candidate against tuberculosis.

R2762 Serotype distribution among bacteraemic pneumococcal pneumonia in adults in Germany

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Objectives: *Streptococcus pneumoniae* remains a leading cause of pneumonia, sepsis and meningitis and disproportionately affects young children and the elderly. In July 2006, vaccination with pneumococcal conjugate vaccine was generally recommended by the German Health authorities for all children up to the age of 24 months. In this study, we present the serotype distribution among adults with bacteremic pneumococcal pneumonia before and after the start of childhood vaccination.
Methods: The National Reference Center for Streptococci has monitored the epidemiology of invasive pneumococcal disease (IPD) in adults in Germany since 1992. Cases of IPD in adults are reported by a laboratory-based surveillance system, including 265 laboratories throughout Germany. The present analysis includes only bacteremic pneumococcal pneumonia cases documented between 2002 and 2010. Species confirmation was done by optochin testing and bile solubility testing. All isolates were serotyped using the Neufeld Quellung reaction.
Results: In the pneumococcal season 2006–2007 the most prevalent serotypes among bacteremic pneumococcal pneumonia in adults were serotypes 14 (19.1%), 3 (10.9%), 1 (8.6%), 7F (6.7%) and 9V (6.5%). In 2009–2010 serotypes 3 (18.5%), 7F (12.4%), 19A (10.9%), 1 (9.6%) and 22F (5.9%) were most prevalent. The coverage of the 23-valent polysaccharide vaccine in 2009–2010 was 86.5%. The serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F, 19A and 23F cover 68.6% of all bacteremic pneumonia cases in 2009–2010.
Conclusions: The burden of pneumococcal pneumonia among German adults is considerably high. The most prevalent serotypes have changed after the start of childhood vaccination and are currently serotypes 3, 7F, 19A, 1 and 22F.

R2763 Incidence and case fatality of invasive pneumococcal disease among adults in the United Kingdom: a systematic review

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Objective: Pneumococcal infection is a potentially life-threatening condition, which can also cause lifelong sequelae such as neurologic deficits. Since 2003, vaccination with a pneumococcal polysaccharide vaccine has been recommended in the UK for those aged 65 years and over, in addition to those in a clinically at-risk group over two years of age (in place since 1992). A pneumococcal conjugate vaccine was introduced into the childhood vaccination scheme in 2006. Although many studies have estimated invasive pneumococcal disease (IPD) incidence among various population subgroups, including adults, a synthesis of estimates has not been performed. The objective here was to estimate age-specific incidence and case fatality rates of IPD among adults in the UK.
Methods: A systematic review was conducted in MEDLINE and EMBASE in August 2010. Two independent reviewers extracted data from articles describing adult pneumococcal disease incidence and mortality in the UK, published from the year 2000 to present. Data were synthesized according to clinically relevant age categories for adult pneumococcal infection and stratified according to type of pneumococcal disease (IPD overall, or its subgroups of bacteraemia/septicaemia or meningitis). These data were supplemented with hand-searching of reference lists.
Results: The search strategy identified 2,444 abstracts for screening; 20 satisfied the inclusion criteria, and presented estimates from 1980 to 2005. Age-specific estimates of the incidence of IPD and its subgroups are provided in the table. Overall, incidence of IPD and its subgroups increased with age. For IPD, incidence rates ranged from 2.7 to 10 cases per 100,000 among those <65, to 18.8 to 70 cases per 100,000 among those >65 years of age. Overall case fatality due to IPD and its subgroups also generally increased with age. Four studies reported overall IPD case

fatality rates between 21% and 34%; and age-specific estimates from 2% to 35% among adults aged <65 years, and 30% to 43% among older adults.

Conclusions: The epidemiologic burden of IPD is greater among older adults. These synthesized estimates may therefore serve as a baseline measure against which to evaluate the cost-effectiveness of new strategies for preventing pneumococcal infection.

Mean annual incidence rates due to pneumococcal disease among adults, stratified by age (per 100,000 population for each age band)

	Condition	Age groups (years)			
		18-64	65-74	75-84	≥85
Incidence	Invasive pneumococcal disease	27-10.0	18.8-36.2	40-45.7	70.0
	Pneumococcal bacteraemia/septicaemia	10.4		78.0	27.5
	Pneumococcal meningitis	0.7-0.7	0.4-1.7	0.7	

R2764 Immunomodulatory properties of live attenuated *Bordetella pertussis* vaccine candidate BPZE1

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Objectives: New vaccines against pertussis are needed to evoke long lasting protection and immunological memory starting from the first administration in neonates. A novel live attenuated *B. pertussis* vaccine strain, BPZE1, has been developed by eliminating or detoxifying three important *B. pertussis* virulence factors: pertussis toxin, dermonecrotic toxin and tracheal cytotoxin. With the aim to evaluate BPZE1 immunomodulatory properties in human pre-clinical models, we used an ex vivo model based on monocyte-derived (MD) dendritic cells (DC) challenged with BPZE1.

Methods: Human immature MDDC were obtained by culturing purified monocytes in the presence of GM-CSF and IL-4 for 6 days. MDDC were challenged with BPZE1 or its parental wild type counterpart, BPSM. After 48 h MDDC were harvested for immunophenotypic analysis and the supernatants collected for cytokine measurement by ELISA. Resistance of BPZE1-challenged MDDC to apoptosis was assessed by AnnexinV/Propidium Iodide staining. Chemokine-driven chemotaxis was also evaluated. Co-culture experiments with T lymphocytes were performed to assess antigen-presenting activity, T-helper cell polarizing ability and induction of functional suppressor T cells.

Results: BPZE1 is able to induce phenotypic maturation of human MDDC which are resistant to apoptosis. BPZE1-primed MDDC produce a broad spectrum of proinflammatory and regulatory cytokines, and very efficiently migrate in vitro in response to the lymphatic chemokine CCL21, due to the inactivation of the pertussis toxin enzymatic activity. BPZE1-primed MDDC acquire antigen-presenting capacity, drive a mixed Th1/Th17 polarization, and induce functional suppressor T cells. Suppressing activity requires cell contact rather than the production of soluble factors.

Conclusion: Our findings support the potential of BPZE1 as a novel live attenuated pertussis vaccine strongly activating the maturation of DC with full-blown activity. BPZE1-challenged DC acquire the capacity to survive death signals and migrate from the site of infection to the lymph nodes. BPZE1-committed DC have the ability to orchestrate a broad spectrum of Th1/Th17 responses, protective in experimental *B. pertussis* infection, and regulatory T cell responses, likely balancing each other to restore local homeostasis.

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R2765 Low immunogenicity of seasonal trivalent influenza vaccine among patients receiving docetaxel for a solid tumour

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Background: Patients receiving cytotoxic therapy for solid tumors are at increased risk of severe influenza, Data on flu vaccine immunogenicity are needed in those patients to improve the vaccine coverage.

Methods: In a multicentric prospective study, 25 patients with breast (n = 13) or prostate (n = 12) cancer received one dose of a trivalent inactivated influenza vaccine the same day that the IV administration of docetaxel. Serum hemagglutination-inhibition (HI) antibody response was assessed 21 days after vaccination.

Results: The median age of the study population was 65 years (min-max, 33–87); 52% were female. At baseline, the percentages of patients with titers of vaccine strain-specific HI antibodies ⩾1:50 were 100%, 72%, and 100% against H1N1, H3N2 and B, respectively. Immunogenicity results at day 21 show that this vaccine is poorly immunogenic (see Table 1).

No serious adverse events (AE) have been reported during the first 3 weeks after vaccination. All the reported AE were from mild to moderate intensity.

Conclusions: The results show that the trivalent inactivated influenza vaccine triggers a low immunogenicity in adults receiving docetaxel for solid tumors, although it demonstrated a good safety profile. Strategies using more immunogenic influenza vaccines have to be evaluated in patients with cancer.

Table 1. Immunogenicity of influenza vaccine

	A/H1/Solomon Islands/3/06	A/H3/Wisconsin/67/05	B/Malaysia/2506/04
Number of tested patients	24	24	24
Geometric mean titer (GMT) (95% CI)	794.4 (609.4–1036)	257.2 (169.3–390.5)	315.2 (221.40–448.9)
Seroprotection rate (SPR) n (%)	24 (100)	19 (79)	24 (100)
Seroconversion rate (SCR), n (%) (95% CI)	7 (28) (23.1–33.3)	2 (8) (7.7–8.3)	4 (16) (7.7–25)
Geometric mean fold rise (seroconversion factor) (95% CI)	2.16 (2.10–2.22)	1.3 (1.26–1.34)	1.58 (1.45–1.73)

SPR: percentage of patients with a post-vaccination HI titer ⩾1:50.
SCR: percentage of patients showing a significant increase in antibody titer defined as a pre-vaccination titer ⩾1:50 and at least a fourfold increase in postvaccination titer.
Geometric mean fold rise: geometric mean of the within-subject ratios of the post-vaccination reciprocal HI titer to the Day 0 reciprocal HI titer.

R2766 Evaluation of cytokine profile following immunisation with *Brucella abortus* S99 lipopolysaccharide-*Neisseria meningitidis* serogroup B outer membrane vesicle conjugate as a new brucellosis vaccine candidate

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Objective: The development of an efficacious vaccine for brucellosis has been a challenge for scientists for many years. Our previous studies demonstrated that LPS+OMV conjugates was elicited high level of anti-LPS IgG titers. In this study, the efficiency of LPS-OMV conjugates to promote IFN-g and IL-4 evaluated to determine the pattern of T-helper population activation.

Method: *B. abortus* LPS was extracted and used for their conjugation to *N. meningitidis* serogroup B outer membrane vesicle as carrier protein as discribed previously. Groups of ten BABL/c mice were injected subcutaneously with 10 μg of LPS alone, combine and conjugated on 0, 14 and 28 days. Sera were taken before and 14 days after each injection. Levels of IFN-g, IL-4 and IL-10 in the sera of immunized animals assayed by ELISA.

Results: Immunization with *B. abortus* LPS significantly induced high level of IFN-g in comparison to the other groups immunized with LPS-OMV conjugate and LPS+adjuvant ($P < 0.05$. In contrast, lower levels of IL-4 and IL-10 were elicited by LPS in all of the immunized animal models. Although Immunization with *B. abortus* LPS-*N. meningitidis* serogroup B OMV conjugate demonstrated significant increase of IL-4 and IL-10 in comparison with the immunization with *B. abortus* LPS ($P < 0.05$, the titer of IFN-g is still significantly higher than IL-4 and IL-10 in the sera of this group's animal models ($P < 0.05$).

Conclusion: The raise of IFN-g production following the immunization with all of the compounds (LPS, LPS-OMV conjugate and LPS+adjuvant) indicates the induction of Th1-type response that would be correlated to the clearance of the organism due to the proliferative response of Polymorphonuclear cells. Low levels of IL-4 and IL-10 following the immunization with all compounds would be a sign of Th1 responses dominancy or inhibition of Th2-type response proliferation and activity. Since Th1-type response would be indicated the efficiency of this brucellosis conjugate vaccine, Th2 responses basically have no role in the immune responses against brucellosis and may lead to the

persistence of intracellular infection. These results indicate that the conjugated LPS obtained by us, can be used as a brucellosis vaccine after further investigation.

Internet and electronic resources

R2767 Improving empirical antibiotic therapy in orthopaedic-related infections using ViResiST, a computerised decision support system

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Introduction and Objectives: Inappropriate antibiotic prescribing is common in orthopaedic-related infections, and the consequences are severe. Commonly used guidelines give empirical antibiotic treatment recommendations that are general and not tailored to local prevalence patterns. We developed a microbiologic decision support tool (ViResiST: www.viresist.org) founded on our local bacterial susceptibility data augmented by expert infectious disease logic. We compared the effectiveness of current antimicrobials prescribed to the antimicrobials recommended by the program.
Methods: We retrospectively enrolled all inpatients with a positive culture at our institution with a surgical site infection after implantation of a joint prosthesis or internal fixation between January 2008 and August 2009 and determined the current empiric therapy before and after the result of culture. ViResiST incorporate the most likely infectious organism and its antimicrobial resistance pattern using a multivariate time-series analysis to provide treatment recommendations. A cross-table was used to compare the effectiveness rate of empiric therapy.
Results: The microbiology laboratory recorded 19 patients/25 pathogen related with surgical site infection over orthopaedic device during the study period that met inclusion criteria. Physicians initiated effective empiric therapy in 13 out of the 19 patients, for an effectiveness rate of 68%. The computer-guided therapy was effective in 19 of the 19 events for a rate of 100% ($p < 0.0001$ by Fisher's exact test).
Conclusion: We found that ViResiST would potentially improve the rate of effectiveness of empirically chosen antimicrobials and could enhance the current targeting of empiric antimicrobial therapy by tracking potential pathogens and their evolving antimicrobial resistance profiles.