Community-acquired infections: focus on unusual epidemics and update on S. pneumoniae

**P430** Community-acquired pneumonia caused by 'atypical' pathogens: clinical discrimination with a predictive model and scoring system

M. Masía, F. Gutiérrez, J.C. Rodríguez, S. Padilla, G. Royo, J.M. Ramos, A. Ayelo, A. Martín-Hidalgo

Elche, Alicante, E

Objective: To develop a bedside predictive model and a scoring system to identify patients with atypical community-acquired pneumonia (CAP), in order to select candidates for tailored antibiotic therapy with macrolides.

Methods: Data from a prospective study of CAP conducted at a Spanish hospital during two consecutive periods, October 99–September 00, and October 00–September 01, were analysed. An extensive non-invasive microbiological investigation was performed in all patients, including detection of Legionella pneumophila and Streptococcus pneumoniae urinary antigen, and acute and convalescent serologic testing. During the first 12-month study period, serum biochemical markers of bacterial infection (C-reactive protein, procalcitonin and lipopolysaccharide-binding protein (LBPs)) were also performed. Clinical, radiological and laboratory data of patients with atypical CAP were compared with data from patients with non-atypical CAP by univariate and multivariable analysis. With the best-discriminating variables from the multivariable analysis, a scoring system model was devised. Each variable was assigned 1 point. ROC curves were used to describe sensitivity and specificity of the model. Two separate models were run.

Results: A total of 493 patients were included. On multivariable analysis, patients with atypical pneumonia were more likely to have air-conditioning exposure (OR 2.64), normal WBC count (OR 2.58), birds at home (OR 2.18), age under 65 years (OR 2.07), non-associated comorbid conditions (OR 2.04), absence of pleuritic chest pain (OR 1.95), and elevated GGT levels (OR 1.87). When the variables age was <65 years, normal WBC, birds at home, elevated transaminase levels, and a negative pneumococcal urinary antigen assay were included in the scoring system, a score of ≥4 captured patients with atypical pneumonia with a sensitivity of 33% and a specificity of 95%. A second scoring system was constructed with the 240 patients from the first 12-month study period. When the variables age <65 years, normal WBC count, birds at home, LBP levels <14 mcg/mL and negative urinary antigen assay were included, a score of ≥4 captured patients with atypical pneumonia with a sensitivity of 49% and a specificity of 95%.

Conclusion: Selected patient factors and additional diagnostic testing can aid to discriminate atypical pneumonia with high specificity. This information could be useful to ensure effective and safe use of macrolides as empirical monotherapy in CAP.

**P432** Effect of initial treatment on bacteraemic Streptococcus pneumoniae pneumonia (BSPP) mortality


Madrid, E

Objectives: Community-acquired BSPP is associated with a high mortality. Recent studies have suggested that monotherapy may be sub-optimal for this infection. The aim of this study was to evaluate the effect of initial antimicrobial therapy on the outcome of BSPP.

Methods: Retrospective study of adult cases (>18 years) of community-acquired BSPP hospitalised at our institution from 1990 to 2003. Severity of pneumonia was assessed using PORT-score. The impact of penicillin susceptibility and initial empirical treatment (combined therapy vs. monotherapy) on the 30-day mortality were assessed by univariate and multivariate analyses.

Results: A total of 343 cases were evaluated. There were 220 males (64%); the mean age was 56.9 years (range, 18–94). Most patients had chronic predisposing conditions and 32% of them had HIV infection. Decreased susceptibility to penicillin (MIC > 0.06 mg/L), cefotaxime (MIC > 0.5 mg/L), and erythromycin (MIC > 1 mg/L) was observed in 34% (108/320), 10% (14/135), and 21% (61/292) of strains. Patients were empirically treated with a beta-lactam (50%), a beta-lactam plus macrolide (26%), or other regimens of monotherapy (10%) or combination therapy (14%). Overall mortality rate was 19%. Mortality was significantly associated with (P < 0.01) with PORT groups: class I: 1/30 (3%), class II = 5/59 (8%), class III = 5/61 (8%), class IV = 25/123 (20%), class V = 28/70 (40%). The mortality rate was higher in patients with penicillin-resistant strains than in patients with susceptible strains (25% vs. 14%; P = 0.03), but was similar in patients receiving monotherapy or combination therapy (16% vs. 22%; P = 0.13). Multivariate analysis showed

**P431** Effects of a pneumococcal vaccination programme in adult invasive Streptococcus pneumoniae disease: a 3-year experience

F.J. Vasallo, A. González-Escalada, V. del Campo, S. Pérez, J. Torres

Vigo, E

Objectives: Streptococcus pneumoniae is a leading cause of serious infections. The prevention of pneumococcal disease has become an important public health issue as a result of the rapid increase in the prevalence of pneumococcal resistance to antibiotics. Our aim was to evaluate the effect of a pneumococcal vaccination programme in elderly people (started in autumn, 1999) in the incidence of invasive disease, and the changes observed in serotypes prevalence and antibiotic resistance during the study period.

Methods: Our institution covers a mainly rural area of approximately 160 000 adult population in the northwestern area of Spain. We retrospectively reviewed all invasive S. pneumoniae (blood, CSF) isolates recovered at our laboratory between 1998 and 2003, 3 years before and after the programme started, named period A and B respectively. Identification and susceptibility testing were performed following standard recommendations. Pneumococci were classified as susceptible, intermediate and resistant (S, I, R) according to 2001 definitions of NCCLS. Serotyping was performed at a national reference laboratory.

Results: A total of 165 invasive pneumococci were recovered during the whole period (period A, 1998–2000: 99 isolates; period B, 2001–2003: 66). The rate of invasive disease dropped from an average of 20.6 cases per 100 000 persons in period A to an average of 13.8 in period B (33% lower). A similar reduction was observed in those 65 years and older (49.2 per 100 000 vs. 37.8 per 100 000, 23.2% reduction). Penicillin susceptibility (S, I, R) in the two periods was: A: 73.4, 18.4, 8.2%, and B: 67.2, 31.2, 1.6% (P 0.05). Cefotaxime susceptibility was: A: 83.7, 13.3, 3%, and B: 85.9, 12.5, 1.6%. Erythromycin susceptibility was: A: 84.7, 1, 14.3%, and B: 68.7, 1.6, 29.7% (P 0.05). The most frequent serotypes were (per cent): period A: 4 (16.9), 3 (15.7), 8 (12), 14 (10.8); and B: 3 (16.7), 14 (16.7), 19 (11.7), 6A (8.3).

Conclusions: A significant reduction (33%) in the incidence of pneumococcal invasive disease was observed after the vaccination programme started. The slight increase in penicillin susceptible strains (26.6% vs. 32.8%) was because of intermediate susceptible ones, with a decrease in those highly resistant (8.2% vs. 1.6%). Erythromycin resistance duplicated in the study period (14.3% vs. 29.7%).
that the presence of septic shock and advanced age (>65 years) were the most important factors associated with mortality.

Conclusions: PORT score accurately identifies the mortality risk of community-acquired BSPP. Decreased susceptibility to penicillin seems to be associated with a worse prognosis. Our study has not confirmed the beneficial impact of empirical combination therapy for BSPP.

P433 Comparative, randomised, open, multicentre trial assessing the efficacy and safety of intravenous/oral azithromycin compared with intravenous/oral clarithromycin for the treatment of community-acquired pneumonia due to Legionella pneumophila


Objective: To evaluate the efficacy and safety of intravenous (i.v.) /oral (p.o.) Azithromycin (AZM) compared with i.v./p.o. clarithromycin (CLA) for the treatment of community-acquired pneumonia (CAP) caused by Legionella pneumophila.

Study design: Randomised, open-label, multicentre phase III clinical trial.

Inclusion criteria: Subjects over 18 years with clinical and radiological findings consistent with CAP with no requirement for hospitalisation in ICU and positive determination of Legionella pneumophila urinary antigen.

Treatment: (A) Patients (pts) not requiring hospitalisation and therefore, will receive only oral treatment: AZM 500 mg/day during 3 days or CLA 500 mg every 12 h during 10–14 days. (B) The hospitalised subjects will receive: (1) AZM: 500 mg once a day, by i.v. route, during at least 2 days and for a maximum of 5 days. After the second day the investigator will assess whether the therapy can be switched to oral route. The treatment will continue to complete a total of 7 days. (2) CLA: 500 mg twice a day, i.v. route for at least 2 days and for a maximum of 5 days. After the second day the investigator will assess when to switch to the oral route at doses of 500 mg every 12 h. Total treatment will be 10–14 days.

Results: During the period of study 50 patients were recruited, 25 in each group (40 male, 10 female). The mean age of the study participants was 57 years (27–83 years), mean bodyweight was 76 kg (50–130 kg) and mean height was 168 cm (141–182 cm). No difference was found in demographics between groups. Twenty-four pts (96%) received initially i.v. treatment in AZM group vs. 22 pts (88%) in CLA group. Mean duration in i.v. treatment was 3 days in both groups. In the intend-to-treat (ITT) population, clinical success rates both at end of treatment (EOT) and end of study (EOS) were 95.8% for AZM and 100% for CLA. Bacteriological eradication rates at EOS and EOT were 100% in both groups for patients cured or improved. The incidence of treatment-related adverse events (AEs) is similar in both groups and most events were of mild to moderate severity. Incidence of phlebitis was seven episodes (28%) for AZM vs. 13 episodes (52%) for CLA.

Conclusions: AZM i.v./p.o. for the treatment of CAP caused by Legionella pneumophila is as efficacious as CLA i.v./p.o. Both treatments were well tolerated but incidence of phlebitis was higher in the group receiving CLA (P = 0.08).

P434 Pseudomonas aeruginosa as a risk factor in acute exacerbations of COPD

M. Allewelt, S. Balk, H. Lode
Berlin, D

Objectives: Pseudomonas aeruginosa is a frequent cause of severe bacterial exacerbation in advanced stages of COPD. Its role in a complicated course of the disease and for an unfavourable outcome were evaluated.

Methods: Ambulatory and hospitalised subjects presenting with an acute exacerbation of COPD were prospectively evaluated for clinical and radiological parameters, for functional impairment, and for life expectancy. From all patients valid sputum samples were obtained at presentation.

Results: A total of 193 patients with a history of COPD and an acute exacerbation were included. In 114 subjects, potentially pathogenic microbes were isolated, Pseudomonas aeruginosa in 12 cases (10.5%). In two patients, bronchiectasis was demonstrated by high-resolution CT scan. Seven individuals infected with Pseudomonas aeruginosa died within 1–21 months after initial presentation. Between subjects who did or did not die, there were no significant differences with respect to age, frequency of underlying diseases, smoking status, or treatment with systemic steroids. All individuals who succumbed had stage III disease (ATS classification: FEV1<35% predicted). On average, these had a worse lung function (VC 49.1 ± 9% vs. 78.8 ± 18.9% predicted, FEV1 26 ± 6.5% vs.43.5 ± 13.4% predicted, absolute FEV1 0.8 ± 0.2L vs. 1.0 ± 0.3L). Emphysema (5/0), a medical history with treatment in an ITU (5/1), and with preceding mechanical ventilation (3/1) were more frequent in subjects with a fatal outcome.

Conclusion: Presence of Pseudomonas aeruginosa in individuals presenting with an acute exacerbation of COPD is associated with a high fatality rate short-term outcome. Particularly severe underlying airflow obstruction and a medical history of treatment in an ITU or mechanical ventilation is associated with an unfavourable outcome.

P435 Community outbreak of Legionella in Hospitalita (Spain). Usefulness of the epidemiological and molecular data to identify the source

Barcelona, Badalona, E

Legionella is considered responsible for 2–13% of cases of community-acquired pneumonia requiring hospitalisation. Colonisation of cooling towers and evaporative condensers by Legionella, with the subsequent production of polluting aerosols, has been associated with community outbreaks of Legionnaires' disease. To date, epidemiological data and microbiological/molecular studies are both necessary in the investigation of an outbreak.

Objective: To describe a community outbreak of pneumonia caused by Legionella in Hospitalita. Catalonia (Spain) and the usefulness of the DNA chromosomic analysis to find the source of the outbreak.

Methods: An observational prospective study was performed. Data from affected patients was requested and water from suspected environmental sources was cultured for Legionella. Isolates of Legionella from patients and water samples were subtyped by Pulsed Field Gel Electrophoresis. From each positive environmental plate five colonies were picked up and re-seeded on BCYE, in order to know the clonal distribution of Legionella.

Results: From August 16–December 16 (2002), 13 definitive cases of pneumonia caused by Legionella were reported. The median age of the patients was 72 years: 92% were males. Of the cases, 11 were hospitalised. The case fatality rate was of 7.7%. The attack rate of the outbreak was 0.029%. The majority of cases were located within a radius of 200 m around the implicated cooling tower (HC) with a risk ratio of 17.4 (CI 95% 3.8–80.6). Of the 17 samples taken from cooling towers and evaporative condensers, nine were positive for L. pneumophila. A single DNA subtype was observed among the three clinical strains (subtype A). Two different subtypes were found in the HC cooling tower, one of them (subtype A) matching exactly with the clinical subtypes. Several subtypes were found in the other cooling towers none of them matching the clinical subtypes. Moreover another cooling tower had its own subtypes of Legionella. After closing the HC cooling tower no new cases of pneumonia caused by Legionella appeared.

Conclusions: The results of epidemiological and microbiological data suggested the HC cooling tower as a source of a community outbreak of LD. The diversity of DNA subtypes even in such a small area facilitate the identification of the outbreak source.
P436  Significance of early *Mycoplasma pneumoniae* serodiagnosis in hospitalised patients with community-acquired pneumonia

A. Kuhnke, M. Bender, M. Allewell, A. Roth, H. Mauch, H. Lode
Berlin, D

**Objectives:** The use of serological methods in early diagnosis of pneumonia is controversial due to delayed production of antibodies. Therefore the temporal course of specific serological response was investigated in hospitalised patients with *Mycoplasma pneumoniae* CAP. P.

**Methods:** Hospitalised patients with suspected CAP were prospectively recruited for the study. The aetiology of *Mycoplasma pneumoniae* was serologically tested by particle-immuno-assay (PIA). A positive serological diagnosis was made if the acute phase serum titre was more than 1:160 or paired samples taken 2–4 weeks apart showed a fourfold rise in serum titre. The PIA does not differentiate between IgM and IgG. Furthermore respiratory specimens were investigated by PCR. In patients with predefined clinical parameters *Mycoplasma pneumoniae* was diagnosed in cases of positive PIA and/or positive PCR.

**Results:** *Mycoplasma pneumoniae* was identified as a causative agent in 50 patients (25 female, 25 male). 41 patients (82%) had a positive serology and 45 patients (90%) had a positive PCR result. In 28 patients (56%) significant PIA titers were detected on hospital admission. On day 3 of hospitalisation cumulative significant titers were measurable in 34 cases (68%) and on day 4 in 42 cases (96%). A correlation of these laboratory results was proofed with clinical findings. 48 patients (96%) were complaining about cough, 43 (86%) about fever, 28 (56%) about dyspnoea and expectation and 26 (52%) had auscultatory findings.

**Conclusion:** The clinical course of mycoplasma pneumonia in patients requiring hospitalisation justifies the early use of serological methods (PIA) in acute diagnosis.

P437  Prevalence and degree of *Legionella* colonisation in cooling towers

M. Garcia-Nunez, S. Ragull, E. Junyent, M.L. Pedro-Botet, N. Sopena, M. Sabria
Badalona, E

Cooling towers have been frequently implicated in community *Legionella* outbreaks. Since July 2003, Spanish regulation obligate test water from cooling tower systems for *Legionella* and perform a chlorine shock if colonisation are above 1000 CFU/L. This last measure cause important logistic and economic consequences troubles. Other guidelines increase the cut-off to above a chlorine shock if colonisation are above 1000 CFU/L. This last measures could be overestimated.

**Colonisation** is not known, the established cut off to initiate shock measures should be probably considered.

P438  Epidemiology of community-acquired pneumonia revisited: a large population-based prospective study in the Mediterranean coast of Spain

Elche, Alicante, E

**Objectives:** Over the last few years there have been significant advances in microbial diagnosis and therapy of community-acquired pneumonia (CAP). The impact of these changes on the epidemiology of CAP is unknown. The aim of this study is to provide a comprehensive overview on current epidemiological features of CAP.

**Methods:** A 2-year population-based prospective study conducted from October 1999 through September 2001 in consecutive adults with CAP at a teaching hospital in the Mediterranean cost of Spain. An extensive non-invasive microbiological investigation was performed, including detection of Legionella pneumophila and Streptococcus pneumoniae urinary antigens, and acute and convalescent serologic testing to detect antibodies against *atypical* and viral pathogens.

**Results:** A total of 493 patients (62.5% men, mean age 56 years) were included. The annual incidence rate of CAP was of 1.03 cases per 1000 inhabitants. In 265 (53.7%) patients there was one or more underlying diseases. 75.1% were included in Fine’s categories I-III. 361 (73.2%) were admitted to hospital, 6 (1.2%) of them to the ICU. A total of 276 microorganisms (69 bacteria, 105 atypical pathogens, and 92 virus) in 250 (50.7%) patients were identified. In 243 (49.3%) cases the microbial aetiology remained unknown. In 20% of the cases, the microbial diagnosis was made by detection of urinary antigens. The most frequent organisms were *S. pneumoniae* (38%), *M. pneumoniae* (18%), *L. pneumophila* (10.4%), *C. pneumoniae* (9.6%), influenza virus (8.8%) and Gram-negative bacilli, including *Pseudomonas* species (14.8). In 30 (12%) cases, infection was considered mixed. The most frequent combination was *S. pneumoniae* with *M. pneumoniae* or *L. pneumophila* (three cases each). Microbial diagnosis of CAP varied according to age, site-of-care and co-morbidity. A total of 27 patients died with an overall mortality rate of 4.9%. During the second year of the study there was a decrease in the proportion of patients admitted to hospital (79.2% vs. 67.2%, P = 0.03) and in the mortality rate (7.1% vs. 2.8%, P = 0.03).

**Conclusions:** Incidence of CAP in this study at the Mediterranean coast of Spain was lower than previously reported. New technologies allowed a rapid etiological diagnosis of CAP in many cases and disclosed a significant proportion of mixed infections. Mortality rate of CAP may be decreasing. The results of this study may aid in the management of antibiotic treatment in patients with CAP.

P439  Factors influencing treatment outcome and length of stay in patients hospitalised for community-acquired pneumonia

A. Neiss, D. Elkharrat, S. Kohno, J. Schentag, S. Decker-Burgard, K. Roscher
Munich, D; Paris, F; Nagasaki, JP; Buffalo, USA; Bad Soden am Taunus, D

**Objectives:** To identify factors affecting treatment outcome and length of hospital stay in patients hospitalised with CAP.
Methods: Data were obtained retrospectively by chart review from 2183 patients with discharge or death diagnosis of CAP during a 12-month period in Germany (D), France (F), Japan (J) and the USA. Exploratory analyses were performed to evaluate predefined prognostic factors affecting treatment success (logistic regression; clinical success = patients discharged [/+ antibotics]; microbiological success = all CAP-relevant organisms were or were presumed to be eradicated) and length of hospital stay (linear regression and analysis of covariance).

Results: Mean age ranged from 63.9 years (D) to 69 years (F). Median hospitalisation time varied from 14 days (USA) to 14 days (J). Antibiotic treatment, Fine score, age and social status were identified as prognostic factors at the P = 0.05-level in final models. Appropriate antibiotic treatment during hospitalisation increased treatment success in J (odds ratio [OR] 0.2, microbiological success in D and J (OR 0.5, 0.3, respectively) and reduced hospitalisation time in D and J (Cox relative risk [RR] 1.3, 1.5). In all countries, monotherapy with antibiotics other than penicillins and derivatives reduced hospitalisation time (RR 1.3–1.7) and a high-risk Fine score (class V) reduced treatment success rates compared with low risk scores (classes I–III) (OR 2.4–8.7). Longer hospitalisation was related to high-risk Fine score in F, and USA (RR 0.4–0.6) and to greater age in D, F, and J (RR 0.8–0.9 per 10 years). In F, ‘living in nursing homes’ correlated with lower treatment success rates than ‘living with families’ (OR 4.5).

Conclusions: The importance of risk factors varied between countries and resulted in different success rates and lengths of stay. Appropriate antibiotic treatment for CAP covering the spectrum of common causative pathogens — even monotherapy with antibiotics other than penicillins and derivatives — and a low risk assessment at the time of hospitalisation were identified as the most important prognostic factor for successful treatment and a short hospitalisation time.

Bacteriological outcomes in hospitalised patients with community-acquired pneumonia treated with i.v. azithromycin plus ceftriaxone vs. i.v. ceftriaxone plus clarithromycin or erythromycin

Basle, CH; Perugia, I; Cape Town, ZA; Guadalajara, E; New York, USA; Milán, I; Utrecht, NL

Objectives: Several treatment recommendations, such as the updated IDSA Guidelines, include a beta-lactam/macrolide combination for hospitalised CAP pts. We compared clinical efficacy and safety of sequential therapy with i.v. ceftriaxone (CEF; 1–2 g q.d.) and azithromycin (AZM; 500 mg q.d.) followed by oral AZM (500 mg q.d.) vs. CEF (1–2 g q.d.) and either clarithromycin (C; 500 mg q12h) or erythromycin (E; 1 g q8h) followed by oral C (500 mg q12h) or E (1 g q8h) in pts with moderate to severe CAP.

Methods: This international multicentre study was a randomised and open label clinical trial. Total length of therapy: CEF/AZM was 7–10 days and CEF/E or C was 7–14 days. Clinical and bacteriological outcomes were assessed at end-of-treatment (EOT; days 12–16) and at end-of-study (EOS; days 28–35).

Results: A total of 135 and 143 pts were treated in the CEF/AZM and the CEF/C or E groups, respectively. 52.6 and 51.1% of pts in the CEF/AZM and CEF/C or E groups, respectively, were classed within the Fine risk categories IV and V. The most frequently isolated baseline pathogens were Streptococcus pneumoniae, Haemophilus influenzae, and Staphylococcus aureus; their results are presented below. The number of pts with other pathogenic organisms was too small, based on this study alone, to allow any definitive conclusions to be drawn about the efficacy of CEF/AZM or the comparator treatment. The MITT clinical success rates at EOT were 84.3% for AZM and 82.7% for CEF/C or E; EOS success rates were 81.7% for AZM vs. 75.0% for CEF/C or E.

Conclusion: In a relatively sick patient population, AZM/CEF followed by oral AZM showed comparable bacteriological efficacy to CEF/C or E followed by oral C or E in the treatment of hospitalised patients with CAP.
Atypical pathogens in pathogenesis of bronchial asthma in children and efficacy of azithromycin as a part of complex therapy

O. Lasitsa, I. Ochotnicova, S. Lomonosov
Kyiv, UKR

Aim: The aim of this study was to evaluate the prevalence of atypical pathogens such as Mycoplasma pneumoniae (MP), Chlamydia pneumoniae (CP) and Chlamydia trachomatis (CT) in children with bronchial asthma and assess the efficacy of azithromycin (Sumamed, Pliva, Croatia) in acute asthma exacerbation as a part of complex therapy.

Methods: Children with acute asthma exacerbation were enrolled in the study. In all children PCR (sera and sputum), serological tests (IgM and IgG) for MP, CP and CT, detection of MP antigen (AG) in nasal aspirate by using immunofluorescence method (IF) were performed.

Results: A total of 76 children, aged 6 months–14 years, with acute asthma exacerbation were enrolled in the study. 29 of them have mild asthma, 28 – intermediate and 19 – severe asthma. Previous history of the disease was 1 month–10 years. Acute respiratory infections were the triggers of asthma exacerbation in majority (88.2%) of children. The prevalence of MP, CP and CT infections among children found to be very high (54–89%). As a result of high prevalence of MP, CP and CT infections in children, with asthma exacerbation, 5-day azithromycin was added to the complex antiasthmatic treatment. Clinical success of the treatment was achieved in majority of children (91.3%), regardless the severity of asthma exacerbation. We found rapid regression of fever and symptoms of intoxication after 2–3 days of onset of treatment. During the follow-up period (6 months) we found that 79.1% of children did not suffer from acute infections and asthma exacerbations.

Conclusions: The prevalence of MP, CP and CT infections among children included in the study found to be very high. The results suggest that such patients may have benefit with short courses of azithromycin. Nevertheless, further studies are needed in order to assess the role of azithromycin in chronic persistence of atypical pathogens in respiratory tract in children with asthma.

A case-control study on acute respiratory infections of patients in general practices in the Netherlands, October 2000 – September 2003

A.B. van Gageldonk-Lafeber, A. Bartelds, M-L.H. Heijmen, M. Peeters, C.M. van der Plas, S.C. de Greeff, B. Wilbrink, Bilthoven, Utrecht, Tilburg, NL

Objectives: Acute respiratory infections (ARI) are very common in the general population: In the Netherlands, yearly 3.2 million patients visit general practitioners with symptoms of upper respiratory disease. To reduce diagnostic deficit, we studied the incidence and aetiology of ARI. Additionally, we studied ARI risk factors, healthcare demand and burden of disease.

Methods: From October 2000 to October 2003, the incidence of influenza-like illness (ILI) and other ARI in general practices and the role of a broad range of pathogens in the Netherlands were studied. General practitioners from a Dutch sentinel network registered all patients consulting them for ILI and other ARI. Weekly, general practitioners randomly sampled one of these patients (case) and one patient of the same age group, visiting the general practitioner for non-respiratory symptoms (control). Samples were analysed for respiratory viruses and bacteria. Participating patients completed a questionnaire on risk factors and burden of disease.

Results: Forty practices registered all patients consulting them for ILI or other ARI. Twenty practices participated in the case-control study. Per year on average, 1600 consultations for ILI and 5000 for ARI were registered, leading to an incidence of 130 and 410 per 10 000 persons respectively. In total 647 cases and 559 controls were employed. In more than 65% of the ILI cases and 50% of the ARI cases, viruses were detected, compared with 20% in controls. Bacteria were detected in more than 30% of both cases and controls.

Conclusion: Despite three unusual calm influenza seasons, influenza was the most common pathogen in ILI-patients. Rhinovirus was most often detected in ARI patients. Incidence for both ILI
and ARI was highest for 0–4 year olds. More data on the incidence of ILI and ARI and associated pathogens will be presented.

**P445 Hospitalised community acquired pneumonia: characteristics, aetiology and outcome**

É. Székely, C. Kösa, Z. Vármai, E. Ludwig
Budapest, HUN

**Objective:** To analyse characteristics of patients with community acquired pneumonia (CAP) at the Department of Internal Medicine in the Central Infectious Disease Hospital during a 3-year period (2001–2003).

**Methods:** All the patients with CAP were included. Recorded data were sex, age, PORT score, presence of underlying diseases, main clinical features and symptoms, radiological appearances and outcome. Blood samples for culture from all patients, sputum when it was possible and serological tests were performed for *Chlamydia pneumoniae, Mycoplasma pneumoniae* and *Legionella* spp. In some cases direct antigen detection (urine or sputum) and serology tests for viruses and other pathogens (influenza, RSV, covidiella etc.) were carried out.

**Results:** One hundred and fifty-nine consecutive patients were included with a mean age of 54.2 years (range 15–96), 98 male and 61 female. The distribution of patients according to the PORT score was: class I, 20%; II, 29%; III, 25%; IV, 20%, V, 6%. In 45% one, in 36% two or more diseases considered as risk factors were present. The most prevalent of them were chronic pulmonary diseases, alcohol abuse and chronic uraemia. Admission to ICU was necessary in 10 cases (mean age 42.6, PORT score class III, I; IV, 8; V, 1). Pathogens were identified in 62% of patients. The most frequent were *Legionella* spp. (34%), *S. pneumoniae* (15%), *M. pneumoniae* (15%), *C. pneumoniae* and *psittaci* (15%). All of the invasive *S. pneumoniae* strains were fully penicillin-sensitive. Overall mortality was 9.8%, directly attributable to pneumonia in 7%. All of the fatal outcomes with detected pathogens were caused by *Legionella pneumophila* infection.

**Conclusions:** The leading causes of hospitalisation of patients with mild pneumonia were fever and/or extrapulmonary clinical symptoms. More thorough ambulatory examinations of patient (X-ray, blood count etc.) would have decreased the number of hospital admissions. The causative pathogens were revealed in 62%, most of them were ‘atypical’. The unusual distribution of pathogens probably results from the special situation of our hospital. Nevertheless, more frequent examination of sputum and BAL would result in the increase of ‘typical’ pathogens. The PORT score was useful to identify patients with poor outcome.

**P446 Comparison of additional antibacterial usage in patients with community-acquired pneumonia receiving telithromycin or clarithromycin: results from two double-blind, randomised clinical trials**

Dundee, UK; Mineola, Amherst, Brookline, USA; Quebec, CAN; Bridgewater, USA

**Objectives:** This analysis was undertaken to compare use of additional respiratory-related antibacterials in patients with community-acquired pneumonia (CAP) treated with the ketolide telithromycin (TEL) or the macrolide clarithromycin (CLA).

**Methods:** Data from two similar, comparative, double-blind, randomised trials were pooled for this analysis. Adult outpatients with mild to moderate CAP were randomised to receive either TEL 800 mg once daily for 5, 7 or 10 days or CLA 500 mg twice daily for 10 days. In both studies, clinical outcome was assessed in the per-protocol (PP) population at the post-therapy/test of cure (TOC; days 17–24) visit. Patients were followed to days 31–36 (late post-therapy visit) to assess the use of additional respiratory-related antibacterials in the intent to treat population (TEL, n = 612; CLA, n = 411).

**Results:** Clinical cure rates in the PP population were equivalent in the two treatment groups (88.8% [428/482] for TEL and 90.1% [272/302] for CLA; 1.3, 95% CI (–6.0, 3.4)). A total of 155 patients required additional respiratory-related antibacterial therapy: 14.2% (87/612) of patients treated with TEL and 16.5% (68/411) of CLA-treated patients (2.3, 95% CI (–7.0, 2.4)). Shorter courses of TEL were not associated with increased requirements for additional antibacterials (14.0% [27/193] for 5 days TEL vs. 14.3% (60/419) for 7/10-day TEL; –0.3, 95% CI (–6.6, 6.0)). Cephalosporins, macrolides and quinolones were the most common additional antibacterials used, accounting for more than half of supplementary respiratory-related antibiotics in both treatment groups. Duration of additional antibacterial therapy was 174 days/100 patients with 5-day TEL, 145 days/100 patients with 7/10-day TEL and 181 days/100 patients in CLA-treated patients. For those patients who received additional treatment with intravenous (i.v.) antibacterials, the duration of additional treatment was 41 days/100 patients with 5-day TEL, 28 days/100 patients with 7/10-day TEL and 54 days/100 patients with 10-day CLA.

**Conclusions:** TEL 800 mg once daily for 5, 7 or 10 days is as effective as CLA 500 mg twice daily for 10 days for the treatment of CAP. The tendency towards a reduced frequency and shorter duration of use of additional antibiotics, particularly i.v. therapy, suggests a potential for cost savings when TEL is used to treat patients with CAP.

**P447 Seroprevalence of Chlamydia pneumoniae infection in patients with chronic stable asthma**

M. Aslan, K. Güven, G. Sağlam, S. Altun, S. Sarıbas, B. Müsellim, B. Gemicıgioğlu, B. Kocayezbek
İstanbul, TR

**Objectives:** Asthma is an inflammatory disease in which the airways become blocked or narrowed, constriction in the bronchi and bronchioles and a feel of tightness in the chest. *Chlamydia pneumoniae* like genetic and environmental factors contribute to the development of asthma. This study was designed to investigate the presence of *C. pneumoniae*-specific IgG, IgA and IgM antibodies in sera samples of 50 adults with a clinical history of chronic stable asthma and 50 healthy individuals as control group.

**Methods:** The correlation between *C. pneumoniae*-specific antibodies in chronic stable asthma cases and eosinophilia cationic protein (ECP) levels were also evaluated. 50 stable chronic asthma cases (12 male, 38 female) between the ages 14 and 70 were evaluated for FEV (forced expiratory volume), ECP, allergic state and smoking habits. *C. pneumoniae*-specific IgG, IgA and IgM antibodies were also investigated for this group.

**Results:** Healthy control group was matched with patient group for age, gender, locality and smoking habits. *C. pneumoniae* seropositivity (a past *C. pneumoniae* infection) was found in 68% (34) and 58% (29) of stable asthma cases and control groups, respectively (P > 0.05). *C. pneumoniae*-specific IgA was found higher in 34% (17) and 18% (9) of stable asthma and control groups respectively (P > 0.05). The indication of chronic *C. pneumoniae* infection (IgG > 1/512 and IgA > 1/40) was found in 28% (14) and 10% (5) of stable asthma cases and control group, respectively (P < 0.05). A statistically significant difference was not found between chronic stable chronic asthma and control groups for chronic *C. pneumoniae* seropositivity and smoking. Also a statistically significant difference was not found between ECP positive and negative patients for chronic *C. pneumoniae* infection seropositivity (P > 0.05).

**Conclusions:** As conclusion, this study supports that there can be a relationship between chronic *C. pneumoniae* infections and stable asthma cases.
**P448** Severe community-acquired pneumonia: impact of empirical antimicrobial treatment on outcome

M. Daganou, P. Bakakos, A. Sakellaropoulos, M. Kompoti, A. Paridou, M. Klena, A. Rassidakis

*Ann Arbor, USA*

**Objectives:** To assess the impact of empirical antimicrobial therapy on the outcome of severe community-acquired pneumonia (SCAP).

**Methods:** Prospective observational study of patients with SCAP admitted to the ICU of a Hospital of Chest Diseases since January 1998. Immunocompromised patients and patients with a known caustive microorganism at the time of admission were excluded. After initial laboratory testing empirical antimicrobial treatment was started by the attending physician.

**Results:** Sixty-seven patients were studied (45 male, mean age 56 ± 18 years, mean APACHE II score 15 ± 6), fifty-one of whom needed mechanical ventilation (76%). Empirical antimicrobial regimens included combination of two to four antibiotics. A combination of a beta-lactam with a macrolide or a quinolone was administered to 54 patients (80%). Twenty-seven (40%) received an aminoglycoside, 16 (24%) received antistaphylococcal agents and 14 (21%) antibiotics against anaerobic microorganisms. Strict adherence to management guidelines of ATS and/or BTS was noted in 39 cases (58%). In most cases (72%), non-compliance consisted of using more antimicrobial agents than those recommended. Overall mortality was 43%. Patients treated with a beta-lactam+macrolide or quinolone combination had significantly lower mortality compared with those treated with other regimens (37% vs. 77%, P = 0.01). Concomitant use of other agents had no impact on outcome. adherence to management guidelines was not shown to influence mortality.

**Conclusions:** Non-adherence to management guidelines was frequently encountered among ICU patients with SCAP. Overuse of aminoglycosides and/or antistaphylococcal agents was the main cause of divergence from guidelines. However, this was not shown to affect mortality. Use of a combination of beta-lactam+macrolide or quinolone was associated with lowest mortality rate.

**P449** Efficacy of linezolid for the treatment of pneumonia caused by penicillin (PRSP) or multi-drug resistant (MDRSP)

*Streptococcus pneumoniae (SP)*

K. Tack, M. Ijzerman, R. Croos-Dabrera

*Ann Arbor, USA*

**Objective:** SP is becoming resistant to many antimicrobials commonly used to treat pneumonia, a common and potentially fatal infection. Linezolid is a new antimicrobial with bactericidal activity against SP. The objective of this analysis was to assess the efficacy of linezolid for the treatment of pneumonia caused by SP, including PRSP and MDRSP.

**Methods:** A meta-analysis was performed pooling data from seven studies evaluating linezolid for the treatment of community- or hospital-acquired pneumonia. Patients were required to have symptoms compatible with pneumonia and a chest X-ray showing an acute infiltrate. Adults received 600 mg linezolid i.v./p.o. q12 h, and children received 10 mg/kg q12 h, for a mean of 11 days. For this analysis, patients with SP isolated from sputum and/or blood were considered; these patients were included in the modified intent-to-treat (MITT) population. Patients who met stricter criteria constituted the microbiologically evaluable (ME) population. Clinical cure rates in these two groups were 85 and 90%, respectively, among patients whose outcomes could be assessed. There were 34 MITT and 25 ME patients with PRSP, with clinical cure rates of 74 and 84%, respectively. In bacteremic ME patients with SP the clinical cure rate was 61/66 (92%) and in those with PRSP it was 7/8 (88%). Amongst the ME patients with PRSP and whose isolates were resistant to at least one other class of antimicrobial (MDRSP), clinical cure rates and microbiological eradication rates ranged from 75 to 100%.

**Conclusions:** Linezolid is an effective agent for the treatment of pneumonia caused by SP, including strains resistant to other antimicrobials.

**P450** Pathogenic role, epidemiology, and susceptibility to antimicrobial agents of *Neisseria meningitidis* isolated from lower respiratory tract secretions of adult patients

E. Cercenado, B. Padilla, P. Martín-Rabadán, N. García-Escribano, O. Pérez-Olaso, E. Bouza

*Madrid, E*

**Objectives:** Meningococcal pneumonia has been recognised clinical syndrome for over 20 years. Because of the nasopharyngeal carriage of *N. meningitidis* (NM), the ability to establish the diagnosis based on the culture of respiratory samples (RS) alone is hazardous. To assess the incidence of respiratory infections caused by NM, the serogroups, and the antimicrobial susceptibility of the isolates, we reviewed the 20 cases of NM isolated from RS of adult patients which occurred at our institution from 1999 to 2003.

**Methods:** Ours is a large teaching institution (1800 beds) serving a population of 640 000 inhabitants. All significant RS of patients suspected of having pneumonia were quantitatively cultured onto blood agar, chocolate agar, and MacConkey agar. Isolates were identified by standard procedures. Susceptibility testing was performed by the broth microdilution method with 5% lysis horse blood. Beta-lactamase production was detected using the nitrocephin test. Serogroups were determined at the National Reference Laboratory for Meningococci in Spain.

**Results:** Over the study period, 15 003 RS were processed. Among these, 40.7% were positive. NM represented 0.4% of all positive samples. The evolution of cases was: 1999, two cases (0.03/1000 admissions); 2000, 0 cases (0/1000); 2001, seven cases (0.11/1000); 2002, two cases (0.03/1000), 2003, nine cases (0.14/1000). The origin of isolates was bronchial aspirate (13 cases), bronchoscopic specimens (4), and sputum (3). Serogroups were B (17); C, Y and 29E, one case each. 80% of the isolates were non-susceptible to penicillin (MICs 0.12–0.25 mg/L). None of the isolates produced beta-lactamase and all were fully susceptible to cefotaxime, rifampin and ciprofloxacin. Fifteen patients were hospitalised in ICUs. Nine patients were diagnosed of pneumonia (four monomicrobial and five polymicrobial) and 11 were colonised. None developed meningococcal bacteraemia.

**Conclusions:** The pathogenic role of NM in lower respiratory tract infections of adults is probably underestimated because its isolation is difficult. In our area the most frequent serogroup was B, and we found a high incidence of penicillin-resistant (non-susceptible) isolates.

**P451** Efficacy of a 7-day course of oral telithromycin 800 mg once daily in community-acquired pneumonia caused by resistant *Streptococcus pneumoniae*

C. Fogarty, D. Rensburg van, C. Salvo de, M. Rangaraju, R. Nusrat

*Spartanburg, USA; Witbank, ZA; Federal Capital, AR; Romai­ville, F; Bridgewater, USA*

**Objectives:** The ketolide telithromycin has a targeted spectrum of antibacterial activity for the treatment of community-acquired pneumonia.
respiratory tract infections, that provides coverage of all relevant pathogens, including atypical/intracellular organisms and *Streptococcus pneumoniae* resistant to penicillin (PRSP) and/or erythromycin (ERSP). Previous studies have shown that oral telithromycin 800 mg once daily for 7–10 days is an effective treatment for patients with community-acquired pneumonia (CAP). The efficacy and tolerability of 7-day telithromycin in patients with CAP, including those with CAP caused by PRSP and/or ERSR, were investigated further in this multicentre multinational study.

Methods: A total of 858 patients (aged ≥13 years) with CAP received telithromycin 800 mg once daily for 7 days in an open label, non-comparative study that aimed to recruit a high proportion of patients with pneumococcal aetiology. Clinical and bacteriological outcomes were assessed in the clinical and bacteriological per-protocol populations (PPc, n = 723; PPa, n = 274) 10–17 days post-therapy [test of cure (TOC) visit].

Results: Clinical cure and bacteriological eradication were achieved in 15/16 (93.8%) patients infected with pneumococci: 14/15 (93.3%) patients with PRSP and 7/7 with PRSP. Overall, satisfactory bacteriological outcome was achieved in 87.6% (240/274) of patients at TOC, with 94.6% (122/129) of *S. pneumoniae*, 87.8% (108/123) of *Haemophilus influenzae*, 80% (16/20) of Moraxella catarrhalis and 80.8% (21/26) of *Staphylococcus aureus* strains being eradicated/presumed eradicated. In total, 89.3% (646/723) of patients were assessed as clinically cured at the TOC visit. Telithromycin treatment was well tolerated. Overall, 15.7% (135/858) of patients experienced treatment-emergent adverse events (TEAEs) classified as possibly related to study medication, the majority of which (occurring in 112 patients) were mild in intensity. The most common possibly treatment-related TEAEs were diarrhoea [4.7% (40/858)] and nausea [3.3% (28/858)].

Conclusion: A 7-day regimen of oral telithromycin 800 mg once daily is an effective and well-tolerated first-line treatment option for CAP, including infections caused by resistant pneumococci.

**P453** Epidemiology of hospitalised patients with AECB (acute exacerbation of chronic bronchitis) and Gram-negative bacilli in the sputum


Objectives: To describe the epidemiological, clinical and microbiological characteristics of patients admitted to hospital with an acute exacerbation of chronic obstructive pulmonary disease (COPD) and Gram-negative bacilli in the sputum.

Methods: During a 6-month period, we studied prospectively all patients admitted to hospital with an acute exacerbation of COPD and Gram-negative bacilli in the sputum. Diagnosis of COPD and acute exacerbation were defined following GOLD and Anthonisen criteria, respectively. Age, gender, smoking history, pulmonary function (FEV1), presence of bronchiectasis, previous use of antibiotics, previous hospital admission, Emergency Room visits and microbiological data at entry were recorded.

Results: From July to November 2003, 111 patients were admitted to hospital for AECB. Thirty patients (27%) had Enterobacteriaceae or *P. aeruginosa* in the sputum. There were 28 men, mean age 75.2 years (10), 22 were ex-smokers and four were current smokers and 46% of the patients had been exposed to antimicrobials in the previous 3 months. Nineteen had been admitted to hospital and another 10 had been in the Emergency Room in the past year, respectively. Of the 22 patients who received antimicrobial therapy on admission, treatment was modified in six (20.7%) according to the sputum microbiological findings. The mean length of stay of 12.3 days (6.95). Mean FEV1 during stable phase of the illness was 805 mL (47.6) (40.89% of predicted). *P. aeruginosa* was isolated in 15 of the 30 patients (50%), multiple Enterobacteriaceae in 12 patients, *S. marcescens* in one patient and *K. pneumoniae* in two patients. Bronchiectasis were present (thoracic scanner) in 75% of patients.

Conclusions: The prevalence of bronchial colonisation/infection with Gram-negative bacilli in COPD patients with AECB and mean FEV1 values of approximately 40%, are higher than those reported previously in retrospective studies. More than 2/3 of the patients had been admitted to hospital during the past year and half had been treated with antimicrobials in the recent past. A striking finding was the prevalence of underlying bronchiectasis, present in 75% of this population.

**P454** Procalcitonin and neopterin correlates with aetiology and severity in adults with pneumonia


Clinical outcome of pneumonia depends of a multifaceted treatment approach. Not only diagnostic methods but also early indicators of the degree of inflammatory response can aid in therapeutic decisions. The aim of this study was to evaluate the
ultrafiltration shows a concordance of 100%. (2) The centrifugal concentration is an easy and rapid system (15–30 min). Therefore, in less than 1 h we could obtain the result of Legionella antigen detection. (3) The concentration of urine samples by centrifugation did not represent a decrease in the sensitivity and specificity of the antigen detection.

**P455** Rapid centrifugal method for Legionella antigen concentration in urine samples

J. Domínguez, S. Blanco, C. Prat, R. Riveló, M.D. Sánchez, M.A. Pallarés, V. Ausina

Badalona, E

The aim of the present study was to evaluate the utility of the antigen concentration method by centrifugal ultrafiltration (Amicon Ultra-4, Millipore Corporation, Bedford, MA, USA) for detecting Legionella pneumohila antigen in urine samples by immunochromatographic (ICT) method, comparing it to the passive selective ultrafiltration (Urfil-10. Millipore Corporation).

**Materials and methods:** Group 1: we studied 35 urine samples from patients with pneumonia caused by *L. pneumophila*. Legionnaires’ disease was diagnosed by detection of soluble antigen from *Legionella pneumophila* serogroup 1 in urine samples by Binax EIA. The second group consisted of 35 urine samples from patients with pneumonia of other aetiologies (15 *Streptococcus pneumoniae*, 15 *Mycoplasma pneumoniae*, and five *Chlamydia pneumoniae*). The third group consisted of 15 urine samples from patients with no clinical or radiological signs of pneumonia, the patients had urinary tract infections (10 *Escherichia coli*, one *Pseudomonas aeruginosa*, two *Proteus mirabilis*, one *Klebsiella pneumoniae*).

**Results:** The results of the ICT test using processed urine samples concentrated by passive and centrifugal ultrafiltration were identical in the 35 samples from group 1 patients (100% agreement). Urine samples, concentrated by both methods, from patients with pneumonia of other aetiologies or no clinical symptoms or signs of pneumonia but with urinary tract infection (groups 2 and 3) were all negative by ICT. The overall agreement between both the concentration methods, considering the three patient groups, was 80.8% (P = 0.010).

**Conclusion:** Both the ICT test and ICT test using processed urine samples by passive and centrifugal ultrafiltration can be considered a method for the diagnosis of *L. pneumophila* in urine samples with a 100% agreement.
efficacy and safety in AECB-patients, documented by signs, symptoms and sputum colour.

Methods: In a post authorisational survey during the season 2002/2003 a total of 1,297 outpatients (median age 59.8 years) were treated with Levofloxacin 500 mg tablets o.d. for 7 days. 1,228 patients suffered from AECB. Concomitant diseases were documented in 49% and concomitant medication for 69% of the patients. Two AECB episodes (median) occurred during the previous 12 months with a median time interval of 15 weeks between the previous and the actual episode. The change of sputum colour characterised by a pretrial defined colour table based on the Stockley criteria was documented by the patients on a daily basis during the treatment.

Results: A purulent sputum was found in 95% of the 1,228 patients before treatment, but at the end of treatment in only 15% (P < 0.001). For 1,034 patients sputum colour was rated. At day 5 a change from green/dark yellow to clear/white was observed in 45% of the patients and in 76% at day 7, end of treatment. Body temperature decreased in 94%, improvement was observed for cough in 93%, for breathing difficulties in 83%, for auscultation findings in 88%, and for general physical condition in 86% of the patients (each P < 0.001). At the end of observation the clinical outcome was rated as successful in 98% of the patients (60% cured, 38% improved). A complete release of symptoms was reached after 6.6 days (mean) and normal everyday activities were resumed after 6.9 days (mean). Adverse drug reactions were reported in only two patients (0.15%).

Conclusions: The clinical outcome of a 7-day treatment with Levofloxacin, assessed by signs, symptoms and the change of sputum colour as an additional marker, demonstrates a high efficacy in the present population of elderly AECB-patients with various concomitant diseases, accompanied by an excellent safety profile, resulting in a subsequent resumption of normal activities at the end of treatment.

### P459 Comparative assessment of moxifloxacin and macrolides in acute exacerbation of chronic bronchitis: clinical efficacy and influence on the long-term prognosis

S. Yakovlev, L. Dvoretsky, V. Nonikov, A. Strekachev, N. Dubrovskaya
Moscow, RUS

Objectives: Comparative clinical studies usually show similar clinical effectiveness of different antibiotics in acute exacerbation of chronic bronchitis (AECB). In connection with this it seems useful to estimate the influence of antibiotics on the long-term prognosis for patients with AECB, i.e. the length of symptom-free period after disease exacerbation.

Methods: It was the open randomised comparative study. Patients with AECB who had at least two exacerbations per year were included into the study. One group of patients with AECB were treated by moxifloxacin (MXF) 400 mg per day during 5 days and the other group by macrolide antibiotic (azithromycin, clarithromycin, or spiramycin) during 7 days. After treatment the patients were followed up for 12 months.

Results: A total of 60 patients with AECB were included into the study, 29 of which were treated by MXF, and 31 by macrolide antibiotic. The average age of the patients was correspondingly 58.9 and 57.3 years, length of chronic bronchitis – 10.8 and 11.8 years. Number of AECB in previous year was similar in both groups. Clinical cure rate estimated a week after the end of treatment was 96.6% for MXF and 93.5% for macrolides. The minimal period to the next exacerbation of chronic bronchitis was 107 days in the group of MXF and 14 days in the group of macrolides. During the follow-up period (6 and 12 months) the incidence of AECB (requiring prescription of antibiotics) was 3.6 and 15.4% in the group of patients who were prescribed MXF, and 17.2 and 44.0% in the group of patients who were treated by macrolide antibiotics. Average length of symptom-free period after MXF treatment was 49 days longer than in the macrolide group.

Conclusion: MXF was superior to macrolides in long-term prognosis in AECB and prolongs the period till the following exacerbation.

### P458 Outcome of clinical differences between pneumococcal pneumonia with and without bacteremia

F. Jover, J.M. Cuadrado, L. Andreu, M. Rodríguez, R. Cañízarre, S. Martínez, V. Ortiz, P. Roig
Alicante, E

Background: Few attempts have been made to compare bacteraemic and non-bacteraemic pneumococcal pneumonia, mainly because the difficulties to gain agreement on which cases represent non-bacteraemic pneumococcal pneumonia. Recently, immunochromatographic assay for detection of S. pneumoniae urinary antigen has been successfully evaluated for the diagnosis of pneumococcal pneumonia.

<table>
<thead>
<tr>
<th>Bacteremic</th>
<th>Nonbacteremic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age</td>
<td>39.8</td>
<td>70.8</td>
</tr>
<tr>
<td>COPD</td>
<td>18%</td>
<td>40%</td>
</tr>
<tr>
<td>Liver Disease</td>
<td>16%</td>
<td>0%</td>
</tr>
<tr>
<td>CHF</td>
<td>7%</td>
<td>32%</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>40%</td>
<td>16%</td>
</tr>
<tr>
<td>Former Smoker</td>
<td>17%</td>
<td>48%</td>
</tr>
<tr>
<td>Alcohol Abuse</td>
<td>30%</td>
<td>8%</td>
</tr>
<tr>
<td>Drug Abuse</td>
<td>19%</td>
<td>0%</td>
</tr>
<tr>
<td>HIV Infection</td>
<td>23%</td>
<td>0%</td>
</tr>
<tr>
<td>Days of Intra-Antibiotic Treatment</td>
<td>6</td>
<td>4.5</td>
</tr>
<tr>
<td>Temperature ≥ 38.5° C</td>
<td>41%</td>
<td>20%</td>
</tr>
<tr>
<td>Length of Hospitalisation</td>
<td>7.9</td>
<td>6.8</td>
</tr>
</tbody>
</table>

COPD: Chronic obstructive pulmonary disease. CHF: congestive heart failure. HIV: human immunodeficiency virus
Bacteraemia and endocarditis

P460  Human leptospirosis in a regional lepto spiria laboratory, Piemonte (Italy)
L. Franzin, D. Cabodi, N. Bonfrate
Turin, I.

Objectives: Human leptospirosis is underdiagnosed in Italy, and often unrecognised because of the difficulty of clinical and laboratory diagnosis. The aim of the study is to report clinical and epidemiological features of human leptospirosis in a North-West area of Italy with some professional risk factors (agriculture, rice cultivation, animals farm, abattoirs).

Methods: Samples from 292 patients with suspected Leptospira infections were examined. The serodiagnosis was performed by microscopic agglutination test, using cultures of 20 reference Leptospira interrogans strains (kindly provided by National Centre for Leptospirosis, Rome, Italy). Cultures were performed on EMJH medium (with and without 5-fluorouracil) and incubated at 30°C for 2 months.

Results: Diagnosis of leptospirosis was confirmed in 42 (14.4%) patients. The clinical symptoms were: fever (100%), jaundice (76.2%), meningitis (9.5%), renal failure (73.8%), enlarged liver (73.8%) and conjunctival suffusion (14.3%). Cultures were positives in two patients, who later died. Leptospira strains were isolated from blood of a 88-year-old patient and from both blood and urine of a 35-year-old patient. The strains were identified as L. interrogans serogroup icterohaemorrhagiae, serovar icterohaemorrhagiae by National Centre for Leptospirosis.

The largest number of infections was ascribed to occupational activities (45%). Water contaminated with animal urine emerged as probable source of infection (77%). The typical leptospiral seasonal trend, with a peak during the summer months, was observed.

Conclusion: The results confirmed leptospirosis as an important cause of infection in our region. However, strict laboratory diagnosis protocol must be followed in order to recognise this rare but very severe re-emerging infection. Clinicians should be aware of leptospirosis, especially in area with high risk factors.

P461  Streptococcus pneumoniae bloodstream infections (SPBI): a clinical study of 460 episodes in the era of penicillin resistance
Madrid, E.

Objectives: SPBI with decreased susceptibility to penicillin are increasing in prevalence in most European countries. The aim of this study was to evaluate the clinical spectrum of disease in adults and the influence of penicillin susceptibility on the outcome of SPBI.

Methods: Retrospective study of adult cases (>18 years) of SPBI diagnosed at our institution from 1990 to 2003. The impact of penicillin susceptibility and initial empirical treatment on the 30-day mortality were assessed by univariate analyses.

Results: A total of 460 episodes of SPBI were evaluated. There were 290 males (63%) and the mean age was 57.6 years (18–94). Most patients had chronic predisposing conditions and 28% of them had HIV infection. Decreased susceptibility to penicillin (MIC > 0.06 mg/L), cefotaxime (MIC > 0.5 mg/L), and erythromycin (MIC > 1 mg/L) was observed in 36% (154/433), 9% (17/196), and 21% (83/400) of strains. The most common form of SPBI was pneumonia (81%), followed by bacteraemia without focus (7%), meningitis (6%), peritonitis (1%), and others (5%); 46 episodes (10%) were nosocomially-acquired. Patients were empirically treated with a beta-lactam (50%), a beta-lactam plus macrolide (20%), or other regimens of monotherapy (9%) or combination therapy (21%). Overall mortality rate was 22% and was similar among patients with pneumonia (21%), bacteraemia (27%), meningitis (23%), or peritonitis (20%). Mortality rate was higher (P < 0.05) in patients with penicillin-resistant strains (27%) than in cases with susceptible strains (18%), and in nosocomial infections (54%) than in community-acquired infections (18%). The mortality rate was similar in patients receiving monotherapy or combination therapy (21% vs. 23%).

Conclusion: Community-acquired pneumonia remain as the most common cause of SPBI, but an increasing proportion of the cases (10%) are hospital-acquired infections. Decreased susceptibility to penicillin and hospital-acquired infections are associated with a worse prognosis in most clinical forms of SPBI.

P462  Streptococcus bovis bacteraemia: clinical characteristics of 41 episodes
S. Higuet, B. Byl, O. Denis, M. Struelsen, F. Jacobs
Brussels, B.

Objectives: To assess the clinical and the therapeutic aspects and the outcome of S. bovis bacteraemia occurring in our 858-bed academic hospital during the last 11 years.

Methods: We reviewed the clinical files of all pts with S. bovis bacteraemia (‘1 blood culture) from January 1990 to December 2001.

Results: S. bovis bacteraemia (mean positive blood cultures 2.7 per pt) was detected in 41 pts (26 male, 15 female, mean age 64.5 years, range 27–90). All except four episodes were community-acquired infections. Thirty-one of the 41 patients (75%) had at least one underlying disease: neoplasia (12), cirrhosis (12), diabetes (7), haemodialysis (two) and corticosteroids (eight). Eleven had valvular disease (nine with prosthesis). A primary focus of infection was found in only eight pts (19%) (six angiocholitis and two gynaecological infections). Transthoracic and/or transoesophageal echography, performed in 30 of the 36 patients who survived more than 7 days, confirmed endocarditis (BE) in 22 (73%). All nine pts with valvular prosthesis had BE (>12 months after surgery). Metastatic foci of infection were found in 10 of the 22 pts with BE (spondylodiscitis (4), cerebral (4), splenic (3) and renal (3) emboli). Valvular replacement was performed in two pts. In 11 pts (27%), no primary focus of infection or BE was found (primary bacteraemia). Colonoscopy (performed in 25 of the 36 pts who survived more than 7 days) showed one or more lesions in 23 of them (92%): polypt (16), carcinoma (3), diverticulitis (5) and ulcer-jaehemorrhagic colitis (1). Thirteen pts died during hospital stay (31%), nine because of S. bovis infection. Pts with BE more frequently suffered from neoplasia or cardiac diseases, digestive symptoms, secondary focus of infection and lesions of the colon and presented with longer duration of symptoms before diagnosis (P < 0.05) as compared with patients without BE.

Conclusion: Bacteraemia caused by S. bovis was mainly associated with endocarditis or primary bacteraemia. Colonoscopy should be performed in all pts considering the high rate of colic lesions. Morbidity and mortality associated with S. bovis was high in this group of elderly pts with underlying diseases.

P463  Infective endocarditis caused by Leptospira grippotyphosa
J. Benes, O. Dzupova, M. Kabelkova, R. Ondrejck, R. Feuereisl, B. Horova
Prague, CZ

Case: A 42-year-old man with no significant previous medical history was hospitalised for fever lasting 9 days, chills, headache, arthralgia, vomiting, diarrhea, and dehydration. On admission, a marked cardiac murmur and hepatomegaly were the only pathological findings. Few days later the patient developed conjunctival
Objectives: To determine the clinical characteristics, site of involvement, microbiological findings, and outcome of infective endocarditis (IE), and to identify factors associated with an increased risk of mortality.

Methods: All cases of IE observed between January, 1980, and December, 2001, were reviewed, and cases which satisfied the Duke definition for definite endocarditis were evaluated. Data were collected with regard to demographic, clinical and laboratory characteristics, blood culture results, HIV serostatus, and outcome. HIV test was not available for patients admitted before 1985.

Results: There were 262 episodes in 246 patients (165 males). The age of patients was 37.9 ± 14.6 years (range 16–83). 145 (58.9%) patients were intravenous drug users (IDUs), 51 had a history of valvular heart disease, and 22 had a prosthetic heart valve. 80 patients were HIV-infected, and 76 of them were IDUs. The most common organism was S. aureus, isolated in 43.1% of cases, followed by streptococci in 23.3%. In 14.1% of cases blood cultures were negative. The left side of the heart alone was involved in 52.7% of cases, the right side of the heart alone in 38.9%, and both sides in 4.2%. Tricuspid and valve involvement was present in 37.4% of cases, compared with mitral and aortic valve involvement in 27.5% and 20.6%, respectively. More than one valve was involved in 8.8% of cases. Staphylococci more frequently affected IDUs (77.6%), patients younger than 30 years (44.6%), and the right side of the heart (53%). In contrast, streptococci more frequently affected patients with valvular heart diseases (39.3%), older than 50 (29.5%), and the left side of the heart (80.3%) (P < 0.001). Overall mortality rate was 16.2%. In multivariate analysis negative blood cultures, left sided and multivalvular IE were associated with an increased risk of death. The mortality rate among HIV-positive patients was 25.3% compared with 11.9% in HIV-negative patients with an attributable mortality of 13.4%.

Conclusions: Clinical characteristics of IE in our institution probably reflect the predominance of IDUs. The causative organisms were significantly associated with the predisposing condition, age of patients, and site of heart involvement. Negative blood cultures, and left side or multivalvular heart involvement were strong risk factors for mortality. Work funded by Ricerca Corrente IRCCS.

P465: Clinical characteristics of infective endocarditis: descriptive review of 262 episodes and risk factors for death

S. Cicalini, C. Angeletti, E. Boumis, F. Palmieri, N. Petrosillo
Rome, I

Objectives: To determine the clinical characteristics, site of involvement, microbiological findings, and outcome of infective endocarditis (IE), and to identify factors associated with an increased risk of mortality.

Methods: Febrile patients with skin rash or eschar satisfying eligibility criteria were randomly assigned to a single 500-mg dose of azithromycin group, a single 1000-mg dose of azithromycin group, 17; doxycycline group, 17) were serologically assessed for eligibility. Seventy-three patients were randomly allocated to compare the efficacy. The mean time to defervescence was shortest for single 1000-mg dose of azithromycin (23.3 h), shorter for single 500-mg dose of azithromycin (26.2 h) and longest for doxycycline (29.4 h), but statistically not significant (P > 0.05). There were no treatment failure or relapses after completion of therapy.

Conclusion: A single 500-mg dose of azithromycin is as effective as a single 1000-mg dose of azithromycin or conventional doxycycline therapy for the treatment of mild scrub typhus in South Korea.
persons without any other recorded comorbidity (adjusted OR = 4.9, 95% CI: 3.7–6.6). The case-fatality in diabetic patients compared with non-diabetic patients was 17.3% vs. 13.4% after 30 days, and 23.6% vs. 19.5% after 90 days. After adjustment for gender, age, comorbidity, and focus of infection, the 30-day mortality rate ratio for diabetic patients was 1.4 (95% CI: 1.0–2.0) compared with the non-diabetic patients.

Conclusions: Diabetes seems to be a strong risk factor for community-acquired bacteraemia caused by *E. coli* and other Enterobacteriaceae. Furthermore, patients with diabetes appear to have a higher case-fatality in this severe infection than non-diabetic patients.

**P467 Predictors of bacteraemia in hospitalised patients with infectious cellulitis**

J. Carratalà, N. Fernández-Sabé, B. Rosón, E. Marcos, L. Calatayud, V. Isern, J. Ribes, F. Gudiol
*L'Hospitalet de Llobregat, Barcelona, E*

Objectives: Among patients with cellulitis, the development of bacteraemia is associated with a high risk of adverse outcomes. We sought to identify predictors of bacteraemia at the time of presentation to the hospital emergency department in patients with cellulitis.

Methods: Review of medical charts of all adult patients hospitalised for community-acquired bacteraemia (Jan 95–Dec 02) who had blood cultures performed on admission. Cellulitis complicating diabetic foot ulcers, orbital cellulitis, HIV-infected patients, and drug addicts were excluded. For the purpose of the study, patients with bacteraemia were compared with patients without bacteraemia.

Results: There were 329 cases of cellulitis (184 female); mean age of 61.5 years. The infection was microbiologically documented in 115 cases (53 bacteraemia, 65 needle aspiration, and 20 surgical aspiration). The organisms isolated most frequently were *Staphylococcus aureus* 44, *Streptococcus pyogenes* 25, groups B, C, and G streptococci 19, *Pseudomonas aeruginosa* 115 cases (53 bacteraemia, 65 needle aspiration, and 20 surgical aspiration). The infection was microbiologically documented in 61.5 years. The infection was microbiologically documented in 37 subjects. It comprised a total of 37 patients with brucellosis. Control subjects were 30 healthy individuals with no history of Brucella infection. Brucella were identified according to the positive blood culture and raised Brucella antibodies in serological tests in addition to compatible clinical symptoms. Cytokine profile analysis was performed according to the Immulite chemiluminescent enzyme immunometric assay. Nitrites/nitrates are representatives for NO. Their serum level was measured by Griess method.

**P470 Brucellar spondylitis (clinical manifestations and outcome of treatment in 32 cases)**

M.R. Hasanjani Roushan, S.M. Smalinejad Ganji, A. Ahmadi Ahangar
Babol, IR

Objectives: Brucellar spondylitis is common in endemic regions of brucellosis. The purpose of this study was to assess the clinical manifestations and outcome of treatment in brucellar spondylitis.

Methods: From September 1998 to June 2003, 32 cases of brucellar spondylitis who attended the Department of Infectious Diseases,
Babol Medical University were studied. All cases were treated with cotrimoxasol plus rifampin or doxycycline plus rifampin for 4 months and all cases were followed for 1 year after completion of therapy. Clinical manifestations and outcome of treatment were recorded.

**Results:** Thirty-two cases (24 male, eight female, mean age, 46 ± 17 years, ranged 18–77 years) were evaluated. Disease was acute and subacute in 28 (87.5%) cases. Severe back pain, sweating and fever were the most clinical symptoms and were seen in 100, 62.5 and 47% cases respectively. Involvement of lumbar, dorsal and cervical regions were seen in 26, 2 and 4 cases, respectively. Seventeen and 15 cases were treated with cotrimoxasol plus rifampin or doxycycline plus rifampin for 4 months, respectively. Only one case in doxycycline plus rifampin treated group had relapse (P > 0.05).

**Conclusion:** Severe back pain, sweating and fever were the most clinical symptoms. Four months of therapy is sufficient in the treatment of brucellar spondylitis.

**P471 Successful treatment of Brucella melitensis endocarditis with antibiotic combination**

O. Ural, N. Dikici, D. Findik
Konya, TR

In July 2003 a 22-year-old woman presented at the hospital with fever, shivering and weight loss. The agglutination test for *Brucella* was positive at a titer of 1/320. A transoesophageal examination showed vegetations and *Brucella* endocarditis was suspected. A blood culture growth of *Brucella melitensis* biotype 1 confirmed our diagnosis. The treatment was started with 600 mg/day rifampin, 2 × 100 mg/day doxycycline and TMP-SMX 160/800 mg twice daily. Two months later TMP-SMX therapy ceased, rifampin and doxycycline therapy continued a further 6 months. At the clinical follow up, there were no signs of heart failure or periphere embolism. On the seventh day of therapy the fever decreased. After treatment a repeat transoesophageal echocardiography showed that the vegetations at the aorta valve disappeared. At 1-year follow up, the patient was healthy. If there is no history of congestive cardiac failure and prosthetic valve involved and only moderate extravascular cardiac involvement medical treatment may be a valid alternative to surgical therapy as advised in some literature where the patient’s illness is not prolonged.

**P472 Clinical features and prognosis of B melitensis vertebral osteomyelitis. A descriptive study of 148 cases**


**Objectives:** To describe current clinical, radiological, diagnostic and therapeutic characteristics and prognosis of Brucelar Vertebral Osteomyelitis (BVO).

**Methods:** We carried out a prospective, descriptive study of 446 patients with Vertebral Osteomyelitis (VO) from January 1983 to October 2005 in two tertiary hospitals. Diagnosis of VO was established by the presence of spinal pain unrelieved by rest or fever and spinal pain on physical examination, together with imaging compatible with VO. Diagnosis of BVO was established according to one of the following criteria: (1) *Brucella* spp isolation in vertebral, paravertebral, or epidural tissue or a psoas sample. (2) *Brucella* spp isolation in blood cultures, high titers of *Brucella* antibodies or seroconversion in the presence of a compatible clinical and radiological picture of VO. All patients were treated with doxycycline 100 mg b.i.d. for 3 months plus streptomycin 1 g i.m. q.d. for 3 weeks or doxycycline 100 mg plus rifampin 900 mg q.d. both for 3 months.

**Results:** One hundred and forty-eight patients of the total sample (32.2%) had BVO. 109 (73.6%) were male and 39 (26.4%) female. The mean age was 51.4 ± 13.6 years. Cervical level was involved in 14 cases (9.5%), dorsal or dorso-lumbar in 32 (21.6%), lumbar or lumbo-sacral in 98 (66.2%) and multiple levels were affected in four cases (2.7%). The mean duration of symptoms prior to diagnosis was 3.2 months. Eighty per cent of patients had fever, 91.9% inflammatory spinal pain and 41.3% had some neurological deficit. Blood cultures were positive in 42% of cases and B melitensis was isolated in all of them. The mean number of affected vertebral bodies was 2.1 (range 1–5). Paravertebral masses were detected in 62 cases (42.9%), epidural masses in 44 (29.7%) and psoas abscesses in 14 (9.5%). Fifty patients (33.8%) required surgical treatment. The rate of therapeutic failure, relapse and attributable mortality were 14.9, 2 and 1.2% respectively. The mean hospital stay was 36.6 days and 29 patients (19.6%) had severe functional sequelae. A high diagnostic delay, cervico-dorsal level and more than two vertebral bodies affected were associated with a worst prognosis.

**Conclusions:** BVO represent a high percentage of VO in endemic areas. In this type of VO, non-invasive diagnostic yield is very high. Although BVO mortality is very low, BVO frequently requires surgical treatment, long hospital stay and results in severe functional sequelae.

**P473 Psychotic disorder related with Brucella infection: three case reports**

A. Celikbas, G. Bayam, S. Eren, O. Pazardjoglu, N. Baykam, B. Dokuzoguz
Ankara, TR

**Objective:** Three patients who were brought to hospital because of psychotic symptoms and diagnosed as brucella infection and treated with antibiotics were presented.

**Cases:** Two of the cases were hospitalised in psychiatry clinic and one of them was hospitalised in infectious disease clinic. The average age of three cases is 33. All of the patients had a history of cheese eating. One case had a history of treatment and as acute psychotic attack 4 years ago. All of them had shared symptoms of fever, headache, sweating, weight loss, back pain, vertigo, irritability and restlessness. In psychiatric examination patients were evaluated as irritable, sleep disturbance, impaired orientation, attention and memory, difficulty in cooperation, acceleration and deceleration in thought flow and auditory and visual hallucinations. In one case there were symptoms of neuralgic disorders like nystagmus, ataxia and strabismus. All cases have positive *Brucella* agglutination test in 1/640 titer. Two cases had positive BOS culture for *Brucella* and all of them positive blood culture for *Brucella*. After the antibiotic treatment patients’ clinic and laboratory states came to normal. Psychiatric symptoms disappeared.

**Conclusion:** Acute psychotic presentation and increasing psychotic symptoms can be seen in neurobrucellosis. In all of the cases who have psychotic symptoms and also fever and infection; neurobrucellosis should be considered in differential diagnosis.

**P474 Pulmonary involvement in brucellosis**

C.A. Hatipoglu, G. Bilgin, N. Tüleker, U. Kosar
Ankara, TR

**Objectives:** Brucellosis is a multisystem infection that may present with a broad spectrum of clinical presentations. Pulmonary involvement is extremely rare in the course of brucellosis with an estimated rate of <1–5% of cases. A variety of pulmonary manifestations have been documented in the literature, including bronchitis, bronchopneumonia, lung abscess, empyema, pleural effusion, granulomas, solitary nodules, hilar and paratracheal lymphadenopathy. The aim of this study was to determine the incidence and forms of pulmonary involvement in the course of brucellosis.

**Methods:** A prospective study was carried out in 110 patients with brucellosis who were admitted to the Infectious Diseases and
Clinical Microbiology Department, Ankara Training and Research Hospital, between October 2001–December 2003. All the patients were questioned about their pulmonary symptoms including cough, expectoration, chest pain, dyspnoea and haemoptysis. All the patients underwent a thorough physical examination, chest radiography and when pulmonary pathological findings were present, underwent additional diagnostic evaluations including computerised tomography of the thorax and pulmonary function tests.

Results: Of these 110 patients, 11 (10%) patients (six female, five male) presented with pulmonary involvement. Eight (72%) patients had pulmonary symptoms including dyspnoea, dry cough and productive cough with expectoration. Six patients had chest radiography findings but two had not. Three patients had no pulmonary symptoms but had findings in chest radiography. Chest radiography findings were compatible with computerised tomography findings of the thorax. Radiological findings were as follows: Parenchymal nodules in eight patients, lobar pneumonia in one, paratracheal lymphadenopathy in one, parenchymal nodules, lobar pneumonia and minimal pleural effusion in one patient. Of 11 patients with pulmonary involvement, four (36%) patients had coexisting chronic obstructive pulmonary disease. All the patients were treated with a combination of rifampin and doxycycline or streptomycin and doxycycline. Clinical and radiological findings of pulmonary involvement were recovered in all patients except four patients who had coexisting chronic obstructive pulmonary disease.

Conclusion: Pulmonary involvement is a rare event in the course of brucellosis but the rate could be higher than estimated. In endemic regions, brucellosis should be considered as a causative agent in patients with pulmonary symptoms.

P475 Same involvement of brucellosis in two brothers: epididymoorchitis

G. Tuncer Ertem, C. Ataman Hatipoglu, N. Tüleksen
Ankara, TR

Objectives: Brucellosis is a very polymorphic disease and could affect any organ. Epididymoorchitis occurs in up to 2–20% of patients with brucellosis. In this report, two brothers with epididymoorchitis were presented.

Case 1: A 43-year-old male sheep breeder was admitted with a 6-week history of fever, sweating, headache, pain in his shoulders and neck. Six weeks ago he had applied to an urologist with a history of painful swelling and redness of the left testis and fever. In ultrasonographic examination increasing of testicular diameter, thickening of scrotal wall and testicular hypoechogenity had been detected. After 10 days of antibiotic therapy, his testicular symptoms had improved. On physical examination restricted mobility of the neck and hepatomegaly were evident but testis was normal. In physical examination restricted mobility of the neck and hepatomegaly were evident but testis was normal. The complete blood cell count and biochemical findings were normal. Standard tube agglutination testing (SAT) was positive at titer of 1/640. Brucella melitensis was isolated in both of blood and bone marrow culture. Magnetic resonance imaging revealed cervical spondylitis. Cerebrospinal fluid examination revealed findings of meningitis and SAT was positive at titer of 1/16. Osteoarticular and neurological involvements of brucellosis were diagnosed and rifampicin plus doxycycline (24 weeks) plus seftiraxone (4 weeks) treatment was administered. The clinical and laboratory findings improved with this treatment.

Case 2: A 45-year-old male sheep breeder was admitted with a 3-month history of pain in his right shoulder and wrist. Before these symptoms, painful swelling and redness of the right testis and fever had developed and he had applied to an urologist. His symptoms had improved with antibiotic therapy. On physical examination there was only hepatomegaly. WBC was 10,500/mm³, other blood cell count and biochemical findings were normal. Serum SAT was positive at titer of 1/640. Blood and bone marrow culture were negative. Rifampicin plus doxycycline (6 weeks) treatment was administered. His clinical findings improved with this treatment.

Conclusions: In both brothers, presenting symptoms of the brucellosis were epididymoorchitis. So that brucellosis must be considered in the differential diagnosis in endemic areas. Although orchitis is a rare complication of brucellosis, it was observed as same involvement in both brothers. These cases suggest the possibility of genetic basis for the occurrence of brucellosis and/or epididymoorchitis.

P476 Human brucellosis: a retrospective evaluation of 75 cases

N. Elaldi, I. Dokmetas, M. Bakir, M.Z. Bakici, M. Sencan
Sivas, TR

Objectives: Brucellosis is an important zoonosis worldwide, mainly in the Mediterranean countries, including Turkey. The annual national incidence of the disease in Turkey is 0.59 per 100,000 persons. The aim of this study was to evaluate the epidemiological, clinical and laboratory findings, therapeutic features and outcomes of patients with brucellosis retrospectively.

Methods: This study was carried out at Cumhuriyet University Hospital, Department of Clinical Bacteriology and Infectious Diseases, Sivas, Turkey, between January 1998 and November 2003. Seventy-five brucellosis patients were included in the study.

Results: Thirty-nine (52%) were female patients and 36 (48%) were male. The mean age was 41 ± 1.8 (range 16–70 years). Clinical form of the disease was acute in 51 cases, 18 sub-acute, five chronic, and one asymptomatic. The most possible source of brucellosis was the consuming of unpasteurised dairy products, especially raw milk and fresh cheese (68%). Malaise (53%), fever (53%), back pain (45%) and anorexia (43%) were the most common presenting symptoms, and fever and hepatomegaly were the most common initial clinical findings among the patients. Elevated serum C-reactive protein levels was determined in 27 of 33 patients and elevated erythrocyte sedimentation rate in 56 of 75 patients. Cultures (blood 36, cerebrospinal fluid 1, joint fluid 1) were positive in 38 (51%) patients and all strains identified as Brucella melitensis. Various treatment regimens were used, mainly doxycycline plus rifampicin. There was no therapeutic failure. Relapse occurred in five of the 75 patients (6.7%).

Conclusion: Brucellosis is still endemic zoonosis in the Central Anatolian region of Turkey. So, we think that the effective heating of dairy products and other potentially contaminated foods is main prevention method of the disease and others.
Diagnostisc methods - 1

P477 Comparison of proficiency testing among governmental and private sector hospital microbiology laboratories in Tehran
M. Rahbar
Tehran, IR

Objective: The aim of this study was to evaluate proficiency testing of microbiology laboratories in governmental and private sector hospitals.

Methods: Two species of bacteria were sent to microbiology laboratories (71 governmental and 35 private sector) included Staphylococcus epidermidis (ATCC 12228) and Burkholderia cepacia (ATCC 2541). We asked all laboratories for identification of each bacteria and susceptibility testing of S. epidermidis. Scoring of results performed according of WHO criteria (maximum score of point for identification 3 and for susceptibility testing 5).

Results: Of 111 microbiology laboratories, we received answer from 87 (75%) laboratories. The mean score of points for identification of S. epidermidis in governmental hospital microbiology laboratories was 2.8% (SD = 0.64). The mean score of point in private hospital microbiology laboratories was also 2.8 (SD = 0.73/6). Statistically there was not a significant difference between two groups of laboratories for identification of S. epidermidis (P-value = 0.712). Of 55 governmental hospital microbiology laboratories, 22 laboratories identified B. cepacia and obtained 3 score of points. Eleven laboratories obtained 0.05-2.5 score of points. The mean score was 1.67 (SD = 1.2). Of 30 private sector hospital microbiology laboratories five laboratories produced correct answer for identification of B. cepacia and obtained 3 score of points, 18 laboratories zero score of points and other laboratories obtained 0.5-2.5 score of points. The mean score was 0.81 (SD = 1.119; P-value = 0.001). The mean score of points for susceptibility testing of S. epidermidis in governmental-related hospitals microbiology laboratories was 4.8 (SD = 0.38) and in private sector was 4.66 (SD = 0.751).

Conclusion: It is concluded that private sector hospital microbiology laboratories in our country in comparison with governmental hospital microbiology laboratories have poor proficiency for identification of microorganisms such as B. cepacia. It may be due to lack of some culture media reagents and unskilled personnel in this sector.

P478 Implementation of particle analysers in the detection and description of biofilm formation
R. Kadlec, J. Plocková, F. Ruzicka, V. Holá
Brno, CZ

The growth in a biofilm form enables bacteria to survive in the environment and in human organism. Biofilm bacteria are highly resistant to host immunity and to antibiotic therapy. The ability of bacteria to form biofilm is considered to be an important factor of the pathogenicity and represents a serious medical problem. The proof of the ability and the evaluation of the dynamics of biofilm formation can be helpful for optimal therapy of the infection. The phenotypical methods of the biofilm detection are related with subjective evaluation of the obtained results. Therefore, we tried to find a new method for the detection and description of biofilm formation. The Staphylococcus epidermidis strains isolated from blood cultures of patients with bacteremia were used in this study. Isolates were grouped on the basis of the presence of intact ica-operon, determined by PCR reaction, into two groups, ica-operon positive and ica-operon negative. The phenotypical slime-positivity or negativity was performed by two methods by Christensen tube method in brain heart infusion and by the typical growth on agar with Congo red. Bacteria with uniform (monodisperse) microparticles were cultivated under constant stirring for different time periods. The growth of bacterial biofilm layer on the microparticles was monitored as an increase of microparticle size. Various microparticles of diameter ranging from 10 to 50 μm were used. The size of biofilm-covered microparticles was measured using different independent measuring principles. Time-of-transition (TOT), low angle laser light scattering (LALLS), gravitational field flow fractionation (GFFF) and dynamic shape analysis. The total biofilm increase given by the number of particles can reach several tens of cm². Therefore, the changes in size distribution resulting from the biofilm growth are statistically significant. This approach also allows determination of the influence of various cultivation parameters (e.g. pH and chemical composition of the medium, properties of the particles, physical conditions and bacteria species) on the growth and detachment of biofilms. The measurement of microparticle increase as a result of biofilm formation has been shown to be an effective tool for investigation of biofilms.

P479 Laboratory variables in selected neuroinflammatory diseases
J. Bednarova, P. Stourac, H. Stroblova, A. Sevcikova
Brno, CZ

Objectives: The intrathecal synthesis of specific IgG antibodies against the viruses of measles, rubella and varicella zoster, called MRZ reaction (M-measles, R-rubella, Z-varicella zoster) is typical for chronic inflammatory autoimmune diseases of the nervous system. MRZ reaction is present in 89% patients with multiple sclerosis (MS), while in patients with neuro borreliosis (NB) it occurs very seldom (<0.1%). The aim of our study was to detect MRZ reaction and intrathecal synthesis of specific antiborrelia IgG antibodies and to evaluate the relevance of specific antibody indices from first diagnostic lumbar puncture for the differential diagnosis between MS and NB.

Methods: We investigated a cohort of 50 patients: 21 patients were diagnosed as multiple sclerosis, 19 patients were diagnosed as neuroborreliosis and 10 patients with OND (other neurologic diseases) served as negative controls. Serum and CSF samples were analysed at each patient. The diagnostic kit of Human Company, Germany (measles, rubella, varicella-zoster virus human ELISA IgG antibody test) and the diagnostic kit of Test-Line Company, Clinical Diagnostics, Czech Republic (EA Borrelia garinii IgG) were used for the detection of specific IgG antibodies. The intrathecal synthesis was evaluated as specific antibody index (AI according to Reiber’s method). Values of AI > 1.4 indicated the intrathecal synthesis.

Results: Intrathecal synthesis of IgG antibodies against measles, rubella and/or varicella zoster viruses (AI > 1.4) was detected in 95% patients with MS. Antibody index against measles was positive in 85.7% patients, against rubella in 52.4% patients and against varicella zoster virus in 38% patients. All these patients had negative intrathecal antibody synthesis against Borrelia burgdorferi (Bb), i.e. negative Bb-IgG-AI. In patients with NB 89.5% had positive Bb-IgG-AI. One patient (5%) only had positive antibody against Bb. OND patients had negative MRZ reaction and Bb-IgG-AI. The statistical significance of MRZ reaction vs. Bb-IgG-AI positivity was confirmed by Spearman rank coefficient and Wilcoxon’s test.

Conclusion: Positive MRZ reaction is the most valuable diagnostic parameter in diagnostically equivocal cases of MS and NB with neurological first symptoms. Our data emphasise the importance of MRZ reaction and Bb-IgG-AI in differential diagnostics of MS.
and NB in early stages when other methods (MRI, oligoclonal IgG bands) do not provide definite diagnosis.

**P480** Bactericidal effect of endox against various pathogens

C. Cassanelli, S. Roveta, F. Cavallini, A. Marchese, E.A. Debbia, R. Armanino

**Genoa, Italy**

**Objectives**: Endox is an instrument used for the sterilisation of the root canal and/or periapical infections after the endodontic treatment. The instrument applies high frequency alternating current (HFAC) and, for each impulse, generates an electromagnetic field for a short period time (140 ms) in the site of infection. To better understand the mechanism by which sterilisation occurs, the instrument was used to test the bactericidal effect on different bacterial suspensions exposed to the above electromagnetic field.

**Methods**: Bacterial strains included Enterococcus faecalis, Staphylococcus aureus, Actinomyces spp., Escherichia coli, Pseudomonas aeruginosa and among fungi it was tested with Candida albicans. A sample of 20 mL of a saline bacterial suspension was exposed to the electromagnetic field for three times. Survivors were then counted by standard procedure.

**Results**: Bactericidal effect of 99.99% was found in E. faecalis, S. aureus, E. coli and P. aeruginosa, instead in Actinomyces spp. and C. albicans the reduction of bacterial concentration was lower (99.9%).

**Conclusions**: Sterilisation effect obtained with Endox was very remarkable (99.99%) on bacteria and, even in less extent, on fungi (99.9%). These different results may be explained by the different constitution of cell wall. The Actinomyces, in comparison with other bacteria has shown a less marked reduction of bacterial concentration, this fact can be attributed to its characteristic to produce ‘sulphur grains’, crystalline and proteinic formations that defend the membrane cell from the electromagnetic field. The extension of the area and the mode of action of the electromagnetic field are under investigation.

**P481** Evaluation of Helicobacter pylori stool antigen test for the detection of H. pylori infection and comparison with other methods

M. Ozdemir, R. Kesli, B. Baysal, M. Baykan, E. Kayacetin, M. Serdengecti

**Konya, TR**

**Objective**: Helicobacter pylori is the main cause of gastritis, peptic ulcer and gastric cancer in adults. The measurement of H. pylori antigens in human stools has been proposed as a valuable, noninvasive diagnostic tool. The aim of this study was to evaluate the usefulness of H. pylori stool antigen (HpSA) in diagnosis of H. pylori infection in adult patient.

**Methods**: Seventy-eight patients aged 21–68 years who were admitted to Department of Internal Medicine, Division of Gastroenterology with symptom of dyspepsia for whom the indication of upper gastrointestinal endoscopy was present were admitted to Department of Internal Medicine, Division of Gastroenterology with symptom of dyspepsia for whom the indication of upper gastrointestinal endoscopy was present were enrolled in Lyon, France. The ‘gold standard’ was a combination of results obtained by thin blood smear film microscopic examination and QBC. PCR and anti-malarial drug assays were performed only on discrepant samples.

**Results**: One hundred and nine (19.6%) patients had proven malarial attacks. Sensitivity was 96.3% for NowICT malaria P.f./P.v. and 79.8% for OptiMAL™ IT (P = 0.0001), and specificity was, respectively, 98.8 and 98.4%. Likelihood ratios for positive tests were, respectively, 86.1 and 50.9 for NowICT malaria P.f./P.v. and OptiMAL™ IT. Of 80 P. falciparum cases, NowICT malaria P.f./P.v. missed two infections. The test detected all 13 P. vivax infections. Five false positive results were observed in patients with a recent history of fever, self-treated for malaria. OptiMAL™ IT misdiagnosed 10 P. falciparum infections with parasitaemia up to 0.1%. Two P. vivax infections were not detected. We observed seven false positive cases, with no evidence of previous malaria attacks in six cases. During patient follow-up, NowICT malaria P.f./P.v. can persist positive for at least 7 days. OptiMAL™ IT turned negative within an average of 3 days being more likely to reflect parasite vitality.

**Conclusions**: Rapid diagnostic tests for malaria could be helpful as an adjunct test, but could not replace microscopic examination of blood films, which remains the gold standard, including more-quantitative techniques.

**P482** Comparison of the NowICT malaria P.f./P.v. and the OptiMAL™ IT rapid diagnostic tests for malaria in a nonendemic area

F. Peyron, F. De Monbrison, P. Gérôme, J.F. Chaulet, M. Wallon, S. Picot

**Lyon, France**

**Objectives**: Malaria patients require a rapid and accurate diagnosis. Microscopic examination of thin or thick blood smear remains widely used; however, such tests are time-consuming and require experience – especially in case of low parasitaemia often encountered in nonendemic areas. Since last decade, malaria rapid diagnosis tests based on the detection, in blood, of soluble parasitic antigens have been marketed. The aim of our study was to compare the performance of NowICT malaria P.f./P.v. (Binax, Portland, ME, USA) and OptiMAL™ IT (DiaMed, Cressier, Switzerland), two new versions of rapid diagnostic tests for malaria.

**Methods**: A total of 556 consecutive patients were prospectively enrolled in Lyon, France. The ‘gold standard’ was a combination of results obtained by thin blood smear film microscopic examination and QBC. PCR and anti-malarial drug assays were performed only on discrepant samples.

**Results**: One hundred and nine (19.6%) patients had proven malarial attacks. Sensitivity was 96.3% for NowICT malaria P.f./P.v. and 79.8% for OptiMAL™ IT (P = 0.0001), and specificity was, respectively, 98.8 and 98.4%. Likelihood ratios for positive tests were, respectively, 86.1 and 50.9 for NowICT malaria P.f./P.v. and OptiMAL™ IT. Of 80 P. falciparum cases, NowICT malaria P.f./P.v. missed two infections. The test detected all 13 P. vivax infections. Five false positive results were observed in patients with a recent history of fever, self-treated for malaria. OptiMAL™ IT misdiagnosed 10 P. falciparum infections with parasitaemia up to 0.1%. Two P. vivax infections were not detected. We observed seven false positive cases, with no evidence of previous malaria attacks in six cases. During patient follow-up, NowICT malaria P.f./P.v. can persist positive for at least 7 days. OptiMAL™ IT turned negative within an average of 3 days being more likely to reflect parasite vitality.

**Conclusions**: Rapid diagnostic tests for malaria could be helpful as an adjunct test, but could not replace microscopic examination of blood films, which remains the gold standard, including more-quantitative techniques.

**P483** Identification of yeasts and yeast-like microorganisms with a colorimetric card newly developed for the VITEK™ 2 system

D. Pincus, J. Mills, B. Knysak, A. Bassel, A. Fothergill, M. Rinaldi

**Hazelwood, San Antonio, USA**

**Objectives**: A new colorimetric card (YST; this new card is not yet available for commercial use) was developed for rapid automated identification of yeasts and yeast-like microorganisms using the VITEK™ 2 system. A database created from testing well-characterised strains was used to optimise the computer-assisted algorithm to achieve a high level of performance accuracy.

**Methods**: The YST card, containing 46 tests that measure enzymatic, acidification and alkalisation activities, was tested with 1304 isolates representing 13 genera comprised of 54 different species. Strains included 246 recent clinical isolates tested at the University of Texas at San Antonio Health Science Center with the remainder of isolates being stock cultures tested at bioMerieux. Cards were filled with organism suspensions made in 0.45% aqueous NaCl to a turbidity equivalent to a McFarland#2 standard. Inoculated cards were inserted into the VITEK™ 2 for approximately 18 h and a computer-assisted algorithm was optimised and used to generate test and identification results.

**Results**: Of the 1304 isolates tested, 1249 (95.8%) gave a correct identification with 138 (10.6%) low discrimination results [requiring supplemental testing and/or microscopic observation to...
discriminate between multiple (up to three) choices. Twenty-six (2.0%) of the isolates gave an incorrect identification and 29 (2.2%) were unidentified. Results of the subset of 246 recent clinical isolates showed 97.2% correct (including 8.9% low discrimination), 2.0% incorrect and 0.8% unidentified. This slightly higher performance was due to a higher frequency of isolates of the more common species.

Conclusion: The new YST colorimetric card used with the VITEK® 2 system provides an accurate, rapid, and automated method for the identification of yeasts and yeast-like microorganisms.

P485 New improved VITEK 2 card for identification of Gram-negative bacilli

B. Blanc, M. Babolat, L. Barbier, B. Celliere, L. Cordier, M.C. Deluermoz, M. Guicherd, S. Petre, S. Chatellier, D. Monget
La Balme les Grottes, F

Objectives: A new VITEK 2 Gram-negative card (GN; this new card is not yet available for commercial use) has been developed recently to increase identification accuracy of key clinical isolates and expand the number of nonfermentative bacteria claimed by the current ID-GNB card on the VITEK 2 system. The GN card contains 47 biochemical tests enabling the identification of 88 species or groups of fermentative bacteria and 47 species or groups of nonfermentative bacteria.

Methods: A total of 2806 well-characterised isolates from the bioMérieux stock collection were used to build the database. Cards were filled with organism suspensions made in 0.45 aqueous NaCl to a turbidity equivalent to a McFarland #0.5 standard. Inoculated cards were incubated in the VITEK 2 instrument and a computer-assisted algorithm was optimised and used to generate kinetic identification results.

Results: Of the 2806 isolates tested, 2737 (97.6%) gave a correct identification with 123 (4.4%) low discrimination results (requiring one additional testing). Fifty-seven (2.0%) of the isolates gave an incorrect identification and 12 (0.4%) were unidentified. All the results were obtained within 2–10 h. Overall correct identification is 98.4% for Enterobacteriaceae and other fermentative bacteria. For nonfermentative bacteria, 95.7% of the isolates gave correct identification.

Conclusions: The new GN card used with the VITEK 2 system provides an accurate and rapid method for the identification of a wide range of Gram-negative bacteria. This updated card provides several improvements compared with the current ID-GNB card that include:

• high level (93.2% vs. 77.5%) of one choice identification without any additional testing required and 97.6% vs. 96.9% overall correct when additional testing is included;
• more species claimed for nonfermentative bacteria 47 (vs. 24);
• elimination of mixed taxon ‘various nonfermenting Gram-negative bacilli’ comprising 26 taxa on the current card.

P486 Identification of Gram-positive bacteria with a new card developed for the VITEK 2 system

La Balme Les Grottes, F

Objectives: A new card (GP; this new card is not yet available for commercial use) was developed for rapid automated identification of Gram-positive bacteria using the VITEK 2 system. An expanded database was generated by testing well-characterised strains from clinical and industrial origin to achieve a high level of performance.

Methods: The GP card, containing 43 biochemical tests that measure enzymatic, acidification and alkalinisation activities, was tested with 1916 routinely and less frequently encountered bacterial isolates distributed into 122 species. Organisms were grown on three different isolation media and cards were filled with bacterial suspensions made in 0.45 aqueous NaCl to a turbidity equivalent to a McFarland #0.5 standard. Inoculated cards were incubated in the VITEK 2 instrument and the optimal algorithm was established to generate kinetic identification results.

Results: Of the 1916 isolates tested, 1882 (98.2%) gave a correct identification with 99 (5.2%) low discrimination results. Twenty-eight (1.5%) of the isolates gave an incorrect identification and six (0.3%) were unidentified. All the results were obtained within 2–5 h.

Conclusion: The new GP card used with the VITEK 2 system provides a rapid and accurate method for automated kinetic identification of a wide range of aerobic Gram-positive species. Compared with the existing ID-GPC card, the main improvements include:

• more species claimed: 122 vs. 57;
• higher level of single-choice identifications for the clinical species of coagulase negative staphylococci: 95.8% vs. 85%;
• less inaccurate results for nonreactive organisms.

P487 The IDEIA Norwalk-like virus enzyme immunoassay – a rapid method for screening outbreaks of nonbacterial gastroenteritis

L. Butcher
Norwich, UK

Norwalk-like viruses (NLVs), initially known as small round structured viruses (SRSVs) and now classified as noroviruses (NoVs), are a group of genetically diverse, single stranded RNA viruses belonging to the family Caliciviridae that are recognised as a major cause of nonbacterial gastroenteritis. This study was performed with a view to implementing the DakoCytomation IDEIA NLV EIA within a routine diagnostic laboratory in order to provide a more rapid response to outbreaks of gastroenteritis within the region.
**Objective:** To evaluate the DakoCytomation IDEIA NLV EIA as an alternative to screening by electron microscopy (EM) and the Lordsdale SRSV EIA.

**Methods:** A faecal suspension of each clinical sample was prepared according to the manufacturer’s instructions. A 100 μL of faecal suspension was added to coated microwells and incubated with NLV-specific genogroup 1 or 2 conjugate. After incubation, the microwells were washed and the presence of specifically bound conjugate was determined by the addition of a chromogen and enzyme substrate. Clinical specimens with an absorbance value greater than the cut-off values were regarded as positive. All samples were sent to Bristol for confirmation by EM, Lordsdale SRSV EIA and reverse transcription polymerase chain reaction (RT-PCR). Discrepant samples were sent to a second laboratory and resubmitted to Bristol for further analysis by RT-PCR.

**Results:** A total of 93 faecal samples collected from patients involved in 28 outbreaks of gastroenteritis were tested. The DakoCytomation EIA detected 24 positives and 45 negatives, confirmed by EM, Lordsdale SRSV EIA and RT-PCR. Detection of NLV antigen in 13 faecal specimens by the DakoCytomation EIA were not confirmed by Bristol. Seven faecal samples found to be negative by the DakoCytomation EIA were positive by the Lordsdale SRSV EIA. Further analysis of these discrepant samples was sought by RT-PCR at a second laboratory. The results obtained were in agreement with those obtained by the DakoCytomation EIA. Of the original 20 discrepant samples, 17 were resubmitted to Bristol for RT-PCR. The results obtained for 11 samples were consistent with those obtained by the DakoCytomation EIA and RT-PCR at the second laboratory. However, six samples remained discrepant.

**Conclusions:** The results of the study indicated that the DakoCytomation IDEIA NLV EIA is a rapid, reliable alternative to EM and the Lordsdale SRSV EIA. The assay was subsequently introduced into routine testing.

**P489** Diagnostic and prognostic value of serum adenosine deaminase activity in scrub typhus


**Objective:** Scrub typhus is a common endemic febrile illness together with haemorrhagic fever with renal syndrome (HFRS) and leptospirosis in Korea. The aim of the present study was to assess the diagnostic and prognostic value of serum adenosine deaminase (ADA) activity in scrub typhus.

**Methods:** Serum ADA activity was determined by colorimetric method. A total of 108 cases of serologically confirmed scrub typhus were compared with 16 cases of HFRS and 16 cases of leptospirosis. Indirect immunofluorescence assay was performed for scrub typhus and leptospirosis, while HFRS was diagnosed by particle agglutination test.

**Results:** Serum ADA levels were significantly higher in scrub typhus (92.46 ± 33.89 U/L) than HFRS (44.19 ± 12.36 U/L) or leptospirosis (24.92 ± 7.92 U/L) (P < 0.001). Serum ADA2 isoenzyme levels also were higher in scrub typhus (70.15 ± 21.65 U/L) than HFRS (33.16 ± 10.47 U/L) or leptospirosis (18.96 ± 9.27 U/L) (P < 0.001). Five patients who died of scrub typhus have more high levels of serum ADA than survived patients (151.3 ± 60.62 U/L vs. 89.6 ± 29.7 U/L; P = 0.002). Severe scrub typhus patients (n = 32) complicating with renal failure, respiratory failure, shock, encephalitis and myocarditis have higher levels of serum ADA than nonsevere patients (115.78 ± 47.04 U/L vs. 82.64 ± 19.83 U/L; P < 0.001).

**Conclusion:** Serum ADA value is sufficiently useful in early differentiation of scrub typhus from other febrile illness and may prove to be useful as prognostic marker of scrub typhus.
Antimicrobial susceptibility in Gram-negative bacteria - I

P492 The activity of some quinolones against Stenotrophomonas maltophilia and Pseudomonas aeruginosa clinical isolates in the presence of pump inhibitors

A.E. Laudy, I. Wojtal, A. Orzeszko, B.J. Starosciak
Warsaw, PL

Objectives: The most common cause of multidrug resistant strains is the efflux mechanism. The presence of such efflux systems was described for Pseudomonas aeruginosa and Stenotrophomonas maltophilia. In P. Aeruginosa, the presence of three efflux systems (Mex-AB-OprM, MexCD-OprJ and MexEF-OprN) responsible for quinolones resistance was described. These MDR pumps belonging to the RND family are inhibited by Phe-Arg-b-naphthylamide. Additionally, it is possible that in case of S. maltophilia, reserpine acts antagonistically to ciprofloxacin. The other studied pump inhibitors did not change generally the MIC of both quinolones. Unlike the inhibitory activity of Phe-Arg-b-naphthylamide agents as reserpine and omeprazole increased slightly the MIC of tested quinolones for some strains of S. maltophilia.

Methods: In our study, activity of some quinolones (nalidixic acid, ciprofloxacin) against S. maltophilia and P. aeruginosa clinical isolates in the presence and absence of pump inhibitors was determined. The following pump inhibitors in two concentrations (20 mg/L and 80 mg/L) were used: Phe-Arg-b-naphthylamide, reserpine and omeprazole. We also looked for a new inhibitors among newly synthesised compounds, 6-(adamant-1-yl)pyrimidines.

Results: From the studied inhibitors only Phe-Arg-b-naphthylamide affected the susceptibility of tested strains to quinolones, first of all to nalidixic acid. Generally, the presence of higher concentration of inhibitor pump (80 mg/L) increased most effectively sensitivity to nalidixic acid both S. maltophilia and P. aeruginosa. In 90% of S. maltophilia and 80% of P. aeruginosa the MIC decreased from threefold to 20-fold in the presence of Phe-Arg-b-naphthylamide. On the contrary, this inhibitor affected the MIC of ciprofloxacin only for a few strains. Moreover, Phe-Arg-b-naphthylamide alone has shown the activity against three strains of P. aeruginosa (MIC 20, 40 and 160 mg/L). The other studied pump inhibitors did not change generally the MIC of both quinolones.

Conclusions: Our data confirm that opposed to reserpine the second tested agent Phe-Arg-b-naphthylamide inhibited efflux pumps not only on P. aeruginosa but also on S. maltophilia. Moreover, obtained results indicated that depending on the structure of antibiotics the quinolones are maybe transported with different effectiveness through the efflux pumps. Additionally, it is possible that in case of S. maltophilia reserpine act antagonistically to ciprofloxacin.
Conclusions: The activity of HS alone cannot be relied upon to eradicate clinical isolates of HI because the majority of HI are SR. FQs were the most intrinsically bactericidal AMs against HI and only MFX was active against all HI. Most other classes of AM were poorly bactericidal or merely bacteriostatic in the absence of active HS. Eradication of HI may be important for the treatment of chronic infections and the use of highly bactericidal agents unaffected by serum susceptibility such as the FQs may be beneficial in this regard.

Conclusions: The activity of HS alone cannot be relied upon to eradicate clinical isolates of HI because the majority of HI are SR. FQs were the most intrinsically bactericidal AMs against HI and only MFX was active against all HI. Most other classes of AM were poorly bactericidal or merely bacteriostatic in the absence of active HS. Eradication of HI may be important for the treatment of chronic infections and the use of highly bactericidal agents unaffected by serum susceptibility such as the FQs may be beneficial in this regard.

Results: The majority of isolates (82.1%) were found to be SR. The sample subset of five SS isolates was all killed by HS alone without AM (data not shown). Average AUC data for AM at 4x MIC against the four SR HI with HS and for all nine HI with I-HS are shown below. All AM were bactericidal against the SR HI in the presence of HS, with the fluoroquinolones (FQs) MFX and LFX being the most active. For one SR HI, only MFX was bactericidal. In contrast, CLA and AMC were not bactericidal and TEL showed weak kills with I-HS. MFX and LFX remained the most active AMs even with I-HS.

<table>
<thead>
<tr>
<th>AUC</th>
<th>MFX</th>
<th>LFX</th>
<th>TEL</th>
<th>CLA</th>
<th>AZI</th>
<th>AMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS (SR only)</td>
<td>20.1</td>
<td>19.4</td>
<td>10.1</td>
<td>8.1</td>
<td>15.1</td>
<td>17.6</td>
</tr>
<tr>
<td>I-HS (SR &amp; SS)</td>
<td>14.1</td>
<td>15.1</td>
<td>2.0</td>
<td>0</td>
<td>8.9</td>
<td>0</td>
</tr>
</tbody>
</table>

**P495** Bactericidal activity and synergy of rifampin alone and in combination against pan-resistant *Acinetobacter baumannii*

M.E. Pachón- Ibáñez, J. Paneque, F. Docobo-Perez, L. Arroyo, M. Ruíz, C. Llanos, A. García-Curiel, M. Rodríguez
Seville, E

Objectives: The purpose of this study was to know the bactericidal activity and synergy of rifampin (RMP) alone and in combination with imipenem (IMP) and sulbactam (SB) against two clinical strains of panresistant *Acinetobacter baumannii*, including resistance to colistin.

Methods: MIC and MBC (mg/L) were performed using microdilution method (NCCLS). Time-kill curves were used to evaluate the bactericidal activity and the synergy of antimicrobial combinations (RMP + IMP, RMP + SB and IMP + SB) against the strains 99 and 113. For the time-kill curves antibiotics concentrations used were equivalent to the respective MIC and the Cmax of RMP, IMP and SB obtained in C57BL/6 mice (RMP 25 mg/kg, IMP 30 mg/kg, and SB 60 mg/kg) in time points from 10 to 150 min after a single dose; antimicrobial levels were determined by bioassay method. Antibiotics were considered to be bactericidal when there was a reduction of the original inoculum f3 log CFU/mL. Synergy was defined as f2 log decrease in CFU/mL when using the drug combination, relative to the most active component alone.

Results: MIC/MBC: RMP (>256/>256) for both strains; IMP (>128/>256) for the strain 99 and (256/>256) for the strain 113; SB (>256/>256) for both strains. Cmax: RMP (13.4 mg/L), IMP (16.7 mg/L) and SB (81.5 mg/L). Bactericidal activity: RMP (MIC) was bactericidal for both strains and not with Cmax. IMP was not bactericidal for any of the strains using MIC or Cmax. SB (MIC and Cmax) was bactericidal for the strain 99 and it was not bactericidal against the strain 113. The following combinations were synergistic: RMP + IMP (MIC and Cmax) and RMP + SB (MIC and Cmax) for both strains.

Conclusions: The combination of RMP plus IMP or SB is synergistic against selected clinical strains of panresistant *A. baumannii*. These results suggest that these combinations may be useful in the treatment of experimental infections caused by this agent.

**P496** Synergistic activities of nontraditional antibiotic combinations against multiresistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains

F. Can, F. Ergin, M. Demirbilek, O. Kurt Azap, H. Arslan
Ankara, TR

Objectives: Multiresistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains are increasingly cause of life-threatening infections and leads to limitation of the therapy. This study was designed to determine the synergistic activity of colistin with rifampicin, doxycycline, meropenem and azithromycin against multidrug resistant (MDR) *P. aeruginosa* and *A. baumannii* clinical isolates.

Methods: The synergistic activity in combination of colistin with rifampicin, doxycycline, meropenem and azithromycin was investigated against randomly selected five *A. baumannii* and five *P. aeruginosa* isolates by using checkerboard titration method.

Results: The combination of colistin and rifampicin was fully synergistic against four of *A. baumannii* and two of *P. aeruginosa* strains. Colistin with meropenem and colistin with azithromycin combinations showed synergistic activity against three of *A. baumannii* isolates, while resulted in additive or indifferent effects on *P. aeruginosa* strains. Colistin and doxycycline combination was
generally partially synergistic and additive effects against all of the isolates.

Conclusions: The results of this study demonstrate that against MDR *P. aeruginosa* and *A. baumannii*, synergy may occur between nontraditional antibiotics. As *P. aeruginosa* and *A. baumannii* have become to resistant to commonly used antibiotics, it is necessary to test various drugs alone and in combinations to find treatment choices for the infections that these organisms can cause.

**P497** Cell surface hydrophobicity and adherence of *Pseudomonas aeruginosa* to abiotic surfaces: effect of subinhibitory concentrations of pipercillin/tazobactam


**Porto, Lisbon, P**

**Objectives:** *Pseudomonas aeruginosa* is a human opportunistic pathogen that colonises biotic or abiotic surfaces and has been emerging as the primary source of nosocomial infections. Cell surface hydrophobicity (CSH) of bacteria is a very important physico-chemical feature, which has a great influence on the ability of bacteria to adhere to the surface of host cells or medical devices. It has been reported that subinhibitory concentrations (sub-MICs) of antibiotics are able to affect the bacterial surface properties and various phenotypic traits. In this study, the effects of sub-MICs of *Pipercillin/Tazobactam (P-T)* on bacterial surface hydrophobicity as well as the effect on bacterial adhesion were analysed, using *P. aeruginosa* strains.

**Methods:** *In vitro* antimicrobial activities were evaluated by micro-dilution method (NCCLS) against three reference strains (ATCC 27853, PAO1 and AK1), three defined PAO1 mutants with deviating surface characteristics (MT1562, PT623 and PAO1alG) and five *P. aeruginosa* clinical isolates (Cl). Selection of Cls was based on minisatellite-primed MSP-PCR fingerprinting of 100 isolates obtained from patients hospitalised at a Portuguese Central Hospital. The hydrophobicity assay was performed by growing the 11 strains in LB in presence and absence of P-T. The changes on CSH were estimated by calculating the percentage of cells adhering to n-hexadecane (1). The effects of 1/2 MIC on bacterial adhesion (1 h) were studied using a modified microtiter-plate assay (2).

**Results:** There was a significant decrease in CSH of all the strains tested that could explain a decrease in adhesion values in Cls and controls (reference and mutant strains). Treatment of the bacterial cells with subinhibitory concentrations (1/2 MIC) of P-T changed significantly the CSH towards the hydrophilic state compared with nontreated cells, and was found to be strain dependent.

**Conclusion:** As CSH and Adhesion ability are considered pathogenic traits, these data indicate the potential effectiveness of sub-MIC P-T for the treatment of patients with *P. aeruginosa* infections.

**Acknowledgements:** This study was supported by a research grant from Fundação Calouste Gulbenkian.

**References**


**P498** *In vitro* activity of antimicrobial drugs against *Brucella melitensis* strains in an endemic area

N. Elaldi, M.Z. Bakici, M. Bakir, I. Dokmetas, M. Sencan

**Sivas, TR**

**Objectives:** In human beings, brucellosis caused by *Brucella melitensis* is the most important clinically apparent disease and remains a major problem in the Mediterranean region including Turkey. Despite clinical and laboratory studies, the optimum antibiotic therapy for brucellosis is still unclear. There are few reports (mainly from endemic regions) in the literature about the sensitivity studies of *Brucella* spp. Furthermore, these studies still have not been standardised and interpretative standards are not available. *In vitro* activities of antimicrobial drugs against *B. melitensis* strains isolated from blood and body fluids cultures of the patients with brucellosis were investigated in an endemic area. A total of 63 *B. melitensis* strains were isolated between January 1998 and November 2003 from 63 patients with brucellosis at Cumhuriyet University Hospital, Sivas, Turkey. Only one strain per patient was included. The isolates were tested for susceptibility to various antimicrobial agents by using the Sceptor (Becton Dickinson Diagnostic Instrument Systems, Towson, MD, USA) automatic system. This is a broth microdilution system that uses plastic microtiter plates with doubling dilutions of desiccated antimicrobial agents.

**Results:** The aminoglycoside-structured antibiotics generally had good activity. Ceftriaxone had active against 59 of 63 (93.6%) strains. Carbapenems (imipenem, meropenem) showed good activity towards all strains of *B. melitensis* with MICs of 4 μg/mL. All strains tested were susceptible to cefepime (MIC < 8 μg/mL) and 62 (98.4%) of 63 strains were susceptible to ciprofloxacin (MIC < 1 μg/mL). Fifty-nine (93.6%) strains were inhibited by rifampicin at 1 mg/mL. Sixty-one (96.8%) were susceptible to tetracycline and 58 (92.1%) were to trimethoprim-sulfa-methoxazole at concentrations of 4 and 0.5/9.5 μg/mL, respectively. Strains were also tested with other antimicrobials. Conclusion: In order to reduce the incidence of complications of brucellosis and the development of drug resistance by the pathogen, it is necessary that proper treatment be instituted, following antimicrobial susceptibility testing.

**P499** Thermophilic *Campylobacter* resistance to five antimicrobial drugs

B. Miljkovic-Selimovic, M. Mraovic, B. Potkonjak, T. Babic, B. Kocic, L. Ristic

**Nis, Belgrade, CS**

Although *Campylobacter enterocolitis* is often self-limited diseases, in prolonged diarrhoea, severe clinical presentation, immunocompromised patients and postinfective sequelae, treatment is necessary. However, appearance of *Campylobacter* spp. strains resistant to erythromycin and also increasing resistance to quinolones may be treat to efficient therapy. The aim of the study is to evaluate sensitivity of thermophilic *Campylobacter* strains to drugs used in therapy of enterocolitis as well as to nalidixic acid used in identification. We investigated sensitivity of 76 thermophilic campylobacters. This work is a part of the project 'The role of *Campylobacter jejuni* in etiology of some autoimmune diseases' (1612) and supported by the Ministry of Science, Technology and Development, Republic of Serbia.
**P500**  
**In vitro activity of 12 anti-anerobic agents against clinical Bacteroides fragilis group strains isolated over a 7-month period**  
L. Alcalá, T. Peláez, N. García-Escribano, Ó. Pérez-Olaso, J. Martínez-Alarcón, J. Guinea, E. Bouza  
Madrid, E  

**Objectives:** Surveillance for antimicrobials resistance of clinical Bacteroides fragilis group isolates is necessary for help guide empiric therapy of anaerobic infections. We determine the antimicrobial susceptibility pattern of clinical B. fragilis group strains isolated in our institution from May to November 2003.  

**Methods:** Susceptibility testing was performed using a microdilution method according to document M11-A5 (NCCLS). Antimicrobials tested were amoxicillin (AM), amoxicillin-clavulanate (AC), piperacillin (PI), piperacillin-tazobactam (PT), cefoxitin (CE), imipenem (IM), chloramphenicol (CH), clindamycin (CL), moxifloxacin (MO), tetracyclin (TE) and vancomycin (VA).  

**Results:** A total of 182 strains from 127 specimens were tested. The percentage of intermediate resistant strains within each species tested were:  

<table>
<thead>
<tr>
<th>Species (number)</th>
<th>AM</th>
<th>AC</th>
<th>PI</th>
<th>PT</th>
<th>CE</th>
<th>IM</th>
<th>CL</th>
<th>MO</th>
<th>TE</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. fragilis (76)</td>
<td>100</td>
<td>79</td>
<td>28</td>
<td>9</td>
<td>2.6</td>
<td>6.6</td>
<td>1.3</td>
<td>46.1</td>
<td>1.3</td>
</tr>
<tr>
<td>B. thetaiotaomicron (38)</td>
<td>100</td>
<td>79</td>
<td>28</td>
<td>9</td>
<td>2.6</td>
<td>52.6</td>
<td>0.2</td>
<td>73.7</td>
<td>2.6</td>
</tr>
<tr>
<td>B. uniformis (18)</td>
<td>94.4</td>
<td>0</td>
<td>16.7</td>
<td>0</td>
<td>16.7</td>
<td>55.6</td>
<td>0</td>
<td>72.2</td>
<td>6.7</td>
</tr>
<tr>
<td>B. vulgatus (15)</td>
<td>100</td>
<td>6.7</td>
<td>40</td>
<td>0</td>
<td>13.3</td>
<td>46.7</td>
<td>0</td>
<td>46.7</td>
<td>8.0</td>
</tr>
<tr>
<td>B. ovatus (14)</td>
<td>100</td>
<td>28.6</td>
<td>50.0</td>
<td>14.3</td>
<td>64.3</td>
<td>0</td>
<td>71.4</td>
<td>0</td>
<td>50.0</td>
</tr>
<tr>
<td>Other (21)</td>
<td>100</td>
<td>23.8</td>
<td>57.1</td>
<td>1.3</td>
<td>42.4</td>
<td>0</td>
<td>71.4</td>
<td>0</td>
<td>33.3</td>
</tr>
<tr>
<td>Overall (182)</td>
<td>99.5</td>
<td>10.4</td>
<td>33.5</td>
<td>2.7</td>
<td>27.5</td>
<td>0.5</td>
<td>57.7</td>
<td>1.1</td>
<td>36.3</td>
</tr>
</tbody>
</table>

**Conclusion:** PT, IM, ME and CH were the most active agents tested, with at least 97% of susceptible strains. Moreover, AC and CE had also a good activity with a susceptibility rate ranging from 72.5 to 90.0%. The remaining antimicrobials had a poor activity. In spite of the growing use of broad-spectrum antimicrobials, most of these agents remain active against clinical strains of B. fragilis group isolated in our institution.

**Conclusions:** The findings indicate that only sub-MIC of CIP caused decrease in the percentage of clones sensitivity to K1A phage.  

**Reference**  

---

**P502**  
**Antibiotic susceptibilities and extended-spectrum β-lactamase production of Enterobacteriaceae from urinary tract infections**  
B. Kocić, S. Antić, B. Todorovíc, T. Babić, L. Ristić, V. Mladenovic  
Nis, YU  

**Objectives:** ESBL producing Enterobacteriaceae have compromised therapy with β-lactam antibiotics, including third generation of cephalosporins. The objective of this study was to determine the occurrence of ESBL phenotypes among different isolates of Enterobacteriaceae and their susceptibility to antimicrobial agents.  

**Materials and Methods:** A total of 1000 strain of Enterobacteriaceae were isolated from urine samples during 1-year period. Suspected strains are presumptively defined as ESBL producers according to result of disk diffusion method, using ESBL marker antibiotics—ceftazidim, ceftriaxion and cefotaksim. Those isolates were retested with double-disc synergy test (DDST)—CAZ, CTR, CTX and amoxicillin-clavulanici acid disks implementation. Enhancement of inhibition zone (or so-called ghost zone) indicated presence of ESBL strain. Antimicrobials susceptibility to β-lactam antibiotics, aminoglikosides, quinolones and trimetoprim-sulfametozaxol evaluated by disc-diffusion method, and the ESBL detection was performed by the DDST, according to NCCLS criteria (2002).  

**Results:** The species distribution as follows: Escherichia coli (63.9%), Klebsiella spp. (30%), Enterobacter spp. (11.1%), Proteus vulgaris (2.3%), P. mirabilis (11%), Providencia spp. (0.5%), Morganella morgani (0.2%) and Citrobacter spp. (2.6%). Total number of isolates (21.4%) was multiresistant for more than three groups of antibiotics: E. coli were 44.39%, Klebsiella spp. 8.41%, Enterobacter spp. 26.63%, P. vulgaris 5.14%, P. mirabilis 10.28%, Providencia spp. 2.33%, M. morgani 0.46% and Citrobacter spp. 35%. Thirty-three per cent of all isolates were from hospital samples; 43.5% of all isolates were producing ESBL, and included four different species. Escherichia coli (4.06%), Klebsiella (23.3%), Enterobacter (8.1%) and Proteus mirabilis (0.2%). The majority of producers were from clinical specimens (63%).  

**Conclusions:** Gram-negative rods were responsible for high percentage of urinary tract infections. Escherichia coli was the most common uropathogen. Multiresistant strain represent 21.4% from Enterobacteriaceae implicated in UTI. The resistance to ampicillin was the most frequent and concerned 62.6% of isolates of all indicated species.

---

**P503**  
**The effect of sub-inhibitory concentration (sub-MIC) of amikacin and ciprofloxacin on the loss of capsular antigen K1 by Escherichia coli strains**  
D. Wojnicz, A. Cisowska, S. Jankowski  
Wrocław, PL  

**Objectives:** The aim of this investigation was to examine the influence of 1/2 MIC of amikacin (AN) and ciprofloxacin (CIP) on the loss of capsular antigen K1 by Escherichia coli strains.  

**Methods:** Four E. coli K1 strains (315, 353, 418 and 662) isolated from urine of children with urinary tract infections were used. The bacteriophage K1A for detecting the capsular antigen K1 was used. The MICs of AN and CIP for each strain in Mueller–Hinton broth were determined by using microdilution method. The frequency of occurrence of surface antigen K1 among 100 clones of each E. coli K1 strains (without antibiotics and with 1/2 MIC of AN and CIP) was detected by the method described earlier (1).  

**Results:** In controls, all tested E. coli K1 strains revealed the occurrence of 93–96% clones with K1 antigen. The exposure of E. coli K1 strains to 1/2 MIC of CIP significantly decreased the percentage of clones with K1 antigen. Only 13% clones of E. coli 418 strain possessed K1 antigen. In cases of E. coli strains 353, 662 and 315, the presence of K1 antigen was found in 28, 37 and 40% per 100 clones, respectively. We observed that after exposure all E. coli K1 strains to 1/2 MIC of AN, the percentage of clones with K1 antigen corresponded to the percentage observed in control.

**Conclusions:** The findings indicate that only sub-MIC of CIP caused decrease in the percentage of clones sensitivity to K1A phage.

**Reference**  

---

**P502**  
**Agreement between disc diffusion and E-test methods to assess the carbapenem susceptibility of four Gram-negative nosocomial pathogens**  
B. Çakır, S. Unal, O. Uzun  
Ankara, TR  

**Objectives:** The aim of this study was to assess the carbapenem susceptibility of four nosocomial pathogens and to evaluate the reliability of the susceptibility results determined by E-test and disc diffusion (DD) methods.  

**Methods:** Escherichia coli (n = 73), Klebsiella pneumoniae (n = 60), Pseudomonas aeruginosa (n = 70) and Acinetobacter spp. (n = 70) isolated from nosocomial infections in 2002–2003 were included in the study. Thirty-five per cent of the strains were isolated from intensive care units. After determining antimicrobial susceptibility against imipenem and meropenem by DD (10 μg; Oxoid, UK) and Etest (AB Biodisk, Solna, Sweden) methods, the results were categorised as susceptible (S), intermediate (I) and resistant (R) according to the NCCLS criteria. For statistical analyses, the
intermediate group was included in the resistant category because of the low numbers of bacteria in the former group.

**Results:** None of *E. coli* or *K. pneumoniae* strains were resistant to carbapenems, whereas, resistance reached up to 59.0% in *Acinetobacter* spp. and *P. aeruginosa* isolates. By either method, the pattern of the susceptibility of the four bacteria was not statistically significantly different for imipenem vs. meropenem. Total agreement of DD and E-test methods for susceptibility to imipenem was 95.7%, and 90.0% in *Acinetobacter* spp. and *P. aeruginosa*, respectively; and susceptibility to meropenem was 90.0% for both bacteria. However, the difference of the results obtained by either method was statistically significant for *Acinetobacter* spp.

**Conclusion:** Study results suggest a high resistance rate for *Acinetobacter* spp. and *P. aeruginosa* strains against carbapenem antibiotics in our hospital. Further studies are needed to clarify whether E-test should be used to confirm meropenem resistance of *Acinetobacter* spp. and *P. aeruginosa*. E-tests are needed for susceptibility testing.

---

**Table 1.** Susceptibility of *Acinetobacter* spp and *P. aeruginosa* strains to imipenem and meropenem by comparing results of DD and Etest Methods.

<table>
<thead>
<tr>
<th>Strain</th>
<th>DD method</th>
<th>Etest method</th>
<th>McNemar test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>R/1</td>
<td>S</td>
<td>0.250</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>s</td>
<td>37</td>
<td>0.25</td>
</tr>
<tr>
<td>s</td>
<td>300</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>R/1</td>
<td>34</td>
<td>1.000</td>
</tr>
<tr>
<td>R/1</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>meropenem</td>
<td>1/2</td>
<td>S</td>
<td>0.021</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>s</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>30</td>
<td>0</td>
<td>0.344</td>
</tr>
</tbody>
</table>

---

**P504**

Gram-negative nosocomial pathogens in Estonian intensive care units

K. Löövukene, E. Sepp, V. Adamson, Ü. Kallandi, K. Otter, P. Naaber
Tartu, Tallinn, EST

**Objective:** While the most important reasons of mortality and morbidity in intensive care units (ICUs) are nosocomial infections caused by Gram-negative pathogens, our objective was to evaluate susceptibility pattern of those pathogens comparatively in Estonian ICUs by similar protocol. To clear up methodological discrepancies, data of E-test and disk diffusion method were compared.

**Methods:** During April–November 2003, a total 105 *Acinetobacter baumannii*, 92 *Pseudomonas aeruginosa* and 96 *Klebsiella pneumoniae* strains were collected from clinical specimens from ICUs of North Estonian Regional Hospital, East Tallinn Central Hospital and Tartu University Clinics. For susceptibility testing, E-tests and antibiotic disks (meropenem, imipenem, ampicillin/sulbactam, cefepime, amikacin, piperacillin/tazoabactam, ciprofloxacin and ceftazidime) were used accordingly to NCCLS guidelines.

**Results:** Ninety-five per cent of *A. baumannii* strains were sensitive to meropenem, 98% to imipenem, 61% to ampicillin/sulbactam, 56% to cefepime and 72% to amikacin (MIC50/90 values, respectively, 1/3, 0.75/2, 6/32, 8/32 and 6/64); 80% of *P. aeruginosa* strains were meropenem, 70% imipenem, 78% piperacillin/tazoabactam, 68% ciprofloxacin, 75% ceftazidime and 98% amikacin sensitive (MIC50/90 values 1/16, 3/32, 6/256, 0.25/12, 1.5/64 and 4/12). The susceptibility of *K. pneumoniae* isolates to meropenem and imipenem was 99%, to cefepine 92% and to amikacin 97% (MIC50/90 values 0.025/0.19, 0.19/0.5, 0.025/1 and 2/3). Generally, *MIC50/90* for meropenem was 0.38/4, imipenem 0.75/6, ampicillin/sulbactam 6/32, cefepime 8/32, amikacin 3/8, piperacillin/tazoabactam 6/256, ciprofloxacin 0.125/1.5 and ceftazidime 1.5/64. In all three ICUs, the sensitivity among *A. baumannii*, *P. aeruginosa* and *K. pneumoniae* strains was similar, except higher resistance to cefepime of *A. baumannii* strains in Tartu University Clinics. Discordance between E-test and disk-diffusion was pathogen specific. In *K. pneumoniae*, one major and seven minor errors were found, whereas in *A. baumannii*, 3/8 major and 69/33 minor errors occurred. Carbapenems test results correlated better than comparisions of other agents.

**Conclusions:** Most active agents against all pathogens were carbapenems and amikacin, whereas meropenem and ciprofloxacin had lowest MICs than others. For empirical treatment meropenem is preferred due to high activity against all Gram-negative pathogens and the lowest MIC values. In case of *A. baumannii* and *P. aeruginosa*, E-tests are needed for susceptibility testing.

---

**P505**

Effects of subminimal inhibitory concentrations of three antimicrobials on the virulence factors and growth of *Proteus mirabilis*

M. Ucar, S. Ozden, Y. Dogan, S. Kirdar, Ö. Yilmaz, H. Baskın
Izmir, TR

**Objectives:** Proteus is the second bacteria only to *Escherichia coli* as a cause of nonhospital acquired urinary tract infections. The enzyme urease causes the pH of urine to rise, allowing unchecked growth of the bacteria. Adherence to uroepithelial cells and the organisms rapid motility are also involved in the pathogenesis of the urinary tract infections. Antimicrobial treatment is often chosen after determination of the minimal inhibitory concentrations (MIC). Although antibiotics are often present in subinhibitory concentrations (sub-MICs) and may still be effective in reducing bacterial virulence.

**Methods:** Subminimal inhibitory concentrations of ciprofloxacin, gentamicin and ampicillin-sulbactam at 1/2–1/32 MIC levels on growth, adherence, urease and swarming characteristics of *Proteus mirabilis* (ATCC 14153) were studied.

**Results:** Minimal inhibitory concentrations values were 0.125 µg/mL for ciprofloxacin, 0.25 µg/mL for ampicillin-sulbactam and 1 µg/mL for gentamicin. These three antimicrobials had no significant effect on swelling and urease production at the sub-MIC levels. Ciprofloxacin and ampicillin-sulbactam had no inhibitory effect on growth at sub-MIC levels whereas gentamicin inhibited the colony counts at 1/2 X MIC level. We also observed that ciprofloxacin, gentamicin, ampicillin-sulbactam inhibited the adherence to uroepithelial cells at 1/2–1/8 X MIC.

**Conclusion:** *Proteus mirabilis* causes urinary tract infections in the complicated urinary tracts, especially in indwelling catheters, presence of the structural abnormalities or in elder patients. Ciprofloxacin (quinoalone), ampicillin-sulbactam (β-lactam) and gentamicin (amino glycoside) have different effect mechanisms, and side effects. In this study, it was interesting to see that there were no differences on the swarming and urease production of these three antibiotics whereas gentamicin inhibited bacterial growth at 1/2 X MIC levels. Gentamicin was more effective in 1/2–1/4 X MIC levels on adherence of the pathogen that is known to be the most important virulence mechanism. In conclusion, we may suggest that gentamicin is more effective antibiotics among these three agents.

---

**P506**

*In vitro* susceptibility of *Pseudomonas aeruginosa* isolated in a burn centre in south Iran, to silversulfadiazine and silver nitrate

M. Hayati, A. Japoni, A. Alborzi, M. Kalani
Shiraz, IR

**Objectives:** Development of microorganisms resistant to antibiotics may increase with the widespread use of these agents. Silver salts and compounds (AgNO₃, silversulfadiazine) are among these agents. Silversulfadiazine (SSD) is extensively used in our burn centre in south of Iran (Ghotbedin Hospital, Shiraz). This study was carried out to determine and compare the susceptibility of
Pseudomonas aeruginosa isolated from burned patients, burn wards and nonburn patients to SSD and AgNO₃.

Methods: Three groups of P. aeruginosa were isolated including the strains from burned patients (group I), environmental strains from burn centre (group II) and strains isolated from nonburn patients (group III or control). The MICs of SSD and AgNO₃ for these strains were determined by agar dilution method in TYE agar without NaCl in dark. The results were compared by Fisher exact test and correlation between MICs was determined as well. Susceptibility of these strains to SSD was also evaluated by agar cup plate method.

Results: From 63 strains in group I, 60 strains were resistant to SSD which 40 of them were highly resistant (MIC >10 mM) and five of them were resistant to AgNO₃. In group II, eight strains of 15 were resistant to SSD with the same range of MIC as group I but non of them showed resistance to AgNO₃. In group III, all the strains were sensitive to SSD and AgNO₃. The differences between MIC of SSD in these groups were significant (p < 0.001). Correlation between MIC of SSD and AgNO₃ was not significant in group I. The results of agar dilution tests were confirmed by agar cup plate method.

Conclusion: Pseudomonas aeruginosa, the epidemic cause of infections in our burn centre could develop a high level of resistance to SSD (MIC >10 mM) which is an important threat for burned patients and warns to revise the effectiveness of this drug. Only five strains of 60 SSD resistant were cross-resistant to AgNO₃ (MIC 0.75–1 mM).

P507 Antimicrobial resistance among Streptococcus pneumoniae and Haemophilus influenzae from Africa and the middle-east: 2002/2003 winter season

A. Shibl, J. Daniels, J. Sievers and the SOAR in AME Study Group

Objectives: Antimicrobial resistance among respiratory pathogens exists worldwide, affecting empirical prescribing choices. Quality surveillance data are needed to monitor the prevalence and spread of resistance to commonly prescribed antimicrobials.

Methods: Respiratory tract isolates of Streptococcus pneumoniae (Sp) and Haemophilus influenzae (Hi) were collected from patients in three African countries, seven middle-eastern countries and Pakistan in the 2002/2003 winter season. MICs for various antimicrobials were determined using E-test, and susceptibility assessed based on NCCLS breakpoints, where applicable. Quality control strains were tested on each day of testing. Not all antimicrobials were tested in all countries, or against all isolates.

Results: A total of 1154 Sp and 1091 Hi isolates were collected. In Africa 58.6% (136/232) and in the middle-east 42.5% (349/822) of Sp were penicillin susceptible and 4.3% (10/232) and 8.3% (68/822), respectively, were penicillin resistant (PRSP). The highest PRSP prevalence was in Tunisia (17.8%). No PRSP were identified in Egypt, Jordan, Kuwait or Pakistan. In Africa 10.8% (25/232), in the middle-east 12.5% (99/808), and in Pakistan 13.0% (13/100) of Sp were azithromycin resistant. The lowest regional prevalence of Sp resistance to a cephalosporin was to cefprozil [Africa: 2.2% (5/232); middle-east: 1.9% (16/822)], and the highest was to cefaclor [Africa: 10.8% (25/232) and cefdinir [middle-east: 24.3% (113/465)]. Only 0.4% (1/232) of Sp in Africa and 0.5% (4/821) in the middle-east were resistant to amoxicillin/clavulanic acid. In Africa, 50.0% (5/10) of PRSP were co-resistant to azithromycin, as were 22.4% (15/67) of PRSP from the middle-east. Of Hi isolates, 14.9% (33/221) (Africa) and 21.6% (166/768) (middle-east) were β-lactamase positive. All Hi from Africa and Pakistan and 99.9% from the middle-east were amoxicillin/clavulanic acid susceptible.

Conclusions: Despite an overall prevalence of PRSP in Africa and the middle-east <10%, the higher prevalence of PRSP in countries such as Tunisia may complicate empirical prescribing in these areas. Of further concern is the prevalence of macrolide resistance and PRSP macrolide co-resistance. The high prevalence of β-lactamase production in Hi in the middle-east may make β-lactamase-unstable antimicrobials unsuitable in this region. Agents such as azithromycin/clavulanic acid, to which both Sp and Hi remain susceptible, may be most appropriate for empirical prescribing in these areas.

Antifungal susceptibility studies

P508 Comparative evaluation of AFST-EUCAST method and Sensititre YeastOne Colorimetric Antifungal Panel with NCCLS reference method for susceptibility testing of Candida species

M. Yucesoy, C. Ergon
Izmir, TR

Objectives: This study was carried out to compare the performance of two alternative methods, Antifungal Susceptibility Testing Subcommittee of the European Committee on Antimicrobial Susceptibility Testing (AFST-EUCAST) method and a commercially prepared Sensititre YeastOne Colorimetric Antifungal Panel with NCCLS M27-A2 microdilution method.

Methods: Two quality controls, seven reference strains of ATCC and two clinical isolates of fluconazole-resistant C. glabrata were included. Susceptibility for amphotericin B, fluconazole, itraconazole and ketoconazole were performed with AFST-EUCAST, Sensititre and NCCLS microdilution methods while the susceptibility of 5-flucytosine was investigated with Sensititre and NCCLS methods. AFST-EUCAST method was performed with RPMI-1640 supplemented with 2% dextrose, inoculum size of 0.5–2.5 × 10⁸ CFU/mL and flat-bottom plates. Sensititre method was carried out according to the manufacturer’s instructions with an inoculum of 1.5–8 × 10⁸. The endpoints were determined visually for amphotericin B, visually and spectrophotometrically at the wavelength of 450 and 492 nm for azoles after 48 h in the NCCLS method. The results were read spectrophotometrically at the same wavelengths in the AFST-EUCAST method and only visually in the Sensititre method. The results were compared according to the agreement of MIC values within ±2-fold dilutions and susceptibility categories.

Results: When the agreement between the results of NCCLS and Sensititre method after 48 h was considered 8–9 of 10 strains were within ±2-fold dilutions for amphotericin B, fluconazole and itraconazole and 6–7 were for ketoconazole. According to the results of NCCLS method and AFST-EUCAST method, at 24 and 48 h 8–10 of the strains were within ±2-fold dilutions for all of the agents. When the susceptibility categories were considered there was no very major and major errors.

Conclusion: It can be concluded that both AFST-EUCAST and Sensititre methods are potentially good alternatives for antifungal susceptibility testing of Candida species for many antifungal agents when tested with reference strains.
**P509** Determination of antifungal activity of caspofungin using flow cytometry

S. Costa-de-Oliveira, C. Pina-Vaz, C. Tavares, A. Gonçalves Rodrigues
Porto, P

Caspofungin is an echinocandin that blocks the synthesis of β-(1,3)-D-glucan of the fungal cell wall, which is an essential component of the wall of numerous fungal species. The inhibition of its synthesis may result in a fungistatic effect, from blockade of the cell wall synthesis, or in a fungicidal effect, from changes in the integrity of the cell wall. Cytometric methods allow the early establishment of a susceptibility profile and give the possibility to evaluate functional and morphologic changes of the fungal cells (1).

**Objectives:** To determine susceptibility of clinical isolates of yeast to caspofungin by cytometric methods.

**Methods:** Two strains of *Candida albicans* with low MIC to caspofungin (determined accordingly M27 A protocol by NCCLS) and one *C. guilliermondii* and one strain of *Cryptococcus neoformans* both with high MIC were studied. The strains were grown overnight in Sabouraud broth and then incubated with caspofungin (Merck), at serial dilutions (MIC, MIC/2, 2x MIC and 4x MIC) during 1, 3 and 5 h, in phosphate buffer saline (Sigma). The suspensions were washed and resuspended with propidium iodide (PI; Sigma) (a marker of cell death by cell membrane lesion), FUN-1 (molecular probes) an indicator of metabolically integrity of yeast cells and SYTO 16 (molecular probes) a green fluorescent nucleic acid stain. The cells were analysed on a flow cytometer (Beckman Coulter XL-MCL): the morphology (scattergram) and the intensity of fluorescence of the stained cells [FL3 (red) for PI; FL2 (green) for FUN-1 and FL2 for SYTO 16] were evaluated.

**Results:** Obvious changes of the scatter were noticed from after 1 h of incubation, which increased with increasing incubation time. Five hours of incubation with 4x MIC were necessary to stain sensitive yeast strains with PI. Resistant strains did not stain with PI after 5 h incubation with the antifungal while fungistatic effects were reproducible, medium dependent in extent, noted in macro- and micro-dilution and on agar containing drug (but not when drug concentrations were not constant, as in agar diffusion), not seen in other Candida species or with other echinocandins, and not due to destruction of drug in tubes with the effect or to mutations in resistance-associated regions of the glucan synthase complex. Co-operative enhancement of inhibition by a second drug could eradicate the effect. Extensive studies of relationship to azole resistance mechanisms suggest a weak association, at most. Occasional isolated clear tubes on subculture yielded a few viable cells in a ring-like pattern, suggesting random distribution, in some strains, of few cells with propensity to grow in the presence of drug.

**Conclusion:** We postulate high drug concentrations derepress or activate resistance mechanisms. The ability of subpopulations to survive at high drug concentrations could have in vivo consequences.

**Reference**

---

**P511** Biofilms of *Candida albicans* on silicon catheters could be reduced by caspofungin in vitro

C. Coccaud, S. Imbert-Bouyer, M.-H. Rodier, C. Imbert-Pottiers, F

**Objectives:** Some manifestations of candidiasis are associated with the formation of biofilms on inert surfaces, and the intrinsic resistance of *C. albicans* biofilms to the most commonly used antifungal agents has been demonstrated. We studied here the effect of caspofungin on biofilms of *C. albicans*.

**Methods:** Calibrated sections of silicone catheters were incubated with *C. albicans* yeasts to obtain biofilms of 2, 24 and 48 h of maturation. Ten strains of *C. albicans* were used: five strains susceptible to fluconazole in *vitro* and five strains resistant to this antifungal. We report on the effect of two concentrations of caspofungin (MIC and 2 mg/L) on these biofilms. The influence of caspofungin on *C. albicans* biofilms was determined by evaluating a significant decrease or increase (<0.0001) in the metabolic activity of yeasts.

**Results:** The results showed that caspofungin (MIC) had no effect on *C. albicans* biofilms, whatever the strains and the maturation status of fungal biofilms. The efficiency of caspofungin (2 mg/L) was observed independently of (i) the susceptibility of yeasts to fluconazole, and (ii) the maturation status of fungal biofilms.

**Conclusion:** Caspofungin (2 mg/L) could represent a good candidate in the prevention of candidiasis associated with silicone medical devices.

---

**P510** Paradoxical caspofungin effect: reduced activity against *Candida albicans* at high concentrations

D.A. Stevens, T.C. White, D.S. Perlin, M. Espiritu, R. Parmar
San Jose, Seattle, Newark, USA

**Objective:** Resistance problems with caspofungin, an echinocandin inhibitor of fungal cell wall glucan synthesis, have been rare. We noted and investigated paradoxical turbid growth of *Candida albicans* isolates in some high, supra-MIC, concentrations of caspofungin.

**Methods:** Broth and agar dilution, checkerboard analysis of drug interaction, DNA sequencing, enzyme expression analysis.

**Results:** Among isolates submitted for susceptibility testing and described in NCCLS document M44-P in Mueller–Hinton agar (Difco Laboratories, USA) supplemented with 2% glucose and methylene blue. Fluconazole (25 μg) and voriconazole (1 μg) disks were obtained from Becton Dickinson (USA). Plates were inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of 0.5 McFarland standard, incubated at 37°C and read at 24 h. Interpretive criteria for fluconazole were: (i) susceptible, zone diameter of 19 mm or more; (ii) susceptible-dose dependent, zone diameter of 15–18 mm; (iii) resistant, zone diameter of <14 mm.

---

**P512** In vitro activities of fluconazole and voriconazole against Spanish bloodstream isolates of *Candida glabrata* and *Candida krusei*

G. Quindós, L. Sánchez, M. Villar, P. Ahuad, E. Eraso, J. Hernández
Bilbao, E; Mexico City, MEX; Buenos Aires, AR; Barakaldo, E

**Objective:** To evaluate the in vitro susceptibility to fluconazole and voriconazole of all the *Candida glabrata* and *Candida krusei* blood isolated during a 14-year period (1990-2003) at a tertiary care hospital (University Hospital of Cruces, Barakaldo, Spain).

**Patients and Methods:** Twenty-eight *C. glabrata* and 15 *C. krusei* blood isolates were tested. Disk diffusion was performed as described in NCCLS document M44-P in Mueller–Hinton agar (Difco Laboratories, USA) supplemented with 2% glucose and methylene blue. Fluconazole (25 μg) and voriconazole (1 μg) disks were obtained from Becton Dickinson (USA). Plates were inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of a 0.5 McFarland standard, incubated at 37°C and read at 24 h. Interpretive criteria for fluconazole were: (i) susceptible, zone diameter of 19 mm or more; (ii) susceptible-dose dependent, zone diameter of 15–18 mm; (iii) resistant, zone diameter of <14 mm.
Interpretive breakpoints have not yet been established for voriconazole on a zone diameter of <13 mm was considered as an indicator of in vitro resistance. Quality control was performed by using C. albicans ATCC 90028, C. krusei ATCC 6258 and C. parapsilosis ATCC 22019. E-test (AB Biodisk, Sweden) and Sensititre YeastOne (AccuMed International, USA) were used for testing all resistant and susceptible-dose dependent isolates and a representative number of susceptible isolates.

Results: 14.3% C. glabrata had a decreased susceptibility to fluconazole, as two C. glabrata were resistant and other two isolates were susceptible-dose dependent. All C. krusei were resistant to fluconazole. Voriconazole was active against 27 (96.4%) C. glabrata and 14 (93.3%) C. krusei. Both voriconazole resistant isolates were also resistant to fluconazole and were isolated one (C. krusei) in 1991 and other (C. glabrata) in 1993. The fluconazole and voriconazole susceptibility patterns were constant during this 14-year period. These susceptibility results to both triazoles were confirmed by the E-test and Sensititre YeastOne methods.

Conclusion: Voriconazole was very active in vitro against C. glabrata and C. krusei blood-stream isolates.

Acknowledgements: This work was financed in part by grant 1/UPV 00093.327-E-14645/2002 from the Universidad del País Vasco.

P513 Comparison of the in vitro activity of anidulafungin with amphotericin B, caspofungin, fluconazole, itraconazole and voriconazole against a panel of 780 yeast isolates obtained from five European centres

E.M. Johnson, B.P. Goldstein, K.G. Davey, M.A. Fraser
Bristol, UK; Pen, USA

Objectives: To compare the in vitro activity of the echinocandin agent anidulafungin with that of five other systemically active antifungal agents against a total of 780 yeast isolates obtained from five European countries.

Methods: Isolates of Candida albicans (505), C. glabrata (89), C. krusei (53), C. parapsilosis (41), C. tropicalis (65) and other Candida species (23) were obtained from superficial and deep infections of patients in the UK, France, Germany, Italy and Spain. All isolates were tested by the microtitre plate modification of method NCCLS M27-A2 with results recorded after 24 and 48 h. A subset of 50 isolates was also tested by the EUCAST method with reading after 24 h.

Results: The 24 and 48 h MIC50, MIC90 and range (mg/L) are presented for each drug against all isolates tested by the NCCLS method. Results of the NCCLS method read after 24 or 48 h and results obtained by the EUCAST method read after 24 h differed by no more than a doubling dilution. Anidulafungin was the most potent agent overall against the panel of yeasts tested. MICs of anidulafungin were similar for azole-susceptible and azole-resistant isolates.

Conclusions: Anidulafungin was highly active in vitro against Candida isolates from five European countries. These data are consistent with previous findings in smaller studies and in a large US survey.
performed with NCCLS-recommended broth microdilution and STC-colorimetric methods in parallel. The colorimetric method using STC was identical to the broth microdilution method with two exceptions: that STC was added to RPMI 1640-MOPS medium with antifungal agents at a final concentration of 50 μg/mL, and that the solubilising agents were added at 48 h of incubation and then the plates were incubated for 2 h. The wells with fungal growth were pink to red after addition of the agent.

**Results:** Among 24 strains, 18 and nine strains, respectively, demonstrated the trailing phenomenon with ketoconazole and itraconazole in the broth microdilution method. In contrast, trailing growth was not seen in the STC-colorimetric method, and, for 22 (92%) and 20 (83%) of the 24 strains, the ketoconazole and itraconazole MICs, respectively, were within two dilutions of those obtained by the NCCLS method. Furthermore, the colorimetric method allows stringent endpoint designation, and there is no difference between visual observation before extraction and visual and spectrophotometric reading after extraction.

**Conclusions:** The colorimetric method using STC was objective and easy to interpret and showed high levels of agreement with the NCCLS method for ketoconazole and itraconazole. I think that the STC-based colorimetric method is easily applicable for the antifungal susceptibility test.

**P516** Antifungal activity of *Juniperus turbinata* on species of *Candida* and dermatophytes

E. Pinto, C. Cavaleiro, L. Salgueiro, A. Palmeira, M. Gonçalves, C. Pina-Vaz, A. Gonçalves Rodrigues, S. Costa-de-Oliveira, C. Tavares, J. Martínez-de-Oliveira

Porto, Coimbra, P

Dermatophytosis and candidiasis are common superficial infections that can be found all over the world. Recently, our group demonstrates that some essential oils (*Thymus* spp., *Origanum* spp. and *Lippia* spp.) can be useful as antifungal agents (1–4).

**Objectives:** Continuing our research on the antifungal activity of essential oils, we report now the activity of *Juniperus turbinata* leaves and berries’ essential oils in order to support its application as therapeutic agents in the treatment of superficial mycoses.

**Methods:** Two samples of leaves oils from plants collected at Algarve (A) and at Alentejo (B) and one sample of berries oil from Alentejo (C) were assayed. Essential oils were isolated from fresh material, by water distillation (3 h) in a Clevenger type apparatus (5) and their compositions investigated by GC and GC-MS, as previously reported (6). Leaves oils are dominated by monoterpenic hydrocarbons (77.1–89.4%), but quantitative important differences were found in the main compounds (α-pinene 27.8% vs. 48.2% and β-phellandrene 28.8% vs. 23.1%, respectively, for samples A and B). The main constituents of the berries oil were α-pinene (66.7%) and β-phellandrene (6.4%). Antifungal activity on *Candida* and dermatophytes strains was evaluated by determination of the minimal inhibitory concentration (MIC), according to the NCCLS protocol, M 27-A and M 38-P, respectively.

**Results:** Important inhibitions of the growing of dermatophytes were observed, with MIC values ranging 0.08–0.32, 0.63–1.25 and 0.32–1.25 μL/mL for *T. rubrum* oils (A, B and C, respectively). For *Candida* strains the oils have low activity with MIC values ranging 0.32–2.5, 1.25–10.0 and 5.0–20.0 μL/mL for samples A, B and C, respectively.

**Conclusion:** The antifungal activity of *J. turbinata* essential oils on dermatophytes, may justify future clinical trials to validate their use as therapeutic alternatives for dermatophytosis treatment.

**Acknowledgements:** FCT, POCTI and FEDER (POCTI/40167/ESP/2001) for financial support.

**References:**

**P517** The effect of onion extract on ultrastructure of *Trichophyton mentagrophytes* and *T. rubrum*

M. Shams, M. Godarzi, T. Tarihi, M. Razaghi Abyaneh

Tehran, IR

Dermatophytes are a specialised group of fungi able to use the keratinised tissue of skin, nail and hair as the sole nutritional source. These fungi are classified in three major genera namely *Microsporum*, *Trichophyton* and *Epidermophyton*. The various species in each genus are the causative agents of dermatophytosis in human and animals. Dermatophytopses usually appear as chronic infections and do not respond well to current antifungal drugs. These drugs also have numerous side effects and their continued administration causes resistance against their therapeutic effects. So there have been several attempts to discover new agents with antidermatophytic effects and less side effects. This survey was conducted to evaluate the effect of onion extract on growth and ultrastructure of two important dermatophytes *T. rubrum* and *T. mentagrophytes*. The fungi were cultured in the presence of aqueous onion extract in sabouraud dextrose broth and the cultures were incubated for 5, 10 and 15 days. Mycelial dry weight was used as the index of fungal growth rate and a portion of mycelia was processed for electron microscopy as mentioned in the Materials and Methods. The results showed that aqueous onion extract can inhibit the growth of *T. rubrum* and *T. mentagrophytes* in a dose and time dependent manner. This inhibition is more revealed for *T. mentagrophytes* compared with *T. rubrum* and the maximum inhibition of growth was observed for both dermatophytes in 6.25% concentration of aqueous onion extract. Study of the effect of 3% (v/v) aqueous onion extract on fungal ultrastructure showed massive changes as deformation and swelling of mycelia, disruption of the mycelial cell wall, separation of filamentous material from mycelial cell wall, severe degeneration of mycelia and conidia and disruption of intracellular organelles especially nuclei and mitochondria. These morphologic changes were also greater for *T. mentagrophytes* than *T. rubrum*. It is assumed that there are some factors in *T. rubrum* causing its resistance to antifungal agents. On the whole it can be concluded that aqueous onion extract can inhibit the growth of *T. rubrum* and *T. mentagrophytes*. This effect is probably caused by disruption and deformation of the cell wall structure and intracellular organelles. Therefore, aqueous onion extract can be used in antifungal preparations with future determination of its effective substances.

**P518** Effects of 5-hydroxytryptamine on virulence properties of *Candida albicans* in vitro

A. Mayr, G. Hinterberger, M.-P. Dierich, C. Lass-Flörl

Innsbruck, A

**Objectives:** In human beings selective serotonin reuptake inhibitors (SSRIs) modify the concentration of 5-hydroxytryptamine (5-HT) and lead to an increase of 5-HT during therapy with SSRIs. Recently, we found that 5-HT has antifungal activity against *Candida* spp. in vitro. Therefore, we investigated the direct influence of 5-HT against clinical isolates of *C. albicans* (CBS 5982) with regard on direct effects on virulence properties of this fungal pathogen in vitro. We examined the influence of 5-HT on enzymatic activity with regard to extracellular phospholipases and the production of secreted aspartyl proteinases (SAPs).

**Methods:** Serial dilutions from 25–0.09 mg/mL 5-HT were used for testing extracellular phospholipase activity and SAPs. The extracellular phospholipase activity of *C. albicans* was measured by the egg yolk agar method. The assay for *Candida* secreted aspartyl proteinases was assessed by a modified version of the original protocol by Ollert et al.

**Results:** An interaction between 5-HT and virulence properties of *C. albicans* in vitro could be clearly demonstrated. A significant decrease (P < 0.05) on phospholipase activity and SAPs of *C. albicans* at 5-HT concentrations of 25–0.09 mg/mL compared with positive control was observed. At a range of 25–12.5 mg/mL 5-HT the most strongest effect on phospholipase activity was
Studies on \textit{in vitro} antimicrobial activity of vitreous substitutes against \textit{Candida albicans}


\textbf{Objectives:} Vitreoretinal surgery is one of the most rapidly developing fields of ophthalmology. However, it may also be responsible for serious blindness as a result of complications including fungal endophthalmitis. Silicone oil (PDMS 5000), perfluorodecaline (PFCL) and perfluorohexyloctane (F6H8) have been used as internal tamponading agents in vitreous surgery. The aim of the study was to evaluate and compare possible antimicrobial properties of PDMS 5000, PFCL and F6H8 \textit{in vitro} against \textit{Candida albicans}, which is considered one of the major causative agents of postoperative fungal endophthalmitis.

\textbf{Materials and Methods:} The clinical isolate of \textit{C. albicans} was selected. The fungus was separately inoculated into PDMS 5000 (produced by AcriMed, Germany), PFCL (produced by AcriMed, Germany) and F6H8 (produced by Fluoron, Germany). Control inoculations into physiological saline and sugar broth were performed. The fungal suspensions in each vitreous substitute, physiological saline and sugar broth were diluted according to serial dilution procedure and plated in Petri dishes with Sabouraud medium. After 48 h incubation fungal CFUs were counted.

\textbf{Results:} \textit{Candida albicans} CFUs decreased significantly in all used vitreous substitutes. CFUs of \textit{C. albicans} in PDMS 5000 decreased up to the fifth day of the study; afterwards no growth was observed. Fungal growth was inhibited on the medium inoculated with \textit{Candida} suspension in PFCL up to the third day of the study but single colonies appeared after the fifth day. For fungal inoculations in F6H8 single colonies on Sabouraud medium were present during the whole period of the study. No total elimination of the fungal growth was observed for PFCL and F6H8. CFUs of \textit{C. albicans} declined slightly in physiologic saline. A growth pattern similar to the growth curve of microorganisms was observed in sugar broth.

\textbf{Conclusion:} Our study indicates that silicon oil, perfluorodecaline and perfluorohexyloctane could have antifungal properties against \textit{C. albicans}, which is considered one of the major causative agents of postoperative fungal endophthalmitis. Additionally, silicon oil seems to be the most efficient vitreous substitute inhibiting fungal growth.

\textbf{P520} Antifungal resistance patterns among oral \textit{Candida} species from patients receiving anticancer therapy

I. Glazar, M. Abram, S. Pezelj-Ribaric, B. Miletic, I. Brekalo Prso, B. Matica Rijeka, Zagreb, HR

\textbf{Objectives:} Oral fungal infections are frequent complications in immunocompromised patients. This study was conducted to understand the current status of yeast resistance to available antifungal agents among patients receiving anticancer therapy.

\textbf{Materials and Methods:} Oral swabs were collected from 216 hospitalised patients receiving chemotherapy or radiotherapy treatment for malignant disease. No patients in this series had previous episodes of oral candidiasis or had received any prophylactic antifungal therapy. Yeast isolates were tested for their susceptibility to five antifungal agents (ampoterhin B, 5-flucytosine, flucanazole, itraconazole and ketoconazole) by the commercially available E-test, using RPMI + 2% glucose + MOPS agar inoculated with 0.5 McFarland yeast suspension in saline and incubated at 35°C/ambient in bag, for 24 and 48 h. For interpretation we used NCCLS M-27-A2, 2002 recommendation.

\textbf{Results:} At time of sampling, 46 (21.3\%) patients were found to be colonised with yeasts, of which 42 (91.3\%) were \textit{Candida albicans} and only four (8.7\%) non-albicans \textit{Candida} species. Antifungal susceptibility patterns showed that 100\% of isolates were susceptible to amphotericin B (mean MICs=0.098 \(\mu\)g/mL), 5-flucytosine (mean MICs=0.11 \(\mu\)g/mL) and flucanazole (mean MICs 1.64 \(\mu\)g/mL) while 91.3\% were susceptible to itraconazole (mean MICs 0.06 \(\mu\)g/mL) and ketoconazole (mean MICs 0.029 \(\mu\)g/mL). Of five resistant yeasts, three were non-albicans species showing simultaneous resistance to both drugs.

\textbf{Conclusion:} The frequency of resistant \textit{Candida} is still very low in cancer patients at the Clinical Hospital in Rijeka. However, it is important to follow continuously the distribution and susceptibility patterns of yeasts, which should contribute in developing optimal prophylactic strategies, as well as, in reducing clinically detectable oral candidiasis in this group of patients.
for determination of MICs for AZ and CL by E-test on Columbia agar, incubated for 24 h at 37°C in 5% CO₂.

Results: After exclusion of one subject in the AZ- and two in the CL-treated group who took antibiotics before completion of the trial and correction for baseline differences, the number of macrolide-resistant streptococcal isolates (expressed by the ratio Col/E/Col) did not differ significantly between the groups. Although higher MICs of isolates from CL-treated individuals compared with those from the AZ-treated group on days 32, 48 and 62 (but not earlier or later) were observed, these differences were not significant. Two subjects from the AZ-treated group were excluded from this analysis because no growth was found on Col + E at day 0 or 90.

Conclusion: The long half-life of AZ does not induce higher rates of antimicrobial resistance in oral streptococci compared with CL with a shorter half-life and lower mucosal concentrations.

**P522** Unchanged susceptibility of key respiratory pathogens to telithromycin postintroduction in Germany

R. Reinert, A. Rodloff, D. Felmingham – The PROTEKT Study Group

Objectives: PROTEKT – a global, longitudinal, international surveillance programme established in 1999 to study the antimicrobial susceptibility of common bacterial pathogens associated with community-acquired respiratory tract infections (RTIs) – has now completed its third year. This analysis was undertaken to track and assess the susceptibility of community-acquired RTI isolates to the ketolide antibacterial telithromycin since its introduction in Germany in October 2001.

Methods: MICs of community-acquired RTI isolates collected with surveys of pathogens susceptible to telithromycin (TEL) against these isolates. Nitrocefin test was used to detect BLA production. Method following NCCLS M100-S12 recommendations. Breakpoints approved by NCCLS SAST, January 2003). A total of 238 isolates of S. pneumoniae were also collected in this study, of which 73.1% (174) were resistant to macrolides (erythromycin MIC = 1 mg/L). TEL maintained high in vitro activity against S. pneumoniae, with 100% of isolates susceptible to TEL at concentrations 1 mg/L.

Conclusions: Approximately 19% of H. influenzae isolates collected from children in Japan were BLNAR (and therefore co-resistant to a number of other antibiotics including amoxicillin–clavulanate, ampicillin–sulbactam and first and second generation cephalosporins), with a further 12.9% BLNAI and 7% BL+. TEL has high in vitro activity against those pathogens targeted in the empiric treatment of CARTIs in Japan.

**P524** Susceptibility of recent paediatric respiratory isolates of Streptococcus pneumoniae and Haemophilus influenzae in Spain


Objectives: Resistance to penicillin and/or erythromycin in Streptococcus pneumoniae, and β-lactamase (BLA) production in Haemophilus influenzae are well-known predictor factors for treatment failure of acute otitis media in children. It is therefore critical to monitor rates of resistance in the community in order to tailor empiric therapeutic recommendations.

Methods: A prospective, multicentre (25 hospitals in 13 Autonomous Communities, CCAA) antimicrobial survey was carried out between November 2001 and October 2002. A total of 373 consecutive S. pneumoniae and 438 H. influenzae isolates from children with community-acquired respiratory tract infections were collected and sent to a central laboratory for further processing. Susceptibility testing was performed by a semiautomated microdilution method following NCCLS M100-S12 recommendations. Breakpoints for penicillin and erythromycin were ≥2 and ≥1 mg/L, respectively. Nitrocefin test was used to detect BLA production.

Results: Excluding those CCAA with less than 18 isolates (11 paediatric clinical isolates: 36 β-lactamase producing; 21 penicillin resistant; and 38 H. influenzae: 21% BLA producers) for the sake of accuracy, mean penicillin resistance was 20% (95% CI 14–26), whereas erythromycin resistance was 48% (95% CI 36–60) for S. pneumoniae, and the MLSB phenotype was dominant (90%). As for H. influenzae, BLA production was 14% (95% CI 9–19). Rates by CCAA are given in the table.
Conclusions: BLA production in paediatric respiratory H. influenzae was below 20% and around 15%. Currently, resistance to penicillin does not seem to keep increasing among paediatric respiratory isolates and remains around 20%. However, resistance to erythromycin among paediatric pneumococcal isolates is alarmingly high, with regions showing more than 60%. Only Cantabria had <20%.

Results: Beta-Lactamase production was below 20%. Macrolides do not provide adequate coverage against the two key bacterial pathogens involved in infantile respiratory infections in Spain and therefore, in the absence of sounding reasons, empirical prescription of macrolides should be avoided.


A. Garcia-Perea, C. Garcia-Rey, C. Garcia-Riestra, R. Landinez, M. De-la-Rosa, P. Alomar, J. Garcia-de-Lomas, L. Aguilar – The Spanish Surveillance Group for Respiratory Pathogens

Objectives: The role of Haemophilus influenzae (and of Streptococcus pneumoniae) in exacerbations of chronic bronchitis, acute otitis media and community acquired pneumonia along with their capability to produce beta-lactamase (BL) are the rationale to add clavulanate to amoxicillin, to use second generation cephalosporins or a respiratory fluoroquinolone to treat these infections. Monitoring of its rate of BL production and of the phenotype BL-negative ampicillin resistant (BLNAR) is strongly recommended.

Methods: A prospective, multicentre (25 hospitals) antimicrobial survey was carried out between November 2001 and October 2002. A total of 2207 consecutive H. influenzae isolates from adult patients with community-acquired respiratory tract infections were collected and sent to a central laboratory for further processing. Susceptibility testing was then performed by a semiautomated microdilution method following NCCLS M100-S12 guidelines and breakpoints against antibiotics commonly used. Chromogenic nitrocefin was used to test BL production.

Results: Beta-Lactamase production was detected in 466 of 2207 (21.1%) isolates (95% CI 17.5–24.4). Additionally, there was 4.0% of BLNAR isolates (95% CI 2.7–6.4). Coamoxiclav, cefuroxime, cefonicid, ciprofloxacin and azithromycin displayed an excellent in vitro activity. In contrast, full susceptibility to cefaclor and clarithromycin was found only in 82.1 and 72.3% of isolates, respectively.

Conclusions: Beta-Lactamase production seems to be decreasing compared with previous surveillances done in Spain and currently stands around 20%, although there are hot spots in the south-east of the peninsula (Valencia and Murcia) with rates around 35–40% and in the north (Vizcaya) with 30%. BLNAR phenotype appears stable around 4% of isolates, but there also were centres with rates between 15 and 19% (Ciudad Real in the centre and Vizcaya) that must be closely watched. The best tested oral anti H. influenzae agents from an in vitro point of view were coamoxiclav, cefuroxime, azithromycin and ciprofloxacine. Clarithromycin and cefaclor displayed the worst susceptibility rates and should be avoided, as better options are available.


K. Fickweiler, U. Fickweiler, A.C. Rodloff
Leipzig, D

Objective: Streptococci belong to the most frequent causative agents of infections in the ENT area. In recent years, resistance of this group of organisms against penicillin and macrolides has increased worldwide. It was the aim of this study to monitor the resistance of beta-haemolytic streptococci, pneumococci and viridans streptococci isolated from infected patients of the ENT department of the University Hospital in Leipzig.

Methods: Since 1999, all microbiological results for patients of the ENT department were recorded according to the clinical diagnosis. In addition, MIC determinations were made for all isolates and penicillin, cefuroxime and roxithromycin. MIC values were established employing E-test strips according to the recommendations of the manufacturer. In order to study possible trends in resistance development, results of strains tested in 1999 were compared with those obtained during 2002/2003.

Results: During the study periods, altogether 262 strains of streptococci were isolated, mostly from patients with peritonsillar and neck abscesses, acute otitis media and acute sinusitis and rhinitis (1999 n = 112, 2002/2003 n = 150). Fifty-three were identified as group A streptococci, 97 were pneumococci and 112 were other viridans streptococci.

Results: All group A streptococci and 99% of the pneumococci were susceptible for penicillin (MIC ≤ 0.125 mg/L). An increasing resistance rate (MIC ≥ 2 mg/L) was observed for viridans streptococci (9% in 1999 vs. 3% in 2002/2003). All streptococci tested were susceptible for cefuroxime (MIC < 2 mg/L). Interestingly, the resistance rate for group A streptococci and roxithromycin (MIC ≥ 8 mg/L) was decreasing (15% in 1999 vs. 4% in 2002/2003), while an increase was observed for pneumococci (4.5% in 1999 and 15% in 2002/2003) and for viridans streptococci (2.5% in 1999 vs. 10% in 2002/2003).

Conclusion: We confirmed that there is no penicillin resistance in group A streptococci. In spite of worldwide reports on rapidly increasing resistance rates for penicillin and macrolides in pneumococci, we observed only limited alterations in the resistance rate of pneumococci and other viridans streptococci. Surprisingly, a large number of viridans streptococci were recovered from patients with abscesses. The pathogenic role of these isolates requires further analysis.

P527 The comparative in vitro activity of moxifloxacin against respiratory tract pathogens isolated during 2003 from Libra-targeted surveillance

I. Morrissey, A. Colclough, L. Viljoen, K. Dowling, M. McKeon, T. Velman
London, UK

Objectives: To assess the antimicrobial agent susceptibility of Haemophilus influenzae (HI) and Streptococcus pneumoniae (SP) isolates causing community-acquired respiratory tract infections from worldwide locations during 2003.

Methods: A total of 35 centres in seven countries submitted 1530 HI and 1541 SP. Bacteria were re-identified and their susceptibility to penicillin G (PEN, SP only), ampicillin (AMP, HI only), amoxicillin-clavulanate (AMC), azithromycin (AZI), ceftriaxone (CTX), levofloxacin (LFX), gatifloxacin (GFX) and moxifloxacin (MFX) was determined using the NCCLS broth microdilution method and breakpoints at a central laboratory.

Results: All HI were fully susceptible to AMC, CTX, LFX, GFX and MFX. AMP resistance in HI was: France (43.0%), Germany (20.2%), Italy (11.3%), Spain (13.8%), Mexico (27.5%), South Africa (6.4%) and USA (34.7%). Eleven AZI nonsusceptible HI strains were found overall (0.7%). SP resistance (number of centres, number of isolates per country) is shown below. Of the 1541
SP, 293 (19.0%) were resistant to two or more of PEN, AMC, CTX, LFX and AZI (GFX and MFX were omitted to avoid duplication). These multi-resistant (MDR) SP were 98.3, 92.5, 27.3, 10.9, 3.4 and 1.4 resistant to PEN, AZI, AMC, CTX, LFX and MFX, respectively.

Conclusion: For HI, resistance to AMP was prevalent in many countries but full susceptibility was seen with MFX and other agents. With SP, AZI and PEN resistance was very high in many countries. Relatively high AMC and CTX resistance was also found in South Africa. Resistance to LFX and GFX was higher than MFX, where <1% resistance was seen in all countries. Virtually all MDR SP were resistant to PEN and AZI and over 1/4 resistant to AMC. MFX was the most active agent against both HI and SP causing community-acquired respiratory tract infection worldwide including MDR SP. MFX is therefore a valuable option for the treatment of community-acquired respiratory-tract infection.

New drugs and novel therapeutic approaches

**P528 In vitro activity of synthetic peptides and mechanisms of resistance to colistin in pan-resistant Acinetobacter baumannii**


**Objectives:** Acinetobacter baumannii colistin resistant constitute a severe chemotherapeutical threat for nosocomial infection. Membrane-active antibiotic peptides were proposed as a good alternative. Among them synthetic hybrids cepcorin A-mellitin hybrids (CAMEs) were reported among those with highest activity.

**Objectives:** To test the in vitro activity of CAMEs against pan-resistant A. baumannii (AbPr) as putative alternative to colistin, and to study the mechanism of AbPr colistin resistance.

**Methods:** Peptides: A = CA(1–8)M(1–18), B = CA(1–7)M(2–9), C = Octanol-CA(1–7)M(2–9) and D = CA(1–7)M(5–9). MIC/MBC (NCCCLS) against 13 AbPr clinical isolates. Bacterial activity (time-killed cultures using 1 MIC, 2 MIC and 4 MIC concentrations) with four strains (NCCCLS): 208628, 201630, 183280R and 183280L. LPS-CAME affinity was determined by displacement of dansyl-LPS bound to LPS. Spheroplasts from a colistin-susceptible (ATCC19606) and two colistin-resistant (208628 and 201630) S. pyogenes strains from a colistin-susceptible (ATCC19606) and two colistin-resistant (208628 and 201630) S. pyogenes strains were prepared according to Dathe et al. (2002); their lysis induced by the peptide, were monitored by decrease in A450.

**Results:** MIC50/MIC90 (mg/L) of colistin and peptides A, B, C and D: 32/64, 4/8, 2/4, 4/4 and 4/4, respectively. MBC50/ MBC90 (mg/L) of peptides A, B, C and D: 4/8, 2/4, 4/4 and 4/8, respectively. Time-killing curves: the four peptides were bactericidal against the four strains; ‘A’ was bactericidal with all concentrations against the four strains; ‘B’ and ‘D’ were bactericidal with all concentrations against strains 183280R and 183280L, and from concentration > MIC against strain 183280L. ‘C’ was bactericidal with all concentrations against strains 208628, 201630 and 183280R, and from concentration > MIC against strain 183280L. LPS affinity for CAMEs were very similar for all of them and much higher than that for colistin, independently of the strain used to obtain LPS the same resulted with permeabilisation of inner membrane. Both, peptide A and colistin, permeabilised spheroplasts at a similar rate, independently of their colistin susceptibility. Thus, the differences in colistin activity were solely located at the outer membrane, mostly to LPS changes.

**Conclusions:** All CAMEs were bactericidal against pan-resistant clinical isolates of A. baumannii, being CA(1–8)M(1–18) the best one. Resistance to colistin is due to changes in outer membrane. CAMEs act by permeabilisation of the inner membrane, and it was achieved at much lower concentration than colistin.

**P529 New antimicrobial peptide active against methicillin-resistant Staphylococcus aureus, multi-resistant coagulase negative staphylococci, and β-haemolytic streptococci**

A. Jasir, F. Kasprzykowski, V. Lindstrom, C. Schalen, A. Grubb Lund, S; Gdansk, PL

**Objectives:** The main objective was to develop antimicrobial compounds active against methicillin-resistant Staphylococcus aureus and multi-resistant coagulase negative staphylococci and streptococci and to elucidate the mode of action of a new antibacterial oligopeptide.

**Methods:** The synthesis of Cystapep has been outlined previously and was prepared from Boc-L-valinol according to the general procedure. The antibacterial activity of Cystapep was tested by agar well diffusion. For determination of MIC and MBC concentrations, a broth dilution method was used.

**Results:** The derivative, here called Cystapep, displayed antibacterial activity against several clinically important Gram-positive bacteria. It displayed MIC and MBC of about 16 µg/mL for both S. aureus and S. pyogenes. In radial agar diffusion assays, groups A, B, C and G streptococci as well as staphylococci were generally susceptible to the action of Cystapep, whereas pneumococci and enterococci were less susceptible. Cystapep also showed high activity against methicillin-resistant S. aureus (MRSA) and multi-antibiotic resistant coagulase negative staphylococci (CNS), suggesting its mechanism of action to differ from those of most currently used antibiotics.

**Conclusion:** Cystapep was apparently as effective against various antibiotic resistant staphylococci and streptococci as against antibiotic susceptible strains of these species. In particular, in a large collection of MRSA comprising many strains with additional resistance properties, the susceptibility to Cystapep proved invariably high. Similarly, a substantial number of CNS clinical isolates involving many multi-resistant strains also showed high susceptibility to Cystapep. Presently, these staphylococci represent leading agents in nosocomial and biomaterial-associated infections posing significant therapeutic problems due to shortage of effective antibacterial agents. In addition, the susceptibility of β-haemolytic streptococci to Cystapep may prove important due to treatment problems both for invasive and superficially located infections. Although several hundreds of isolates have been studied, we have not so far observed any strains of staphylococci or β-haemolytic streptococci resistant to Cystapep. Furthermore, the possibility to select resistant mutants by repeated passages in media containing Cystapep is currently being investigated but no resistant mutants have so far been obtained.
**P530** Comparison of azithromycin and amoxicillin for treatment of adult patients with solitary erythema migrans

S. Lotric-Furlan, M. Logar, V. Maraspin, J. Cimperman, T. Jurca, F. Strel
Ljubljana, SI

Objective: To evaluate the effectiveness and side effects of azithromycin and amoxicillin for treatment of adult patients with solitary erythema migrans.

Methods: Consecutive adult patients with typical erythema migrans were enrolled in a prospective study on early Lyme borreliosis at the Department of Infectious Diseases in Ljubljana during 1997. Patients receiving antibiotics at their first visit, having clinical evidence of disseminated Borella burgdorferi s.l. infection, and/or being pregnant were excluded. They were randomised to receive either azithromycin 500 mg b.i.d. for the first day, followed by 500 mg once a day for the following 4 days (AZT) or amoxicillin 1000 mg t.i.d. for the first 5 days, followed by 500 mg t.i.d. for the following 10 days (AMO). Basic epidemiological data were obtained by means of questionnaires. Serum IgM and IgG antibody titre against B. burgdorferi s.l. were determined by IFA without absorption. Titres equal and/or greater than 1:256 were considered positive. In all patients skin biopsy had been accomplished prior to the institution of antibiotic treatment and specimen cultured in MKP medium.

Results: A total of 133 patients, 77 (57.9%) females and 56 (42.1%) males, aged 16–83 (median 49) years were included in this study. Sixty-five patients were evaluated in AZT group and 68 patients in AMO group. No differences in epidemiological and pretreatment characteristics were present comparing the two groups. Median duration of skin lesions after the institution of treatment was 7 (1–60) days in the AZT group and 7 (2–180) days in the AMO group (P = 0.325). During the follow-up of 12 months none of the patients developed major late manifestations of Lyme borreliosis but in six patients severe minor manifestations appeared: in two (3.1%) from AZT group and in four (5.9%) included in AMO group. Isolation rates of B. burgdorferi s.l. from skin before treatment (25/65 vs. 33/68; P = 0.319) as well as 2–3 months after therapy (0/25 vs. 0/33) were comparable for the two groups. Three (4.6%) AZT group patients and one (1.5%) patient from AMO group reported mild gastrointestinal discomfort (P = 0.358).

Conclusions: Treatment of adult patients with solitary erythema migrans with two different antibiotics exhibited equal effectiveness and comparable side effects. The outcome of borrelian infection after one year was favourable in both treatment groups.

**P531** Comparative in vitro activity of ABT-492 and six other antimicrobial agents against anaerobic bacteria

E. Sillerström, E. Wahlund, C.E. Nord
Stockholm, S

Objectives: ABT-492 is a new quinolone active against aerobic and anaerobic bacteria involved in respiratory tract infections, urinary tract infections, blood stream infections, and skin and soft tissue infections. ABT-492, 1-(6-amino-3,5-difluorophenylamino-2-y)-8-chloro-6-fluoro-(3-hydroxyazetidin-1-yl)-4-oxo-1,4-di hydroquinolone-3-carboxylic acid, is more potent in vitro than other new quinolones. The present investigation determined the in vitro activity of ABT-492 against anaerobic bacteria recently isolated from human infections. The activity was compared with that of moxifloxacin, piperacillin, cefoxitin, imipenem, clindamycin and metronidazole.

Methods: The 369 anaerobic strains investigated were isolated from respiratory tract infections, gastrointestinal infections, gynaecological infections, and skin infections. All strains were identified using morphological tests, biochemical tests and gas-liquid chromatography. The antimicrobial susceptibility tests were performed by the agar dilution method according to NCCLS. The testing was performed on Brucella agar supplemented with 5 mg haemin and 1 mg vitamin K per litre and 5% laked sheep blood. The plates were read after 48 h of incubation at 37°C in anaerobic jars. Four control strains were used for monitoring the antimicrobial susceptibility tests: Bacteroides fragilis ATCC 25285, B. thetaiotaomicron ATCC 29741, Clostridium perfringens ATCC 13124 and Eubacterium lentum ATCC 43055.

Results: ABT-492 and imipenem were the most active antimicrobial agents tested: Peptostreptococci (52 strains) had the following minimum inhibitory concentrations: ABT-492, range 0.008–0.25 mg/L; imipenem, range 0.016–0.064 mg/L. Propionibacterium acnes (32 strains): ABT-492, 0.032–0.125 mg/L; imipenem, 0.002–0.05 mg/L. Clostridium perfringens (50 strains): ABT-492, 0.008–0.032 mg/L; imipenem, 0.016–0.5 mg/L. Clostridium difficile (50 strains): ABT-492, 0.008–0.45 mg/L; imipenem, 8 mg/L. Bacteroides fragilis (100 strains): ABT-492, 0.032–0.125 mg/L; imipenem, 0.064–0.25 mg/L. Porphyromonas and Prevotella species (55 strains): ABT-492, 0.008–0.5 mg/L; imipenem, 0.016–0.25 mg/L. Fusobacterium nucleatum (30 strains): ABT-492, 0.008–0.125 mg/L; imipenem, 0.008–0.064 mg/L.

Conclusions: ABT-492 is active against anaerobic bacteria and might be useful in the treatment and prophylaxis of anaerobic infections. Clinical trials are therefore warranted.

**P532** Iclaprim, a novel diaminopyrimidine antibiotic: synergy studies with different classes of antibiotics

L. Weiss, D. Gillessen, S. Hawser, K. Islam
Münchenstein, CH

Objectives: Iclaprim (formerly AR-100) is a novel broad-spectrum diaminopyrimidine antibiotic that exerts its antibacterial action through the specific and selective inhibition of bacterial dihydrofolate reductase. The in vitro synergistic potential of Iclaprim with thirty different antibiotics was evaluated using several Gram-positive and Gram-negative pathogens.

Methods: MICs were performed using the NCCLS micro dilution methods. Pathogens used were: Staphylococcus aureus, S. pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and Klebsiella pneumoniae. Checkerboard experiments were used to determine the potential synergy or antagonism with 30 antibacterial agents. Synergism was calculated using the FIC index. [Synergy was defined whereby the SigmaFIC were <0.5, indifference (no synergy or antagonism) whereby SigmaFIC was >4].

Results: Iclaprim showed potent MIC against most of the pathogens used in this study with MICs ranging from 0.063 to 16 μg/mL. Iclaprim was also active against Trimethoprim-resistant strains of S. aureus and S. pneumoniae. In terms of the synergistic potential, Iclaprim was highly synergistic with the two sulphonamides tested, namely sulphamethoxazole and sulphadiazine against the majority of isolates used. By contrast, Iclaprim showed no synergy with the other 28 antibiotics including macrolides, aminoglycosides, quinolones, penicillins, trimethoprim, rifampicin, tetracycline and vancomycin. Importantly, no antagonism was observed between Iclaprim and the 30 antibiotics used in this study.

Conclusions: Iclaprim was synergistic with sulphonamides and showed neither synergy nor antagonism with other classes of antibiotics.

**P533** Anti-pneumocystis activity of iclaprim, a reliable therapeutic alternative against Pneumocystis pneumonia

Lille, F; Münchenstein, CH

Objective: Available drugs effective against severe forms of Pneumocystis pneumonia (PCP) are limited; often induce adverse reactions with treatment failure common. Moreover, recent reports suggest the emergence of P. jiroveci resistance to sulphapyridine, the most commonly used drugs to treat pneumocystosis. The unmet medical need is that no reliable therapeutic alternative to Trimethoprim/Sulfamethoxazole (TMP/SMX) is available to
physicians; pentamidine exhibits significant toxicity and atovaquone is currently used only against mild forms of PCP. Iclaprim (formerly AR-105), a novel broad-spectrum diaminopyrimidine compound that exhibits antimicrobial activity through inhibition of dihydrofolate reductase, may represent a new therapy for the treatment of this unmet medical need. For this reason the objective of the present work was to test the activity of Iclaprim against P. carinii using highly efficient in vitro and in vivo models.

Methods: The activity of Iclaprim against P. carinii was tested both in vitro, using an axenic culture system, and in vivo using P. carinii endotracheally inoculated corticosteroid-treated rats, the most reproducible PCP model available. Animals were orally administered with Iclaprim (5, 25 and 50 mg/kg/day), Iclaprim/SMX (5/25, 25/125, 50/250 mg/kg/day), TMP (50 mg/kg/day) or TMP/SMX (50/250 mg/kg/day) once a day for 10 consecutive days.

Results: Iclaprim showed in vitro a high anti-Pneumocystis activity, with an EC50 value of 20.3 μg/mL. Iclaprim/SMX combination (proportion 1/5) showed a significant synergistic activity with an EC50 value of 13.2/66 μg/mL. TMP/SMX was the least potent compound (EC50 of 51/255 μg/mL). In vivo, although Iclaprim and TMP showed a similar activity, the Iclaprim/SMX combination was more potent (98.5 ± 0.9% of inhibition for 25/125 mg/kg/day) than TMP/SMX (86.6 ± 7.1% of inhibition for 50/250 mg/kg/day).

Conclusions: These data suggest that Iclaprim may constitute a reliable therapeutic alternative for treating severe forms of PCP.

### Table 1. In vitro activity of LB11058 and selected agents against invasive NM strains

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC50</th>
<th>MIC90</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB1 1058</td>
<td>&lt;0.008</td>
<td>&lt;0.008</td>
<td>&lt;0.008</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>Penicillin</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Gatifloxacine</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Rifampin</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
</tr>
</tbody>
</table>
Results: The MICs of BAL 9141 for strains producing β-lactamases of groups 1 (CMY-2, CMY-7, DHA-1, FOX-1, FOX-2 and LAT-1), 2b (FIM-1, LX-1, SHV-1, TEM-1, TEM-2 and TEM-90) and 2a (TEM-30 to TEM-36) as defined by Bush et al. (1) ranged from 0.06 to 2 mg/L. However, the MIC of LAT-2 producing K. pneumoniae strain N10 was 32 mg/L. BAL 9141 displayed inconsistent activity against extended spectrum β-lactamase (ESBL)-producing strains (group 2b β-lactamases) of E. coli, Klebsiella and P. mirabilis. The MICs of BAL 9141 for E. coli strains harbouring ESBLs SHV-2, SHV-4, TEM-3, TEM-21, or TEM-50 as well as those of P. mirabilis strain 33 producing TEM-52 and K. oxytoca hyperproducers of K1 β-lactamase (range of MICs 8–64 mg/L) exceeded those for E. coli strains producing ESBLs SHV-3, TEM-5 to TEM-10, or TEM-20 (range of MICs 0.06–2 mg/L). BAL 9141 had also inconsistent activity against E. coli producing OXA enzymes. MICs were 4–8 mg/L for OXA-5 and OXA-7 compared with 0.06–0.125 mg/L for OXA-1 to OXA-4. Pseudomonas aeruginosa strains producing carbencillin- or oxacillin-hydrolysing enzymes (group 2c and 2d) were associated with MICs ranging from 1 to 64 mg/L, whereas an MIC of 0.06 mg/L was recorded for the Aeromonas hydrophila strain producing AER-1. In general, MICs of BAL 9141 were comparable with those of aztreonam.

Conclusions: BAL 9141 displayed good activity against Gram-negative bacteria producing various types of β-lactamase. In addition to its anti-MRSA activity, BAL 9141 maintains the potent activity of third generation cephalosporins and aztreonam.

Reference

P537 MIC determination of the anti-pneumococcal activity of BAL 9141 compared with other agents
P. Appelbaum, D. Hoellman, M. Jacobs
Hershey, Cleveland, USA

Background: Pneumococcal drug resistance has become a worldwide problem.

Objective: This study examined the anti-pneumococcal activity of BAL9141, a new broad-spectrum intravenous cephalosporin, compared with those of amoxicillin, imipenem, etrapenem, ceftizoxime, cefotaxime, cefuroxime, levofloxacin, moxifloxacin, azithromycin, clarithromycin, linezolid, quinupristin/dalfopristin, daptomycin, vancomycin and teicoplanin.

Methods: The MICs of all β-lactams rose with those of penicillin G, BAL9141 had the lowest MICs of all cephalosporins tested. Using NCCLS IV cephalosporin nonmeningeal pneumococcalbreakpoints, 98.3% of strains were S, 1.3% L, and 0.3% R to BAL9141; compared with 73.2% S, 20% I, and 6.7% R with ceftriaxone and 70.9% S, 24% I, and 5% R with cefepime. All strains were S to quinupristin/dalfopristin, daptomycin, vancomycin and teicoplanin, and 93.3% S to telithromycin (breakpoint 0.5 μg/mL).

Conclusions: BAL 9141 had the lowest MICs (similar to carbapenems) of all IV cephalosporins tested against pneumococci irrespective of a strain’s β-lactam, macrolide or quinolone susceptibility.

P538 Efficacy and safety of pharmacokinetically enhanced amoxicillin/clavulancate 2000/125 mg in adult patients with acute bacterial sinusitis in Hungary
L. Tamas, P. Kovacs, G. Horvai, M. Twynholm
Budapest, HUIN; Harlow, UK

Objectives: Recent increases in the prevalence of antimicrobial resistance among common respiratory pathogens have caused concern worldwide. Pharmacokinetically enhanced amoxicillin/clavulanate 2000/125 mg was designed using pharmacokinetic/pharmacodynamic principles to achieve eradication of common respiratory pathogens, including penicillin-resistant Streptococcus pneumoniae (PRSP, penicillin MICs ≥2 mg/L) with amoxicillin/clavulate MICs of up to and including 4 mg/L.

Methods: In this multicentre, international, open-label, noncomparative study, patients with acute bacterial sinusitis (ABS) were given amoxicillin/clavulanate 2000/125 mg as two 1000/62.5 mg tablets twice daily for 10 days. Diagnosis of ABS was based on clinical and radiological findings. Patients were required to have sinus aspiration for bacteriological assessment at screening and, for patients in whom treatment failed, at the time of treatment failure. Treatment success was based on eradication of the initial infecting pathogen or, in the absence of an evaluable repeat sample, clinical evidence of eradication. Data from a subset of patients recruited in Hungary are presented here.

Results: A total of 222 patients received study medication. The mean age of patients was 42 years and the majority of patients were female (59.5%). Overall, 127 isolates were cultured from 109 patients [bacteriology intent-to-treat (bITT) population]. Streptococcus pneumoniae was the most frequently isolated pathogen, identified in 40.4% (44/109) of patients in the bITT population. Success in the bITT population at follow-up (days 17–28, primary efficacy endpoint) was 87.2% (95/109). Of 14 patients who were not successes, seven were confirmed bacteriological failures and seven had a response of ‘unable to determine’. In patients with S. pneumoniae infection, 93.2% (43/44) were successes at follow-up. Two patients had PRSP identified at screening, and both patients were successes at follow-up. Adverse events (AEs), due to any cause, were reported by 25.2% (56/222) of patients. Diarrhoea was reported as an AE by 12.6% (28/222) of patients. The majority of AEs were mild to moderate in severity. Only 2.7% (6/222) of patients withdrew from the study due to AEs.

Conclusion: Amoxicillin/clavulanate 2000/125 mg was highly effective in treating patients with ABS, particularly cases caused by S. pneumoniae, including two patients with PRSP infection, and was generally well tolerated.

P539 An in vitro evaluation of the antimicrobial activity of a novel fluoroquinolone
M.J. Robbins, D. De Rubeis, C. Dencer, L. Williams, M. Harrison, A. Bryskier, D. Fleming
London, UK; Romsneville, F

Background: Since the introduction of the fluoroquinolones, ciprofloxacin and ofloxacin over 10 years ago, there has been a small but significant increase in the number of resistant clinical
isolates of species previously susceptible to these agents. More recent fluoroquinolones have better activity against Gram-positive species while retaining activity against Gram-negative species. The prepulse method in vitro activity of the novel fluoroquinolone WCK 1152A was determined against a range of clinical isolates.

Methods: MICs were determined for 1007 clinical isolates, the majority using the NCCLS agar dilution method. The micromethod was performed when testing isolates of Streptococcus pneumoniae, Haemophilus influenzae and Moraxella catarrhalis.

Results: MICs for WCK 1152A, moxifloxacin and ciprofloxacin for respiratory tract pathogens are summarised below. Generally, the in vitro activity of WCK 1152A was similar to that of moxifloxacin. Against isolates of S. pneumoniae of defined genotype, and ‘atypical’ respiratory pathogens, WCK 1152A was some twofold to fourfold more active than moxifloxacin.

Conclusions: Improved activity against multi-resistant isolates of S. pneumoniae and ‘atypical’ pathogens indicate a potential role for WCK 1152A in the treatment of respiratory tract infections.

<table>
<thead>
<tr>
<th>MICs/L</th>
<th>WCK1152A</th>
<th>Moxifloxacin</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species (n)</td>
<td>50%</td>
<td>90%</td>
<td>90%</td>
</tr>
<tr>
<td>S. pneumoniae multi-resistant</td>
<td>0.03</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>S. pneumoniae FLQ 1/R (5)</td>
<td>0.25</td>
<td>1.00</td>
<td>1.02</td>
</tr>
<tr>
<td>H. influenzae (24)</td>
<td>0.015</td>
<td>0.03</td>
<td>0.015</td>
</tr>
<tr>
<td>M. catarrhalis (14)</td>
<td>0.015</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>L. pneumophila spp. (25)</td>
<td>0.015</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>M. pneumoniae (12)</td>
<td>0.015</td>
<td>–</td>
<td>0.12</td>
</tr>
<tr>
<td>C. pneumoniae (5)</td>
<td>range 0.03-0.06</td>
<td>range 0.12-0.25</td>
<td>All at 4</td>
</tr>
</tbody>
</table>

**P540 Intracellular penetration and activity of cethromycin against Legionella pneumophila in a monocytic cell line (Mono Mac 6)**

I. García, S. Ballesta, E.J. Perea, Á. Pascual
Seville, E

Objectives: Antimicrobial intracellular penetration and activity are important parameters in the therapy of infections due to intracellular pathogens. The penetration of the ketolide cethromycin into the mature monocytic cell line Mono Mac 6 and its intracellular activity compared with telithromycin against Legionella pneumophila was evaluated.

Methods: Uptake of radiolabeled cethromycin by Mono Mac 6 cells was determined by a radiometric assay. The cells were incubated with different ketolide extracellular concentrations (0.1–10 mg/L). After 48 h of incubation, intracellular bacteria were released and plated on BCYE agar.

Results: The uptake of cethromycin by Mono Mac 6 cells was rapid and not saturable. At extracellular concentration of 2 mg/L, the intracellular concentration of cethromycin was 40 times higher than extracellular one. Cethromycin was rapidly released from loaded Mono Mac 6 cells (after 30 min incubation in antimicrobial-free medium, only 20% of accumulated-drug remained cell associated). MIC of cethromycin and telithromycin against L. pneumophila ATCC 33152 were 0.008 and 0.015, respectively. At the extracellular concentrations evaluated, cethromycin impaired significantly the intracellular growth of L. pneumophila. At an extracellular concentration of 0.1 mg/L (10XCM), the percentage of bacterial inhibition for cethromycin was 90%. Only at higher extracellular concentrations (around 1 mg/L), a similar bactericidal effect against intracellular bacteria was achieved by telithromycin.

Conclusions: Cethromycin penetrates into the mature monocyte cell line Mono Mac 6, reaching high intracellular concentrations, while it remains active intracellularly. The intracellular activity of cethromycin against L. pneumophila was significantly higher than that of telithromycin.

**P542 Linezolid in the treatment of skin and soft tissue infections caused by methicillin-resistant Staphylococcus aureus**

N. Allikadic, D. Smrke
Ljubljana, SI

Objectives: LinezOLID, the first member of a new class of synthetic antibacterial agents (the oxazolidinones), has demonstrated Gram-positive spectrum of activity, involving all strains of staphylococci, including methicillin resistant Staphylococcus aureus (MRSA) and methicillin resistant Staphylococcus epidermidis (MRSE), enterococci, including vancomycin resistant enterococci (VRE), and pneumococci (including penicillin-intermediate and penicillin-
resistant strains). To assess the efficacy, safety and tolerance of intravenously and orally administered linezolid in the treatment of patients with documented MRSA skin and soft tissue infections (SSTI), our department participated in the international multicentric clinical trials.

Methods: The study was conducted in 16 patients with confirmed MRSA surgical SSTI. Patients were randomised to receive one of the following regimens: linezolid iv 600 mg every 12 h for the entire treatment period or switched to linezolid orally 600 mg every 12 h; vancomycin iv 1 g every 12 h for the entire treatment period (dose may have been adjusted by serum level determination to maintain therapeutic level). Along with linezolid the surgical procedures were performed, when necessary. Clinical and microbiological assessments were performed throughout the study. After completion of therapy all patients were short-term follow-up.

Results: The treatment with linezolid resulted in resolution of clinical signs and symptoms as well as laboratory signs of infection in all patients enrolled. No serious adverse events related to the medication were noted. The efficacy of linezolid was comparable to that of vancomycin, while its microbiological success rate at the end of treatment was significantly higher and on the follow-up.

Conclusion: Our trials suggest that linezolid is an effective, safe and well-tolerated option for the treatment of SSTI caused by methicillin resistant Staphylococcus aureus. It may be regarded as a valuable antimicrobial agent to control the increasing occurrence and spread of infections caused by resistant Gram-positive strains or in patients who cannot tolerate standard antimicrobial therapy.

P543 Linezolid tolerance among multiresistant nasopharyngeal isolates of Streptococcus pneumoniae

A. Malm, I. Korona-Glowniak, A. Kalasiewicz
Lublin, PL

Objectives: Linezolid, a synthetic compound belonging to a new class of antibiotics called the oxazolidinones, shows antipneumococcal activity. Nasopharyngeal isolates of S. pneumoniae from healthy children are useful for predicting antimicrobial susceptibility of pneumococci in a given population. We assess in vitro activity of linezolid against multiresistant strains of S. pneumoniae isolated from nasopharynx of healthy children.

Methods: 58 strains of S. pneumoniae were tested, including 22 isolates sensitive to penicillin (PSSP) and 36 – relatively resistant to penicillin (RRSP). All strains were resistant to at least three antimicrobials, including erythromycin, clindamycin, tetracycline, chloramphenicol or cotrimoxazole. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values for linezolid were determined by broth microdilution method. The bacteriostatic or bactericidal effects of linezolid were assessed by a time-kill assay. Sensitivity of isolates to optochin and their bile solubility were tested by conventional methods.

Results: Linezolid inhibited all isolates tested with MICs ranging from 0.25 to 1 mg/L, showing similar activity against PSSP and RRSP strains. This indicates that all strains, including those resistant to all antimicrobials used, were sensitive to linezolid. Although the majority of the tested isolates were sensitive to bactericidal effect of linezolid with MBC = 8 mg/L or less, three of them (1 RRSP, 2 PSSP) were killed at high concentrations of linezolid – MBC = 16 or 32 mg/L and had a high MBC/MIC ratios 32 or 64. Linezolid tolerance was confirmed by monitoring viability of these strains during exposure to 20 mg/L of this antibiotic, that is similar to its maximal serum concentration after standard dosing. All tolerant strains were sensitive to optochin and lysed with desoxycholate, which indicates autolysis production. The prevalence of linezolid tolerance among the tested multiresistant pneumococci was 5.17%.

Conclusions: Although it has not been found linezolid resistance in S. pneumoniae, some pneumococcal strains were insensitive to bactericidal effect of this antibiotic. Linezolid tolerance in clinical isolates may represent a potential risk, especially in patients with suppressed immune response.

P544 New penams (6-APA) and cephems (7-ACA) with bulky T-shaped side-chains: synthesis and antibiotic activities

Brussels, B

Objectives: Discovered more than 50 years ago, beta-lactam antibiotics remain of large interest because of their potentially large spectrum and low intrinsic toxicity. A large number of semi-synthetic derivatives have been obtained, but few have explored the possibility to use bulky side chains in an attempt to more fully block the catalytic crevice in PBPs. In this context, we have synthesised penam and cephem derivatives bearing two morpholine rings attached to their side-chains. We report here on two typical compounds (DEMO-Pen and DEMO-Cef; see structures in the figure) modelled after benzylpenicillin and the corresponding methoxy-acetyl-cephem.

Methods: DEMO-Pen and DEMO-Cef were formed via the mixed anhydride method. The whole side chain was first obtained by bis-alkylation of methyl 3,5-dihydroxybenzoate with N-chloroethylmorpholine, and made into a pentafluorophenyl ester. The latter was coupled with 6-APA and 7-ACA to give the corresponding penam and cephem derivatives. MIC’s were determined by common agar dilution method in comparison with ampicillin and cefadroxil against both collection strains and clinical isolates.

Results: The structures and stabilities of DEMO-Pen and DEMO-Cef were confirmed by 1H NMR, 13C NMR, IR, and high-resolution mass spectrometry. DEMO-Pen was active against Gram (+) organisms (S. aureus, S. pneumoniae, S. pyogenes). DEMO-Cef was more active (8x) than cefadroxil against S. pneumoniae.

P545 In vitro and in vivo activities of novel compounds active against multiresistant strains of Mycobacterium tuberculosis

U. Möllmann, L.P. Martinova, S. Rütsch-Gerdes, V.A. Makarov
Jena, D; Moscow, RUS; Borstel, D

Objectives: During the past two decades the WHO indicated an increasing number of TB patients infected by strains of M. tuberculosis resistant to most of the available drugs. In contrast it has been nearly 30 years since the introduction of a novel compound for the treatment of TB. We synthesised series of analogues of our newly discovered class of antimycobacterial compounds to enhance in vitro and in vivo activity by structure activity relationship studies.

Methods: Molecules were derived by specific methods of classical synthesis. In vitro activity was determined in a first screen against
a variety of microorganisms (Gram-positive and Gram-negative bacteria, yeast, fungi) including fast growing mycobacteria, than against sensitive strains and drug resistant clinical isolates of Mycobacterium tuberculosis. In vivo activity was tested in a murine model of TB infection. Acute and chronic toxicity were checked in mice.

Results: While starting compounds were active against Gram-positive bacteria, mycobacteria, fungi and yeast, in the course of the investigations activity was focused on bacteria and mycobacteria. Various analogues demonstrated high in vitro activity against M. tuberculosis including clinical isolates and MDR strains. MIC's of the most advanced compounds for M. tuberculosis H37Rv and clinically isolated MDR strains were <0.78 to <0.063 µg/mL. The compounds were therapeutically active after oral application in mice infected with M. tuberculosis H37Rv with 100% survival rates. The LD50 in mice after oral application was >500 mg/kg for all compounds. Additionally, synthesis of the compounds is efficient and inexpensive.

Conclusions: Considering the activity of the novel compounds against MDR strains of M. tuberculosis the mechanism of action must be different to that of the existing therapeutics. Because of the narrow spectrum of activity and the low toxicity this new class of antimycobacterial compounds represents a promising lead candidate for low cost drugs to overcome MDR-TB with reduced side effects.

**Methods:**
- **Results:** The search for the novel antiviral substances active against Epstein–Barr virus (EBV) is a topical problem since the persistent EBV infection alters immune status promoting the development of adenocarcinomas and lymphoproliferative diseases. Moreover, EBV like other herpesviruses affects central and peripheral nervous system being involved in the pathogenesis of meningoencephalitis, arachnoencephalitis and meningitis. Anti-EBV activity of the substances was assessed in EBV-infected lymphoblastoid Raji cells. The substances were assayed within broad concentration ranges. The activity of drugs was studied at its dosing simultaneously with infected, 24 h prior to infected and in 24 h after to infected. The maximally tolerable concentrations for the cell line being assayed amounted to 150 µg/mL.
- **Conclusions:** To summarise, the substances under study possess significant anti-EBV activity and may be the advantageous for the therapy of EBV-associated diseases.

**Abstracts**

**P546** Basidiomycete metabolites attenuate virulence properties of Candida albicans in vitro

L. Pleyer, S. Ressler, A. Berg, M.P. Dierich, R. Würzner
Innsbruck, A; Jena, D

Objectives: The previously used stragem of targeting a singular cellular metabolic or biosynthetic process in antifungal drug design has proven inadequate, especially with regard to the increasing drug-resistance. We therefore studied the recently discovered basidiomycete protease inhibitors Aureoquinone and Laccardiones A and B for their ability to block fungal adhesion and to inhibit candidial secreted aspartate protease (Sap) release.

Methods: Inhibition of adhesion was tested on endothelial and epithelial cells, Sap antigen concentrations by specific ELISA and their activity by enzymatic cleavage of bovine serum albumin.

Results: The inhibition of C. albicans adhesion to the epithelial cell line Hela S3 was shown to be dose dependant and highly significant (24% inhibition with Aureoquinone, 35% with Laccardione A and 56% with Laccardione B at 10 µg/mL, respectively), clearly marking Laccardione B as the front runner. The inhibitory effect of Laccardione B on candidal adherence to the endothelial cell line EAhy 926 was an even greater (highly significant 66%). Concerning Sap-release Laccardione B also proved to be the most effective among the substances tested, showing a significant 50% reduction in concentration and a reduced activity at 10 µg/mL. The inhibitory effects observed were shown to be the result of an inhibition of Sap-production and/or -release and not due to a direct interaction of the basidiomycete metabolites with Sap. For both Sap-release and activity a single application of the drug resulted in a much less pronounced effect than regular drug addition over a period of 8 days.

Conclusion: Animal studies will show whether Laccardione A and especially Laccardione B, or derivatives thereof, may also represent the 6 g tolevamer TTROD was 3.0 days for both groups. There was no statistically significant difference in TTROD between vancomycin and 6 g tolevamer in either primary or recurrent CDAD patients. The 3 g TTROD was 4.0 days, showing a tolevamer dose response. In the

**P547** Search and assessment of novel substances active against Epstein–Barr virus

S. Zagorodnya, N. Dyachenko, N. Nesterova, V. Atamaniuk, A. Novik, S. Rybalko, G. Baranova
Kyiv, UKR

Objectives: The aim of the study was to assay the anti-EBV activity of several substances prepared from the raw material of the plant origin, namely Proteflasid in the miscellaneous modified forms (drug '1' and drug '2'). Proteflasid (Ecopharm Research and Production Company, Kyiv) represents the quecetin-containing herbal extract of wild grasses Deschampsia caespitosa L. and Calamagrostis epigeos L.

Methods: PCR. An inhibition of reproduction of EBV in cell culture by Proteflasid was determined by reduction of number of genome equivalents of EBV DNA on a cell in treated vs. untreated cells. To determine it, a quantitative PCR was applied using primes and reagents of 'AMPLY-Senc-100R' (Russia) and programme 'Biomet'.

Results: The search for the novel antiviral substances active against Epstein–Barr virus (EBV) is a topical problem since the persistent EBV infection alters immune status promoting the development of adenocarcinomas and lymphoproliferative diseases. Moreover, EBV like other herpesviruses affects central and peripheral nervous system being involved in the pathogenesis of meningoencephalitis, arachnoencephalitis and meningitis. Anti-EBV activity of the substances was assessed in EBV-infected lymphoblastoid Raji cells. The substances were assayed within broad concentration ranges. The activity of drugs was studied at its dosing simultaneously with infected, 24 h prior to infected and in 24 h after to infected. The maximally tolerable concentrations for the cell line being assayed amounted to 150 µg/mL. Is rotined, that the dosing drug '2' in a syrup gives the best outcomes as contrasted to by drug '1' – about it the values chemotherapeutic indices of an indices testify. Specially good outcomes were obtained on medical operating preparations in two solvents chemotherapeutic indices 350 (1) and 1250 (2). But also preventive the processing lymphoblastoid of cages by a drug (24 h prior to contamination) gives high enough parameters ~ 250 (1) and 400 (2). The inhibition of EBV reproduction was assessed by PCR technique estimating the number of EBV DNA genomic equivalents. Minimal effective concentrations amounted to 0.1 mg/mL for Proteflasid for both modifications.

Conclusion: To summarise, the substances under study possess the significant anti-EBV activity and may be the advantageous for the therapy of EBV-associated diseases.

**P548** A phase 2 study of the toxin binding polymer tolevamer in patients with C. difficile associated diarrhoea

D. Davidson, J. Peppe, T. Louie - The Tolevamer Working Group

Background: Tolevamer sodium is a novel, nonabsorbed, nonantibiotic polymer that binds C. difficile toxins A and B, and is being developed to treat C. difficile-associated diarrhoea (CDAD). C. difficile is the most common cause of infectious nosocomial diarrhea, affecting ~1% of hospitalised patients. C. difficile proliferates when normal colonic flora are altered, typically by antibiotics. Pathogenic strains of C. difficile produce two toxins, A and B, that induce colonic inflammation and fluid loss. CDAD treatment with metronidazole or vancomycin also disrupts normal flora, and CDAD recurs in 20% of treated patients. We have completed a phase 2 trial demonstrating that tolevamer and vancomycin have similar efficacy in the treatment of mild-moderate CDAD.

Primary objective: To demonstrate noninferiority vs. vancomycin with respect to time to resolution of diarrhoea (TTROD).

Methods: A randomised, double-blind, double-dummy, active-controlled phase 2 trial was conducted to determine the safety and efficacy of monotherapy with 1 or 2 g tolevamer TID vs. a standard oral dose of 125 mg vancomycin QID. 289 patients with a first episode or recurrent CDAD were enrolled at 58 US, UK and Canadian sites.

Results: Preliminary data showed that the median TTROD in the per protocol population (PP) was 2.0 days with vancomycin, and 2.5 days with 6 g tolevamer. Noninferiority testing demonstrated that the 1 g tolevamer TTROD was noninferior to the standard regimen (PP 0.015), and that the 6 g tolevamer TTROD risk ratio relative to vancomycin was 0.98 (95% CI: 0.68-1.41). In the ITT population, the median TTROD was 3.0 days for both groups. There was no statistically significant difference in TTROD between vancomycin and 6 g tolevamer in either primary or recurrent CDAD patients. The 3 g TTROD was 4.0 days, showing a tolevamer dose response. In the
PP, the definitive recurrence rate (DRR: confirmed by a positive toxin assay) was 19% with vancomycin and 10% with 6 g tolevamer. In this average did not achieve statistical significance (P = 0.185). In the PP treated with recurrent CDAD at enrollment, the DRR was 27% with vancomycin and 0% with 6 g tolevamer (P = 0.07). Overall rates of serious and nonserious adverse events were similar between groups.

Conclusion: Tolevamer dosed at 6 g/day rapidly resolved CDAD similarly to vancomycin and demonstrated a trend towards reduced recurrence. Tolevamer may provide a nonantibiotic alternative for the treatment of this antibiotic induced disease.

**P549** A novel antimicrobial system to treat methicillin-resistant and glycopeptide-resistant staphylococci

B. Cookson, M. Embleton, S. Nair, M. Wilson

**London, UK**

**Objectives:** Methicillin-resistant *Staphylococcus aureus* (MRSA) pose a worldwide public health problem. It has long been a major cause of nosocomial infections and strains causing serious complications. The infections have, recently emerged. MRSA and indeed coagulase negative staphylococci (CNS) are often multidrug resistant and can rapidly acquire resistance to new antimicrobials such as linezolid, making infections difficult to treat, costly and life-threatening. Hence, there is a real need to develop alternative treatments. We have previously shown that MRSA strains can be killed by photodynamic therapy (PDT) using targeted photosensitizer conjugates. We describe the use of nonstrain-specific phage-photosensitizer conjugates to target and kill MRSA, glycopeptide resistant MRSA and CNS.

**Methods:** The *S. aureus* phage 75 was conjugated to the photosensitizer tin (IV) chlorin e6. The conjugate was then added to a suspension of MRSA and this was exposed to red light (λ 633 nm). The phototoxic phage-photosensitizer conjugates, producing highly reactive singlet oxygen which can exert a bactericidal effect. Following irradiation, survivors were enumerated.

**Results:** 99.99% of MRSA in suspensions containing 1 x 10^7 cfu/mL were killed by the treatment using a conjugate/bacteria ratio of 1:1. Furthermore, the photophotosensitizer conjugate conjugate could kill bacteria in exponential and stationary phases of growth. By increasing the ratio of conjugate to bacteria to 10:1, it was possible to achieve 100% kills. Controls showed that at the concentrations used phage, photosensitizer or laser alone did not produce significant kills. We were also able to kill Glycopeptid resistant MRSA and CNS. Calcium neutralisation showed that these effects were because of nonspecific phage adhesion.

**Conclusions:** The phage-photosensitizer conjugate was very effective at killing multidrug-resistant MRSA and CNS. Because *Staphylococcus aureus* phages have the ability to adsorb to any staphylococci, they make ideal targeting systems for PDT. Therefore, phage-targeted PDT is an excellent candidate for a new treatment against such infections. By selecting different phages and photosensitizers, it may be possible to use this technique against many species of bacteria.

**P551** A novel antiviral based on oral antibodies: clinical benefits in paediatric upper respiratory infections

A.V. Martyushev-Poklad, V.F. Uchaikin, M.P. Kotelnikova, J.L. Dugina, O.I. Epstein, S.A. Sergeeva

**Moscow, RLS**

**Objectives:** High prevalence of viral upper respiratory infections (URI) and inefficacy of anti-influenza vaccination for noninfluenza and mixed URIs reveal an unmet need in managing URI, most urgent in pediatric population. A novel antiviral and immunomodulating drug developed and currently marketed in Russia – Anaferon (AF) – contains antibodies to interferon gamma (IFNg) in ultra-low doses intended for oral use. Animal studies showed its therapeutic and preventive action in influenza and herpetic infections. On course oral treatment AF significantly (three to six times) enhanced IFNg ex vivo secretion in peripheral mononuclears.

**Methods:** Efficacy and safety of AF for influenza and other URI was studied in a multicentre randomised placebo-controlled trial that involved over 400 pediatric in- and outpatients aged 6 months–14 years. AF (oral tablets) was given three to seven times daily starting on day 1–2 of URI onset, as add-on therapy to symptomatics and/or antibiotics (if indicated). Major clinical signs of URI and possible adverse drug reactions were monitored daily. In a randomised study of AF as a prophylactic for URI, over 400 patients (aged 6 months–4 years) received AF/placebo, 1 oral tablet daily for 3 months. In cases of URI, AF was given according to treatment schedule. Points to consider were occurrence and severity of URIs, rate of complications, tolerability.

**Results:** Treatment with AF provided considerable (P < 0.05) reduction in duration and severity of major signs of URIs, and rate of complications. Duration of fever reduced by 35–40%, of intoxication – by 40–50%, of coryza – by 20%, of cough – by 30%. None of AF patients showed any adverse drug-related events (ADREs). Prophylactic AF provided a solid prophylaxis in occurrence of URIs. The part of children who avoided URI within 3 months of treatment rose from 3% in placebo to 24.7% in AF group. The use of AF provided a 1.5–2-fold reduction in duration of major signs of URI. Typical pediatric URI complications (otitis and purulent rhinitis) were 2.3 and 2.1 times less frequent (correspondingly). No ADREs were registered on AF.

**P550** Novel ‘ethiological’ therapies for AIDS-related Kaposi’s sarcoma, and possible laboratory monitoring

R. Manfredi, L. Calza, F. Chiiodo

**Bologna, I**

HAART changed the natural history of HIV disease, but AIDS-related malignancies still occur, including Kaposi sarcoma (KS), whose multifactorial pathogenesis include a definite pathogenetic role of HHV-8, HIV infection itself, and underlying immunodeciency. Five consecutive male homosexual patients (p) diagnosed with AIDS because of cutaneous disseminated and/or visceral KS, received HAART combined with an alternating weekly schedule of liposomal doxorubicin or daunorubicin, and the antiviral drug cidofovir at 5 mg/kg (preceded by probenecid administration).

Our p received four to 13 cycles of alternating chemotherapy-cidofovir administered every other week, which led to a significant improvement of number, size, and activity of KS lesions, obtained in all treated p at all body sites in the second-third therapeutic cycle. However, the role of concurrent HAART-related immune recovery (45–395% increase of CD4+ lymphocyte count, compared with baseline), has to be taken into account, as well as the hypothetic direct or indirect activity of antiretroviral therapy on KS evolution. Only one p with a prior, recent myocardial infarction received only four cycles of daunorubicin, because of contraindications indicated for p suffering from coronary heart disease; he continued cidofovir alone, administered every other week. Four p needed concurrent HIV-CSF administration to control drug-related neutropenia. A drop of quantitative plasma HHV-8 viraemia was demonstrated in four treated p, and strictly paralleled clinical improvement. Both liposomal doxorubicin and daunorubicin were approved for the treatment of HIV-related KS, demonstrating a comparable effect (but less toxicity), of combined chemotherapy based on vincristine, adriamycin, and bleomycin. The confirmed pathogenetic role of HHV-8 in KS prompts the introduction of cidofovir (an antiviral drug with extensive activity towards all Herpesviruses), in the ethiological KS treatment, but neither validated schedules of administration of both drugs are available, nor criteria for selection of a specific drug over the other one, because of the lack of data from controlled studies. After paying careful attention to heart-kidney toxicity of doxorubicin-daunorubicin, and cidofovir too, this cytotoxic-antiviral combination therapy may become the first-line therapeutic choice for HIV-associated KS, waiting for controlled data. The decay of HHV-8 viraemia needs further investigation, as a predictor of response to combined cytotoxic-antiviral therapy.

**Clinical Microbiology and Infection, Volume 10, Supplement 3, 2004**

125
**Conclusion:** AF is effective and safe in prophylaxis and treatment of influenza and other URIs in children aged 6 months and older. Moreover, pilot studies have shown AF efficiency in infectious mononucleosis and haemorrhagic fever with renal syndrome. Russian Health Ministry recommends AF as one of choice remedies for pediatric URIs.

**P552 Disruption of the interaction between the HCMV DNA polymerase subunits: towards new anti-HCMV inhibitors**

A. Loregian, B. Appleton, J.M. Hogle, H.S. Marsden, D.M. Coen, G. Palu
Padova, I; Boston, USA; Glasgow, UK

**Objectives:** The human cytomegalovirus DNA polymerase is composed of a catalytic subunit, UL54, and an accessory protein, UL44. The observations that both UL54 and UL44 are essential for HCMV DNA replication, and that antisense inhibition of UL44 synthesis in HCMV-infected cells strongly inhibits viral DNA replication raises the possibility that the UL54/UL44 interaction might be a valid target for antiviral drugs.

**Methods and Results:** To investigate this possibility, overlapping peptides spanning residues 1161–1242 of UL54 were synthesised and tested for inhibition of the interaction between purified UL54 and UL44 proteins. A peptide, LPRRLHLEPAFLPYSVKAHECC, corresponding to residues 1221–1242 at the very C-terminus of UL54, disrupted both the physical interaction between the two proteins and specifically inhibited the stimulation of UL54 by UL44. Moreover, to define individual residues in UL44 and UL54 that are crucial for interacting with each other, we have engineered several mutations both in the C-terminal region of UL54 and in a region of UL44 identified in the crystal structure as the ‘connector loop’. Substitution of alanine for Ile135 in UL44 or for Leu1227 or Phe1231 in UL54 greatly and specifically impaired the UL54–UL44 interaction in both pull-down assays and assays of long-chain DNA synthesis, identifying these residues as crucial for subunit interaction.

**Conclusions:** Thus, a few specific side chains appear to be crucial for UL54/UL44 interaction, suggesting that small molecules targeting the relevant side chains could interfere with this interaction. This information may aid in the discovery of new drugs for the treatment of HCMV infection.

**MRSA and staphylococci: epidemiology**

**P553 MRSA outbreaks in nursing homes in Central Norway**

K.W. Larssen, G. Iversen, T. Jacobsen, K. Bergh, O. Scheel
Trondheim, N

**Introduction:** Norway has a low prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA), with a rate of less than 1% of all staphylococcal infections. Reports from the Norwegian Notification System for Infectious Diseases (MSIS) show an increase in the number of reported cases of MRSA from 63 in 1998 to 142 in 2002. Most cases are now contracted in Norway and outside hospital. Although there have been several outbreaks of MRSA in hospitals, this is the first report of MRSA outbreaks in Norwegian nursing homes (NH).

**Material/methods:** Since March 2003 there has been an outbreak of MRSA at two different NH in Central Norway located 111 km from each other. The initial detection of MRSA was performed at a local hospital. Positive strains were sent to St Olav University hospital for confirmation and further investigation. Strains from 24 persons (Both infection and carrier strains) were analysed by Pulsed Field Gel Electrophoresis (PFGE), and one strain from each NH was analysed by Multi Locus Sequence Typing (MLST).

**Results:** NH A had 10 cases among inmates and five among health care workers. NH B had nine cases, all among inmates. MRSA strains were isolated from nostrils or wounds, in some case from both locations. The nine cases in NH B had an identical PFGE pattern. The strains from NH A were also similar except from one band difference. The strains from NH A differed by one or two bands from the NH B strains, thus all strains were closely related using PFGE. By MLST both strains were found to be ST 45. The SCCmec gene has not yet been determined. (The international strains Berlin is at ST 45 type IV).

**Discussion:** No exchange of personnel or inmates had occurred between the two NH, and no obvious cause of spread was found. The close relation of the strains makes us suspect a common source. Although both NH are served by the same hospital, this hospital had not experienced any outbreak of MRSA. Our results show the potential for spread of MRSA within NH. Several authors report MRSA within NH to be an increasing problem, and NH residency has been proven to be an independent risk factor for MRSA carriage upon hospital admission. It is important that health care workers at NH are aware of this. Proper infection control must be implemented not only in hospitals but also in NH.

**P554 Prevalence and risk factors for colonisation with methicillin-resistant Staphylococcus aureus (MRSA-C) in residents of long-term-care facilities in Greece**

Athens, GR

**Objectives:** LTCF are becoming a major component of the health care delivery system. The high prevalence of antimicrobial-resistant organisms in some LTCF has been documented for many years. The prevalence of colonisation or infection with MRSA may be as high as 20–30%. MRSA-C in LTCF often precedes invasive infection and represents a reservoir for dissemination in other residents or facilities. A survey was conducted to determine the prevalence and main risk factors for MRSA-C in residents of LTCF in Greece.

**Methods:** 18 LTCF were randomly selected from the public sanitation list of Attic province. Nasopharyngeal (N) and wound (W) samples were collected from 561 residents; from each LTCF, we chose randomly 30% of the existing population (minimum sum 25 residents). Cultures and susceptibilities were performed, following NCCLS guidelines. Information was collected on facility and resident demographic data. Univariate and multivariate analyses were performed.

**Results:** 587 samples were collected and 22 (3.75%) MRSA were isolated, 18 (2%) from N and 4 (0.6%) from W specimens. The most common site was W (four of 26, 15.4%), followed by N (18 of 561, 3.2%). Variables associated with MRSA-C by univariate analysis: recent (previous 30 days) antimicrobial use (RR 3.8, \( P = 0.017 \)), indwelling urinary catheter (RR 2.2, \( P = 0.02 \)), recent (previous 120 days) hospitalisation (RR 2.5, \( P = 0.011 \)), poor functional status (RR 3.6, \( P = 0.013 \)) and feeding tube (RR 4.4,
Risk factors for nasal carriage of methicillin-resistant
Staphylococcus aureus among patients in long-term care
facilities in Korea

Seoul, KOR

Objective: As populations in Korea age, increasing numbers of
older individuals reside in long term care facilities (LTCF) such as
nursing homes and chronic disease hospitals. As antimicrobials
are frequently prescribed in LTCF, the emergence of antimicrobial
resistant organisms is a serious problem. But, little is known about
antimicrobial resistance of LTCF in Korea. Among various anti-
microbial resistant organisms, Methicillin resistant Staphylococcus aureus
(MRSA) is one of the most common pathogens causing nosocomial
infections and widely prevailing in Korean hospitals. We designed
this study to investigate the prevalence of MRSA and risk factors
associated with nasal carriage of MRSA in LTCF.

Methods: The study was performed among 632 residents in eight
geriatric care hospitals from July to August 2002. Samples were
obtained by swabbing both nares. Manitol salt agar containing 6
mg/mL of oxacillin was used for isolation of MRSA. The anti-
microbial resistance of isolated strains was determined by the disk
diffusion method, according to the recommendation of NCCLS.
We reviewed all medical records of 632 residents to analyse varia-
ties as risk factors including demographic, hospitalisation-related
factors, antibiotic usage and comorbid conditions.

Results: Overall prevalence of nasal carriage of S. aureus was 50.2%
(317 of 632). Among 317 isolated S. aureus, 64.1% was resistant to
oxacillin. Recent infections (OR, 2.79, P = 0.01) and use of anti-
microbials (OR, 2.787, P = 0.01) contributed to isolation of MRSA.
We found that MRSA were spreading widely in Kor-

ean LTCF. Multiple risk factors including recent infections and
the use of antimicrobials, indwelling devices and the existence of
wounds or bedsores were associated with the nasal carriage of
MRSA in geriatric care hospitals in Korea.

The prevalence of nasal colonisation with MRSA
among residents of long-term care facilities in South Korea

B.-S. Kim
Seoul, KOR

Objectives: The long-term care facilities (LTCFs) patients are those
with serious underlying disease, poor functional status, wounds
such as pressure sores, invasive devices of urinary catheters. Resi-
dents of LTCFs are at risk for colonisation with multidrug-resistant
bacteria including methicillin resistant S. aureus (MRSA). More
than 70% of S. aureus isolated in tertiary hospitals in Korea was
methicillin resistant. But the prevalence of antimicrobial resistance
data in elderly population has not been known yet in Korea. To
determine the prevalence of nasal MRSA colonisation in LTCFs, we
investigated the rates of methicillin resistance among the nasal iso-
lates of S. aureus isolated from provincial hospitals for the elderly.

Methods: Nasal swab specimens were obtained from 632 patients
of eight provincial hospitals for elderly from July to August 2002.

Swab specimens were cultured on staphylococcal broth for enri-
\footnote{In multivariate analysis, only recent hospitalisation
(OR 2.5, P = 0.01), poor functional status (OR 3.6, P = 0.01) and
feeding tube (OR 4.4, P = 0.01) were independently associated
with MRSA-C. This model had a sensitivity of 95% and an area
under the ROC of 76%.

Conclusions: MRSA-C is relatively low in Greek LTCF. According
to our survey, independent risk factors for MRSA-C in LTCF are
hospitalisation, poor functional status and usage of feeding tubes.

Clinical Microbiology and Infection, Volume 10, Supplement 3, 2004 127

Basic hospital infection control methods reduced the
isolation rate of methicillin-resistant Staphylococcus aureus

E.S. Kim, E. Heo, D.H. Whang, H. Yum, B. Shin, H. Ko, B.H. Jun
Seoul, KOR

Objectives: Methicillin resistant Staphylococcus aureus (MRSA) is
highly prevalent in hospitals in Korea. Hospital infections by
MRSA are causing serious problems, so every hospital is trying to
control MRSA by applying effective methods. Seoul Paik Hospital,
tertiary teaching 430 bed hospital, applied relatively stricter infec-
tion control methods than previous ones in March 2002. This
study was designed to evaluate the difference of the isolation rate
of MRSA from all clinical specimens before and after the applica-
tion of the infection control methods.

Methods: Each month, data of the number of MRSA was gathered
and sorted; the results of the study were reported to every ward.

Results: After starting restricting system of antibiotics, glycopep-
tides and carbapenems were prescribed 15% (81 vs. 69 vials/1000
patient-days, P < 0.01) and 35% (37 vs. 24 vials/1000 patient-days,
P < 0.01) less respectively, during the same period (September
2001–February 2002) and after (March 2002–August 2003),
applying the new infection control methods.

Conclusions: This study showed that the isolation rate of MRSA
was reduced by applying hospital infection control methods in
the hospital.

Molecular surveillance of clinical methicillin-resistant
Staphylococcus aureus isolates in neonatal intensive care units

Y.-C. Huang, L.-H. Su
Kaohsiung, TW

Objectives: Methicillin-resistant Staphylococcus aureus (MRSA) has
become an important nosocomial pathogen in our neonatal
intensive care units (NICUs) and accounts for almost 100% of all
**P559** Meticillin-resistant *Staphylococcus aureus* bacteraemia in neonatal intensive care units: genotyping analysis and case–control study

P.-W. Chung, Y.-C. Huang, C.-Y. Lee, L.-H. Su, T.-Y. Lin
Kaeilsan, TW

**Objectives:** To assess the relatedness of meticillin-resistant *Staphylococcus aureus* (MRSA) isolates and to identify the risk factors for the acquisition of MRSA bacteraemia in the infants hospitalised in the MRSA endemic neonatal intensive care units (NICUs).

**Methods:** Twenty-one isolates from the bloodstream of 21 infants hospitalised in NICUs were genotyped by pulsed-field gel electrophoresis (PFGE) and infrequent-restriction-site polymerase chain reaction (IRS-PCR). Other 21 infants stayed in the same NICUs with a same gender, a similar gestational age and a similar birth weight but without MRSA bacteraemia were matched for a case–control study.

**Results:** Of the 21 MRSA isolates, two genotypes (A and C) were identified by PFGE while three genotypes (I, II and III) by IRS-PCR. Genotype C-III in nine isolates and genotype A-I in seven isolates were the two predominant clones in each year. The other four types were minor. Among the 15 infants with multiple isolates, the genotype was usually same if the isolates were from the same episode of MRSA infection, while the genotype was different if the isolates were from distinct episodes.

**Conclusion:** There were two predominant MRSA clones prevailing in our NICUs between 1998 and 2000. Infection control measures should be implemented to try to control the spread of MRSA.

**P560** Delineation of the endemic and sporadic clones among meticillin-resistant *Staphylococcus aureus* strains in a Czech hospital

O. Melter, M. Aires de Sousa, K. Laskafeldová, P. Urbášková, M. Wünschová, H. de Lencastre
Prague, CZ; Oeiras, P; Nový Jičín, CZ; New York, USA

**Objectives:** To define the clones among meticillin-resistant *Staphylococcus aureus* (MRSA) strains collected between September 2001 and February 2003 at the regional hospital of Nový Jičín, Czech Republic.

**Methods:** The isolates were characterised by susceptibility tests, HindIII ribotyping, and pulsed-field gel electrophoresis. Representative strains of each clonal type were analysed by multilocus sequence typing and staphylococcal cassette chromosome mec (SCCmec) typing. The prevalence of the major macrolide (ermA, ermB, ermC and mrsA) and aminoglycoside (aac6'-aph2'', aph3' and ant4') resistance genes was evaluated as well.

**Results:** The presence of two international MRSA clones was documented in the Czech hospital: (i) the Iberian clone (ST24: SCCmec IA: PFGE A: ribotype H2) endemic in the hospital and associated with a single multiresistant phenotype and (ii) clone EMRSA-15 (ST22: SCCmec IV: PFGE H: ribotype H7) detected since the beginning of 2002 and associated with three phenotypes. These two clones could be distinguished by the distribution of macrolide and aminoglycoside resistance genes (ermA, aac6'-aph2'', ant4' and ermC plus mrsA in a few isolates, respectively) and the presence of enterotoxin A in the Iberian clone.

**Conclusions:** Two clones could be delineated among the strains studied. The combination of the molecular characterisation with chronological epidemiological data enabled following the spread of EMRSA-15 clone in the hospital.
P562 Epidemiological analysis of the incidence of MRSA infections in hospitals in the Czech Republic

B. Macková, O. Melter, P. Urbášková
Prague, CZ

Objectives: Infections caused by MRSA (methicillin-resistant Staphylococcus aureus) have been historically considered as hospital infections. Nevertheless, MRSA is becoming increasingly involved in community infections as well. The incidence of MRSA infections varies widely with the regions and population groups.

Methods: Since 2000 the Czech Republic has been taking part in the European Antimicrobial Resistance Surveillance System monitoring the incidence of five pathogens in invasive isolates from blood. One of the causative agents under surveillance is Staphylococcus aureus. The incidence of MRSA has been monitored in blood specimens from 89 hospitals of the Czech Republic. Data on patients, hospitals, and phenotypes of antibiotic resistance in the bacterial strains isolated have been recorded. Basic statistical methods are used for epidemiological analysis.

Results: Between 2000 and 2002, 2804 Staphylococcus aureus strains were analyzed. Out of these strains, 3.5% were MRSA (see Table). The incidence of MRSA varied with types of hospitals, hospital wards, age groups and time. Geographical distribution of MRSA strains in the Czech Republic was mapped. Thanks to collaboration of 45 laboratories, highly valid data covering 82% of the Czech population have been available.

<table>
<thead>
<tr>
<th>Year</th>
<th>S. aureus</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>525</td>
<td>20 (3.8%)</td>
</tr>
<tr>
<td>2001</td>
<td>109</td>
<td>53 (5.8%)</td>
</tr>
<tr>
<td>2002</td>
<td>1166</td>
<td>70 (5.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>2804</td>
<td>153 (5.5%)</td>
</tr>
</tbody>
</table>

Conclusion: Surveillance of MRSA strains as a basis for active antibiotic policy has become of increasing concern to both health care providers in hospitals and community general practitioners. There is a need for better awareness of MRSA infections among both health care professionals and the public. The incidence of MRSA infections in the Czech Republic shows a slightly increasing trend. The development of the incidence of MRSA infections and factors involved in their spread will be the subject of further study.

P563 Evolution of two different clones of methicillin-resistant Staphylococcus aureus (MRSA) isolated in Italy

F. Campanile, D. Bongiorno, S. Borbone, V. Cafiso, F. Di Bassiano, S. Stefani
Catania, I

Objectives: The origins of the major MRSA clones are still poorly understood. Previous reports have suggested a common origin for all MRSA from a single ancestral S. aureus strain that acquired the mec complex. Recent studies have shown that some MRSA strains are very divergent, implying that mecA has been transferred among S. aureus lineages at different times in the past.

Methods: S. aureus MLST, together with BURST analysis and SCCmec-typing, has been used to probe population biology of bacteria and to predict ancestral genotypes and evolutionary descendents within groups of related genotypes.

Results: We explored the origin and the evolution of two novel MRSA clones: the Rome and the Italian clones. The Rome clone, circulating in a Rome hospital from 1997, had a characteristic clonal type (II:PH:C) and was susceptible to erythromycin, clindamycin, spectinomycin, vancomycin and teicoplanin. During the last few years, a variant of this clone has appeared and undergone evolution, consisting in the acquisition of two copies of Tn554, the integration of the pUB110 plasmid downstream of the mecA gene with the evolution of the SCCmec type I to IA and the consequent modification of mecA polymorph II to I, maintaining the sequence type ST247 (3-3-1-12-4-4-16; CC8) and the PFGE C pattern. The acquisition of extra-resistance genes determined erythromycin, spectinomycin and clindamycin resistance; moreover, these strains showed heteroresistance to glycopeptides. On the contrary, the Italian clone has always shown the same phenotypic (susceptible to tetracycline and rifampin) and genotypic features (II-E-E; ST228: 1-4-1-4-12-24-29; SCCmec I), suggesting the immediate success of these strains in the environment.

Conclusions: Our results unambiguously indicate that the Rome clone and its variant derive from the same MRSA clone as the ‘Archaic’, the ‘Iberian’ and the ‘Brazilian’ ones (CC8). Moreover, the ‘Italian’ clone closely correlates with that of several S. aureus including MSSA, MRSA and GISA strains (CC5), confirming the horizontal mecA transfer among different S. aureus ancestral lineages. This ancestral MRSA became the successful clone that is spreading in Italy.

P564 Methicillin resistance in Staphylococci: results of a survey in a Northern Italian region

R. Bandettini, M. Lemmi, L. Pescetto, A. Scaramuccia, E. Debbia
for Ligurian AMCLI group
Genoa, I

Objectives: Methicillin-resistance (MR) in staphylococci represents a major therapeutic treat in the management of nosocomial Gram-positive infections. This parameter therefore, need a periodical evaluation.

Methods: In this study of the Italian Clinical Microbiologist Association (AMCLI), MR was assessed in nine Centres localised in Ligurian area (Northern Italy). During April 2003, 476 staphylococcal strains were collected from hospitalised patients in Medical Department (64.5%), Surgery (12.2%) and high risk of infection wards (23.3%). Samples were obtained from wound swabs (22.3%), infections of upper (17.0%) and lower respiratory tract (15.8%), blood (11.6%), vascular devices (8.0%) and other (25.3%). MR was evaluated by NCCLS suggested guidelines.

Results: The strain collection included 358 S. aureus (75.2%) and 118 CNS (24.8%). S. epidermidis were 56.8% of this latter group of strains. MR S. aureus (MRSA) accounted for 63.7% and in CNS this trait was 66.1%. Many isolates were also resistant to other classes of antibiotics. In particular, some representative pathogens (among MRSA) exhibited together with MR, unsusceptible to amnoglycosides, MLS, fluoroquinolones, and tetracycline (46.5%). All strains resulted susceptible to vancomycin and teicoplanin. Among CNS, concomitant resistance to methicillin and other antibiotics were detected in 33.3% of these pathogens. No strain was found resistant to vancomycin and teicoplanin.

Conclusions: The present findings indicate that antibiotic resistance in nosocomial staphylococci is largely diffused and need a continuous programme of surveillance.

P565 Methicillin-resistant Staphylococci in a surgical hospital: incidence, antimicrobial susceptibility, molecular typing

A. Zilevica, T. Tracevska, R. Treimane, R. Paberza, I. Vingre Riga, LV

Objectives: Monitoring of the incidence rate of methicillin-resistant Staphylococcus aureus (MRSA) and coagulase-negative Staphylococci (MR CoNS) in hospitalised patients in a surgical hospital, characterising of their multiresistant nature, slime production in MR CoNS and molecular types in MRSA.

Methods: A total of 165 strains of S. aureus and coagulase-negative Staphylococci collected from clinical specimens of different hospitalised patients within 2002 were analysed. Microbial strains were
isolated and characterised by conventional methods. An automated Crystal system was used for species' identification. Antimicrobial susceptibility testing was performed by the disk diffusion method (NCCLS) and detection of the minimal inhibitory concentration (MIC) in SCEPTOR panels. Methicillin resistance was tested by oxacillin disks with the potency 1 μg of oxacillin and confirmed by detection of the mec A gene by PCR, the latex agglutination test and the E-test (Bio Disk). Slime production in CoNS was tested using the tube test with tryptic soya broth (TSB) and glucose. Molecular typing was performed by the randomly amplified polymorphic DNA test (RAPD).

Results: The incidence rate of MRSA and MR CoNS in 2002 was 1.64 and 30.6%, respectively. MRSA were resistant to erythromycin (99%), clindamycin (71.5%), trimethoprim (35.8%), gentamicin (25%), ciprofloxacin (17.9%). MR CoNS were resistant to clindamycin (94%), erythromycin (92%), trimethoprim (66%), gentamicin (40%), ciprofloxacin (28%). The result among different species of CoNS varied. 74 CoNS strains (52 S. epidermidis sensu stricto, 12 S. haemolyticus, 4 S. hominis, 3 S. capitis, 1 S. cohnii) were tested for slime production. Twenty-three of them were methicillin-sensitive, 51 – methicillin-resistant. Fifty-one (69%) of the investigated strains did not produce slime, 10 strains (13.5%) produced slime with a high intensity, 13 strains (17.5%) – with a moderate intensity. Slime production in MRs was more intensive than in MSS strains. Thirty-six strains of MRSA were examined by the RAPD typing method, and the main genetic groups were differentiated.

Conclusions: The incidence rate of methicillin resistance in Staphylococci in our hospital is not high. MRs are multiresistant. 13.5% of CoNS are active producers of slime, 17.5% are moderate producers, 69% do not produce slime. Methicillin-resistant Staphylococci produce slime more actively than methicillin-sensitive strains. The RAPD method is a sensitive and reliable molecular typing method.

P566 | Initial molecular characterisation of the first MRSA outbreak at a clinical university hospital

E. Miklasevics, A. Balode, U. Dumps, A. Martinsons
Riga, LV

Objectives: Methicillin-resistant S. aureus (MRSA) is a well known pathogen which causes severe nosocomial infections. First confirmed case of MRSA at P. Stradins Clinical University Hospital (CUH) was registered in March 2003. The aims of this study were comparison of bacteriological and molecular identification of staphylococci isolates as well as their initial characterisation and typing.

Methods: *Staphylococcus* isolates were collected at CUH in period March–September 2003. Presence of the cflA, femB, mecA and PVL genes were detected by PCR amplification. rep-PCR and RFLP were used for MRSA typing.

Results: Screening of 86 isolates revealed 16 MRSA (cflA+, femB+, mecA+). This result was in concordance with the one obtained by bacteriological methods. Twelve of these strains were typed by rep-PCR and RFLP. Application of these methods generated three groups including nine, two and one isolates, respectively. The last, single isolate was the only one positive for Panton-Valentine leukocidin toxin.

Conclusions: The local ICU outbreak was registered and confirmed by molecular methods. Maintenance of vigorous surveillance and prevention measures are strongly required.

P567 | Clonal spread of borderline oxacillin-resistant *Staphylococcus aureus* in a dermatological hospital unit

M.K. Thomsen, M. Rasmussen, K. Fuursted, L.N. Pedersen, M. Deleuran, J.K. Møller
Aarhus, DK

Objectives: The aim of this study was to describe and investigate a clonal spread of mecA gene-negative *Staphylococcus aureus* strains with decreased sensitivity towards oxacillin (BORSA) among patients in a dermatological hospital unit.

Methods: The medical records from patients in the dermatological hospital unit and clinical samples received in the Department of Clinical Microbiology were reviewed, retrospectively, from November 2000 to October 2001. Susceptibility to oxacillin (1 μg disk, Oxoid) was examined with either the disc diffusion method or Etest on Iso-Sensitest Agar or Columbia agar (with 4.5% NaCl) at 35°C. The presence of the coa gene and absence of the mecA gene was examined with a polymerase chain reaction (PCR) method. BORSA was defined as phenotypic oxacillin-resistant *S. aureus* being mecA-negative. All isolates were phage-typed and genotyping was performed with pulsed field gel electrophoresis ('enzyme').

Results: Fifteen isolates from fifteen patients were evaluated. All the strains carried the coa gene, were of phage-type 95u and the PFGE results confirmed that all the isolates constituted a clone. The median zone size from disc diffusion testing was 6 mm (range 6–19 mm) while the median oxacillin MIC was 3.0 mg/L (ranged from 0.25 to 6.0 mg/L). None of them carried the mecA gene. Ten of the patients received systemic immuno-suppressive medications, and four others used topic immuno-suppressive agents (chloromethine and group III steroids). At least 10 of the patients received low-dosis penicillinase-stable penicillins for longer periods of time.

Discussion: We describe for the first time a clonal outbreak of BORSA among patients in a dermatological hospital unit. The clonality was confirmed by means of both phenotyping (phage-type 95u) and gene-typing (same PFGE type). It could not be clarified whether the 'BORSA clone' spread directly from one patient to another or whether it was spread by a health care worker or through shared objects. One of the patients had a history with visits to the out-patients clinic only which may suggest indirect transmission. We believe that the spread of BORSA in our dermatological hospital unit was facilitated by longtime (low-dosis beta-lactam) antibiotic pressure, close relationships between 2 patients during admission, and immuno-suppressive treatment. The BORSA clone seems to have disappeared after changes in hygienic measures and discussions of the antibiotic policy in the department.

P568 | The use of molecular epidemiology to monitor the nosocomial dissemination of methicillin-resistant *Staphylococcus aureus* in a tertiary care university hospital during a 10-year survey: 1991–2001

A.L. Beretta, P. Trabasso, R.B. Stucchi, M.L. Moretti
Sao Paulo, BR

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a severe clinical threat for patients worldwide and has been the cause of major outbreaks and epidemics among hospitalised patients, with high mortality and morbidity rates.

Objectives: To study the genomic diversity of MRSA strains isolated from patients with nosocomial infection assisted in a tertiary care university hospital during a 10-year survey (1991–2001).

Methods: The study comprised two periods: the period I from 1991 to 1993 and period II from 1995 to 2001. The DNA typing analysis was performed by pulsed field gel electrophoresis and the similarity among the MRSA strains was determined by cluster analysis.

Results: In the period I, 73 strains presented five distinctive DNA profiles: A, B, C, D and E. Profile A was the most frequent DNA profile (13.7%) closely-related profiles and 18% of the strains isolated in period II were considered to be from the same clone. The molecular monitoring of MRSA strains permitted to
determine the clonal dissemination and maintenance of a dominant endemic strain during a 10-year period and the presence of closely and possibly related patterns to the endemic profile A. Conclusion: Further studies on virulence factors of this MRSA endemic profile and the reinforcement of strict measures of hand hygiene and environment cleaning are necessary to improve the understanding and control of the dissemination of the endemic profile in our hospital.

P569 Epidemiology of methicillin-resistant Staphylococcus aureus at a German university hospital
H. Haefner, C. Bauer, S. Koch, F. Huenger, S.W. Lemmen Aachen, D

Background: Many risk factors for acquisition of methicillin-resistant Staphylococcus aureus (MRSA) infections or colonisation during hospital stay are described in the literature. In our study we evaluated the risk factors of patients identified as MRSA positive at the University hospital Aachen.

Methods: During January–November 2003 all medical records of inpatients identified as MRSA positive from clinical species were reviewed prospectively for demographic data and risk factors. Whenever possible the patients were interviewed also about hospital stay in the last 6 months, being a nursing home resident, being known as MRSA positive, previous antibiotic therapy and underlying diseases. Division for infection or colonisation was determined by GP.

Results: During the study period 148 S. aureus strains (7.8%) were identified as MRSA. Fifteen isolates were detected in outpatients; 133 strains in inpatients, 20 (15%) of which were known as MRSA positive at time of admission. Until now the data of 114 (85.7%) (19%) patients were colonised and 23 (13%) available for 7% (17%) and tip of central vascular catheter (19%). Seventy per cent of patients were colonised and 23% infected, no information was available for 7%. Lethality rate was 30%. The demographic data were: male: 70%, female: 30%, age 60 years (mean 64); hospital service: surgery 48%, internal medicine 31%, other wards 21%; 44% were detected in any ICU; major admission diagnosis: trauma (25%), acute infection (21%), previous surgery (13%), cardiovascular disease (11%), malignancy 10%; known chronic diseases (malignancy, cardiovascular disease, diabetes etc.) on admission 67%; previous antibiotic therapy 79%; central venous catheter 59%; hospital stay days 47 (mean 39), time until MRSA detection 26 days (mean 20), hospitalisation in the last 6 months 34%, transmitted from other hospitals 29%, nursing home residents 6%. Conclusions: The MRSA-rate of 7.8% in our hospital is low. The late detection of MRSA after average hospital stay of 26 days shows that most patients were colonised or infected during their hospitalisation either by strain transmission or strain selection because of shown risk factors.

P570 Nosocomial meningitis due to methicillin-resistant Staphylococcus aureus (MRSA): review of eight cases

Objectives: To evaluate MRSA meningitis cases in our hospital between 1999 and 2003. Patients and method: We evaluated the hospital charts of eight patients who had culture proven MRSA meningitis retrospectively.

Results: Patients were six men, two women, aged 4–70 years (mean 39). All had postneurosurgical state and two had shunt infections. All patients were evaluated as hospital-acquired meningitis. Fever, leucocytosis, disturbances in the consciousness were the most common clinical and laboratory findings. One patient had mixed infection (MRSA + Enterococcus spp.) whereas seven were infected only with MRSA. One patient was treated with vancomycin alone and three with teicoplanin alone. One patient was treated empirically with cefazolin and died during this treatment while awaiting the CSF culture results. One patient was treated with vancomycin followed with teicoplanin + meropenem because of tubulo interstitial nephritis. The last two were treated with combined regimens one with vancomycin + chloramphenicol, and one with teicoplanin + chloramphenicol. Mean duration of treatment was 27.5 days (range 3–60 days). Mortality rate was 12.5%.

Conclusions: MRSA meningitis is a rare but hard to manage nosocomial infection. Although IV vancomycin is the mainstay of therapy, the fact that five of these eight cases were successfully treated with teicoplanin (alone or in combination) shows that it may be an alternative treatment option.

P571 Effect of an intervention programme on the MRSA outbreak in an Aberdeen infirmary
J.-M. Lopez-Lozano, A. Beyaert, F.M. MacKenzie, R. Wilson, D. Stuart, I.M. Gould Orihuela, Murcia, E; Aberdeen, UK

Objectives: Aberdeen Royal Infirmary (ARI) has experienced an outbreak of Methicillin resistant Staphylococcus aureus (MRSA) since 1997. We previously reported the relationship between hospital use of third generation cephalosporins (3GC), macrolides (MAC) and fluoroquinolones (FQU) and the emergence of MRSA. In May 2001, an intervention programme was introduced in the intensive care unit (ICU) involving admission screening and body decontamination. This study evaluated the effect of this programme on the overall hospital outbreak.

Methods: ARI is a 1200 bed teaching hospital with 16 ICU beds. Monthly nonduplicate MRSA data and antibiotic use data were collected for the ICU beds and the non-ICU beds, for the period January 96 to March 03. Time series dynamic regression models were adjusted to evaluate the intervention effect.

Results: ICU-MRSA evolution preceded the non-ICU MRSA by a 1-month lag. ICU-MRSA was dependent on past ICU-MRSA values as well as lagged ICU use of MAC, TGC and FQU. The intervention decreased the per cent monthly ICU-MRSA by the value 10.6. The impact of the ICU intervention on the non-ICU MRSA was 5.6%, thus breaking the increasing trend of the MRSA epidemic.

Figure 1. Monthly %MRSA for ICU and Non-ICU beds. Aberdeen Royal Infirmary, January 1996-March 2003

Conclusion: The ICU can influence the prevalence of nosocomial infections in the rest of the hospital because of the continuous flux of patients. This study promotes the benefits of interventions other than reducing antimicrobial use in the control of MRSA.
**P572** How dangerous is the environment of a patient with respect to transfer of MRSA

S. Maxwell, M. Wiggins
Stockport, UK

**Background:** Methicillin resistant *Staphylococcus aureus* (MRSA) is a dangerous and persistent hospital pathogen. It is accepted that the major source of this organism within the hospital environment is infected and colonised patients’ themselves. However, there is little documented fact on the relative importance of the patients’ environment in the spread of this organism.

**Objectives:** To assess the degree of contamination of the patient’s immediate environment and to estimate the likelihood of spread of MRSA from this environment to Health Care Workers and via them to other patients.

**Method:** A 5-week prospective study was carried out on a variety of wards. The environments of 29 colonised patients were sampled, including 29 curtains both sides, 59 hard surfaces, and 58 samples of the gloved hands of the investigator after samples had been taken. Curtains were sampled by direct indentation of the curtains on to selective agar. Hard surfaces were swabbed and hands were sampled using the finger streak method. Ten control environments containing patients who were not known to be colonised were also sampled.

**Results:** MRSA was isolated in 15 of the 29 environments. It was found on the sampler’s hands on eight occasions out of the 29.

**Conclusion:** MRSA is a frequent contaminant of the patients’ environment especially soft furnishings such as curtains. It is readily transferred to the hands after minimal contact. These findings need to be taken into consideration when cleaning protocols are devised and are especially important in terminal cleaning after patients’ infected or colonised with MRSA have been discharged.

---

**P573** Antibiotic usage and environmental reservoirs maintain methicillin-resistant *Staphylococcus aureus* on an intensive care unit

K.J. Hardy, P.M. Hawkey, B.A. Oppenheim, F. Gao
Birmingham, UK

**Objectives:** To determine the rate of colonisation and the incidence of transmission of methicillin resistant *Staphylococcus aureus* (MRSA) on an Intensive Care Unit (ICU) and control MRSA transmission.

**Method:** A nine bed ICU was studied for an initial observation period of 8 months, all patients admitted to ICU for >24 h were screened for MRSA within 48 h then three times a week. Demographic data and antibiotic usage was recorded. An intervention period of 8 months when antibiotic prescribing was restricted followed. A second observation period (6 months) was instituted. Monthly environmental screening (29 sites) with swabs from three areas in each bed space (bed floor, monitor and workstation) plus two from the nurses workstation took place. Patient and environmental isolates were typed using pulse field gel electrophoresis (PFGE).

**Results:** Fifty-seven of 215 patients (26%) were colonised with MRSA during the initial phase, 50% of these acquired MRSA on ICU. Eighty-eight per cent of patients received >1 antibiotic, cefuroxime and metronidazole being the most heavily prescribed primarily as prophylaxis. During the intervention period prophylactic antibiotics were restricted to one dose and the need for treatment antibiotics reviewed daily. Postintervention the total antibiotics used was reduced from 132.5 to 104.1 DDD/100 patient days and a reduction in metronidazole/cefuroxime from 33.1 to 12.0 and 20.6 to 3.8 DDD/100 patient days respectively. Despite this reduction in the number of patients colonised with MRSA on ICU, but the percentage of patients acquiring MRSA on ICU fell to 43.2%. Results from 19 environmental screens of 29 sites in the ICU yielded MRSA from 1 to 11 sites on every screen (mean 4.6). On two occasions no patients on ICU were colonised with MRSA, but MRSA was isolated from two and five environmental sites respectively. Typing showed EMRSA-15 variants were predominant in patients and environment with the environmental isolates reflecting both the current patient types and previously discharged patients.

**Conclusion:** Despite reducing the total antibiotic usage the rates of MRSA colonisation remained the same. The continuous isolation of MRSA from the environment together with our typing data indicates that environmental sources of MRSA have a role to play and are important in controlling the endemic MRSA infection in hospitals.

---

**P574** Monitoring outbreaks of MRSA in a university hospital, Innsbruck by automated ribotyping

K. Grif, D. Orth, R. Würzner, M. Dierich, F. Allerberger, C. Lass-Flörk
Innsbruck, A

**Objectives:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of hospital- and community-acquired infections. The aim of this epidemiological study was to elucidate the spread of MRSA clones by means of molecular typing in the University hospital of Innsbruck.

**Methods:** Positive MRSA cultures collected from clinical specimens, such as blood cultures, cerebrospinal fluids, sputum, drains and various swabs isolated from patients admitted to the University hospital of Innsbruck were investigated from March to November 2003. All MRSA strains (initial isolate per patient) were typed by automated ribotyping according to manufacturer instructions using EcoRI as restriction enzyme.

**Results:** 116 patients acquired MRSA in the hospital and 49 were in intensive care units. Seventy-one MRSA strains were investigated and classified in 14 different ribotyping patterns (RP 1–RP 14) using EcoRI. RP 10 was identified in 24, RP 1 in 18, RP 3 in 10, RP 7 in 7, RP 4 and RP 2 in two patients. Further eight MRSA isolates yielded unique RFs. In the neurology intensive care unit RP 1 strains and in the medical intensive care unit RP 10 strains occurred constantly over a period of seven and four months, respectively. These strains were related to colonisation (n = 22) and infections (n = 20). Overall, 41 and 30 patients showed colonisation and infections because of MRSA, respectively.

**Conclusions:** Automated ribotyping successfully fulfilled our aim to study epidemiological aspects of MRSA spread. The majority of MRSA strains were limited to three clonal groups. RP 1, 3 and 10 strains were predominant in intensive care units, yet spread and persistence was also found within other wards. Furthermore this study shows the importance of stringent infection control measurements and the necessity of guidelines for antibiotic use to avoid selection of antibacterial resistance.

---

**P575** MRSA acquisition on an intensive care unit (ITU)

S. Dancer, M. Coyne, A. Speekenbrink, S. Samavedam, J. Kennedy, P. Wallace
Glasgow, UK

**Objectives:** The aim of this study was to investigate MRSA acquisition within a seven-bedded ITU in a tertiary hospital over a period of 5 months.

**Methods:** Data was collected from all patients admitted into ITU, including microbiological results and dependency scores. This enabled the distinction to be made between patients who were admitted with MRSA and those who acquired it whilst in ITU. Staffing levels for trained, auxiliary and agency nurses were plotted against bed occupancy rates and acute admissions; student attendance and nurses from other wards were also included. From these, nurse-patient ratios, workload and MRSA colonisation pressures were calculated and modelled against the timing of MRSA clusters. Standardised environmental screening was performed throughout the study using commercial dipslides.
Results: Of 162 patients admitted into ITU, 28 (17%) were found to have MRSA. Twelve of 28 (43%) acquired MRSA on the unit in four clusters involving three patients. Each cluster occurred within a 5-day period and was preceded by enhanced workload, because of a shortage of trained nurses and increased bed occupancy. There was also an association with surface level hygiene throughout the study. Of 160 sites screened, 37 (23%) produced quantitative growth of 2.5–12 cfu/cm² and 26 of 37 (70%) were from hand touch sites. MRSA was found in the environment during the most intense period of activity. Some of the strains appeared to be related within and between clusters, and were particularly associated with upper respiratory sites.

Conclusions: Over a 5-month period, 12 of 162 (7%) patients acquired MRSA in this ITU, less than half of the patients shown to have MRSA overall. Clusters of MRSA acquisition were associated with shortages of trained nurses, increase in workload and hygiene failures predominantly involving hand-touch sites.

P576 Control of methicillin-resistant Staphylococcus aureus transmission in an intensive care unit: evaluation of the efficacy of control practices

G. Guerrier, L. Bodin, S. Males, J.M. Ekherian, D. Resiere, J.J. Rouby
Paris, F

Objectives: Fighting against the dissemination of methicillin resistant Staphylococcus aureus (MRSA) infections depends on the understanding of the use of antibiotics and also the prevention of cross contamination. We evaluate the importance of hygienic measures in order to prevent MRSA spreading in a surgical intensive care unit.

Methods: We compared the incidence of MRSA carriage and infections before and after starting preventive measures among ill patients who often experience infections caused by MRSA. Environmental measures (technical and geographical isolation, hand washing) and decontamination of ill patients carrying MRSA may help improve the care of many at risk patients.

Results: Incidence of cases of nasal MRSA is relatively decrease from 28 to 5% and 30 to 2% for acquired MRSA infections over 10-year period. Risk factors to develop pneumonia because of MRSA is stable for chronically ill carriers through the time despite of nasal and skin decontamination.

Conclusion: Observation of hygienic measures by the medical team is a key to the prevention and control of the hyperendemic nature of MRSA. However, it seems useful a global strategy to fight against MRSA, but the benefit of each measure of a global control programme is difficult to evaluate.

P577 Emergence of a VISA strain in a patient with osteomyelitis: first isolation reported from Austria

A. Wechsler-Fördoß, N. Isufosvki, F. Geppert, T.R. Walsh
Vienna, A; Bristol, UK

Introduction: MRSA with reduced susceptibility to vancomycin was first described 1997 by Hiramatsu in Japan. So far, no MRSA strains with intermediate resistance to glycopeptides have been reported from Austria. We present a patient with osteomyelitis caused by MRSA developing intermediate resistance to glycopeptides during therapy.

Case report: In June 1998 W.P., a male, aged 59, presented with a polymicrobial diabetic foot infection requiring the amputation of two toes of the right foot. After initial clinical improvement MRSA fully susceptible only to glycopeptides and fusidic acid was isolated repeatedly from the wound as well as from the bone in the summer of 1998. As the patient was willing to comply with a long-term outpatient intravenous therapy teicoplanin was administered three times a week, which resulted in improvement clinically and by MRI investigations. Additionally, in May 2000 a bone graft impregnated with vancomycin was implanted resulting in local healing and decreased inflammatory activity in MRI controls.

Conclusion: Detection of VISA is a challenge for the routine clinical laboratory and requires a high level of suspicion. Prolonged use of glycopeptides promotes the development of glycopeptide resistance as seen in this case and in many others and should prompt further investigation in order to identify VISA and to enable adequate isolation precautions and efficient antimicrobial treatment.

P578 Six lethal cases of community-acquired methicillin-resistant Staphylococcus aureus infections in young adults in Montevideo, Uruguay

A. Galiana, W. Pedroza, K. Hiramatsu, X. X. Ma, T. Ito, O. Bertaux, I. Constenla, I. Christophersen, H. Bagnulo
Montevideo, UY; Tokyo, JP

Objective: To describe six severe sepsis (in 6-month period) in young immunocompetent hospitalised at ICU during an outbreak of community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA) infections.

Methods: Retrospective study between July and December 2003 of CA-MRSA severe sepsis syndrome cases. Two haemoculture sets (HEM) at admission (FAN Bact-Alert), culture of traqueal aspirate, pleural fluid(PF), drains(D) and skin lesions if present.

Results: Three females (16, 16, 45 years) and three males (15, 20, 37 years) except 1, none presented health care associated risk factors. Four patients with necrotizing pneumonia and respiratory sepsis (RS) three complicated with empyema and during period of nosocomial incidence of Influenza virus. One sepsis secondary to skin infection (SI) and one surgical site infection (SSI) sepsis. Four of them with previous superficial SI. All had severe respiratory distress syndrome and haemoptysis that require ventilatory assistance, refractory septic shock, kidney and haematology failure, four liver failure, two coagulopathy. Initial empiric antimicrobial treatment.
therapy (AT) for respiratory sepsis: Ceftriaxone and Azythromycin iv, for skin associated sepsis: Cefadroxine iv and for SSL Cefadroxine and Gentamicyn iv. Outcome: four died in the first 48h; three RS and SI before bacteriologic culture results were available and in the other two (RS and SSL) AT change to Vancomycin 2 gr iv bid. These patients died 8–45 days after. CA-MRSA (PBP2a positive) were isolated from all patients HEM, three PF from four RS and from one D. All susceptible to VA, CIP, SXT, GM, CLI, and resistant to OXA, E. Gene mecA positive, SCCmec type IV, Panton Valentine leucocidine gene + (LPV), enterotoxin A-B gene – and PFGE indistinguishable and closely related. Conclusions: In our country like others, in a short period CA-MRSA infections were associated with unusual rate of severe and rapidly mortal cases mainly respiratory sepsis in young people, probably clonally related all LPV + that challenges the current empiric antimicrobial guidelines. Because their high virulence, this unsuspected emergent pathogen, constitutes a health care problem nonresolved yet.

P579 S. aureus community-acquired infections. Antibiotic resistance rates and macrolide resistance phenotypes
Sp. Fokas, St. Fokas, F. Markatou, E. Lauranou, M. Kalkani, M. Dionysopoulou
Sparta, GR

Objectives: To estimate the antibiotic resistance rates of S. aureus strains isolated from community-acquired infections and to determine the macrolide resistance phenotypes.

Methods: We examined retrospectively 152 S. aureus strains isolated over a 2-year period (2001–02) from 152 clinical specimens received from patients with community-acquired infections as follows: 99 strains (65.1%) from abscesses, 18 strains (11.8%) from wound infections, 15 strains (9.9%) from skin and soft tissue infections and 20 strains (13.2%) from various other infections. Conventional methodology was used for identification to the species level and the susceptibility tests were performed using the disk diffusion method according to the NCCLS procedure (2000). MRSA detection was achieved by oxacillin 1 Mcg disk and detection of PBP 2a by a slide latex agglutination assay. The macrolide resistance phenotypes were determined by the double disk method using erythromycin and clindamycin disks.

Results: A total of 44 (28.9%) MRSA strains were found, the penicillin resistance rate was 86.8% (132 strains) and we found no vancomycin or teicoplanine resistant S. aureus isolates. The resistance rates to other antibiotics were as follows for MRSA and MSSA respectively: ciprofloxacin 22.7–3.7%, erythromycin 20.5–13.9%, clindamycin 15.9–3.7%, cotrimoxazole 29.5–13.9%, fusidic acid 84.1–25.9% (CA-FSM 1996 guidelines), gentamicin 11.4–1.8%. Resistance to gentamicin was clearly associated (< 0.05) with methicillin resistance. The macrolide resistant phenotypes for MRSA were cMLSb 78% – cMLSb 22% and for MSSA were cMLSb 27% – iMLSb 73%. Multiresistant strains were isolated among MRSA (10 strains, 22.7%).

Conclusions: In our area aminopenicillins are not useful in the empirical treatment of community-acquired S. aureus infections and their combinations with beta lactamase inhibitors must used with caution. Erythromycin, clindamycin and cotrimoxazole can be used in selected cases. The high prevalence of inducible macrolide resistant phenotype in MSSA raises questions about the use of clindamycin in infections caused by these S. aureus strains.

P580 An epidemic European fusidic acid resistant strain of Staphylococcus aureus carries the fusB determinant
Leeds, UK; Copenhagen, DK

Objectives: Fusidic acid-resistant epidemic clonotypes of Staphylococcus aureus causing impetigo have recently been reported in several European countries. The genetic basis of fusidic acid resistance in these strains has not been determined, and it is unknown whether they constitute a single epidemic strain undergoing inter-country spread. To address this, representative epidemic strains were typed by Pulsed-Field Gel Electrophoresis (PFGE) and the genetic basis for their reduced susceptibility to fusidic acid was established.

Methods: PFGE-typing was performed according to the HARMONY protocol. Strains were examined for fusidic acid resistance polymorphisms in the fusA drug target by PCR amplification and DNA sequencing. For detection of the acquired staphylococcal fusidic acid-resistance determinant, fusB, southern hybridisation was employed to probe both total DNA and purified plasmid DNA preparations. Conjugal transfer capabilities were examined by filter-mating.

Results: PFGE-analysis of epidemic fusR clonotypes established that strains from Sweden, Norway, the United Kingdom, Denmark and Ireland represent a single clone. No mutations were detected in the fusA genes of this clonotype. Strains were positive for fusB in both total and purified plasmid DNA preparations, indicating that fusB is associated with a plasmid. Further characterisation of this replicon revealed that it was ~42 Kb in size, and incapable of transfer by self- mobilisation. Development of a sensitive and specific PCR-based assay for probing strains for fusB enabled rapid detection of the fusB determinant in further members of this clonotype.

Conclusions: The epidemic fusidic acid-resistant clonotypes of S. aureus described in several European countries actually constitute a single clonotype that is spreading in Europe. Fusidic acid resistance in this clonotype is mediated by carriage of the fusB determinant on a large, nonconjugative plasmid.

MRSA and staphylococci: laboratory aspects

P581 Susceptibility of pulse field characterised methicillin-resistant Staphylococcus aureus to daptomycin
R. Smyth, G. Kahlmeter
Växjö, S

Objectives: Daptomycin, the first antibiotic in the lipopeptide class, is in clinical development for the treatment of serious infections caused by Gram-positive pathogens. Daptomycin possesses potent in vitro and in vivo bactericidal activity against Staphylococcus aureus isolates, including those strains resistant to methicillin (MRSA), vancomycin (VRSA), and linezolid. This study is the first to test the potency of daptomycin against a set of clinical MSSA and MRSA isolates in Sweden.

Methods: The strains comprised 100 S. aureus from a reference collection at the Swedish Institute for Infectious Disease Control (SMI), 25 of which were MSSA (mecA-) and 75 of which were MRSA (mecA+). The MRSA strains were clinical isolates from single cases or minor outbreaks in Sweden (1998–99) and had had their Harmony-type and pulse field patterns characterised. Susceptibility testing was performed by the NCCLS broth microdilution methodology using commercial lyophilised panels.

Results: Daptomycin had a MIC90 of 1 mg/L for all strains tested regardless of their susceptibility to methicillin. No strain exhibited an MIC above 2 mg/L.

Conclusion: The results of this study demonstrate that daptomycin has potent activity against Swedish S. aureus isolates, regardless of the presence of the mecA gene. This suggests that daptomycin...
may provide an alternative to the limited antimicrobial agents available for the treatment of serious *S. aureus* infections.

### P582 Usefulness of mec-associated dru sequences in monitoring the spread of highly clonal epidemic methicillin-resistant *Staphylococcus aureus* isolates in Scotland


**Objectives:** The epidemic methicillin-resistant *Staphylococcus aureus* strains, EMRSA-15 and -16, initially observed in England in the early 1990s, spread to Scotland by the mid 1990s. Since that time, reports of MRSA in Scotland have risen dramatically from 565 in 1995 to over 12,000 in 2001. EMRSA-15 and -16 account for 70 and 20% of these isolates, respectively. While PFGE typing has identified a number of EMRSA-15 and -16 clonal variants, epidemiological tracking is difficult because c. 50% of EMRSA-15 and 35% of EMRSA-16 isolates are indistinguishable by PFGE (pulsotypes PF15a and PF16a, respectively) and other typing methods. We evaluated the usefulness of mec-associated dru sequences as more sensitive approach to tracking the persistence and spread of these 'clonal' epidemic MRSA isolates in Scotland.

**Methods:** EMRSA-15 and -16 cultures were collected from hospitals throughout Scotland. Sixty-nine isolates with PFGE pulstype PF15a and PF16a were selected for analysis of the mec-associated dru region. DNA sequences were aligned and interrelationships analyses using BioNumerics v. 3.5 (Sint-Martens-Latem, Belgium).

**Results:** Analysis of dru sequences allowed separation of the 69 PF15a and PF16a isolates into 19 specific subtypes. While some types were found in multiple hospitals, dru sequence comparisons identified instances of specific strain movement between hospitals in a given geographic region (i.e. hospital-specific types).

**Conclusions:** The mec-associated dru region has the potential for extreme variability both in sequence and in number of bp tandem repeats. However, specific sequence types appear to be very stable over time. Analysis of dru sequences thus appears very promising as a means of identifying and tracking specific subtypes of otherwise indistinguishable epidemic MRSA isolates in Scotland. The ability to potentially monitor the specific movement of these epidemic MRSA within and between hospitals is a welcome addition to ongoing public health and infection control efforts to control the persistence and spread of these problem organisms.

### P584 Use of polyvalent anti-staphylococcal bacteriophages for the biocontrol of methicillin-resistant *Staphylococcus aureus* and other staphylococci

A. Coffey, S. O’Flaherty, W. Meaney, M. Elbreki, R. Ross

**Objectives:** The emergence of drug resistant staphylococci has prompted the need for alternate controls other than antibiotics. The objectives of this study were to isolate and characterise anti-staphylococcal bacteriophages and to test their host-range against a broad range of staphylococci including antibiotic-resistant *S. aureus* strains as MRSA and VRSA.

**Methods:** General bacteriophage isolation procedures including enrichment, filtration, plaque assay and broth-culture bacterial challenge assays were performed. This was followed by restriction analysis and electron microscopy according to standard protocols. Challenge experiments were carried out *in vitro* and anti-microbial efficacy was evaluated by plate count.

**Results:** A number of anti-staphylococcal phages, which effectively kill documented typed isolates of MRSA, GISA, VISA, VRSA and teicoplanin resistant *S. aureus* were successfully collected and characterised. These phages exhibiting broadest host range and largest plaque size were retained for further use. These termed ‘polyvalent’ phages were phage K, phage CS1 and phage DW2. In the case of phage K, the host range extends well beyond the species *S. aureus* to include the coagulase-negative staphylococci *S. caprea, S. hyicus, S. epidermidis, S. captis, S. haemolyticus* and *S. chromogenes*. On the basis of genomic and electron microscopic analysis, the three phages fall into the myoviridae family in the classification scheme of the International Committee on Virus Taxonomy. As expected, these phages have no effect whatsoever on bacteria outside of the genus Staphylococcus nor do they affect enterococytic cells. In general, phage K had the broadest host range. In cases where phage resistance occurred in staphylococci, it was demonstrated that this was because of indigenous staphylococcal restriction-modification systems. Phages could be modified to circumvent these systems. Infusion of the phage into a bismuth-based cream resulted in strong anti-staphylococcal activity from...
the cream. Similarly phages were incorporated into handwash where they also exhibited a strong anti-staphylococcal activity.

Conclusions: The results indicate that the phages used in this study are capable of significantly reducing the numbers of recently-emerged antibiotic-resistant staphylococci from Irish hospitals.

**P585**  Evaluation of cefoxitin MIC determination to detect low-level methicillin-resistant *Staphylococcus aureus* (MRSA) by the automatic system Phoenix

A. Felten
Paris, F

Background and objectives: Phenotypic detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA) may fail when relying only on oxacillin susceptibility, regardless of whether determined by disk diffusion or by broth dilution. In a previous study, testing of cefoxitin by disk diffusion proved to be a powerful tool to detect MRSA. The aim of this study was to evaluate the possibility to identify a cefoxitin concentration which would enhance the ability to detect MRSA.

Methods: Seventy-seven *Staphylococcus aureus* (SA) isolates and seven SA reference strains were tested by oxacillin disk diffusion methods and by the Phoenix System panel PMIC/ID25 for staphylococci which measures broth dilutions MICs including cefoxitin MICs (FOX-MIC) in the range of concentrations from 1 to 64 mg/L. Isolates were classified as being MRSA or not according to the presence or absence of the mecA gene.

Results: Eleven SA were mecA negative: nine were methicillin-susceptible and two borderline. Oxacillin MICs (OXA-MIC) and FOX-MICs ranged respectively from ≤0.25 to 1 mg/L and from 2 to 4 mg/L. Seventy-three SA were mecA positive: 38 with OXA-MICs ≥4 mg/L and 35 with low-level OXA-MICs from 0.5 to 2 mg/L. For 70 of 72, FOX-MICs ranged from 8 to >64 mg/L, for two of 72 FOX-MIC was 2 mg/L. Therefore, a 8 mg/L cefoxitin cut-off value allowed detection of 100% of the MRSA detected by OXA-MICs and of 94% of those with low-level OXA-MICs (<4 mg/L).

Conclusions: Cefoxitin MIC determination increases the rate of detection of low-level MRSA. A >4 mg/L cefoxitin cut-off MIC is 100% predictive of MRSA in low-level OXA-MICs SA according to a yearlong practice in our laboratory.

**P586**  The stability of mecA genes and MIC values of methicillin in passage-selected vancomycin-resistant EMRSA strains isolated in the UK

I. Alshami, S.A.H. Awad, J.P. Burnie
Manchester, UK

Objectives: Nosocomial outbreaks of methicillin resistant *Staphylococcus aureus* (MRSA) strains are associated with significant morbidity and mortality. Such strains have been characterised by their phage type and antibiogram and, in the UK, are termed epidemic MRSA (EMRSA). Seventeen epidemic MRSA strains (EMRSA 1-17) have been documented. The purpose of the present study was to ascertain if representatives of strains of EMRSA 1-17 could grow in increasing concentrations of vancomycin and whether this was associated with consistent changes in genotype and phenotype.

Methods: Vancomycin susceptible isolates previously reported and identified to be EMRSA (1-17) and their vancomycin-resistant derivatives were examined. Vancomycin-resistant derivatives were obtained by serial passage of the parental strains in nutrient broth with increasing concentrations of vancomycin producing vancomycin-resistant isolates. Antibiotic sensitivities (vancomycin, methicillin) were determined by E-test performed according to the manufacturers recommendations. The stability of mecA genes was examined by using PCR. Cell wall changes were demonstrated by transmission electron microscopy.

Results: Six strains became vancomycin resistant, three became vancomycin intermediate and seven remained susceptible. The vancomycin MICs for the vancomycin resistant clones ranged from 24 to 32 μg/mL, and were associated with decreased methicillin susceptibilities and increased cell wall thickness. Four out of the six vancomycin-resistant derivatives became sensitive to methicillin (MIC 0.75–1 mg/mL) (P < 0.05) and the mecA gene could not be detected using PCR.

Conclusions: In conclusion, we found that, in vitro, decreased susceptibility to vancomycin was readily inducible following exposure to sub-inhibitory concentrations of vancomycin. This may be a strain specific phenomenon. Development of vancomycin resistance affects resistance to other antimicrobial agents, including methicillin and affects the stability of the *S. aureus* mecA gene. Southern hybridisation of mecA should be investigated to confirm the points and size of the deletion.

**P587**  Susceptibility of methicillin-resistant strains of *S. aureus* isolates from nasal carrier to mupirocin and bacitracin in a Tehran hospital, Iran

M. Rahbar, K. Bahar, M. Yayghobi, A. Reza Shajari
Tehran, IR

Objective: The aim of this study was to determine prevalence of nasal colonisation with *Staphylococcus aureus* and susceptibility of isolates to mupirocin and bacitracin.

Methods: Of 1000 Health Care workers (HCWs) of Milad hospital 774 participated in our study nasal swabs were taken from both of nose of all participants. All specimen were processed in microbiology laboratory within 2 h. Culture performed on Manitol salt agar. Suspected colonies of *S. aureus* were subcultured on sheep blood agar. Identification of *S. aureus* was based on the morphology of colonies, a positive coagulate test and other tests. We performed susceptibility testing by disk diffusion method as recommended by National Committee for Clinical Laboratories Standards (NCCLS).

Results: We screened 774 HCWs for nasal carriage of *S. aureus*. Among HCWs 241 (31%) were colonised by *S. aureus*. We found significantly more male HCWs with *S. aureus* (34.9% vs. 27%, P < 0.05). In some department there was a high frequency for nasal carriage of *S. aureus*. For example in Postintensive Care Units (PICU) and general operating room 53 and 37% were carriage of *S. aureus* respectively. About 7% of all isolates of *S. aureus* were resistant to Methicillin. All strains of *S. aureus* were susceptible to mupirocin (Mast Diagnostic, Mupirocin5) and bacitracin. Mupirocin could be used as nasal ointment for eradication of MRSA.

**P588**  Aminoglycoside-resistance genes and phenotypes in multiresistant nosocomial strains of *Staphylococcus aureus*

M. Malossi, S. Cresti, S. Pollini, C. Cellesi, G. Amicosante, G.M. Rossolini
Siena, L’Aquila, I

Objectives: Aminoglycosides are potent bactericidal agents that play a role in chemotherapy of serious staphylococcal infections. In this study, multidrug-resistant (MDR) nosocomial strains of *Staphylococcus aureus* from an Italian hospital, were analysed for susceptibility to several aminoglycosides and for the presence of aminoglycoside resistance genes.

Methods: Studied strains: a collection of 28 MDR strains of *S. aureus*, isolated from nosocomial infections at the Siena University Hospital (Italy), that included representatives of genotypically
different (as per results of spa and coa typing) *S. aureus* strains circulating in the hospital (some of which had caused nosocomial outcomes). In vitro susceptibility testing was determined by the microdilution method according to NCCLS guidelines. Aminoglycoside resistance genes were detected by dot-blot hybridisation and PCR analysis.

**Results:** Of the 28 MDR strains of *S. aureus* included in this study, 18 were methicillin-susceptible (MSSA) and 10 methicillin-resistant (MRSA). The resistance rates to aminoglycosides were: gentamicin and tobramycin, 50% (22% in MSSA and 100% in MRSA); amikacin, 18% (11% in MSSA and 30% in MRSA); netilmicin 7% (0% in MSSA and 20% in MRSA). All the 14 aminoglycoside-resistant (AR) strains harbored the aac(6′)-le-aph(2′) resistance gene, encoding the bifunctional AAC(6)-APH(2′) enzyme; of them, 10 (71%) also carried the aph(3′)-Ila aminoglycoside phosphotransferase gene, and 4 (29%) the ant(4′)-la aminoglycoside nucleotidyl-transferase gene. Multiple resistance genes were present in most AR strains (three of four MSSA AR strains, and all 10 MRSA strains). Resistance genes were never detected in aminoglycoside susceptible strains.

**Conclusions:** High resistance rates to gentamicin and tobramycin were observed in MDR nosocomial strains of *S. aureus*, especially in MRSA strains. Netilmicin was the most effective anti-staphylococcal aminoglycoside, and retained activity against most gentamicin- and tobramycin-resistant strains, including MRSA. The aac(6′)-le-aph(2′) gene was the most common resistance determinant, followed by the aph(3′)-Ila and ant(4′)-la. The latter genes were never observed alone. The distribution of aminoglycoside resistance genes revealed differences in comparison with other epidemiological settings.

**Acknowledgements:** This work was supported by a research grant from Essex Italia S.p.a.

---

**P589** Frequency of glycopeptide intermediate and heteroglycopeptide intermediate *Staphylococcus aureus* among methicillin-resistant strains isolated in 2002 in a Warsaw university hospital

G. Mlynarczyk, A. Mlynarczyk, M. Luczak
Warsaw, PL

**Objectives:** The problem of glycopeptide intermediate *Staphylococcus aureus* (GISA) and hetero-GISA was first found by Hiramatsu group. They showed, that in the case of infections caused by GISA strains, glycopeptide antibiotics were not effective. The inefficacy of glycopeptides against h-GISA strains was not fully confirmed, but they were presumably precursors of GISA. Because the usage of glycopeptides in hospitals is still very high, it seems that monitoring of both types of *S. aureus* strains is necessary.

**Methods:** In the presented work, the 103 methicillin-resistant strains of *S. aureus* (MRSA) isolated during 1 year (2002) were examined. All strains were isolated from patients from different wards of the one of Warsaw University hospitals, the Center of Injuries Treatment. The reference Keiichi Hiramatsu strains Mu50 and Mu3 were also used, as well as reference susceptible strain FDA 209P. First, the preliminary selection was performed, using a sector of BHI-agar plate with 4 mg/L of vancomycin and undiluted suspension of strain. For all strains that showed growth, MICs for vancomycin were examined, performing β-tests. Strains with MIC values of vancomycin <8 mg/L were examined if they are h-VISA. The population analysis and modifications of the method were performed. The consecutive dilutions of bacterial strain were plated on the growing concentrations of vancomycin. At the same time reference strains were examined.

**Results:** From investigated 103 MRSA only 18 strains grew in the preliminary selection. The MICs of vancomycin for them were lower than 8 mg/L, but higher than 1 mg/L. For all of them the population analysis was performed. The course of population analysis curve in the case of five of the examined strains suggested that they are h-VISA. In the case of some clinical as well as standard strains (susceptible, GISA and especially h-GISA) the start inoculum significantly influenced a shape of the growth curve obtained as a result of the population analysis. The obtained results of frequency of VISA and h-VISA in 2002 were compared with results obtained for *S. aureus* strains obtained in former years in the same laboratory.

**Conclusions:** The method of detection of h-VISA still requires improvements. There was observed no increase in the frequency of VISA and h-VISA in the investigated hospital in last years, despite that consumption of glycopeptides was not reduced.
at a standard (0.5 McFarland) inoculum on Mueller-Hinton (MHA), or high (2.0 McFarland) inoculum or BHIA, respectively. Any MSSA isolate yielding <50 CFU (representing <10^2 of the plated inoculum) on either BHIA containing 2 mg/L of vancomycin (V2-BHIA) or 5 mg/L of teicoplanin (T5-BHIA) at 48 h at 37°C was considered as fully susceptible to vancomycin or teicoplanin, respectively. All MRSA isolates were typed by pulsed-field gel electrophoresis (PFGE).

**Results:** The proportion of MRSA isolates yielding >50 CFU on either V2-BHIA or T5-BHIA significantly (P < 0.01) increased from seven of 94 (7.4%) or eight of 94 (8.5%) in period A to 16 of 95 (16.8%) or 14 of 95 (14.7%) in period B, respectively. Vancomycin Etests MICs on MHA were of lower sensitivity (<65%), but high specificity (99%) on period B isolates compared with those on V2-BHIA. Vancomycin Etests MICs on BHIA were of a higher sensitivity (81%) but lower specificity (65%) compared with those on V2-BHIA, because of an unexpectedly high number of false positive isolates (28/95). Eighty-three per cent of period B isolates (including the potential GISA isolates) analysed by PFGE belonged to a single predominant MRSA clontotype that essentially replaced all four major MRSA clontypes formerly present during period A.

**Conclusions:** Screening of decreased susceptibility to vancomycin or teicoplanin on V2-BHIA or T5-BHIA, respectively, may represent simple low-cost alternatives to Etest MICs, minimising the risk of missing potential GISA isolates.

---

**P592 Sequence analysis of the polymorphic region X of protein A in a methicillin-sensitive Staphylococcus aureus population isolated from the airways of cystic fibrosis patients**

B. Kahl, A. Mellmann, S. Deivick, G. Peters, D. Harmsen

**Munster, D**

**Objectives:** Recently, we demonstrated a high prevalence and long-term persistence of methicillin-sensitive *Staphylococcus aureus* (MSSA) in the airways of cystic fibrosis (CF) patients. Pulsed-field gel electrophoresis (PFGE) distinguished six prevalent clonal lineages (Dice similarity coefficient >85%; 36 isolates) and 35 individual clones. Single locus DNA-sequencing of the *S. aureus* polymorphic region X of protein A (spa) was used to evaluate a faster and more feasible method. The region X consists of a variable number of 21–27 bp repeats. The goals of the present study were: (i) to determine the spa-types of persistent MSSA strains in a defined patient group and (ii) to compare PFGE and spa-typing as a tool for molecular typing for long-term observations.

**Methods:** Seventy-one MSSA isolates collected during a 6-year longitudinal study from 50 patients were analysed. The following primers were used for amplification and sequencing: spa-1113f and spa-1496r. Spa-types were determined with the Ridom StaphType® software (Ridom GmbH, Wu¨rzburg, Germany), which automatically assigns numeric spa-repeat and -type codes. The software synchronizes with an accompanying website (http:// www.ridom.de/spaserver) to ensure a uniform terminology code.

**Results:** In total, 48 spa-types were distinguished by sequencing all 71 MSSA isolates. Twenty-four spa-types occurred within the 6 -by-PFGE- defined prevalent clonal lineages (36 isolates). The remaining 35 individual isolates showed 24 different spa-types. As the overall composition of spa-types within a clonal lineage was very similar, it is conceivable that the differing spa-types could be explained by micro-evolution of the spa-region: deletion of single or several repeats (11 strains), duplication of repeats (three strains) or point-mutations (three strains). However, four strains displayed totally different spa-typings. As the overall composition of spa-types within a clonal lineage was very similar, it is conceivable that the differing spa-types could be explained by micro-evolution of the spa-region. Therefore, if spa-typing is used as a molecular typing method, micro-evolutionary events have to be taken into account when analyzing the data, especially if isolates from long-term observations are to be compared.

**Conclusions:** Spa-typing showed a high diversity in a MSSA population isolated from a defined patient group. The discriminatory power of spa-typing was comparable with PFGE results, and overall the same clonal lineages were detected. However, gains, losses of repeats, and point-mutations occurred in strains within the six prevalent clonal lineages indicating micro-evolution of the spa-region. Therefore, if spa-typing is used as a molecular typing method, micro-evolutionary events have to be taken into account when analyzing the data, especially if isolates from long-term observations are to be compared.

**P593 Ridom StaphType software: facing the challenge of inter-laboratory evaluation of sequence-based MRSA typing**

A. Mellmann, D. Harmsen, A.W. Friedrich, J. Rothganger

**Munster, Wurzburg, D**

**Objectives:** *Staphylococcus aureus* is a major pathogen that causes a wide range of infectious diseases. Since its first identification in the early 1960s, methicillin-resistant *S. aureus* (MRSA) has become a major concern. In order to manage increasing MRSA numbers effective typing protocols have to be applied. Typing of MRSA by analysis of protein A (spa) gene repeat sequences is reproducible; it has a high discriminatory power; the data generated are highly portable (digital data management), and delivers results congruent to other typing methods (e.g. PFGE). One major drawback of repeat typing is that automatic Internet based repeat code assignment has not been available until now.

**Methods:** In our study, we used the recently developed Ridom StaphType® software (Ridom GmbH, Würzburg, Germany; version 1.0) which meets these requirements. The performance of the software was evaluated using spa sequences obtained from 220 independent MRSA isolates, which were collected at the two German university hospitals in Würzburg (n = 107, collected from 7/2001/6/2002) and Münster (n = 113, 1/2002–12/2002).

**Results:** Assignment of spa-types by Ridom StaphType was possible for all 220 isolates tested. In total, three predominant spa-types, two of them identical in both institutions, accounting for about 50% of all isolates were observed. The remaining isolates showed sporadic and different spa-types in both hospitals. The use of Ridom StaphType greatly restricted time needed for generation of data. Furthermore, the software allowed for easy sequence chromatogram editing and flexible data management and retrieval.

**Conclusion:** In conclusion, automatic repeat assignment by Ridom StaphType overcomes shortcomings of current spa-typing and will promote wide-spread use of the method and inter-laboratory exchange of data. Finally and most important the software helps to take evidence based actions (e.g. infectious disease control measurements) in hospital settings.

**P594 Intracellular killing of drug-resistant Staphylococcus aureus by different antibiotics**

F. Rozgonyi, C. Gemmell

**Budapest, HUN; Glasgow, UK**

**Objectives:** The emergence of epidemic multiple resistant *Staphylococcus aureus* (EMRSA) and vancomycin intermediate susceptible (VISA) strains has heightened concerns about the treatment of associated infections. It has also been suggested that intracellular survival of *S. aureus* in phagocytic cells play an important role in the pathogenesis of associated infections, moreover it may contribute to failure of antimicrobial chemotherapy. Therefore intracellularly active antibiotics may be of value in treatment. This study was aimed to investigate the effect of linezolid, moxifloxacin and vancomycin on the intracellular survival of *S. aureus*.

**Methods:** Two clinical isolates of methicillin resistant *S. aureus* (EMRSA 16 also resistant to macrolids and VISA 3759.v with intermediate susceptibility to vancomycin) were tested in J774 macrophage cell line. Susceptibility of the strains was determined by NCCLS broth microdilution method. Cells were infected with bacteria opsonised with 10% normal pooled human serum in Hank’s balanced salt solution supplemented with 1% gelatine. After 2 h of incubation the supernatant was discarded and cells were washed three times in the buffer. Thereafter, antibiotics were
added to the cells at the following concentration: 0 × MIC, 1/2 × MIC, 1 × MIC, 2 × MIC in triplicate. Samples were taken 1, 2, 3, 4 h after antibiotic addition. Cells were washed three times with phosphate buffered saline, then lysed with distilled water. Bacterial count of the cell lysate was determined by the microtitration followed by blood agar plating. Each test was performed three times.

Results: In the presence of vancomycin, intracellular killing was not enhanced, as linezolid and moxifloxacin facilitated the clearance of live intracellular bacteria even at sub-MIC concentrations.

Discussion: Whether this enhancement is due to inhibition of intracellular bacterial multiplication, or due to an effect on the host cells’ killing mechanisms, or both remains to be seen. According to our observation linezolid and moxifloxacin have been shown bioactive intracellularly against MRSA.

Acknowledgement: The project was supported by the FEMS.

P595  MRSA with Panton-Valentine-Leukocidin in Germany

W. Witte, C. Braulke, C. Cuny, B. Strommenger, G. Werner Wernigerode, D

MRSA containing lukS-lukF (Panton-Valentine-Leukocidin) have been reported as community acquired MRSA from US, Australia and Europe (France and Switzerland). The European cMRSA are clearly different by genomic background from isolates from other continents. Here we report on emergence and of lukS-lukF MRSA in Germany.

Methodology: Typing of MRSA from hospital and community all over Germany as National reference center by means of Smal-macrorestriction patterns, MLST (according to www.mlst.net) and spa-sequence (www.ridom.de). PCR demonstration of lukS-lukF, of resistance genes and the agr locus.

Results: From autumn 2002 until December 2003 there were nine sporadic infections in hospitals and 10 cases of deep seated skin infections with lukS-lukF MRSA in the community in different geographical areas of Germany. The isolates exhibited an unique typing pattern with regard to SmaI-macrorestriction, MLST (ST80) and spa (type 46). The exhibited resistance to oxacillin, ciprofloxacin, oxycetracycline (tetM) and fusidic acid (far-1 coded efflux).

They were negative for the agr-locus.

Conclusion: The typing pattern of lukS-lukF MRSA from until now sporadic cases of infections corresponds to that known for MRSA from France and Switzerland. Further characteristics are far-1 mediated fusidic acid resistance (not in other MRSA from Central Europe) and deletion of agr.

P596  Characterisation of methicillin-resistant Staphylococcus aureus isolated at a policlinic in Bari, Italy


Objectives: To evaluate several methods for the detection of methicillin resistance of Staphylococcus aureus (MRSA) isolated in Bari, South Italy and to characterise the strains by genotyping methods.

Methods: Forty-eight strains of S. aureus isolated from clinical samples and different wards of the Policlinico Hospital (Bari, South Italy) were evaluated for methicillin resistance by the PBP latex agglutination test (Oxoid, Milan, Italy), the oxacillin-salt agar screen test, the results of an automated system (Microscan Pans-P, Dade Behring, Milan, Italy), the determination of MIC values to methicillin by the agar dilution and the detection of the gene MecA by PCR. The strains were also analysed by PCR for the mec-associated hypervariable region (HVR-PCR) and by Random Amplified Polymorphic DNA (RAPD) analysis. Thirty-one strains were also tested for the production of enterotoxins A, B, C and D by the Reverse Passive Agglutination Assay (RPLA, Oxoid).

Results: All the 48 strains of S. aureus resulted MRSA by the oxacillin agar screen test, the PBP2a latex agglutination test, the automated system and by the mecA-PCR. The MIC90 for oxacillin was >256 mg/L. Both RAPD and HVR-PCR clustered the strains in three main genotypes. Eight different resistotypes were found.

The strains isolated in the surgical units belonged to the same RAPD and HVR-PCR group and displayed the same resistotype. Fifteen of the 31 strains (48.4%) of S. aureus tested resulted enterotoxins producers (11 were producers of the enterotoxin A, three of the enterotoxin B and one of the enterotoxin D).

Conclusion: In previous studies MRSA accounted for the 40.1% of the S. aureus isolated at Policlinico hospital. In this survey all the methods used for MRSA detection produced concordant results with mecA-PCR that is considered the gold standard for methicillin detection. In addition RAPD and HVR-PCR resulted well correlated to each other in order to cluster the strains and to correlate the groups to the different hospital wards. The methods described and the pattern of antimicrobial susceptibility may be useful for a rapid and inexpensive typing of MRSA in the hospital, especially for the comparison of the strains isolated from different wards and for identification of clonal spread within a hospital.

Parasitic diseases

P597  Detection of Giardia lamblia in stool samples by enzyme immunoassays

N. Miladinovic-Tasic, S. Tasic, A. Tasic, D. Zdravkovic, I. Tasic Nis, CS

Background: Giardia lamblia (GL) protozoa type is the most frequent parasite in the digestive tract. Transmission of GL is faecal-oral, and interhuman contact has the greatest significance in bad hygienic and sanitary conditions. We made comparison of two methods for determination of GL cyst in stool.

Methods: Three successive stool samples were examined by direct microscopy of native preparations with Lugol and after applying of formaline-ethyl acetate concentration technique. Second method for detection of GL antigens in stool specimens was enzyme immunoassays (EIAs) (Ridascreen Giardia, R-Biopharm, Germany). Investigation was performed in July 2003 on risk group of patients in Special hospital for retarded children in Kulina. Stool samples of 104 patients were examined.

Results: Stool samples of all patients were examined by conventional microscopy examination (CME), and by EIAs. From total score of examined samples 101 was negative to GL, using both methods. GL was detected at three stool samples using both methods. Three stool samples were positive using EIAs, and negative using CME. In repeated examination of stool samples using CME, there was no change in results. We conclude that EIAs method is more sensitive for determination of GL cyst in stool.

In risk group of retarded children is hard to perform examination of at least three successive stool samples using CME on presence of GL. By using a more sensitive method one can obtain results examining one stool sample, which has great diagnostic and epidemiological importance.
**P598** Epidemic features of intestinal parasitosis in a children's hospital in Athens

I. Varzakakos, H. Damaskopoulou, A. Makri, L. Papavasileiou, H. Papavasileiou

*Athens, GR*

**Background:** Intestinal parasitosis is a major problem in children, which is responsible for diarrhoea and nutritional deficiencies. Environmental, socioeconomic, demographic and health related behavior is known to influence the transmission and distribution of these infections.

**Objective:** Our goal was to determine the prevalence of intestinal parasitic infections among patients in a children’s hospital in Athens and its possible association with demographic and socioeconomic parameters.

**Material and methods:** During a period of 6 years (27/9/97–27/9/03) a total of 3022 samples were examined in our laboratory (1720 of stool specimens and 1302 of scotch tests). The study population (both Greeks and immigrants from developing countries) was children between 3 and 15 years old, which either examined in the outpatient’s clinic or hospitalised. These patients had one or more of the following symptoms: diarrhoea, abdominal pain, eosinophilia, pruritus. All specimens were examined in direct microscopy. In addition for all stool specimens the formalin – ether technique and trichromic stain were used.

**Results:** Of the 3022 children examined, 212 (7% were found positive for various intestinal parasites. Six (6) different species of helminthes and protozoa were found among the samples. By far the highest frequency 162 cases (76.5%) was noted for Enterobius vermicularis, followed by Giardia lamblia 30 cases (14.2%), Entamoeba histolytica 10 cases (4.7%), Ascaris lumbricoides six cases (2.8%), Trichuris trichiura two cases (0.9%) and Taenia saginata two cases (0.9%).

**Conclusion:** The frequency of intestinal parasitic infection in Greece, although it is relatively low, it is not rare. Fifty-five per cent of the 212 cases that were found positive belonged to immigrants coming from developing countries. The above results indicate that education policies should be taken and domestic and personal hygiene should be improved as well.

**P599** Survey of intestinal parasitic infections among physical and mental retardees in a maintenance centre, Taft

M.H. Anvari Tafti, A.A. Jafarri Nodoushan, A. Fattahi Bafghi

*Yazd, IR*

**Introduction:** There are more than 453,070 individuals suffering from physical and mental retardation in Iran, those who need expensive health services and maintenance facilities. Because of their mental, physiological and physical problems in handling personal care and also living in mass population, they are always at high risk in acquiring contagious infections. In order to decrease their infection rate and then to treat them, it seems that searching their intestine for parasites found together with epidemiological data as gender, age and its possible association with demographic and socio-economic parameters.

**Material and methods:** The current descriptive and cross-sectional study was performed on 86 mental and physical retardation cases. Following completion questionnaire form of cases, three stool samples were collected from each case for direct examination using wet-mount and formaline-ether concentration methods. Data was analysed using SPSS software.

**Result:** 54.7% of cases included female, 34.9% had more than 20 years old and 65.8% needed the camping observation. 45.3% of cases had previous history of infectious disease, that highest ratio was related to cutaneous fungal disease (25.6%). In total, 48.8% were infected with different types of intestinal parasites and 18.6% had more than one parasite. The parasite frequency, which were detected is followed: Entamoeba coli 32.6%, Giardia lamblia 18.6%, Chlamastix mesnili 11.6%, Lodamoeba butschlii 8.1%, Blastocystis hominis 1.2% and oxyour 1.2%. There was not seen any statistical significant differences of infection rate between male and female cases. The 20-year-old and more age group showed the highest rate of parasitic infection (16.5%). There was seen a statistical significant differences in infection rates between camping and noncamping individual (P < 0.05).

**Conclusion:** Fifty per cent infection suggest needs of control and supervision for health care services and facilities to improve their personal health and also prevention of contagious contacts.

**P600** Intestinal parasites in a paediatric hospital population in Madrid (Spain), January 2002 to October 2003

A. Morente, V.M. Martinez, R. De Julián, M. Baquero, M. Subirats

*Madrid, E*

**Objectives:** Parasitic diseases are important causes of chronic diarrhoea and low growth in childhood. The aim of this study was to analyse and present the incidence of fecal parasites in pediatric population in our area.

**Methods:** From January 2002 to October 2003 in our laboratory 2710 faecal samples were examined for the presence of parasitic pathogens. We studied 721 consecutive outpatient (mean age 4.8 years, range 0.1 months–16 years) who were referred to our Paediatric Department and considered for recruitment into this analysis.

**Results:** The following parasites were detected. Blastocystis hominis (105); Chilomastix mesnili (10); Dientamoeba fragilis (3); Entodinamoeba histolytica nana (88); Entamoeba coli (103); Entamoeba hartmanni (24); Entamoeba histolytica (54); Enterobius vermicularis (3); Giardia intestinalis (156); Schistosoma mansoni (1); Hymenolepis nana (89); Iodamoeba butschlii (12); Isospora belli (1); Paragonimus sp. (2); Strongyloides stercoralis (10); Taenia sp. (2); Trichuris trichiura (41); Hookworms (5); Ascaris lumbricoides (22). Parasitologic findings were proved in 19.5% of all examined samples (29.54% of total patients). No significant differences was observed when we analysed per sex. In spite of the greater number of samples was found in the range of 2 years old or less, the highest positive rate was found in the range of 7–10 years old. Of the total number of faecal samples, in 383 (53.7%) pathogen parasites were detected, while in 330 (46.2%) nonpathogen parasites were found. Giardia intestinalis was the most commonly pathogen isolated, followed by Hymenolepis nana and Entamoeba histolytica, whereas Blastocystis hominis and Entamoeba coli were the main non-pathogen parasites detected.

**Conclusion:** From these results, we could conclude intestinal parasitic diseases are important causes of morbidity in childhood. We observed discrepancies between age ranges in which diarrhoeas because of parasites are mainly suspected and age ranges in which positive rates are higher.

**P601** A 3-year descriptive study of intestinal parasite infections in outpatients in Madrid, Spain (2000–02)


*Madrid, E*

**Background:** Intestinal parasite infections have increased in our country mainly because of easier access to international travel and immigration.

**Objective:** To know the prevalence of intestinal parasite infections in outpatients attending to a health area (Area 1, Madrid, Spain).

**Patients and methods:** 11 016 faecal samples (10 346 stools, 604 perianal swabs and 66 adhesive tapes for diagnosis of pinworms) were processed from 5757 patients between January 2000 and December 2002. Origin of samples and seasonal incidence of the parasites found together with epidemiological data as gender, age and geographical origin of infected subjects were analysed. Stools were concentrated using a disposable parasite concentrator with formalin-ethyl acetate (Biosepar, Germany). Coccidian oocytes were screened on direct and concentrated faecal smears stained by a modified Kinyoun acid-fast staining.

**Results:** 53.3% of parasitised patients were from foreign origin, mainly from South America (91.7%); of which 79.8% were Ecuado-
Enteropathogenic bacteria isolated from patients with diarrhoea: frequency of isolation and susceptibility testing to commonly used antibiotics in a Tehran hospital: a 1-year study

B. Kiadarbandisari
Tehran, IR

Objectives: The aim of this study was to determine bacterial aetiology of the diarrhoea, frequency of isolated various enteric pathogens and susceptibility to commonly used antibiotics.

Methods: During 1 year study from November 2001 to November 2002, in total 2291 stool specimens were examined microscopically in microbiology laboratory. All specimens inoculated to routine microbiological cultures media including: XLD, Selenit F Mac-Konky agar Hekton Enteric and SS agar. All isolated bacteria identified by biochemical tests and stereotyped by relevant antisera (Bahar Afshar Company). Susceptibility testing performed by disk diffusion method as recommended by NCCLS.

Results: Of 2991 stool sample 123 enteropathogenic bacteria isolated. The frequency of isolated bacteria was: Shigella spp. 58 (47.8%) strains, enteropathogenic Escherichia coli (EPEC) 28 (22.2%) and Salmonella spp. 28 (22.8%). Shigella sonnei was the most prevalent serotype with 43 (74.5%) isolates followed by Shigella flexneri 11 (12%) and Shigella dysenteriae and Shigella boydii each two strains. Salmonella group B and Salmonella typhi. Susceptibility of Shigella isolates to ciprofloxacin, ceftrixone, amikacin, nalidixic acid co-trimoxazol, ampicillin and tetracycline was 98.2, 91, 88.3, 20.4, 19.3 and 15.1% respectively. All Salmonella isolates were susceptible to cefotaxim, followed by gentamycin 99.6% chloramphenicol 89.6% co-trimoxazol 80% and ampicillin 19.34% and tetracyclin 15.1%. About 60% of all EPEC were susceptible to ciprofloxacin, gentamycin, chloramphenicol co-trimazol and cefotaxim. All isolates were resistant to ampicillin.

Conclusion: This study reveals that Shigella sonnei was the predominant serotype among Shigella isolates. The majority of Shigella spp. and EPEC were resistant to ampicillin and co-trimoxazol. The rate of resistance among Salmonella spp. isolates was not high except for ampicillin.

Intestinal parasites found in native and foreign workers during a 7-year period in Greece

J. Spiroipoupolou, M. Palta, V. Nickolas, V. Petrochelou-Paschou
Athens, GR

Infections with intestinal parasites constitute both a medical and a public health problem. The improvement of sanitation since 1960 led to a significant reduction of parasitosis in Greece. However, the high numbers of foreign workers entering the country have contributed to the increased numbers of faecal examination and isolation of intestinal parasites in our laboratory. The objective of this study was to monitor the number of people carrying intestinal parasites and to compare them with those of earlier years.

Material and methods: During a 7-year period (1997–2003) samples from 1879 individuals (natives and foreigners) who were examined as outpatients at the hospital, in order to obtain a health clearance certificate, were included in the study. Single stool specimens obtained without purgatives were subjected both to macroscopic and microscopic examination: (1) direct unstained smears, (2) iodine stained direct smears, (3) unstained wet films after centrifugation (sedimentation method: Ritchie).

Results: Out of 1879 individuals who were examined, 1061 were natives and 818 foreigners. Parasites were found in 17 natives (16.6%) and in 97 foreigners (11.8%). The species of isolated parasites were: (a) Protozoa [Giardia lambia (34), Blastocystis hominis (9), Entamoeba histolytica/E. dispers (6), nonpathogenic Amoeba (77)] (b) Intestinal Nematodes [Ascaris lumbricoide (5) and Enterobius vermicularis (3)]. In 23 individuals a mixed infection with two species of parasites was found while in one, three species were present.

Conclusion: The alertness of public health services is critical in order to constrain the spreading of parasitic infections to indigenous populations by locating and treating, if possible, foreign carriers.
**P606** Cryptosporidiosis in children


**Cadiz, E**

**Objectives:** During an outbreak of acute watery diarrhoea among the residents children of Cadiz (Spain) occurred from August 12 through November 18 2003, Cryptosporidium parvum oocysts were identified on 22 stool specimens from these patients.

**Methods:** Stool specimens were collected from patients with diarrhoea, and were preserved in sodium acetate-acetic acid-formaldehyde. Stool were sedimented by centrifugation. The microscopic examination of a direct smear and the cold acid-fast Kinyoun stain were realised. An extensive questionnaire with demographic, clinical and epidemiologic characteristics was defined.

**Results:** Of the 22 patients with cryptosporidiosis, a 59% was male, a 27% required hospitalization, 21 were immunocompetents and one was HIV positive. Their mean age was 4 years. The clinical manifestation included watery diarrheaa (100%), anorexia (63%), abdominal cramps (54%), vomiting (35%), and fever(13%). The median duration of diarrhoea was 13 days. Cryptosporidium parvum was identified as the unique pathogen in 86% of the cases.

**Conclusion:** Cryptosporidium parvum was not a common cause of gastroenteritis in immunocompetent children resident in Cadiz. However, we recommend that clinicians and laboratories consider performing routine stool test for Cryptosporidium in people with watery diarrhoea. This outbreak highlights the importance of surveillance for cryptosporidiosis and the need for guide for the prevention of infections among HIV infected persons. Further studies are needed to determine the prevalence and spectrum of the clinical patterns of this parasitic disease.

**P607** Human and animal trichinellosis in Belgrade

Z. Dakić, Z. Kulisic, B. Antonijević, R. Dimitrović, M. Jovanović, M. Petrović

**Belgrade, CS**

**Objectives:** To determine the number of humans infected with Trichinella spiralis in the area of Belgrade and the kind and source of meat contaminated with the parasite.

**Methods:** Epidemiologic data on the number of human trichinellosis in Belgrade in the period from 1996 to 2000 were collected from the Institute for Infectious and Tropical Diseases and Institute of Public Health of Belgrade and the data on pig trichinellosis from the Ministry of Agriculture and Forestry. We collected data on the connection between the infected humans and source of meat from the Food and Drinks Centre.

**Results:** In the period of investigation on the territory of Belgrade 399 individuals were infected with T. spiralis that makes 12.3% of the infected individuals in Serbia. The greatest number of infected people was in 1997 and 2000 (morbidity: 6.0/10 000 and 5.1/10 000 respectively). The disease had seasonal character and appeared in epidemics (42 epidemics with 306 patients). In this period 77 pigs were found to harbour the parasite. Fifteen of them were the source of infections, while the others were eliminated thanks to the timely action of veterinary inspection. It makes 30.61% of the total of 49 pigs infected with T. spiralis that were the source of infection for inhabitants of Belgrade. The other pigs (69.39%) originated from the other parts of the country. The smallest number of infected individuals was observed in central parts of the town and the greatest number of them was in three suburbs, where was also found the greatest number of pigs infected with T. spiralis.

**Conclusion:** 30.61% of all pigs that were sources of epidemics of inhabitants of Belgrade originated from the territory of Belgrade. There was topographic correlation between trichinellosis of pigs and humans. The greatest number of infected humans and pigs was observed on the territory of three suburbs, that implicates the foci of infections in these areas.
relapsing fever and also haemorrhagic fevers. The observed blood parasites in the studied rodents show that these animals can be potential reservoir hosts for some zoonoses in Bandar Abbas, especially in quarters with low sanitation.

**P609** Epidemiology of *Blastocystis hominis* and other intestinal parasites in female marriage emigrants in Taiwan

J.W. Shin, H.S. Cheng
Taiwan, TW

**Background:** There were 139,735 foreign marriage immigrants (exclude the Mainland China) until the end of 2002 in Taiwan and 58.2% of them came from Vietnamese. There were several studies mentioned about the prevalence of parasitic infection among foreign workers but none did concern with these marriages immigrants. This is the first study about the prevalence of intestinal parasitic infections in the female marriage immigrants from Vietnam in Taiwan.

**Methods:** The female marriage immigrants from Vietnam who were required to take a complete physical examination for the residence approval July 1998 to June 2001 in southern Taiwan were included in this study. Examination for intestinal parasites used the merthiolate-iodine formaldehyde concentration method. Student’s t-tests and multiple regressions were used to test for significance and for statistical adjustment.

**Results:** The prevalence of intestinal parasite infection of 1434 female Vietnamese marriage immigrants was 37.3% and there was a significant increase from 1999 to 2001 in statistically (P < 0.0001) but decreasing trend in the prevalence by age in statistically (P < 0.0001). There were 20 species of intestinal parasites were found in the study. 30.5% for 12 species transmitted via faecal–oral route, 11.8% for three species from soil-mediated route, and 0.7% for five species by food-bone infection. The prevalence of *blastocystosis* (20.4%) and hookworm infection (9.7%) was remained high in the protozoa and helminthes infection in the immigrants. The results of the prevalence of intestinal parasite examinations were aged adjusted by using multiple regression analysis. This also showed significant differences in the prevalence of intestinal parasite infection in different annuals in statistically (adjusted with age, P < 0.001).

**Conclusion:** The results provide systematic information on intestinal parasitic infection among female marriage immigrants in Taiwan and advise appropriate health care after parasite infection confirmed in these migration communities.

**P610** Characterisation of casein kinase 1 in *Trichomonas vaginalis*

P. Tang, C.H. Huang
Taoyuan, TW

Casein kinases (CK) are important regulators of many cellular processes in higher eukaryotes; however, casein kinase related genes has never been identified in *Trichomonas vaginalis*. The full length cDNA of two casein kinase 1 (CK1) partial cDNA clones isolated from a *T. vaginalis* expressed sequence tags (EST) library were obtained by 5′ rapid amplification cDNA ends (5′-RACE). The complete open reading frames of *TvCK1.1* and *TvCK1.2* were 1467 bp and 1572 bp. The *Trichomonads* CK1-like gene encodes a predicted molecular weight of 60 and 63 kDa, respectively. The expression of *TvCK1s* were determined in synchronised cell division cycle. Results from quantitative real-time PCR showed that *TvCK1.1* are highly expressed during the cell division cycle but not *TvCK1.2*.

**P611** Comparison of direct microscopy and in vitro cultures in detection of *Trichomonas vaginalis*

G. Sömmez Tamer, D. Öztürk Dünder, S. Caliskan, E. Doger Kocaeli, TR

**Objectives:** The parasitic protozoan *Trichomonas vaginalis* is a common pathogen that causes trichomoniasis and has been linked to preterm birth, acquisition of human immunodeficiency virus, infertility and nongonococcal urethritis. Diagnosis is made by identifying motile unicellular flagellates on a vaginal saline specimen, by using different culture media and serological and molecular methods. This clinical study performed to evaluate wet mount microscopy and two broth culture methods for the detection of *T. vaginalis* in swab specimens obtained from female patients.

**Methods:** A total of 128 women, ages between 18 and 48 years with abnormal vaginal discharge who applied to Obstetrics and Gynecology Department were enrolled to this study. The samples of vaginal secretions from the posterior fornix collected on a sterile cotton tipped swab. The smears were examined using wet-mount preparations and culturing on cystein pepton maltose (CPLM) medium and tripicase yeast extract maltose (TYM) medium in 1 h after specimen collection. We determined the optimal days on which to read culture tubes by inoculating aliquots of secretions in to each medium and reading the tubes 1, 2, 3, 4 and 7 days later and evaluated the test performance criteria of three methods.

**Results:** Of the 128 patients 12 (9.37%) had positive results for *T. vaginalis*. All the 12 positive cases were detected in TYM medium. TYM medium is accepted as gold standard. CPLM medium detected only nine of these 12 positive cases. Sensitivity, specificity, positive predictive values and negative predictive values were 75, 100, 100 and 97% respectively. Wet mount examination detected only seven of the 12 positive cases. Sensitivity, specificity, positive predictive values and negative predictive values were 58, 100, 100 and 96% respectively. One CPLM negative case was positive in wet mount examination. Optimal growth observed in 2 days for CPLM and in 4 days for TYM medium.

**Conclusions:** Culturing on TYM medium was the most sensitive technique which we used in *T. vaginalis* diagnosis. Although vaginal saline wet mount is an easy and low-cost technique, culture is more sensitive than the direct examination and TYM medium is superior to CPLM medium for growth of *T. vaginalis*. We project to expand this study with large numbers of clinical specimens.

**P612** Seroprevalence of *Toxoplasma gondii* infection among some risk groups in Erzurum, Turkey

A.E. Aktas, H. Yazgi, M. Ertek, A. Ayyildiz
Erzurum, TR

**Objectives:** Toxoplasmosis is an infection caused by a single-celled parasite called *Toxoplasma gondii*. The parasite is found throughout the world. Toxoplasmosis can be transmitted to humans by ingestion of tissue cysts in raw or inadequately cooked infected meat or in uncooked foods that have come in contact with contaminated meat, by inadvertent ingestion of oocysts and sporozoites in cat faeces, or transplacentally. Our objective was to determine the seroprevalence of *T. gondii* antibody among some risk groups to ascertain whether they have an increased risk through occupational exposure.

**Methods:** The blood samples collected from three different risk groups including 30 veterinarians, 43 butchers, 43 slaughterhouses worker and, 100 healthy people as control groups, and obtained sera were stored at −20°C until used. Anti-toxoplasma IgG and IgM antibodies were determined by using ELISA (Trinity Biotech USA). Data and results were analysed by software Microsta (hypothesis test for two proportional from independent groups).

**Results:** The percentage of *Toxoplasma* Ig G seropositivity were 61.2% in the risk groups and 38% in control group. The differences between risk and control groups was found to be significant (P < 0.001). Toxoplasma Ig G seropositivity rates were 53.5% in veterinarians, 60.5% in butchers, 67.4% in slaughterhouses workers. Toxoplasma Ig M was negative in all groups.
**P613** Comparison of DNA extraction methods and PCR assays for detection of *Toxoplasma gondii*

B. Edvinsson, S. Jalal, B. Evengård on behalf of the ESGT

**Objectives:** The use of PCR for detection of *Toxoplasma gondii* is sensitive and more relevant to use than serological techniques as a diagnostic tool in immunocompromised hosts. There are different DNA extraction methods and PCR assays available. We compare different DNA extraction methods and three different PCR assays for detection of *T. gondii*.

**Methods:** DNA from *T. gondii* tachyzoites extracted either with QIAamp DNA mini Kit or MagNa pure DNA extraction methods was analysed with LightCycler SYBR green 1. Sensitivity of a real-time PCR TaqMan assay was determined using dilution series of extracted DNA. Also, DNA from 2 mL blood samples spiked with 10 to 100 tachyzoites per sample was extracted using the two extraction methods and analysed with conventional PCR, PCR in combination with oligochromatography or real-time PCR SYBR green 1 or TaqMan. All assays targets the B1 gene.

**Results:** The two DNA extraction methods showed no difference in extracting DNA from *T. gondii* tachyzoites. Analysis of spiked blood samples revealed no difference in sensitivity between the two DNA extraction methods when followed by PCR oligochromatography or real-time PCR TaqMan. Conventional PCR was more sensitive when DNA was extracted using QIAamp DNA mini Kit. Detection limit of the TaqMan assay was one parasitic genome in a run using dilution series of pure parasitic DNA. When analysing DNA extracted from 2 mL spiked blood samples a less sensitive detection limit was observed. Real-time PCR SYBR-green 1 was unable to detect parasitic DNA in all spiked blood samples.

**Conclusions:** The two DNA extraction methods are equally efficient in extraction of DNA from *T. gondii* tachyzoites. LightCycler PCR SYBR green 1 yielded a high background signal when analysing blood samples making the detection signal undistinguishable. Presence of blood cell DNA also altered the detection limit of the TaqMan assay. Conventional PCR and PCR oligochromatography were more sensitive than real-time PCR TaqMan for spiked blood samples. Our results also show that conventional PCR was more sensitive in the spiked blood samples using QIAamp DNA mini Kit, suggesting that the choice of extraction method may affect different PCR assays differently.

**P614** Evaluation of the ELISA IgE test for diagnosis of acute toxoplasmosis

P. Kodym, V. Tolarova, A. Lehmovcova, M. Maly
Prague, CZ

**Objectives:** Usefulness of the IgE ELISA test (TEST-LINE Brno, Czech Republic) for the detection of acute toxoplasmosis was evaluated by comparing the course of quantitative and qualitative results after infection, and also the sensitivity, specificity, positive and negative predictive value with parameters of other tests.

**Methods:** 545 sera samples taken from Toxoplasma-infected patients with known clinical status and duration of the infection were, besides the IgE test, tested also with ELISA IgA, IgM (TEST-LINE, BIO-RAD), the complement-fixation test (CFT-Seva-pharma, Prague) and IgG avidity test (in-house, NRL TOXO). As a criterion of acute toxoplasmosis, the presence of clinical symptoms was considered.

**Results:** Clinical signs persist for longer than 4 months in only 30% of patients. While low avidity of IgG prevails in samples taken up to 4 months after onset of symptoms, IgE ELISA is predominantly positive up to month 6, IgA ELISA up to month 8, and IgM ELISA up to month 12, like CFT (titres up to 256). Sensitivity of the IgE test (94.3%) is lower than that of IgM ELISA (98.1%), but the specific and predictive value of a positive test for IgE (91.7%; 75.6%, respectively) are superior when compared with the same parameters of IgM (65%; 43.3%). No relation of IgE positivity with an allergy in patients was found. The IgE test showed the lowest rate (7.7%) of false positive plus false negative results evidencing the best correlation with clinical features.

**Conclusions:** Similarly like the IgG avidity test, the IgE ELISA TEST-LINE is a highly specific test which can be very useful in combination with some sensitive test (IgM, CFT). As the increase in IgG avidity is strictly related to the time since infection, this method is preferred when the duration of the Toxoplasma infection is needed to be known. However, in some patients, high avidity after 4 months can be accompanied by persisting clinical symptoms of toxoplasmosis. If data reflecting the clinical state of the patients are preferred, the IgE test is the method of choice.

**P615** Effect of testing for IgG avidity for serodiagnosis of toxoplasmosis in pregnant women

D. Findik, U. Arslan, O. Ural, I. Tuncer
Konya, TR

**Objectives:** Measurement of *Toxoplasma gondii* immunoglobulin G (IgG) avidity (binding strength) is a powerful tool for distinguishing recent toxoplasma infection from past. This study was planned to determine Toxo IgM and IgG specific antibodies against *T. gondii* and IgG toxo avidity in pregnant women.

**Materials and methods:** Blood samples were taken from all 1363 female patients attending the gynecology and obstetric department of Selcuk University Meram Faculty of Education’s Research and Practice Hospital during the period 2001–03. The presence of specific *T. gondii* antibodies was determined using VIDAS system; screening test, test for screening IgM and IgG specific antibodies and IgG toxo avidity test (bio-Merieux, France).

**Results:** Out of 1363 pregnant women 410 (30.08%) were found IgG toxo antibodies positive. Thirty-two (7.8%) pregnant women have both IgM and IgG positive tests. Four (1%) have equivocal IgM toxo antibodies tests. Low IgG avidity (<0.200, may be seen in acute primary infection with *T. gondii*) was found in 10 (2.4%) and high IgG avidity (>0.300 excludes primary infection within last 16 weeks) was found in 398 (97.1%) of the samples (toxo IgG positive). In two (0.5%) sera the avidity of IgG antibodies was borderline (0.200 to <0.300) indicating possible primary infection during the last 6 months. In 87.5% of IgG positive and IgG positive pregnant women, we determined high IgG avidity (showing low risk pregnancy) reducing unnecessary pregnancy terminations. Besides of this; in 1.1% of IgG positive and IgM negative women we determined low IgG (showing high risk pregnancies that were not defined before). A relation of IgG positivity with an allergy in patients was found. The IgG test showed the lowest rate (7.7%) of false positive plus false negative results evidencing the best correlation with clinical features.

**Conclusions:** Toxo IgG avidity test is more precise test in showing high risk and low risk pregnancies.
Methods: V. cholerae strains (V. cholerae O139 from the outbreak in Bangladesh 1993, the seventh-pandemic V. cholerae O1 El Tor-Inaba strain N16961, the sixth-pandemic V. cholerae O1 Classical-Ogawa strain 395, and the nonepidemic strain V. cholerae O54) were co-cultivated with A. castellanii for 16 days to examine whether an interaction could be established. Each day the number of live bacteria as well as amoeba was estimated. Intracellularly growing bacteria were distinguished from extracellularly growing bacteria by gentamycin treatment to show the intracellular growth of bacteria. Bacteria were located to different compartments of amoebae, which could be mirrored by microscopy.

Results: When V. cholerae strains and A. castellanii were co-cultured it was found that V. cholerae O139, V. cholerae O1 El Tor-Inaba, and V. cholerae O54 multiplied intracellularly from 0 to 105 cfu/mL, while V. cholerae O1 Classical-Ogawa strain 395 could not grow in Acanthamoeba cells. All V. cholerae strains tested stimulated growth of amoebae during the co-culturing period, as the number of amoebae increased. Approximately 2 weeks after infection of amoebae, bacteria could be found in the inter-space between walls of the amoeba cysts. These data show a role of free-living amoebae as hosts for growth of V. cholerae and that this interaction can play an important role in the ecological niche of the bacterium.

Conclusions: Besides showing a new ecological niche of V. cholerae, this study redirects the bacterium from being pure extracellular parasite to the constellation of facultative intracellular bacterium that, thus, opens new medical aspects of the disease. This will have implications on the cholera epidemiology, immunology, and pathogenicity.
than 700 malaria cases are detected each year by microscopy, which is an insensitive technique.

**Materials/subject & methods:** In this study, 229 patients with the microscopic diagnosis of *P. falciparum* infection were monitored with microscopy, ICT Malaria *Pf/Pv* test and OptiMAL assay to detect persistent antigenemia in patients negative for asexual-stage parasitaemia following antimalarial treatment.

**Results:** Following successful antimalarial chemotherapy, the ICT Malaria *Pf/Pv* test detected persistent antigenemia in 160 of the 229 (70%) patients on day 7. 144 (63%) of the patients reacted with histidine rich protein 2 (HRP2) antigen and 110 (48%) reacted with malarial antigens (PMAs). However, the reactivity to HRP2 antigen and PMAs dropped to 35 and 23% respectively on day 14. A higher proportion of patients were positive for HRP2 antigen than for PMAs. The majority of the patients were positive for both HRP2 antigen and PMAs however, 16 (7%) patients were HRP2– but PMAs+. Compared with ICT Malaria *Pf/Pv* test, the OptiMAL assay detected significantly less number of post-treatment persistent reactions. Levels of parasite-specific lactate dehydrogenases (pLDH) and malarial pLDH, as detected by the OptiMAL assay, were shown to decline in parallel with clearance of asexual-stage parasitemia. On day 7, persistent pLDH reactivity was detected in 89 (39%) and malarial pLDH in 34 (15%) cases that dropped significantly to 21 (9%) and 14 (6%) respectively on day 14.

**Conclusion:** Thus OptiMAL may be superior to ICT Malaria *Pf/Pv* in monitoring therapeutic responses. However, a further improvement in quantification of current antigens is required to further enhance the sensitivity and specificity these assays.

**Acknowledgements:** Financially supported was granted by Kuwait University Grant MI 109.

**P620** Drug resistance to malaria in Orissa

P. Bishnu Prasad  
Bhubaneswar, IND

**Objective:** To find out the resistance status of malaria parasite to 4-aminoquinolin in Orissa.

**Methods:** The place of study is selected on the basis of predominance of *P. falciparum* cases and deaths because of malaria. The team follow the method of 28 days *in vivo* study prescribe by the World Health Organization (WHO).

**Results:** The team carried out total 14 studies from 1998 to 2002 over a span of 5 years (see Table).

**Table 1. The total number of test case were 2033**

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>RI</th>
<th>RII</th>
<th>RIII</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1722</td>
<td>147</td>
<td>121</td>
<td>43</td>
</tr>
<tr>
<td>%</td>
<td>34.60</td>
<td>7.20</td>
<td>5.90</td>
<td>2.10</td>
</tr>
</tbody>
</table>

**Conclusion:** We get RI & RII resistance in all most all studies. The summary findings for last 3 years shows there is increasing of RII & RIII in persistent areas of transmission. As per the drug policy on malaria in the country, change of drug in resistance areas only required when there is 25% increase of RII & RIII put together. So these areas do not warrant change of chloroquine to some second line drug in the region.

**P621** *In vitro* recrudescence of *P. falciparum* parasites suppressed to dormant state by atovaquone alone and in combination with proguanil

M. Thapar, P. Gil, A. Bjorkman  
Stockholm, S

**Objective:** The primary objective was to study the viability of *Plasmodium falciparum* parasites reappearing during long-term follow up cultures after repetitive exposures to various concentrations of atovaquone and proguanil.

**Methods:** Two *P. falciparum* parasite strains were used for the *in vitro* experiments, i.e. F32 and FCR3 originating from Tanzania and Thailand respectively. The parasite strains were kept in continuous culture according to known method of Trager and Jensen, 1976.

**Results:** Parasites (F32 and FCR3) exposed to 100–5000 nM atovaquone for 96 h were reduced to ≤ 5% of initial parasitaemia but recrudesced after 16–21 days. Similarly, parasites exposed to 1000 nM atovaquone for 48, 72, 96 and 144 h recrudesced after 9, 14, 21 and 23 days respectively. After the removal of drug exposure, only one to three parasites and only schizonts were consistently found per 10 000 RBCs, apparently unable to produce trophozoites and thus possibly adopting a ‘dormant state’. Parasites (F32 and FCR3) exposed to 500 nM atovaquone for 72 h, recurred after 14 days. These recrudescent parasites were then similarly re-exposed and suppressed by atovaquone in three consecutive follow up experiments. They then reappeared after 10, 9 and 6 days respectively. No known point mutations in cytochrome b gene (cytb), associated with atovaquone resistance, were however detected in any recrudescent parasites. Finally, parasites (F32) exposed to various concentrations of atovaquone and proguanil in combination for 72 h reappeared after 9–17 days. Relatively low concentrations of proguanil (0.2 times EC90) were required in combination with atovaquone to kill more than 95% of the parasites. The baseline susceptibilities of the parasites to both individual drugs were similar before and after recrudescence in all experiments.

**Conclusions:** In *in vivo*, the ‘dormant state’ parasites may represent a small fraction of cytostatic parasites, unable to grow and unsuseptible to further treatment with the drug. Return from ‘dormant state’ to normal growth within few days after removal of the drug pressure may represent the failure of drug action.

**P622** Typhoid and paratyphoid fever in the Czech Republic, 1993–2003

H. Ambrozov, J. Vanista, M. Reisingerova, C. Benes  
Prague, CZ

**Objectives:** Typhoid fever is endemic in many developing countries. Number of the cases is estimated between 12 and 33 million/year in the world. In the Czech republic these diseases occur very rarely. Since the World War II, the occurrence has decreased. While in 1951 the incidence rate was more than 14/100 000 inhabitants, in 2002 was only 0.01/100 000. The incidence rate of paratyphoid fever B decreased from 0.75/100 000 in 1951 to 0/100 000 in 2002. Almost all cases are imported. Between the years 1993 and 2003 50 cases of enteric fever were reported in the Czech republic. In our department we admitted 20 patients with imported typhoid or paratyphoid fever during this time. Seventeen patients had typhoid fever and three paratyphoid fever A. Seventeen patients were from the Czech republic, three were foreigners. Men prevailed women (16:4). Majority of cases were from the age group between 20 and 30 years. Eighty-five per cent of all patients were infected in Asia, the rest in Africa and Europe. The highest risk of infection was in India, where 13 patients travelled.

**Methods:** All patients were physically examined and diagnosis was verified by haemoculture, stool culture and Widal’s test. In all patients blood count, liver function test and other biochemical examination were examined.

**Results:** Clinical picture was usually mild or moderate, only one patient had severe course with confusion. Fever occurred in 100%, hepatosplenomegaly in 75%, diarrhea in 70% and headache in 68.4% of all patients. The other signs (hypotension, abdominal pain, bradycardia) were less frequent. Leucopenia was found only in 10% of all patients. 94.5% of patients had leucopenia, 40% leucocytosis. Complications occurred very rarely, relapse was seen only once. Four patients had dual infection. Diagnosis was usually made by haemoculture (90% positive), stool culture was positive only in 35%. Fifty per cent of all strains were resistant to antibiotics. Multiresistant strain occurred only
Imported dengue fever in the Czech Republic

P. Chalupa, N. Sojkova, J. Januska
Brno, Prague, Ostrava, CZ

Two institutions in the Czech Republic possess the facilities for the diagnosis of dengue fever (DF): the National Reference Laboratory for Arboviruses, Ostrava, and the Department of Virology, Teaching Hospital Na Bulovce, Prague. During 1997–2002, 417 patients were examined for suspected DF. Serological evidence, i.e. the presence of anti-dengue IgM and IgG antibodies, was based on ELISA using PANBIO kits. Epidemiological data on DF have been collected by the National Reference Center for Epidemiological Data Analysis in Prague since 1997. Up till now, 15 DF cases (diagnostic code A90) and one case of dengue haemorrhagic fever (DHF, diagnostic code A91) have been registered.

Conclusions: Typhoid and paratyphoid fever are very rare in the Czech Republic, usually are imported. In our department were treated 41% of all cases occurring in the Czech Republic between the years 1993 and 2003. The highest risk of infection was in Asia (India) followed by Africa and Europe. Fifty per cent of all strains were resistant to antibiotics. Fluoroquinolones were the drug of choice and treatment was successful.

Discussion:

A 34-year-old man presented with a 4-week history of fever, malaise, poor feeding and abdominal pain. Past medical history was unremarkable. Physical examination revealed high fever (39.2°C) with pallor, hepatomegaly and splenomegaly. Lymph nodes were not enlarged and vital signs were normal. The laboratory findings showed pancytopenia and elevated erythrocyte sedimentation rate; serum triglycerides were increased to 267 mg/dL with normal cholesterol. Transaminase activity and total bilirubin were high, whereas serum fibrinogen was low with elevated circulating fibrin degradation products. There was no serological evidence for infection with cytomegalovirus, Epstein-Barr virus and toxoplasmosis. Blood cultures were negative. The patient symptoms deteriorated within 24 h and he was referred to intensive care unit where intestinal bleeding with disseminated intravascular coagulation occurred. The patient died 48 h after admission in the ICU. Bone marrow and liver biopsy performed after death were compatible with diagnosis of HLH, whereas peripheral blood culture for parasites yielded Leishmania infantum, identified as zymodeme MON1 which is predominant in our country.

Conclusion: Leishmaniasis should be considered when discussing the cause of haemophagocyticosis in countries where the disease is endemic, such as Tunisia. When revealed by haemophagocytosis, diagnosis may be difficult particularly in adults.

Contributions of PCR in laboratory diagnosis of visceral Leishmaniasis

Thessaloniki, GR

Background: Visceral leishmaniasis (VL) is a severe zoonotic and often life-threatening disease caused by the protozoan Leishmania spp. The disease is widespread in the Mediterranean region and also endemic in many parts of the world. The aim of this study was the evaluation and the contribution of PCR in the early diagnosis of VL and also the comparison of PCR to the serological tests and the direct detection of parasite in bone-marrow aspirate (BMA).

Materials and methods: A total of 24 patients (36 whole blood and sera samples) with confirmed VL were assessed in this study. The patients were divided in to two groups: In group 1, 11 patients (16 whole blood and sera samples) with definite VL confirmed by the demonstration of the parasite in BMA were included. In group 2, 13 patients (20 whole blood and sera samples) with clinical manifestations of VL (fever, enlargement of spleen, pancytopenia), positive results in serological tests (indirect immunofluorescent antibody test, IFA, indirect haemagglutination test, IHA) and negative direct examination of BMA were included. Twenty patients with fever of other aetiology in whom Leishmaniasis was evaluated as part of the differential diagnosis and 20 healthy persons were also included in the study, as a control group. PCR was performed in all samples. In PCR amplification two different pairs of primers and the highly repetitive kinetoplast DNA as target were used. PCR amplification revealed products of 120- and 145-bp. IFA and IHA methods were used for the detection of the specific IgG and total antibodies of Leishmania spp.

Results: The two PCR methods produced positive results in all 24 patients (group 1, 2). Both serological tests gave positive result in 17 of 24 patients, while only one of the two serological tests was positive in six patients (group 1, 2). There was also one patient with positive direct examination of BMA and low serological reactivity (group 1). All healthy persons and patients of other infections were found negative for Leishmaniasis by serological methods and PCR.

Conclusions: PCR methods contribute in the early and definitive diagnosis of VL in areas where the disease is endemic, such as southern Europe. The demonstration of the parasite in the direct examination of BMA is essential for establishing the diagnosis, but presents low sensitivity. The use and the combination of serological tests are useful in the diagnosis of VL.

Detection of Leishmania major in visceral infection by a nested-PCR assay

M. Karamian, M. Motazedian, K. Gholami
Shiraz, IR

Leishmania infantum is the typical agent of visceral leishmaniasis in Iran and middle east, but a few reports suggested that L. tropica could cause this type of leishmaniasis too. In this study, we detected L. major in bone marrow and lymph node samples of a village dweller from booshehr, who had kala-azar signs. A 30-year-old man with kala-azar symptoms was bedridden in Namazi hospital. Four bone marrow smears and two paraffin-embedded blocks of him were tested. At first, DNA extraction from smears performed by proteinase k and from blocks DNA extracted by boiling and proteinase k. Then variable regions of Leishmania KDNA minicircles amplified by CSB1XR and CSB2XF primers in step 1, 13Z and LiR primers in step 2 of nested-PCR assay. We determined the species of parasite by electrophoresis of PCR product on agarose gel and comparing between their bands with marker and standards. Leishmania major has a 560 bp variable region. PCR was performed several times rigorously, and in all of samples only
**Abstracts**

L. major has been detected. All clinical signs suggested to kala-azar and leishman bodies were seen in lymph nodes and skin lesions of patients. The skin lesion has been appeared at the left forearm of this man 10 years ago and did not be cured by plastic surgery, but began to cure contemporary with kala-azar therapy. According to increasingly spreading of L. major in Iran, if this parasite can cause visceral symptoms, it could be reckoned to a major common health problem in this country. Therefore, it is necessary to carry out widespread molecular studies in typing of various agents of leishmaniasis.

**P627** Study of glucantime resistance in cutaneous leishmaniasis by PCR-RFLP method in Shiraz, Iran

M. Motazedian, M. Karamian, S. Ardehali
Shiraz, IR

Glucantime is an effective drug for cutaneous leishmaniasis (CL) therapy in endemic regions of CL including Iran. In recent years, there are several reports of resistance to it. In this study, PCR-RFLP analysis of 19 fragments length was performed on the left forearm used for determination of any relationship between the genome and drug resistance of causative agents of CL in Shiraz in southern Iran. For this purpose, Giemsa-stained positive slides of 102 patients with different degrees of amastigote density were used. Nineteen slides belonged to healed patients who had been treated by glucantime and 28 slides have been prepared from unhealed patients. The surface materials of these slides scraped and DNA extraction was carried out by Proteinase K and amplified by CSB1XR and CSB2XF primers in step 1, 13Z and LiR primers in step 2 of nested-PCR amplification method. The products were characterised by agarose gel electrophoresis in comparison with reference strains. The amplified fragments digested by restriction enzymes were electrophoresed and produced schizodeme patterns were analysed. Among 19 samples from healed patients, six were Leishmania tropica and 13 were L. major. Among 28 samples from unhealed patients, 14 were L. tropica and 14 were L. major. Analysis of these results showed that patients infected with L. tropica have more drug resistance. Those infected with L. major have more drug sensitivity to glucantime in this region. Schizodeme analysis showed high genomic diversity in L. tropica and L. major in Shiraz. The genomic diversity of L. tropica was considered higher than L. major. The results of this study determine a relationship between genomic diversity and incidence of drug resistance in patients infected with L. tropica in Shiraz.

**P629** First cases of Acanthamoeba keratitis in Slovakia

F. Ondriska, M. Mrva, M. Lichvar, P. Ziak, Z. Murgašova, E. Nohynkova
Brtislava, Martin, Nitra, SK; Prague, CZ

**Objectives:** Two dominant risk factors are responsible for initiating the amebic keratitis: wearing of the contact lenses and corneal trauma. Both these factors are associated with the first isolations of the Acanthamoeba species as the causative agents of human keratitis in the Slovak Republic. Three cases report are presented here.

**Results:** The first case of amebic keratitis manifested in the right eye of a 53-year-old man after the eye injury. Amebae were identified as Acanthamoeba sp. of group III. The course of the disease was influenced mainly by seeing a physician at a late stage and discovering the aetiology of disease no sooner than 10 months after the eye injury. The disease progress has not been staunched either by itraconazole (Sporanox), or by corneal transplantation and the patient had to undergo enucleation. Acanthamoeba lugdunensis was identified as a causative agent of amebic keratitis in the second case. A 39-year-old man wearing contact lenses visited thermal swimming pool. A month later, the first disease symptoms occurred. The disease has manifested as herpetic keratitis of the left eye with cloudy cornea, circular infiltrate and deterioration of vision. The cultivation of the eye swab has revealed polyresistant strain of Pseudomonas aeruginosa and the cultivation of corneal scraping has revealed amoebids. Due to immediate clinical and laboratory diagnosis the propamidine-iseithionate gtt. (Brolene) therapy has significantly improved the eye condition. Wearing contact lenses is probably connected also with the third case. A 15-year-old woman worn the contact lenses during bathing in various swimming pools and in the sea. She even cleaned the contact lens case under tap water regularly. In the used contact lens, a disfecting solution, there were found cysts of Acanthamoeba sp. of group II and we assume that it was the way of the eye infection. The Brolene therapy was successful.

**Conclusion:** Presented cases suggest probably the glacier phenomenon of the occurrence of amebic keratitis in the Slovakia. Thanks to the first record of this disease, the amebic keratitis has attrac-
A 45-year-old farmer from Espand village (located in west of Iranshahr county, Sistan and Baluchistan Province, south-east Iran) with severe headache, vertigo, nosebleed, oedema in face and agitation symptoms, referred to the Khatam-Al-Anbia Hospital in Iranshahr. CT scan showed an extensive oedema in nasal and paranasal sinuses. Endoscopy consideration revealed the presence of numerous larvae lodged inside the nasal cavity. In the operating room, on 6 May 2002 about 60 larvae of third instar fly larvae were pulled out under the general anaesthesia condition.

Full-grown larvae crawled into the soil and developed to adult flies later on. The larvae and adult flies were identified according to James (1947), Zumpt (1965) and Spradbery (1991) keys. Our precise identification indicated that the flies were the Old World Screw-worm (Chrysomya bezziana). Six adult flies and six full grown larvae have been deposited in the collection of Entomology Museum, School of Public Health, Tehran University of Medical Sciences. The patient’s life history implied that he usually was resting near the goats in his farm at mid-days. On the contrary, most of the goats usually being attacked with myiasis larvae.

**Imported case of brucellosis complicated by liver abscess**

C. Mutini, M. Di Carlo, L. Coppolaro, A. Di Girolamo,
E. Pizzigallo
Chieti, IT

Hepatic involvement is a common feature of brucella infection, but liver abscess is a less frequent complication. We report a case of brucellar hepatic abscess in a 42-year-old man with a 4 week complaint of fever, abdominal pain, anorexia and weight loss. Forty days earlier, he was back from a 1 week stay in Albania. A detailed epidemiological history showed that he consumed, about 2 weeks before the onset of symptoms, fresh unpasteurised cheese. At admission the patient was febrile (39°C), and tachycardic (100 bpm). Abdomen was diffusely painful, especially in the right upper quadrant. Liver was 2 cm below the costal margin. Blood tests showed mild neutrophilia (6800 cells × mm³), anaemia (haemoglobin 10.6 g/dL) and hypoalbuminaemia (3034 mg/dL). ESR 94 mm/h and C-reactive protein 15.1 mg/dL. Abdominal ultrasound scan showed an abscess within the VI hepatic segment of about 55 mm in diameter which was promptly drained. Blood cultures were positive for *Streptococcus anginosus*, but Wright test for *Brucella* was also positive at high titre (>1600). Clinical course was complicated by right sided pleural effusion that needed evacuation. Ciprofloxacin i.v., doxycycline p.o., and metronidazole p.o. were initially administered. Antibiotic regimen was then switched to ciprofloxacin i.v., doxycycline p.o., and metronidazole p.o. for 6 weeks, accomplishing a progressive improvement of general conditions. *Escherichia coli* and *S. anginosus* were isolated from the abscess drainage. Any attempt of isolating *Brucella* spp., including biomolecular approaches, failed. Wright serology was repeated at discharge, showing a decrement in titres. A review of published cases was done, finding a brucellar hepatic abscess incidence of some 1%. As the isolated strains in this patient did not clearly indicate a brucellar aetiology, some question could be raised on the real involvement of *Brucella* spp. in this case. The possibility are that normal intestinal flora could reach, by contiguity, the liver. Otherwise, the initial brucellar colonisation could have been replaced, during abscess evolution, by superinfecting strains that made the specific diagnosis impossible. The latter mechanism could also explain the low incidence of confirmed brucellar liver abscesses in other series.

**Evaluation of available stains for detection of Acanthamoeba sp. from brain specimen: an assessment**

G. Gonzalez Mediero, P. Santiago, M. Seijo, G. Visvesvara
Vigo, Pontevedra, E; Atlanta, USA

Objectives: *Acanthamoeba* sp. and others free-living amoebae are rare cause of opportunistic cerebral and mucose disease above all
Results: An indirect immunofluorescence and culture in non-nutrient agar were performed. Escherichia coli growth was not observed, but it could be grown on malt extract agar. Then a positive culture can facilitate the identification, diagnostic sometimes probably, additional cultures are required for the identification. As all a but a few isolations from human tissue have yield it is generally believed that most human infections have been due to free-living amoebae. The tissue can have presence of amoebae and others without it. Fast stains including trichrome, haematoxylin-eosin, Gomori methenamine silver and others.

Conclusions: We think as others authors that GAE is not diagnostic - meningitis probably, special cultures are required for the identification. As all a but a few isolations from human tissue have yield it is generally believed that most human infections have been due to free-living amoebae. The tissue can have presence of amoebae and others without it. Fast stains including trichrome, haematoxylin-eosin, Gomori methenamine silver and others.

Methods: A patient, 33-year-old was admitted to the hospital in late January 1998 with a 2 days history of headache, confusion and left field visual defect. A cranial computed tomography scan revealed a mass in the right occipital lobe. Toxoplasmosis was suspected. A biopsy specimen was obtained which multiple double walled cyst-like structures and granulomatous reaction were seen. We never has seen visualised this image but we think in Acanthamoeba sp. or Balamuthia mandrillaris. Six weeks after, a right parieto-occipital craniotomy was performed and microbiological examination, wet and stained preparations was carried out, Kop Color (Innogenetics), periodic acid-Schiff (PAS), trichrome, haematoxylin-eosin, Comori methenamine silver and others.

Results: An indirect immunofluorescence and culture in non-nutrient agar plates precoated with Escherichia coli culture and incubate at 37°C about 8 days was identified as Acanthamoeba castellanii. This is the first case of GAE identified from Spain.

Conclusions: We think as others authors that GAE is not diagnostic having been due to free-living amoebae. The tissue can have presence of amoebae and others without it. Fast stains including trichrome, haematoxylin-eosin, Gomori methenamine silver and others.

Methods: During 2002 and 2003, we treated 46 patients with neurocysticercosis. Neurocysticercosis was the most frequent parasitosis of the central nerve system. Parenchymal form was seen in 60-90% of patients; Leptomeningeal and spinal form are rare. We treated four patients with this form of disease. The objective of our work was to present clinical course and therapeutic outcome in patients with chronic meningitis during neurocysticercosis.

Methods: We present clinical, laboratory and neuroradiological findings and therapeutic outcome in these patients.

Results: Our patients had pleocitosis in cerebrospinal fluid (CSF), average 74 ± 20 cells, CSF proteins was elevated, more than 6.4 g/L, and CSF glucose levels were 0–1.5 mmol/L. The pathological findings were present in prolong time period (more than 6.4 g/L, and CSF glucose levels were 0–1.5 mmol/L. The pathological findings were present in prolong time period (6 months to 2 years). In neurological findings, all of them have meningeval symptoms, with neurological deficiencies (paresis, ataxia, etc.). Duration of which was not correlated with CSF findings. Pathological changes in CSF were present longer then 6.4 g/L, and CSF glucose levels were 0–1.5 mmol/L. The pathological findings were present in prolong time period (6 months to 2 years). In neurological findings, all of them have meningeval symptoms, with neurological deficiencies (paresis, ataxia, etc.). Duration of which was not correlated with CSF findings. Pathological changes in CSF were present longer then neurological problems. Serological conformation of cysticercosis was performed by ELISA test from blood and CSF. Intrathecal synthesis was positive. Tests for echococcosis and toxoplasmosis were negative. Magnetic resonance imaging (MRI) was reviled in two cases parenchimal and leptomeningeal form of neurocysticercosis. One patient has only leptomeningeal form, and one patient has parenchimal, leptomeningeal and spinal form of neurocysticercosis.

Conclusions: Leptomeningeal form of neurocysticercosis was rare (8%). All of our patients have chronic meningitis during 6 months to 2 years. Neurological and laboratory findings are correlating with MRI. Repeated treatment (3–5) was necessary for successful cure. We used albendasol with corticosteroids. Side effects were mild and well tolerated.
Meningitis

P637 Demographic, clinical and laboratory data in 140 meningococcal disease cases

A.R. Emami Naenini
Isfahan, IR

Introduction and Objective: Meningococcal diseases occur in a worldwide distribution as endemic or in epidemics. The overall mortality is 8–10% with deaths occurring mainly in patients presenting with signs and symptoms of meningococcemia. Several investigators have devised scoring systems using clinical and laboratory parameters available at the time of presentation to prognosticate the outcome of the infection. We conducted this survey to determine the prevalence of demographic, clinical, laboratory data in our patients.

Methods: This was a prospective descriptive study performed upon patients with definite diagnosis of meningococcal infection (blood or cerebrospinal fluid positive culture) in St Zahra Hospital, Isfahan (centre of Iran) during October 1996 till September 2001. The subjects were comprised 140 patients [99 (70.7%) males and 41 (29.3%) females] aged 1–50 years. The data were achieved by checklist and analysed by using SPSS software.

Results: Upon this study, 99 (70.7%) were males and 41 (29.3%) females, and among them 75 (53.6%) were aged between 11 and 20 years. In the study population, 57 (40.7%) were students, 39 (27.8%) workers, 20 (14.3%) infant and children and 24 (17.2%) other jobs. Seasonal frequency: winter 49 (35%), spring 37 (26.4%), summer 30 (21.4%), fall 24 (17.2%). Iranian 92 (65.7%) and Afghan refugees 48 (34.3%). Clinical presentation: pure meningitis 40 (28.6%), meningitis with meningococcemia 62 (44.3%), meningococcemia alone 33 (23.5%) and meningoencephalitis five (3.6%). The five most clinical symptoms and signs were: fever 131 (93.6%), vomiting 107 (74.6%), neck stiffness 105 (75%), headache 96 (68.5%), skin rash 92 (65.7%). Paraclinical data: leucocytosis 99 (70.7%), normal range 25(17.8%), thrombocytopenia 27 (19.5%). Abnormal prothrombin time 46 (32.8%). The predominant white blood cell was polymorphonuclear 134 (95.7). Thrombocytopenia 27 (19.5%).

Conclusion: Meningococci is still a killer, it affects males more than females, the highest age-related attack rate occurs in teenagers and young adults. Afghan refugees were a source of infection in our survey that may be due to crowded and low socioeconomic state of them. Central nervous system is a target organ in meningococcal infection. Our mortality was higher than what to be suggested.

P638 Laboratory surveillance of meningococcal disease in Portugal

M.J. Simões, L. Brum, C. Furtado on behalf of the Laboratory Meningococcal Disease Network

Objectives: In 2002, the control of meningococcal disease (MD) was considered a priority in Portugal and a new surveillance system was introduced. Since October 2002, a laboratory-based surveillance system for MD (VigLab MD) started based on a laboratory network, which includes all laboratories from hospitals with MD inpatients. Laboratories notify to the National Institute of Health (INSA) all suspected cases received for laboratory confirmation, make Neisseria meningitides isolation and send the strains for further characterisation (antigenic and molecular). Besides, they also should notify all culture negative cases of CSF having previously established criteria, and sent them to INSA where the confirmation is made by non-cultural methods (PCR). The aim of this study was to determine the number of clinical or laboratory suspected cases of MD in which N. meningitides was detected.

Methods: Neisseria meningitides strains were isolated in hospital laboratories according to their own protocols. Direct DNA detection in clinical samples with negative cultures was performed by real-time PCR (Light Cycler System, Roche, Germany) amplifying a 111-nucleotide sequence of gene ctra. In a screening test, we used the fluorochrome SYBR Green and, in a confirmatory test, a fluorescent dye-labelled-specific probes. The melting temperature was used for identification of amplicons. Strain group determination was performed by direct agglutination (Difco) or, when non-reactive, by PCR using primers specific of siaD gene for group B, C, Y and W135, and orf-2 gene for group A. Group determination of noncultural strains present in clinical samples, was also performed by PCR. For serotyping and subtyping, we used monoclonal antibodies from National Institute for Biological Standards and Control in an ELISA technique.

Results: In a 14-month period, we received 181 clinical samples and 118 strains. In 155 cases, we had confirmed MD and characterised serogroups as follows:
- 144 – culture negative + PCR negative;
- 37 – culture negative + PCR positive;
- 118 – culture positive.

P639 Multidisciplinary research of meningococcal invasive disease in the Czech Republic

Pilsen, Prague, Hradec Kralove, Ostrava, Brno, CZ

Objectives: Changing severity of MD in the Czech Republic (CR) being related to the high incidence of invasive strain of Neisseria meningitidis (NM), belonging to ET-15/37 complex, constrains to elaboration of diagnostic, therapeutic and severity assessment algorithms. High incidence of sepsis initiated our intensive study of prognostic factors of MD on admitting to the hospital, properties of NM and other factors of MID. Methods: A new grant project being further to our previous grant researches started in 2002, concentrates complex of MID data from eight departments in CR to the database including haematological and genetic research results. The research of NM in NIPH – identification of electrophoresis genotype ET by multilocus electrophoresis (MLEE) and identification of sequence type (ST) by multilocus sequence typing (MLST) is carried out in all patients as well. NM strains were isolated from patients and/or from PCR products in patients with negative cultivation. Belonging to hyper-virulent complexes (HC) is assessed together with prognostic factors and contextualised with the genetic familiar predisposition to the pathological pathway of fibrinolysis (due to mutation of promoter gene for PAI-1), and polymorphism C308A in the promoter gene for tumour necrosis factor (TNF). Within 15 months, we have got data from 78 patients (53.4% of MID in the CR). Group characteristics: median of age 17.5 years, male:female = 42:36, mortality = 10.3%.

Results: Data analyses show 6.4% incidence of benign meningococcemia, increasing incidence of sepsis (33.3%) with mortality 30.7%, increasing incidence of serogroup B (42.5%) with 53% of HC (20.6% without), increasing incidence of serogroup C (34.6%) with HC in 92.5%. Serogroup was not identified in 16.6%. HC could not be proved in two cases with group C and in seven cases with group B due to the PCR detection only. Relationship among three above-mentioned areas of investigation is evaluated.
Conclusions: High incidence of hyper-virulent strains proved almost in all cases with NM group C and in majority of cases with group B does not correspond with only 33.3% incidence of sepsis. High incidence of proved benign meningococcaemia indicates good awareness of MID in the CR. Complete multidisciplinary approach to the MID research can contribute to get explanation for not yet answered question, why MID sometimes kills and sometimes has a benign course.

Acknowledgements: This study was supported by grant IGA Ministry of Health NF-7109-3 (http://www.neisseria.mlst.net).

P640 Community-acquired enterococcal meningitis caused by Enterococcus casseliflavus: first case report

A. Iaria, G. Stassi, R. Di Leo, A. Toscano, A. Cascio Messina, I

Background: Enterococcal meningitis accounts for only 0.3–4.0% of cases of bacterial meningitis. Enterococcus faecalis and E. faecium are the most frequent meningal isolates accounting for 76–90 and 9–22%, respectively. Enterococcus casseliflavus is a motile enterococcus that produces a yellow pigment in agar and has a VanC phenotype (intrinsin low level resistance to vancomycin and susceptibility to teicoplanin). It has been implicated in a wide variety of infections in human beings, especially immunocompromised persons, but never in meningitis.

Case Report: A 77-year-old female presented for evaluation of fever, stupor, diarrhea and vomiting of 3 days duration. There was no history or head injury or any surgical procedures. She had been suffering from rheumatoid arthritis for 30 years for which she was in treatment with steroids and methotrexate, diabetes in treatment with insulin and moderate renal failure. On admission she had a temperature of 38.0°C. She was alert but not oriented to time and place. Her neck was stiff, and she had a positive Kernig’s sign. WBC 15 100/mm3, 7 0% neutrophils and 23% lymphocytes. CSF was opalescent, glucose: 14 mg/dL, protein: 472 mg/dL, white cell counts: 200/mm3. CSF was processed using BACTEC 9120. Enterococcus casseliflavus was identified using the VITEK-2 system and on the basis of motility and yellow pigmentation testing. The isolate was (i) susceptible to penicillin, ampicillin, ampicillin-sulbactam, imipenem, teicoplanin, tetracyclines and linezolid; (ii) low level resistant to vancomycin (MIC > 8 mg/L), trimethoprim-sulfamethoxazole, levofloxacine, ciprofloxacin and quinupristin-dalfopristin; and (iii) high level resistant to gentamicin, streptomycin and kanamycin and clindamycin. Echocardiogram revealed no isolates. Colonoscopic examination revealed two ulcerative lesions covered by fibrin in the rectal mucosa and multiple punctuate erosions in the sigma mucosa. She was successfully treated with meropenem and ampicillin-sulbactam.

P641 Factors predicting fatal outcome of purulent meningitis

N. Dzupova, J. Benes, M. Helcl, J. Prihodova Prague, CZ

Objectives: To define the most accurate factors predicting in-hospital mortality of patients with purulent meningitis.

Methods: Retrospective study of patients hospitalised between 1997 and 2001 in an infectious diseases department of a tertiary care hospital. Records of 149 consecutive patients, older than 15 years with community-acquired purulent meningitis were reviewed. The following data were selected and further analysed: age, sex, duration of symptoms to diagnosis, underlying debilitating condition, GCS score, APACHE II score, cerebrospinal fluid (CSF) leucocyte count, CSF protein level, CSF glucose level, CSF/blood glucose ratio and aetiology. Each parameter was tested in univariate logistic regression analysis and furthermore all significant variables were tested in multivariate analysis.

Results: There were 30 fatal cases with the overall mortality rate 20.1%. Neisseria meningitidis meningitis possessed the lowest mortality rate (2/37 = 5.4%), compared with Listeria monocytogenes (2/10 = 20%) and Streptococcus pneumoniae (12/39 = 30.8%). The risk of death was significantly higher in older age (P = 0.002), presence of underlying condition (P = 0.005), lower GCS score (P < 0.001), higher APACHE II score (P = 0.001), lower CSF/blood glucose ratio (P = 0.001). Conclusion: Significant prognostic factors indicated by univariate logistic regression analysis were age, underlying debilitating condition, GCS score, APACHE II score and CSF/blood glucose ratio. Sex, duration of symptoms to diagnosis, CSF leucocyte count, CSF protein level and CSF glucose level had no significant prognostic value. In multivariate analysis only age and APACHE II score were significant predictors of fatal outcome. These results are in agreement with previously published studies.

P642 Dexamethasone therapy for bacterial meningitis in southeastern Anatolia region of Turkey

C. Ayaz, M.K. Celen, S. Hosoglu, M.F. Geyik, M. Ulug Diyarbakir, TR

Objectives: Routine use of steroids as adjunctive treatment of bacterial meningitis remains controversial. We have carried out a retrospective, placebo-controlled, double-blind study of dexamethasone in 145 adult with acute bacterial meningitis in Dicle University of Turkey.

Methods: The patients were randomly assigned to receive either ceftriaxone (n = 72) or dexamethasone (n = 73) in addition to optimum antibiotic treatment (4 g every day ceftriaxone). Dexamethasone therapy (16 mg every day) was started 10 min before the first dose of ceftriaxone and has given every 6 h for 3 days.

Results: Baseline demographic, clinical and laboratory features of the two groups were similar. The mean age of the patients was 30.2 ± 15.3 years. The sex of the patients was 91 males and 54 females. We have growth in 23 patients (15.9%) Streptococcus pneumoniae, in 12 (8.3%) Neisseria meningitidis, in three (2.1%) S. aureus and 107 (73.7%) patients had no growth in cerebrospinal fluid cultures. CSF glucose concentration significantly increased in dexamethasone therapy than the other group after 24 h treatment (P = 0.01). However, other indices of inflammation showed similar changes in both groups. Addition of dexamethasone did not affect the rate at which CSF became sterile. When we compared two group the fatality rate was observed in patients with acute bacterial meningitis which were receiving dexamethasone; only seven of 73 patients died, 12 of 72 patient died which were not receiving dexamethasone.

Conclusion: Acute bacterial meningitis still remains a serious infection. Early diagnosis and treatment may reduce fatal outcome and improve the course of the disease. We conclude that dexamethasone is beneficial in the treatment of adults with bacterial meningitis, particularly in preventing deafness.

P643 Serologic evidence of Borrelia burgdorferi infection among patients hospitalised with tick-borne encephalitis

S. Grygorczuk, M. Kondrusik, S. Pancewicz, J. Zakowska, T. Hermanowska-Szapokowicz Bialystok, PL

Objective: The purpose of this work was to evaluate serum and cerebrospinal fluid presence of antibodies against Borrelia burgdorferi of patients with diagnosed tick-borne encephalitis.

Methods: Sera and cerebrospinal fluid of 90 patients with diagnosed TBE were analysed. The diagnosis of TBE was based on amnnesia (reported tick bites or endemic area dwelling) clinical symptoms (fever, headache and neurological signs) and results of laboratory tests (inflammatory changes of CSF parameters, serologically confirmed serum/CSF presence of IgM, IgG antibodies against TBE virus). Patients were divided into two groups: group I: 28 patients with severe clinical course of TBE with paresis and
unconsciousness; group II: 62 patients with mild course of TBE. Serological diagnosis of IgM, IgG antibodies against *B. burgdorferi* was performed with use of ELISA recombinant kit. CSF and sera were collected during first days of hospitalisation when diagnosis was established.

**Results:** In group I, IgM antibodies against *B. burgdorferi* in CSF were detected in two (7.14%) patients and IgG in six (21.42%) patients. One patient showed presence of IgM and IgG simultaneously. In group II, IgM in CSF was not present in any patient but IgG was found in CSF of eight (12.9%) patients. Serum IgM antibodies among patients from group I were detected in two (7.14%) patients and IgG in seven (25%) patients. Patients with CSF antibodies against *B. burgdorferi* showed their presence in serum as well. In sera of group II patients, presence of IgM antibodies was detected in four (6.45%) patients and IgG in 12 (19.35%).

**Conclusion:** The diagnosis of tick-borne encephalitis should not exclude infection with *B. burgdorferi*. Patients with severe course of TBE showed more frequent presence of antibodies against *B. burgdorferi* in CSF and serum.

---

**P644** Spinal epidural abscess in the MRI age

F.J. Fernández-Fernández, S. Pérez-Fernández, J. de la Fuente-Aguado, B. Sopena-Pérez Argüelles, C. Martínez-Vázquez

**San Juan, PR**

**Objectives:** To evaluate the clinical, microbiological and prognostic characteristics of patients who were seen in our hospital with spinal epidural abscess (SEA).

**Methods:** A retrospective analysis of ART-experienced patients and ART failure. Seventy-three per cent of patients had resistance to NRTI, 55% to PI, and 46% to NNRTI. Success was not predicted by the number of sensitive drugs nor the number of new drugs in the patient’s ART.

**Results:** During the period of study 11 patients (nine males, mean age 58 ± 14 years) were diagnosed of SEA. The most common symptoms at presentation were fever (100%) and radicular pain (82%). There were signs of cord compression in 55%. In seven patients, the origin was community acquired. Seven patients had comorbid conditions (three alcoholism, two diabetes mellitus and two cancer); two cases presented a potential source of infection (vascular catheter); and in three cases, there was a direct spread from contiguous sources (epidural catheter, previous laminectomy and thoracic empyema). The isolated microorganisms were *Staphylococcus aureus* (six patients), *Streptococcus* (two patients) and polymicrobial (one patient). The aetiological agent was not identified in two patients. Bacteraemia was documented in six patients. The lumbar spine was most commonly involved (seven patients), followed by thoracic (five patients) and cervical (two patients) regions. In three patients, there were multiple segments affected. In five patients, the abscess was circumferential, and in four cases was posterior. Spondylodiscitis was associated in five patients. The treatment was laminectomy and antibiotics in eight cases, and three patients were treated only with antibiotic therapy. Two patients died. In the univariated analysis the advanced age was the only variable associated with worse outcome.

**Conclusions:** In our experience the SEA is essentially produced by *S. aureus*. A high index of clinical suspicion should be kept in mind in patients with fever and spinal pain to avoid the progression and development of cord compression. Less than half of the cases have spondylodiscitis associated. In selected patients, the antibiotics alone can be effective.

---

**P645** Genotype-guided treatment change in previously heavily antiretroviral treatment HIV patients


**San Juan, PR**

**Objective:** To study HIV resistance in Hispanic antiretroviral therapy (ART)-experienced patients, and response after genotype (GT)-guided ART changes.

**Methods:** A retrospective analysis of ART-experienced patients seen in an outpatient clinic in Puerto Rico, assessment of resistance by GT, and evaluation of virological response after GT-guided ART change.

**Results:** A total of 84 patients, all ART-experienced Hispanic men, had 92 GTs performed for ART failure. Seventy-three per cent had >1 prior change in their ART, 89% were using >3 ART drugs. Prior exposure to all three drug classes was seen in 35% patients, to NRTI + PI in 38%, to NRTI + NNRTI in 18% and to only NRTI in 9%. Mean ART length was 38 (range 360) months. Mean VL was 40000 (81750000). Resistance by GT to all three drug classes was seen in 26% of patients, to two classes in 48% and to one class in 14%. Twelve per cent of patients had wild-type virus; 86% had resistance to NRTI, 55% to PI, and 46% to NNRTI. Fifty-eight patients had a GT-guided ART change. VL decrease >0.5 log was seen in 57% patients within 16 weeks. Thirty-four patients reached VL <400. New ART included two sensitive drugs in 69% patients, only 26% had >2 sensitive drugs. Success in patients having used <3 drugs in the past was 100%, those having used 3–7 drugs 59%, and those having used >7 drugs 20% (P = 0.05). Patients with resistance to <2 drug classes were more likely to have virological success vs. those with higher resistance (71% vs. 57%), but not significantly so. Success did not depend on the number of sensitive drugs or on the number of new drugs added.

**Conclusions:** Genotype-guided ART change was associated with 59% virological success in this clinic, despite having a heavily ART-experienced and heavily resistant population. Success was predicted by the number of drugs the patient had previously used. There was a trend to better success in those with <2 ART drug class resistance. Success was not predicted by the number of sensitive drugs nor the number of new drugs in the patient’s ART.

**P646** Lipodystrophy as paradoxically marker of efficacy of antiretroviral therapy in AIDS patients: our experience

L. Terra, S. Pellican, R. Calzone, A.C. Del Giudice, D. Tetti, F. Rombola, S.N. Bertuccio, L. Terra

**Crotone, I**

**Background:** After antiretroviral therapy are described lipidic alterations of metabolism and lipodystrophy with peripheral loss of fat and central accumulation.

**Objective:** To evaluate prevalence of lipodystrophy and its correlations with lipid alterations.

**Patients and Methods:** Between 1998 and 2002, 203 AIDS patients, 125 males (62%), 78 females (38%), age 23–71 (mean 47 ± 24) years were hospitalised.

**Results:** Seventy-three patients (46%) had lipid alterations: 35 patients (22%) had hypercholesterolemia, 29 patients (18%) hypertriglyceridemia and nine patients (6%) both. Fifty patients (68%) had lipodystrophy. Hypercholesteraemia was associated at age, lamivudine (P = 0.033), ritonavir (P = 0.002), whereas hyper-
triglyceridaemia at age, sex, time of diagnosis, lamivudine, stavudine, ritonavir and saquinavir.

Conclusions: In our study, lipidic alterations were associated with lipodystrophy especially but there was a better immune-virological answer to antiretrovirals. Lipodystrophy paradoxically can be considered marker of efficacy of therapy.

**P647** Head-to-head comparison between first-choice HAART in antiretroviral-naïve patients with HIV infection: lopinavir/ritonavir versus efavirenz-based therapy

R. Manfredi, L. Calza, F. Chiordo
Bologna, I

The updated international guidelines of antiretroviral therapy pose lopinavir/ritonavir (L)- or efavirenz (E)-based HAART as the first-choice line in naive patients. Aim of our study is to review retrospectively the efficacy and tolerability of L- vs. E-based HAART in 67 naïve patients who started HAART since 2002; 36 consecutive patients treated with L plus two nucleoside analogues (NA) were compared with 31 consecutive patients who received E and two NA. At baseline, the two study groups were matched as to demographic and epidemiological features, as well as mean viral load (4.5 ± 1.3 vs. 4.3 ± 1.7 Log 10 HIV-RNA copies per millilitre, for L and E). However, the L group included a greater number of patients with prior-concurrent AIDS (P < 0.005), and showed a lower mean CD4⁺ count at baseline (P < 0.004). The number of early (first month) interruptions due to poor tolerability proved similar: five cases in the L group vs. four among E-treated patients, although untoward events involved the gastrointestinal tract and the CNS, respectively, for L and E. During the subsequent follow-up (9–21 months), laboratory examinations were performed at least quarterly, and showed a comparable virological response (as to mode of decay, and time and rate of viral suppression), in the presence of only one case of virological failure in the E group. Conversely, a more rapid immune recovery occurred in L-treated patients, regardless of the more compromised mean initial CD4⁺ count of this last patients group. Mid-term toxicity was significantly different, with L-treated patients who experienced an altered serum lipid profile in over 40% of cases vs. <10% recognised in the E group. The overall need of change of antiretroviral regimen due to toxicity, poor adherence, patient’s request, or failure, was comparable in the two examined patient groups. When considering our experience on 67 antiretroviral-naïve patients treated with either L- or E-based HAART, more potent and rapid immunological effects were achieved in the L group, which started from a deeper immunodeficiency, while E-treated patients experienced more rare adverse events and had less compliance problems with pill burden and subjective tolerability. Virological efficacy did not prove remarkably different between L- and E-treated patients. Pending the approval of other agents useful for first-line HIV infection therapy, the selection of L- vs. E-based regimens has to take into account of the initial immunological and disease status, while more data are needed on long-term outcome, role of emerging resistance and toxicity, as well as targeted pharmacoeconomic evaluation.

**P648** Polysaturated fatty acid ethyl ester as therapy of hypertriglyceridaemia related to HIV infection treated with antiretrovirals

R. Manfredi, L. Calza, F. Chiordo
Bologna, I

Aim of our study is to assess prospectively the efficacy and safety profile of polysaturated fatty acids ethyl ester (PFAEE) in the control of hypertriglyceridaemia complicating antiretroviral-treated HIV disease. Forty-three patients aged 38–61 years (29 males) with a diet- and exercise-resistant hyperlipidaemia and a mean triglyceridaemia of 298.3 ± 42.2 mg/dL, received PFAEE at 1 g twice daily, and were followed quarterly for at least 1 year. A hypercholesterolaemia (mean value 245.0 ± 29.6 mg/dL), contributed in 11 cases only. The dyslipidaemia was prompted by a combined highly active antiretroviral therapy (HAART) lasting from 16 to 119 months (mean 47.2 ± 21.7 months), and based on protease inhibitors in 30 cases, and non-nucleoside reverse transcriptase inhibitors in 11 patients. Continued PFAEE administration led to a significant decrease of triglyceridaemia of 24.1, 34.3, 36.8 and 39.2%, after 3, 6, 9 and 12 months, respectively (P < 0.001 vs. baseline levels), while negligible changes occurred in serum cholesterol levels. Normal serum triglyceride levels (<172 mg/dL) were reached by 14 patients (34.1%), who continued PFAEE at 500 mg/day after 6–9 months, while five patients had persistently elevated triglyceridaemia (<300 mg/dL), and after 6 months significantly benefited from increased PFAEE dosage up to 1.5 g/day (reaching a mean 31.2% reduction of serum triglyceride levels vs. baseline). Mild and transient gastrointestinal disturbances (possibly attributable to concurrent medications) were referred by 12 patients, but no treatment discontinuation became necessary. Treatment with PFAEE prevented from changes of antiretroviral regimen and its composition, directly caused by persisting dyslipidaemia. Dyslipidaemia is a mounting problem in long-term management of HIV infection, and cardiovascular damage is of particular concern. In our experience (1–3), all administered fibrates and statins showed a similar efficacy in the therapy of HIV-associated dyslipidaemia, but significant side effects and drug–drug interactions may occur, especially when antiretroviral drugs and other underlying pharmacological therapies are of concern. For isolated or predominant hypertriglyceridaemia, PFAEE may represent an effective and safe alternative to fibrates and/or statins, to be confirmed in enlarged, randomised, dose-finding comparative trials.

References

**P649** Pharmacokinetics of non-nucleoside inhibitors of HIV-1 reverse transcriptase of the DATA/DAPY classes of compounds


Objectives: Diaryl-triazine (DATA) and diaryl-pyrimidine analogues of DATA/DAPY are potent nonnucleoside inhibitors of HIV-1 reverse transcriptase (NNRTIs) in cell-based assays on wild-type virus and also in a large panel of clinically relevant single and double mutants. Most NNRTIs assume a butterfly conformation within the lipophilic binding pocket of reverse transcriptase (RT). In the case of DATA/DAPY compounds, the two wings of the butterfly conformation are composed of aromatic rings, while the central part contains hydrogen bridge forming fragments. Hence, it is not surprising that these compounds are highly lipophilic with log P-values often in excess of 5. In spite of their lipophilicity, these compounds show moderate to good bioavailability in animal species and man. As can be expected, they are also absorbed to some extent through the lymphatic pathway.

Methods: We have investigated the possible causes of this seemingly paradoxical relationship between antiviral activity, lipophilicity and bioavailability. This comparative pharmacokinetic study of DATA/DAPY compounds involves various cell-based assays and physicochemical as well as computed parameters.

Results: A surprising finding is that highly potent and bioavailable DATA/DAPY analogues also form aggregates with radii between 34 and 100 nm. This has been confirmed by means of
**P650** Restoration of complement-mediated phagocytosis in HIV-1 infected macrophages by 2',5'-dideoxyadenosin

E. Doischer, A. Jaworowski, U. Frank, S. Crowe
Freiburg, D; Melbourne, AUS

**Background:** Using cAMP analogues, evidence has been obtained that complement-mediated phagocytosis (CMP) by human monocyte-derived macrophages (MDM) is impaired by cAMP. During HIV-1 infection, increased intracellular concentrations of the nucleotide cAMP may occur which could have negative effects on CMP in macrophages.

**Objective:** To examine the effect of an activator of adenyl cyclase on CMP and the effect of inhibition of adenyl cyclase on CMP by MDM infected with HIV-1.

**Materials and Methods:** Using a colorimetric assay, sheep-erythrocytes (E) opsonised with human serum as a source of complement components were used as targets to quantify CMP by MDM infected with a laboratory adapted, M-tropic strain of HIV-1 for 7-10 days. Complement receptors were activated using 200 nm phorbol myristyl acetate for 15 min, and E uptake measured in the presence of varying concentrations of forskolin, a potent stimulator of adenylate cyclase, or dideoxyadenosin (ddAD), an inhibitor of adenylate cyclase. HIV-1 infection was assessed by retroviral reverse transcriptase activity, whereas immunolabelling of p24 viral antigen and the cell surface marker CD64 allowed quantification actively infected macrophages by microscopy and digital imaging.

**Results:** Consistent with results obtained using cAMP analogues, forskolin (100 μM) strongly inhibited CMP by 80% (P < 0.0016) in uninfected MDM. A significant increase in phagocytic activity was observed when erythrocytes were incubated with incrementing levels of 2',5'-ddAD (up to 10 μg/mL) in both HIV-1 infected cells and controls (57 ± 20%, and 30 ± 6%, respectively). In the presence of 10 μg/mL 2',5'-ddAD, particle ingestion by HIV-1-infected macrophages reached levels (97%) of complement-mediated phagocytosis of untreated, HIV-negative controls.

**Conclusions:** In HIV-1 infected MDM, integrin-dependent phagocytosis can be restored by 2',5'-ddAD to levels of uninfected macrophages suggesting that elevated cAMP levels in these cells may contribute to decreased complement receptor function.

**P651** CD4+ T-lymphocytes counts at AIDS among patients treated by highly active antiretroviral therapy or other antiretroviral regimens

Lyon, F

**Objectives:** Opportunistic infections (OI) seem to occur at higher CD4 count, as the use of HAART. We reported and compared the CD4 count at four AIDS-defining events for patients receiving HAART or other regimens. The risk to present OI at higher CD4 count was increased for AIDS-defining illnesses and CD4-based HAART commencement. CD4 counts since the use of HAART emphasises the need of appropriate follow-up to detect and prevent OI. Results from other cohorts would be helpful to conclude.

**Method:** Through database extraction, we identified 1066 patients who sought treatment for the first time at our Special Immunology Unit between 1995 and 2002. Of these, 806 (80.1%) with no history of AIDS-defining illnesses more than 3 months prior to presentation and no prior antiretroviral exposure are included in this analysis. Data were analysed using t-test for independent groups and chi-square (SPSS version 11.0).

**Results:** Six hundred and thirty-two (78%) of our patients were men. Women had higher CD4 counts and lower HIV RNA than men at baseline (mean: 388 ± 282 vs. 310 ± 242 cells/μL, P = 0.003, and 89 396 ± 168 223 vs. 126 537 ± 200 540 copies/mL, P = 0.048, respectively). The proportion of entrants with CD4 cell counts <200/μL did not differ significantly between sexes (37.8% vs. 30.4%, P = 0.11). There was no difference in the proportion of patients presenting with category C AIDS-defining illnesses, category B symptomatic conditions and STD history between men and women (15.8% vs. 12.0%, P = 0.23; 27.8% vs. 29.8%, P = 0.63; and 17.7% vs. 14.9%, P = 0.42, respectively).

**P662** Gender differences in HIV RNA and CD4 counts, but not in AIDS-defining illnesses and CD4-based HAART initiation threshold, in new entrants to HIV care in Cleveland, Ohio

Cleveland, Ridgebury, USA

**Objectives:** To examine possible differences between HIV-infected men and women new to HIV care at University Hospitals of Cleveland between 1995 and 2002.

**Methods:** Through database extraction, we identified 1066 patients who sought treatment for the first time at our Special Immunology Unit between 1995 and 2002. Of these, 806 (80.1%) with no history of AIDS-defining illnesses more than 3 months prior to presentation and no prior antiretroviral exposure are included in this analysis. Data were analysed using t-test for independent groups and chi-square (SPSS version 11.0).

**Results:** Six hundred and thirty-two (78%) of our patients were men. Women had higher CD4 counts and lower HIV RNA than men at baseline (mean: 388 ± 282 vs. 310 ± 242 cells/μL, P = 0.003, and 89 396 ± 168 223 vs. 126 537 ± 200 540 copies/mL, P = 0.048, respectively). The proportion of entrants with CD4 cell counts <200/μL did not differ significantly between sexes (37.8% vs. 30.4%, P = 0.11). There was no difference in the proportion of patients presenting with category C AIDS-defining illnesses, category B symptomatic conditions and STD history between men and women (15.8% vs. 12.0%, P = 0.23; 27.8% vs. 29.8%, P = 0.63; and 17.7% vs. 14.9%, P = 0.42, respectively).

**Table 1.** Median and mean CD4 counts at OI onset by treatment groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Median CD4</th>
<th>Mean CD4</th>
<th>Median CD4</th>
<th>Mean CD4</th>
<th>Mean CD4</th>
<th>Mean CD4</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAART</td>
<td>7.7</td>
<td>9.7</td>
<td>2.4</td>
<td>3.0</td>
<td>10.0</td>
<td>12.0</td>
</tr>
<tr>
<td>B + M</td>
<td>9.7</td>
<td>12.0</td>
<td>3.0</td>
<td>4.0</td>
<td>13.0</td>
<td>15.0</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

(1)Mean comparison

(2)Median comparison
P < 0.001), but there was no difference in hepatitis C Ab (18.8% vs. 15.6%, P = 0.40).

Conclusions: In Cleveland, female new entrants to HIV care had higher CD4 cell counts, lower HIV RNA levels and were less frequently seropositive for hepatitis B infection. Whether women here seek HIV care earlier in the course of disease or whether early markers of HIV disease differ between sexes in this population remains to be determined.

P653  Incidence and predictors of opportunistic infections and mortality in HIV/AIDS patients admitted to a university hospital: a 16-year follow-up
F. Sain Güven, N. Aydemir, B. Cakir, O. Uzun, S. Ünal
Ankara, TR

The study aimed to determine the incidence and significant risk factors of opportunistic infections in HIV/AIDS patients followed in the Department of Internal Medicine between 1986 and 2002, and to investigate predictors of mortality in this group. The charts of all HIV-AIDS patients (n = 110) were retrospectively examined. Data were collected on patients’ sociodemographic characteristics, morbidity features at admission, presence and type of opportunistic infections, antiretroviral treatment modalities used and outcomes as of 31 December 2002. The mean (±SD) age was 37.1 ± 12 years and 68% were men. Heterosexual contact was the major route of transmission (68.2%). According to CDC-93 classification (modified in 1997), 36, 38 and 33 patients were at categories A, B and C, respectively, and 36, 38 and 33 patients had CD4 cell count of <200/mm³ at the first visit. Forty-two patients experienced at least one opportunistic infection. The most common AIDS indicator condition was pneumocystis carinii pneumonia (n = 17, 15.5%), followed by tuberculosis (12.7%), CMV infection (9.1%), toxoplasmosis (8.2%), cryptococcal meningitis (2.7%) and PML (1.8%). Ninety-one patients (82.7%) received at least one antiretroviral drug. Twenty-four (21.8%) patients died during therapy. Cox proportional hazards modelling was conducted to identify statistically significant predictors of opportunistic infections: adjusting for initial CD4 cell count, antiretroviral therapy and route of transmission, patient’s age at admission (HR = 1.03, 95% CI 1.01–1.06) was the only significant predictor of opportunistic infection. In the study group, mortality was significantly associated with age at admission (HR = 1.06, 95% CI 1.02–1.10) and presence of at least one opportunistic infection (HR = 2.58, 95% CI 1.01–6.58). Study findings confirmed that opportunistic infections are significant predictors of mortality in HIV/AIDS patients. In the study, age at admission was indicated as a significant predictor of both occurrence of opportunistic infections, and subsequent mortality.

P654  Venezuelan HIV patients and prevalence of HPV infection in anal samples
M. Correnti, M.E. CavaZZa Porro, R. Alfonso, E. Arias, M.P. Avila, M. Uribe
Caracas, VE

Objective: The aim of the study was to characterise HPV infection in a group of HIV positive male patients to determine the association between HIV infection, anal squamous intraepithelia lesions (ASILs) and HPV genotypes.

Methods: Twenty HIV positive and 9 HIV negative men were enrolled after informed consent and complete physical examination. Anal exfoliated cells were collected for PCR assay. The HPV typing were performed by using FRLP (HPV fast, Pharmagen, Spain).

Results: In the group of HPV positive patients, 10% had ASCUS lesions, 35% low grade anal squamous intraepithelia lesions (LgASIL), 15% high grade ASIL (HgASIL) and 5% presented HgASIL/invasive anal cancer. In the HIV negative patients 67% had normal cytology, 11% with ASCUS and 22% with LgASIL. The overall prevalence of HPV infection was higher in HIV positive group (95%) the negative HIV patients (78%) (P < 0.001). The HPV type 16 was the most frequent genotype in HIV positive male (55%) associated with ASCUS, LgASIL and in all cases of HgASIL/anal cancer; only 33% of HIV negative patients presented this HPV type. A high spectrum of HPV genotypes in the HIV positive group: 6, 11, CP8304, 70, 58, 31, 33, 61 and 83. A high prevalence of oncogenic HPV types (30%) was found in the group of HIV positive patients with normal anal cytology.

Conclusions: The results in this study shown both high rate anal HPV infection and anal disease in the HIV positive patients (95%). The HPV 16 is the most frequent genotype in our HIV positive men population and another common feature of HPV infection in this group was the mix viral genotypes observed in the anal samples. In the HIV positive patients with normal anal cytology, we found a high prevalence of high-risk oncogenic types of HPV. This group represents a high-risk population for development anal diseases.

Acknowledgements: This study was supported by grant FONA-CIT-S1200000643.

P655  HCV viraemia and the degree of hepatic affection in HCV-HIV co-infected patients
Valladolid, E

Objective: We study the relationship between HCV viraemia (determined by quantitative-PCR) and the degree of hepatic fibrosis in patients with HCV-HIV co-infection prior to treatment.

Materials and Methods: Twenty-three sera from 23 patients with chronic hepatitis C were studied. The level of RNA HCV was determined by PCR, previous reverse transcription RNA-DNA complementary (Cobas Amplicor; Roche Diagnostics). The degree of hepatic fibrosis was studied by histological activity rate (HAR-Metavir) in hepatic biopsy.

Results: Nine (39.2%) patients have RNA levels lower than 500 000 IU/mL; five of these have a severe hepatic affection; three have a severe HAR-Metavir (3) and two have cirrhosis HAR-Metavir (2). In the remaining four patients, three have a moderate HAR-Metavir (2) and one has a slight HAR-Metavir (1). Eight (34.8%) patients have RNA levels from 500 000 to 1 000 000 IU/mL: one patient has a slight HAR-Metavir (1), four patients have a moderate HAR-Metavir (2) and three patients have a severe HAR-Metavir (3). Six (26%) patients have RNA levels higher than 1 000 000 IU/mL: two patients have a slight HAR-Metavir (1), and four patients have a moderate HAR-Metavir (2).

Conclusion: In patients HCV-HIV co-infected there is an association between viraemia and degree of hepatic affection (HAR-Metavir).

Table 1. Distribution of patients by viremia level and degree of hepatic affection

<table>
<thead>
<tr>
<th>Q-PCR (IU/mL)</th>
<th>Slight (1) No.</th>
<th>Moderate (2) No.</th>
<th>Severe (3) No.</th>
<th>Cirrhosis (4) No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–500 000</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>500 001–1 000 000</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>&gt;1 000 000</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Spearman’s Rho: *r* = 0.522
Prevalence of GBV-c/HGV in HIV-infected patients and potential influence of co-infection on the course of the disease

V. Aster, J. König, M. Stanková, H. Roszypal, B. Prochazka
Prague, CZ

Objectives: Assessment of prevalence of GBV-c/HGV infection in HIV-infected patients and evaluating of a possible influence of GBV-c/HGV on the course of HIV infection by assessment of immunological and virological markers of progression of HIV infection.

Methods: We have investigated sera of 273 HIV-infected patients from AIDS Center of the University Hospital Na Bulovce Prague during 2002–2003. Our target was to assess the presence of markers of GBV-c/HGV infection by semiquantitative HGV PCR evaluation and anti-E2 antibodies by ELISA testing. 271 of serum samples were tested for HGV PCR and 269 samples were tested for anti-E2 antibodies. HIV viral load and CD4 count were tested concurrently. We used Spearman’s test to rule out the dependency of CD4 count and HIV viral load on HGV infection.

Results: Eighty-nine (33.3%) of patients were positive in PCR HGV test and 101 (38.5%) of patients were positive in anti-E2-ELISA. No statistically significant effect of GBV-c/HGV infection was observed on CD4 count and HIV viral load in our cohort of patients.

Conclusion: The effect of GBV-c/HGV infection on predictive laboratory markers of HIV infection was not confirmed in our study. Further investigations regarding this subject seem to be necessary.

Acknowledgements: Our study is constituent of the grant project no.7509-3/2003 IGA MH CR.

Pancreatic abnormalities during HIV infection. What about epidemiology, management and outcome?

R. Manfredi, L. Calza, F. Chiodo
Bologna, I

HIV-infected patients on HAART are exposed to direct and indirect (hyperlipidemia-mediated) pancreatotoxicity and multiple predispelling conditions (biliary-liver disorders, alcoholism). In order to assess frequency and significance of pancreatic anomalies, a case–control study involved 976 HIV-infected patients followed for 1.5 years. Octreotide ± gabexate administration was carried out in patients with severe, prolonged or symptomatic abnormalities. In our single-centre cohort, 349 of 976 (35.7%) patients experienced altered amylase and/or lipase at least once. Compared with the remaining 627 patients without pancreatic alterations a multivariate analysis detected a relationship with duration of HIV infection, AIDS, a CD4+ count <200 cells/μL, duration of a protease inhibitor-based HAART, hypertriglycerideraemia and underlying acute-chronic hepato-biliary diseases (P < 0.01–0.001), while no association was found with nucleoside analogues. Only 36 of 349 (10.3%) patients had signs and symptoms of pancreatic involvement and a frank pancreatic crisis occurred in only nine (2.6%) patients. A specific treatment with gabexate (46 patients), octreotide (17 patients), or both (35 patients) was performed when pancreatic enzymes were greater than threefold normal values for >6 months, and when signs-symptoms of pancreatitis became evident. A drop of pancreatic enzymes >50% vs. baseline, combined with improvement-cure of clinical-instrumental picture, was achieved in 68 of 98 (69.4%) patients treated for 13–35 days, in absence of untoward events. A more rapid and effective response was obtained in the 35 patients treated with gabexate + octreotide vs. 63 patients who received a single drug (P < 0.05). A further case–control study compared the 98 treated patients with 76 HIV controls with matched pancreatic abnormalities, but followed with dietary-conservative measures: a significantly better short- and long-term evolution was seen in patients treated pharmacologically as found by a more frequent-rapid drop of pancreatic enzymes, improvement of clinical-instrumental alterations, reduced relapse rate, and better HAART tolerability. Limited literature data are available about pancreatic abnormalities and their management during HIV disease and HAART. However, a crude 35.7% rate of patients in our cohort had laboratory alterations, while pathogenetic pathways are broadening, owing to emerging dysmetabolism and mitochondrialopathy. Further studies are needed to give reliable estimates of both frequency and evolution of HIV-associated pancreatic abnormalities, their consequences on HAART administration and specific treatment.

Laboratory and clinical pancreatic abnormalities during HIV infection treated with antiretroviral therapy

R. Manfredi, L. Calza, F. Chiodo
Bologna, I

The epidemiological and clinical features of HIV-associated pancreatic abnormalities are changing in the HAART era. Their frequency, risk factors and clinical and therapeutic features were assessed in an observational case–control study involving 986
patients, assessed for pancreatic abnormalities in a case–control comparison including the whole follow-up period of each patient; 149 patients with high and prolonged laboratory abnormalities underwent comprehensive metabolic assessment. Comparing the 352 patients (35.7%) who experienced greater than one episode of confirmed pancreatic laboratory abnormality had a longer duration of seropositivity, exposure to protease inhibitors, a more frequent immunodeficiency, AIDS diagnosis, liver-biliary disease, and hypertriglyceridaemia (P < 0.04–0.001), while no correlation was found with type and duration of antiretroviral combinations (as well as specific nucleoside analogue use). Among these 352 patients, high and prolonged laboratory alterations eventually associated with signs of organ involvement occurred in 149 cases, and seemed related to the administration of didanosine, stavudine, lamivudine, pentamidine, cotrimoxazole, or anti-tubercular therapy, substance or alcohol abuse, opportunistic infections, liver-biliary disease, a protease inhibitor-based HAART, and hypertriglyceridaemia (P < 0.02–0.001). However, no difference was noticed between the 39 patients with clinical and/or imaging evidence of pancreatic involvement and the remaining 110 asymptomatic patients, as to the same risk factors. Although recurrences of enzyme alterations involved >70% of patients, in only 33.8% a change of antiretroviral or antimicrobial therapy became necessary. An acute but uncomplicated pancreatitis occurred in eight of 29 overall symptomatic patients (27.6%). A 2–4 weeks gabexate and/or octreotide administration (performed in 68 patients of 149), attained a significant laboratory, clinical and instrumental cure or improvement in 73.5% of patients, with a better success rate of combined versus single therapy, a reduced tendency to disease relapses in the subsequent 6–42-month follow-up, and a better tolerability of antiretrovirals (P < 0.05–0.004). Epidemiologic and pathogenetic studies are warranted to re-evaluate pancreatic abnormalities in the HAART era, and their consequences on continued anti-HIV and antimicrobial therapy. The management of antiretroviral therapy and the indication to gabexate and/or octreotide administration in the different clinical and laboratory settings, deserve controlled investigation.

**Objective:** The fat redistribution syndrome and its variably associated metabolic abnormalities, emerged as a result of the administration of potent anti-HIV combinations. Local fat accumulation may present as central adiposity, increased breast dimension, gynecomastia, lipomastia and the so-called buffalo hump. Lipomas and other benign tumours of fatty tissue have not been yet reported during HIV disease, and the HAART era.

**Methods and Results:** Eight of approximately 1000 HIV-infected patients (p) experienced multiple lipomas since the year 2000. All p suffered from ultrasonography-confirmed multiple lipomatous lesions (3 to >20), predominantly involving limbs, thorax, and anterior abdomen, associated with limited local discomfort. Six p were male and two were females, with age ranging from 36 to 58 years, and duration of seropositivity between 38 and 116 months. Risk factors for HIV disease included iv drug use and heterosexual contacts in three p each, and homo/bisexual transmission in two p. At the time of onset of lipomas, all p were on a protease inhibitor (PI)-based HAART regimen since 17-56 (mean 24 ± 14) months. Our p experienced four to nine different anti-HIV therapeutic lines: almost all available PI and nucleoside analogues (NA) had been used, while these p never took non-NA reverse transcriptase inhibitors. Laboratory markers of HIV disease tested satisfactory: mean viral load 3.1 ± 0.6 log10 HIV-RNA copies/mL, and mean CD4+ count 432 ± 146 cells/μL. Lipomatosis was present in six of eight p, associated with central adiposity in four p, while no localised fat accumulation was present (i.e. breast enlargement, buffalo hump). Increased triglyceridaemia, cholesterol- olemia, and glycaemia were detected in five, three, and one p, respectively, with some correlation with the onset of signs of the fat redistribution syndrome. The 15–31-month follow-up allowed us to identify the appearance of further lesions in four p, and a substantially stable disease in the remaining four p, while spontaneous regression never occurred, as well as resort to surgery.

**Conclusion:** The relationship between lipomas, HIV infection, and HAART is a novel entity, but the frequent association with other clinical and metabolic disturbances possibly related to antiretroviral therapy should prompt further epidemiological, pathogenetic, and clinical studies. Malignant degeneration should be rare, but careful surveillance seems recommendable. The possible pathogenetic role carried out by antiretrovirals, and the concomitant occurrence of lipodystrophy and dysmetabolism, warrant investigation.

**Antiretroviral treatment and gynecomastia: which correlations?**

R. Manfredi, L. Calza, F. Chiodo

Bologna, I

Aim of our study was to recognise all episodes of gynecomastia occurring during anti-HIV therapy in our patient (p) cohort, and to search for all correlations with several demographic–epidemiologic variables, clinical–laboratory markers, prior-underlying therapy, metabolic anomalies, and subsequent evolution. A cross-sectional survey of 988 p treated with antiretrovirals for >1 year (661 males:66.9% of p), allowed us to retrieve all p with ultrasonography-confirmed, true gynecomastia, considered after exclusion of all potentially involved conditions. Particular attention was deserved to metabolic alterations, including the lipodystrophy syndrome, dysmetabolism, and administered antiretrovirals. A complete hormonal workout failed in detecting significant abnormalities (i.e. hypogonadism) in all p but one, and hyperprolactinemia was not found. Fifteen p of the 513 evaluable males (35.7%), developed gynecomastia when aged 12–58 years. The duration of HIV-infection, antiretroviral therapy, and HAART, varied significantly in our p group, and no correlation was found with clinical–laboratory markers of HIV disease, but five of 15 p (33.3%) never received protease inhibitors (PI), while an efavirenz-based therapy apparently prompted gynecomastia in four p who were naïve for PI, and worsened this sign in three more p who switched from a PI-based HAART towards efavirenz. One p developed gynecomastia while on prolonged, isolated didanosine nucleoside analogue (NA) therapy, without administration of PI and non-NA reverse transcriptase inhibitors. A concurrent lipodystrophy was present in all p who developed gynecomastia, while hypertriglyceridaemia, -cholesterolemia, and -glycaemia were found in 11, six, and three p, respectively. Among NA, stavudine represented the more frequently used drug, administered during a more prolonged time in all p with gynecomastia. During the subsequent follow-up (7–23 months), no significant clinical amelioration of gynecomastia was observed, despite therapeutic changes (determined by regimen failure and/or toxicity), but surgery never proved necessary. Gynecomastia, as an emerging untoward event of HIV infection treated with antiretrovirals, warrants investigation, from an epidemiologic, clinical, and pathogenetic point of view. The apparently frequent association with other metabolic anomalies suggests some common pathway with other HIV- and antiretroviral-associated disturbances, so that special attention should be deserved to anti-HIV therapy, and the role of single and associated antiretroviral compounds.
and cholesterol. About 4% stopped taking Kaletra because of side effects. Alopecia is a well-recognized adverse effect of chemotherapy, but it is uncommon with antiretroviral therapy. Alopecia, generally involving the scalp, has been reported in patients with HIV infection treated with indinavir but not with Kaletra. Hair loss has been linked to certain other drugs. We present a 62-year-old man with HIV infection, stage B2, CD4 count 432 and plasma viral load <50 copies/mL experienced alopecia totalis of his scalp, eyebrows and eyelashes beginning 18 months after initiating antiretroviral treatment including Kaletra. No hair loss of arms, legs and pubic area was observed. Our patient’s drug regimen consisted of Lopinavir/Ritonavir (four caps bid), Efavirenz (600 mg qd) and Stavudine (40 mg bid); in addition, the patient was receiving treatment for diabetes with Glivencamide and Metformin for the last 3 years. These drugs have not been shown to cause alopecia. The alopecia reversed completely 2 months after Kaletra substitution by Nelfinavir without any other change of treatment and his eyelashes and eyebrows grew back as well. To our knowledge, alopecia totalis has not been reported in patients with HIV infection treated with Kaletra. In conclusion, the course of alopecia related to Kaletra seems to be reversible.

P663  Modification of lipid parameters during structured interruptions of treatment in HIV chronically infected patients

M. Montes de Oca Arjona, R. García Juárez, A. Martin-Aspas, R. Pérez-Canó, F. Brun Romero, C. Fernandez Gutierrez, J. Giron-González

Cadiz, E

Objective: Analysis of the modifications of anthropometric, biochemical and lipid parameters as well as those molecules implying in the lipid metabolism (leptin, tumor necrosis factor alpha – TNF-z) in patients with chronic infection by human immunodeficiency virus (HIV) during structured interruptions of antiretroviral treatment (STI).

Patients and Methods: Forty chronically HIV infected patients were evaluated (CD4 cell count, nadir 374; at the beginning of STI 968; viral load, VH nadir 41521; at the beginning of the STI < 50 copies; the median duration of antiretroviral treatment at entry of the study was 60 months). The STI consisted of HAART 2 months, nontreatment 1 month. Patients were evaluated at the end of each period and after four STI, with particular attention to the following parameters: CD4 cell count, viral load, waist and hip circumference, tricipital fold, arm circumference and serum concentrations of cholesterol, triglycerides, leptin levels, TNF and its soluble receptors (TNFR1 and TNFR2).

Results: There were no significant modifications in lymphocyte T CD4 counts or in the viral load at the end of 4 STI. Viral load persisted undetectable in the patients who completed the study. Likewise, significant differences were not detected between values observed at the beginning of STI and at the end of the intervention with reference to the anthropometric parameters (waist circumference 84 vs. 87; hip circumference 92 vs. 100 cm; tricipital fold 14 vs. 21 cm; arm circumference 31 vs. 31 cm, P > 0.05 in each case), serum cholesterol (173 vs. 226 mg/dL) or triglycerides levels (124 vs. 144 mg/dL). Nevertheless, a significant increase in the concentration of leptin (15 vs. 66 pg/ml, P 0.003) and a diminution of the TNFR1 (100 vs. 80 vs. pg/ml, P 0.0017 ) and TNFR1 (253 vs. 185 vs. pg/ml, P 0.093) levels were detected.

Conclusions: The STI is associated to significant modifications in lipid markers receptors (TNF and leptin), suggestive of an increase in lipogenesis without significant changes in the anthropometric parameters during the 12 months of follow-up.

P664  Metabolic changes in HIV-infected patients on HAART

E. Valadas, H. Chaves Ramos, J. Cruz, C. Ribeiro, L. Caldeira, F. Antunes

Lisbon, P

Objective: The use of highly active antiretroviral therapy (HAART) has dramatically improved the prognosis of HIV infected patients, but it has been associated with various adverse effects. A number of metabolic disorders have been reported among patients on HAART, such as high serum lipids and elevated liver enzymes. However, results are still limited and controversial. To assess the impact of NNRTI on serum lipids and liver enzymes in HIV infected patients.

Methods: The study was conducted during the year 2001 at a large outpatient clinic, in Lisbon (Portugal). Clinical charts from 695 consecutive HIV infected patients were analysed. Total and high-density lipoprotein (HDL) cholesterol, triglyceride levels, and AST/ALT were determined. Values represent the average of at least four time-points through the 12 months of the study. Data from patients who were either taking nevirapine or efavirenz-based regimens was compared with patients treated with other combinations, as well as with nontreated HIV infected patients.

Results: Data from 25 patients was excluded from analysis, because of the poor quality of the data. Complete records were available from 670 patients: 175 patients (26.1%) were on a combination of NNRTI and NRTI: 68 patients were taking efavirenz (10.1%, Group EFV), and 107 patients were taking nevirapine (16.0%, group NVP); 418 patients (62.4%) were on other drug combinations (group O), and 77 HIV patients (11.5%) were not taking antiretroviral treatment (group XI (Table 1). No age or gender differences between the groups were noted. Group NVP had lower triglyceride levels and higher HDL cholesterol when compared with group O (triglyceride: 1.63 vs. 1.70, P < 0.001); HDL cholesterol: 1.36 vs. 1.07, P < 0.001), and higher HDL cholesterol when compared with group X (1.36 vs. 1.03, P < 0.001).

Group EFV had lower triglyceride levels and higher HDL cholesterol when compared with group O (triglyceride: 1.63 vs. 1.70, P < 0.001); HDL cholesterol: 1.36 vs. 1.07, P < 0.001), and higher HDL cholesterol when compared with group X (1.36 vs. 1.03, P < 0.001).

Conclusion: Our data show that there is an association between the use of NNRTI and better lipid profiles (higher HDL cholesterol and lower triglycerides). No increase in liver enzymes was found on association to NNRTI. The use of nevirapine improved the lipid profile both by reducing the triglycerides and increasing the protective HDL fraction. These changes are expected to contribute to the reduction of cardiovascular disease in HIV-1-infected patients on HAART.

P665  Nephrolithiasis induced by indinavir plus boosting dose of ritonavir in a Belgrade study population

G. Dragovic, D. Jevtovic

Belgrade, CS

Objectives: Efficacy of Indinavir (IDV) has been known for more than 6 years. Its major disadvantages are limited absorption, a short elimination half-life and adverse events such as nephrolithiasis. IDV in combination with ritonavir (RTV) is believed to ameliorate these disadvantages. We investigate if the use of IDV singly or with boosting dose RTV in combination with nucleoside reverse transcriptase inhibitors (NRTIs) was associated with patients treated with antiretroviral treatment including Kaletra. No hair loss of arms, legs and pubic area was observed. Our patient’s drug regimen consisted of Lopinavir/Ritonavir (four caps bid), Efavirenz (600 mg qd) and Stavudine (40 mg bid); in addition, the patient was receiving treatment for diabetes with Glivencamide and Metformin for the last 3 years. These drugs have not been shown to cause alopecia. The alopecia reversed completely 2 months after Kaletra substitution by Nelfinavir without any other change of treatment and his eyelashes and eyebrows grew back as well. To our knowledge, alopecia totalis has not been reported in patients with HIV infection treated with Kaletra. In conclusion, the course of alopecia related to Kaletra seems to be reversible.
probability of developing nephrolithiasis was estimated by Univariate and stepwise Multivariate logistic regression.

**Results:** There were 189 patients; 99 patients were included in the IDV arm and 90 patients in the IDV + RTV arm. Nephrolithiasis developed in 38 (26.57%) patients in total. The prevalence of nephrolithiasis was 26.97% on IDV arm and 25.93% on IDV + RTV arm, respectively (P < 0.001; d.f. = 1). Multivariate logistic regression shown that the relative risk of developing nephrolithiasis is 1.9-fold greater (RR = 1.9; 95% CI 0.88-2.46) in IDV arm and 6.6-fold greater in IDV + RTV arm (RR = 6.2; 95% CI 1.68-17.12).

**Conclusion:** We demonstrate that boosting IDV with RTV increased the risk of developing nephrolithiasis by 6.2-fold. We support the need for therapeutic drug monitoring in patients using IDV with or without RTV in order to monitor the number of patients who discontinued IDV due to toxicity.

---

**P667** Prevalence of resistance mutations in subtype F strains isolated from Romanian naïve children

Bucharest, RO

With the advent of highly active antiretroviral therapy (HAART) the life expectancy of HIV infected patients have been substantially prolonged. However, it is now clear that mutations accumulate in treated patients, limiting the efficacy of the therapy and requiring a change in the existing medication. Resistance genotyping has become a SOC in HIV infection management. Furthermore, resistance mutations are more and more encountered in recently diagnosed/newly infected individuals. Information has accumulated so far mainly on type M clade B strains which are dominantly circulating in Western Europe and North America. Sporadic communications have suggested that resistance mutations can occur spontaneously in the genome of viruses of other subtypes isolated from untreated patients. In this study we present data coming from strains of Romanian children obtained before the onset of the treatment. Most of the samples had viral loads over 10 000 copies/mL. The genotyping has been performed with the VirosolQM (Applied Biosystems) kit according to the manufacturer’s recommendations. Previous anecdotal information suggested that subtype F strains are rather common in Romania. Our findings have confirmed these observations (all strains tested so far belonged to the clade F), although the strains displayed several dissimilarities with subtype F strains available from databases in order to show the relatedness of the Romanian and international strains phylogenetic trees are being displayed. Bearing in mind that resistance mutations are more readily selected in subtypes other than B, we evaluated several genomic positions belonging to the RT and protease genes. Our results reveal that while the reverse transcriptase gene are relatively stable in respect to the resistance mutations, the frequency of the resistance mutations is significantly higher in the protease gene. Some of the genomic positions seem more prone to evolve towards a resistant genotype. These findings suggest that some resistance calculation algorithms of clinical interest might need to be revised for other subtypes than B – taking in consideration that the virological response of these patients was good.

---

**P668** Comparison of line probe assay vs. sequence analysis for detection of human immunodeficiency virus type 1 (HIV-1) mutations conferring resistance to antiretroviral drugs

S. García-Bujalance, C. Ladron de Guevara, A. Sánchez-Maroto, A. Gutiérrez
Madrid, E

**Objective:** To compare the line probe assay (LiPA) vs. sequence analysis for detection of mutations conferring resistance to nucleoside (NRTI) and non-nucleoside inhibitors (NNRTI) of HIV-1 reverse transcriptase and protease inhibitors (PI).

**Material and Methods:** In a retrospective study, 54 plasma samples from 54 HIV-1-infected patients were analysed for drug resistance. All the patients were followed in our hospital. Baseline characteristics of the patients were studied. Forty-nine patients received antiretroviral therapy. The more frequently treatment was two NRTI plus a PI in 42.6%, and two NRTI plus a NNRTI in 33.3% of the patients. Sequencing of the protease (PR) and reverse transcriptase (RT) gene was performed by the TruGene HIV-1 assay (Visible Genetics, Canada). The new version of the LiPA test, LiPA HIV-1 RT and Protease assay v 2.0 (Innogenetics, Belgium) was compared with the reference method: the sequence analysis. The LiPA assay allows the study of wild-type and mutant sequences at codons 41, 69, 70, 74, 75, 184, 215, 103, 106 and 181 of the RT gene (LiPA RT) and at codons 30, 46, 48, 50, 54, 82, 84 and 90 of the PR gene (LiPA P). A descriptive study was carried out with the aid of the statistical program SPSS for Windows version 9.0. Each codon was scored as wild type, mutant, a mixture of both or uninterpretable results. Concordance was defined as the same interpretable results obtained by the two methods. Discordances were defined as minor or major.

**Results:** LiPA gave uninterpretable results for 36 (59.5%) of 605 analysed codons in the RT gene and for 13 (3.7%) of 344 analysed codons in the PR gene. The concordance between LiPA and sequence analysis was 97.6% for codons of the RT gene and 96.7% in the PR gene. Minor discordancces was 1.2% in the RT gene and 2.4% in the PR gene.

**Conclusions:** (1) The LiPA HIV-1RT and Protease assay v 2.0 give a high rate of codon hybridisation failures and it’s do not improve the previous version. It is unacceptable in the clinical practice. (2) LiPA to detect more minority mutant-wild-type mixtures than DNA sequencing. It is useful for the screening of primary HIV-1 resistance.
**P669 Evaluation of the Roche COBAS TaqMan 48 HIV-1 test**

J. Djupsjöbacka, K. Sopenlehto, H. Pipparinen, M. Lappalainen, J. Suri

**Helsinki, FIN**

**Objectives:** Analytical and clinical performance of the real-time based Roche COBAS TaqMan 48 HIV-1 Test was evaluated and compared with the Roche COBAS AMPLICOR HIV-1 MONITOR UltraSensitive Test v1.5.

**Methods:** HIV-RNA purification for the COBAS TaqMan HIV-1 test was conducted by using glass fibre-based High Pure System Viral Nucleic Acid Kit. Plasma panels for intra-assay, linearity and analytical performance studies were obtained from AcroMetrix Corporation. Genotype panels (A–H) were obtained from Boston Biomedica Inc. Plasma specimens for specificity, linearity and competitive sensitivity for low viral titres and high titre viral quantitation of clinical performance were obtained from our previously well-characterised HIV-1 positive plasma panel deposit. For analytical performance the specimens were tested by both methods either in duplicate or triplicate. Clinical performance samples for specificity and linearity were tested in singlicate, for competitive sensitivity in duplicate and for high titer quantitation in quadruplicate.

**Results:** Intra-assay results showed good correlation (R = 0.996) between the two methods and CV% was similar, 2–44% within detection range of the assay. Similar correlation and CV% were obtained in linearity studies. However, COBAS TaqMan HIV-1 test proved to give slightly higher copy numbers, especially within low copy number range. This was observed also when studying competitive sensitivity for low viral titres in clinical performance. Testing quantitation equality across different genotypes COBAS TaqMan HIV-1 Test showed slightly better sensitivity in all genotypes compared with the COBAS AMPLICOR HIV-1 MONITOR UltraSensitive Test, v1.5. The specificity of the COBAS TaqMan HIV-1 Test, when testing negative sample panel, was 100%. In quantitation of high titer specimens the CV% varied between 6 and 19%. When using COBAS TaqMan HIV-1 Test there was no need for reruns.

**Conclusion:** The analytical and clinical performance of the Roche COBAS TaqMan HIV-1 Test is comparable with the Roche COBAS AMPLICOR HIV-1 MONITOR UltraSensitive Test, v1.5 depicting better sensitivity especially with low copy numbers. Wider detection range of 40–10 × 10⁸ copies/mL and shorter assay run time of the COBAS TaqMan HIV-1 Test are considerable improvements in HIV RNA-quantitation.

**P670 Comparison of two commercial assays for the quantification of HIV-RNA**

L. Valdés, S. García-Bujalance, M.P. Romero, C. Ladrón de Guevara

**Madrid, E**

**Objectives:** In this study we compared two of the commercially available assays for the measurement of HIV-1 RNA, the QUANTIPLEX HIV RNA 3.0 (Bayer) which uses the branched DNA signal amplification technique (v3bDNA) and the AMPLICOR HIV-1 MONITOR TEST 1.5 (Roche) which uses reverse transcription (RT-PCR) to assess their quantitative relationship. It is useful to find a quantitative relationship between these two assays because patients may be monitored with more than one assay over the course of their treatment.

**Methods:** Total of 44 plasma samples from 44 antiVH-1 infected patients monitored at the specialised unit of HIV at our hospital, were tested with v3bDNA and RT-PCR. Both are ultrasensitive assays with lower detection limits of 50 copies/mL.

**Statistical analysis:** for the correlation analyses, the intraclass correlation coefficient was calculated. Wilcoxon test was used to determine the statistical significance. Assays values were transformed to common (log10) logarithms and expressed as log 10 v3bDNA or log10 RT-PCR. These assays were also expressed by groups. Three groups (A, B and C) were classified based on numbers of copies/mL.

**Results:** The data are shown in the following table:

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1000</td>
<td>27</td>
<td>2</td>
<td>4</td>
<td>33</td>
</tr>
<tr>
<td>1000–4000</td>
<td>2</td>
<td>7</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>&gt;40000</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Totals</td>
<td>29</td>
<td>11</td>
<td>44</td>
<td>84</td>
</tr>
</tbody>
</table>

The undetectable samples were 19 (43,18%) tested by RT-PCR and 24 (54,5%) tested by v3bDNA. The two assays were found to be significantly correlated (P = 0.002) coeff. = 0.976. Statistical significant differences were observed among the A group (P < 0.035) and B group (P < 0.021). No statistical significant differences were observed in C group (P > 0.263).

**Conclusions:** (1) Both test are highly correlated and are used to monitor patients. (2) In patients with low viral load it is not recommended to mix both techniques. (3) These results indicate that variability between assays increases as values approach the lower detection limit.

**P671 Evaluation of a simplified protocol of the Versant bDNA HIV 3.0 for HIV-1 viral load quantitation**

F. Salvá, M. Rotger, E. Ruiz, H. Machado, M. Gaya, C. Alonso, J.L. Perez

**Palma de Mallorca, E**

**Objectives:** In this study we evaluate the validity of a new protocol that simplifies and shortens the standard procedure of the Versant bDNA HIV-1 RNA 3.0 for viral load (VL) quantitation using the System 340 instrument (S340).

**Methods:** A total of 252 samples were studied in parallel by the standard (SP) and alternate (AP) procedures. Frozen aliquots stored at ~70 C were used for performing the AP. AP implies manual vortexing of RNA extracts (x96), incubation for 2 h in thermal block, and then run the S340 HIV program; in contrast, only direct incubation of the nucleic acid extracts in the S340 was required for AP, but running the HCV program (the same. Samples were allocated in groups A–F according to their respective VLs determined by SP: A (n = 50) <50 RNA copies/mL (c/mL); B (n = 50) 50–100 c/mL; C (n = 47) 100–1000 c/mL; D (n = 45) 1000–10 000 c/mL; E (n = 30) 10 000–100 000 c/mL; F (n = 30) >100 000 c/mL.

**Results:** Mean log-transformed values between SP and AP differed in 0.05 log units. As for groups B–F, these differences were –0.14, 0.01, 0.07, 0.06 and 0.04, respectively. Eight samples in group A (below the limit of quantitation of the SP) gave detectable VL with the AP (range 51–143 c/mL); conversely, seven samples from group B (range 53–75 c/mL) were undetectable by AP. An excellent correlation between SP and AP values were observed, either considering the numeric VLs (R = 0.936; P < 0.001) or log-transformed values (R = 0.987; P < 0.001). The AP numeric values of the VL appear to be an overall 1.5% lower than the corresponding SP quantitations.

**Conclusions:** (1) Both procedures gave comparable VL values, but the alternate protocol is simpler and less time-consuming than the standard procedure. (2) A trend towards lower values was observed for the alternate procedure. (3) Overall differences were far below the 0.2 log units accepted as the normal technical variability.
HIV-1 viral load by NucliSens EasyQ HIV-1 v1.1 in combination with the NucliSens mini MAG instrument

P. van Deuren, T. Oosterlaken, T. Cuypers, A. Verhoeven, P. de Bie, R. Bosch, I. Berghuis, M. Jacobs, P. van de Wiel
Boxtel, Amsterdam, NL

Objectives: bioMérieux has developed a new nucleic acid isolation method (NucliSens Magnetic Extraction Reagents) that uses Boom chemistry in combination with magnetic silica particles. The NucliSens mini MAG instrument is used to wash and collect the silica particles in a user friendly and efficient way. In principle, the extraction method is generic and can be applied for a broad range of different sample types. The objective of this study was to verify this new extraction platform for the isolation of HIV-1 RNA from plasma samples in combination with the NucliSens HIV-1 v1.1 assay.

Methods: Spiked plasma samples were used to measure the analytical sensitivity of NucliSens EasyQ HIV-1 v1.1 in combination with the new extraction method. The extraction performance on clinical samples was measured by using 123 EDTA plasma samples obtained from HIV-1 infected individuals. As a reference extraction method the NucliSens Extractor was used. Testing was performed on two different sites. In this study also carry over was addressed by processing high positive samples (spiked with >1 x 000 000 HIV-1 IU/mL) and negative samples obtained from healthy blood donors in the same run.

Results: The analytical sensitivity of NucliSens EasyQ HIV-1 was 53 IU/mL and 92 IU/mL for the new extraction method and the reference method, respectively. In the clinical samples, HIV-1 RNA was detected in 121 of 123 (98%) and 115 of 123 (93%) of the clinical samples for new extraction and the reference method, respectively. In addition, for both extraction methods, excellent correlation was found in the quantification results, the overall R value was 0.94. No case of sample-to-sample carry over during extraction was observed. With the new extraction method 24 samples were processed within 90 min.

Conclusion: The NucliSens mini MAG instrument was successfully used to isolated HIV-1 RNA from plasma samples for determination of viral load. Compared with the reference extraction method (NucliSens Extractor) an improved analytical sensitivity was obtained in combination with NucliSens EasyQ HIV-1 v1.1, and a good clinical reactivity was measured. The sample throughput is <90 min for 24 samples. Moreover, not a single case of sample-to-sample carry over was detected.

Comparative study of two techniques to measure HIV-1 viral load in a French commercial laboratory

G. Kreplak, R. Legouge
Paris, F

Objectives: The aim of the study was to compare the performance of the NucliSens EasyQ HIV-1 V1.1 assay (bioMérieux, Boxtel, The Netherlands), a real time NASBA amplification assay for the quantitative determination of HIV-1 RNA in clinical specimen with the Versant HIV-1 RNA 3.0 (bDNA, Bayer Diagnostics) technique routinely used in our laboratory.

Methods: A total of 221 plasma samples were collected from HIV positive/AIDS patients. For each sample, HIV-1 viral load was measured using the NucliSens EasyQ HIV-1 V1.1 assay and the assay was used for routine i.e. Versant HIV-1 RNA 3. An eight-member calibration panel from Versant was used to check for proper set-up and functioning of both systems.

Results: Concordant results were obtained in 202 of 221 samples (91.40%). Seventeen results were found to be discordant. Among them, six were negative with Versant HIV-1 RNA 3 and quantified with EasyQ HIV-1 V1.1 and 11 were negative with EasyQ HIV-1 V1.1 giving a quantification result with Versant HIV-1 RNA 3. These discordant results showed very low values of viral load, always inferior to 2.89 log, corresponding to <776 copies/mL. Two EasyQ HIV-1 V1.1 results were considered as invalid. The results obtained demonstrate a good correlation between the two techniques for both low and high viral loads.

Conclusion: Analysis of a set of 221 clinical routine samples revealed that EasyQ HIV-1 V1.1 has a comparable performance to the Versant HIV-1 RNA 3 assay used in our laboratory. No difference in quantitation was observed between NucliSens EasyQ HIV-1 and Versant HIV-1 RNA 3. These performance results would enable us to switch from our routine method to NucliSens EasyQ HIV-1 without any impact on patient follow-up.

Evolution of hypervariable region 1 of hepatitis C virus in HIV–HCV co-infected patients after treatment with highly active antiretroviral therapy


Objectives: HCV variability is mainly attributed to virus ability to respond to host immune pressure. This study was aimed at studying the effects of highly active antiretroviral therapy (HAART) on heterogeneity HCV quasispecies in HIV/HCV co-infected patients.

Methods: Sixteen HIV/HCV co-infected patients were selected from I.Co.N.A. Cohort. The selected patients harboured HCV genotype 1, were not co-infected with HBV, and had been on HAART for at least 1 year. With respect to HAART, eight patients showed stable or increasing CD4 counts (immunological respondents, I-R) after 1 year of therapy, and eight showed decreasing CD4 counts (I-NR); 11 patients showed HIV viral load <400 cp/mL after therapy (virological responders, V-R), and five showed HIV viral load >10 000 cp/mL (virological nonresponders, V-NR). For quasispecies investigation, plasma samples at baseline (T0) and after 1 year HAART (T1) underwent clonal analysis of HVR-1 region of HCV (10–16 clones per patient per time point). Complexity (Shannon entropy) diversity (mean genetic distance), ratio synonymous to nonsynonymous substitutions (Ks/Ka) was considered, as parameters representative of quasispecies heterogeneity at T0 and T1.

Results: All parameters of quasispecies heterogeneity showed significant correlation, but did not correlate with HCV viral load, as already shown for patients mono-infected with genotype 1 HCV. Mean values of HVR-1 complexity and diversity were comparable at T0 and T1, in patients classified on the basis of either virological or immunological response. However, when analysing the individual variations of complexity, diversity and Ks/Ka a general tendency was observed, with increasing values in V-R and decreasing values observed in V-NR. This behaviour reached high statistical significance for complexity (P = 0.005), was significant for Ks/Ka (P = 0.045), and approached statistical significance for diversity (P = 0.082). No correlation was observed for the variation of heterogeneity parameters according to immunological response.

Discussion: In HIV-infected patients undergoing HAART, control of HIV replication and not CD4 cell count restoration is predictor of increased HVR-1 quasispecies heterogeneity after 1 year of therapy. This is rather surprising, and warrants further investigation on the relationships between HIV and HCV viral replication dynamics.
Development of endogenous resistance by staphylococci to BAL9141 and comparators

S. Heller, E. Marrer, M.G.P. Page, S. Shapiro, L. Thenoz
Basle, CH

Introduction: BAL9141 is a novel broad-spectrum cephalosporin strongly cidal towards homogeneous MRSA and vancomycin-resistant staphylococci. As emergence of resistance to antibiotics inevitably follows their clinical use, we examined the proclivity of MR staphylococci to develop high-level endogenous resistance to BAL9141 and comparators.

Materials and Methods: MICs (mcg/mL) towards linezolid, moxifloxacin, and BAL9141 of three staphylococcal strains were as follows: MRSA strain 745: 4, 0.06, 4; MRSA strain P8(+)-Hom: 1, 0.06, 4; MRSE strain CNS 184: 1, 2, 1. Each strain was serially passaged (10^8 CFU/passage, 48-h intervals, 35°C) on Mueller-Hinton agar (MHA) containing linezolid, moxifloxacin, or BAL9141 for 50 passages or until the MIC stabilised at ≥64 mcg/mL. Cells harvested from the last BAL9141 passage were subsequently passaged on MHA without antibiotic, and their MICs towards the cephalosporin checked periodically by agar dilution. BAL9141-passaged cells were also examined by DNA sequencing of the mec operon to follow the chromosomal location of the resistance mechanism(s). MICs towards BAL9141 fell from 32 to 8 mcg/mL within 1–2 transfers and remained at 8 mcg/mL through 30 serial passages. In contrast, the MICs of cells transferred in the presence of linezolid achieved MICs of ≥64 mcg/mL after 27 (MRSA strain 745), 28 [MRSA strain P8(+)-Hom], and 28 (MRSE strain CNS 184) serial passages. Cells transferred with moxifloxacin achieved MICs of ≥64 mcg/mL after eight (745), 22 [P8(+)-Hom], and four (CNS 184) serial passages. The MICs towards BAL9141 remained at this value through the 50th passage. When isolates of BAL9141-acclimated MR staphylococci were passaged subsequently on antibiotic-free MHA, their MICs towards BAL9141 fell from 32 to 8 mcg/mL within 1–2 transfers and remained at 8 mcg/mL through 30 serial passages in the absence of antibiotic pressure.

Conclusions: The frequency of chromosomal mutations conferring resistance in MR staphylococci follows the order moxifloxacin > linezolid > BAL9141. Emergence of endogenous resistance during a course of treatment with BAL9141 is considered exceedingly unlikely.

Evidence of reserpine-affected mechanism of resistance to tetracycline in Neisseria gonorrhoeae clinical isolates

J. Ruiz, A. Ribera, A. Jurado, F. Marco, M.T. Jiménez de Anta, J. Vila
Barcelona, E

Objective: To analyse the effect of reserpine in the MIC of tetracycline in N. gonorrhoeae.

Methods: Seventeen clinical isolates of N. gonorrhoeae and three strains with a characterised mutation in the mtrCDE operon were analysed. The MIC of tetracycline both in the absence or the presence of reserpine was performed by E-test and read both at 24 and 48 h. The isolates were studied. All tetracycline-resistant strain carried the tetM gene (Dutch variant) whereas none presented the tetA gene. Nor tetA neither tetM genes were present among the intermediate isolates. In all strains the MIC of tetracycline was affected by reserpine. Thus, among intermediate isolates the MIC in presence of reserpine decreased from 4 to >62-fold (from 0.5 to 1 mg/L to <0.016 to 0.25 mg/L), without differences when the plates were read at 24 or 48 h, while those strains carrying tetM gene presented a MIC ranged from 12 to 16 mg/L and 1–1.5 mg/L at 24 h which was determined in the absence and the presence of reserpine, respectively, and of 32 mg/L, and 6–8 mg/mL when read in absence and presence of reserpine at 48 h. The isolates were able to grow on concentrations of reserpine higher than 100 mg/L. The effect of reserpine on mtrCDE system was discharged due to its null effect inhibiting the MIC of erythromycin.

Conclusion: Our results suggest an intrinsic mechanism of resistance to tetracycline in Neisseria gonorrhoeae clinical isolates probably associated with the basal expression of some reserpine inhibitable efflux pump different from mtrCDE.
**P678** Distribution of ermA, ermC, msrA and linA genes in methicillin-resistant staphylococci in the Czech Republic

G. Novotná, J. Janata, J. Spizek

Prague, CZ

Objectives: The aim of this study was to investigate an incidence of different resistance mechanisms to macrolides and lincosamides in methicillin-resistant coagulase-negative staphylococci in the Czech Republic.

Methods: Phenotypes were determined by triple-disc diffusion method. The presence of the genes ermA, ermC (resistance by target site alteration) msrA (efflux resistance) and linA (resistance by antibiotic inactivation) was tested by Southern blot analysis. A bioassay for the detection of lincosamide inactivation mechanism was performed in lincosamide-resistant strains, where none of the resistance genes was detected.

Results: In 99 clinical isolates in vitro resistant to one of erythromycin, lincomycin or clindamycin, triple-disc diffusion method reveals seven different phenotypes corresponding to resistance mechanisms. The resistance was mainly because of the presence of msrA gene, which was detected in 53 strains. Genes ermC and ermA were detected in 42 strains and the linA was detected in 28 strains. In 15 lincosamides-resistant strains no resistance gene was detected. Lincosamides were not inactivated in those strains indicating a new type of resistance different from inactivation.

Conclusion: The dissemination of resistance types differs strongly from the published data. While in other countries cross-resistance to macrolides and lincosamides conferred by ermA and ermC predominates, in the Czech Republic the gene msrA is the most frequent genetic determinant conferring resistance to macrolides only. It follows that one third of macrolide-resistant staphylococci remains lincosamide sensitive. A group of lincosamide-resistant strains with unknown resistance mechanism was newly defined.

**P679** Fluoroquinolone-selected resistance among *Pseudomonas aeruginosa*: impact on susceptibility to imipenem, ertapenem and meropenem

P. Lister, A. Hossain, J.A. Black, E. Smith-Moland, N.D. Hanson

Omaha, USA

Objectives: Fluoroquinolone-selected mutants of *Pseudomonas aeruginosa* can exhibit significant changes in carbapenem susceptibility. However, little is known about which fluoroquinolones are more likely to select for carbapenem resistance. This study assessed the occurrence and mechanism(s) of altered carbapenem susceptibility among *P. aeruginosa* mutants selected with levofloxacin and ciprofloxacin.

Methods: *Pseudomonas aeruginosa* PA01 and a clinical isolate *P. aeruginosa* 164 were the parent strains, and single-step mutants were selected in-agar with 1X–4X MIC of ciprofloxacin and levofloxacin. Confirmed mutants were evaluated for changes in susceptibility to imipenem, ertapenem and meropenem. Mutants with significant changes in carbapenem susceptibility were evaluated for changes in transcriptional expression of four efflux pumps and oprD.

Results: Fifty-six confirmed fluoroquinolone-resistant mutants were selected, with none exhibiting decreases in imipenem susceptibility or changes in expression of mexEF-oprN. In contrast, four mutants demonstrated significantly decreased susceptibility to meropenem and ertapenem. However, these four mutants did not alter their expression of the four efflux pumps or oprD, and they were selected with both fluoroquinolones from both parent strains. The small numbers prevented meaningful comparisons between the two fluoroquinolones. Three additional mutants exhibited hypersusceptibility to imipenem and ertapenem, with associated six- to 11-fold overexpression of mexCD-oprJ. One of these mutants was hypersusceptible to all three carbapenems, and meropenem hypersusceptibility was associated with fourfold decreased mexAB-oprM expression.

Conclusions: Ciprofloxacin and levofloxacin exhibited a propensity to select for both dual resistances to fluoroquinolones and meropenem/ertapenem, as well as carbapenem hypersusceptibility. The mechanism(s) of meropenem and ertapenem resistance remain unknown and warrant further investigation. Furthermore, similar studies using in vitro pharmacodynamic models should be conducted to determine if pharmacokinetically relevant exposure to levofloxacin and ciprofloxacin provides different selection pressures for mutant selection compared the static conditions used in this study.

**P680** High prevalence of macrolide resistance in *Streptococcus mitis* from neutropenic patients

W. Achour, O. Guenni, B. Malbruny, A. Canu, R. Leclercq, A. Ben Hassen

Tunis, TN

Background: The purpose of this study is to determine antibiotic susceptibility of *S. mitis* and to characterise the mechanisms of macrolide resistance.

Material: In 2002, 169 isolates of *S. mitis* were recovered at the Tunisian Bone Marrow Transplant Centre. A total of 120 (70%) strains were nonsusceptible to erythromycin. From this collection, 33 strains were randomly chosen and studied.

Methods: Susceptibility of erythromycin-resistant isolates to antibiotics was tested by agar dilution technique (CA-SFM). Amplification of ermB and mefE genes was carried out by multiplex PCR technique. For strains harbouring ermB genes, amplification of int-Tn genes encoding the integrase of the Tn916–Tn1545 family of conjugative transposones was carried out by PCR technique.

Results and Discussion: The rate of macrolide resistance was impressive and remarkably higher than those in previous studies. In neutropenic cancer patients, exposure to antimicrobial pressure is high and selection of resistant strains may occur. Of the 33 erythromycin-resistant strains studied, 14 were cross resistant to erythromycin, spiramycin and lincomycin, including 10 strains containing ermB genes and four containing a combination of ermB and mefE genes. Fourteen strains displayed M phenotype containing mefE genes. Five strains were apparently susceptible to lincomycin but resistant to spiramycin, four contained ermB genes and one a combination of ermB and mefE genes. In our study and in Canada, ermB and mefE genes are prevalent. However, ermB genes are prevalent in Japan and mefE genes in Spain. The duplicity of genes has not been described in *S. mitis* but already been identified in *S. pneumoniae*, *S. agalactiae*, *S. oralis* and *S. pyogenes*. A correlation was found between lower erythromycin MIC (4–64 mg/L) for isolates carrying mefE and higher level (MIC of 4 to >1024 mg/L) for those carrying ermB genes. The int-Tn gene, was detected in all strains harbouring ermB genes indicating that this gene is disseminated by conjugative transposition to different strains.

Conclusion: This study shows high erythromycin resistance rate among *S. mitis* isolates with prevalence of mefE and ermB genes in our centre, which argue against the clinical usefulness of erythromycin to prevent viridans group streptococcal bacteraemia in neutropenic cancer patients.

**P681** Frequency and stability of mutants selected with fluoroquinolones from *Klebsiella pneumoniae* containing the plasmid-mediated resistance determinant QNR


Seville, E

Objectives: To evaluate the frequency and stability of mutants derived from a porin-deficient *Klebsiella pneumoniae* (Kp) strain KpIMP17 (wild-type strain) containing the Ser33Phe mutation in gyrA and its derived transconjugant KpIMP22 containing plasmid pMG252, which codes for the plasmid-mediated fluoroquinolone-resistant determinant QNR.

Methods: Multiple single-step mutants selected with ciprofloxacin and levofloxacin were recovered from the wild-type KpIMP17 and its derived transconjugants. The frequency of mutants was determined by comparing MIC dilutions between the wild-type and mutants selected. Stability of the mutants was determined by the number of colonies able to grow in the presence of the fluoroquinolone.

Results: The frequency of mutants selected with ciprofloxacin from the wild-type KpIMP17 was 0.1%, and 0.001% from the transconjugant KpIMP22. The frequency of mutants selected with levofloxacin from the wild-type KpIMP17 was 0.01%, and 0.0001% from the transconjugant KpIMP22. The stability of the mutants selected with ciprofloxacin from the wild-type KpIMP17 was 98%, and 99% from the transconjugant KpIMP22. The stability of the mutants selected with levofloxacin from the wild-type KpIMP17 was 99%, and 99% from the transconjugant KpIMP22.

Conclusions: The frequency of mutants selected with ciprofloxacin and levofloxacin from the wild-type KpIMP17 and its derived transconjugant KpIMP22 were low, and the stability of the mutants was high. The results suggest that the plasmid-mediated resistance determinant QNR is stable in the environment of the wild-type KpIMP17 and its derived transconjugant KpIMP22.

Abstracts
(FQ) resistance determinant qnr. MICs (mg/L) of ciprofloxacin (CIP), levofloxacin (LEV) and moxifloxacin (MFX) against KpIMP17 and KpIMP22 were 0.5/0.5/0.25 and 4/4/8, respectively. Moreover, the presence of mutations in the genes gyrA and parC, in these mutants, were studied.

Methods: Mueller–Hinton (MH) agar plates containing 4x MIC of CIP, LEV or MOX were inoculated with bacteria grown *in vivo* (pneumonia model in mouse) or *in vitro* (MH broth), and incubated at 35–37°C for 48 h. Mutants (up to eight from each plate) were subcultured at least twice in MH agar without antibiotics, and MICs of the selecting quinolones were determined by microdilution (NCCLS guidelines). True mutants were defined as those for which the MIC has increased greater than or equal to fourfold with respect to the parental strain. Mutations in the quinolone resistance determining region (QRDR) of the genes gyrA and parC were evaluated by PCR and sequencing.

Results: All mutants selected on agar plates containing FQ showed stable increased resistance to the selecting agent. Frequency of mutation of KpIMP17 were 5 x 10^-7 (in vitro grown bacteria) and 5 x 10^-4 (in vivo grown bacteria), respectively. For KpIMP22, these values were 10^-6 and (in vitro grown bacteria) and 10^-3 to 10^-4 (in vivo grown bacteria), respectively. MICs of FQ against mutants from KpIMP17 were 16 to 64-folds higher than against KpIMP17. In the case of KpIMP22-derived mutants MICs increased eight- to 16-fold. None of the mutants presented any additional mutation in the QRDR of gyrA or parC.

Conclusions: In KpIMP22, pMG252 coding for qnr increases 10^-4 to 16-fold) to FQ. This increase is not caused by additional mutations in the QRDR of gyrA or parC. One hundred per cent of the selected mutants presented stable resistance to fluoroquinolones.

**P682** Resistance mechanisms of fluoroquinolone-resistant clinical isolates of *Streptococcus pneumoniae* collected in Belgium, 1999–2003

S. De Craeye, R. Vanhoof, N. Celio, S. Damée, E. Vanbossuyt, J. Content

Brussels, B

Objectives: Study of point mutations in the quinolone resistance determining regions (QRDR) i.e. gyrA and topoisomerase IV, and the role of pmrA efflux or other efflux pumps.

Methods: Different centres collected clinical strains of *S. pneumoniae* during the winters of 1999 (205), 2001 (314) and 2003 (394). These strains were screened for ciprofloxacin (CIP) resistance by the NCCLS microdilution technique. *Streptococcus pneumoniae* R6 was used as a susceptible reference strain. As positive control the strains F4 and J5 from A. Dickens (UK) were used. The QRDR regions of gyrA, parC and parE of each strain were amplified with the corresponding primers by PCR and then sequenced using the ABI PRISM 3100 Avant Genetic Analyzer. To determine the contribution of efflux to the FQ-resistance, the strains were cultured on blood agar, with or without resepsere. E-test for CIP and moxifloxacin (MOX) were used to determine the MIC values on both media. A PCR for the pmrA-gene and its regulator (mta1) was carried out on each strain.

Results: Resistance caused by QRDR mutations: of a total of 913 clinical isolates, 89 (10.2%) were intermediate resistant (I) and 29 (3.2%) resistant (R) to CIP. All the resistant strains, 38 intermediate and four susceptible (S) strains were sequenced (SIR: 1999: 3-15-3; 2001: 1-5-9; 2003: 0-24-17). Of a total of 71 strains, 16.9% had mutations in gyrA, 0% in gyrB, 29.6% parC and 74.6% parE. Combined mutations: 1999: 38.9% with two mutations and 5.6% with three. For 2001: 28.6, 7.1 and 14.3% with 2, 3 and 4 mutations, respectively. For 2003: 7.7, 10.2 and 2.6%, respectively. Only 30.4% of the multiple mutations resulted in I or R to CIP and 8.7% to MOX.

Efflux resistance: All strains were positive for pmrA. An efflux activity for CIP was found in 46 strains (64.8%) while only one

**P683** Inducible metronidazole resistance in nim-positive clinical *Bacteroides fragilis* group strains

M. Hedberg, H. Fang, C. Edlund

Stockholm, S

Objectives: To survey the incidence of nitroimidazole resistance (nim) genes among 1502 clinical strains of *Bacteroides fragilis* group species originating from a pan-Europe study. Selected nim-positive metronidazole susceptible strains were tested for inducibility of metronidazole resistance.

Methods: All strains were screened for nim-genes by PCR. Determination of specific nim-genes (A-F) was carried out by RFLP. Presence of insertion sequence elements in the nim-positive strains was detected using PCR. Seven randomly chosen nim-positive clinical strains (*B. fragilis, B. thetaiotaomicron* and *B. ovatus*) with low initial MIC values (0.25–8 mg/mL) were assayed for induction using metronidazole E-test strips. Micro colonies were picked within the ellipse area of the E-test strips for three passages, followed by testing of the stability of the resistance by inoculation on blood agar plates without exposure to metronidazole over three additional passages. *Bacteroides fragilis* ATCC 25285 *B. thetaiotaomicron* CCGU 29741 and three clinical strains (*B. fragilis, B. thetaiotaomicron* and *B. ovatus*) all lacking nim-genes, were used as control strains.

Results: Two per cent (n = 30) of the isolates tested harboured nim-genes. These strains belonged to four species, *B. thetaiotaomicron, B. vulgatus* and *B. ovatus* and to the highest extent by the clinically most important species *B. fragilis*. The nimA was detected in 16 of the strains two had nimB, two nimC, seven nimD, two nimE and one nimF. In 23 of the 30 nim-positive strains an IS element possibly involved in regulation of the nim-gene expression was identified. All seven nim-positive strains tested for inducibility of metronidazole resistance could be induced to express high levels of resistance (MIC 64 to ≥256 mg/mL) after three passages on subinhibitory concentrations of metronidazole. After three subsequent passages without metronidazole, the resistance was maintained at the same induced level only in one strain. The other six strains yielded a lower stability of resistance and had lower MIC-values after the three final passages. However, four of these strains still had elevated MIC-values compared with preinduction. In contrary, the five nim-negative control strains demonstrated no significant increase of resistance.

Conclusion: The fact that nim-positive strains were easily induced to high levels of resistance is of great clinical concern and emphasises the importance of acknowledging metronidazole resistance in the clinical setting.

**P684** Diversity of the mechanisms involved in imipenem resistance in *Acinetobacter baumannii*

S. Bourdon, M.L. Joly-Guillou

Angers, F

Objectives: *Acinetobacter baumannii* (Ab), a nosocomial pathogen, is often resistant to multiple antibiotics now including ip. At this time, ip resistance represents about 5% of the strains, when several mechanisms have been described already. These strains are more often involved in hospital outbreaks. The diversity of the mechanisms involved in ip resistance was studied in nine clinical isolates of Ab.
Methods: The minimal inhibitory concentrations (MICs) of ip (MSD Laboratory) were determined by E-test method (AB Diagnostics). The serial dilutions (1–128 mg/L) in Muller–Hinton broth (MHB). Susceptibility testing of seven beta-lactams (amoxicillin, piperacillin, piperacillin-tazobactam, ticarcillin, ticarcillin-clavulanate, ceftriaxone, cefazidime) was performed by the agar diffusion method in accordance with the French committee of antibiogram guidelines. Including cloxacillin in agar medium that is known to inhibit cephalosporinase revealed cephalosporinase activity. The presence of a metallo-enzyme responsible for imipenem resistance was studied, comparing the MICs of ip with and without EDTA. Enzymes probably produced by the strains were characterised by isoelectic-focusing method with precast polyacrylamid gels containing ampholines with pH range 3.5–9.5 (Amersham Bioscience).

Results: MICs of ip, obtained with E-test method, were ranged from 4 to 128 mg/L. Discordance was noted between E-test method and susceptibility testing in MHB: four strains had ip MICs first considered superior or equal to 32 mg/L because of the presence of mutants when MICs in MHB were 4 mg/L. Other beta-lactams also showed a decreased activity. Cephalosporinase activity was involved in imipenem resistance in two strains for which, inhibition of the cephalosporinase activity by cloxacillin not only restore activity to ureidopenicillin, carboxypenicillin or cephalosporins but also to ip. Imipenemase activity was found in four strains: the MIC of ip plus EDTA was lower than the MIC of only ip. Electro-focusing showed one or two enzymes with alkaline pl, consistent with those described for IMP-1 and IMP-2 (pl 8–9). For three strains no enzyme were produced. A two modification of the PLP or an impermeability process. SPM-1 was purified from a recombinant plasmid using a periplasmic preparation followed by a two-stage purification process. SPM-1 was 98% pure, confirmed by SDS-PAGE. Beta-lactamase assays for pH 4.5–5.5 were carried out in acetate buffer, pH 6.0–7.5 in cadoxylate buffer and pH 8.0–9.0 in Tris–HCl buffer. The purified enzyme was transferred to the relevant pH by ultrafiltration and enzyme concentration determined for each preparation. Kinetic parameters (kcat/Km) were determined for penicillin G, cefuroxime, meropenem and nitrocefin at each pH from the initial rates of hydrolysis for different substrate concentrations.

Results: Substrate inhibition is seen for penicillin G and cefuroxime at high substrate concentrations under acidic conditions and is particularly marked for penicillin G. As the pH values increase, substrate inhibition is reduced. No substrate inhibition is observed between pH 6.0 and 7.5 but once again becomes apparent for penicillin G and cefuroxime in the alkaline pH range. No substrate inhibition is seen for cefuroxime or meropenem. The hydrolytic efficiency (kcat/Km ratio) of SPM-1 for all beta-lactams is optimal between pH 6.0 and 6.5. The hydrolytic efficiency of SPM-1 decreases towards the acidic and alkaline pH ranges. Overall, pH has a limited effect on the hydrolytic activity of SPM-1. The greatest effect being seen for meropenem where a 20-fold order of magnitude between pH 4.5 and 6.5, and pH 9.0 and 6.5 is observed.

Conclusion: SPM-1 shows substrate inhibition for penicillin G and cefuroxime at acidic and alkaline pH only, indicating a mechanistic explanation for these effects. The lower hydrolytic efficiency at acid and alkaline pH’s has previously been seen for both IMP-1, with similar orders of magnitude to SPM-1, and BCL where greater orders of magnitude were seen between pH 4.5 and 6.5. In contrast to these enzymes, the effects of pH on the efficiency of SPM-1 are substrate dependant.

P685 Cross-resistance between voriconazole and fluconazole due to the expression of efflux pumps
S. Costa-de-Oliveira, C. Pina-Vaz, A. Gonçalves Rodrigues
Porto, P

Voriconazole (Vor) is a new triazole antifungal agent, structurally related to fluconazole (Flu), with improved potency and spectrum of activity.

Objective: To evaluate the activity of Vor on clinical resistant strains to Flu.

Methods: Thirty-six resistant and 20 susceptible strains to Flu were used. Minimal inhibitory concentration (MIC) to Flu and Vor were determined according the NCCLS protocol M27-A. Flow cytometry analysis of conidia stained with FUN-1 after 1 h incubation with Flu (1) and 2 h with Vor allowed us to determine a staining index (SI), a ratio between the intensity of fluorescence of treated conidia and the control (nontreated conidia). These assays were repeated after incubating the conidia with sodium azide and with four modulators of efflux pumps (verapamil, β-estradiol, progesterone and ibuprofen), at a concentration able to block efflux pumps. High MIC values to Flu, a SI < 1 and a reversion of resistance following the reduction of the energetic pool with sodium azide and with the modulators was suggestive of resistance of efflux pumps (2).

Results: Sixteen resistant strains to Flu showed very high MIC to Vor (>4 μg/mL), and evidence of active efflux. Low MIC values to Vor and no evidence of efflux were found on the remaining resistant strains to Flu, including five C. krusei strains (known as intrinsically resistant to Flu). All susceptible strains to Flu showed low MIC to Vor.

Conclusions: Vor is a more active compound on Candida strains than Flu. The presence of efflux pumps seems to be responsible for the cross resistance between the two antifungals.

References

P686 Substrate inhibition of the Pseudomonas aeruginosa metallo-beta-lactamase, SPM-1, in acidic and alkaline conditions
T.A. Murphy, J. Spencer, R.N. Jones, T.R. Walsh
Bristol, UK; North Liberty, USA

Objectives: To date, there is limited information on the mechanism of hydrolysis for metallo-beta-lactamases (MBLs) with therapeutic beta-lactams. Recently, we described a new-type of MBL, SPM-1, which has unusual kinetics. Accordingly, in order to provide an insight into the mechanism of hydrolysis by SPM-1, a detailed kinetic analysis under different pH conditions was undertaken.

Methods: SPM-1 was purified from a recombinant plasmid using a periplasmic preparation followed by a two-stage purification process. SPM-1 was 98% pure, confirmed by SDS-PAGE. Beta-lactamase assays for pH 4.5–5.5 were carried out in acetate buffer, pH 6.0–7.5 in cadoxylate buffer and pH 8.0–9.0 in Tris–HCl buffer. The purified enzyme was transferred to the relevant pH by ultrafiltration and enzyme concentration determined for each preparation. Kinetic parameters (kcat/Km) were determined for penicillin G, cefuroxime, meropenem and nitrocefin at each pH from the initial rates of hydrolysis for different substrate concentrations.

Results: Substrate inhibition is seen for penicillin G and cefuroxime at high substrate concentrations under acidic conditions and is particularly marked for penicillin G. As the pH values increase, substrate inhibition is reduced. No substrate inhibition is observed between pH 6.0 and 7.5 but once again becomes apparent for penicillin G and cefuroxime in the alkaline pH range. No substrate inhibition is seen for cefuroxime or meropenem. The hydrolytic efficiency (kcat/Km ratio) of SPM-1 for all beta-lactams is optimal between pH 6.0 and 6.5. The hydrolytic efficiency of SPM-1 decreases towards the acidic and alkaline pH ranges. Overall, pH has a limited effect on the hydrolytic activity of SPM-1. The greatest effect being seen for meropenem where a 20-fold order of magnitude between pH 4.5 and 6.5, and pH 9.0 and 6.5 is observed.

Conclusion: SPM-1 shows substrate inhibition for penicillin G and cefuroxime at acidic and alkaline pH only, indicating a mechanistic explanation for these effects. The lower hydrolytic efficiency at acid and alkaline pH’s has previously been seen for both IMP-1, with similar orders of magnitude to SPM-1, and BCL where greater orders of magnitude were seen between pH 4.5 and 6.5. In contrast to these enzymes, the effects of pH on the efficiency of SPM-1 are substrate dependant.

P687 Class 1 and class 2 integrons among ESBL and non-ESBL Escherichia coli isolates recovered from nosocomial and community environments
E. Machado, R. Cantón, J.C. Galán, A. Rollán, L. Peixe, F. Baquero, T.M. Coque
Madrid, Porto, E

Objectives: To analyse the overall prevalence of integrons among E. coli (EC) isolates from different environments, phylogenetic groups and susceptibility profiles.

Methods: We studied 135 isolates: (i) 52 PFGE ESBL (+) clonal types from hospital setting ([16 TEM, nine SHV, 22 CTX-M-9, one CTX-M-14 and four CTX-M-10] (1998–2000); (ii) 43 ESBL (+) isolates causing bacteraemia (1998–2000); and (iii) 40 ESBL (+) isolates from faecal samples of healthy volunteers (HV) living in Madrid (2000–2001). EC phylogenetic groups were determined by a multiplex PCR assay. Class 1 and 2 integrons were detected by PCR, typed by RFLP using AluI and HaeIII as restriction enzymes, respectively, and identified by sequencing (one per RFLP type).

Results: Class 1 were most prevalent than class 2 integrons (48% vs. 14%). Class 1 integrons were more commonly found among ESBL than non-ESBL from blood or HV isolates (69, 40 and 30%) due to the high prevalence of EC containing blaCTX-M-9, which
is located in an In6-like class 1 integron. Class 2 integrons were more common among community than nosocomial isolates [18, 7 and 10% for ESBL−/βIV, blood ESBL− and ESBL+, respectively]. A number of integrons did not contain gene cassettes (nine of 65 of class 1 and one of 19 of class 2). Presence of integrons was more frequent among group D than A, B1 or B2 phylogenetic EC groups (39, 25, 11 and 25% for class 1, and 61, 22, 11, and 6% for class 2). Seven class 1 integron types and three class 2 integron types were detected. Among them, class 1 integrons adaA1 (15 of 56), adaA2 (12 of 56) and dfrA16aadA2 (13 of 56) were the most frequently found. Class 2 integrons were mainly associated to Tn7 (15 of 18).

Conclusions: Integrons are widely distributed among EC isolates from both community and nosocomial environments, being frequently associated to EC group D. The low diversity of integrons found might indicate a wide dissemination of other elements (plasmids or transposons) in which they are located. The capture of genes as blatCTXM-9 from the metagenome community pool might be facilitated by the potentially adaptive functions encoded in its genetic neighbourhood.

Induction of the Acinetobacter baumannii RND efflux pump AdeB with ciprofloxacin and gentamicin

H. Seifert
Cologne, D

Objectives: The Acinetobacter baumannii RND efflux pump AdeB has been shown to confer resistance to gentamicin (GEN) and is involved in reduced sensitivity to the fluoroquinolones. AdeR is the putative regulator of adeB, however, expression levels of the genes encoding the pump and regulator is at present unknown. We investigated the effect of ciprofloxacin (CIP) and GEN on expression of these genes by growing isolates in subinhibitory concentrations of the agents and looked at mRNA expression levels quantitatively by RT real-time PCR.

Methods: The standard A. baumannii laboratory strain ATCC 19606 (CIP/GEN MICs 1 and 4 mg/L, respectively) and a multi-drug resistant clinical isolate SB13 (CIP/GEN MICs 8 and 128 mg/L, respectively) were used for this study. Log-phase cells were challenged with a subinhibitory (1/2) MIC of CIP or GEN and aliquots extracted at time t = 0, 1, 3, 5 and 10 min. RNA was stabilised before extraction and treatment with DNase. cDNA was synthesised and amplicons of the desired size were sequenced by a ABI sequencer. PCR amplicons were obtained for CTX-M-type and OXA-type and often more than one primer set was required. PCR primers used were designed on the conserved regions of the genes and often more than one primer set was required. PCR quantitative real-time PCR was performed in a LightCycler with primers for internal sequences of adeB and adeR and aliquots extracted at time t = 0, ATCC 19606 expressed a higher level of adeB than SB13, 1.7E + 04 vs.1E + 03 transcripts/ng RNA. Levels of adeR were lower than those of adeB by 1E + 02. Ten minutes after addition of CIP there was no difference in adeB levels with ATCC 19606. However, strain SB13 showed a 13-fold increase in adeB transcripts over 10 min. ATCC 19606 adeR levels dropped 80% after 3 min and increased back up to prechallenge levels by 10 min. With SB13, levels of adeR decreased by 50%. A similar but weaker response in adeB expression was seen after challenge with GEN; no change with ATCC 19606 and a sixfold increase with SB13. ATCC 19606 adeR levels decreased by 50% but rose two-fold with SB13.

Conclusions: The gene encoding AdeB is induced in a multidrug resistant clinical isolate by ciprofloxacin and gentamicin but is not induced in a sensitive strain. Total levels of adeB or adeR transcripts may not be an indicator of reduced sensitivity whereas inducibility is. This suggests that other factors such as stability of transcripts may play a greater role in A. baumannii drug resistance.

Detection of carbapenemases in Pseudomonas aeruginosa clinical isolates resistant to imipenem

E. Sevillaño, M. Canduela, A. Umaran, F.E. Calvo, L. Gallego
Bilbao, E

Objectives: The aim of this study was to detect and identify the presence of carbapenemases in clinical isolates of Ps. aeruginosa resistant to imipenem.

Methods: The study included all resistant and intermediate isolates (33 and 15, respectively) obtained at a Hospital from Bilbao (Northern Spain) during 2002. Phenotypic detection was carried out using the Hodge and EDTA tests. Genetic experiments to detect bla-OXA 40, bla-IMP and bla-VIM genes were performed by DNA amplification with the primers P1/OXA: 25'TG-ACLAATACCGGTGTAAG-T3' and P2/OXA: 25'TTCCCTTTAACAGT-GATTACCGGTT-3' and BLAIMP: 25'CTACCGCAAGCAGTCTTT-TG-3' and BLAIMP: 25'ACCATGCCTGACACCAT-3' and VIM-DIA: 1F 5'CGAATTCCGCGAGTGGTGTTTGG-3' and VIM-DIA: 1R 5'AGGTG-TGCCCATTCGCCGACA-3'; VIM-1upv 5'GTCGCAAGTCTGGTTATTCC-TAGCCCCCCAA-3' and VIM 2-upv 5'GATTCATGCGGTGATATC-GG-3'. To detect class 1 integrons, primers 3'CS and 5'CS were used in amplification experiments.

Results: Results with Hodge and EDTA tests were as follows: (a) Hodge+ and EDTA+, nine isolates; (b) Hodge+ and EDTA−, six isolates; and (c) Hodge− and EDTA+, four isolates. The inhibition of the growth of the control strain and the mucoidity of the clinical isolates tested did not allow the correct interpretation of the results in many experiments. All bla-IMP experiments were negative but some fragments were obtained when the bla-VIM genes were amplified. OXA-40 gene was detected in two isolates (both were Hodge+ and EDTA−), which also bore integrons of 1500 and 850 bp.

Conclusions: Phenotypic methods did not allow the correct detection of carbapenemases in the majority of the Ps. aeruginosa isolates tested. Genetic experiments showed the presence of OXA-40 gene in two isolates resistant to imipenem, enzyme firstly identified in A. baumannii isolates from the same hospital.

Nonenzyme-mediated imipenem resistance in a clinical isolate of Citrobacter freundii from Trondheim, Norway

M. Castanheira, B. Haldorsen, A. Sundsfjord, J. Jacobsen, J. Afset, T. Walsh
Bristol, UK; Tromso, Trondheim, N

Objectives: Citrobacter freundii K2-23 was recovered from a surgical site infection of a 59-year-old kidney-transplanted male during treatment with imipenem and was shown to possess high-level resistance to third and fourth generation cephalosporins as well as imipenem (MIC 8 mg/L). Initial phenotypic screening for extended spectrum beta-lactamases (BL) using Etest (AB biodisk) showed that the resistance was not sensitive to clavulanic acid. Therefore, we molecularly investigated the mechanism of this broad-spectrum beta-lactam resistance.

Methods: Genetically screening for extended spectrum beta-lactamases (BL) using Etest (AB biodisk) showed that the resistance was not sensitive to clavulanic acid. Therefore, we molecularly investigated the mechanism of this broad-spectrum beta-lactam resistance. PCR amplicons of the desired size were sequenced by a ABI sequencer. Crude cell extracts for BL hydrolytic activity were prepared by sonication and centrifugation. Assays were carried out in a Perkin Elmer Lambda 35 spectrophotometer with a range of β-lactams. Isoelectric focusing (IEF) was determined using a Novex (Invitrogen, CA, USA) vertical gel system. Outermembrane preparations were carried out on K2-23 and 5 C. freundii β-lactam sensitive strains by standard extraction methods and visualised using SDS-PAGE (Invitrogen). N-terminal protein sequencing was performed on those outermembrane differentially expressed in K2-23.

Results: PCR amplicons were obtained for CTX-M-type and CMY-type BL genes but only the sequence of the latter was creditable and that was of the nascent C. freundii AmpC. IEF revealed
only a single weak band with a pl of 8.0 and the BL assays demonstrated no significant hydrolysis with cefotaxime, cefazidime, cephaloridine, or imipenem. SDS-PAGE of C. freundii isolates revealed that only K23 had a band missing at a molecular weight of 43 kDa. Another protein of a similar size also appeared to be weakly expressed. Preliminary sequencing of the N-terminus of this and revealed the amino acid sequence of AEYKN. These sequencing residues showed similarity with the ompK35 of Klebsiella pneumoniae indicating that the protein missing in strain K2-23 is likely to be an OMP-like outer membrane protein.

**Conclusion:** The data arising from these studies would indicate that key beta-lactam resistant determinant from C. freundii K2-23 is not over-production of the nascent AmpC but down-regulation in one or more of its outer membrane proteins.

**P691** Extended spectrum beta-lactamase (ESBL) producers among enterobacteriaceae from patients in Bulgarian hospitals

Sofia, Pleven, Stara Zagora, BG

**Objective:** To preliminary characterise the main types of ESBLs among Bulgarian Enterobacteriaceae strains and to determine their rate of resistance.

**Methods:** A total of 427 Enterobacteriaceae (K. pneumoniae – 238, K. oxytoca – six, E. coli – 129, C. freundii – five, E. cloacae – eight, E. aerogenes – seven, Proteus – five, Serratia – three, Salmonella – one) strains identified as ESBL producers were collected from seven medical centres in Sofia, Pleven and Stara Zagora during 1996–2003. They were confirmed by phenotypical tests (DDS, NCCLS method). MICs were determined by an agar dilution technique (NCCLS, 2002). Conjugal plasmid transfer was performed, followed by an isoelectric focusing according to Mathew/Bauernfeind. The hydrolytic activity of the bands was proved by Bioassay (Bauernfeind).

**Results:** The rate of resistance was: amoxicillin/clavulanate – 86%, cefotaxime – 85%, cefotaxime – 95%, ceftriaxone – 94%, aztreonam – 74%, cefoxitin – 14%, cefditoren – 19%, cefepime – 37%, imipenem – 0%, tobramycin – 95%, gentamicin – 84%, amikacin – 41%, ciprofloxacin – 56%, tetracycline – 86%, co-trimoxazole – 53% chloramphenicol – 58%. MIC of cefazidime (CAZ) ranged 1 to >512 mg/L and of cefotaxime (CTX) – 2–512 mg/L. The strains were divided into two main groups: the first one: MICCAZ > MICCTX – 251 strains and the second: MICCAZ < MICCTX – 161. In all strains sulbactam in combination with CAZ and CTX resistance was transferable in 91 from 100 mating experiments. IEF analysis of 91 strains showed the presence of three clusters. One cluster (42 strains Klebsiella and 4 E. cloacae) presents CAZ hydrolytic bands with isoelectric points (pl) 8.2 (SHV type). The second cluster (18 strains Klebsiella, two C. freundii, 1 E. coli) demonstrated enzymes focusing at pl 6.3, which suggests TEM type. The strains with MICCAZ > MICCAZ – (11 K. pneumoniae, 12 E. coli, 1 S. marcescens) showed CTX hydrolytic bands with pl 8.4 or 8.8 (CTX-M type). The pl data were from transconjugants and wild type strains.

**Conclusion:** The SHV type was predominant among ESBLs Enterobacteriaceae producers in Bulgarian hospitals. TEM type was proved in one hospital. CTX-M types ESBLs have increased from 2001 after their first detection and become emerging problem in Bulgaria. All strains were highly polysensitive.

**P692** Temporal trends of Streptococcus pneumoniae isolates with dual resistance to beta-lactam and macrolide antibiotics

J. Fuller, J. Powis, P. Tang, K. Green, D. Low and the Canadian Bacterial Surveillance Network

**Objectives:** Streptococcus pneumoniae (SPN) is a leading cause of pneumonia, meningitis, and otitis media. The evolution of antimicrobial resistance and concomitant clinical failures has become a global concern. Recent mathematical modelling predicted that the resistance to both penicillin and erythromycin antibiotics will increase significantly faster than resistance to either agent alone. We examined the longitudinal changes in dual resistance to these agents in clinical isolates collected by an ongoing Canadian surveillance programme.

**Method:** The Canadian Bacterial Surveillance Network (CBSN) is comprised of private and hospital-affiliated laboratories from across Canada. Laboratories were asked to collect a defined number of consecutive clinical isolates followed by all sterile site isolates of SPN. In vitro susceptibility testing was performed by broth microdilution using NCCLS guidelines.

**Results:** In our population, penicillin-nonsusceptibility and erythromycin resistance in SPN is presently 16.25% and 16.46%, respectively. The proportion of these isolates dually resistant to these antibiotics has slowly increased at a rate of approximately 1% per year since 1993. Analysis of the subpopulation of penicillin-nonsusceptible isolates revealed that erythromycin resistance has increased dramatically (rate approximately 5.5% per year). Conversely, among erythromycin-resistant isolates, the acquisition of penicillin nonsusceptibility occurred at a much slower rate (rate approximately 1.3% per year).

**Conclusion:** Ten years of surveillance of clinical isolates in Canada indicates that the increase in penicillin and erythromycin dual resistance in SPN is largely attributed to an increased propensity for penicillin-nonsusceptible isolates to acquire resistance to macrolide antibiotics. At the present rates of increase, all penicillin-nonsusceptible isolates will be erythromycin-resistant within several years, at which point additional increases in dually resistant isolates will be limited by the increase of SPN isolates resistant to penicillin alone.

**P693** CdeA of Clostridium difficile, a new multidrug efflux transporter of the MATE family

J. Tankovic, L. Dredi, F. Barbut, D. Decré, J.-C. Petit
Paris, F

**Introduction:** Clostridium difficile is a Gram-positive spore-forming anaerobic bacterium that is the major cause of nosocomial diarrhea. Its habitat is the human gastrointestinal tract, an environment rich in lipophilic inhibitors such as bile salts and fatty acids. Furthermore, this species is intrinsically less susceptible to antibiotics, notably to beta-lactams, fluoroquinolones, chloramphenicol, and lincosamides, than the other clostridia. It is likely that active efflux may be a crucial mechanism implicated in survival in the gastrointestinal tract and intrinsic antibiotic resistance of this species.

**Methods:** We tried to clone in Escherichia coli and Clostridium perfringens an efflux gene from total DNA of C. difficile strain 714 responsible for norfloxacin or ethidium bromide resistance. Ethidium bromide accumulation was measured by a whole-cell fluorometric assay in the presence or in the absence of sodium ions.

**Results:** We cloned a gene, cdeA, that made E. coli and C. perfringens resistant to ethidium bromide and acriflavin but had no effect on the susceptibility of the hosts to the antibiotics tested: norfloxacin, ciprofloxacin, gentamicin, erythromycin, tetracyclin, and chloramphenicol. It caused ethidium bromide energy-dependent efflux in whole cells of E. coli. The deduced protein was homologous to the protein sequences of known efflux pumps from the third cluster (the so-called DinF branch) of the multidrug and toxic compound extrusion (MATE) family. Efflux activity was stimulated by addition of Na+ ions suggesting that CdeA, like other pumps of the MATE family, is a Na+-coupled efflux pump.

**Conclusion:** CdeA is the first multidrug efflux transporter of the MATE family identified in gram-positive bacteria. It did not cause significant resistance to antibiotics when cloned in E. coli and C. perfringens. Gene inactivation would be helpful to appreciate its exact role in C. difficile but this experiment could not be performed due to incapacity of the transformation in that species.
**P694** First appearance of the CfiA metallo-beta-lactamase gene in Norway and its association with a novel insertion element

M. Toleman, A. Sundsfjord, A. Onken, B. Haldorsen, T. Walsh
Bristol, UK; Tromsø, Oslo, N

Objectives: A Bacteroides fragilis blood culture isolate (K2-28) was recovered from a 61-year-old man with severe general atherosclerosis, 7 days after amputation of both lower extremities and during treatment with meropenem. K2-28 was shown to possess high-level resistance (MIC > 128 mg/L) to carbapenems and most other beta-lactam antibiotics. This resistant pattern, indicative of a metallo-beta-lactamase (MBL), is unique in Norway where resistance to antibiotics is extremely low. Accordingly, we molecularly investigated the precise mechanism of this broad-spectrum beta-lactam resistance.

Methods: Production of a MBL was verified using the Etest MBL strips (AB biodisk, Solna, Sweden) plated onto Columbia blood agar with 5% blood. Genetically screening for the B. fragilis MBL gene was undertaken using primers based on the cfiA gene using standard PCR techniques. Primers were also designed against upstream insertion elements known to provide a strong promoter for expression of the cfiA gene. Degenerate primers were designed on the conserved regions of the genes and often more than one primer set was required. PCR amplicons of the desired size were sequenced. The entire sequence of the gene and the insertion element was obtained by the ‘primer-walking’ strategy.

Results: E-test results confirmed the presence of a functional MBL with a reduction in MIC from 256 to 3 mg/L in the presence of EDTA. Sequence analysis of the cfiA revealed it was 100% identical to previously described sequences, presenting with the principal zinc binding sequence of HWHGD. Sequencing of the upstream region of cfiA revealed a novel insertion sequence (IS) element, being most similar (94% identity) to IS612 recently described from Japan. These data designates the element within the IS4 family. The element had a typical imperfect terminal inverted repeat sequences at the distal ends of the IS element. This IS614-like element demonstrates regions homologous to the –10 and –35 promoter regions of the nascent cfiA gene. However, the –10 was most similar to that of IS613 (100%) and the –35 to IS612 (100%) indicating the plasticity of these regions.

Conclusion: This is the first report of a Bacteroides spp. possessing an active cfiA gene within Scandinavia and the unique insertion sequences associated with this gene testifies to the plasticity of these genetic resistant elements.

**P695** Molecular analysis of linezolid resistance in Staphylococcus aureus

T.A. Wichelhaus, S. Besier, V. Brade, A. Ludwig
Frankfurt am Main, D

Objectives: Linezolid, the first oxazolidinone in clinical use, is effective in the treatment of severe staphylococcal infections. Resistance of S. aureus to linezolid recently has been shown to be associated with point mutations within the domain V region of the 23S rRNA gene, which is present in multiple copies in the genome of this pathogen. Here we studied whether there is a correlation between the level of linezolid resistance in S. aureus and the presence of mutations in the different copies of the 23S RNA gene.

Methods: Linezolid-susceptible parental strain was subjected to daily serial subcultivation in Mueller-Hinton in containing increasing linezolid concentrations for 40 days. The minimal inhibitory concentration (MIC) of linezolid was determined for the parental strain and for descendents by the agar dilution method. Domain V of the different 23S rRNA gene copies of the parental strain and of derivative isolates were amplified by PCR and sequenced.

Results: The linezolid MIC was 2 mg/L of the parental strain and increased gradually to 128 mg/L within 40 days of linezolid selection. Derivatives with elevated linezolid MICs revealed the substitution G2447T in the 23S rRNA gene. The level of linezolid resistance within derivatives correlated with the number of the 23S rRNA gene copies carrying this mutation.

Conclusions: The data suggest that the level of linezolid resistance in S. aureus is dependent on the number of the 23S rRNA gene copies carrying a specific resistance-associated point mutation. As linezolid-resistant S. aureus mutants can be selected in vitro under selection pressure, a cautious and judicious use of linezolid in vivo is strongly recommended in order to maintain the valuable status of this class of antibiotic agents.

**P696** Characterisation of the first CTX-M14-producing Salmonella enterica serotype Enteritidis isolate

L. Romero, L. López, L. Martínez-Martínez, B. Guerra, J.R. Hernández, A. Pascual
Seville, E; Berlin, D

Introduction: We describe the first infection caused by a CTX-M-14-producing Salmonella enterica serotype Enteritidis isolate. Although reports of ESBL associated with Salmonella are relatively rare, the number of reported cases in this organism has been increasing in recent years in numerous countries.

Materials and Methods: Clinical samples were processed according routine and identification was carried out with VITEK 2 system. The screening of ESBL was achieved by disk diffusion with discs of third cephalosporins with and without clavulanic acid according to manufacturer’s instructions. Cefotaxime-resistant transconjugants were obtained. MICs were evaluated for the isolates and their transconjugants by microdilution in broth according to NCCLS and 4 mg/mL fixed concentration of beta-lactamase-inhibitor was added to third generation cephalosporins, aztreonam and piperacillin. PGFE was performed for Salmonella strains using XbaI endonuclease. Isoelectrofocusing was realised with Phast System. bla CTX-M gene was amplified from all cefotaxime-resistant isolates and their transconjugants and sequence was analysed.

Results: An susceptible Salmonella isolate (no. 1) was isolated from a blood culture from a 10-month-old girl admitted in Paediatric wards Then, two control faeces samples from the same patient yielded a cefotaxime-resistant Salmonella (isolate nos 2 and 3) and a cefotaxime-resistant Escherichia coli (isolate no. 4). The three resistant isolates and their transconjugants showed synergy with clavulanic acid compatible with ESBL phenotype and they also produced beta-lactamase with a pI of 8.0, which suggests a CTX-M9-type enzyme. It was possible to amplify the blaCTX-M fragment and the analysis of deduced amino acid bla sequences showed that the corresponding gene encoded the CTX-M-14 beta-lactamase. The three Salmonella isolates belonged to serogroup D, serotype Enteritidis, phage type PT1 and showed identical PGFE patterns except for one extra band of 79 kb in the cefotaxime-resistant isolates 2 and 3.

Conclusions: Our findings provide the first evidence of CTX-M-14 associated with Salmonella enterica serotype Enteritidis. The molecular epidemiologic study showed that the child was infected with the same Salmonella strain, which developed ESBL phenotype during the episode. The finding of an intestinal E. coli strain with the same ESBL indicates a possible intestinal bla gene transmission between both species of Enterobacteriaceae.

**P697** OmpK37 and reduced susceptibility to imipenem in Klebsiella pneumoniae

H. Segal, B.G. Elisha
Cape Town, ZA

Objectives: The aim of the study was to investigate the reduced carbapenem susceptibilities in clinical isolates of Klebsiella pneumoniae.
Methods: K. pneumoniae strains were initially isolated from blood obtained from two patients at Groote Schuur Hospital, Cape Town. The isolates were susceptible to amoxicillin-clavulanate, imipenem (MIC, 0.125 mg/L), and meropenem (MIC, 0.032 mg/L) but resistant to amoxicillin, cefuroxime, cefotaxime, cefazidime, ceftipime, and cefoxitin. Extended spectrum beta-lactamase activity was detected using the double disc technique. Both patients were treated with meropenem. Subsequently, K. pneumoniae was isolated from faeces from the same two patients. These isolates displayed a similar susceptibility profile, except that they were resistant to co-amoxiclav, and had reduced susceptibilities to meropenem (MIC, 3–6 mg/L) and imipenem (MIC, 1–2 mg/L). The relatedness of the strains was investigated using pulsed field gel electrophoresis (PFGE). E-test strips were used to detect the presence of metallo-beta-lactamases (MBLs) with activity against carbapenems. To study the porin content of the strains, outer membrane proteins (OMPs) were extracted, separated by SDS-PAGE and, where necessary, identified by Maldi-TOF analysis.

Results: Each of the strains had an identical PFGE profile, showing that the strains were related. No MBLs were detected in the strains with reduced susceptibility to the carbapenems. A comparison of the porin profiles identified a 40 kDa protein in the strains with reduced carbapenem susceptibilities, which was not present in the susceptible isolates. Maldi-TOF analysis indicated that the protein was most similar to the E. coli OmpG. The most likely counterpart of OmpG in K. pneumoniae is OmpK37, the absence of which probably accounts for the reduced susceptibility to meropenem and imipenem. OmpK35 and OmpK36 were not observed in any of the preparations. This finding is consistent with the cefoxitin resistance phenotype of all four strains.

Conclusion: Meropenem susceptible and resistant K. pneumoniae were isolated from blood and faeces, respectively, from two patients. Reduced susceptibility to meropenem and imipenem was associated with the loss of OmpK37. Both patients were treated with meropenem, which may have influenced the selection of OmpK37-deficient strains.

P698 In vitro sequential development of gene mutations in fluoroquinolone-resistant salmonellae
J.M. Ling, Y. Jin
Hong Kong, HK

Objectives: (1) To investigate the development of fluoroquinolone-resistant salmonellae in vitro; (2) to characterise the sequential accumulation of target gene mutations leading to high-level fluoroquinolone-resistance.

Methods: Fluoroquinolone-resistant mutants of Salmonella enterica serotype Typhimurium and S. Hadar were obtained by plating sensitive strains on agar containing increasing twofold concentrations of ciprofloxacin, gemifloxacin, levofloxacin, moxifloxacin, norfloxacin or ofloxacin. Mutants thus obtained were again subjected to selection to obtain nth-step mutants (where n stands for the number of times the mutants had been subjected to selection). Susceptibilities of the mutants were determined and mutations of target genes were detected by multiplex PCR amplimer conformation analysis and confirmed by direct DNA sequencing.

Results: First-step mutants of S. Typhimurium usually harboured a gyrA mutation (Asp87—Asn/Gly/His/Tyr), the MICs were 4–16 times higher than those for the parent strains and were below the sensitive breakpoint of fluoroquinolones. One mutant harboured a gyrB (Glu466—Glu) mutation and the MICs were 2–8 times lower than those for the parent strain. Only three second-step mutants harboured an additional gyrA mutation (Ser83—Phe) while two 2nd-step mutants from the parent with a gyrB mutation harboured a gyrA mutation (Asp87—Gly or Ser83—Phe). Mutations in parC (Glu51—Asp, Gly78—Asp, Ser80—Arg/Le or Glu84—Gly/Lys) were first observed in third-step mutants. Although most fourth-step mutants were resistant to high concentrations of fluoroquinolones as were third-step mutants, no additional mutation was found. Fewer mutations were detected in mutants of S. Hadar: First-step mutants harboured a gyrA mutation (Asp87—Asn/Gly/Tyr or Ser83—Phe) and a gyrB (Glu466—Asp) mutation. No additional mutation was found in second-step mutants although MICs were higher than those for the parents.

Conclusions: Low-level fluoroquinolone-resistant salmonellae which evolved by development of a gyrA mutation became high-level resistant by developing additional gyrA and parC mutations. Such sequential development of fluoroquinolone-resistance varied among Salmonella serotypes.
Molecular bacteriology: detection and identification of agents

P700  Pyrosequencing at the Health Protection Agency, UK

C. Arnold, S.E. Gharbia
London, UK

One of the remits in the Genomics, Proteomics and Bioinformatics Unit (GPBU) of the UK Health Protection Agency is to utilise genomics in infection control by the use of accelerated genomic information and enhanced biotechnological methods to streamline the traditional approaches used in classical molecular genetics to instigate advances in Public Health Protection. Alteration in gene expression patterns or DNA sequence can have profound effects on biological functions and pathological processes. In GPBU, systems are developed for identifying genetic variations and gene expression and our key developmental objectives are to reformat sequence-based typing into fast, high throughput systems, to explore microbial genome sequences for VNTRs and SNP targets as tools for high resolution strain comparison and to provide tools for molecular-based surveillance of high priority infections. To this end, Pyrosequencing technology has been evaluated for its utility in SNP characterisation (including antibiotic/drug resistance) and genotyping in Public Health Microbiology in the UK. An overview of applications will be presented including: HIV-1 – drug resistance – subtyping Hepatitis C virus – genotyping – minor genotype analysis Antibiotic resistance – Neisseria gonorrhoeae – Salmonella enterica – Mycobacterium tuberculosis Mycobacterium tuberculosis – screening from sputum samples – VNTR typing – H antigen typing of Salmonella

P701  Universal 16S rDNA PCR and sequencing in the diagnosis of infective endocarditis directly from heart valve tissue

Madrid, Spain

Background: The microbiological diagnosis of infective endocarditis (IE) is based on positive culture of heart valve tissue or blood culture but cultures remain negative when IE is caused by fastidious micro-organisms or antimicrobial treatment is started before cultures are obtained.

Objective: To evaluate the usefulness of a universal 16S rDNA PCR method followed by direct sequencing in heart valve tissue for IE diagnosis in the routine of a clinical microbiology laboratory, compared with traditional heart valve culture (HVC) and blood culture (BC).

Methods: Heart valves received for culture over a ten-month period were studied by 16S rDNA PCR with primers PSL and P13P. Positive samples were subsequently sequenced for identification. HVC were cultured by conventional methods. BCs were performed by the BACTEC 9240 system. Sensitivity of the assay was assessed by obtaining DNA from 10-fold dilutions of Streptococcus oralis. After molecular analysis, clinical records of patients and results of conventional cultures were consulted.

Results: Twenty-four samples of HV (24 patients) were studied. In 10 patients IE was clinically rejected and their valves were included in the study as negative controls. Their HVC, BCs and PCR were negative. The remaining 14 cases had either proven (13) or possible (1) IE. Overall, BCs were positive in 11 patients but HVC remained positive at the moment of resection in only four patients. Of the 14 cases, 12 were microbiologically documented by conventional cultures. PCR was positive in the 12 confirmed cases. Micro-organisms identified by PCR matched those cultured by conventional cultures except in one case in which the valve was inadequately remitted to the microbiology laboratory. In the 2 cases with IE and with no micro-organisms demonstrated by conventional cultures, PCR was also negative. The median time of analysis to a PCR result was 1 day and to a sequence and bacterial identification in PCR-positive samples, 3 days. The analytical sensitivity of this assay was 100 CFU/mL.

Conclusions: Universal 16S rDNA PCR followed by sequencing applied to resected heart valves seems to be a reliable test for diagnosis of IE. Our study suggests its applicability to patients with conventional negative microbiology, mainly those studied during the course of antimicrobial therapy.

P702  Clinical importance of PCR diagnosis in bacterial meningitis and sepsis

S. Plísek, L. Plískova, V. Dostál, V. Stepanova, E. Pozlerova, J. Blazkova
Hradec Kralove, CZ

Objectives: The importance of the detection of bacterial DNA by PCR in laboratory diagnosis of invasive bacterial infections has been increasing. PCR represents the rapid, sensitive and specific method, which provides the possibility to detect bacterial DNA in cases of application of few doses of ATB therapy. In such cases the classical microbiology methods often fail.

Methods and Materials: After DNA extraction PCR for determination of N. meningitidis, Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus and Listeria monocytogenes was used. In 2001–2003, 92 patients (CSF, blood and urine) with suspected invasive bacterial infection (sepsis or bacterial meningitis) were examined for the presence of mentioned bacterial DNA in case of negative microscopy and agglutination examinations.

Results: Invasive meningococcal disease (IMD) was diagnosed by clinical and laboratory methods in 20 of 60 examined patients. In 10 patients N. meningitidis was detected by classical microbiology methods, in 10 patients N. meningitidis DNA was found only by PCR (7x in CSF; 3x both in CSF and blood; 1 only in urine). In 50% of patients with the diagnosis of IMD the presence of N. meningitidis was not detected by classical microbiology methods, only by PCR. Of the rest of 72 patients bacterial DNA was detected in 25 patients (4x Haemophilus influenzae, 15x Streptococcus pneumoniae, 6x Listeria monocytogenes and 3x Staphylococcus aureus).

Conclusion: The decline of positive findings of bacterial DNA by conventional methods (microscopy, agglutination, cultivation) may be caused by the administration of the first doses of ATB in the onset of non-specific symptoms of disease. The detection by very sensitive PCR enables to detect DNA not only from live but also disinTEGRATED bacteria. The significance of PCR diagnosis in these cases has increased very much because of its speed, sensitivity and specificity.

P703  A new scoring method for molecular diagnostic kit users

H. Staines, P. Wallace, R. Pogathota, E. Buultjens
Dundee, Glasgow, UK

Objectives: A proposed score provides informative, intuitively appealing feedback to molecular diagnostic kit users. The colour-coded score [green (= highly satisfactory) to red (highly
unsatisfactory) is presented for individual samples and overall performance. Simple graphical presentations compare performances within and between amplification methods.

Methods: The score uses the log copies/mL reported from quantitative assays. Analysis of Variance using the amplification method for each panel sample is used to: check for normality, identify outliers and estimate the standard deviation. The difference between the participant’s result and target value is divided by the standard deviation. A participant’s score for an individual sample is defined as the integer part of the absolute value of this value (max 3). Scores of 0, 1, 2 and 3 are colour-coded green, yellow, amber and red, respectively, for participant feedback. Assuming independence, normality and no amplification method effect, the distribution of scores are approximately 68, 27, 4.5 and 0.5%. The overall performance for an individual participant is the sum of the individual samples scores. Monte Carlo methods determine the probability distribution of the overall performance score. These scores are colour coded from green to red in proportions approximately equal to individual samples (e.g. 68% green). Feedback for a participant’s overall performance is the same as for individual samples.

Results: Scores have been found for the 2002 QCMD panels for hepatitis B, C and HIV. For each sample log results were normally distributed within each amplification method with few outliers detected. Scores frequencies varied amongst individual samples within each panel. For many samples, the proportion of scores were not consistent with that predicted by independent observations from a normal distribution. This was confirmed by ANOVA that showed significant differences in the mean log observations amongst amplification methods. Graphical representations showed pronounced differences amongst the methods within and across panels.

Conclusions: The new scoring scheme is based on well-known statistical properties and techniques. The colour coded performance scores are readily interpretable and simple graphical output also allows participants to gauge their performance against other users of the same method and to help choose amongst methods.

P704 Application of TaqMan probes in end-point fluorimetry for detection of pathogenic bacteria by polymerase chain reaction

K. Oravcová, E. Kaclíková, T. Kuchta
Bratislava, SK

Objectives: 5’-Nuclease polymerase chain reaction in a conventional thermal cycler and a subsequent fluorescence measurement in a 96 well fluorimeter were developed and successfully carried out in flat-bottom microtubes.

Methods: Complete reaction system was simply transferred from real-time PCR to new instrumental conditions. Specific primers and specific TaqMan probes (fluorescent dye 6-FAM, quenching dye TAMRA) for Salmonella sp. and E. coli strains were used. When finishing the PCR, the fluorescence was measured in end-point mode in fluorescence reader equipped with an excitation filter with a pass maximum of 492 nm and an emission filter with a pass maximum of 520 nm from the bottom orientation. To define the positivity threshold, three negative control samples (containing no DNA template) were used. Mean value and the standard deviation (SD) were calculated and the positivity threshold was set to (mean + 2 SD).

Results: In these conditions, consistent results were obtained when PCR was done with purified Salmonella enteritidis and E. coli DNA or with culture lysates. When lysates of series of decimally diluted cultures were analysed a detection limit of 10^4 CFU/mL was determined, the same sensitivity as with real-time PCR and the same or one order of magnitude higher than with the gel electrophoresis were determined.

Conclusion: The method proved to be a fast and contamination-free alternative to gel electrophoresis and an inexpensive alternative to real-time PCR.

P705 Usefulness of the MicroSeq 500 16S rDNA-based method for identification of bacterial isolates unidentified by commercial automated systems

C. Fontana, M. Favaro, M. Pelliccioni, E.S. Pistoia, C. Favalli
Rome, I

Objective: Reliable automated identification and susceptibility testing of clinically relevant bacteria is very important for routine microbiology laboratories, thus improving patients’ care. Examples of automated identification systems are: the Phoenix (Becton&Dickinson) and the Vitek2BioMerieux). Both systems claim to provide accurate and rapid identification as well as susceptibility results with substantially easy workflow. However, more and more frequently microbiologists isolate ‘difficult’ strains, particularly those isolates from patients who have undergone repetitive antibiotic treatment and which consequently exhibit biochemical characteristics that do not fit into patterns of any known genus and species. The identification of these pathogens is normally failed by automated systems. An alternative could be the genetic identification: the latter based on the 16S rDNA sequencing and analysis, as 16S is highly conserved within a species and among species of the same genus. Aim of the present work is to evaluate the possible use of the MicroSeq (Applera), sequencing 16S rDNA, as the new gold standard for identification of isolates whose identification is not obtained or results inadequate with conventional systems.

Methods: In the present work we have analysed 83 difficult isolates: 25 Gram+ and 58 Gram– strains. The isolates were contemporaneously identified by using both automated systems such as: Vitek2 and Phoenix. The phenotypic identifications were confirmed by genetic analysis performed by using MicroSeq system.

Results: The results have shown that the phenotypic identification provided by Vitek2 and Phoenix was remarkably similar, in particular: 74% of the identification obtained for the Gram–, and 80% for the Gram+ resulted to be in concordance with both systems, and was also concordant with genetic characterisation. The exception was represented by 15 Gram– and nine Gram+ isolates whose phenotypic identifications were contrasting or not conclusive. For these strains the use of MicroSeq demonstrated to be fundamental to achieve the species identification.

Conclusion: The use in clinical microbiology of MicroSeq, particularly for those strains with ambiguous biochemical profiles (including slow-growing strains), results to be helpful allowing to better achieve identifications as compared with conventional systems. Moreover, the system appears easy to use and cost effective, making MicroSeq reasonably applicable also in clinical laboratory.

P706 Facts, feasibility and future of bacterial load of bloodstream infections

Amsterdam, Groningen, NL

Real-time PCR is becoming more and more a standard technique in many laboratories for the detection of micro-organisms in clinical samples. This closed PCR system enables rapid clinical molecular diagnostics in the medical microbiological laboratory. In blood samples, pathogen-specific PCR is of additional value to conventional blood culture in case of prior use of antibiotics or when slow-growing organisms like mycobacteria are involved. Although there are many reports on pathogen-specific applications, a more general approach with broad-range detection based on the 16S gene has only been described a few times, with interesting results. None of these broad-range studies has been described so far with real-time PCR that enables quantification of initial DNA with deduction of a ‘bacterial load’ that can possibly be used for monitoring antimicrobial therapy. In this study real- time PCR (TaqMan 7000) was combined with an automated DNA isolation robot (MagNA Pure LC) in order to standardise en optimise DNA isolation and subsequent amplification. Ninety
blood samples from patients with fever were evaluated. PCR showed amplification of 10/13 samples with positive concurrent blood cultures, the three that remained negative in PCR all grew coagulase-negative staphylococci. Other PCR results were in concordance with blood culture outcome and/or clinical data. However, sequence-analysis showed cross-contamination with Burkholderia species DNA that could be traced to buffers of the DNA isolation kit, although a second (mixed) sequence could be recognised. The concordance of clinical data and PCR results may be explained by the presence of blood components related to infection that function as PCR-enhancer, like serum-proteins. In conclusion, real-time PCR seems a promising addition to the spectrum of diagnostic possibilities for bloodstream infections, especially in case of pathogen-specific detection of bacteria. To develop a real-time broad-range PCR for detection of bloodstream infections with determination of a ‘bacterial load’, attention should be given to optimisation of DNA isolation and DNA-free nucleic acid isolation kits and PCR reagents.

**P707** Broad-range polymerase chain reaction based bacterial and fungal molecular assays improve the diagnostic ability of the Duke scheme in suspected cases of infective endocarditis

M. Slany, M. Grijalva, J. Cerny, T. Freiberger
Brno, CZ

**Objectives:** The diagnosis of infective endocarditis (IE) may become somewhat difficult, particularly when blood cultures are negative or in cases in which the course of the disease is insidious. The Duke scheme has improved the diagnostic ability of clinicians dealing with suspected cases of IE. However, reaching a precise microbiological diagnosis may be a challenge in clinical situations such as those mentioned above. Molecular approaches have helped on this task and a few reports have been published regarding the clinical validation of these methods and techniques in IE settings. We hypothesised that the molecular microbiological diagnosis of IE may improve the diagnostic ability of the Duke scheme.

**Methods:** To test this hypothesis, we defined a group of IE patients in which microbiology results from traditional methods (blood and valve cultures) as well as from molecular testing (broad-range PCR-based 16S rDNA bacterial detection and 28S-5.8S rDNA fungal detection assays from valve samples) were available. Then we compared the diagnostic performance (in terms of Duke classification) of the Duke criteria alone and after the inclusion of microbiological molecular data. Forty-nine suspected IE patients were included in the study.

**Results:** The Duke scheme alone classified the patients as follows: 25 (51%) as ‘definite IE’, 22 (45%) as ‘possible IE’, and two (4%) as ‘rejected IE’. When we applied the molecular microbiological data as an additional major criterion, 19 ‘possible IE’ cases were reclassified as ‘definite IE’ and the two ‘rejected IE’ cases were reclassified as ‘possible IE’. Overall, 43 patients (88%) were considered as ‘definite IE’ and six as ‘possible IE’, while no cases remained classified as ‘rejected IE’.

**Conclusions:** Broad-range bacterial and fungal PCR-based assays, when considered as part of the Duke scheme improve its diagnostic ability and might be a useful tool in the microbiological diagnosis of infective endocarditis. This study was supported by the grant of MZ CR No. CEZ MZ 1000209775.

**Target**

<table>
<thead>
<tr>
<th>Probe</th>
<th>IS</th>
<th>CTR</th>
<th>CPN</th>
<th>CPS+</th>
<th>Cy5</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>600</td>
</tr>
<tr>
<td>C. trachomatis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>1-2</td>
</tr>
<tr>
<td>C. pneumoniae</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>2-4</td>
</tr>
<tr>
<td>C. psittaci</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>2-4</td>
</tr>
<tr>
<td>C. abortus</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>2-4</td>
</tr>
<tr>
<td>C. felis</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>2-4</td>
</tr>
</tbody>
</table>

**Detection limit, copies/reaction**

<table>
<thead>
<tr>
<th>Copies/Reaction</th>
<th>IS</th>
<th>CTR</th>
<th>CPN</th>
<th>CPS+</th>
</tr>
</thead>
<tbody>
<tr>
<td>600</td>
<td>1-2</td>
<td>2-4</td>
<td>2-4</td>
<td>2-4</td>
</tr>
</tbody>
</table>

**Results/conclusion:** With regard to the 220 bacterial isolates and the 75 isolates from blood cultures tested the hybridisation assay so far showed excellent results. Differentiation results were obtained in about 4 h from the time the blood cultures gave a positive signal, including DNA extraction, PCR and the hybridisation procedure.

**P709** Development of a multiplex real-time PCR for detection and differentiation of Chlamydiaceae species which are pathogenic for humans

I. Edelstein, A. Narezkina, M. Edelstein
Smolensk, RUS

**Objectives:** Real-time PCR (RT-PCR) offers many advantages over conventional PCR methods for detection of microbial pathogens. The aim of our study was to develop and evaluate the performance of a multiplex 5'-nuclease-based RT-PCR assay for direct detection and differentiation of *Chlamydia trachomatis* (CTR), *Chlamydophila pneumoniae* (CPN) and zoonotic agents (*Chlamydophila psittaci* (CPS), *Chlamydophila abortus* (CAB) and *Chlamydophila felis* (CFe)) in clinical specimens.
Methods: The 5’-end sequence of the omp1 gene which is well characterised in all chlamydial species was selected as a PCR target. It was amplified using the family-specific primers CM1 and CM2 (H. Yoshida et al., 1998) on a Rotor-Gene 2000 system (Corbett research). Three probes containing different fluorescent dyes, JOE, ROX and Cy5 (Biosearch Technologies) were designed to target the signature sequences in the amplified omp1 region, which are highly conserved within CTR, CPN and zoonotic agents and are distinctive between them. The fourth FAM-labelled probe was used for the detection of a heterogeneous internal standard (IS). The analytical sensitivity of the assay was determined by testing peripheral blood leucocyte specimens spiked with chlamydial elementary bodies or recombinant plasmids containing omp1 fragments of the following strains: CTR L2, CPN Kajaani 7, CPS 6BC, CAB B577 and CFE Felp. In addition, a panel of 219 genital swab specimens was used to assess the sensitivity and specificity of CTR detection using RT-PCR in comparison with a commercial PCR assay targeting the cryptic plasmid of this species.

Results: As shown in the table, the multiplex RT-PCR was able to detect specifically and reproduce single DNA copies of each chlamydial species in the presence of IS and excess of human DNA. When compared with monoplex PCRs, multiplexing of four probes did not decrease sensitivity, while no cross-detection between CTR, CPN and zoonotic species was observed. In testing clinical specimens, RT-PCR detected CTR DNA in 44 of the 46 samples that were positive and in one sample that was negative by commercial PCR. The lack of amplification of IS indicated the presence of inhibitors in two samples. Consequently, with commercial test used as a reference, the sensitivity and specificity of RT-PCR were 95.7% and 99.4%, respectively.

Conclusion: The developed method enables rapid, sensitive and specific detection of all members of Chlamydiaceae which are pathogenic for humans.

Objectives: To determine the suitability of TriPath SurePath™ medium for detection of Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC) using the Gen-Probe APTIMA (R) Combo 2 (AC2) Assay.

Methods: Because the cervix is a site of infection for CT and GC, Pap specimens may also be appropriate for detection of these sexually transmitted bacteria. By testing Pap specimens for CT and GC with AC2, multiple test results (CT, GC, Pap) can be obtained from one specimen. Preservcvt medium was evaluated for the detection of CT and GC with the AC2 assay using a protocol in which an aliquot is transferred to a tube containing Gen-Probe transport medium. The AC2 assay was then performed on the Preservcvt/transport medium sample using the standard AC2 protocol used for endocervical swab and urine specimens. For analytical sensitivity, 14 CT serovars and 20 GC clinical strains were diluted in the Preservcvt medium to 0.01 CT IFU and 0.5 GC CFU per AC2 reaction. Specificity was evaluated with three Chlamydia and 51 Neisseria nontarget species. Stability of samples in the Preservcvt vial and in the Preservcvt/transport medium mixture were monitored at 4–35°C. Cross-contamination due to Pap sample processing on the Cytyc ThinPrep2000 (TP2K) instrument was determined by processing alternating negative and high titre GC samples and then running them in the AC2 assay. Potentially interfering substances evaluated included whole blood and commonly used feminine hygiene products at usage levels higher than those expected in normal usage.

Results: All CT serovars were detected at 0.01 IFU/AC2 reaction and all GC strains were detected at 0.5 CFU/AC2 reaction. Specificity was 100%, with no cross-reactions observed with non-CT and non-GC species evaluated. Stability study test results indicated that CT and GC can be detected from Preservcvt and Preservcvt/transport medium mixtures stored at 4–35°C for 30 days. Following TP2K processing of high titre positives, false-positive results in known negative samples were <1%. None of the potentially interfering substances affected assay results.

Conclusion: These results indicate that the PreservCyt Pap medium is compatible with the APTIMA Combo 2 Assay for CT and GC detection.
the presence of Cp DNA and mRNA in aortic wall biopsies, obtained at surgery, from 24 patients with stable angina pectoris (SAP) and 20 patients with unstable angina pectoris (UAP).

Methods: DNA and RNA were extracted from the same biopsy using the RNA/DNA mini kit (Qagen). cDNA was made using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). Real time PCR directed against the MOMP gene was used to detect the presence of Cp DNA and mRNA in the biopsies. Patient sera were tested for Cp-specific IgM, IgG and IgA antibodies by the microimmunofluorescence technique.

Results: Thirteen (30%) of the biopsies (six SAP; seven UAP) were positive for Cp DNA, eight (18%) were positive for Cp mRNA (five SAP; three UAP) and six (14%) were positive for both DNA and mRNA (4 SAP; 2 UAP). All biopsies but one were positive for human cDNA encoding the GAPDH gene. That biopsy was negative also for Cp DNA and Cp mRNA. Results from the serology will be presented on the poster.

Conclusion: We have demonstrated the presence of Cp in the aortic wall of patients suffering from stable (SAP) or unstable (UAP) angina pectoris. Also, we have demonstrated Cp mRNA in 17% of these patients indicating that the bacteria were metabolically active. There were no significant differences in the frequencies of positivity for Cp DNA or Cp mRNA between the SAP and UAP groups. The results support the hypothesis of an active role for Cp in the pathogenesis of atherosclerosis.

P713 Rapid identification of HACEK group of bacteria by 16S rRNA gene PCR and restriction fragment length polymorphism analysis

Y. Kodama, M. Sasaki, S. Tajika, Y. Ohara-Nemoto, C. Yamaura, Y. Shimoyama, S. Kimura
Moriko, JP

Objectives: Although infective endocarditis due to HACEK group of bacteria (Haemophilus spp., Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens and Kingella spp.) is a rare occurrence, the identification of the organisms is still important diagnostically for the specific therapy. The HACEK bacteria are classified as fastidious Gram-negative coc-cobacilli, and the biochemical characteristics resemble each other. Thus, the identification of HACEK bacteria has been rather hard and sometimes inconclusive. In this study, we developed a rapid and highly sensitive identification method for HACEK bacteria by means of 16S rRNA gene PCR amplification followed by restriction fragment length polymorphism analysis (PCR-RFLP).

Methods: H. aphrophilus ATCC 33894, A. actinomycetemcomitans ATCC 33384, C. hominis ATCC 12826, E. corrodens ATCC 23834 and K. kingae ATCC 23330 were used. DNA samples were prepared by a DNA purification kit. After PCR amplification using the primers corresponding to Escherichia coli 16S rRNA gene, the PCR products were digested with 4 U of either HinfI and MspI at 37°C for 1.5 h. The samples were then separated on 1.8% agarose gel, and the restriction patterns were recorded.

Results: The RFLP patterns of five species of HACEK bacteria obtained by combing use of HinfI and MspI digestion were readily distinguished from each other and from other pathogens of infective endocarditis including viridans streptococci. Furthermore, the PCR-RFLP analysis yielded a definitive identification of C. hominis from one of the blood samples of the patients with infective endocarditis in which causative pathogens could not be unidentified by biochemical identification kits. The result was confirmed by a 16S rRNA gene sequence analysis of the isolate.

Conclusion: The PCR-RFLP analysis developed in this study was a rapid and highly sensitive identification method for HACEK group of bacteria, and could be applicable for a definitive diagnostic detection of HACEK group of bacteria.

P714 Same day molecular diagnostic test result for atypical and difficult to grow respiratory pathogens including M. tuberculosis complex by combining automated DNA extraction with real-time PCR

K. Jaton, C. André, A. Wenger, G. Prod’hom, J. Bille
Lausanne, CH

Background: Real-time PCR is a powerful method for detecting bacteria in clinical samples, but DNA extraction is still a crucial and cumbersome step when performing such tests.

Objectives: (i) Establish a 1-day TAT result for the molecular diagnosis of tuberculosis using a combination of an automated DNA extraction method with real-time PCR (RT) (ii) test its performance for other bacterial respiratory pathogens (M. pneumoniae, C. pneumoniae, L. pneumophila, B. pertussis and B. parapertussis (iii) assess its performance on clinical samples for M. tuberculosis.

Methods: (i) The automated DNA extraction procedure, MagPureR, Roche (MP) was compared with a conventional homemade extraction procedure (Boom et al.) using silica particles (SI) for 10 stored specimens positive by culture for M. tuberculosis. The extracted DNA were amplified and the amplicons detected by real-time PCR (RT) using the ABI 7700, Applied Biosystems; (ii) MP and SI were compared for diluted positive-specimens for the other bacteria; (iii) to assess the performance for the diagnosis of tuberculosis on clinical samples, two different periods of 14 months were compared in terms of sensitivity, specificity, PPV and NPV using culture results as gold standard. A: (March 2001–May 2002) 710 specimens (513 patients) with 76 positive cultures for M. tuberculosis (10.7% with SI-RT); B: (May 2002–July 2003) 817 specimens (619 patients) with 88 positive cultures for M. tuberculosis (10.7%) with MP-RT.

Results: (i) Eight of 10 specimens were positive for M. tuberculosis with SI-RT and nine of 10 with MP-RT (the three discordant results were weak-positive specimens upon culture); (ii) 100% concordance between the two procedures for the five different bacterial respiratory specimens, even better for the MP-RT when considering the cycle threshold (Ct) results; (iii) the A vs. B (A/B) results were very similar (%): global sensitivity: 90/89; specificity for the Ziehl-Neelsen (ZN)-positive samples: 100/100; sensitivity for the ZN-negative samples: 77/80; specificity: 99/99; PPV: 95/95 and NPV: 99/99.

Conclusions: One-day TAT molecular diagnostic test results are now possible for several atypical or difficult to grow respiratory bacterial pathogens including M. tuberculosis complex by combining automated DNA extraction with real-time PCR.
**P715** Reliable and rapid detection of clinically relevant *Mycobacterium* and *Nocardia* species using a multiplex-PCR with genus- and species-specific primers

A. Roth, O. Landt, S. Andrees, H. Mauch
Berlin, D

We report a rapid and reliable multiplex PCR assay to identify 13 clinically relevant *Mycobacterium* species (including *M. bovis*) and seven of the most frequently occurring *Nocardia* species. The method includes two genus-specific and six species-specific primer mixtures for identification to the species level. Additional genus-specific primers for closely related actinomycetes such as *Streptomyces* and *Tsukamurella* were included also. The primers were designed from nucleotide sequences of the 16S rDNA, the 16S–23S rRNA intergenic spacer region and part of the 23S rRNA. The established multiplex PCR identification scheme was applied to the identification of 131 reference strains and clinical isolates that were previously identified to the species level by 16S rDNA sequencing or 16S–23S rRNA spacer RFLP. The new scheme was very reliable and specific concerning the intra-species stability of the primers: all strains were identified correctly, although not all strains within a few genetically heterogenous taxa such as *M. kansasii* or *N. abscessus* were detected by this PCR method. This will make the inclusion of further primers necessary for some groups. Furthermore, we tested this multiplex PCR with 45 specimens from the respiratory tract from patients with suspected mycobacteriosis or nocardiosis (42 specimens were positive for acid fast rods, three contained branched Gram-positive rods). Since only microscopically positive specimens were tested, the negative predictive value and the sensitivity were very high. This multiplex-PCR represents a cost-effective, rapid and easy to perform method for the identification of mycobacteria and nocardia from cultures and for the direct detection of these rare but important pathogens in microscopically positive clinical specimens.

**P717** Evaluation of the BD ProbeTec™ ET *Mycoplasma pneumoniae* amplified DNA assay

S.S. Nielsen, S.A. Uldum, B. Dohn, J.S. Jensen
Copenhagen, DK

Objectives: New assays for diagnosing atypical pneumonia caused by Chlamydiaceae family, *Legionella pneumophila* and *Mycoplasma pneumoniae* from throat swabs and lower respiratory samples are currently under evaluation on the BD ProbeTec™ ET system. These assays are based on real-time homogenous strand displacement amplification (SDA) and detection technology. It has been reported that molecular methods have increased sensitivity and shorter time to results vs. culture. For this study, we took part in the evaluation of BD ProbeTec ET *Mycoplasma pneumoniae* (MP) Assay with previously collected throat swabs expressed in 2SP medium and stored frozen.

Methods: Specimens (78) were included in this study – 15 specimens were negative by PCR and 63 specimens were previously confirmed positive for *Mycoplasma pneumoniae* by our in-house PCR method. Nineteen of the PCR-positive specimens were cultured positive for *Mycoplasma pneumoniae*. Sensitivity of the MP assay was calculated against the gold standard (culture) and against our in-house PCR method. For samples with discrepant results, the PCR assay was repeated from the original 2SP media if available and also from the BD ProbeTec ET processed samples.

Results: Gold standard (culture): Of 19 culture-positive specimens, 16 were MP Assay positive (sensitivity 84.2%). The three culture-positive MP Assay negative specimens could not be tested by PCR on the original 2SP media due to insufficient volume, but were tested from the BD ProbeTec ET processed sample. Only one of the three specimens was positive by PCR.

PCR: Of 63 PCR-positive specimens, 52 were MP Assay positive (sensitivity 82.5%) and 11 were negative. Three of these 11 specimens were culture positive, but could not be tested by PCR as mentioned previously. Eight of the 11 PCR-positive MP Assay negative specimens were tested by PCR only one of the 11 specimens was positive by PCR.

Conclusion: The BD ProbeTec ET MP Assay is a sensitive and specific assay for the rapid identification of *M. pneumoniae* in throat swabs expressed in 2SP media. The MP assay may be useful in situations where culture is time consuming and difficult to perform.

**P716** Identification of *Mycobacterium gordonae* clinical isolates with the use of four molecular methods

Larissa, Crete, Athens, GR

Objective: The aim of our study was the evaluation of the PCR-RFLP analysis of hsp65 and rpoB genes, a PCR-based assay and the Accuprobe tests (Gene-Probe Inc.) for the identification of clinical *M. gordonae* isolates.

Methods: Thirty seven, of the 42 *M. gordonae* isolates studied, were recovered from clinical specimens of different patients and five strains obtained from the collection of the Greek National Mycobacterium Reference Unit. A segment of the RNA polymerase gene (rpoB) was amplified by PCR and the products (342 bp) were digested with the restriction enzymes *Hae* III, *Mva* I and AccI. A segment of the heat shock protein gene (hsp65) was amplified by PCR and the products (440 bp) were digested with the restriction enzymes *Hin* dII and *Bst* EI. The digested products were electrophoresed and the results were analysed using the PCR-RFLP algorithms. PCR-assays were performed using a pair of *M. gordonae*-specific primers that amplified a fragment of 152 bp of the internal transcribed spacer region. The results of identification were compared with those obtained by conventional biochemical and AccuProbe tests.

Results: All the 42 *M. gordonae* isolates included in this study have been correctly identified by the four molecular methods tested. The PCR-RFLP analysis of rpoB gene generated the typ-
Quantification of Salmonella by 5’-nuclease polymerase chain reaction targeted to fimC gene

E. Kačiková, D. Pangallo, T. Kuchta
Bratislava, SK

Objectives: A new 5’ nuclease PCR system for the quantification of Salmonella spp. using the primers and the probe oriented to Salmonella-specific region of the fimC gene was developed.

Methods: The sequence of the fimC gene was carefully checked and Salmonella-specific region was identified, and primers and a 5’-nucleotide probe oriented to it were designed with a theoretical melting temperature of 60°C. To determine the exclusivity of the primers, 45 non-Salmonella strains were tested by conventional PCR. To determine the inclusivity of the PCR system consisting of the primers and the probe, 48 Salmonella clinical and food isolates of 34 various serotypes were tested by real-time PCR. For quantification purposes, calibration lines were constructed for three Salmonella strains pure cultures and with other bacteria background, respectively.

Results: The PCR system is specific and sensitive with 100% inclusivity and 100% exclusivity. Calibration lines constructed for three Salmonella strains were very similar to each other and facilitated quantification in the range from 10^3–10^9 CFU/mL. Escherichia coli (10^6) and Citrobacter freundii (10^6) background had no effect on Salmonella quantification by the system.

Conclusion: Presented highly specific real-time PCR system represents a good tool for quantification of Salmonella sp. in clinical, food and environmental materials.

Experiences with Chlamydia testing using the BD ProbeTecET and BD Viper focusing on inhibition in urine samples

G. Lisby, H. Westh
Heideløv, DK

Objectives: On June 1, 2003 our laboratory changed the Chlamydia trachomatis (CT) testing platform from PCR/EIA to real-time strand displacement amplification (SDA) using the BD Viper sample preparation robot and two BD ProbeTecETs. The lab performs 70 000 CT analysis per year. The wet swab for cervix and urethra, and urine testing for men was introduced. We evaluated the inhibitory effect in different specimen types and analysed the effect of washing urine samples prior to testing.

Methods: We evaluated the first 6 months of using SDA (33 000 samples). Information was obtained from our LIS. A patient was only included once and a positive test result had priority over negative results. We calculated changes in gender sampling, sampling site, age-specific positive sample rate and age-specific percentage of women tested in Copenhagen. After washing 91 negative urine samples and 90 positive urine samples were retested without the washing step.

Results: The average positive sample rate increased from 4.4 (EIA) to 6.4% (SDA). The age-specific positive rate for women increased for all ages over 18 years. The positive rate from female urine n = 130 was 5.4% and from male urine, n = 2220, 15%. CT samples from men were increased by 53% and positive rate from 12.5–13.7%. Inhibition was seen in 0.7% from cervix, 0.16% from female urethra, 0% from female urine, 0.24% from male urethra and 0.14% from male urine. Reportable results were obtained from 88/90 (97.8%) unwashed urines that were positive after washing. Two of 90 had inhibition (2.2%) and three of 88 were negative.

Conclusion: The positive sample rate was increased by 45% in women and 9.6% in men with male sampling increasing by 53%. Inhibition was only seen in 0.46% of samples, primarily from cervix samples. Washing of urine samples removes virtually all inhibition. Inhibition rate for unwashed urines was significantly higher in negative urines than positive urines P = 0.02. Omitting the washing step (with a CT prevalence of 15%) will reduce the time spent on washing by about 66%. This will delay laboratory answers on the inhibited samples by one day due to washing and re-analysis of approximately 10% of the urine samples.
P721 Early diagnosis of leptospirosis by polymerase chain reaction (PCR)

Thessaloniki, GR

Leptospirosis is a worldwide zoonosis that affects wild and domestic animals and humans. The disease is characterised by various clinical manifestations, ranging from asymptomatic disease to fatal icterohaemorrhagic forms, while the diagnosis is mainly based on the detection of serum-specific antibodies (serological methods). The aim of the study was the contribution of PCR amplification in early diagnosis of leptospirosis and its evaluation in parallel to the results of the serological tests.

Material and methods: In this study, 58 patients (78 whole blood and sera samples) with probable leptospirosis were examined over a 4-year period, 2000–2003. Another group of five patients (five whole blood and sera samples) with fever of other aetiology (rickettsiosis, syphilis, brucellosis, tuberculosis) was assessed in order to evaluate the specificity of PCR. 20 healthy persons (20 whole blood and sera samples) were included in the study, as a control group. PCR methods were performed in all samples using two pairs of primers that amplify the rrs (16S) gene and insertion sequence of IS1533 region in Leptospira genome. An enzyme-linked immunosorbent assay (ELISA) was used for the detection of specific antibodies against Leptospira spp. (IgM, IgG). Only PCR techniques were performed in samples of control group.

Results: The two PCR methods produced positive results in 25 of 58 patients (rate 43%) while serological tests gave positive results in 22 patients of 58 (rate 38%). The remaining 33 patients were all negative by PCR and only two patients demonstrated low serological reactivity (cross-reaction). All patients of other infections and the control subjects were found negative by PCR and ELISA. Both PCR methods revealed identical results in all groups.

Conclusions: The diagnosis of leptospirosis is often based on serological tests, but the specific antibodies against Leptospira spp. are detected 8–10 days after the onset of the disease, while cross-reactions may be present. PCR is a highly sensitive and specific method that contributes to the early diagnosis of the disease. The combination of PCR and serological tests can substantiate the definitive diagnosis of leptospirosis.

P722 Rapid detection and identification of Aspergillus spp. and Candida spp. by real-time PCR

M. Torres, J. Aznar, E. Martin, M. Ruiz, M. Ramirez, J.C. Palomares
Seville, E

Objectives: To develop and evaluate a real-time PCR assay, based in the Light Cycler technology, amplifying a highly conserved sequence of the multicopy 18S rRNA gene and using specific probes for genus-level identification of Aspergillus spp and Candida spp.

Methods: Aspergillus and Candida strains obtained from the Spanish Collection of Type Cultures (CECT) including A. flavus, A. fumigatus, A. nidulans, A. niger, A. terreus and C. albicans, C. dublinensis, C. glabrata, C. guilliermondii, C. krusei, C. lusitaniae, C. parapsilosis, C. tropicalis and C. sake, were used. Specificity of the assay was assessed by using DNA extracted from a collection of pathogenic and nonpathogenic bacteria and fungi. The analytical sensitivity of the process was evaluated with of different inocula (10^1–10^9 CFU/mL), and serially diluted DNA of A. fumigatus and C. albicans.

Results: Reactions using genomic DNA from other species resulted in negative results, indicating that specificity of that assay was a 100%. Analytical sensitivity was 60 fg using DNA and 15 conidia using conidial suspensions for Aspergillus fumigatus; while for Candida albicans were 100 fg and three cells. The linear range of the assay was from 6 to 6 × 10^7 fg for Aspergillus DNA and from 10 to 10^7 fg for Candida DNA. Species identification was determined by analysing the melting curves obtained with the specific probes, the Tm ranged from 67.34°C to 70.7°C for Aspergillus spp. and from 51.3°C to 64.5°C for Candida spp.

Conclusions: We therefore developed a rapid, quantitative, sensitive, and specific real-time PCR assay to detect Aspergillus and Candida species. The PCR assay designed and tested as described here provides a high sensitivity and specificity for the detection of fungal DNA and rapidly identifies most of clinically relevant Candida and Aspergillus species.

P723 Molecular genetic identity of blood and oral isolates of viridans streptococci

J. Dušková, Z. Broukal, T. Janatová, G. Novotná, J. Janata
Prague, CZ

Prevention of haematogenic dissemination of oral bacteria belongs to the significant issues of current emergency care of health-compromised individuals. The aim of study to ascertain the molecular genetic relationships of oral and blood culture isolates of viridans streptococci (S. salivarius, S. mitis, S. mutans and S. sanguis groups).

Methods: An attempt was made to test the similarities of the relationships in a group of 30 patients with positive haemocultures harbouring these organisms. Isolates were preliminarily identified by means of colonial morphology and biochemical properties and then genotyped by means of the PCR (spacer 16S and 23S rDNA) and AP-PCR.

Results: Strain identity of blood and oral isolates has been proved in S. salivarius in two of four cases, in S. mitis in eight of 10 cases, in S. mutans in two of two cases and in S. sanguis in 15 of 16 cases. Clonal identity of blood and oral isolates has been proved in 50% of isolates. Oral microbial flora is thus the significant source of viridans streptococci in positive haemocultures.

Discussion: Based on these findings it should be recommended, when managing oral health care of a patient at risk, to eradicate not only the oral infectious foci but also to introduce the oral home-care regime reducing substantially plaque and mucosal oral flora.

Supported by the grant Min. of Health, Czech Republic No. 0002377901.