carried a class 1 integron harbouring dfrA12 + orfF+aadA2 and dfrA1 + aadA1 arrangements, respectively.
Conclusion: A high polymorphism was detected in the blaCMY gene of clinical and commensal Citrobacter isolates with high clonal divergence among them. The variant blaCMY-2 was infrequently detected in the studied collection.

R2472 Utilising population analysis to investigate piperacillin/tazobactam differences between broth microdilution and agar dilution for a set of *Escherichia coli*

C. Shubert, J. Slaughter, D. Creely, G. Zambardi, D. Shortridge (Hazelwood, US; La Balme, FR)*

Objective: An abbreviated population analysis (PA) was done on 42 *Escherichia coli* (EC) isolates, in order to determine if heterogeneity of resistance expression was a factor in piperacillin/tazobactam (TZP) MIC differences between agar dilution (AD) and broth microdilution (BMD), observed for some of the isolates.
Methods: BMD and AD testing for TZP was performed in triplicate for 42 EC isolates, and a composite MIC (i.e. voted based on the three results) was determined for each method. PA was performed utilizing a range of agar plates prepared with piperacillin at doubling-dilutions from 1 to 256 mcg/mL and tazobactam at a fixed concentration of 4 mcg/mL. PCR for tem, shv, oxa, plasmid-mediated ampC, and ctx-m β-lactamases was done on all isolates.
Results: Twenty EC isolates differed by interpretive category between BMD and AD, utilizing the composite MICs for comparison. When BMD and AD were not in agreement, higher MICs were usually observed for BMD. Seventeen isolates were TZP resistant (R) by both BMD and AD, and five were TZP susceptible (S) by both BMD and AD. Of the 20 isolates that differed between BMD and AD, 85% (17/20) were heterogeneous, and three were homogeneous, by PA. Of the 17 R by both BMD and AD, 65% (11/17) were homogeneous, and six were heterogeneous, by PA. Of the five S by both BMD and AD, three were heterogeneous, and two (including QC EC ATCC 35218) were homogeneous. For the 22 total in category agreement between BMD and AD, 13 were homogeneous (59%), and nine were heterogeneous (41%). The rates of heterogeneity and homogeneity for the respective sets (BMD vs. AD not in agreement, and BMD vs. AD in agreement) were significantly different based on Chi-square analysis p-values of 0.003 and 0.010, respectively. Most isolates (28 of 42) harbored only tem-1.
Conclusion: The majority of isolates with differences between BMD and AD demonstrated heterogeneous growth by PA. When BMD and AD were in agreement, the majority of isolates demonstrated homogeneous growth. PA data support the conclusion that TZP MIC differences between BMD and AD are primarily due to heterogeneity of resistance expression. Further study of the mechanism of TZP resistance for EC harboring tem-1 is in progress.

R2473 Analysis of the mechanisms of resistance to azithromycin and its transferability in clinical isolates of *Escherichia coli* and *Shigella* spp. clinical isolates from Lima, Peru

C. Gomes, D. Durand, A. Lluque, S. Mosquito, M.J. Pons, A. Prada, T.J. Ochoa, J. Ruiz (Barcelona, ES; Lima, PE)*

Objective: To analyse the mechanisms of resistance to azithromycin (AZM) and its transferability in *Escherichia coli* and *Shigella* spp. clinical isolates from Lima, Peru.
Methods: The Minimal Inhibitory Concentration (MIC) to AZM was determined in 71 clinical isolates (62 *E. coli* and nine *Shigella* spp.) exhibiting a halo to AZM <15 mm. The role of efflux pumps was tested establishing the MIC levels in presence of Phe-Arg-beta-Naphtylamyde (PAN), an inhibitor of efflux pumps. Point mutations in rplD and rplV genes were observed by PCR and sequencing. The presence of 10 established mechanisms of resistance to macrolides (ereA, ereB, msrA, ermA, ermB, ermC, mefA, mefB, mphA and mphB) was searched. Conjugations assays were performed in order to evaluate if plasmid mechanisms are transferable.
Results: The MICs of AZM ranged between 32 and >256 mg/L among *E. coli* isolates and 4 e 8 mg/L among *Shigella* spp. When PAN was added to the media the MICs levels decreased from 1 to eightfold. In seven strains were found substitutions in the rplV gene (K82-N, D-94H, K98-N; L46-Q in two isolates; S101-T, I103-L; I4-L, K6-Q and P80-S in two isolates of *Shigella* spp.). The most frequent plasmid mediated gene was mphA (present in 59% of *E. coli* isolates and 11% of *Shigella* spp.). Also the genes mphB, ermA, ermB, ereA and mefA were found among the isolates. The conjugation assays showed that 25.8% of *E. coli* isolates were able to transfer the AZM-resistance. Thus three genes (mphA, ermA and ermB) were present within conjugative elements. One *E. coli* isolate without any of the searched transferable AZM-resistance mechanisms was able to transfer the AZM-resistance to recipient strain.
Although no analysed plasmid mediated mechanism of resistance was found among *Shigella* spp.
Conclusions: PAN-inhibitible efflux pumps play a role in development of AZM resistance. The possible association between detected mutations in rplV gene and resistance to azithromycin may not be ruled out and need to be confirmed by further experiments. In the area several transferable AZM-resistance mechanisms are present, being the mphA the most disseminated. The AZM should be carefully used in Lima.

R2474 Phenotypic and molecular characterisation of CMY-46 and CMY-50, two novel plasmid-mediated AmpC beta-lactamase carried by *Escherichia coli*

V. Manageiro, D. Louro, E. Ferreira, M. Caniça (Lisbon, PT)*

Objectives: The identification of isolates containing AmpC β-lactamases is epidemiologically and clinically relevant. With this study we performed the phenotypic and molecular characterization of two new CMY-2-types, designated CMY-46 and CMY-50, encountered among a total of 1664 clinical non-duplicate isolates of various *Enterobacteriaceae* species.
Methods: *E. coli* INSRA1169 and INSRA3413 were isolated from the urine of patients with 77 years and 7 months old, hospitalized in the ward and in pediatrics, respectively. The blaCMY genes were cloned in the plasmid pBK-CMV and transformed into electrocompetent *E. coli* DH5 alpha delta ampC by electroporation. Antimicrobial susceptibility (MIC) was determined by a microdilution method. *E. coli* INSRA6015, a CMY-2-producer, was used for phenotype comparison. PCR-mapping of the genetic environment of new blaCMY genes was performed using primers for known antibiotic and mercury resistance genes.
Results: Antimicrobial susceptibly tests showed that all isolates and respective transformants were nonsusceptible to amoxicillin, amoxicillin plus clavulanic acid, cephalothin, cefoxitin, ceftazidime and cefotaxime. INSRA1169 and INSRA6015 were also nonsusceptible to ciprofloxacin and to trimethoprim. Regarding gentamycin, only INSRA1169 was resistant. Its noteworthy that the transformants EcDH5a(pBK-CMY-2) and EcDH5a(pBK-CMY-46) exhibited higher values for extended-spectrum cephalosporins than the respective isolates. All strains were susceptible to cefepime and imipenem, showing synergy between cloxacilin and cefoxitin and/or ceftazidime. No phenotypic alterations were found comparing the new CMY-type with the parental CMY-2. The genetic characterization of CMY-46 and CMY-50-encoding genes revealed a *Citrobacter freundii* chromosome-type structure, encompassing a blc-sugE-blaCMY-2-type-ampR platform in both isolates. In addition, a sul1-type class 1 integron and a truncated mercury resistance operon were encountered.
Conclusion: Although the CMY-type enzymes studied conferred resistance to extended-spectrum cephalosporins, the susceptibility to cefepime lead us to assume that those enzymes are not extended-spectrum cephalosporinases. Otherwise, the presence of three genetic resistance-encoding regions is of great concern, namely the truncated

mercury resistance operon, which may help to promote antibiotic resistance through indirect selection.

Resistance surveillance

R2475 First report of KPC beta-lactamase in *Klebsiella pneumoniae* isolate from Croatia

B. Bedenic, A. Mazzariol, V. Plecko, Z. Bosnjak, D. Sijak, G. Cornaglia (Zagreb, HR; Verona, IT)*

Objectives: The aim of the study was to characterize carbapenem resistance in *K. pneumoniae* from Zagreb, Croatia.
Material and Methods: In February 2011. A 78 old male patient was admitted to Clinical Hospital Center Zagreb with subdural haematoma. He was previously diagnosed with acute myeloblastic leukemia. After surgical removal of haematoma he developed purulent meningtis. *K. pneumoniae* with reduced susceptibility to carbapenems was isolated. The patient died from intracerebral bleeding in April 2011.
The antimicrobial susceptibility to a wide range of antibiotics was determined by broth microdilution method in Mueller-Hinton broth and 96 well microtiter plates according to CLSI guidelines. A double-disk-synergy test was performed to detect ESBLs.
Modified Hodge Test (MHT) was used to screen for production of carbapenemases. MBL E-test was used to screen for production of metallo-β-lactamases. The transferability of meropenem resistance was determined by conjugation (broth mating method) employing *E. coli* A15R-strain resistant to rifampicin. Transconjugant was selected on the combined plates containing meropenem (1 mg/L) and rifampicin (128 mg/mL). The presence of genes encoding ESBLs (blaSHV, blaTEM, blaCTX-M), plasmid mediated ampC β-lactamases and carbapenemases blaKPC, blaOXA-48, blaOXA-NDM, blaVIM and blaIMP was determined by PCR.
Results: The isolate showed resistance or intermediate susceptibility to expanded-spectrum cephalosporins, beta-lactam combinations with inhibitors, carbapenems and gentamicin but remained susceptible only to ciprofloxacin and colistin. Modified Hodge test was consistent with the activity of carbapenemases. The MBL test for metallo-beta-lactamase was negative indicating the absence of metallo beta-lactamase. Imipenem resistance was not transferred to *E. coli* recipient strain by conjugation. PCR revealed the presence of blaKPC, blaTEM genes and blaSHV genes. Sequencing of blaKPC gene revealed the presence of KPC-2 beta-lactamase. Neither plasmid-mediated AmpC beta-lactamase nor OXA-48 beta-lactamase were found. The strain was found belong to ST37 clone by MLST.
Conclusions: Infection control efforts limited the spread of KPC-producing clone of *K. pneumoniae* in our hospital so far. KPC-2 beta-lactamase with similar properties was previously reported from USA, United Kingdom, Israel and Greece. To our best knowledge, this is the first report of KPC beta-lactamase from Croatia.

R2476 Evaluation of the antimicrobial resistance of *Pseudomonas aeruginosa* at a clinical hospital Osijek, Croatia

V. Atalic, M. Bogdan, D. Vukovic (Osijek, HR)*

Objectives: *Pseudomonas aeruginosa* (PA) is an important nosocomial pathogen causing a wide varriety of infections. The aim of this study is to evaluate the resistance rate of PA isolates between years April 2003 and October 2009, from patients hospitalised at the Clinical Hospital Osijek (1250 beds), Croatia.
Methods: Of 1.531 isolates of PA were tested during 2003/2004, and 1.369 during 2009/2010. The antimicrobial susceptibility was determined by the Kirby-Bauer disc diffusion method and E-test when necessary. Results were interpreted according to CLSI. The isolates were tested for amikacin (AN), gentamicin (GM), ciprofloxacin (CIP), piperacillin/tazobactam (TZP), ceftazidime (CAZ), imipenem (IPM) and meropenem (MEM). The statistical analysis was performed using the chi-square test.
Results: After comparing the resistance rate of PA in the period of April 2003–October 2009, the increasing rate of resistance with statistical significance for tested antimicrobial agents were found as follows: AM 10.84%/18.19% ($p < 0.01$), CIP 25.08%/30.39% ($p < 0.05$), CAZ 1.82%/3.21% ($p < 0.05$), IMP 6.92%/19.28% ($p < 0.01$), MEM 6.92%/17.82% ($p < 0.01$), TZP 1.96%/6.72% ($p < 0.01$). The resistance decreased only for GM 45.33%/39.01% ($p < 0.05$).
Conclusion: According to the mentioned ongoing increasing resistance rate, we can conclude that the number of therapeutic options has continuously been decreasing. Ceftazidime is still the best therapeutic agent for PA infections, due to its low resistance rate. On the other hand, fluoroquinolones and karbapenems show substantional resistance increase which we can contribute to the excessive and irrational usage of broadspectrum antibiotics. The decrease in gentamicine resistance was because gentamicine was not used in the therapy of PA and it was generally used less in last few years because of its high resistance, so it recovered susceptibility. Although there is the increasing rate of resistance, we can still be satisfied that the resistance rate for PA is lower if compared to some other European countries. It is important to continue the monitoring of the antimicrobial resistance in treatment of pseudomonas infections with effective infection control measures, in order to limit the development and spreading of resistance, followed by good clinical practise.

R2477 Fluoroquinolone-resistant urinary isolates of *Escherichia coli* from nosocomial vs. community-acquired infections

S. Baka, I. Tsouma, E. Kouskouni (Athens, GR)*

Objectives: Community-acquired (CA) and healthcare-associated (HA) urinary tract infections (UTI) have been associated with high rates of morbidity, and pose a significant economic burden to healthcare systems all over the world. *Escherichia coli* is the primary causative agent of UTI but its susceptibility profile has changed over the last decade. Fluoroquinolones, such as levofloxacin, norfloxacin or ciprofloxacin, are now recommended for the empirical treatment for UTI. However, the increased use of fluoroquinolones has resulted in the rapid emergence of fluoroquinolone-resistant *E. coli*, making the medical community skeptic as to whether fluoroquinolones should remain the drugs of choice for UTI. The purpose of this study was to evaluate the prevalence of fluoroquinolone-resistant *E. coli* in CA-UTI and compare it to cases of HA-UTI.
Methods: We studied all episodes of CA-UTI and HA-UTI, diagnosed in our hospital, due to *E. coli* during the period January 2009 to September 2011. HA-UTI was defined as those UTI affecting patients hospitalized for two or more days. Urine samples were obtained from clean-catch mid-stream urine or from urinary catheters and cultured on Blood agar and MacConkey agar followed by incubation for 24 hour at 37°C. Positive urine cultures were defined by bacterial grow (10^5 colony forming units/mL. Patients with polymicrobial urine cultures were excluded from the study. Identification of *E. coli* was performed by means of standard methods and susceptibilities to ciprofloxacin, norfloxacin and levofloxacin were tested by agar disk diffusion method according to the CLSI criteria. Intermediate and resistant *E. coli* strains to either of the antimicrobials studied were grouped together for data analysis.
Results: We obtained 119 *E. coli* isolates from an equal number of hospitalized patients and 321 from patients attending the Outpatient Clinic of our hospital. Out of the 119 *E. coli* strains isolated from HA-UTI, the resistance to ciprofloxacin, norfloxacin and levofloxacin was 16.8%, 15.1% and 17.6%, respectively. The respective percentages for the 321 *E. coli* strains isolated from CA-UTI were 14.0%, 14.0% and 13.1%, respectively.
Conclusion: Although fluoroquinolones are considered an optimal therapeutic choice in UTI, care should be given before initiating empirical treatment with these agents, at least until antimicrobial susceptibility tests become available for the clinicians.