Molecular bacteriology for *Helicobacter pylori*

P724 cagA, vacA and babA2 genotypes of *Helicobacter pylori* strains from patients with gastritis or peptic ulcer in Spain

J.A. García-Campos, D. Domingo, T. Alarcón, E. Aznar, J. Díaz-Regañón, M. López-Brea
Madrid, E

Objectives: In this study we wanted to examine the prevalence of cagA, vacA and babA2 status in *Helicobacter pylori* (Hp) isolates from patients with gastritis or peptic ulcer; to compare them and to know if there were any relationships between those virulence factors in each group.
Methods: Gastric biopsy specimens from 44 Hp positive patients with peptic ulcer (25 cases) and gastritis (19 cases) were studied. DNA was extracted and PCR performed to detect cagA, vacA s1/s2 alleles and babA2 gene.
Results: Gastritis: In 74% of strains, the expected cagA fragment was amplified by PCR; 68% carried the s1-allele and 32% the s2-allele; babA2 gene was detected in 37% of strains. Peptic ulcer: 84% of strains were cagA+; 72% were vacA s1-allele and 28% were vacA s2-allele; babA2 gene was detected in 36% of strains. No significant differences in the prevalence of cagA, vacA or babA2 were found in both groups. Neither of them showed relationship between the presence of babA2 gene and cagA gene or vacA s1/s2-alleles.
Conclusions: Although the risk of developing more serious gastric lesions increased as the number of virulence factor genes are accumulated in a given Hp strain, we did not find any significant differences or relationship in the cagA, vacA or babA2 status between the Hp isolates from patients with gastritis or peptic ulcer in this study. A low percentage of babA2 gene was found in both groups.

P725 FISH detection of clarithromycin-resistant *Helicobacter pylori* cells in clinical and wastewater samples

P. Piqueres, Y. Moreno, A. Jimenez, J. Hernández, M. Ferrús
Valencia, E

Introduction: The presence of viable but non-cultivable *Helicobacter pylori* cells in environmental samples may underestimate the importance of this way for its transmission. The determination of resistance to antibiotics in these strains is important to a better understanding of the epidemiology of the infection.
Objectives: We have evaluated the use of a fluorescent in situ hybridisation (FISH) assay directly from biopsies and wastewater to detect *H. pylori* and simultaneously its macrolide resistance genotype.
Methods: A total of 26 gastric biopsies samples from ulcer-patients were homogenised in 2 mL of selective broth, and a 500 μL aliquot was used for FISH detection. Twenty-nine wastewater samples collected from different treatment plants were centrifuged and subsequently fixed with 4% paraformaldehyde solution for 4 h at 4°C and then washed with 1% PBS buffer. HPY probe, a 16S rRNA targeted FITC-labelled oligonucleotide sequence was used for the detection of all *H. pylori* strains. In addition to CLA1–3, a set of three CY3-labelled probes was used for the detection of 23S rRNA mutations associated with resistance to clarithromycin. Hybridisation was performed with 35% formamide at 46°C for 2 h.
Results: FISH allowed the detection of *H. pylori* in 20 out of 26 clinical samples and 12 samples were positive in wastewater. The 35% of the positive biopsies showed the presence of clarithromycin resistant strains and 16.6% of the positive wastewater samples yielded resistance genotype to this macrolide. By using a double filter set we could observe directly the clarithromicyn resistant *H. pylori* organisms in the samples and its morphology in the different types of environments. The predominant cells' morphology in both clinical and wastewater samples was of helicoidal form.
Conclusions: The FISH is a specific and rapid culture-independent method to determine directly the presence of clarithromycin-resistant *H. pylori* cells in clinical and environmental samples. Results showed the presence of macrolide resistant cells in water and, therefore, water must be considered a potential route of *H. pylori* transmission.
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P726 Effect of the therapy of patients with *Helicobacter pylori* related gastritis is correlated with organisation of CAG pathogenicity island

V. Simanenkov, D. Bovaeva, O. Solovjova, A. Suvorov
St Petersburg, RUS

Objectives: *Helicobacter pylori* is a leading cause of various gastrointestinal diseases such as atrophic gastritis and gastroduodenal ulcer. The cagA gene product CagA is directly injected into the bacteria-attached host cells and deregulates intracellular signalling pathways and thereby initiates pathogenesis. cagA gene is located on pathoginicty island but the function of other genes on the island is unknown. The goal of the work was to evaluate the impact of cag island genotype on the outcome of the therapy.
Materials and methods: Three groups of 25 patients each with total number of 75 patients were investigated. First group was taking a typical antibiotic therapy (amoxicillin, claritromycin, rabeprasol), patients in the second group were treated with the same antibiotics together with probiotic Laminolact (*E. faecium* strain L-3 in the form of bon-bons together with pectin, soy bean amino acids and sea weed), and the third group was taking only Laminolact without any antibiotic. The genotype was determined by PCR with DNA primers against three *H. pylori* genes ureB, cagA and cagH. cagH was used as a marker cag island integrity and ureB was a marker of *H. pylori* presence.
Results: Five different genotypes were determined: ureB+,cagA−cagH−, ure+,cagA−cagH+, ureB+,cagA+cagH+, ureB+,cagA−cagH+ and ureB+,cagA+cagH−. Treatment with antibiotics alone was leading to 68% of eradication. The best eradication percentage (84%) took place in the second group where classical antibiotic treatment was taken together with probiotics. Interestingly, probiotic treatment alone was giving 48% of eradication. Results of the therapy were highly consistent with cag genotype. Patients were found to be statistically less susceptible ($P < 0.05$) to the therapy in case when the entire cag regulon was present regardless of the therapy used. This fact suggests immunosuppressant function of CagA or other proteins encoded by the genes on cag pathogenicity island.
Conclusion: The effect of *H. pylori* eradication depends on cag pathogenicity island genotype. Probiotics including *E. faecium* L-3 might significantly improve the anti-*H. pylori* treatment.

P727 AP-PCR genotyping of clinical isolates of *Pseudomonas aeruginosa*

D.H. Binnet, G. Uraz, E. Senol, N. Akar
Ankara, TR

Objectives: Arbitrarily Primed Polymerase Chain Reaction (AP-PCR) has been used for the epidemiological evolution clinical isolates of *P. aeruginosa*. This method is recommended for first-pass

screening of *P. aeruginosa* isolates. This study was done to determine if the *P. aeruginosa* strains isolated from the cultures of patients hospitalised in the Infectious Diseases Unit were from an individual strain. This technique was preferred because it is cheap and provides a rapid detection opportunity.
Methods: 45 samples obtained from the clinical specimens of the patients and from the hands of the staff of the Infectious Diseases Unit were cultured. Of the 45 samples, 15 were isolated from blood, six from sputum, 10 from drainage and 14 from the hands of the medical staff. *P. aeruginosa* identification was made by API 20NE system. DNA was extracted from the culture material by phenol–chloroform extraction method. AP-PCR was performed by using the primer 5′-GTT GCG ATCC-3′, and subjected 8% PAGE. Band patterns were visualised by silver staining.
Results: In none of the isolates of the hospital staff *P. aeruginosa* was cultured. Out of the 31 clinical samples of the patients, 15 different genotypes were determined.
Conclusions: The *P. aeruginosa* strains of the patients were individual strains, neither related to the staff of the department nor to a specific patient.

P728 *Pseudomonas aeruginosa* – microarray for rapid determination of antibiotic susceptibility and virulence factors

J. Weile, M. Susa, T. Bachmann, C. Knabbe
Stuttgart, D

Objectives: *Pseudomonas aeruginosa* is a leading cause of nosocomial infections, particularly pneumonia or sepsis, on intensive care units. Its high intrinsic antibiotic resistance and the ability to develop multidrug resistance pose, especially for critically ill patients, serious therapeutic problems. Since, culture based techniques for pathogen identification and resistance determination requires at least 2 days, a calculated antibiotic therapy may harbour the risk of an increase in antibiotic resistance and therapy failure. Therefore, the development of a fast and reliable identification and antimicrobial susceptibility test is essential for the improvement of the therapy. The aim of the present study was to develop an oligonucleotide-array for a quick, genotypic test of antibiotic susceptibility combined with the determination of relevant virulence factors.
Methods: DNA from different clinical specimen was isolated with a modified QIAmp DNA Blood Mini Kit. Template DNA was amplified and simultaneously labelled with Cy3 during multiplex PCR. 146 oligonucleotide capture probes (17–24mer), containing a poly-T(15)-spacer at the 5′-end, were spotted on epoxy-slides to build an array covering regulatory genes of multidrug efflux pumps (mexR, mexT, nfxB), alginate synthesis (mucA), metallo-beta-lactamases (bla-vim, bla-imp), aminoglycoside modifying enzymes (aac, aad, aph) and virulence factors (exoU, exoS, exoT).
Results: 12 of 15 clinical *P. aeruginosa* isolates could be correctly genotyped. Three isolates displayed a hybridisation pattern that could be assigned neither to wild-type nor to known mutations. A sequence analysis of these isolates revealed an unknown mutation in mexR and nfxB. Hybridisation with DNA from other non-fermenter or enterobacteriacea showed no cross-reactivity. Genotypic resistance profile of *P. aeruginosa* deduced from the array data correlated fully with the susceptibility pattern obtained by standard tests. The sensitivity of the array was 100 genome equivalent even with an 10exp7-fold excess of non-pseudomonas DNA. The whole analysis, including DNA processing, array hybridisation and data evaluation could be performed in less than 5 h.
Conclusions: Due to the good correlation with standard procedures, the *Pseudomonas*-array may be used for a rapid susceptibility test even directly from clinical samples. Combined analysis of antibiotic resistance and virulence factors may improve the outcome of an antimicrobial therapy.

P729 Phenotypic and genotypic analysis of *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients

S. Scutera, T. Allice, S. Bianco, M.G. Chirillo, M. Zucca, D. Savoia
Orbassano, I

Objectives: Because of a high prevalence of *Pseudomonas aeruginosa* infections in cystic fibrosis (CF) patients, we conducted a study to assess 60 *P. aeruginosa* isolates collected over 10 years from the sputa of 38 CF adult patients attending an Italian CF centre. Some phenotypic characters of bacteria (O-serotype, motility, production of enzymes and resistance to antibiotics) and their PFGE genotypic patterns were evaluated to analyse for the presence of epidemic strains. Moreover some sequential isolates collected from 15 CF patients were investigated to look for the chronicisation of the infection.
Methods: The strains were identified biochemically. The O-serotype was determined by slide agglutination; the production of enzymes (protease, elastase, gelatinase, haemolysin, beta-lactamase) and motility were detected using specific techniques. The antibiotic susceptibility was analysed by the Vitek AMS System and disc diffusion method. PFGE was used to discriminate the genotypes of *P. aeruginosa*.
Results: In our hands, O serotyping failed to identify 26.3% of isolates, considering the bacteria collected at the onset of colonisation; the most frequent serotypes were O: 10, O: 6 and O: 3. Moreover, the percentages of protease, haemolysin, gelatinase and elastase production were respectively 78.9, 52.6, 55.3 and 39.5, whereas 42.1% of the microorganisms were non-motile. PFGE allowed the typing of all strains except one. The heterogeneity of isolates indicated that cross-infection is unusual; we also observed in several strains isolated in the last years a predominant pattern. Some CF patients were harbouring the same *P. aeruginosa* genotype in sequential isolates and the susceptibility of bacteria to antibiotics tested varies greatly, also in strains belonging to the same PFGE profile.
Conclusion: Our results indicate no relationship between genotype and phenotype suggesting that the phenotypic variability is due to an adaptation of the microorganism to the host. Moreover, the presence of several strains with the same genotypic profile suggests a possible cross-colonisation in CF patients due to the circulation of a transmissible strain.

P730 Construction of unmarked mutants in gene coding for Ser/Thr protein kinases and phosphoprotein phosphatase of *Pseudomonas aeruginosa* and analysis of their properties

J. Nedvedova, P. Lnenicka, K. Hercik, P. Branny
Prague, CZ

Objectives: *Pseudomonas aeruginosa* is an opportunistic pathogen that causes infections in eye, urinary tract, burn, and immunocompromised patients. Three genetic loci of *P. aeruginosa* which encodes Ser/Thr protein kinases were identified. Two of them, ppkA and stk1, were also characterised but little is known about their function in cell signalling. Gene stp1 localised upstream of stk1 encodes Stk1 cognate phosphoprotein phosphatase. A possible relationship between quorum sensing and protein phosphorylation in Gram-negative bacteria has already been described. The aim of this work was to prepare unmarked deletion mutants in ppkA, stk1 and stp1 genes and to find out if the linkage between quorum sensing and protein phosphorylation in *P. aeruginosa* exists.
Methods: To prepare the unmarked deletion mutants an improved method for gene replacement in *P. aeruginosa* which employs a broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally located DNA sequences was used. The phosphorprotein pattern and biochemical properties of the mutants were examined. Double mutant in homoserine lactone synthase genes (lasI and rhlI) was also subjected to phosphoprotein pattern analysis.

Results and conclusion: stk1, stp1 and a double mutant stk1/stp1 were prepared. No differences were found in either biochemical properties or phosphoprotein pattern. Deletion of ppkA gene failed due to the integration of vector into the unknown, but specific site of *P. aeruginosa* genome. The comparison of phosphoprotein patterns of lasI, rhlI double mutant and wild type strain showed important differences. This result suggested that phosphorylation circuit operating in *P. aeruginosa* is related to quorum sensing system(s).

P731 Application of real-time PCR assay to detect fragments of the *Clostridium botulinum* types A, B and E neurotoxin genes for public health microbiology

D. Akbulut, K. Grant, M.M. Brett, J. McLauchlin
London, UK

Objectives: Botulism is a rare but potentially fatal disease generally caused by the neurotoxin produced by *Clostridium botulinum*. Symptoms of the disease include paralysis which is due to BoNT inhibiting neuro-transmitter release. Laboratory diagnosis of botulism relies on detecting BoNT in clinical or food specimens using *in vivo* tests. Diagnosis also includes isolation and identification of the bacterium which again relies on *in vivo* tests for detection of toxin production from the bacterium growing *in vitro*. We previously described the development of real-time PCR assays for BoNTA, B and E gene fragments, and here presented further evaluation data.
Methods: DNA was extracted from faeces, enrichment cultures of naturally contaminated food and clinical samples and from colonies growing on agar plates. TaqMan-based assays for BoNTA, B and E gene fragments were performed using a 7700 Sequence Detector (Applied Biosystems). The assays were performed as a duplex reaction for BoNTA and B using FAM and VIC labelled probes, respectively, and as a monoplex for BoNTE using a single FAM labelled probe. All samples were tested by using the conventional bio-assay and results were compared with real-time PCR assay results.
Results: PCR and bio-assay were found to be consistent in all samples except those that contained 'silent B' neurotoxin genes in addition to BoNTA genes. The samples tested comprised: direct examination of six faecal samples, 18 enrichment cultures for six clinical and 12 foods and 34 pure culture growing *in vitro*.
Conclusion: This study is the first to report the successful identification of different *C. botulinum* toxin types for wild type BoNTA, B and E by using Taq-Man real-time PCR assay. This assay has already provided a useful adjunct to *in vivo* tests for the rapid identification of bacteria containing BoNT genes in wild type *C. botulinum*.

P732 Evaluation of variable number tandem repeats (VNTRs) polymorphisms for genotyping '*Rickettsia conorii* complex' strains

L. Vitorino, R. De Sousa, L. Zé-Zé, F. Bacellar, R. Tenreiro
Lisbon, P

Introduction: Mediterranean spotted fever (MSF) is an acute, febrile tick transmitted rickettsiosis caused by strains of *Rickettsia conorii* complex. MSF is endemic in Portugal and is an obligatory notifiable disease. During 1989–2000 the annual incidence rate of the disease was 9.8/105 inhabitants. In Portugal, MSF is caused by two strains: *R. conorii* Malish and Israeli tick typhus (ITT). This strain was isolated, for the first time in 1997, from a patient. Moreover, data from the National Institute of Health points out that half of MSF cases occurring in Portugal are caused by ITT, showing a similar prevalence of infection as *R. conorii* Malish.
Objective: In this work we present a PCR-based method to detect VNTR sequences that enables amplicon size differentiation between *R. conorii* Malish and ITT human isolate. VNTRs have a high discriminatory capacity not only because they contain greater diversity but also because they often vary in copy, therefore they are being used for molecular typing of many bacteria species.
Methods: Human strains were isolated by Shell-vial technique from patient's total blood. DNA from tick isolates and reference strains were also used for comparative purposes. VNTR loci were identified by the Tandem Repeats Finder software within the *R. conorii* genome. The VNTR65 locus was selected based on the following criteria: repeat units 50 nucleotides in length, 95% nucleotide sequence identity between individual repeat units, and two or more copies of the repeat unit. Primers flanking this sequence were designed to enable VNTR-PCR amplification. The clinical isolates' identification was also confirmed by ompA gene sequencing.
Results: The VNTR sequence chosen was highly informative since it possesses different repeats of the consensus pattern among the strains tested, namely *R. conorii* Malish and ITT. The former contains five tandem repeats and the later only have three repeats of the 65 bp motif unit, which can be easily detected by agarose gel electrophoresis. Therefore, the polymorphism observed enabled discrimination between these two strains. These results are in agreement with ompA gene sequence.
Conclusion: This PCR-based method provides a useful and rapid way for genotyping *R. conorii* Malish and ITT isolates (*R. conorii* complex) which are responsible for MSF in Portugal. OmpA and gltA gene amplification are currently the most widely Rickettsiae detection method used. However, strain identification is only accomplished by sequencing.

P733 Monitoring the ability of the human intestinal microflora to become re-established after antibiotic treatment using T-RFLP

C. Jernberg, Å. Sullivan, C. Edlund, J. Jansson
Huddinge, Stockholm, S

Objectives: To study the composition of the human normal faecal microflora during administration of clindamycin and a probiotic or placebo product. Since only a small portion of the faecal flora is cultivable the samples were primarily analysed using a culture independent molecular fingerprinting technique, terminal-restriction fragment length polymorphism (T-RFLP).
Methods: The study included eight healthy volunteers. All subjects received clindamycin orally for 7 days. Four subjects received a probiotic yoghurt concomitantly containing 10 exp 8 CFU/mL of the strains *Lactobacillus* F19, *Lactobacillus acidophilus* NCFB 1748 and *Bifidobacterium lactis* Bb12. The placebo group received ordinary yoghurt. Faecal samples were taken before the administration (day 0), the last day of administration (day 7) and 14 days after the administration (day 21). The samples were analysed both by conventional cultivation and by T-RFLP. Both universal bacterial primers and *Lactobacillus* specific primers were used when analysing the samples using T-RFLP. The areas of the different Terminal Restriction Fragments (TRFs), each of which theoretically corresponds to one or a group of closely related species, were used to calculate the relative abundance values for the TRFs. These values were used for Principal Components Analyses (PCA) and UPGMA analyses to compare the microbial flora at the three different time points.
Results and conclusions: In the group ingesting the probiotic, the microflora in three out of four subjects became re-established close to their original compositions 2 weeks after antibiotic treatment ceased. By contrast, only one subject out of four in the placebo group had an intestinal microflora that showed tendencies towards normalisation during the same time period. These findings were in accordance with the results from the culture-based analysis. T-RFLP was also used to monitor specific bacterial populations that were either positively or negatively impacted by clindamycin. For example, one of the dominating populations, belonging to the *Clostridium coccoides* subgroup, was highly negatively impacted by clindamycin administration in all subjects. When using lactobacilli specific primers, *L. acidophilus* and *Lactobacillus* F19 were the two dominating populations in the group

receiving the probiotic. T-RFLP was shown to be a reproducible technique for analyses of antibiotic and probiotic induced alterations in the normal intestinal microflora.

P734 Exploring the potential for the rapid identification of *Campylobacter* species using MALDI-TOF mass spectrometry

D. Dare, H. Sutton, C. Keys, H. Shah, M. Lunt, G. Wells
Manchester, London, UK

Laser interrogation of bacteria by matrix assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry (MS) reveals unique fingerprint patterns of biomarkers. These patterns are reproducible for a given set of conditions and can be used as the basis for bacterial identification against a database of known bacterial spectra. Manchester Metropolitan University in collaboration with the Health Protection Agency (UK) and Waters Corporation have created a MALDI-TOF mass spectral database of clinical, environmental and food borne pathogens. These pathogens are all supplied from the UK National Collection of Type Cultures (NCTC). The database spectra are therefore representative of organisms from a world-renowned collection. This database has continued to grow over the last 3 years from the initial 300 to currently over 3000 spectral entries covering ~100 different genera. Bacterial identification using this database is often conclusive with the top five matches suggesting the same genera/species. However for identification to be robust, the strains within the database must be well characterised and their identity well established.

For *Campylobacter* the number of representative strains in the database has increased significantly from around 10 to 170 over 3 years. The species covered within this taxa are: *C. coli*, *C. consicus*, *C. curvus*, *C. fetus*, *C. gracilis*, *C. helveticus*, *C. hyolei*, *C. hyointestinalis*, *C. jejuni*, *C. lari*, *C. rectus*, *C. sputorum* and *C. upsaliensis*. This study presents the results of analysing the same datasets for 21 *Campylobacter* strains against the expanding databases containing 300, 1099, 2159 and >3000 mass spectral entries respectively.

The results demonstrate a significant improvement (i.e. 29–100%) in the number of *Campylobacter* sp. correctly identified as the number of representative strains increase. Therefore MALDI-TOF MS provides a potential rapid identification system for *Campylobacter* sp.

P735 Application of 23S-5S intergenic spacer sequencing for the detection and molecular differentiation of *Legionella* species

F. Grattard, C. Ginevra, S. Riffard, A. Ros, J. Etienne, B. Pozzetto
Saint-Etienne, F

Objectives: Among the more than 40 species of *Legionella* that have been identified so far, 21 have been reported to be pathogenic for humans. By now, the precise identification of clinical isolates in reference laboratories needs the use of monoclonal antibodies or of molecular markers such as 16S rRNA-, mip-, rpoB- or dotA-gene sequencing. We developed a rapid and convenient technique based on the sequencing of the 23S-5S intergenic spacer using non-degenerated primers specific for *Legionella* spp.
Methods: We tested 37 Legionella species (reference and clinical isolates), including 15 serogroups of *L. pneumophila* subsp. *pneumophila*. The amplification step was performed by using a real-time PCR (LightCycler, Roche Diagnostics) and sequencing was performed on the Ceq8000 sequencer (Beckman). The comparative analysis of the sequences was done with the computer program MEGA and the dendrograms obtained by the neighbour-joining method.
Results: The phylogenic tree of the 23S-5S intergenic spacer sequences was found able to clearly differentiate all *Legionella* species at the subspecies level. Actually three subspecies of *L. pneumophila* (subsp. *pneumophila*, subsp. *fraseri* and subsp. *pascullei*) were clearly distinguished. Species sharing the same autofluorescence properties and ubiquinone and fatty acid composition were shown to be phylogenetically related. In addition to rpoB sequencing that was shown previously to exhibit similar results, our technique was found able to detect and identify strains present in clinical or environmental specimens that could not be cultured on agar medium. Although this tool was not discriminatory enough to differentiate all strains of *L. pneumophila* subsp. *pneumophila* at the serogroup level, it was used in two different outbreaks to demonstrate rapidly the identity of the sequences between strains responsible for severe human infection and those isolated in the hot water reservoir, suggesting a common origin.
Conclusion: The 23S-5S intergenic spacer sequencing was found to be suitable for rapid detection and powerful identification of *Legionella* species in clinical settings.

P736 Cerebral Whipple's disease diagnosed by PCR. The first case reported from Greece

M. Papdopoulou, M. Rentzos, V. Ioannidou, A. Ioannidis, C. Nicolaou, S. Chatzipanagiotou
Athens, GR

Whipple's disease (WD) is a rare multisystemic bacterial infection, with variable clinical manifestations occasionally involving the central nervous system. As the cultivation of the aetiologic agent, *Tropheryma whippelii*, is difficult, the laboratory diagnosis is usually based on histological methods. In the last few years, molecular detection of the bacterial 16SrRNS genes by the polymerase chain reaction (PCR) with two primer sets, has greatly contributed to the diagnosis. We present a cerebral case of WD in a 48-year-old male, successfully diagnosed by PCR of *T. whippelii* in the blood and the faeces. As far as we know this is the first case reported from Greece. For the diagnosis of WD histological examination of duodenum biopsy for diastase resistant, non-acid fast, periodic acid Schiff (PAS)-positive inclusions in macrophages, and molecular detection of the 16SrRNA genes of by PCR in CSF, blood and faeces were performed. The histological detection was negative. PCR was positive in the blood and the faeces of the patient and negative in the CSF. Seven months after the onset of antimicrobial therapy, PCR was negative in all three clinical specimens. In conclusion, the application of PCR proved to be an invaluable tool for the recognition, the differential diagnosis and the early start of the antimicrobial therapy of WD, a generally fatal disease, if it remains untreated.

P737 *Corynebacterium amycolatum* ATCC 49368 reference strain is atypical for clinical *Corynebacterium amycolatum* isolates with respect to biochemical properties, fatty acid analysis and DNA analysis

K. Martienssen, A. Becker, D.H. Forster, K. Pelz, A. Sander, E. Kniehl
Karlsruhe, Freiburg, D

Objective: *C. amycolatum* is an emerging pathogen selected by antimicrobial chemotherapy. The aim of our study was to investigate clinical *C. amycolatum* isolates, which were tested biochemically, by fatty acid analysis and by 16S rRNA gene sequencing. Clinical isolates were thereby compared with the *C. amycolatum* ATCC 49368 reference strain.
Methods: 182 clinical isolates of *C. amycolatum*, isolated from 158 in-patients of a tertiary hospital, were identified by API Coryne (bioMèrieux, Marcy L'Etoile, France) and additional tests. Fatty acid analysis was performed from all isolates subcultured on Columbia agar and from 33 isolates subcultured on TSB agar. Specimens were prepared by standard methods and analysed by gas chromatography (Hewlett Packard 5890A; Sherlock Microbial Identification system version 4.0). The first 500 base pairs of the 16S rRNA gene of eight representative isolates were sequenced in both directions using the Microseq 500 Kit (Applied Biosystems, Foster City, CA, USA) on an ABI Prism 310 Genetic Analyser (Applied Biosystems). Consensus sequences were compared with those available in GenBank, EMBL, DDBJ and PDB.

Results: Only one clinical isolate had the same API code profile as the reference strain. Fifteen per cent of clinical strains were tested urease positive, as was the reference strain. By fatty acid analysis, clinical isolates could be separated in four different groups (I–IV), containing 77, 98, 5 and 1 isolates, respectively. ATCC 49368 was grouped to group II. Sequences were obtained from three strains of groups I and II, respectively, and from one strain of groups III and IV, respectively.

Comparison of the determined eight sequences with public databases showed the greatest similarity score with *C. asperum* (X82050.1) with values between 98.7 and 100%. *C. asperum* and *C. amycolatum* are considered as synonyms, because they exhibit a level of DNA-DNA relatedness greater than 90% (Ruimy R *et al. Int J Syst Bacteriol* 1995; 45: 740). Homology with *C. amycolatum* ATCC 49368 (X82057.1) was only between 97.1 and 98.3%. Sequencing of the *C. amycolatum* reference strain yielded 100% homology with the published sequence (X82057.1).

Conclusions: Our data confirm the hypothesis that ATCC 49368 is atypical for clinical *C. amycolatum* strains. Furthermore, our data are in concordance with the observation, that by pyrolysis-gas-liquid chromatography *C. amycolatum* isolates can be separated in two different groups (Voisin S *et al. Res Microbiol* 2002; 153: 307).

P738 Detection of mutations associated with resistance to tetracycline and clarithromycin in *Helicobacter pylori* using the Pyrosequencer

A. Lawson, C. Arnold, R. Owen
London, UK

Objectives: Clarithromycin and tetracycline are key components of *H. pylori* eradication therapy. Resistance to clarithromycin occurs due to single nucleotide mutations in 16S rDNA and an assay to detect these was amongst the first to be developed for the Pyrosequencer. Recently it has been shown that resistance and reduced susceptibility to tetracycline occur due to single, double or triple mutations in 23S rDNA. The aim of this study was to develop a single multiplex assay using the Pyrosequencer to determine susceptibility to clarithromycin and tetracycline from *H. pylori* isolates and direct from gastric biopsy samples.

Methods: Pyrosequencer assays to detect mutations conferring tetracycline and clarithromycin resistance were designed to work singly and in multiplex. The assays were evaluated using 20 isolates with fully characterised 16S and 23S rDNA sequences. Subsequently, DNA extracts from 30 clinical isolates and 20 *H. pylori*-positive human gastric biopsies – all of unknown antibiotic susceptibility – were examined and the results compared with those achieved by conventional culture-based techniques, namely antibiotic disc diffusion and Etest.

Results: The Pyrosquencer multiplex assay correctly determined the 16S and 23S rDNA sequences of the 20 characterised control isolates. When applied to DNA extracted from clinical isolates and gastric biopsy samples, the Pyrosequencer assay was in agreement with the clarithromycin and tetracycline susceptibilities determined by culture-based analysis.

Conclusion: The Pyrosequencer assay allowed rapid determination of clarithromycin and tetracycline susceptibility from both *H. pylori* isolates and gastric biopsy samples. The sequence data obtained for the mutations occurring in each strain may provide useful epidemiological information and guide patient management.

P739 Detection of *Helicobacter pylori* infection by ELISA and Western blot techniques and evaluation of anti CagA seropositivity in adult dyspeptic patients

N. Sen, Ö. Yilmaz, A.A. Küpelioglu, I. Simsek
Izmir, TR

Objectives: *Helicobacter pylori* is a common human pathogen implicated in certain gastrointestinal diseases and extra gastrointestinal diseases. The aim of this prospective pilot study was to detect IgA, IgG and anti-CagA antibody status and to evaluate the correlation with anti- *H. pylori* IgA, IgG Western blot and ELISA tests in adult dyspeptic patients.

Methods: Upper gastrointestinal endoscopy, two from gastric antrum and two from corpus, was performed in 56 patients (mean age ±46.41) with dyspeptic symptoms. *H. pylori* was assessed by rapid urease test and by histopathologic examination in these biopsy specimens. Patients' sera were tested by anti- *H. pylori* IgA, IgG Western blot, IgA, IgG ELISA and anti-CagA-IgA, IgG ELISA (EUROIMMUN Medizinische Labordiagnostika, Lübeck) tests.

Results: A total of 56 patients were evaluated and *H. pylori* infection was diagnosed in 48 (85.71%) patients by rapid urease test and/or histopathology. Serological anti- *H. pylori* test results were shown as below (Table 1). Twenty-eight (50%) of 56 adult dyspeptic patients sera were positive for anti-CagA-IgG ELISA and 17 (30.35%) were positive for anti-CagA-IgA ELISA.

Table 1. Correlation of westernblot/ELISA test results of 56 adult dyspeptic patients sera

		Westernblot IgG				Westernblot IgA		
		Positive	Borderline	negative		Positive	Borderline	Negative
ELISA IgG	Positive	40	4	2	ELISA IgA Postive	15	4	8
	Borderline	3	–	–	Borderline	1	1	5
	Negative	2	5	–	Negative	3	–	19

Conclusion: Infection with *H. pylori* results in the production of local and systemic antibodies. Cag A is the important pathologic marker with high immunogenic power. A set of serological tests may give more accurate determination of *H. pylori* infection than one test detecting specific antibody or bacterial antigen. It seems that there is a good correlation with Western blot and ELISA test results and gold standards.

Acknowledgement: This work was supported by EUROIMMUN Medizinische Labordiagnostika, Lübeck, Germany.

P740 Susceptibility of *Helicobacter pylori* isolates to the anti-adhesion activity of a high-molecular-weight constituent of cranberry

H. Shmuely, O. Burger, I. Neeman, J. Yahav, Z. Samra, Y. Niv, N. Sharon, E. Weiss, M. Tabak, A. Athamna, I. Ofek
Petach Tiqva, Haifa, Rehovot, Jerusalem, Kfar Qaraa, Tel Aviv, IL

Background: Previous studies have shown that a high molecular mass non-dialysable constituent derived from cranberry juice inhibited the adhesion of *Helicobacter pylori* to human gastric mucus and to human erythrocytes. The aim of the present study was to determine the sensitivity of a large number of both antibiotic-resistant and susceptible clinical isolates of *H. pylori* to the anti-adhesion effect of the cranberry constituent.

Material and methods: Confluent monolayer of gastric cell line in wells of a microtitre plate was exposed to bacterial suspensions prepared from 83 *H. pylori* clinical isolates, including 17 from patients after treatment failure. Adhesion was estimated by the urease assay to calculate the percent inhibition of adhesion by the non-dialysable material. Antibiotic susceptibility of *H. pylori* isolates to metronidazole, tetracycline and amoxicillin were tested by the Etest.

Results: In two-thirds of the isolates, adhesion to the gastric cells was inhibited by 0.2 mg/mL of the non-dialysable material. All isolates were susceptible to amoxicillin and tetracycline and 35 isolates (42%) were resistant to metronidazole. There was no relationship between the anti-adhesion effect of the cranberry material and the resistance to metronidazole in isolates from either the antibiotic-treated or untreated patients. Most important, only 13 isolates (16%) were resistant to both non-dialysable material and metronidazole and 30 isolates (36%) were resistant to the

non-dialysable material alone. No cross-resistance of the isolates to cranberry constituent and metronidazole was found.
Conclusions: The data suggest that a combination of antibiotics and a cranberry preparation may improve the eradication of *H. pylori*.

P741 *Helicobacter pylori* infection in Venezuela. Public health problem

M.E. Cavazza Porro, M. Correnti, M.I. Urrestarazu, J. Vivas, M.E. Avila, D. Ortiz, V. Lecuna, R. Piñero, M. Perrone
Caracas, San Cristóbal, VE

Objectives: (1) Evaluation of the seroprevalence of *Helicobacter pylori* infection in different counties of Venezuela and its association with grastroduodenal diseases. (2) Analyse the presence of the gene cagA and VacA in the *H. pylori* isolates from cancer endemic area (San Crsitobal) and Metropolitan area of Caracas.
Methods: For the seroprevalence study a total of 1041 people of different states from the country were evaluated: 370 symptomatic and 406 asymptomatic adults; 27 symptomatic and 238 asymptomatic children. The determination of specific IgG antibodies was made by commercial ELISA. The presence of the gene cagA was evaluated in 133 patients of the metropolitan area and the Center of Gastric Cancer Control of San Cristóbal (endemic zone of gastric cancer). The detection of VacA was determined in 29 biopsy from patients of San Cristóbal and 36 biopsy from patients of the metropolitan area. The biopsies were analysed by different methods for diagnosis of *H. pylori*: culture, urease test, polymerase chain reaction and RAPDS for genotyping the *H. pylori* isolates.
Results: The percentage of asymptomatic children with values of specific IgG antibodies anti-*H. pylori* (over 300 U) varies from 30 to 60% (Metropolitan area vs. San Cristóbal). In symptomatic adults groups, the seroprevalence was between 68 and 93% according to the studied geographic area. A decreased title of IgG antibodies anti-*H. pylori* was observed in patients with diffuse antral gastritis associated with metaplasia type II. In the group of endemic cancer area the titles of IgG anti-Hp were elevated in patients with antral diffuse gastritis. The cagA gene was detected in 46% of patients of the Metropolitan Area unlike the group of patients of San Cristóbal a smaller frequency was observed (26.41%) ($P < 0.001$). A high incidence of S1a and m2 genotype was observed in the *H. pylori* isolated from the patients of endemic gastric cancer area (40%), unlike what we observed in the metropolitan *H. pylori* isolates where an elevated prevalence of S1b and m1 genotypes was found.
Conclusions: In our country we observed a high prevalence of *H. pylori* infection in adults and children. The determination of the cagA and VacA genes of *H. pylori* demonstrates a high genetic variability of this bacterium in our population.
Acknowledgement: Grant FONACIT Scientific Millenium 4572-B.

P742 Primary antibiotics susceptibility of *Helicobacter pylori* in a general hospital

N. Montiel, J.M. Navarro, J.L. Prada, J. De La Torre, A. Del Arco, C. De Sola, I. Lopez
Marbella, E

Objectives: To determine *H. pylori* primary resistance to different antibiotics in our area.
Methods: From patients diagnosed of dyspepsia in the Gastroenterolgy Unit of Costa del Sol Hospital, we have studied 148 samples from gastric antro. No current resident in our area and patients treated previously with eradicated therapy have been excluded. Samples were homogenised and cultured in blood-agar, chocolate-agar, pylori-agar and tioglicolate broth. It was incubated to 37°C in microaerofile atmosphere during 5–7 days. We studied the susceptibility to: amoxicillin (Am), claritromicin (Ch), metronidazole (Mz), tetracycline (Te) and ciprofloxacin (Cp) by detection of IMC by E-test (Biodisk®). We have followed NCCLS criteria for antibiogram lecture.
Results: From 148 samples, 60 were males and 88 females. We found the follow primary resistance; Ch 16 (10.8%), Mz 52 (35.1%), Te 8 (5.4%), Am 2 (1.4%), Cp 23 (15.5%) and 10 samples (6.8%) with a mix resistance to CH and Mz. Ch and Mz resistance are more common in females, but the difference is only statistically significant for Mz (P: 0.037).
Conclusions: There is a progressive increased antibiotic resistance in *H. Pylori* in our area. This may be related with a raised used of antibiotics for other indications. CH resistance data agree with other Spanish and multicentre European studies, which show a foremost rate in the Mediterranean area. The Mz resistance is higher than other Spanish works. Our high prevalence of resistance supports the idea of avoiding imidazol therapy as primary choice treatment.

P743 The prevalence and consequences of antibiotic resistance in Danish *H. pylori* strains isolated with an interval of 10 years

S. Hartzen, H. Colding
Charlottenlund, Copenhagen, DK

Objectives and Background: The treatment of *H. pylori* (HP) infections is complex and the use of combination therapy is imperative. The choice of the antibiotics is often made exclusively on empirical basis although resistance to many therapeutically relevant antibiotics has been described. The mainstay of HP treatment in Denmark is various combinations of normally two of the following antibiotics: metronidazole, amoxicillin, tetracycline and clarithromycin. To clarify whether these compounds were to remain the drugs of choice we decided to determine the susceptibilities of metronidazole, clarithromycin, tetracycline, and amoxicillin against 180 HP strains recently isolated from patients with duodenal ulcer. The results were compared with results previously obtained by us in 1993 using a similar methodology.
Materials and methods: Strains: 360 Danish HP strains, 180 from about 1990–93, 180 from 2000–02. Susceptibility testing: E-test AB Biodisk®,Sweden. Antibiotics: Amoxicillin, metronidazole, clarithromycin, tetracycline. Medium: Isosensitest® agar with 10% horse blood. Inoculum: 10^6 CFU/mL. Microaerobic incubation for 72 h.
Results: Amoxicillin: 1993 MIC_{50} 0.02 mg/L, MIC_{90} 0.03 mg/L, 2002 MIC_{50} <0.02 mg/L, MIC_{90} 0.03 mg/L. Metronidazole: 1993 MIC_{50} 2 mg/L, MIC_{90} 64 mg/L, 2002 MIC_{50} and MIC_{90} >256 mg/L. Tetracycline: 1993 MIC_{50} 0.25 mg/L, MIC_{90} 0.5 mg/L, 2002 MIC_{50} 0.125 mg/L, MIC_{90} 0.5 mg/L. Clarithromycin: 1993 MIC_{50} 0.03 mg/L, MIC_{90} 0.06 mg/L, 2002 MIC_{50} 0.02 mg/L, MIC_{90} 0.06 mg/L.
Conclusions: Over a period of 10 years only the development of resistance to metronidazole appears to constitute a problem. Otherwise HP has remained remarkably susceptible to these therapeutically relevant antibiotics. On the basis of our results we recommend that surveillance of especially metronidazole resistance in Denmark is markedly intensified, e.g. by increasing the use of diagnostic methods of HP infections that allow susceptibility testing. In cases where treatment with metronidazole is considered, susceptibility testing is of course of major importance, if not downright necessary.

P744 Prevalence of *Helicobacter pylori* resistance to clarithromycin in Turkey

F. Can, M. Demirbilek, H. Selcuk, H. Arslan, S. Boyacioglu
Ankara, TR

Objectives: *Helicobacter pylori* is the main causative agent of peptic ulcer disease. Clarithromycin resistance of *H. pylori* is the common reason of failure of the eradication therapy, which includes amoxicillin–clarithromycin and proton pomp inhibitor. The aim of this study was to determine the prevalence of clarithromycin resistance among *H. pylori* strains isolated from gastric biopsies obtained during routine endoscopies at the Baskent University Medical Faculty in Ankara, Turkey.
Methods: *H. pylori* strains were isolated from antral biopsy specimens taken from dyspeptic patients. Antibiotic susceptibilities of the isolates to clarithromycin were performed using the NCCLS approved agar dilution and the E test methods.
Results: 78 *H. pylori* isolates were included in the study. Clarithromycin resistance was found in 16 (20.5%) of the isolates. The resistance rates were similar by the E test and agar dilution methods.
Conclusion: The percentage of the clarithromycin resistance among *H. pylori* strains in our population is significantly high. This information is important to monitoring the eradication therapy and defines regional treatment policies.

P745 Low seroprevalence of anti-Cag A in spite of high seroprevalence of anti-*Helicobacter pylori* antibodies in a rural Egyptian community

S. Zakaria, E. El Sherbini, M. El Raziki, S. Saad El Din, M. Mubarak, R. Salama, S. Zakaria
Cairo, Giza, EGY

Introduction: *Helicobacter pylori* has been the subject of many studies that contributed to a better understanding of its epidemiology and its clinical importance in the pathology of the upper gastrointestinal tract, being an important cause of duodenal, gastric ulcers and a definite cause of gastric adenocarcinoma in human.
Objectives: to determine the seroprevalence of *H. pylori* among population living in rural community, in relation to the epidemiological aspect, and to study the seroprevalence of anti-CagA as a virulence factor in a step that might be helpful in studying the magnitude of *H. pylori* infection. Also, to determine a cut-off value among the population in this community.
Subjects and methods: This is a community based, field study which was performed on 605 randomly chosen subjects representing villagers of eight villages in Giza governorate Egypt. Serological testing for anti- *H. pylori* and anti-Cag A were performed by ELISA.
Results: The overall seroprevalence of anti- *H. pylori* IgG was 91.7% with different degrees of positivity: 40.8% mild, 39.2% moderate and 11.7% high. Anti-Cag A was present in 10.6%. There was a significant agreement between the presence of the two antibodies; however, on studying the relation of anti-*H. pylori* IgG level with anti-CagA no statistically significant relation was found denoting that the level of infection even if mild does not rule out the possible association of virulent strain of *H. pylori*. No age or sex difference was noted as regards anti-*H. pylori* seropositivity but subjects seropositive for anti-Cag A had a statistically significant higher mean age. When relating the seroprevalence of anti-*H. pylori* to type of community, it was found to be the same in semi-rural communities and rural ones and when investigating the respective conditions in both communities it was found that the prevalence is rather related to pattern of life, socioeconomic status and to other possible vehicle of transmission as animals or flies than faecaly contaminated water which is not considered the only vehicle for *H. pylori* transmission in our study.
Conclusion: *H. pylori* is holoendemic in Egypt; however, infection by virulent strains is not common.

ESBL and metallo-beta-lactamases - I

P746 Extended-spectrum beta-lactamases detection in the clinical microbiology laboratory

H. Moraitou, I. Galani, S. Kanavaki, S. Karabela, M. Makarona, L. Choraiti, H. Giamarellou
Athens, GR

Objectives: Extended-spectrum beta lactamases (ESBLs) are an increasing cause of resistance in Enterobacteriaceae. Unfortunately, the laboratory detection of ESBLs can be complex and, at times, misleading. The aim of this study was to determine whether routine methods performed in a clinical microbiology laboratory of a tertiary care Hospital, are adequate for detecting emerging ESBL producing clinical isolates.
Methods: To evaluate the ESBL confirmation protocol, we collected 29 Enterobacteriacae strains, isolated in our Laboratory. Each isolate met the NCCLS screening criteria for potential ESBL producers (ceftazidime or cefotaxime MICs were $\geq$2 for all isolates). We tested 13 *Kl. pneumoniae*, five *Ent. cloacae*, two *Ent. aerogenes*, five *E. coli* and four *Pr. mirabilis* strains, by methods routinely used in our laboratory. Initially, the isolates were tested for clavulanic acid effect by disk diffusion method and all were analysed by the Vitek2 automated system (bioMerieux, France), which performs a susceptibility testing, by determining the MIC breakpoints. The Advanced Expert System (AES) of Vitek2 was set on the phenotypic resistance knowledge-based system and the panel GN020 was used. In parallel, the isolates were tested by the ESBL E-test with ceftazidime and cefotaxime plus beta lactamase inhibitor (AB, Biodisk, Sweden). In order to confirm the ESBL production, all strains were tested by isoelectric focusing (IEF) followed by PCR for blaTEM, blaSHV, blaOXA, blaIBC and blaCTX genes.
Results: Twenty-one out of 29 isolates proved to produce ESBLs by molecular methods. All *Enterobacter* strains and one *Proteus mirabilis* were not ESBL producers. No blaOXA or blaIBC genes were detected. The PCR detection of ESBL genes results were compared with the double disk diffusion, Vitek2 and ESBL E-test to estimate the sensitivity, specificity and the predictive value of the methods tested. The sensitivity of the methods was 84.6, 85.7 and 70.5%, respectively, the specificity 62.5, 87.5 and 66.6%, respectively, and the predictive value 64.7, 93.3 and 75%, respectively.
Discussion: Given the increasing incidence of ESBL producing clinical isolates, it is important that ESBL screening is incorporated into routine diagnostic testing. The backup of the simple disk diffusion method by the automated Vitek2 system increases the possibility of identifying ESBL activity of clinical strains in the Hospital Microbiology Laboratory setting.

P747 Rapid genotyping of the ESBL TEM beta-lactamases using DNA-microarrays

V. Jung, S. Ezaki, M. Susa, C. Knabbe, R. Schmid, T. Bachmann
Stuttgart, D

Objectives: Extended-spectrum beta-lactamases (ESBL) are plasmid-mediated beta-lactamases and most of them are mutant of TEM or SHV beta-lactamases. ESBLs have been associated with

clinical failures due to serious interpretive problems of standard laboratory tests. Detection of ESBLs remains a challenge for the laboratory, since routine tests for monitoring a susceptibility to oxyimino-cehalosporins and aztreonam have not been sensitive enough to detect ESBL strains and require up to 2 days. We describe an oligonucleotide array for rapid identification of single nucleotide polymorphisms (SNPs) of the ESBL TEM beta-lactamases.
Methods: Plasmid DNA was amplified and Cy5 labelled during PCR with consensus primer pair flanking the blaTEM gene. Oligonucleotide arrays were constructed with 168 oligonucleotide capture probes. The probes were designed with the SNP at the central base of the probe sequence for maximum perfect match/mismatch discrimination.
Results: 40 of 41 SNP positions were correctly identified. The signal intensity values ranged up to 20 000 for the perfect match probes. The discriminatory power of the array expressed as relative intensity of mismatches (RIMM) remained for 99% of the mismatches below 0.4. A perfect match was considered as correctly identified, if RIMM did not exceed 0.7. Analysis of the array reproducibility revealed that in analysed blaTEM-1 samples all 41 SNP positions could be identified. The mean RIMM values varied, but 95% remained below 0.4. In DNA isolated from clinical samples all mismatches in blaTEM were identified without ambiguity, and 91% of them remained below the RIMM limit. Since the reduction of the array-hybridisation time to 30 min had no influence on RIMM (RIMM limit less than 0.6 for 95% mismatch positions), the assay may be performed within 3.5 h while keeping its discriminatory power.
Conclusion: The blaTEM gene variants could be amplified by the use of a single consensus primer pair. Using DNA-array we were able to discriminate SNPs in 102 of the 106 TEM variants. SNP mismatches could be analysed by array within 3.5 h enabling the identification of the corresponding ESBLs or inhibitor resistant TEMs.

P748 A simple, relevant and cost-effective ESBL testing protocol for a routine clinical microbiology laboratory: a tertiary care hospital study

A. Guleri, S.R. Alcock, G.D. Corcoran, S. Samavedam, J. Robinson, J. Wilson
Glasgow, UK

Background: Routine, cost effective and clinically relevant testing for ESBL/AmpC beta lactamases is of growing concern. A pilot study was conducted to establish the prevalence of ESBL strains in clinical isolates from high-risk units (ITU, HDU, nephrology, oncology, haematology) and to develop an optimal protocol for routine ESBL testing.
Methods: 72 clinical isolates of Gram-negative rods resistant (NCCLS disc test) to cefotaxime and ceftazidime were tested for ESBL/Amp-C beta lactamases. Three test panels were used: (1) ESBL E-test (AB Biodisk) panel: cefepime, ceftazidime and cefotaxime, each ± clavulanate; (2) ESBL combination discs (Oxoid) conventional panel: cefpodoxime, ceftazidime, and cefotaxime, each ± clavulanate, and cefoxitin alone; (3) Combination discs extended panel: Cefpirome (Oxoid) and cefepime (MAST), each ± clavulanate. Simultaneously MICs of meropenem, ertapenem, piptazobactam, ciprofloxacin, gentamicin and co-trimoxazole were estimated by E-test.
Results: Of the 72 isolates, 20 produced ESBL only, 23 AmpC β-lactamase only and 29 produced both. The isolates were *Enterobacter cloacae* (26), *Escherichia coli* (15), *Klebsiella. pneumoniae* (12), *Serratia marcescens* and *Cit. freundii* (four each), *Serratia liquefaciens* (three), *Cit. koserii*, *Morganella morganii*, *Salmonella virchow* (two each), *Cit. youngae* and *Enterobacter aerogenes* (one each). Of the overall 49 ESBLs, the E-test panel detected one additional isolate compared with the extended combination disk panel but missed three. The conventional combination disc panel missed detecting ESBL in four isolates, which were detected by the extended disc panel. MIC ranges (mg/L) were: meropenem 0.008–0.125, ertapenem 0.006–0.5, piptazobactam 0.5–256, ciprofloxacin 0.006–32, gentamicin 0.125–256 and co-trimoxazole 0.064–256.
Conclusion: Use of more than one substrate for screening, and a combination of ESBL combination discs including cefepime and cefpirome (each ± clavulanate) for confirmation appears to make ESBL testing more sensitive. ESBL E-test strips are simpler to use and almost as sensitive as the combination of discs. All isolates were sensitive to meropenem and ertapenem, but showed varying degrees of resistance to piptazobactam, ciprofloxacin, gentamicin and co-trimoxazole. Details with illustrations to be discussed.

P749 Characterisation of Enterobacteriaceae producing extended-spectrum beta-lactamase enzymes in Scotland

F.M. MacKenzie, A. Leslie, I.M. Gould
Aberdeen, UK

Objectives: To characterise Scottish strains of Enterobacteriaceae producing Extended Spectrum β-lactamase (ESBL) enzymes and to characterise the ESBL enzymes produced by these strains.
Methods: Sixty-four isolates from six hospitals throughout Scotland were screened positive for ESBL production and sent to our laboratory for confirmation. All isolates were screened for ESBL activity by disc diffusion, the Jarlier Double Disc test and the NCCLS confirmatory test. Thirty isolates were then selected comprising a representative of each species from each hospital. These underwent MIC testing and isoelectric focusing (IEF) to presumptively identify the β-lactamases present.
Results: 53 of the 64 isolates confirmed as ESBL producers by the Jarlier Double Disc test and/or the NCCLS confirmatory test. Of these, one confirmed by the Jarlier Double Disc test only and four by the NCCLS confirmatory test only. The 53 ESBL positive isolates underwent disc diffusion testing; 100% appeared susceptible to imipenem, 62% to piperacillin/tazobactam, 32% to cefotaxime, 31% to ceftazidime, 28% to co-amoxiclav, 9% to cefuroxime, 7% to cefpodoxime and 0% to ampicillin. MICs were carried out on 25 ESBL positive isolates; 100% appeared susceptible to imipenem, 40% to cefpirome, 32% to cefotaxime, 32% to ceftazidime and 0% to cefpodoxime. By IEF 0–7 bands were detected (mean, median and mode = four bands). Eleven of 25 isolates produced both TEM and SHV-like enzymes, 10/25 produced TEM-like enzymes and 3/25 produced SHV-like enzymes. TEM-like enzymes were most prevalent in *E. coli* and *Klebsiella* species, whereas SHV-like enzymes were most prevalent in *Enterobacter* species.
Conclusions: ESBL enzymes are produced by a wide variety of species in Scotland. By disc diffusion and MIC testing, ESBL positive isolates appeared falsely susceptible to the third-generation cephalosporins. The IEF results were diverse and complex and showed that TEM- and SHV-like enzymes are both prevalent in Scotland.

P750 Occurrence and transferability of beta-lactam and aminoglycoside resistance in clinical isolates of the family Enterobacteriaceae obtained from different wards of a hospital in Nitra (Slovakia)

M. Vrabelova, P. Dianiskova, D. Michalkova-Papajova, A. Liskova, M. Kettner
Bratislava, Nitra, SK

Objectives: The aim was to study the occurrence, mechanisms and transferability of beta-lactam and aminoglycoside resistance in the set of 34 clinical isolates (10 *Serratia marcescens*, six *Citrobacter freundii*, six *Enterobacter cloacae*, five *Klebsiella pneumoniae*, three *Morganella morganii*, two *Escherichia coli*, one *Proteus mirabilis*, one *Providencia rettgeri*) obtained from 13 different wards of the Hospital Nitra (Slovakia) in 2001.
Methods: The level of beta-lactam and aminoglycoside resistance was determined by standard agar dilution method according to

the NCCLS recommendations. The production of extended-spectrum beta-lactamases (ESBL) was detected by double diffusion test. The presence of blaTEM gene was determined by PCR method. Transferability of resistance determinants was studied by bacterial conjugation.
Results: 70.5% of the clinical isolates were resistant to ampicillin (AMPI); 76.5% to cefoxitine (CFOX); 55.8% to cefotaxime (CTAX); 55.8% to ceftazidime (CTAZ); 41.2% to ceftriaxone (CIAX); 14.7% to cefepime (CEPI); 58.8% to azthreonam (AZTR); 5.8% to meropenem (MERP); 53.0% to gentamicin (GEN); 41.0% to tobramycin (TOB); 8.8% to netilmicin (NET); 5.8% to amikacin (AMI); 8.8% to isepamicin (ISE); 55.8% to ciprofloxacin (CIP). A total of 61.8% of clinical isolates were identified as ESBL producers. The presence of blaTEM gene coding for TEM-type beta-lactamases was detected in 82.4% of clinical isolates tested. Resistance determinants to all antibiotics tested, with only one exception of MERP, were transferable by bacterial conjugation to the recipient strain *Escherichia coli* K-12 3110. Frequency of transfer ranged from 7.1×10^{-9} to 1.2×10^{-1}.
Conclusions: The occurrence of resistance to beta-lactam antibiotics was very high. The most efficient beta-lactams were the carbapenem meropenem and the fourth-generation cephalosporin cefepime. Aminoglycoside antibiotics netilmicin, amikacin and isepamicin had high efficiency, too. On the other hand, more than one half of the clinical isolates tested were resistant to the fluoroquinolone ciprofloxacin. Beta-lactam resistance was due to the production of ESBL and to the presence of the blaTEM gene in the majority of clinical isolates. Transferability of beta-lactam and aminoglycoside resistance determinants by bacterial conjugation is important from the epidemiological point of view.

P751 Clinical effectiveness of cefepime in case of third-generation cephalosporins treatment failure in nosocomial infections caused by Enterobacteriaceae producing extended-spectrum beta-lactamases

O. Romashov, S. Yakovlev, S. Sidorenko, A. Berezin
Moscow, RUS

Objectives: Today there are very few significant data on the effectiveness of cephalosporin antibiotics in nosocomial infections caused by the microorganisms producing ESBL.
Methods: The cases of nosocomial infections caused by Enterobacteriaceae with a proved ESBL production were analysed. ESBL producing Enterobacteriaceae strains were assayed for susceptibility to different antimicrobials and MICs were determined by a broth microdilution method. To determine molecular typing of ESBL genes polymerase chain reactions and sequencing reactions were used. Patients received initial empiric intravenous antibacterial therapy with third-generation cephalosporin (cefotaxime). In case of failure cefepime 4 g a day was prescribed. Results of those infections treatment with third- and fourth-generation cephalosporins were assessed depending on MIC.
Results: ESBL production with specific SHV and CTX oligonucleotids was proved for six strains of Enterobacteriaceae in four patients with nosocomial pneumonia (in two cases mixed infection took place), among them four strains were *Klebsiella* spp. and two strains *E. coli*. The analysis of the dependence of MIC on the results of the treatment gave the following results (Table 1). It may be stated that with the proved Enterobacteriaceae ESBL production MIC values for third-generation cephalosporins of the majority of strains were within the resistance range (more than 32 μg/mL), and these antibiotics were not effective in all cases. As for cefepime, MIC showed intermediate sensitivity (32 μg/mL) to the drug only in 33.3% cases; the rest of the strains (MIC = 1–8 μg/mL) were sensitive. The therapy with cefepime was effective in three of four patients.
Conclusion: Effectiveness of third-generation cephalosporins correlates with existing NCCLS criteria: if MIC value is high the therapy is not effective. MIC value for fourth-generation cephalosporins does not have much effect on the drug clinical effectiveness: cefepime preserves clinical effectiveness for ESBL producers with MIC range from 1 to 32 μg/mL inclusively.

Table 1.

			Initial therapy			Effective theraphy		
Patient	Pathogen	ESBL	Antibiotic	MIC	Outcentre	Antibiotic	MIC	Outcome
Z	*E. coli*	SHV	Cefotaxime	16	Failure	Cefepime	2	Cure
	K. pneumoniae	SHV		128			32	
H	*E. coli*	CTX-UNI.A		128			32	Cure
	K. pneumoniae	SHV		16			1	
T	*K. pneumoniae*	SHV		64			8	Cure
P	*K. pneumoniae*	SHV		128			2	Failure

P752 High prevalence of CTX-M type extended-spectrum beta-lactamases in members of Enterobacteriaceae in Turkey

Z. Gulay, G. Terek, B. Eraç, Ç. Bal, D. Gur, I. Köksal, C. Ozakin, M. Ozinel, B. Sumerkan
Izmir, Istanbul, Ankara, Trabzon, Bursa, Kayseri, TR

Objective: To find the prevalance and predominant types of CTX-M type beta-lactamases in Enterobacteriaceae isolated in different regions of Turkey.
Methods: Seventy clinical isolates recovered between November 2002 and July 2003 at seven medical centres located in six cities, were taken into the study. Isolates were chosen randomly on a 10 isolate per centre basis. The isolates consisted of 34 *Escherichia coli*, 23 *Klebsiella pneumoniae*, eight *Enterobacter* spp., two *K. oxytoca*, two *Proteus mirabilis* and one *Shigella sonnei*. All isolates were extended spectrum beta-lactamase (ESBL) producers according to the NCCLS criteria and confirmatory tests. B-lactamases were characterised by PCR amplification using specific primers. Conjugation experiments and isoelectric focusing was also performed. PCR products of 20 isolates (two to three isolates per centre) were sequenced in both strands.
Results: The prevalance of CTX-M type beta-lactamases was 50–100% at different centres. Enzyme production was detected in 76.5, 82.6 and 50% of *E. coli*, *K. pneumoniae* and *Enterobacter* isolates, respectively. One *K. oxytoca* and the *Shigella* isolate also produced CTX-M type ESBL(s). None of the *P. mirabilis* isolates were CTX-M producers. The beta-lactamases were transferred to the recipient *E. coli* K-12 (SmR) from 66% of the donors. Sequencing results revealed CTX-M-3 production in all isolates.
Conclusion: This study shows that CTX-M type enzymes and particularly CTX-M-3 is widely disseminated in microorganisms belonging to the family of Enterobacteriaceae in Turkey.

P753 Prospective evaluation of carriage and colonisation with extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae among newly admitted patients in medical departments

B. Rudensky, D. Raveh, E. Zartzer, E. Broidie, A.M. Yinnon
Jerusalem, IL

Objectives: To determine the colonisation rate of ESBL producing Enterobacteriaceae among patients newly admitted to departments of internal medicine, the rate of acquisition of these organisms during hospitalisation, and risk factors for both.

Methods: During a 5-month period, a representative sample of 167 newly admitted patients was enrolled. A nasal swab and a rectal swab were obtained on admission and subsequently every 3–4 days until discharge or death. ESBL was tested by double disk method for cefotaxime and ceftazidime with and without clavulanic acid. In addition, nasal swabs were tested for presence of methicillin resistant *Staphylococcus aureus* (MRSA).
Results: On admission, 15 patients (9%) were nasal carriers of MRSA; in addition, five (3%) and 13 (8%) patients were nasal and rectal ESBL carriers, respectively. Bivariate risk factors for rectal ESBL carriage included: male gender, functional dependency, recent antibiotic treatment, and concomitant nasal carriage of MRSA or ESBL ($P < 0.05$ to $P < 0.005$). Multivariate indicators were male gender and recent antibiotic treatment. During hospitalisation, rectal carriage increased from 13 (8%) to 35 patients (24% of the cohort, P for trend <0.001). Bivariate risk factors for acquisition included: older age, functional dependency, impaired cognition, recent antibiotic treatment, chemotherapy, being mechanically ventilated, and nasal MRSA carriage ($P < 0.05$–0.001). Multivariate indicators were older age and recent antibiotic therapy ($P < 0.05$ to $P < 0.01$). Out of 35 rectal ESBL carriers, six (21%) died, compared with 8/83 (9%), who were not ESBL carriers ($P = 0.1$).
Conclusion: Nasal and particularly rectal ESBL colonisation occurs in a significant percentage of patients admitted to our medical departments. Acquisition of rectal ESBL+ organisms increases to 25% of all patients within 2 weeks. Risk factors for both phenomena were identified. Isolation and cohorting of colonised patients, although in theory the way to prevent spread of ESBL organisms among hospitalised patients, may be difficult to implement because of the large number of colonised patients. We fear an imminent increase in infections due to ESBL+ strains with attendant increased use of broad-spectrum antimicrobials.

P754 Extended-spectrum beta-lactamases in isolates of Enterobacteriaceae from inpatients and outpatients in Northern Italy

F. Luzzaro, M. Perilli, E. Dell'Amico, S. Stefani, G.M. Rossolini, G. Amicosante, A. Toniolo
Varese, L'Aquila, Siena, Catania, I

Objectives: Extended-spectrum beta-lactamases (ESBL) are important resistance traits for nosocomial isolates of Enterobacteriaceae and their spread in the community generates increasing concern. This study was performed to investigate the prevalence of ESBL producers in *Enterobacteria* isolated from both inpatients and outpatients in three different geographic areas of Northern Italy.
Methods: 2600 consecutive nonreplicate isolates of Enterobacteriaceae were collected at three Clinical Microbiology Laboratories (Novara, Varese, and Verona; 1850 isolates from inpatients and 750 from outpatients) from June to November 2003. ESBL production was screened for by MIC analysis and confirmed by the double-disk synergy test. ESBL determinants of the TEM-, SHV-, CTX-M-, and PER-type were investigated by molecular analysis (colony-blot hybridisation, PCR and DNA sequencing).
Results: The average prevalence of ESBL producers was 7.5% among isolates from inpatients (range 4.8–8.5% in different hospitals) and 2.5% among outpatient isolates (range 0.8–5.6% in different geographic areas). Approximately 70% of the ESBL-producing strains belonged to just three species: *Proteus mirabilis* ($n = 45$), *Escherichia coli* ($n = 34$), and *Klebsiella pneumoniae* ($n = 31$). Most ESBLs were of the TEM- or SHV-type, the TEM-52 and SHV-12 enzymes showing the highest prevalence. CTX-M type enzymes appeared to emerge, with most of these resistance traits detected in *E. coli* isolates. Enzymes of the PER-type were not found.
Conclusions: ESBL producers are widespread in different hospitals of Northern Italy and are also present in the community, though at lower rates. A variety of ESBL has been detected in several different species of Enterobacteriaceae and their expression is usually associated with multi-drug resistance. Thus, surveillance of ESBL dissemination appears to be important also for community acquired isolates.
Acknowledgement: Supported by an educational grant from Wyeth-Lederle SpA, Italy.

P755 CTX-M is the dominating extended-spectrum beta-lactamase type in Norwegian clinical ESBL-producing isolates of *Escherichia coli* and *Klebsiella pneumoniae*

S. Tofteland, B. Haldorsen, K. Dahl, G. Simonsen, M. Steinbakk, T. Walsh, A. Sundsfjord
Tromsø, Akerhus, N; Bristol, UK

Background: We have limited knowledge regarding the prevalence and molecular epidemiology of extended spectrum betalactamase (ESBL)-producing clinical isolates of Enterobacteriaceae in Norway.
Objectives: (i) Evaluate methods to detect ESBL-production in Norwegian clinical isolates of Enterobacteriaceae. (ii) Perform ESBL-genotyping.
Methods: Consecutive isolates of Enterobacteriaceae with reduced susceptibility to third-generation cefalosporins and/or azetreonam were collected during March to October 2003 from 18 Norwegian laboratories. The routine antimicrobial susceptibility method was used in each laboratory to screen isolates using MIC breakpoint >1 mg/L for reduced susceptibility. Further analyses included: (i) MIC determinations − Etest + Etest ESBL. (ii) Disc-approximation test with five substrates + amoxicillin–clavulanate. (iii) Combined disc method. (iv) TEM-/SHV-/CTX-M PCRs and sequence-typing.
Results: A total of 90 *E. coli* and 29 *K. pneumoniae* strains were detected among 191 Enterobacteriaceae-isolates. ESBL-production was confirmed in 55 *E. coli* and 22 *K. pneumoniae* isolates. Several strains had low ceftazidime and/or cefotaxime MIC-values (0.5–2 mg/L). ESBL-positive *E. coli* and *K. pneumoniae* was detected by Etest ESBL (53/55 and 22/22), by disc-approximation (53/55 and 22/20) and by combined discs (51/55 and 20/22). ESBL-producing *E. coli* and *K. pneumoniae* expressed reduced susceptibility towards cefpodoxime (54/55 and 19/22), cefotaxime (52/55 and 16/22), ceftazidime (37/55 and 19/22). ESBL-genotyping has shown both TEM (type 42) and SHV (types 2a, 28 and 48) ESBLs, but the CTX-M-types dominate. A total of 41/44 *E. coli* and 5/13 *K. pneumoniae* isolates examined were CTX-M PCR positive. Sequence analyses of 35 different CTX-Ms have shown type-15/-28 ($n = 25$), −9/9a ($n = 4$), −1 ($n = 3$), −3/−22 ($n = 2$) and −2/20 ($n = 1$).
Conclusions: (i) Using MIC breakpoint >1 mg/L for reduced susceptibility to third-generation cephalosporins we detected ESBL-producing *E. coli* and *K. pneumoniae* with low MIC-values (0.5–2 mg/L). (ii) Cefotaxime-hydrolysis was the dominating profile in ESBL-positive *E. coli* strains whereas ceftazidime was the most sensitive substrate for detection of ESBL-production in *K. pneumoniae*. (iii) The different methods showed almost the same sensitivity in detecting ESBL production assuming that more than one substrate was used, i.e. both cefotaxime and ceftazidime. (iv) CTX-M was the dominating ESBL-type.

P756 Simultaneous, bi-clonal outbreak of urinary tract infection by *E. coli* O25 strains with CTX-M-15: community and hospital effects in two English health districts

R. Warren, A. Doroshenko, R. Carr, D. Patel, P. O'Neill, G. Harvey, A. Pearson, N. Woodford, J. Turton, E. Ward, T. Cheastey, D. Livermore, C.J. Munday, V.M. Ensor, P. Hawkey
Shrewsbury, Colindale, Birmingham, UK

Objectives: English ESBL resistance, hitherto in Klebsiella, is now emerging in *E. coli*.
Methods: Urine culture on chromogenic agar (not nationally recommended CLED); susceptibility testing, serotyping, PFGE, sequencing of beta-lactamase genes; policy review.

Results: Two *E. coli* O25 strains with CTX-M-15 ESBL and resistant to quinolones and trimethoprim caused simultaneous 'community' and hospital UTI outbreaks with >100 cases stabilising at 4% of UTI. Blood and surgical wounds also yielded strains. Quinolone-resistant ESBL carriage was 4% in diarrhoeal samples. The strains, one gentamicin-susceptible and with an insertion sequence between promoter and bla ctx-m-15, the other resistant and without the insertion sequence, were 78% related by PFGE and were largely distinct from other UK CTX-M-15 producers. They were susceptible to nitrofurantoin, carbapenems, and amikacin; the former two were used therapeutically. Most 'community' cases had associations with healthcare in the previous 3 years but spread into community hospitals and nursing homes developed during the outbreak. Cases exceeded hospital containment isolation facilities, necessitating risk prioritisation scoring for single rooms, but co-infection with MRSA and *C. difficile* was noted. Descriptive epidemiology identified no common source but a wider region reported earlier and simultaneous *Klebsiella* and *E. coli* with CTX-M-15. Hospital and community antibiotic policies were changed: quinolone and cephalosporin reporting was reduced; carbapenems (inc. ertapenem) were substituted in empirical treatment.
Conclusion: ESBL-positive *E. coli* have epidemic potential, justify urgent major change to chemotherapy, and can overwhelm limited containment facilities. Focussed national surveillance, changes to low-cost national methods, early intervention and investment in containment are needed to prevent establishment of this rapidly evolving resistance class.

P757 Increased incidence of ESBL in ICU isolates from West Midlands region, UK

V.M. Ensor, C.J. Munday, W-E. Liu, P.M. Hawkey
Birmingham, UK

Objective: The incidence of Enterobacteriaceae producing extended spectrum beta-lactamase (ESBL) causing infections in ICU patients in the UK is currently unknown. Surveillance was conducted in the West Midlands to assess current rates of antibiotic resistance and ESBL production on ICUs.
Methods: Consecutive, non-duplicate Enterobacteriaceae were collected from 12 hospitals over a 6-month period in 2003. Isolates were identified using MAST ID or API 20E. MICs were determined by BSAC agar dilution method. ESBL producing strains were confirmed by clavulanate synergy testing.
Results: A total of 343 (no. ESBL+) isolates consisting of 98 (26) *E. coli*, 38 (10) *K. pneumoniae*, 18 (2) *K. oxytoca*, 83 (37) *Enterobacter* spp., 34 (6) *Serratia* spp., and 72 (13) others. MICs were determined to 16 antibiotics. Ninety-five strains showed at least a fourfold decrease in MIC with a third-generation cephalosporin in the presence of clavulanate, giving an ESBL rate of 28%. The rates of resistance were as follows: cefoxitin 58%; cefuroxime 56%; co-amoxiclav 53%; cefotaxime 41%; aztreonam 36%; moxifloxacin 28%; ceftazidime 27%; ciprofloxacin 24%; gatifloxacin 22%; piperacillin/tazobactam and cefpirome 19%; gentamicin 13%; meropenem 0.3%.
Conclusion: In a UK survey in 1991 the ESBL rate was 1%. The BSAC bacteraemia survey, 2001–2002 reported ESBL rates of 5 and 9% in *E. coli* and *K. pneumoniae*, respectively. The ESBL rate in this study was 28% which is the highest to be reported from the UK yet. ESBLs were present in all ICUs surveyed (range 12–39%). About 45% of *Enterobacter* spp. were ESBL producers which was the highest proportion of all species. This emphasises the need to carefully test species other than *E. coli* and *K. pneumoniae* for the presence of ESBLs. *Enterobacter* spp. are not currently included in NCCLS guidelines for detection of this mechanism of resistance.

P758 Molecular characterisation of *Escherichia coli* isolates producing CTX-M-15 extended-spectrum beta-lactamase (ESBL) in the United Kingdom

N. Woodford, E. Ward, M.E. Kaufmann, J. Turton, A. Pearson, S. Harry, R.E. Warren, D.M. Livermore
London, Shrewsbury, UK

Objectives: During 2003, the SRMD received isolates of *Escherichia coli* for confirmation of ESBL production with a phenotype implying a CTX-M-type beta-lactamase, i.e. cefotaxime (CTX) MICs fourfold greater than ceftazidime (CTZ) MICs. Isolates were from hospital patients and, in some instances, from community patients with little or no recent hospital contact. TEM- and SHV-type ESBLs are largely confined to nosocomial isolates, so the apparent spread of CTX-M enzymes in the community is cause for concern. We compared the isolates and investigated the genetic basis of their CTX-M phenotype.
Methods: Isolates were compared by PFGE of XbaI-digested genomic DNA and data were analysed using BioNumerics software. MICs were determined by Etest or agar dilution, and interpreted using BSAC breakpoints. Isolates with a CTX-M phenotype were tested for blaCTX-M alleles by PCR, initially with universal primers, and then with primers specific for various blaCTX-M groups. Selected amplicons were sequenced, either directly or after cloning into pCR2.1. Transfer of CTX-M to *E. coli* J62 was attempted in broth and on agar plates.
Results: Over 100 CTX-M-producing *E. coli* were obtained from more than 20 UK centres. These isolates represented multiple strains, although clusters of related isolates (>80% similarity) were observed, some including isolates from more than one centre. Sequencing confirmed that 12 *E. coli* from 11 different centres all produced CTX-M-15. Most isolates had substantial resistance to CTX (MICs >128 mg/L) and CTZ (MICs >16 mg/L), consistent with CTX-M-15. Isolates ($n = 25$) associated with a large community cluster produced atypically large amplicons with group I CTX-M primers, as did two related isolates from another centre. These isolates were less resistant to CTX (MICs 16–64 mg/L) and CTZ (MICs 1–4 mg/L), and susceptible to gentamicin; sequencing of a representative isolate identified IS26 within the terminal inverted repeat of the ISEcpI element upstream of blaCTX-M-15, separating the allele from its usual promoter, and the spacer between ISEcpI and blaCTX-M-15 had a T/C polymorphism not seen in other sequenced isolates.
Conclusions: We have confirmed the recent multifocal emergence of CTX-M-15 in the community. The isolates represented many different strains, but transfer of the enzyme *in vitro* was not demonstrated. Because of the public health implications, this worrying development merits close monitoring.

P759 The prevalence of ESBL producing *E. coli* and *Klebsiella* strains in Copenhagen, Denmark

A. Kjerulf, D.S. Hansen, N. Frimodt-Møller
Herlev, Copenhagen, DK

Objectives: Four studies were conducted in order to elucidate the frequency of ESBL producing *E. coli* and *Klebsiella* strains in isolates from hospitalised patients.
Methods: Four collections of strains were investigated: (1) 380 consecutive *E. coli* and *Klebsiella* isolates mainly from the urine ($n = 360$) were collected during the period 27 February to 24 March 2003 at H:S Hvidovre Hospital. (2) 200 gentamicin resistant *E. coli* and *Klebsiella* isolates collected from 1998 to 2003 at H:S Hvidovre Hospital. (3) 210 consecutive *E. coli* isolates from blood cultures collected during the period 1 July to 31 December 2001 at Herlev University Hospital. (4) 68 cefuroxime resistant *E. coli* and *Klebsiella* strains mainly from the urine ($n = 44$) collected during the period 1 January 2002 to 30 June 203 at Herlev University Hospital. All strains were screened for susceptibility to cefpodoxime, ceftazidime, ceftriaxone and cefotaxime by the disk diffusion method. Strains with a zone diameter for cefpodoxime <22 mm

were tested by a phenotypic confirmatory test for ESBL production by the Oxoid combination disk method, using the above mentioned cephalosporins ± clavulanic acid; a zone difference of >5 mm for any of these combinations was taken as confirmation of ESBL production.

Results: Number of screening-test positive strains in the four studies – study 1: 11/380, study 2: 20/200, study 3: 3/210 and study 4: 56/68. Number of confirmatory-test positive – study 1: 2/11, study 2: 12/20, study 3: 0/3 and study 4: 41/56. This gives an ESBL prevalence of 0.5% (2/380) in study 1, 6% (12/200) in study 2, 0% (0/210) in study 3 and 60% (41/68) in study 4.

Conclusion: The prevalence of ESBL producing *E. coli* and *Klebsiella* isolates is low in the Copenhagen area of Denmark (0.5 and 0%). These strains, however, deserve attention due to their epidemic potential. Among gentamicin resistant strains the prevalence of ESBL positive strains was 12 times higher so we suggest testing these strains for ESBL production.

P760 Emergence of ESBL-producing *E. coli* and antimicrobial use in a Spanish region

A. Yagüe, D. Sandvang, J.-C. Rodríguez, G. Royo, N. Gonzalo, A. García-Monsalve, P. Campillos, D. Monnet, J.-M. López-Lozano
Orihuela, E; Copenhagen, DK; Elche, E

Objectives: To describe an important outbreak of Extended-Spectrum Beta-Lactamase (ESBL) producing strains of *Escherichia coli* (EBSL-EC) in the Vega Baja sanitary district (250 000 inhabitants region of the South of Spain including a 400-bed general hospital). We studied also antimicrobial sensitivity of the strains, co-resistance to non-beta-lactam antimicrobials and relationship with antibiotic use.

Methods: Data of monthly non-duplicate EBSL-EC and antibiotic use (hospital: DDD/1000 pat-day and community: DDD/1000 inhabitants-day) were collected for January 1999 to October 2003. Time Series Dynamic Regression models were adjusted to evaluate the relationship between the use of antimicrobials and the emergence of the bacteria. Sensitivity testing was determined by microdilution with Gram-negative and Urine panels (MicroScan®). ESBL producing strains were initially selected by screening with MicroScan®Gram-negative and Urine panels (MIC >1 μg/mL for cefotaxime, ceftazidime or aztreonam, and/or a difference of three or more dilutions between ceftazidime and ceftazidime with 2 μg/mL of clavulanic acid). Production of ESBL was confirmed using the Double Disk Screening Test.

Results: 231 of 7678 (3%) strains of non-duplicate *E. coli* isolated from January 1999 to September 2003 were ESBL-EC (71 from inpatients and 160 from outpatients). Monthly aggregation reveals a clear increase of ESBL-EC isolates from January 2001 to the present time, reaching a peak of 11.4% in August 2003. This evolution was clearly preceded by a similar increase, 2 months before, in the hospital consumption of levofloxacin (the rest of antibiotics didn't shown any relationship) (Figure), as well as in the levofloxacin community use (not shown). On the other hand, our ESBL-EC strains have very high percentages of resistance to ciprofloxacin (mean 65.3%), cotrimoxazole (62.3%) and gentamicin (16.45%), in contrast with the non-ESBL-EC (28.2, 34.6 and 10.1%, respectively).

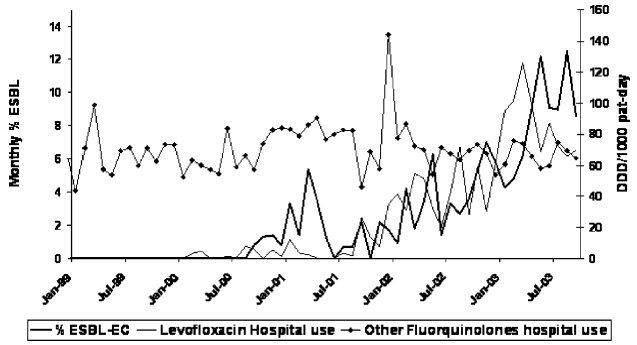

Figure 1. Emergence of Extended specturm Beta Lactamase *E. coli* and use of fluroquinolones. Vega Baja sanitary district (Spain) (1999–2003).

Conclusion: we think we are in the presence of an important outbreak of ESBL-producing *E. coli*, that affects hospital and community settings, and that could be causally related with the great increase in the use of levofloxacin. Fluoroquinolone-resistant strains, selected by antibiotic pressure, could have acquired resistance factors (ESBL production). Moreover, the presence of plasmids that code for resistance (ESBL) could favour the development of resistance to fluoroquinolones by chromosomal mutation.

P761 Risk factors for community-acquired infections due to *Escherichia coli* harbouring extended-spectrum-beta-lactamases

V. Romani, E. Calbo, C. Garcia-Vidal, L. Gomez, M. Xercavins, C. Estrada, L. Lopez-Yeste, S. Quintana, J. Garau
Terrassa, E

Introduction: The prevalence of Extended-Spectrum-B-lactamase-producing *E. coli* (ESBL Ec) is increasing. In Spain, current prevalence rates range from 0.06 to 2.4% of all *E. coli* isolates. ESBLs have been found most often in the hospital setting. However, ESBL Ec have been increasingly recognised in the community. We have conducted a matched case–control study to identify risk factors for community acquired (CA) ESBL Ec non-epidemic infections.

Material and methods: Adult cases and matched controls were identified through records of the clinical microbiology laboratory in a 450 bed acute care teaching hospital with an area of influence of circa 300 000 population. Patients with CA infection for whom culture results were positive for ESBL Ec were eligible. Two different periods were studied: from January 2000 to January 2001, and from August to November 2003. Controls were matched in a 3:1 ratio for age, gender, date of isolation, site of infection and residence in long-term-care facility. Potential risk factors recorded included: patient demographics, comorbidities, site of infection, antimicrobial therapy, bacterial infections in the past year, immunosupression, McCabe Jackson score, abnormalities and/or urinary tract manipulation, recent hospitalisation and contact with the Healthcare System.

Results: In our area, the prevalence of infection of ESBL producing Ec has increased from 0.47% in 2000 to 1.62% in 2003 ($P < 0.001$) with respect to all Ec isolates. CA infection shifted from 47% in the first period to 80% in 2003. Out of the 15 cases of CA-ESBL Ec found were urinary tract infections, 87% were women and the mean age was 65 (range 17–92). On univariate analysis only connective tissue disease ($P < 0.003$), genitourinary pathology ($P < 0.008$), infections in the past year ($P < 0.007$) and previous exposure to second-generation cephalosporins ($P < 0.001$) were factors associated with CA infection due to ESBL Ec. In our regression model, only previous exposure to second-generation cephalosporins was strongly associated (OR 18.25, CI 95% 1.92–175).

Conclusions: In the last 3 years there has been a marked increase in infections due to ESBL Ec, especially from the community. Only previous exposure to second-generation cephalosporins (not to ciprofloxacin, third-generation cephalosporins or aminoglycosides) was predictive of an ESBL Ec CA infection. Strikingly, neither comorbidity nor previous contact with the Healthcare System was risk factors for ESBL Ec.

P762 Emergence of CTX-M-type enzymes in hospital- and community-acquired isolates of *Escherichia coli* from Northern Italy

G. Brigante, F. Luzzaro, M. Perilli, G. Lombardi, G.M. Rossolini, G. Amicosante, A. Toniolo
Varese, L'Aquila, Siena, I

Objectives: Enzymes of the CTX-M family are currently classified as extended-spectrum beta-lactamases (ESBLs). Over the last decade, CTX-M-type enzymes have been increasingly reported from several countries in Europe. The aim of this study was to search for CTX-M-type enzymes in *Escherichia coli* isolates obtained at our Institution (Varese, Northern Italy).
Methods: We studied consecutive *E. coli* isolates recovered over a 2-year period (2000–2002). Stains suspected of producing ESBLs (according to NCCLS criteria) were further investigated. The double-disk synergy test and Etest ESBL strips (AB Biodisk, Solna, Sweden) were used to confirm ESBL production. The Etest method was also used to evaluate MICs of amikacin, gentamicin, ciprofloxacin, and beta-lactams (including last-generation cephalosporins, carbapenems, and aztreonam). ESBL-positive isolates were evaluated for the presence of CTX-M-type genes using specific DNA probes. Patient records were examined to assess risk factors for infections and underlying clinical conditions.
Results: A total of 12 386 consecutive *E. coli* isolates were studied. Overall, 26 out of 124 ESBL-positive strains were found to carry a CTX-M-type gene and to produce a CTX-M-type enzyme. Most isolates (21/26) showed high MIC values for cefotaxime (>32 mg/L) and borderline values for ceftazidime (1–2 mg/L). The remaining five isolates had also high MICs for ceftazidime. CTX-M-positive isolates were obtained both from inpatients ($n = 17$) and outpatients ($n = 9$). Epidemiological analysis showed that most strains were isolated from urinary tract infections, even though some isolates were recovered from the lower respiratory tract, wounds and blood. Most patients (20/26) were treated with immunosuppressive therapy. Recurrent urinary infections occurred in five outpatients.
Conclusions: CTX-M-type enzymes appear to be emerging among *E. coli* isolates in both the hospital and community environments. The analysis of clinical records demonstrated that these microorganisms can cause severe and persistent infections. Therefore, despite the currently low prevalence of CTX-M phenotype, we suggest that a monitoring of this resistance phenotype should be established to avoid the spreading of resistance traits.

P763 First detection of *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates producing acquired beta-lactamases of the CMY-LAT lineage in Italy

L. Pagani, M.M. D'Andrea, R. Migliavacca, E. Dell'Amico, M. Spalla, E. Nucleo, G.M. Rossolini
Pavia, Siena, I

Background and Objectives: Class C beta-lactamases (CBLs) are enzymes that confer broad-spectrum beta-lactam resistance (including penicillin, expanded-spectrum cephalosporins, and cephamycins) and are poorly or not susceptible to commercially available beta-lactamase inhibitors. In strains with reduced outer membrane permeability, they can also provide resistance to carbapenems. A number of these enzymes are chromosomally encoded, but plasmid-mediated CBLs are also known as a cause of acquired resistance to expanded-spectrum cephalosporins and cephamycins in clinical isolates of Enterobacteriaceae. In Italy, only the FOX-3 acquired CBL has previously been reported, in *Klebsiella* spp. In this work we report the first detection of acquired CBLs of the CMY-LAT lineage in *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates from an Italian hospital.
Methods: Ten consecutive non-replicate clinical isolates of *E. coli* (eight) and *K. pneumoniae* (two) resistant to expanded-spectrum cephalosporins and cephamycins were collected, during 2002, at the Laboratory of Microbiology of the S. Matteo Hospital of Pavia (northern Italy). *In vitro* susceptibility testing was determined by a microdilution method according to NCCLS. Beta-lactamase production was investigated by analytical isoelectric focusing (IEF) coupled with a bio-assay. Molecular characterisation of beta-lactamase genes was carried out by a multiplex PCR approach designed for detection of all major lineages of acquired CBLs genes, and by sequencing. Transferability of resistance genes was tested by mating assays in liquid medium.
Results: Two isolates, one of *E. coli* and one of *K. pneumoniae*, were found to be resistant to expanded-spectrum cephalosporins, except for cefepime, and cephamycins (cefoxitin MICs >128 mg/L). Both isolates produced a beta-lactamase of pI >8.4 that showed hydrolytic activity against cefoxitin, cefotaxime and ceftazidime. Molecular characterisation revealed, in both cases, the presence of an acquired CBL gene of the CMY-LAT lineage, which was compatible with blaCMY-2/LAT-3 (the leader peptide-encoding region was not sequenced). The CBL determinant was transferable by conjugation from the *E. coli* isolate, while conjugal transfer was not detected from the *K. pneumoniae* isolate.
Conclusions: These findings reveal that acquired CBLs of the CMY-LAT lineage, which are the most common acquired CBLs, can also be encountered in nosocomial settings from northern Italy.

Enteropathogens

P764 Dissemination of sulphonamide resistance genes: first sul3 found in *Salmonella* from Portugal

P. Antunes, J. Machado, J.C. Sousa, L. Peixe
Porto, Lisbon, P

Objectives: The purpose of this study was to determine the distribution of sulphonamide resistance genes sul1, sul2 and sul3 and class 1 integrons in Portuguese *Salmonella* isolates collected during 2002–2003, from human and nonhuman sources.
Methods: Eight hundred and seventy-five isolates were tested for resistance to 10 antimicrobial agents by the agar dilution method. Sulphonamide resistant isolates were screened for resistance genes sul1, sul2, and sul3 and class 1 integrons by PCR assays.
Results: Resistance was found in 54% and multiresistance in 21% of the isolates. In 151 (17%) sulphonamide-resistant isolates (MICs 512 mg/L), 118 (78%) sul1 genes, 57 (38%) sul2 genes and nine (6%) sul3 genes were detected. In 29 isolates, more than one gene encoding sulphonamide resistance was present: sul1 and sul2 in 22, sul1 and sul3 in three and sul1, sul2 and sul3 in four. Class 1 integrons were found in 77% of those isolates. Among the 116 isolates carrying class 1 integrons, 114 presented sul1 gene, found alone (87 isolates) or simultaneously with sul2 (20) or sul3 (3) and with sul2 and sul3 (4). The two strains with class 1 integrons, which lacked the qacED1 and sul1 genes, carried a sul3 gene. Of the 118 sul1-positive isolates, 114 harboured class 1 integrons.
Conclusion: Class 1 integrons and sulphonamide resistance genes are widespread among *Salmonella*. The newly described sul3 gene

has now been identified in nine *Salmonella* isolates collected from human and nonhuman sources in Portugal.

P765 Characterisation of beta-lactamase production in *Salmonella* from Portugal

P. Antunes, J. Machado, J.C. Sousa, L. Peixe
Porto, Lisbon, P

Objectives: The aim of this study was the characterisation of beta-lactamase production in Portuguese *Salmonella* isolates collected during 2002–2003, from human and non-human sources.
Methods: Eight hundred and seventy-five isolates were tested for resistance to 10 antimicrobial agents by the agar dilution method. A double-disk synergy test for the detection of extended-spectrum beta-lactamase production was performed by disk diffusion method. The identification of beta-lactamases was done in ampicillin resistant isolates by IEF and PCR assays, with primers, which detects genes encoding TEM, PSE-1 and OXA group III enzymes. To evaluate the association of beta-lactamase genes to class 1 integrons, 5′CS–3′CS primers were used in a PCR assay. PCR products were purified and both strands sequenced.
Results: In total, 17% of the isolates exhibited resistance to ampicillin, with MICs 64 mg/L. Resistance to ampicillin was conferred by a TEM-1 beta-lactamase in 99 (68%) of the isolates, PSE-1 in 37 (25%) and OXA-30 in nine isolates. It is to be noted that there is the detection of the extended-spectrum beta-lactamase (ESBL) TEM-52 in one isolate. The TEM-type beta-lactamases was not associated with class 1 integrons. In contrast, all the blapse-1 and blaoxa-30 genes were inserted in 1200 and 2000 bp class 1 integrons, respectively.
Conclusion: A considerable percentage of Portuguese *Salmonella* were resistant to beta-lactams, mostly due to the production of TEM-1 like beta-lactamase and PSE-1 inserted in integrons. The detection of an isolate that produce an ESBL, such as TEM-52, and nine isolates carrying a class 1 integron with OXA-30, are causes of concern due to the possible therapeutic failures with broad-spectrum beta-lactams.

P766 Increasing incidence of *Salmonella typhii* with reduced susceptibility to ciprofloxacin in Kuwait

A.A. Dashti, P.W.J. West, D. Panigrahi
Suleibikhat, KWT

Objectives: To determine the current incidence of reduced ciprofloxacin susceptibility in *Salmonella typhi*, to compare with previous data and to investigate the mechanism responsible.
Methods: 48 isolates of *S. typhi* collected in 2002–2003 were tested for susceptibility to ciprofloxacin and other antibiotics using the Vitek 2 and E-test. Isolates showing reduced ciprofloxacin susceptibility were subjected to PCR to determine if a mutation of the gyrA gene was responsible. PCR was carried out using two primers (ATGAGCGACCTTGCGAGAGAAATTACACCG) and (TTCCATCAGCCCTTCAATGCTGATGTCTTC). Results were compared with those for isolates collected from 1995–1997.
Results: 18 out of 48 (42%) of the isolates were resistant to multiple antibiotics, including ampicillin, chloramphenicol tetracycline and trimethoprim. Of these 12 (67%) showed resistance to nalidixic acid and reduced susceptibility to ciprofloxacin (MIC 0.125–0.38 mg/L). Of the 30 susceptible isolates, seven (23%) showed reduced ciprofloxacin susceptibility. Isolates from 1995 to 1997 showed 11% of 53 multi-resistant strains, but none of 100 susceptible isolates with reduced ciprofloxacin susceptibility. PCR results showed mutations of the gyrA gene.
Conclusion: Reduced susceptibility to ciprofloxacin in multi-resistant *S. typhi* has increased from 11% in 1995–1996 to 67% in 2002–2003 and from 0 to 23% in susceptible strains. Mutation of gyrA is the mechanism responsible.

P767 Comparison of antimicrobial resistance in diarrhoeagenic *Escherichia coli* isolates causing traveller's diarrhoea between two periods, 1994–1997 and 2001–2003

E. Mendez Arancibia, J. Ruiz, R. Cabrera, J. Gascon, J. Vila
Barcelona, E

Objectives: To compare the antimicrobial resistance levels in *Escherichia coli* clinical isolates causing traveller's diarrhoea in two periods, 1994–1997 and 2001–2003.
Material and methods: Presence of enteroaggregative (EAEC) and enterotoxigenic *E. coli* (ETEC) was established by PCR among those isolated from travellers with diarrhoea during the periods 1994–1997 and 2001–2003. Susceptibility to ampicillin (AMP), amoxicillin plus clavulanic acid (AMC), tetracycline (TET), chloramphenicol (CHL), cotrimoxazole (SXT), nalidixic acid (NAL) and ciprofloxacin (CIP) was determined by disk diffusion.
Results: One hundred thirty-two (50 EAEC, 82 ETEC) and 113 (49 EAEC, 64 ETEC) diarrhoeagenic *E. coli* were recovered during two periods, 1994–1997 and 2001–2003, respectively. The levels of resistance of EAEC to all tested antibacterial agents increased in the second period: AMP from 52 to 73%, AMC from 0 to 10%, TET from 64 to 86%, SXT from 48 to 69%, NAL from 6 to 31% and CIP from 2 to 16% ($P < 0.0001$), whereas the leaves of resistance to CHL showed a slight decrease (28–22%) but not statistically significant. In ETEC strains resistance to AMP, NAL, CIP and AMC increased from 43 to 50%; 6 to 17%; 1 to 6%; 0 to 6%, respectively, while resistance to CHL decreased from 20 to 14%. The levels of resistance to TET and SXT did not present greater differences, but suggested a slight increase in the resistance (57–61% and 50–53% respectively).
Conclusions: A trend to an increase in the resistance of EAEC and ETEC to AMP, AMC, NAL, and CIP has been detected, and the decrease of resistance to CIP is worthy of note due to the fact that this antimocrobial agent is considered a first choice treatment for traveller's diarrhoea.

P768 Increasing fluoroquinolone resistance in *Salmonella* isolates in Finland

A.J. Hakanen, S. Pitkänen, A. Siitonen, P. Kotilainen, J. Jalava, P. Huovinen
Turku, Helsinki, FIN

Objectives: Quinolone-resistant *Salmonella* isolates emerged in Finland in the mid-1990s. The main origin of these strains is travellers returning from Southeast Asia. This study was performed to evaluate the incidence and changes of fluoroquinolone resistance in *Salmonella* isolates between 2000 and 2003 in Finland.
Methods: We collected a total of 805 *Salmonella* enterica isolates which were considered to be epidemiologically unrelated. The isolates were divided into two groups (Finnish and foreign isolates) on the basis of travel history. The collection was performed in four phases: each year in 2000, 2001, 2002 and 2003 starting in January, we consecutively collected 100 Finnish and 100 foreign isolates. MICs for nalidixic acid, ciprofloxacin and 10 additional fluoroquinolones were determined by the standard agar dilution method (NCCLS).
Results: During the study period, the number of isolates with decreased ciprofloxacin susceptibility (MIC of ciprofloxacin >0.125 μg/mL) increased from 16 to 34% of all isolates ($P < 0.01$). A similar trend could be seen both among the isolates of foreign and Finnish origin. In addition, within the non-susceptible population the MIC values were increasing. MIC50 of ciprofloxacin increased from 0.25 to 0.5 μg/mL among the isolates with decreased ciprofloxacin susceptibility between 2000 and 2003. The respective figures for MIC90 were 0.5 and 1 μg/mL. All isolates with decreased ciprofloxacin susceptibility had also increased MICs to additional fluoroquinolones.
Conclusion: The number of *Salmonella* isolates with decreased ciprofloxacin susceptibility continues to grow in Finland.

Moreover, the MIC levels of these isolates have increased. This phenomenon might have serious clinical implications.

P769 Bacteraemia caused by ESBL-producing *Salmonella enterica* serovar. *virchow* 6.7:r:1,2 – a cause for concern

A. Guleri, G.D. Corcoran, S.R. Alcock, D.J. Brown
Glasgow, UK

Background: Antibiotic resistance in *Salmonellae* is now common. In developed countries such strains are largely zoonotic and acquire resistance in the animal host before transmission to humans in food. We present our first case of bacteraemic illness with multi-resistant, extended spectrum beta lactamase (ESBL) producing non-typhoidal *Salmonella*.
Case summary: A 34-year-old male, with a history of recent foreign travel was admitted to hospital with a 7–10 day history of gastrointestinal symptoms/fever. On admission he was febrile and splenomegaly was detected. Physical examination was otherwise normal. Biochemistry revealed mildly deranged liver function. *Salmonella enterica* serovar. *virchow* 6.7:r:1,2 was isolated from blood culture. It was sensitive *in vitro* (NCCLS disk test) to ciprofloxacin and gentamicin but resistant to ampicillin, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, co-trimoxazole, nalidixic acid and streptomycin. MIC of ciprofloxacin was 0.094 mg/L. Antibiotic treatment was with ciprofloxacin, to which he responded well.
ESBL detection: The isolate was identified as *Salmonella enterica* serovar. *virchow* 6.7:r:1,2 [API 20 identification system (bioMérieux), serogrouping, serotyping and phagetyping]. The isolate, resistant *in vitro* (NCCLS) to cefotaxime and ceftazidime, was tested for extended-spectrum beta lactamase/AmpC production by phenotypic methods. AB Biodisk ESBL E–tests (cefepime, ceftazidime and cefotaxime, each ± clavulanic acid) and Oxoid ESBL combination disks (cefpodoxime, ceftazidime, cefotaxime and cefpirome, each ± clavulanic acid) and cefoxitin alone were used based on modified NCCLS/manufacturer's guidelines. The isolate tested positive for ESBL production by both ESBL E-tests and combination disks. Molecular typing of the ESBL is awaited.
Conclusion: Invasive infection with *Salmonella virchow* is uncommon. The source of infection in this case appears to have been undercooked chicken. The emergence of resistance to antimicrobial agents within the salmonellae is a worldwide problem that has been associated with the use of antibiotics in livestock. Invasive infection with *S. virchow*, resistant to broad-spectrum beta-lactams, is a cause for concern. If antimicrobial therapy is indicated for travellers with a history of recent foreign travel, physicians should be aware of the possibility of treatment failures and in such cases MICs of third-generation cephalosporins and ciprofloxacin should be determined.

P770 The first outbreak of Shiaga toxin producing *Escherichia coli* (STEC) in Korea: molecular–epidemiological approach

S. Kim, Y. Kim, M. Park, J. Kim, S. Jeon, H. Jang, S. Kim, S.G. Chun, S. Kim, J.-H. Kim, J. Koo, Y. Kang, J. Lee, P. Park, J. Hur, Y. Kim, B. Lee
Seoul, Suwon, KOR

Objectives: Since 1982, in many countries, epidemic or sporadic cases caused by Shiga toxin producing *E. coli* (STEC) were reported. But in Korea, after active surveillance system to STEC was initiated in 1996, only 21 STEC sporadic human infection cases were reported until 2002. But in 2003, 39 strains of STEC were isolated from epidemic or sporadic cases between June and July. We try to elucidate the genetic relations between these 39 STEC isolates in this study.
Methods: O Seorgroup of each STEC isolate was detected by *E. coli* serotyping kit (Laboratorio de Reference de *E. coli*, Lugo, Spain). Antimicrobial susceptibilities test was performed by the disk diffusion method of National Committee of Clinical Laboratory Standards. PFGE was conducted by PulseNet standardised protocol and computer-assisted analysis of the PFGE banding patterns was performed with Fingerprinting II Informatix software (Bio-Rad).
Results: 39 STEC isolates were categorised to 11 O serogroups – O21, O26, O55, O91, O103, O104, O111, O119, O121, O157 and OUT. The most prevailed serogroup was O104 (nine strains) and the next was O55 (eight strains). Antibiogram and PFGE type was closely correlated with O serogroup. Eight of O104 isolates were resistant to ampicillin and ticarcillin, and their PFGE type was identical except one isolate, which showed 96.97% relatedness with other O104 serpgroup isolates. All eight of O55 strains were resistant to chloramphenicol, nalidixic acid, stereptomycin and tetracycline. Five of O55 strains showed identical PFGE type. From the comparison with PFGE types of SETC strains isolated between 1998 and 2002, O104 and O55 serotype strains were not closely related with isolates before 2002.
Conclusions: PFGE types of STEC strains isolated in 2003 were closely correlated with O serogroup and their patterns of antibiotic susceptibility. Thirty-nine cases of STEC infection in 2003 can be differentiated two remarkable outbreaks, caused from O104 and O55 serotype, and some sporadic cases.

P771 Epidemiology of antibiotic resistance of non-typhoidal *Salmonella* isolates originating from non-human sources during an 11-year period in Greece

A. Markogiannakis, E.N. Velonakis, L. Kondyli, M. Mouroutsou, M. Katramadou, M. Lampiri, M. Passiotou, A. Bethimouti, A.C. Vatopoulos
Athens, Halkis, GR

Objective: The aim of the present study was to assess the distribution and the antibiotic resistance rates (ARR) of the various non-typhoidal *Salmonella* serotypes originated from non-human sources in Greece, during a 11-year period (1990–2000).
Material and methods: A total of 753 isolates, belonging to 27 different serotypes, were selected from the collection of National Reference Center for Salmonella and Shigella (NRCSS), in order to reflect the prevalence of these serotypes during the mentioned period. The sample consisted of 382 isolates from animals, 295 isolates from foods, and 76 environmental isolates. Susceptibilities to 10 antibiotics of various classes were determined using MICs broth micro-dilution method.
Results and conclusions: The highest ARR and also the higher incidence of multiresistance have been observed for *S. virhow*, followed by *S. hadar* and *S. typhimurium*. The vast part of

Table 1. Distribution (%) of the main non-typhoidal *salmonella* antibiotic resistance phenotypes originated from non-human sources during an 11-year period (1999–2000)

Serotype	Sens.	A	A/T	A/T/*	A/T/C/*
S. enteritidis ($n = 418$)[(a)]	62.4	18.2	3.3	0.9	0
S. eyphimurium ($n = 157$)	42.7	0	1.9	10.2	32.5
S. virhow ($n = 38$)	31.6	10.5	7.9	28.9	18.4
S. livingstone ($n = 33$)	87.9	3	3	3	0
S. gallinarum ($n = 29$)	93.1	0	3.5	0	0
S. hadar ($n = 27$)	33.3	3.7	7.4	11.1	6
Other serotypes ($n = 51$)	94.1	1.9	0	1.9	0

[(a)]Number of tested isolates.
Sens.: Sensitive isolates, A: ampicillin, T:tetracycline, C: chloramphenicol, *: any other antibiotic.

S. typhimurium isolates was resistant at least to ampicillin, tetracycline and chloramphenicol, while the main resistance phenotype of *S. enteritidis* isolates was the monoresistance to ampicillin (Table). The ARR and the phenotypes of resistance for the isolates of the above four serotypes were similar with the corresponding ones of human isolates as resulted from a recent Greek study derived also from NRCSS (*Eur J Epidemiol* 2001; **17**: 751–755) a fact consistent with possible transfer of antibiotic resistant strains from animals to humans through the food chain. The incidence of resistance for the rest of serotypes was very low. All the examined isolates were susceptible to ceftriaxone and ciprofloxacin. Interestingly, almost all the examined isolates belonged to animals bred at a non-industrial scale (e.g. pigeons) and the environmental isolates were sensitive to all tested antimicrobials, possibly because of the reduced antibiotic pressure in these isolates.

P772 Serotypes and antibiotic resistance patterns of *Salmonella* species isolates in the province of South-Kivu, Democratic Republic of Congo

N. Zozo, O. Vandenberg, B. Bisimwa, A. Dediste, P. Donnen, G. Zissis, J. Butzler
Bukavu, CD; Brussels, B

Objective: *Salmonella* infections represent a major health problem worldwide but particularly in developing countries. To ensure appropriate treatment of salmonellosis in the province of South-Kivu, Democratic Republic of Congo, we conducted a provincial survey to determine the serotypes and antimicrobial resistance of *Salmonella* isolates between September 1997 and November 2003.
Methods: All *Salmonella* isolates received at the Provincial Public Health Reference laboratory during this period were studied for serotyping and resistance to 11 antimicrobial agents, including third-generation cephalosporins and fluoroquinolones.
Results: Among the 182 *Salmonella* spp. studied, *S. typhi* (39.6%) and *S. typhimurium* (30.9%) were the most common serogroups found. Ninety-two per cent were multidrug resistant with the following proportion of resistant strains to: ampicillin (97.9%), chloramphenicol (92.9%), co-trimoxazole (95.7%) and tetracycline (60.5%). The most common resistance pattern seen was ampicillin, chloramphenicol, tetracyclin and co-trimoxazole. All but one strain were sensitive to amikacin, ceftriaxone, ciprofloxacin and nalidixic acid.
Conclusions: Due to the high prevalence of multidrug resistance, third-generation cephalosporins or fluoroquinolones are now the drug of choice to treat severe *Salmonella* infection in the province of South-Kivu. These findings indicate the need to use drugs rationally in order to control the spread of multi-drug resistance in province of South-Kivu.

P773 Antimicrobial resistance of human non-typhoidal *Salmonella* in Ankara, Turkey

I. Mumcuoglu, N. Balaban, I. Baran, Z.C. Karahan, V. Yetener, H. Bodur
Ankara, TR

Objectives: In this study we aimed to investigate the antimicrobial resistance and the epidemiologic characteristics of 423 human non-typhoidal *Salmonella* spp. isolates which were obtained from stool specimens of 11 768 patients between 1996 and 2001.
Methods: Between 1996 and 2001, 423 *Salmonella* spp. isolates were obtained from the community acquired infections in Ankara, Turkey. *Salmonella* strains were identified by standard biochemical reactions and using spesific O and H antisera. Antimicrobial susceptibility for Ampicillin (AMP), trimethoprim–sulfametoxazole (TMP–SXT) and ciprofloxacin (CIP) was determined by the disk diffusion method according to National Committee for Clinical Laboratory Standards.
Results: Species which were identified in this study were 293 (69.2%) *S. enteritidis*, 86 (20.3%), *S. typhimurium* and 44 (10.3%) others. In order to show the differences in epidemiology and antimicrobial resistance, the study was divided into two periods: 1996–1998 and 1999–2001. The isolation rates of *S. enteritidis*, *S. typhimurium* and the others were 74.2, 18.2 and 7.6%, respectively, in the first period. In the second period isolation rates were found 51.6, 29 and 18.4%, respectively. Antimicrobial resistance for (AMP and TMP-SXT) in *S. enteritidis*, *S. typhimurium* and others were found (33.0/11.6%), (31.6/16.6%) and (19.0/8.0%) in the first period and (41.0/17.0%), (42.3/19.2%) and (23.0/15.0%) in the second period, respectively. All the isolates were susceptible to ciprofloxacin except one in other non-typhoidal *Salmonella* group.
Conclusions: The increasing number of ampicillin and trimethoprim–sulfametoxazole resistant *Salmonella* strains makes these drugs unsuitable for empiric treatment.

P774 Epidemiology and antimicrobial resistance of *Shigella* spp. in Ankara, Turkey, during a 9-year period (1992–2000)

N. Balaban, I. Mumcuoglu, A. Ulusoy, N. Hayirlioglu, V. Yetener, H. Bodur
Ankara, TR

Objectives: The aim of this study was to investigate the epidemiology and antimicrobial resistance of *Shigella* spp. in Ankara, Turkey.
Method: The laboratory data were analysed in 978 *Shigella* spp. isolated from stool materials of adult patients over a 9-year period (1992–2000) retrospectively. *Shigella* isolates were identified by standard biochemical reactions and using specific antisera. Antimicrobial susceptibility for ampicillin (AMP), trimethoprim–sulfamethoxazole (TMP–SXT) and ciprofloxacin (CIP) were determined by the disk diffusion method according to National Committee for Clinical Laboratory Standards.
Results: In order to show the differences in epidemiology and antimicrobial resistance, the study was divided into two periods. The first period was from 1992 to1996, and the second period was from 1997 to 2000. A total of 475 *Shigella* spp. isolates were obtained in the first period and 503 isolates in the second period. Isolation rates of the strains in the first and second periods were, respectively, for *Shigella flexneri* 55.7 and 21.5%; for *Shigella sonnei* 30.5 and 60.4%; for *Shigella dysenteria* 8.9 and 11.9%; and for *Shigella boydii* 4.8 and 6.2%. The rates of resistance to (AMP) in the first and second periods were respectively in *S. flexneri* 64.9 and 66.7%; *S. sonnei* 54.5 and 30.5%, *S. dysenteria* 32.4 and 62.0%; *S. boydii* 46.0 and 65.0%. The rates of resistance to TMP–SXT in *S. flexneri* 22.6 and 40.8%; *S. sonnei* 38.0 and 41.3%; *S. dysenteria* 23.2 and 33.7%; *S. boydii* 40.0 and 56.5%. All strains were susceptible to ciprofloksasin.
Conclusions: *S. flexneri* was the most common species isolated in the first period and *S. sonnei* was the common species in the second period. In Ankara, the data showed an increase in the resistance to the commonly used antimicrobial agents are ampicillin and trimethoprim–sulfamethoxazole. Ciprofloxacin seemed to be the best choice for the treatment of Shigelliosis.

P775 Antimicrobial resistance in *Salmonella* and *Shigella* strains isolated from stool cultures over a 2-year period in Kocaeli, Turkey

S. Günde, H. Üçkarde, A. Willke
Kocaeli, TR

Objectives: To determine the antimicrobial resistance in *Salmonella* and *Shigella* strains isolated from stool specimens during a 2-year period, from patients admitted to our clinics with a diagnosis of diarrhoea.
Methods: The identification and susceptibility testing was done by VITEC 2 (bioMerieux, Fr) automated system. The antibiotics

tested for the study were ampicillin, ampicillin–sulbactam, cefotaxime, cefepim, ciprofloxacin, ofloxacin, and trimethoprim–sulfametaxazole.
Results: Nineteen *Salmonella* and seven *Shigella* isolates obtained between 1 January 2002 and 30 November 2003 were tested for their susceptibilities to seven antimicrobial agents. The total numbers of isolates during 1999–2001 (including the year of big Marmara earthquake) were 39. Five of six *Shigella* isolates were *S. sonnei*, one was *S. flexneri*. Thirteen of 19 *Salmonella* isolates were *S. typhimurium*, three were *S. enteritidis*, two were identified as *Salmonella* spp., one was *S. arizonae*. Although all of the isolates were found susceptible to the therapeutic agents, ampicillin susceptibility was decreased to 78% from 100% and trimethoprim–sulfametaxazole susceptibility was decreased to 89% from 100% in *Salmonella* strains during a 2-year period. Only one strain was resistant to cefotaxime. No resistance was found against ofloxacin and ciprofloxacin. All of the *Shigella* isolates were susceptible to all tested antibiotics.
Conclusions: (1) The incidence of *Salmonella* and *Shigella* infections seemed to decrease significantly over a 5-year period. (2) *S. typhimurium* and *Shigella sonnei* are the most commonly identified serotypes. (3) There is no significant change in resistance to 'old' and 'new' antibiotics. (4) All of the isolates showed a very good sensitivity all the antimicrobials tested. (5) A careful rotational use of antibiotics might be the best policy to make old drugs again active, and abuse of new agents.

P776 Transmission and antibiotic resistance of *Campylobacter* sp. in a poultry slaughterhouse

J. Posch, W. Sixl, G. Wüst, S. Schmidt, F.F. Reinthaler, G. Feierl, E. Marth
Graz, A

Objectives: Since 1996 the incidence of human Campylobacteriosis has shown a significant increase in Austria. Consumption of contaminated poultry products is a well known risk factor for human infections. During the slaughter process meat products can become contaminated with intestinal organisms. Furthermore antibiotic resistance is increasing in humans and animals. The aim of the study was to determine the resistance patterns and the transmission routes of *Campylobacter* sp. on the chicken-carcasses along the slaughter line.
Methods: From June 2002 until October 2003, a total of 88 slaughterhouse-equipment swab samples were collected and tested for the presence of *Campylobacter* organisms. Further five measurements of air were conducted during slaughtering, by using the SKC Bio Sampler (two sampling sites). The strains were cultured on mCCDA-agar (microaerophilic, 48 h, 42°C). A total of 130 strains were tested by disc diffusion method for susceptibility to nalidixic acid (NA), ciprofloxacin (CIP), tetracycline (TE) and erythromycin (E).
Results: Among the 88 equipment swabs 65% were positive for *Campylobacter* sp. Species distribution was: 75% *C. jejuni* and 25% *C. coli*. Eight samples contained both, *C. jej* and *C. coli*; 49% of strains were resistant to NA, 48% to CIP and 32% to TE. Further a total of 65 airborne *Campylobacter* sp. were analysed; 52% were *C. jej* and 48% *C. coli* and 68% of them showed resistance against NA and CIP, and 31% to TE. All strains were susceptible to E. The number of airborne *Campylobacter* sp. ranged from 0 to 4×10^4 CFU/m^3.
Conclusion: The study shows significant contamination of *Campylobacter* sp. of both the equipment as well as the air of the slaughterhouse. The slaughter of poultry is a largely mechanical process, so it is evident that intestinal colonised flocks lead to cross-contamination of *Campylobacter*-free carcasses. The present study shows importantly higher concentrations of airborne *Campylobacter* sp. in comparison to previous investigations. So we consider these levels of airborne *Campylobacter* sp. as an underestimated risk factor for transmission. Moreover resistant strains in the food chain can compromise the antibiotic treatment of human infections.

P777 Resistance of *Shigella* spp. in different regions of Russia

O. Kretchikova, A. Ivanov, M. Sukhorukova, L. Stratchounski
Smolensk, RUS

Objectives: Incidence of shigellosis in Russia is high and greater than in Western Europe and USA. In order to be efficacious, to decrease the risk of complications, and to restrict the spread of multiresistant strains in population, antimicrobial therapy of shigellosis should be based on local data on antimicrobial susceptibilities of the causative pathogen.
Methods: The strains were isolated from faeces of in-patients with clinically suspected shigellosis in 1998–2001 in Bryansk (B), St-Petersburg (SP), Smolensk (S), Moscow and Ekaterinburg. The tested antimicrobials were: ampicillin (AM), ampicillin/sulbactam (AMS), cefotaxime (CTX), tetracycline (TE), chloramphenicol (CL), ciprofloxacin (CIP), and co-thrimoxazole (SXT). An agar dilution method was used in the study. All procedures were performed and the results were interpreted according to the NCCLS recommendations (January 2003).
Results: A total of 458 strains were tested. *S. flexneri* (180 strains) and *S. sonnei* (278 strains) were highly resistant to the following drugs (respectively): SXT (92.2 and 91.4%), TE (98.3 and 83.5%), CL (95.6 and 42.8%), AM (96.1 and 26.6%) and AMS (95.6 and 25.2%). No strains were resistant to CIP or CTX. Multiresistance (to four or more drugs) was shown in 96.1% of *S. flexneri* and 23.7% of *S. sonnei* strains. More strains of *S. flexneri* (vs. *S. sonnei*) were resistant in B (78 isolates), SP (50 isolates) and S (281 isolates), respectively, to AM (100 vs. 10.9% in B, 96.6 vs. 38.1% in SP, 95.5 vs. 30.7% in S), CL (100 vs. 26.6% in B, 100 vs. 66.7% in SP, 93.3 vs. 45.8% in S), TE (92.9 vs. 62.5% in B, 100 vs. 66.7% in SP, 97.8 vs. 92.2% in S), and SXT (92.9 vs. 75% in B, 65.5 vs. 95.2% in SP, 96.6 vs. 96.4% in S). As for *S. sonnei*, difference in the resistance prevalence among the centres was statistically significant ($P < 0.05$) for all the antimicrobials; for *S. flexneri*, it was significant for SXT.
Conclusions: In Russia, most strains of *Shigella* spp. are resistant to AM, SXT, TE, and CL. All the strains are susceptible to fluoroquinolones and the third-generation cefalosporins which should be recommended as the drugs of choice.

P778 *Campylobacter jejuni* and *C. coli* resistance in humans, poultry products and farm chickens: an epidemiological and laboratory study

S. Uzunovic-Kamberovic, T. Zorman, L. Herman, I. Berce, S. Smole-Mozina
Zenica, BIH; Ljubljana, SI; Melle, B; Nova Gorica, SI

Objectives: To compare the frequency of isolation and occurrence of antimicrobial resistance among *C. jejuni* and *C. coli* isolated in humans, retail poultry meat and farm broilers in 2002.
Methods: Fifty-three human, 51 retail poultry meat and 15 *Campylobacter* spp. isolates from broiler cloacal swab were investigated for antibiotic susceptibility to 12 antimicrobials by disk-diffusion method. MICs were further determined for erythromycin- and ciprofloxacin-resistant isolates by Etest. To confirm ciprofloxacin-resistance we used a mismatch amplification mutation assay (MAMA) PCR to detect the gyrA mutation. Species were determined by multiplex PCR and genetic diversity by PFGE typing.
Results: *C. coli* isolated in significant proportion in all three sources, 27.8, 56.9 and 53.3%, respectively. Resistance to one or more antibiotic tested was 71.7, 89.5, and 53.8% and multiresistance 30.2, 41.7 and 30.85% in human, retail poultry and farm isolates, respectively. No significant difference was found in the overall resistance rates, and for each antibiotic tested between *C. jejuni* and *C. coli* isolates from all three sources, which is unusual finding. Moreover, they were higher in *C. jejuni*. Given that after the war population in this region were mostly the Muslim, *C. coli* in humans originated from other sources than pigs. Thus, it may suggest that *C. coli* resistance is origin-related. Erythromycin-

and ciprofloxacin-resistance was high and almost equal in all three sources (30.2, 30.6, 38.5%, and 32.1, 26.5, 30.8%, respectively).Imported retail poultry meat (from five countries) had higher resistance rates for erythromycin than domestic one, 38.7 vs. 26.7%, but ciprofloxacin resistance was higher in domestic one, 38.7 vs. 22.2%.
Conclusion: The distribution of *C. jejuni* and *C. coli* species and drug resistance in isolates from chicken and farm animals were similar to that seen in humans, even in the absence of antibiotic pressure (57% of patients were under 6 years of age, and growth promoters ban in Bosnia and Herzegovina), suggesting that poultry may play a role in human infections. When PFGE patterns were considered, they were remarkably diverse, suggesting considerable genetic heterogeneity. It may support the hypothesis that *Campylobacter* spp. from food animals and humans may not be represented by discrete populations but rather, form part of a common population shared by food animals and humans, suggesting related sources of infection.

Linezolid and glycopeptides in Gram-positive bacterial infection

P779 Oral linezolid as alternative therapy in patients with cellulitis referred to an infusion centre

G. Stein, S. Schooley, V. Kak
East Lansing, USA

Objectives: This investigation was designed to study the potential usefulness and economic benefits of oral linezolid as an alternative to outpatient parenteral antibiotic therapy (OPAT) in the treatment of primary cellulitis.
Methods: Patients with moderately severe cellulitis referred to an infusion centre for antibiotic treatment were enrolled into an open, non-randomised, pilot study. After informed written consent, patients were treated with oral linezolid, 600 mg q12 h, in place of their prescribed parenteral antibiotic. Patients were followed with clinic visits and lab monitoring.
Results: A total of 10 patients, five males and five females (mean age, 49 years), were enrolled. Seven were obese (mean weight, 146 kg; range, 101–196 kg), six had lower extremity cellulitis, one had lymphedema, and two were smokers. The average length of linezolid therapy was 12 days (range, 5–27 days). All were compliant with the treatment regimen and had a clinical cure of their infection. Mild side-effects (nausea, loose stools, headache, metallic taste) were reported by four patients. None developed thrombocytopenia or prematurely discontinued therapy. A 12-day course of linezolid therapy (drug costs, clinic visits, and lab monitoring) was found to be less expensive than 4 days of vancomycin treatment (1 g q12 h) in the infusion centre.
Conclusions: In this study, we found that oral linezolid was safe and effective in the treatment of moderately severe cellulitis and could be a suitable replacement for OPAT. Furthermore, oral linezolid has the potential to improve patient satisfaction as well as lower overall treatment costs when compared with OPAT.

P780 Gram-positive bacteria from diabetic foot ulcers and resistance to second-line antibiotics (glycopeptides, linezolid, quinopristin-dalfopristin) in a German university hospital

S. Monecke, S. Vogel, S. Weber
Dresden, Hoyerswerda, D

Objectives: Patients with diabetic foot ulcers are usually treated with various antibiotics for a long time. In poorly circulated or necrotic tissue, permanently low, i.e., sub-inhibitory concentrations of antibiotics might prevail. This gives bacteria the opportunity to develop resistance by gene exchange or by accumulation of mutations. In order to monitor this development, Gram-positive isolates from diabetic foot ulcers were collected and tested. Special emphasis was placed on second-line antibiotics such as glycopeptides, Linezolid and Quinopristin-Dalfopristin.
Methods: Diabetic patients included in the study were admitted to a specialised outpatient department of a German university hospital in November/December 2003. One hundred and seven isolates were selected consecutively from swabs from diabetic foot ulcers. They were identified and tested using MIC microdilution and e-tests.
Results: Of the 107 isolated bacteria, 46 were *Staphylococcus aureus*, 34 coagulase-negative staphylococci (CNS), 18 *Enterococcus faecalis*, one *E. facium*, one *E. avium*, four *Streptococcus* spp., two *Corynebacterium* spp., and one *Micrococcus* sp. Thirteen (28.2%) of the *S. aureus* and 14 (41.2%) of the CNS isolates were methicillin/oxacillin resistant. Neither vancomycin-resistant enterococci nor glycopeptide-intermediate staphylococci were found. Except for *E. faecalis* isolates (naturally resistant), there was no strain resistant to quinopristin-dalfopristin. No resistance to linezolid was detected.
Conclusion: In a population of patients of a German university hospital with diabetic foot ulcers the proportion of staphylococci resistant to methicillin/oxacillin is high. No resistance to second line antibiotics as glycopeptides, linezolid or quinopristin/dalfopristin was detected. Despite these favourable results, a close monitoring of isolates from diabetic foot ulcers is necessary as local conditions in these lesions might favour selection of multiresistant strains.

P781 Effects of oral linezolid treatment in methicillin-resistant *Staphylococcus aureus* colonisation in cystic fibrosis patients

A. Molina, R. Del Campo, L. Máiz, H. Escobar, F. Baquero, R. Cantón
Madrid, E

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) pulmonary colonisation is increasingly recognised in cystic fibrosis (CF) patients. Linezolid (LNZ) has demonstrated relevant *in vitro* activity against MRSA and its oral administration represents an excellent alternative to IV vancomycin, which is currently recommended for CF patients colonised with MRSA.
Material and methods: Oral LNZ (600 mg/12 bid) was administrated in two male CF patients (25 and 29 years) during 14 and 10 days, respectively. *S. aureus* isolates were cultured from different sputum samples recovered before, during, and after LNZ treatment. Antibiotic susceptibility was performed by NCCLS microdilution method using the Wider system (Fco. Soria Melguizo, S.A., Madrid, Spain). PFGE-Sma I was applied to analyse genetic relatedness of *S. aureus* isolates.
Results: In the first patient, a total of 10 isolates were analysed during the studied period; six of them recovered in the previous year to LNZ administration, two isolates during LNZ administration period, and two isolates 4 and 6 months after the end of treatment. With the exception of one isolate that was methicillin-susceptible and recovered during LNZ treatment period, all isolates were MRSA and presented homogeneous antibiotic susceptibility pattern. A single clone, with a subtype variant that included two isolates, was identified in all isolates, except in the

meticillin-susceptible one. In the second patient, two MRSA and one meticillin-susceptible isolates were recovered during 5 months before LNZ therapy. No MRSA were identified in the following CF controls during 8 months, but occasionally a methicillin susceptible isolate was recovered. MRSA isolates shared the same PFGE and antibiotic susceptibility pattern, whereas meticillin-susceptible isolates corresponded to two different clones unrelated with the MRSA clone. Independently of microbiology results, patients' pulmonary function remains unchanged after LNZ administration, although there was a mild improvement of pulmonary symptoms.
Conclusion: Oral LNZ treatment in CF may affect population dynamics of *S. aureus* colonisation, being effective in MRSA eradication. Despite this fact and assuming the brief follow-up period, maintenance or eradication of MRSA colonisation after LNZ treatment seems not to affect pulmonary function, which may be related to the uncertain role of this pathogen in CF patients.

P782 Successful treatment of methicillin-resistant (MRSA), teicoplanin-heteroresistant *Staphylococcus aureus* prosthetic valve endocarditis (PVE) with linezolid

M. Souli, K. Pontikis, Z. Chryssouli, K. Orlandou, I. Galani, D. Katsala, H. Giamarellou
Chaidari, GR

Objective: Vancomycin (V) in combination with rifampin (R) and gentamicin (G) has been the recommended regimen for the treatment of PVE caused by MRSA but intolerance to these agents and emergence of MRSA strains with reduced susceptibility to the glycopeptides create the need for alternative agents. We describe the case of a patient with MRSA tricuspid PVE who was successfully treated with linezolid (L) after failure of glycopeptides.
Case: A 67-year-old man was admitted for persistent MRSA bacteraemia. He had been treated with V, G, R, and trimethoprim/sulfamethoxazole (T/S) for MRSA PVE of the tricuspid valve which had recurred after a 45-day course of V and a 60-day course of T/S. Due to acute renal failure G was discontinued and V was changed to teicoplanin (T). He was transferred to our department because of persistent bacteraemia of 20 days duration despite adequate blood levels of T. Blood cultures revealed a MRSA strain with MICs of V, T, and L of 1, 2, and 0.75 mg/L, respectively. He was started on L (600 mg bid) and R (300 mg tid) and bacteraemia cleared the seventh day of treatment. He completed a 6-week course of L and a 3-week course of R. During his treatment he developed anaemia which was managed with blood transfusions and erythropoetin, mild leucopenia and mild thrombocytopenia. He was discharged afebrile with sterile blood cultures and a TEE showing reduction in the size of the vegetation. The patient remained well and blood cultures were sterile one month later while pancytopenia fully recovered. The MRSA isolate was investigated for heteroresistance to glycopeptides with (1) a simplified and (2) a detailed population analysis profile method. (1) 0.01 mL of a 10^8 CFU/mL bacterial suspension was plated on BHI agar with V (4 mg/L). Subclones that grew after 48 h were submitted to MIC determination. (2) Tenfold serial diluents of an inoculum of 10^8 CFU/mL were plated on BHI agar plates with increasing concentrations of V or T alone or with 4% NaCl. Viable colonies were counted at 48 h and plotted against the antibiotic concentration. Subclones with V MIC 4–6 mg/L were identified, suggesting that the isolate had heterogeneously reduced susceptibility to V which probably explained the failure of V treatment. The population curve showed that 50% of the original inoculum survived on T concentration $\geq$8 mg/L suggesting heteroresistance to T.
Conclusions: L offers an alternative for the treatment of methicillin-resistant–glycopeptide-heteroresistant *S. aureus* PVE.

P783 The possible role of linezolid in the control of methicillin-resistant *Staphylococcus aureus* outbreaks

I. Pulay, D. Varga Szabó, E. Ungvári, K. Kristóf, M. Konkoly-Thege
Budapest, HUN

Objectives: Patients treated on surgical and traumatological wards, on burn and intensive care units are at highest risk of nosocomial infections due to methicillin-resistant *Staphylococcus aureus* (MRSA). The spread of MRSA results in many therapeutic and economic difficulties for these departments. Epidemiological measures and antibiotic treatment play the most important role in the control of MRSA outbreaks. Vancomycin and teicoplanin are widely used for treatment of patients, while a new oxazolidinon – linezolid – has been introduced into the clinical practice only recently. The aim of the study was to determine the effectiveness of linezolid in the control of an MRSA outbreak.
Patients and methods: An MRSA outbreak involving 10 patients (nine men and one woman, mean age 62 years) was registered at the 1st Department of Surgery, Semmelweis University, in a 4-month period in the middle of 2002. In the previous years the incidence of MRSA infections was extremely rare in this tertiary care centre: one to four sporadic cases per year. Seven patients were infected, and three proved carriers. Extensive epidemiological and microbiological investigations were carried out in order to find the source of the outbreak, and characterise the isolates by phage typing and pulsed-field gel electrophoresis (PFGE). All the 10 patients were treated with 2×600 mg linezolid for a mean of 5.6 days intravenously, and 2.6 days per os.
Results: Phage typing revealed that the outbreak was caused by three different MRSA strains (phage type 623, 629, and non-typable). PFGE has confirmed this result. In the seven infected cases the complex treatment was effective: the patients cured. All the three carriers died; however, the fatal outcome was independent from the carrier state. The control nose and throat cultures performed 3–6 weeks after the hospital emission of the patients were MRSA negative in all seven cases. Any toxic or side effect of linezolid has not been experienced.
Conclusions: The MRSA outbreak was stopped by the mandatory epidemiological measures and by linezolid treatment. Linezolid could be effective not only in curing the MRSA infections but in eradication of the carrier state as well.

P784 Pharmacoeconomic advantages of linezolid for the treatment of Gram-positive infections

D. Nathwani, S. Duttagupta
Dundee, UK; New York, USA

Objectives: Gram-positive bacterial infections, in particular methicillin-resistant *Staphylococcus aureus* (MRSA), represent a major economic burden worldwide. In cost-containment environments, it is imperative to demonstrate the true value of innovative medications to physicians, patients, and payers. Clinical studies have demonstrated the efficacy and safety of linezolid (LNZ) for the treatment of Gram-positive infections. Health-economic and outcomes endpoints from three clinical trials were analysed to determine the value of LNZ to healthcare decision makers.
Methods: Out of three randomised, open-label, multinational studies, two compared length of stay (LOS) between patients treated with either LNZ or vancomycin (VAN). The first study compared LOS in hospitalised patients with MRSA infections, while the second study compared LOS in hospitalised patients with complicated skin and soft-tissue infections (cSSTI) due to suspected/confirmed MRSA. The third study compared hospital resource use and cost of treatment between LNZ and teicoplanin (TEI) in patients with severe Gram-positive infections from Europe, South America, and Mexico.
Results: In all three studies, patients treated with LNZ had shorter intravenous antibiotic treatment (IVAT) duration than

patients in comparator groups, which results in increased rates of early patient discharges and reduced use of resources. In two of the studies, patients had significantly shorter mean LOS and greater odds of early discharge from hospital. Indeed, compared with TEI, treatment with LNZ had 66% greater odds of early discharge ($P = 0.049$); a similar early discharge potential was also seen when LNZ was compared with VAN ($P = 0.005$). In select patient populations, such as those with cSSTI due to suspected/confirmed MRSA, reduction in LOS may be even more pronounced in LNZ- vs. VAN-treated patients. For the cost comparison in the third study, total mean adjusted cost was also reduced by US $335 ($P > 0.05$) in the LNZ group compared with the TEI group in patients from South America and Mexico.

Conclusions: Across multiple studies, there is consistent evidence of significant reductions in LOS and IVAT associated with LNZ treatment, with significant differences in the rate of early patient discharge. Therapy with LNZ shows pharmacoeconomic advantages that have the potential to reduce total costs of treatment.

P785 Linezolid vs. glycopeptides for the treatment of suspected or known Gram-positive infections in critical patients

F. Alvarez-Lerma, M. Palomar, F. Bobillo, V. González, J. Solé, A. Blanco, C. León - Study Group for Gram-positive Infections

Introduction: Linezolid is the first of a new antimicrobial class, the oxazolidinones, which has been shown to be active against most isolates of Gram-positive (GP) microorganisms, including those resistant to methicillin and vancomycin. Its main indication is the treatment of GP infections due to multiresistant bacteria.

Objective: To evaluate the efficacy and tolerability of linezolid (LNZ) vs. glycopeptides, either vancomycin (VAN) or teicoplanin (TPN), in critically ill patients who have known or suspected infections due to GP multiresistant bacteria.

Methods: Multicentre, controlled, open and randomised Phase III Clinical Trial. Inclusion criteria: known or suspected multiresistant GP infection of inpatients in Intensive Care Units with an APACHE II between 8 and 24. Study drugs: LNZ 600 mg b.i.d. or VAN, 1 g b.i.d or TPN 400 mg o.d. (the first three doses were administered b.i.d.). Each patient was randomised as follows: 2 LNZ : 1 VAN : 1 TPN. Study populations selected for analysis: Intention to treat population (ITT), clinically and microbiologically evaluable population (CME). Data selected for analysis: End of treatment (EOT). Statistical analysis: chi-square for qualitative variables and Student's *t*-test for quantitative variables. We also included a logistical regression analysis for identification of factors that may have influence in satisfactory response.

Results: 300 patients were recruited (155 LZD, 75 VCN, 70 TPN). We excluded 91 patients from efficacy analysis due to absence of microorganisms or GP isolates. No significant differences were identified within the following variables, both in ITT and CME populations: demographic data, infection severity, infection site, type of infection and type of microorganism isolated. Main findings: Patients discontinuing therapy due to failure of treatment: LZD, 3.7%; VCN, 9.3%; TPN, 12.5% (P = NS). Average days of treatment: LZD, 11.9; VCN, 13.7; TPN, 12.5 ($P = 0.08$). Satisfactory clinical response: LZD, 86.9%; VCN, 74.1%; TPN, 77.1% (P = NS). Individual factors associated with favourable response: absence of abdominal infection (OR 0.11, IC95% 0.020–0.558), days of treatment (OR 1.19, IC95% 1.066–1.324), treatment with LNZ (OR 2.70, IC95% 1.157–6.310).

Conclusions: LNZ was an independent variable for favourable response compared with glycopeptides (vancomycin and teicoplanin) for the treatment of GP infections in critically ill patients.

P786 Linezolid vs.vancomycin for culture-proven complicated skin and soft tissue infections – trends of subjects enrolled in Europe and globally in a global study

M. Dryden, N. Chaudry, A. Reisman, J. Weigelt
Winchester, UK; New York, Milwaukee, USA

Objective: This study examined the clinical efficacy and safety in subjects with culture-proven cSSTIs treated with either LZD or VAN. The aim was to detect trends in efficacy rates between subjects enrolled in the European Union (EU) and overall (globally).

Methods: This was a controlled, multicentre, global study with subjects randomised in a 1:1 ratio to receive open-label LZD 600 mg IV or PO q12 h or VAN 1 g IV q12 h for 7–21 days. Clinical efficacy was determined by investigator's assessment of resolution of signs and symptoms compared with baseline (Day 1) at the test-of-cure (TOC) visit (7 days post-therapy). Primary population was intent-to-treat (ITT), defined as all treated subjects. Clinically evaluable (CE) population included ITT subjects who received >4 days of therapy and completed the TOC visit. Microbiologically evaluable (ME) population included all CE subjects with ≥1 Gram-positive pathogen isolated at baseline. EU countries included Portugal, Spain, and United Kingdom. Global regions included EU, North and South America, and Asia.

Table. Clinical efficacy, ITT, CE and ME population [*N* (%)]

	LZD	VAN	95% CI**	*P*-value**
ITT				
Global	439/476 (92.2)	402/454 (88.5)	−0.11, 7.47	0.0565
EU*	37/40 (92.5)	34/42 (80.9)		
CE				
Global	436/462 (94.4)	394/436 (90.4)	0.53, 7.48	0.0234
EU*	37/39 (94.9)	33/39 (84.6)		
ME				
Global	312/330 (94.5)	278/310 (89.7)	0.69, 9.05	0.0218
EU*	26/28 (92.8)	28/33 (84.8)		

*Included Portugal, Spain, and United Kingdom.
**CI for global is for difference LZD − VAN, EU not sufficiently powered for analysis.

Results: 1200 subjects were enrolled globally, 92 in the EU. Globally, the clinical efficacy rates of LZD were higher than VAN in all populations at TOC (see table). Clinical efficacy rates in EU subjects appeared to be similar for LZD and lower for VAN to global subjects for each treatment group in each population. Methicillin-resistant *S. aureus* (MRSA) was the most isolated baseline pathogen globally (LZD 140; VAN 145). Among global ME subjects with MRSA, LZD demonstrated superior clinical efficacy: LZD, 94.0% (126/134); VAN, 83.6% (112/134) (95% CI, –3.00, 17.89; $P = 0.0108$). Overall incidence of ≥1 treatment-related adverse event was 22.1% for LZD and 20.8% for VAN.

Conclusions: LZD demonstrated higher clinical efficacy than VAN among all global subjects with culture-proven cSSTIs, including MRSA. Clinical efficacy rates of EU subjects appeared to exhibit similar trends to rates of global subjects.

P787 Effects of oral linezolid treatment in methicillin-resistant *Staphylococcus aureus* colonisation in cystic fibrosis patients

A. Molina, R. Del Campo, L. Máiz, H. Escobar, F. Baquero, R. Cantón
Madrid, E

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) pulmonary colonisation is increasingly recognised in cystic fibrosis (CF) patients. Linezolid (LNZ) has demonstrated relevant

in vitro activity against MRSA and its oral administration represents an excellent alternative to IV vancomycin, which is currently recommended for CF patients colonised with MRSA.
Material and methods: Oral LNZ (600 mg/12 bid) was administrated in two male CF patients (25 and 29 years) during 14 and 10 days, respectively. *S. aureus* isolates were cultured from different sputum samples recovered before, during, and after LNZ treatment. Antibiotic susceptibility was performed by NCCLS microdilution method using the Wider system (Fco. Soria Melguizo, S.A., Madrid, Spain). PFGE-SmaI was applied to analyse genetic relatedness of *S. aureus* isolates.
Results: In the first patient, a total of 10 isolates were analysed during the studied period; six of them recovered in the previous year to LNZ administration, two isolates during LNZ administration period, and two isolates 4 and 6 months after the end of treatment. With the exception of one isolate that was methicillin-susceptible recovered during LNZ treatment period, all isolates were MRSA and presented homogeneous antibiotic susceptibility pattern. A single clone, with a subtype variant that included two isolates, was identified in all isolates, except in the meticillin-susceptible one. In the second patient, two MRSA and one meticillin-susceptible isolates were recovered during 5 months before LNZ therapy. Another methicillin-susceptible isolate was recovered after the LNZ therapy and no *S. aureus* were identified in the following CF controls during 8 months. MRSA isolates shared the same PFGE and antibiotic susceptibility pattern, whereas meticillin-susceptible isolates corresponded to two different clones unrelated with the MRSA clone. Independently of microbiology results, patients' pulmonary function remains unchanged after LNZ administration.
Conclusion: Oral LNZ treatment in CF may affect population dynamics of *S. aureus* colonisation, being effective in MRSA eradication. Despite this fact and assuming the brief follow-up period, maintenance or eradication of MRSA colonisation after LNZ treatment seems not to affect pulmonary function, which may be related to the uncertain role of this pathogen in CF patients.

P788 *In vitro* spectrum of linezolid and other agents against clinical isolates of anaerobes

K. Aldridge, C. Manders, S. Broyles
New Orleans, USA

Objectives: Linezolid is an oxazolidinone antimicrobial with established *in vitro* and *in vivo* activity against aerobic Gram-positive cocci. In infections such as wounds these Gram-positive pathogens may be mixed with other pathogens including anaerobes. The role of linezolid as an anti-anaerobe agent has yet to be determined. This study was performed to establish the *in vitro* activity of linezolid and comparative agents against recently isolated anaerobes.
Methods: Approximately 700 anaerobes were tested for susceptibility to linezolid (LZD), ceftriaxone (AXO), cefoxitin (FOX), clindamycin (CL), and metronidazole (MRD) using twofold dilutions (0.06–256 mg/L) of each agent using the NCCLS-recommended broth microdilution method. The sources of test isolates included wounds, abscesses, body fluids, and tissues.
Results: Against all test isolates LZD had an MIC range of 0.06–128 mg/L, a mode MIC of 4 mg/L, and MIC50 and MIC90 values of 2 and 4 mg/L, respectively. LZD activity was judged by percentage of isolates inhibited at 2 and 4 mg/L. Overall LZD inhibited 51 and 96% of isolates at 2 and 4 mg/L, respectively; at 2 and 4 mg/L, respectively, LZD inhibited 35 and 95% of *Bacteroides fragilis* group; 63 and 92% of *Clostridium* isolates; 71 and 97% of *Prevotella* isolates; 100% of *Fusobacterium* isolates; and 100% of *Peptostreptococcus* isolates. By comparison of MIC90 values LZD was 2- to 64-fold more active than AXO, 0- to 16-fold more active than FOX, and 2- to 32-fold more active than CL against these same groups of isolates. LZD and MRD had virtually equal *in vitro* activity. Interestingly, all isolates with MICs of 8 mg/L or higher to LZD had MICs of 2 mg/L or less to MRD, while isolates with MICs of 8 mg/L or higher to MRD had MICs of 4 mg/L or less to LZD.
Conclusions: Based on these results and arbitrary use of NCCLS breakpoints for Gram-positive isolates, we conclude that LZD is highly active against anaerobe pathogens, but this needs to be verified by pharmacokinetic and clinical studies.

Epidemiology of antibiotic susceptibility

P789 Decline in antimicrobial susceptibilities of anaerobic bacteria from the academic hospitals in Bloemfontein, South Africa, 1996–2003

P.O. Smith, M.N. Janse van Rensburg, M. Theron
Bloemfontein, ZA

Objectives: Susceptibility testing of anaerobic infections is not routinely done in all microbiology laboratories and concerns about resistance development are on the increase. The study was conducted to investigate the rate of resistance development in anaerobic bacteria over a period of 7 years.
Methods: The antimicrobial susceptibilities of 130 anaerobic bacteria collected from the Pelonomi and Universitas Hospitals in Bloemfontein, South Africa, from March 2002 to April 2003 were determined. The isolates included 13 *Clostridium perfringens*, 17 *Clostridium* spp., 11 *Peptostreptococcus anaerobius*, 20 *Peptostreptococcus* spp., 41 *Bacteroides fragilis*, 17 *Prevotella* spp. and 11 *Fusobacterium* spp. strains. MICs were determined for 12 antimicrobial agents using the NCCLS agar-dilution method. Susceptibility data were compared with the MICs obtained in a study done on isolates from 1996/1997.
Results: On comparing the MICs from the current study with the results from 7 years ago, an unmistakable and very alarming decline in susceptibility was noted for all the antimicrobial agents tested. The greatest difference in susceptibility was noted for cefoxitin (from 91 to 62%), metronidazole (from 98 to 78%), piperacillin (from 84 to 68%) and amoxicillin (from 74 to 60%). The antimicrobial agents for which <5% decrease in susceptibility was found, included meropenem (from 96 to 93%), clindamycin (85 to 81%) and ciprofloxacin (from 74 to 69%). A great concern, however, was an 8% decrease found in the susceptibility for imipenem (from 96 to 88%).
Conclusions: A decade ago, most anaerobic bacteria were susceptible to antimicrobial agents usually used for infections caused by these bacteria. The results from this study, however, indicate a situation that has undergone some dramatic changes in a relatively short period. It is of concern that the agents most frequently used in the empirical treatment of anaerobic infections, such as metronidazole and the β-lactams such as cefoxitin and piperacillin have shown the most alarming decrease in susceptibility. There is now, more than ever before, a definite need for continuous susceptibility testing of anaerobes and a serious restructuring of the treatment regimes for anaerobic infections.

P790 Antimicrobial resistance of enteropathogenic *Escherichia coli* isolated from rabbits in South Italy

A. Camarda, D. Pennelli, P. Battista, M. Corrente, I. Alloggio
Valenzano, I

Objectives: *Escherichia coli* isolated from fattening-rabbits dead for enteritis were biotypised, tested with PCR for the presence of virulence genes eae and afr2 coding for intimin and the fimbrial adhesin AF/R2 and investigated for antimicrobial resistance.
Methods: Fifty-six strains of *E. coli* isolated in 28 farms were biotypised using the fermentation of sorbose, dulcitol, raffinose, sucrose and L-rhamnose. Detection of drug resistance was determined using the method of Kirby-Bauer on Mueller-Hinton agar with antibiotic disks containing gentamicin (GM10), amikacin (AN30), tetracycline (TE30), erythromicin (E15), spiramicin (SP100), enrofloxacin (ENR5), flumequine (AR30), trimethoprim/sulphametoxazole (SXT), amoxicilline (AMX 25), apramycin (APR30), difloxacine (DFX10), marbofloxacine (MAR5), nalidixic acid (NA30), neomycin (N30), colistin (CL50), streptomycin (S10).
Results: 10 biotypes (B0, B1, B8, B9, B12, B16, B17, B24, B25, B28) were detected: biotypes 8 and 24 were predominant in rabbitries. Eae and afr2 genes were almost observed in *E. coli* strains belonging to them. Results of antibiograms have shown that all the isolates (100%) were E15 resistant. High rate of resistance were also found towards SP100 (98.2%), SXT (92.8%), TE30 (87.5%), S10 (73.2%), GM10 (71.4%), N30 (69.3%). About 91% of *E. coli* tested showed the same susceptibility rate (91.5%) to MAR5 and CL50. Susceptibility to DFX10, ENR5, AR30, NA30, was exhibited by the 80.3, 78.5, 75, 71.4%, of the strains, respectively. Sensitivity against AMX25 was quite high (76.7%). Multiple antibiotic resistance was expressed by all *E. coli* tested. The most prevalent resistotypes were resistant to TE30-E15 SP100-SXT, detected in 47 strains (83.9%), TE30-E15-SP100-SXT-S10, which accounted for over 60% and TE30-E15-SP100-SXT-GM10 detected in 67.8% of isolates.
Conclusions: No significant correlation was observed between enteropathogenic *E. coli* (eae+ and AF/R2+) and pattern of antibiotic resistance. Quinolones have shown very good activity; in particular MAR5, which has been recently adopted in veterinary medicine seems to possess high efficacy. On the other hand, *E. coli* strains exhibited high-level of resistance to antimicrobials. Like human *E. coli*, rabbit strains revealed different patterns of multiresistance, which could make disease control difficult in rabbits and also promote dissemination and increasing of antimicrobial resistance in human strains.

P791 Frequency and antimicrobial susceptibility of bacterial pathogens isolated from bloodstream infections in haematology–oncology patients: results from the Latin America SENTRY Antimicrobial Surveillance Program (1997–2002)

S.S. Andrade, A.C. Gales, H.S. Sader, R.N. Jones, J.B. Silva, A.C.C. Pignatari and The Sentry Participants Group – Latin America

Objective: To determine the frequency and susceptibility patterns of bacterial pathogens isolated from bloodstream (BSI) of haematology–oncology patients hospitalised at Latin American medical centres.
Material and methods: As part of the SENTRY Antimicrobial Surveillance Program, a total of 1587 BSI isolates were recovered from haematology–oncology patients from 1997 to 2002. The isolates were susceptibility tested to >20 antimicrobial agents in a central laboratory using NCCLS broth microdilution method.
Results: The most frequent isolated pathogen was coagulase-negative staphylococci (CoNS; 17.7%), followed by *Escherichia coli* (17.5%), *Staphylococcus aureus* (15.8%), *Klebsiella pneumoniae* (10.3%), *Pseudomonas aeruginosa* (8.9%), *Enterobacter* spp. (6.7%), *Acinetobacter* spp. (4.5%), and Enterococcus spp. (3.1%). Oxacillin-resistance rates were 33.9 and 74.4% among *S. aureus* and CoNS, respectively, isolates. The prevalence of ESBL-producing strains ranged from 9.4% for *E. coli* to 41.1% for *K. pneumoniae*. For *Enterobacter* spp., susceptibility rates were 54.7 and 86.9% to ceftazidime and cefepime, respectively. All Enterobacteriaceae isolates tested were susceptible to carbapenems. The susceptibility of *P. aeruginosa* to imipenem and meropenem was 83.7 and 86.5%, respectively; 82.2 and 92.8% of the Gram-negative bacilli were susceptible to cefepime and meropenem, respectively. Only 4.1% of the *Enterococcus* spp. isolates were resistant to vancomycin.
Conclusions: In contrast to American and European reports, Gram-negative bacilli represented the major cause of BSI among haematology–oncology patients in the Latin American hospitals evaluated. The antimicrobial agents with the best covered against such pathogens were the carbapenems and cefepime. However, none of the evaluated antimicrobial agents inhibited the growth of 100.0% of the Gram-negative bacilli. Thus, continued monitoring by surveillance programs is necessary to determine if the observed trends would continue to be recorded.

P792 Frequency of occurrence and antimicrobial susceptibility profile of pathogens causing bloodstream infections in paediatric patients from Latin America: report of SENTRY Antimicrobial Surveillance Program (1997–2002)

A.C. Gales, S.S. Andrade, H.S. Sader, R.N. Jones, S. Silbert, A.C.C. Pignatari and The Sentry Participants Group – Latin America

Objective: We attempted to verify if the frequency of occurrence (FO) and antimicrobial susceptibility profile (ASP) of bacterial isolates responsible for causing bloodstream Infections (BSI) in paediatric patients varied along the years and age categories.
Methods: A total of 2450 bloodstream isolates were collected from paediatric patients hospitalised in Latin American hospitals through the SENTRY Program between 1997 and 2002. The ASP to various antimicrobials was determined by the NCCLS broth microdilution method. The FO and ASP were studied according to age categories (AC): $\leq$1 year, 2–5 years, and 6–12 years.
Results: Overall, *S. aureus* (SA) was the most frequently isolated pathogen among children $\leq$1 year (18.3%) and 6–12 years (26.8%) followed by coagulase negative staphylococci (CoNS). Among children $\leq$1 year and 2–5 years, *S. pneumoniae* (SPN) ranked among the top five pathogens. In contrast, it has caused less than 5.0% of BSI among children 6–12 years. Curiously, in this age group, *Acinetobacter* spp. and *P. aeruginosa* (5.8%) assumed the fifth position in the rank order of frequency. In general, among SA, the oxacillin resistance (OR) rates were lower in the 6–12-year-old AC (17.2%; $P \leq 0.05$) than in children $\leq$1 year (27.3%) and 2–5 years (25.0%). In contrast, among the CoNS, elevated rates of OR were noticed in all ACs ($\leq$1 year, 82.1%; 2–5 years, 81.2%; 6–12 years, 78.3%; $P > 0.05$). ESBL-producing *K. pneumoniae* were more frequently detected in the AC $\leq$1 year (66.8% of the *K. pneumoniae* isolates) and 2–5 years (64.1%) than 6–12 years (52.0%). On the contrary, ESBL-producing *E. coli* isolates were less frequently encountered among children $\leq$1 year (12.1%) than children $\geq$6 years (21.9%). However, these differences did not reach statistical significance ($P > 0.05$). SPN isolates showing reduced susceptibility to penicillin were detected more frequently in the AC of $\leq$1 year (39.2%) and 2–5 years (32.7%) than in 6–12 years (15.0%; $P \leq 0.05$).
Conclusions: Although only slight differences in the FO of BSI pathogens was noticed along the years and AC, important differences were observed on the ASP of the BSI pathogens according to the age categories, especially for SPN and SA isolates.

P793 Frequency of Gram-positive bacterial pathogens in bloodstream infections and their resistance to antibiotics in the Czech Republic

M. Kolar, T. Latal, B. Heinigeova, N. Bartonikova, P. Cermák
Olomouc, Prague, Jindrichuv Hradec, Zlin, Hradec Kralove, CZ

Objectives: The aim of this prospective, multicentric study was to assess incidence of Gram-positive bacteria in bloodstream infections (BSI) and characteristics of their resistance to antibiotics in the Czech Republic.

Methods: The study was done in 15 sites in the Czech Republic from January to April 2003. Consecutive Gram-positive strains isolated from blood were assessed and their clinical significance was evaluated.
Results: The strains of *Staphylococcus aureus* (39%), coagulase-negative staphylococci (34%), *Streptococcus pneumoniae* (11%) and *Enterococcus* spp. (9%) were identified as the etiologic agent of Gram-positive BSI. The frequency of oxacillin-resistant strains was in *Staphylococcus aureus* and in coagulase-negative staphylococci 10 and 41%, respectively. All *Streptococcus pneumoniae* strains were susceptible to penicillin and chloramphenicol. No strains resistant to glycopeptides were found in enterococci. Clinical significance of isolated Gram-positive bacteria was significantly conditioned by bacterial species ($P = 0.001$) and reached 100% in *Streptococcus pneumoniae*, 87% in *Staphylococcus aureus*, 82% in *Enterococcus* spp. strains and 10% in coagulase-negative staphylococci. Production of bacterial biofilm was shown in 56% *Staphylococcus aureus* strains and in 42% coagulase-negative staphylococci. BSI was the immediate cause of death of the patient in 5%.
Conclusion: We could confirm that presence of artificial material means significant risk factor for BSI. Catheter-related infections were present in 32% of cases. Forty-six per cent of BSI can be characterised as secondary and pneumonias, GIT infections and urinary tract infections were the most common sources. The frequency of *Staphylococcus* spp. with positive finding of biofilm was 49% in this study; this finding supports its clinical significance.
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P794 Trends of *Staphylococcus aureus* susceptibility to antibiotics isolates in a university hospital in France, 1997–2002

L. Gambotti, P. Girardo, C.M. Couris, M.E. Reverdy, J. Etienne, P. Vanhems
Lyon, F

Objectives: To describe the trends of methicillin-resistant *Staphylococcus aureus* (MRSA) strains proportions during 6 years among all *Staphylococcus aureus* (SA) positives isolates diagnosed in a 1100 bed university hospital.
Methods: A total of 6670 *S. aureus* positive sample isolates between 1 January 1997 and 31 December 2002 from the laboratory were analysed. The susceptibility to antibiotics was assessed by antibiogram based on the API system (bioMérieux®) according to the French guidelines (CA-SFM). The SA strains were classified as methicillin susceptible and gentamicin susceptible (gentaS-MSSA), methicillin resistant and gentamicin susceptible (gentaS-MRSA), methicillin susceptible and gentamicin resistant (gentaR-MSSA) or methicillin resistant and gentamicin resistant (gentaR-MRSA). The number of isolates was calculated for 1000 admissions. Means per year were compared using Kruskal Wallis test. The Spearman coefficient (R) was used to calculate the correlation between the proportion of isolates (for each antibiotic resistance profile) and months.
Results: The overall proportion of SA positive samples for 1000 admissions during the study period was: 11.2, 12.0, 12.7, 11.9, 11.4 and 11.8 for 1997, 1998, 1999, 2000, 2001 and 2002, respectively ($R = -0.2$; $P = 0.9$). The percentage of MSSA was 76.2 (75.7 for gentaS, 0.5 for gentaR) and the percentage of MSSA was 23.8 (20.1 for gentaS, 3.7 for gentaR) for the total period. Patients with MRSA were older (57.3 years) compared with patients with MSSA (mean age 41.5, $P < 0.0001$) but patients with gentaR-MRSA were younger (53.2 years) compared with patients with gentaS-MRSA (mean age 58.1, $P = 0.006$). The proportion of gentaS-MSSA for 1000 admissions was similar by time (9.4, 9.0, 9.9, 8.8, 8.2, 9.0, $P = 0.5$) ($R = -0.1$; $P = 0.5$). However, the proportion of gentaS-MRSA strains increased significantly (1.7, 2.4, 2.4, 2.5, 2.9, 2.5, $P = 0.001$) ($R = 0.4$; $P < 0.0001$) while the proportion of gentaR-MRSA strains decreased significantly during the period (0.6, 0.6, 0.3, 0.6, 0.3, 0.3, $P = 0.001$) ($R = -0.4$; $P < 0.0001$).
Conclusion: Although the proportion of SA positive samples for 1000 admissions remains constant during the last 6 years, there is a continuous increasing trend of isolates with gentaS-MRSA and a decreasing trend of isolates with gentaR-MRSA. The age difference between these two sub-groups should be explored.

P795 A map of bacterial frequency and resistance in a Greek Region – Corfu Island

E. Gatsouli, M. Ovrenovits, A. Pasxali, A. Tzanavari
Corfu, GR

Background: In order to assess the regional trends of microbiological resistance pattern, all cultured bacteria isolated in 2003 in our Laboratory were reviewed as to specimen source and susceptibility profile.
Materials and methods: In 2003, 7220 samples were cultured, 75% (5415) of hospitalised patients and 25% (1805) from ambulatory patients. The samples were: 3760 urine, 1276 blood cultures, 580 lesions and 1604 samples of other secretions. Classic culture methods, Vitek system and NCCLS breakpoints were used.
Results: Cultivations were positive in 23% (1661, 1381 adults and 280 children samples). The distribution of bacteria differed according to the types of specimens. The distribution of 1147 Gram(−) was 1003 Enterobacteriaceae and 145 Nonfermentative bacilli. There were 460 Gram(+) cocci and 54 yeasts, too. *E. Coli* predominated in enterobacteriaceae (65%), followed by *Klebsiella* sp., *P. aeruginosa* in non-fermentative bacilli (70%) and *A. baumanii* (29%). Among the Gram(+) *S. aureus* was the most frequent (42.5%), followed by CNS. Ampicillin inhibited growth of 35% for *E. Coli*. Ttime/Sulfa combination could inhibit less than 16% and the second-generation cephalosporins less than 25%, while fluoroquinolons were very effective against enterobacteriaceae strains (more than 95%). Piperacillin inhibited growth of 8% of *P. aeruginosa* and quinolons less than 17%. *Enterococcus* strains were highly sensitive to teicoplanin (100%) and nitrofurantoin (97.9%). MRSA were 31% but GISA were 1%. *A. baumanii* and GISA were in ICU.
Conclusion: A permanent surveillance of frequency and sensitivity levels of the most common pathogens responsible for infectious enables to identify local antimicrobial activity and plays a key role in starting empiric therapy pending bacterial identification and *in vitro* assay.

P796 The epidemiology of antibiotic resistance

M. Heginbothom
Cardiff, UK

Objectives: The biochemistry and genetics of antibiotic resistance are well documented; however, information regarding the medical and social factors that influence its occurrence remains lacking. The aim of this study was to elucidate these latter relationships and to examine the dynamics of their effects.
Methods: Antibiotic resistance data for bacterial isolates obtained from the community was collected from all microbiology laboratories in Wales from 1996 to 2003. Antibiotic prescribing data, practice demographics, deprivation indices, general practitioner demographics, and details of sampling behaviour was also obtained for the same period for all general practices in Wales. Initial analyses exploring the nature of these data and the relationships of the various components were undertaken using Excel and SPSS.
Results: Preliminary analyses indicate that both antibiotic resistance and prescribing varied between practices. For coliform UTIs, there was a clear association between high prescribing and higher levels of resistance, with prescribing accounting for 10–20% of variation in resistance. The correlation between prescribing and resistance was not confined to the urinary coliforms but seen throughout a range of pathogens including those responsible for respiratory and skin infections. There was an association between

resistance and social deprivation exceeding that expected from high prescribing in deprived areas and an apparent association between resistance and the number of practitioners in a practice and the practice list size. Resistance was more common in infections in the young (<10 years), the aged (>60 years) and for some pathogens resistance was significantly greater in males. Multilevel modelling, regression analysis and time series analysis of this complex data set is in progress.
Conclusions: Antibiotic usage appears to affect resistance at practice level and the dynamics of this selection process are currently being investigated. It is hoped that these studies will assist in the design of interventions to limit the future impact of resistance and contribute to our ability to predict their outcomes.

P797 Antimicrobial resistance among *Staphylococcus aureus* and *Escherichia coli* isolates in the Indonesian population inside and outside hospitals

E.S. Lestari, K. Kuntaman, H.A. Verbrugh on behalf of the study group Antimicrobial Resistance in Indonesia: Prevalence and Prevention (AMRIN)

Objectives: Data about the prevalence of antimicrobial resistance in Indonesia are limited. The AMRIN study measured the prevalence of antimicrobial resistance in the Indonesian population inside and outside hospitals.
Methods: 4000 individuals were targeted to be screened constituting four different populations in each of two cities: patients admitted to hospital, patients discharged from hospital, patients visiting primary health centres, and relatives of patients admitted to hospital. Nasal swabs and rectal swabs were taken and cultured using phenol red mannitol agar for the isolation of *Staphylococcus aureus*, and CHROM agar orientation medium for *Escherichia coli*. Susceptibility testing was performed by disk diffusion method recommended by NCCLS.
Results: 3996 individuals were included in the study between July and October 2001 in Surabaya and between January and May 2002 in Semarang equally distributed over the four groups and two cities. *S. aureus* isolates ($n = 298$) were frequently resistant to tetracycline (23%) and oxacillin (7%) without obvious differences between the four populations. None of the oxacillin resistant strains of *S. aureus* harboured mec A gene. *E. coli* isolates ($n = 3284$) showed considerable levels of resistance against a number of commonly used antibiotics. The highest levels of resistance to ampicillin (72.5%), chloramphenicol (42.5%), gentamicin (17.5%), cefotaxim (12.5%), ciprofloxacin (22.5%), and cotrimoxazole (55.5%) were among *E. coli* isolated from patients on the day of discharge from hospitals. Resistance rates were consistently lowest among *E. coli* from relatives of patients on admission to hospital and among patients visiting primary health care centres.
Conclusions: The results show that antimicrobial resistance among common bacterial pathogens has emerged in Indonesia. Among *E. coli* the prevalence of resistance to ciprofloxacin and other antibiotics is remarkable high, especially in individuals after hospitalisation. Although the prevalence of MRSA is low, tetracycline resistance is common among *S. aureus* and not associated with hospital stay.

P798 Surveillance of invasive bacterial diseases in Greenland, Northern Canada, and the US Arctic: 2000–2003

T. Cottle, M. Bruce, J. Butler, D. Parks, T. Tam, S. Deeks, K. Jenson, A. Parkinson
Anchorage, USA; Ottawa, CAN; Nuuk, GL

Objectives: The International Circumpolar Surveillance system conducts population-based surveillance of invasive bacterial diseases in Greenland (GN), Northern Canada (N Can), and in the US Arctic (AK).
Methods: Isolates from patients with invasive diseases caused by *Haemophilus influenzae* (Hi), *Neisseria meningitidis* (Nm), Group A *Streptococcus* (GAS), and Group B *Streptococcus* (GBS) were forwarded to reference laboratories in Alaska (2000–2003), Canada (2000–2003), and Greenland (2001–2003) for confirmation and serotyping. Chart reviews were conducted on confirmed cases to verify illness episode information. Data reported for 2003 are preliminary.
Results: The total numbers of reported cases were 86 Hi, 34 Nm, 126 GAS, and 92 GBS. Crude annual rates of invasive disease per 100 000 population varied by country and organism [Hi (AK = 1.96, N Can = 6.68, GN = 0), Nm (AK = 1.02, N Can = 0.56, GN = 2.95), GAS (AK = 4.15, N Can = 3.53, GN = 0.59), GBS (AK = 2.94, N Can = 2.60, GN = 1.77)]. AK Native and N Can aboriginal people had consistently higher rates of disease (all organisms) than non-aboriginals. Of the 78 Hi cases that were serotyped, 15 (19%) were Hib [AK 11 cases (rate 0.43), N Can 4 cases (rate 0.74)] and age ranged from <1 to 69 years; most Hib disease occurred in persons <2 years of age (AK = 55%, N Can = 100%). Twenty-four (31%) Hi cases were serotype A (Hia) [AK 7 cases (rate 0.36), N Can 17 cases (rate 4.38)]. No Hia cases were reported in AK during 2000 and 2001; in 2002 and 2003, rates in AK were 0.62 and 0.46, respectively. In N Can, Hia cases were reported during each year from 2000 to 2002; rates were 2.99, 7.03, and 3.13, respectively. No Hia cases have been reported in N Can in 2003. Case fatality ratios (CFRs) were higher in AK than N Can and GN for invasive disease caused by both Hi (AK = 22.5%, N Can = 4.2%) and Nm (AK = 13.6%, N Can = 0%, GN = 0%).
Conclusion: Native peoples of AK and N Can have high rates of invasive bacterial disease caused by Hi, Nm, GAS and GBS. Overall rates of Nm disease are higher in GN than AK and N Can. Cases of invasive Hib disease continue to occur in children <2 years of age. Rates of Hia appear to be elevated in N Can and increasing in AK, however, caution needs to be used when interpreting rates due to the small number of cases. This trend merits further surveillance. Elevated case fatality rates in AK for Hi and Nm also warrant further investigation.

P799 Region specificity, frequency of occurrence and pattern of antimicrobial susceptibility of common bacterial infections in Central Illinois

N. Khardori, M. El-Azizi, A. Mushtaq, C. Ball, C. Drake, J. Lawhorn
Springfield, USA

Objectives: For tertiary care hospitals, knowing the local patterns of spectrum and susceptibility at the referring institutes can add significantly to the selection of appropriate antimicrobial therapy. Our objective was to get information regarding the region specificity, frequency of occurrence and pattern of antimicrobial susceptibility of common bacterial infections in Central Illinois.
Methods: We used hospital antibiogram data to assess predominant pathogens and pattern of *in vitro* antimicrobial susceptibility of bacterial infections in the four regions (west, southwest, central and south) of central Illinois from January 2001 to June 2002.
Results: Gram-negative bacteria were predominant in four regions (57, 48, 63 and 52% respectively). In all regions, *E. coli* was the most common organism (20, 22, 32, and 25%) followed by *S. aureus* (17, 21, 14, and 24%). *E. faecalis*, *P. aeruginosa*, and *K. pneumoniae* were also among the five most frequently reported species. On the other hand, the frequency of occurrence of *S. pneumonia*e was 1–3% in the four regions. The pattern of methicillin-resistant *S. aureus* was different in the four regions (27, 53, 34, and 42%) with only 0.1% of the total number of *S. aureus* showing intermediate resistance to vancomycin. *E. faecalis*, 99, 91, 96 and 96%, respectively, were susceptible to vancomycin. Susceptibility of *S. pneumonia*e to penicillin was almost the same in the four regions (67, 74, 64, and 75%). It was not surprising that *P. aeruginosa* was the least susceptible species among Gram-negative bacteria, and this species showed decreased susceptibility to gentamicin (78, 80, 77, and 55%) and to ciprofloxacin (73, 81, 67, and 67%).

Conclusions: Our data show that different communities in central Illinois have variable occurrence and pattern of antimicrobial susceptibility of common bacterial infections. We plan to formulate a regional antibiogram, distribute it to all hospitals in the area, and follow the patterns prospectively with renewal of the antibiogram once a year.

P800 Antimicrobial resistance surveillance of Gram-negative anaerobic bacteria isolated in six Greek hospitals

J. Papaparaskevas, N.J. Legakis, A. Katsandri, A. Avlamis - The Hellenic Study Group for Gram-Negative Anaerobic Bacteria

Objectives: The antimicrobial resistance surveillance of Gram-negative anaerobic bacteria isolated in six Greek hospitals.
Methods: A total of 122 Gram-negative anaerobic clinical strains (72 *Bacteroides fragilis* group, 17 other *Bacteroides* spp. non-*fragilis*, 25 *Prevotella* spp., 4 *Fusobacterium* spp. and 4 miscellaneous) isolated during the period November 2002 to November 2003 were tested using the Etest method on brucella blood agar plates. Incubation in a ChelLab 1.5 Anaerobic Chamber was performed for 48 h and interpretation was according to NCCLS guidelines.
Results: Overall Gram-negative non-susceptible (intermediate and fully resistant) rates to penicillin, ticarcillin + clavulanic acid, cefoxitin, tetracycline, clindamycin, metronidazole, imipenem and ertapenem were 83, 2, 31, 55, 32, 6, 1 and 5%, respectively. *Bacteroides fragilis* group rates were 93, 4, 38, 65, 31, 1, 1 and 7%, respectively. *Prevotella* spp. rates were 68, 0, 8, 32, 36, 16, 0 and 0%, respectively. Overall Gram-negative MIC90s were 256, 2, 64, 128, 256, 2, 0.5 and 1 mg/L, respectively. *Bacteroides fragilis* group MIC90s were 256, 4, 128, 128, 256, 1, 1 and 4, respectively. *Prevotella* spp. MIC90s were 256, 1, 16, 64, 256, 256, 0.125 and 0.5, respectively. Metronidazole resistance was detected among four *Prevotella* spp., one *Bacteroides* spp., one *Porphyromonas* spp. and one *Fusobacterium* spp. isolates. Additionally, a *B. fragilis* strain was found highly resistant (MIC > 32 mg/L) both to imipenem and ertapenem and resistant to all other antimicrobials tested except metronidazole.
Conclusions: Carbapenems, beta-lactam + inhibitor combinations and metronidazole remain the antimicrobial agents of choice against most Gram-negative anaerobes. However, metronidazole resistance seems to be an emerging problem in Greece, especially among *Prevotella* spp. isolates. In that respect species identification and periodic susceptibility surveillance is mandatory. Imipenem and ertapenem activity was comparable, though ertapenem MICs were slightly higher.
Acknowledgements: Members of the Hellenic Study Group for Gram Negative Anaerobic Bacteria are Drs A. Avlamis, C. Koutsia-Karouzou, C. Kontou-Kastelanou, A. Pangalis, E. Papafrangas and E. Trika-Grafakos.

P801 The value of quality control strains in susceptibility tests

K. Huppertz, I. Noll, B. Wiedemann and the GENARS group

Objectives: The goals of a quality control programme are to assist in monitoring the precision and accuracy of the susceptibility test procedure, the performance of reagents used in the test and the performance of persons who carry out the tests and read the results. They are best accomplished by the testing of quality control (QC) strains with known susceptibility to the antimicrobial agents to be tested (NCCLS). Therefore, QC strain measurements done by laboratories taking part in the GENARS-project (German Network for Antimicrobial Resistance Surveillance) were used for a comparison of the performance of three different methods for MIC determination.
Methods: In the GENARS-project two commercial MIC test systems and one manual microdilution system according to NCCLS are used for the determination of antimicrobial susceptibility. The commercial systems are the Vitek 2 (bioMérieux) and the Micronaut system (Merlin Diagnostics) with 384-well microtitre-plates.

Table 1 Number of antibiotics (bold) which are tested in the respective test system with at least one concentartion above and under the MIC at the respective QC strain in relation to the total number if antibiotics tested

MIC test system	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*E. faecalis* ATCC 29212	*S. aureus* ATCC 29213
Micronaut	**2**/32	**10**/32	**11**/32	**11**/32
Vitek 2	**2**/20	**2**/20	**3**/20	**3**/20
Manual microdilution system	**10**/23	**12**/23	**9**/23	**9**/23

QC strains measured by all test systems were evaluated for those antibiotics where a range of ±1 dilution step of the modal value of the respective QC strain is included in the range of concentrations tested. For reliable assessment of the test quality the distance of the modal value from the lowest and highest concentration tested has to be two or more dilution steps.
Results: From a multitude of antibiotics tested only few drugs are tested with a range of concentrations which meets the above mentioned requirements. Table 1 indicates the number of test combinations available for evaluation. The Vitek 2 system offers the shortest ranges of concentrations. However, from the range of concentrations only few are tested, while the others are calculated, e.g. for Gentamicin the range includes six concentrations while only three are measured (AST-P526).
Conclusions: An evaluation of QC strain measurements should be possible for all antibiotics tested. However, due to the concentrations chosen and the short ranges of concentrations available in the different test-systems only few antimicrobial agents can be used for a comparison of the performance of the test methods. Therefore, either the range of concentrations has to be extended, or more suitable QC strains have to be implemented in a way that their MICs fall into the range of concentrations which are sufficient in clinical terms.

P802 Lack of evidence for DNA in antibiotic preparations as a source of antibiotic resistance genes

S.K.P. Lau, P.C.Y. Woo, A.P.C. To, A.T.K. Lau, K.Y. Yuen
Hong Kong, HK

Objective: To investigate the significance of DNA encoding antibiotic resistance genes present in antibiotic preparations in the rapid development of antibiotic-induced antimicrobial resistance.
Methods: A comprehensive study using sequence alignments and phylogenetic analysis of genes encoding antibiotic resistance in antibiotic-producing bacteria and the corresponding ones in non-antibiotic-producing human or animal bacterial isolates [erythromycin resistant methylase (*erm*), aminoglycoside 3′-phosphotransferase (*aph3*), aminoglycoside 6′-phosphotransferase (*aph6*), aminoglycoside acetyltransferase (*aac*), class A beta-lactamase, tetracycline resistance efflux protein, tetracycline resistance ribosomal protection protein and vancomycin resistance proteins (*vanA*, *vanH*, *vanX*) and bacitracin transport proteins (*bcrA*, *bcrB*, *bcrC*)] was carried out. If DNA encoding antibiotic resistance genes present in antibiotic preparations has been important in the development of antibiotic resistance, genes of almost identical amino acid sequences would be expected to be present in antibiotic-producing organisms and other human or animal bacteria, inferring that horizontal transfer of antibiotic-resistance genes had occurred from the former to the latter.
Results: The maximum amino acid identities of genes among different non-antibiotic-producing bacterial isolates were close to 100% for most genes, but those between antibiotic-producing and human or animal bacteria ranged from <28 to <77%. Therefore, recent horizontal transfer of antibiotic resistance genes has not occurred from antibiotic-producing organisms to human or animal

bacteria. On the other hand, frequent horizontal transfer of antibiotic resistance genes was observed among the human or animal bacteria, even if they were phylogenetically distantly related. Moreover, such transfer was particularly common among gastrointestinal tract flora or pathogens.
Conclusion: DNA encoding antibiotic resistance genes in antibiotic preparations has not been an important source of antibiotic resistance genes. DNA decontamination during the process of antibiotic synthesis is probably not necessary. The human gastrointestinal tract has been an important place for bacterial gene exchange. The role of the human gut in the dissemination of antibiotic resistance should be further investigated.

Enterococci and other Gram-positive bacteria

P803 Molecular-biology analysis of vancomycin-resistant enterococci isolated from the community in the Czech Republic

M. Kolar, L. Cekanova, I. Vagnerova, M. Kesselova, P. Sauer, D. Koukalova, P. Hejnar
Olomouc, CZ

Objectives: Enterococci have become a significant problem due to their aetiologic role in bacterial infections. An alarming problem is the increasing rate of enterococci with resistance to vancomycin (VRE) and therefore the source and spreading of these strains are very important epidemiological questions. The aim of the study was the monitoring of VRE in the community of the Czech Republic and molecular-biology analysis of VRE isolated.
Methods: Enterococci were isolated from rectal swabs of healthy people in Olomouc region (population 300 000), Czech Republic, during period September 2002 to September 2003. Enterococci were identified by the evaluation of their biochemical activities. Susceptibility to vancomycin and teicoplanin was detected by the microdilution method in accordance with the NCCLS guideline. Molecular-biology analysis of VRE was performed by analysis of isolated DNA, which was cleaved by restriction enzyme SmaI and separated by pulse field gel electrophoresis (PFGE). Restriction profile was analysed by Gel compare programme.
Results: A total number of 5283 swabs were evaluated and 558 *Enterococcus* sp. strains were isolated during the follow-up period. Nine strains (1.6%) were identified as VRE. Two strains were *E. faecium* phenotype VanA, one strain was *E. faecalis* phenotype VanB and six strains were *E. casseliflavus* phenotype VanC. By PFGE nine different restriction profiles of VRE strains were obtained. The analysis showed closer similarity of *Enterococcus casseliflavus* strains (80–95%) than similarity between *Enterococcus faecium* strains (41%).
Conclusion: It is evident that it is necessary to take into account a possibility of VRE spreading from community into health care facilities. A further opportunity of their spreading occurs in hospital divisions via environmental vectors and due to wide-spectrum antibiotic treatment.
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P804 Prevalence and resistance genotypes of glycopeptide-resistant enterococci from human and animal sources in Styria, Austria

A. Eisner, W. Sixl, G. Feierl, J. Koefer, G. Gorkiewicz, A.J. Grisold, E. Marth
Graz, A

Introduction: Glycopeptide-resistant enterococci (GRE) have emerged as important pathogens since the late 1980s. An important factor associated with the appearance of GRE in the community in Europe has been avoparcin, a glycopeptide antimicrobial drug used for years in many European countries as a growth promoter in food-producing animals. In Europe, evidence suggests that food-borne GRE may cause human colonisation or infection.
Objectives: The objective of this study was to investigate the prevalence and to determine the genotypes of GRE from different human and animal sources in Styria, Austria.
Methods: Stool specimens from each 100 patients with precedent antibiotic therapy and 100 non-hospitalised humans without precedent antibiotic therapy, 166 faecal cattle specimens, 117 faecal pig specimens and 40 faecal poultry specimens were collected in 2003. One millilitre of diluted faeces was added to 9 mL of Enterococcosel Bouillon (BD) for enrichment. After incubation, 100 mL was subcultured on VRE Screen Agar (BD). Species identification was performed with the API STREP systems and Vitek2 (bioMérieux). Resistance to vancomycin and teicoplanin was determined by the E-test method (AB Biodisk). Determination of glycopeptide resistance genotypes (vanA, vanB, vanC1, vanC2/3) was performed by PCR.
Results: 4% of the patients with precedent antibiotic therapy harboured VRE. Among these, two were identified as *E. faecium* vanA, two as *E. gallinarum* vanC1 and *E. casseliflavus* vanC2, respectively. Eight per cent of the non-hospitalised human specimens contained VRE (six *E. gallinarum*, two *E. casseliflavus*). A total of 90 VRE strains were isolated out of the animal samples, 25.6% *E. faecium*, 35.6% *E. gallinarum*, and 38.8% *E. casseliflavus* strains. No resistant *E. faecalis* strains were detected. PCRs confirmed that all *E. gallinarum* were of the vanC1, all the *E. casseliflavus* of the vanC2 and all the *E. faecium* strains of the vanA genotype. About 95.6% of all *E. faecium* vanA strains were isolated out of the poultry samples. One strain was isolated from a cattle sample, no specimen from pigs yielded glycopeptide-resistant *E. faecium*.
Conclusion: The present study indicates that the prevalence of GRE in humans and in pig and cattle husbandry appears to be low, but it reveals a high prevalence of GRE (*E. faecium*) in Styrian poultry 6 years after the use of avoparcin was banned.

P805 Species distribution and antibiotic resistance patterns of glycopeptide-resistant enterococci from food-producing animals in Styria, Austria

A. Eisner, W. Sixl, G. Feierl, J. Koefer, G. Gorkiewicz, A.J. Grisold, E. Marth
Graz, A

Introduction: Glycopeptide resistant enterococci (GRE) have become an increasing problem in the US and in Europe. Enterococci are intrinsically resistant against cephalosporins, aminoglycosides (low-level), polymixins, lincomycin and clindamycin. Furthermore, enterococci are able to acquire resistance to a wide range of antibiotics. There remain concerns that antibiotic use for growth promotion, prophylaxis and therapy in animal husbandry may lead to increased resistance to antibiotics used in human medicine.
Objectives: The aim of this study was to evaluate the species distribution and the antibiotic resistance of GRE isolated from Styrian food-producing animals.
Methods: A total of 90 GRE strains isolated from cattle, pig and poultry faecal specimens in 2003 were collected. The strains were identified using the Vitek2 automated methods (GPC) and the API STREP systems (bioMérieux). Antimicrobial susceptibilities were determined by Vitek 2 P524 card and by disk diffusion (linezolid). The strains were studied for susceptibility to 13 antibiotics: ampicillin (Am), amoxicillin/sulbactame (Amc), ciprofloxacin (Cip), erythromycin (Ery), gentamicin high level (Ge), linezolid (Li), norfloxacin (Nor), penicillin (P), quinupristin/

dalfopristin (Syn), streptomycin high level (Str), teicoplanin (Tp), tetracycline (Te) and vancomycin (Va).
Results: *E. casseliflavus* was the most common GRE species isolated (38.8%), followed by *E. gallinarum* (35.6%) and *E. faecium* (25.6%). All *E. gallinarum* and *E. casseliflavus* were of the vanC, all *E. faecium* of the vanA phenotype. All investigated strains were sensitive against linezolid and gentamicin high level. P and Am resistance (82.6%) and reduced susceptibility to Cip (17.4%) was seen in *E. faecium* only. Ery resistance for *E. faecium* revealed 17.4%, for *E. casseliflavus* 85.7% and for *E. gallinarum* 31.3%. Resistance against Te for *E. faecium* was 91.3%, for *E. casseliflavus* 11.4% and for *E. gallinarum* 68.8%. About 17.4% of *E. faecium* strains were not susceptible to quinupristin/dalfopristin.
Conclusions: Resistance phenotypes to P, Am, Cip, Ery and Te differed among *Enterococcus* species. Resistances found against tetracyclines, quinupristin/dalfopristin and erythromycin are causes of concern. High levels of antibiotic and multidrug resistance were observed among the *E. faecium* strains.

P806 Incidence and *in vitro* antibiotic resistance of streptococci in urinary tract infections

E. Ikonomopoulou, S. Andreatou, T. Podimatas, I. Katsarou, K. Papadopoulos, A. Kolotouros, A. Regli
Patras, Zante, GR

Objectives: To estimate the incidence of streptococci in urinary tract infections (UTI) and also to carry out the *in vitro* antibiotic resistance of streptococci in urinary tract infections.
Methods: From October 2002 to September 2003, 298 (9.4%) streptococci strains were isolated from 3157 positive urine cultures. The identification was carried out by the Vitek system (Biomerieux). The susceptibility test was performed either by the breakpoint system:mini API or the Vitek system (Biomerieux).
Results: 146 (49%) and 152 (51%) streptococci strains were isolated from outpatients' and inpatients' urine cultures, respectively. The distribution by sex was 65% women/35% men in outpatients and 58% women/42% men in inpatients. A total of 157 (53%) of streptococci strains were *Enterococcus faecalis*, 54 (18%) were *Enterococcus faecium*, 10 (3%) were *Enterococcus gallinarum* and 77 (26%) were streptococci group B. The *in vitro* antibiotic resistance of *Enterococci* spp. was: penicillin 38.5% (85/221), ampicillin 23% (51/221), gentamicin 39% (86/221), nitrofurantoin 9.5% (21/221), ciprofloxacin 58% (128/221), tetracyclines 56% (123/221), vancomycin 6% (13/221), linezolid 0%. Eight VRE strains were *Enterococcus faecium*, three were *Enterococcus gallinarum* and two were *Enterococcus faecalis*. The *in vitro* antibiotic resistance of group B streptococci was: vancomycin 0%, nitrofurantoin 5.2% (4/77), ampicillin 6.5% (5/77), penicillin 7.8% (6/77), erythromycin 14.3% (11/77), tetracyclines 61% (47/77).
Conclusions: Streptococci are responsible only for the 9.4% of urinary tract infections. *Enterococcus faecalis* was the most frequent pathogen (53%). *Enterococci* spp. showed high resistance in ciprofloxacin, tetracyclines, gentamicin, penicillin, and ampicillin.

P807 Emergence of *Enterococcus faecium* clinical isolates resistant to quinupristin/dalfopristin

F. Kolonitsiou, E. Petinaki, M. Maniati, G. Dimitracopoulos, I. Spiliopoulou
Patras, Larissa, GR

Objectives: Enterococcal infections are becoming an increasing concern, particularly due to the emergence and spread of resistance to animicrobial agents. We have investigated the phenotypic and genotypic properties of 66 *Enterococcus faecium* clinical isolates, expressing resistance to the combination of quinupristin/dalfopristin, recovered during a 3-year period in the University Hospital of Patras.
Methods: All isolates were characterised at species level by Gram stain, catalase production and by the Crystal ID Gram Positive System (BBL). Minimal inhibitory concentrations (MICs) to ampicillin (Amp), erythromycin (Em), chloramphenicol (Chl), gentamicin (Gm), ciprofloxacin (Cip), vancomycin (Va), teicoplanin (Tp), quinupristin/dalfopristin (Rp) and linezolid (Lin) were performed by the E-test (AB Biodisk) according to NCCLS recommendations. The presence of VanA and VanB genes was investigated by the Evigene commercial kit (Statens Serum Institut), while the presence of vga, vgb and sat1 genes by PCR with specific primers. Clonal types were characterised by PFGE of SmaI DNA digests.
Results: In a collection of 88 *E. faecium*, 66 (75%) expressed MIC of Rp $\geq$ 1 mg/L, and among them 10 isolates (15%) showed MIC > 4 mg/L. High-level resistance to Gm was detected in 27 (41%) isolates, 53 (80%) to Cip, 5 (7.6%) to Chl, 58 (88%) to Amp and 62 (94%) to Em. Forty-three (65%) isolates were vancomycin-resistant, carrying the vanA gene. No isolate was found to carry vga, vgb and sat1 genes. PFGE classified 36 isolates to clonal type A, 11 to type B, 6 to type C and the remaining 13 isolates belonged to 10 more types.
Conclusions: High prevalence of low-level resistance to quinupristin/dalfopristin (MIC: 1–4 mg/L) was detected in this collection of *E. faecium*, with 10 strains expressing higher MIC levels. This was mainly due to the dissemination of certain clones in the hospital.

P808 Patterns of antimicrobial resistance in nosocomial strains of *Enterococcus* spp. isolated from patients with UTI in different parts of Russia

V. Kretchikov, L. Stratchounski, A. Dekhnich, G. Reshedko
Smolensk, RUS

Objectives: To determine current state of antimicrobial resistance in nosocomial strains of *Enterococcus* spp. isolated from urine in patients with UTI in different regions of Russia.
Methods: A total of 87 clinical strains of *Enterococcus* spp. isolated in 2001 from urine in patients with UTI hospitalised in 10 medical institutions in different parts of Russia – two in Central region (Moscow, Smolensk), two in North-West region (St. Petersburg), two in South region (Krasnodar), two in Ural region (Ekaterinburg), two in Siberian region (Krasnoyarsk, Tomsk) were studied. Antimicrobials tested included ampicillin (AMP), gentamicin (GEN), streptomycin (STR), vancomycin (VAN), teicoplanin (TEI), linezolid (LIN), tetracycline (TET), chloramphenicol (CHL), quinupristin/dalfopristin (QND), ciprofloxacin (CIP), levofloxacin (LEV) and moxifloxacin (MOX). Antimicrobial susceptibility testing was performed by agar dilution method. The susceptibility testing and interpretation of the results were performed according

	E. faecalis (N=65)			*E. faecium (N=17)*		
Antimicrobial	1+R, %	MIC_{50}/ MIC_{90}, mg/L	MIC range, mg/L	1+R, %	MIC_{50}/ MIC_{90}, mg/L	MIC range, mg/L
AMP	7.7	2/2	1–64	41.2	2/64	1–128
GEN	50.8	256/4096	8–4096	52.9	32/4096	16–4096
STR	50.8	512/8192	128–8192	52.9	512/8192	32–8192
VAN	0	1/2	0.5–4	0	1/2	0.5–2
TEI	0	0.25/1	0.125–2	0	0.25/2	0.125–2
LIN	0	2/2	1–2	0	2/2	1–2
TET	86.2	64/128	0.5–128	35.3	1/128	0.5–128
CHL	61.5	32/64	4–64	41.2	8/64	2–64
QND	93.8	8/16	0.5–16	35.3	1/16	0.5–16
CIP	58.5	2/4	1–128	76.5	2/16	0.5–32
LEV	9.2	2/2	0.5–64	17.6	2/8	0.5–8
MOX	NA	0.5/2	0.125–32	NA	0.5/2	0.125–8

to the NCCLS recommendations. *E. faecalis* ATCC 29212 was used as a control.
Results: The majority of isolates (78.2%) were collected from patients hospitalised in surgical units. Among 87 strains tested 65 (74.7%) were *E. faecalis*, 17 (19.6%) *E. faecium*, 3 (3.4%) *E. durans* and 2 (2.3%) *Enterococcus* spp. Results of susceptibility testing are presented in the table.
Conclusions: (1) The most active antimicrobials were linezolid and glycopeptides with no resistant strains found. (2) High levels of resistance to ampicillin, quinupristin/dalfopristin and ciprofloxacin in *E. faecium* and to aminoglycosides in both *E. faecalis* and *E. faecium* are the most important problems.

P809 Dynamic population evolution of enterococcal intestinal carriage during ICU admittance

P. Ruiz-Garbajosa, V. Pintado, F. Baquero, R. Cantón, R. Del Campo
Madrid, E

Background: In a previous work, we described the dissemination of RENC1 and RENC4 *E. faecalis* multiresistant clones colonising patients of four different ICUs. The RENC4 clone was frequently found in bacteraemias, suggesting a blood invasion from an intestinal origin. The aim of this study was to analyse the dynamic population evolution of enterococcal intestinal isolates and if the acquisition of epidemic hospital clones may occurs during ICU admittance.
Material and methods: A close follow-up of four patients from the Neurosurgery ICU who were admitted after acute traumatism was performed. Rectal swabs were collected at the admittance and daily until they were discharged from the ICU. Stool samples were seeded in m-Enterococcus agar, eventually supplemented with selective antibiotics, and multiple colonies were analysed in each sample. PFGE-SmaI and the Phoretrix 5.0 software were applied to analyse the genetic relatedness among these isolates and the previously described hospital endemic clones.
Results: Patient 1 and 2 stayed in the ICU for 12 days, and patient 3 and 4 for 7 days. Patient 1 carried along the 12 days the original *E. faecalis* and *E. faecium* clones. Moreover, five *E. faecalis* clones, one identical to the epidemic clone RENC1, and one *E. faecium* clone were acquired during the ICU stay, all of them persisting over the rest of the studied period. Patient 2 presented at admission three *E. faecalis* and two *E. faecium* clones; two *E. faecalis* were lost in 5 days, and *E. faecium* were lost at the second day. Four new *E. faecalis* and one *E. faecium* clones were found during all stays, whereas five more clones were occasionally isolated without persistence. In patient 3 an *E. faecium* clone was identified along all the studied period, and two new *E. faecium* clones were later acquired. Patient 4 had two *E. faecium* clones at admission, one of them being lost after the first day; the second persisted during all 7 days; a new *E. faecium* clone was acquired during the ICU stay.
Conclusion: In general, enterococcal intestinal isolates recovered at the ICU admittance persisted along the ICU stay. However, several multirresistant endemic hospital clones were able to frequently colonise the intestinal tract of ICU patients, coexisting in most cases with the previous resident clones. These facts represent a challenge for the persistence of multiresistant clones within the ICU environment.

P810 Community-acquired urinary tract infection caused by vancomycin-resistant *Enterococcus faecalis* clinical isolate

E. Aznar, T. Alarcón, B. Buendía, E. García-Peñuela, E. Escudero, M. López-Brea
Madrid, E

Background: Vancomycin-resistant *Enterococcus faecalis* is a rare cause of community acquired infections.
Objective: We present a case of urinary tract infection caused by vancomycin-resistant *Enterococcus faecalis*.
Patient and methods: The patient was a 62-year-old woman hysterectomised 15 years ago. She reported four surgical interventions due to a cystocele. The last operation took place 11 years ago and she reported no further admittances at any hospital. In the last years the patient also suffered from repeated urinary tract infections. In the present episode she consulted because of typical UTI symptoms (dysuria, bladder tenesmus) and a urine sample was collected. After 24 h of incubation, a Gram positive coccus was isolated (more than 100 000 ufc/mL). The identification and susceptibility were preliminarily achieved by a commercially available method following manufacturer's recommendations (MicroScan, DADE). Identification was confirmed by API rapid strep system (BioMerieux). To discard *Enterococcus* species intrinsically resistant to vancomycin the absence of motility was observed with direct microscopic detection and the absence of pigmentation was determined by culture on TSA agar. Susceptibility to vancomycin, teicoplanin and ampicillin were assessed by disk diffusion, E-test and broth mcrodilution.
Results: The isolated microorganism was identified as *Enterococcus faecalis* and showed high MICs to vancomycin (>128 mg/L by broth microdilution and 6 mm by disk diffusion) and teicoplanin (8 mg/L by broth microdilution and 10 mm by disk diffusion) but was susceptible to ampicillin (0.5 mg/L by broth microdilution).
Discussion: The transmission ways of vancomycin resistant enterococci in the community and its clinical implications remain uncertain. In USA there is little evidence that VRE transmission may occur in the community. The opposite is true in Europe where these microorganisms have been isolated from different animal sources and healthy individuals. In our country, some studies have demonstrated the presence of VRE in animals and food.
Conclusion: The identification of a VRE strain as the cause of a community-acquired urinary tract infection is an unusual finding and it may lay to important epidemiological implications.

P811 Detection of vanB2-containing *Enterococcus faecium* strains with the same PFGE clonal type in two Spanish hospitals

A. Portillo, S. Escobar, L. Torres, A. Rezusta, M. Revillo, C. Aspiroz, F. Ruiz-Larrea, C. Torres
Logrono, Zaragoza, E

Objective: To characterise the resistance mechanism involved in a series of 11 vancomycin resistant *Enterococcus faecium* (VREF) strains recovered in two Spanish hospitals of the same city, and to determine their clonal relationship.
Methods: A surveillance programme was carried out during a 1-year period in MS-Hospital in order to detect VREF intestinal colonisation. Seven VREF strains were recovered from seven faecal samples which represents <1% of VREF intestinal colonisation. In the same period, four clinical VREF strains, implicated in infectious processes were recovered in MS-Hospital ($n = 3$) and RV-Hospital ($n = 1$). All VREF strains ($n = 11$) were recovered from 11 unrelated patients, most of them previously treated with glycopeptides or broad spectrum antibiotics and diagnosed with severe diseases. Antibiotic susceptibility testing was performed by agar dilution method and vancomycin resistance genes (vanA, vanB, vanC1, vanC-2/3, and vanD) were studied by PCR. vanB amplicons were sequenced to determine the subtype and the vanB cluster of genes were also characterised. Other resistance genes were studied by PCR: aph(3′)-IIIa, ant(6′)-Ia and erm(B). PFGE assays were performed with SmaI digestion.
Results: Nine of the 11 VREF strains (eight of MS-Hospital and one of RV-Hospital) showed a VanB phenotype [MIC (mg/L): vancomycin (16–32) and teicoplanin (0.5)]. The vanB2 gene was detected in these nine strains and in addition, the intergenic vanSB-YB region showed the characteristic mutations of the vanB2 subtype. The vanB2 gene cluster was integrated into the Tn5382-like element in all of them, as it was demonstrated by specific PCRs and sequencing. These strains were resistant to streptomycin, kanamycin and erythromycin and ant(6′)-Ia, aph(3′)-IIIa and erm(B) genes were detected by PCR. All of them were included in the same PFGE clonal type A and two closely related subtypes

were distinguished: A1 (seven strains from both hospitals) and A2 (two strains from MS-Hospital). Both subtypes were found in clinical strains as well as in strains recovered from faecal samples. The remaining two VREF strains, with unrelated PFGE patterns, showed high level glycopeptide resistance (MIC > 64 mg/L) and harboured the vanA gene.
Conclusions: VanB2 *E. faecium* strains with the same PFGE clonal type were recovered from unrelated patients in two Spanish hospitals. To our knowledge, this is the first finding of vanB2 enterococcal strains of human origin in Spain.

P812 Molecular epidemiology of haematology ward related VRE in Poland 2002–2003: from epidemic to endemic

M. Kawalec, R. Ziolkowski, T. Ozorowski, J. Kedzierska, M. Baran, W. Hryniewicz
Warsaw, Poznan, Cracow, PL

At the end of 1996, the first VRE appeared in haematology centres in Poland. To the end of 2000, sporadic outbreaks, *E. faecium* VanB mostly, were noticed in three of them. The next VRE (VanA) appeared in June 2002 in the centre in Poznan that was not the stage for the former outbreaks. Next outbreak was registered in January 2003 in the centre in Cracov, in which one of the previous VanB outbreaks had happened. There was an epidemiological link found between the centres. There was transmission confirmed to non-haematological hospitals.
Methods: MICs of antibiotics were evaluated according to NCCLS guidelines. Molecular typing of the isolates was performed by PFGE. The vanA/vanB genes were detected by specific PCR. For the analysis of Tn1546-like transposons and vanRSHAX regions polymorphism, the L-PCR/RFLP approach was used.
Results: Vast majority of the isolates were MDR *E. faecium* (VRE, HLAR, PenR). Until now (December 2003) 49 isolates of *E. faecium* from Poznan were analysed, 39 from Haematology and 10 from Transplantology. Only three of them were from serious infections, the rest was isolated from carriers. All but one present VanA phenotype and harbour vanA gene. The only vanB harbouring strain was resistant to teicoplanin. The outbreak at Haematology was at the beginning polyclonal (10 PFGE clones) but eventually three of them became predominant in both wards. The outbreak in Cracov centre was spread to two other wards of this hospital (Surgery and Geriatry) with 53, 27 and 7 VREM isolated up to now, respectively. Five were from serious infections, 15 were from wounds in the Surgery, the rest represents for carriers detected during infection-control measurements. All but two of them were VanA phenotype/genotype (2 VanB phenotype/genotype isolates). One predominant PFGE clone was observed, differentiated into 14 PFGE sub-types ('hospital clone'). Five other PFGE clones detected seemed to be unique (one to five isolates). In both outbreaks two basic mechanisms of VRE spread were detected, clonal spread of VRE strains and the VanA-elements horizontal transfer.
Conclusion: After time of VREM presenting VanB phenotype caused sporadic outbreaks two of haematology centres in Poland become the stages of multi-drug-resistant VanA VREM outbreaks, eventually turning into endemic. The colonisation rate was 10–15 times higher than infection in both cases. The danger of transmission to other centres and non-haematological hospitals in the country appears very high in these circumstances.

P813 Local engineering and the evolution of an *Enterococcus faecalis* clone in three Portuguese cities during a 7-year period (1996–2002)

C. Novais, T.M. Coque, F. Baquero, J.C. Sousa, L. Peixe and The Enterococci Study Group

Objectives: To analyse the clonal relationship and the presence of specific virulence and antibiotic resistance elements in vancomycin resistant *E. faecalis* (VREfls) clinical isolates from hospitals of different Portuguese cities.
Material and methods: Thirty-three selected VREfls from different patients at three hospitals (HUC, HSA and HST) in the North and Centre of Portugal (1996–2002) were studied. Susceptibility to 12 antibiotics was performed by the agar dilution method (NCCLS). Isolates were searched for genes coding for resistance to glycopeptides, macrolides, and aminoglycosides. Tn1546 characterisation was done by an overlapping PCR strategy and sequencing when necessary. Clonal relatedness was performed by SmaI-PFGE. Virulence traits (Cyl, Agg, GelE, Esp) were investigated by a multiplex PCR assay.
Results: All VREfls showed VanA phenotype and were mostly resistant to Ery, Cipro, HLRGm, and HLRKm (91, 88, 82, and 82%, respectively). Resistance genes found were vanA, erm(B), aac6-aph2, and aph3′-IIIa. Nine PFGE types were isolated: eight from eight patients and one (clone B) from 25 patients. Clone B was disseminated among the three hospitals for 7 years giving eight PFGE subtypes, each one characteristic of a specific hospital. VSEfls showing PFGE patterns identical to two clone B subtypes were found in HST. Six variants of Tn1546 were found, five of them among isolates of clone B. Tn1546- PP4 was found in all hospitals for 7 years and predominates in HUC and HST. It contains an ISEf1 insertion in the intergenic vanX-vanY region. Tn1546-PP15, only found in HSA, lacks genes involved in transposition. PP5 and PP2 were variants of PP4 and were recovered at HUC. PP16 was a PP-15 variant found in HSA. All VRE but one isolate of clone B were Agg+ and Gel+. Cyl and Esp were present in 43% of the VRE.
Conclusion: Our findings indicate that the dissemination and establishment of successful *E. faecalis* clones in the hospital setting amplify particular genetic determinants in local metagenomes resistant to vancomycin, and therefore influences future evolutionary events. We also report the first Tn1546-variant containing an ISEf1 insertion.

P814 High diversity of Tn1546 among vancomycin-resistant enterococci from Portuguese human, animal and environmental sources

C. Novais, T.M. Coque, J.C. Sousa, F. Baquero, L. Peixe
Porto, P; Madrid, E

Objectives: To characterise the diversity of the genetic element Tn1546 in vancomycin resistant enterococcal (VRE) isolates recovered from different sources in different Portuguese regions.
Methods: VRE were searched in different sources from the North and Centre of Portugal (1996–2003): (i) 99 poultry samples (P) for human consumption, (ii) 37 swine faeces (S), (iii) six river water samples (R), (iv) 32 samples collected upstream and downstream hospital sewage water (SW), (v) 99 faecal samples from healthy volunteers (HV), (vi) clinical isolates from three hospitals in three cities (H). Identification and characterisation of vancomycin resistant genes vanA, vanB, vanC1 and vanC2 were determined by a multiplex PCR. Tn1546-typing was performed by scoring the presence of 10 overlapping fragments, using a PCR assay.
Results: One hundred and two isolates showing vanA were identified (63 *E. faecium*, 35 *E. faecalis* and four *Enterococcus* spp.). Sixteen Tn1546 variants were detected. The most common ones were variant A (indistinguishable to Tn1546), and variant PP4 (with an ISEfm1 insertion in the intergenic region of vanY-vanZ) which were found in 16 and 22% of the isolates, respectively. Variant A was recovered from P, S and H samples while PP4 was only found in human samples. Ten Tn1546 variants lack amplicons with primers corresponding to regions associated with transposition functions and 12 variants had alterations in regions downstream vanA. These include insertions in the vanY-vanZ ($n = 3$) or vanX-vanY ($n = 3$) regions. Four Tn1546 variants have been already described [groups A, D (HV,P,S), E (P) and S (P)]. Nine of 16 Tn1546 types were detected in different enterococcal species. Three Tn1546 types were only found in human, three in animals and humans, one in animals, humans and rivers, two in humans and river and two found in both humans and sewage.
Conclusions: High diversity of Tn1546 was observed among Portuguese VRE. Variable distribution of Tn1546 variants suggests both

local availability of specific types in local metagenomes and dissemination of specific genetic elements among different reservoirs. Reasons underlying Tn1546 diversity needs further research.

P815 Bactericidal activity of daptomycin against European staphylococci and enterococci

A. Fluit, A. Florijn, S. de Vaal , J. Verhoef, D. Milatovic
Utrecht, NL

Objective: Daptomycin is a novel lipopeptide antibiotic with very good *in vitro* activity against Gram-positive cocci. We compared the bactericidal activity of daptomycin with that of other agents against European staphylococci and enterococci including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).
Methods: A total of 18 isolates including methicillin-susceptible *S. aureus* (MSSA), MRSA, vancomycin-susceptible and -resistant *E. faecium* and *E. faecalis* were selected. Killing studies were performed using daptomycin and quinupristin/dalfopristin, vancomycin, linezolid, and oxacillin as comparators. Each isolate was incubated with one, two and four times the MIC of the antibiotic used. Viable cells were determined after 0, 1, 3, 6 and 24 h. Bactericidal activity was defined as a 3 log 10 reduction in viable CFU/mL.
Results: Bactericidal activity of oxacillin against MSSA required at least 2× MIC for 6 h. Vancomycin showed bactericidal activity against both MSSA and some MRSA in 6–24 h for 2 and 4× MIC, but some isolates showed regrowth after 24 h. No bactericidal activity against enterococci was observed. Linezolid was bactericidal after 24 h for three of the four MSSA isolates tested at 4× MIC. A comparable result was obtained for MRSA. No bactericidal activity against enterococci was observed, except for one vancomycin-resistant *E. faecium*. Quinupristin/dalfopristin showed very good bactericidal activity against MSSA within 1 h with 1× MIC, but required longer times for MRSA (up to 24 h). A similar pattern was observed for *E. faecium* where vancomycin-resistant isolates were killed more slowly. Quinupristin/dalfopristin is ineffective against *E. faecalis*. Daptomycin showed excellent bactericidal activity against enterococci. This was slightly more efficient for *E. faecium* than *E. faecalis* isolates. Vancomycin resistance did not have any influence. Bactericidal activity against staphylococci including MRSA was generally achieved within 3–6 h at 2× the MIC of daptomycin.
Conclusion: Daptomycin showed excellent bactericidal activity against enterococci and *S. aureus* isolates including VRE and MRSA. None of the comparators achieved similar results.

P816 Biofilm production and antibiotic resistance of human and veterinary *Staphylococcus* strains

V. Cuteri, L. Bastianini, F. Panzarella, M. Marenzoni, A. Attili, C. Pieramati, C. Valente
Matelica, Perugia, I

Staphylococcus spp. is widely distributed in medical and veterinary pathology and represents one of the most important causes of infection. Many strains are antibiotic-resistant even for the presence of an eso-polysaccharide matrix. The aim of this work was to individuate, among 396 different Staphylococci of human and animal origin, the slime producing strains and to correlate the presence of biofilm to the resistance to eight antibiotics. A total of 185 coagulase negative staphylococci (CNS) and 211 *S. aureus* isolated from different sources and identified with Sceptor System, were tested for antibiotic susceptibility (Kirby Bauer method) and for slime production (Polystyrene plates – stained with Alcian blue – Spectrophotometric reading at 450 nm). The strains were classified as weak, strong and no slime-producing on the basis of OD results. The results were submitted to statistical analysis using Student's *t*-test and chi-square tests. Evaluating the differences of slime production among medical and veterinary strains, we found different statistical frequencies ($P > 0.001$). No statistical differences were obtained between *S. aureus* and the other CNS. Instead, the statistical analysis on *S. epidermidis* vs. the other staphylococci has shown no statistical differences among average values using Student's *t*-test ($P < 0.052$) and significant frequency differences using chi square tests ($P < 0.02$). Finally in the CNS, between *S. epidermidis* and the other strains, no statistical differences were found. The relation between slime production and the origin of strains was evaluated and no correlation was found. About the correlation between antibiotic-resistance and slime production a resistance increment of about 30% was obtained in strongly slime producing strains. *Staphylococcus* spp. is often involved in nosocomial infections as complication of post-surgery wounds, catheters and orthopaedic devices. The presence of antibiotic-resistant strains interferes in the therapy successes and seems to be strictly related to biofilm production beyond that genetically acquired. Human and veterinary strains have shown a similar behaviour towards biofilm production and antibiotic-resistance. The results confirm that *S. epidermidis* is one of the most slime-producer and introduce *S. aureus* as a new high slime-producer.

P817 Phenotypic characterisation of macrolide resistance in *S. agalactiae*

Sp. Fokas, St. Fokas, F. Markatou, M. Kalkani, M. Dionysopoulou
Sparta, GR

Objective: In this study, we aimed to record the current trend regarding macrolide resistance in *S. agalactiae* (GBS) as well as to determine the phenotypes of resistance.
Methods: We investigated a total of 86 non-duplicated *S. agalactiae* strains collected over a period of 2 years (2001–2002). The bacteria were obtained from clinical specimens as follows: 20 strains from vaginal secretions of healthy asymptomatic pregnant women, 30 strains from vaginal secretions of women with the clinical suspicion of vaginitis, 30 strains from urine and six strains from cases of invasive GBS infection. The identification to the species level was achieved by Gram stain, catalase test, colony morphology on blood agar and determination of the group B antigen by latex agglutination technique. The pattern of susceptibility to erythromycin, clindamycin, penicillin G and vancomycin was examined for all the strains performing the disk diffusion method according to the NCCLS (2000) recommendations. The macrolide resistance phenotypes were determined using the erythromycin–clindamycin double disk test.
Results: All the *S. agalactiae* isolates tested were found susceptible to penicillin G and vancomycin while the resistance rate to erythromycin was 8.1% (seven strains). The expression (%) of the macrolide resistance phenotypes among the resistant strains as they were evaluated by the double disk test were: constitutive (cMLSB) phenotype 57% (four isolates) and inducible (iMLSB) phenotype 43% (three isolates). No *S. agalactiae* strain was assigned to the M resistance phenotype. The overall resistance rate to clindamycin was 8.1%.
Conclusions: Our findings demonstrate that *S. agalactiae* remains fully susceptible to penicillin and vancomycin while there are relatively low resistance values to macrolides and lincosamides. The MLSB phenotype predominated among the macrolide-resistant strains, a finding that raises concern about the use of clindamycin instead of erythromycin in prophylaxis or treatment of *S. agalactiae* infection in patients allergic to beta lactams. However, continuing surveillance is needed to detect any change in susceptibility patterns.

P818 Survival of antibiotic-resistant *Propionibacterium acnes* in the environment

A. Qureshi, J.I. Ross, A.M. Snelling, E.A. Eady, J.H. Cove
Leeds, Bradford, UK

Objectives: Antibiotic treatment for acne is aimed at reducing levels of the anaerobe, *Propionibacterium acnes*, on skin. Treatment can

involve long and multiple courses of antibiotics, and patients are often colonised with resistant strains. It is not clear if such strains develop *de novo* or are transferred directly or indirectly between individuals. It has been assumed *P. acnes* do not survive well in the environment. This study aimed to test if this is the case by modelling their survival on surfaces.
Methods: The *P. acnes* type strain, NCTC737, was used to assess survival under different environmental conditions. Approximately 10 exp 7 to 10 exp 8 washed cells were dried onto individual glass coverslips and maintained under different conditions of temperature, relative humidity (RH) with and without oxygen. Triplicate samples were assayed for up to 77 days. Standard conditions (20°C, 31% RH) were used to assess the survival of 10 antibiotic resistant (erythromycin or tetracycline resistant) isolates and one fully suceptible clinical isolate.
Results: *P. acnes* NCTC737 survived in air for >77 days at 20°C, 31% RH (3 log reduction). Survival was adversely affected at 15% RH, 20°C (3 log reduction over 1 day) and 37°C, 31% RH (3 log reduction over 1 day 22 h). Survival was poor at 6°C, 31% RH (3 log reduction over 12 days 15 h) and under anaerobic conditions, 20°C, 31% RH (3 log reduction over 7 days 7 h). Survival of the 10 resistant *P. acnes* isolates at 20°C, 31% RH was variable and eight strains had reduced survival (3 log reduction between just 2–14 days), compared with NCTC737 and the susceptible clinical isolate (3 log reduction >28 days). Two strains, one tetracycline and one erythromycin resistant, were more resilient with 3 log reductions taking >17 days. Viable cells could be recovered after 17 days with all strains.
Conclusions: Although *P. acnes* is sensitive to oxygen, it can survive on surfaces for extended periods at room temperature in air. Antibiotic resistant clinical isolates remained viable on surfaces for at least 17 days. These results suggest strains can be transmitted between acne patients via inanimate objects. Infection control policies in dermatology clinics should take these findings into account.

Molecular and clinical virology

P819 Real-time PCR quantification of enterovirus and adenovirus in longitudinal series of stool samples from babies at the highest genetic risk of type 1 diabetes mellitus

O. Cinek, P. Drevinek, E. Witso, T. Wetlesen, P. Sedlakova, S. Jeansson, K. Ronningen
Prague, CZ; Oslo, N

Background: Effect of enterovirus infection on the risk of type 1 diabetes mellitus has been studied mainly using indirect serological evidence of past infections, or using RT-PCR detection of the virus in plasma. With respect to enterovirus biology, we decided to assess the exposure to enterovirus using real-time RT-PCR detection and quantification from stool samples. This exposure is studied in relation to signs of autoimmune process ultimately leading to type 1 diabetes.
Methods: The study population comes from the Norwegian 'MIDIA' study which screens newborns from the general population for the highest HLA-encoded risk of type 1 diabetes mellitus. The high-risk babies are followed-up by questionnaires, serum samples for markers of beta-cell autoimmunity, and stool samples collected in monthly intervals from month 3 to month 24. The stool samples are collected by parents and mailed to the laboratory where RNA and DNA is co-purified on Qiagen columns together with a low quantity of exogenous control RNA. Enterovirus is quantified by real-time RT-PCR using Armored RNA as a standard. Control RNA is detected in late cycles in the same reaction using a differently coloured probe reporter. Adenovirus quantity is simultaneously investigated as a viral exposure which has not been implicated in triggering type 1 diabetes. Here we present the results of the pilot study.
Results: Parents of 72 high-risk children collected and mailed 791 (93%) out of 850 scheduled monthly samples. Enterovirus RNA was present in 10.4% samples. Longitudinal analysis was performed in 592 samples from 44 subjects who had completed 12 months of age. Out of these children, 36% had enterovirus in two to seven samples, 32% had one enterovirus-positive sample, and 32% remained permanently negative. In children with multiple positive samples there were both prolonged infections, and several isolated infections. Adenovirus was present in 5.2% samples. Both viruses showed marked seasonality, and adenovirus frequency was also dependent on the age of the child.
Conclusions: The testing of viruses from stool samples using real-time PCR is rapid and reliable. Since enteroviruses persist in the gut for up to several months, stool tests provide good information on enterovirus infection, its extent, duration and on type of the virus. The good cooperation of the parents shows that collecting stool samples is perceived as non-invasive and easy.
Acknowledgement: Supported by grant GACR 301/02/d80.

P820 Genotypic distribution of Cytomegalovirus (CMV) envelope glycoprotein in different population groups in Honduras

A. Ferrera Boza, M. Arias, N. Tábora, M. Brytting
Tegucigalpa, HD; Stockholm, S

Objectives: Past studies indicate that genetically distinct strains of CMV, identified by variations in the gene encoding envelope glycoprotein B (gB) occur at variable frequencies and can be associated with different clinical outcomes. The purpose of the study was to determine if there is a distinct distribution frequency of circulating CMV gB genotypes in plasma samples in five different patient population groups in Tegucigalpa, Honduras.
Methods: An analysis of CMV genotypes was conducted in plasma samples of 276 patients belonging to five categories: voluntary blood donors, chronic alcoholics, pregnant women, HIV infected patients and oncology children. The genotyping was based on sequence variations in the gene encoding gB as detected by restriction and sequencing analysis of a semi-nested polymerase chain reaction (PCR)-amplified gB DNA.
Results: The distribution of gB genotypes in patients of all studied groups were similar. Among all samples studied, the distribution of gB types 1–4 was 24, 66, 13 and 3%, respectively, with 5.6% of all samples containing more than one gB type. However, there was a unique distribution of gB genotypes in the HIV infected people, which was distinct from those of all other categories. They were the only group infected by the four CMV genotypes and 15% of them presented mixed infections.
Conclusions: Undoubtedly, there is a high prevalence of this virus in Honduran population suggested by detection of the virus circulating in plasma samples. The predominance of gB2, as described by others, was confirmed. We speculate that the mixed infections encountered indicated that reinfection and reactivation are common traits in HIV infected people.

P821 Prevalence of transfusion transmitted virus DNA in serum samples in Austria

C. Koidl, M. Bozic, R.B. Raggam, J. Berg, E. Marth, H.H. Kessler
Graz, A

Objective: To investigate the endemic situation of transfusion transmitted virus (TTV) infection in the Austrian population.
Methods: A molecular assay for detection of TTV based on automated nucleic acid extraction and real time PCR was developed and used for screening of 103 clinical serum samples. The new assay included a homologous internal control. All sera had been tested for anti HCV and anti HIV-1 antibodies earlier.
Results: The new molecular assay showed an excellent amplification efficiency. The internal control was detected in all negative and weak positive samples. When clinical specimens of four different groups of patients were tested, 84% of patients with both anti-HCV and anti-HIV-1 antibodies, 89% of haemodialysis patients without both anti-HCV and anti-HIV-1 antibodies, 74% of patients aged below 18 years without both anti-HCV and anti-HIV-1 antibodies, and 54% of bone marrow transplant donors without both anti-HCV and anti-HIV-1 antibodies were found to be positive for TTV DNA.
Conclusion: TTV is a common endemic virus in the Austrian population. With the employed assay, 76% of the clinical samples were found to be positive for TTV. There was no significant difference in TTV prevalence between the four groups of patients in this study. The new molecular assay proved to be suitable for routine detection of TTV in clinical samples.

P822 PID-ARI.net and year-round surveillance of 19 respiratory pathogens in children

J.A.I. Weigl, W. Puppe, C.U. Meyer, R. Berner, J. Forster, F. Zepp, H.J. Schmitt on behalf of the PID-ARI.net

Background: No surveillance system, which delivers data for several airway pathogens simultaneously and year round, exists in Germany so far.
Objectives: To establish pathogen-specific surveillance in airway infections in children as comprehensive as possible and to make the data accessible to the medical community as quick as possible.
Method: Nasopharyngeal samples provided by hospitals and offices in Freiburg, Mainz and Schleswig-Holstein including Kiel are tested in the molecular–biological laboratory of our network in Kiel by a 19-valent multiplex-RT-PCR: RSV, hMPV, parainfluenza types 1, 2, 3, 4, influenza A and B, entro- and rhinoviruses, coronaviruses OC43, 229E, reoviruses, adenoviruses, *Bordetella pertussis* and parapertussis, Mycoplasma and *Chlamydia pneumoniae*, *Legionella pneumophila*. This guarantees a centralised and uniform detection of pathogens, which do not colonise the upper airways in general. Children under 16 years of age with lower airway infections treated in hospitals or as outpatients are enrolled. The recruitment sites are located from the south to the north of Germany. Children of a total population of roughly 1.5 million (2%) of German citizens are under surveillance. Since October 2002 the surveillance data are published online weekly via www.pid-ari.net including a web-based early warning system. In an overview section seasonal data since 1999 are presented. In the commentaries epidemiological predictions are made and the findings are explained.
Conclusions: The surveillance system of PID-ARI.net is the first, pathogen-specific, relatively comprehensive and fast surveillance-system for airway pathogens in Germany. Targeting health care interventions in an epidemic-synchronous fashion is facilitated.
Acknowledgements: PID-ARI.net is sponsored by the German Ministry of Research; for collaboration to validate the PCR we thank the Institute of Virology, Erasmus University Rotterdam, Prof. A. Osterhaus.

P823 Genotypes of measles virus in Spain during 2001–2003

M.M. Mosquera, F. de Ory, J.E. Echevarria
Majadahonda. Madrid, E

Objective: According to WHO recommendations for the Plan of Elimination of Measles positive cases found between January 2001 and October 2003 by multiple RT-PCR (measles, rubella and parvovirus B19) were genotyped directly on the specimens to establish the surveillance of this virus in Spain.
Methods: Amplification fragments of a different measles specific nested PCR designed in the variable C terminus of the nucleoprotein gene were sequenced for this purpose. All positive sporadic cases were genotyped and at least one case in outbreak situations.
Results: Eighty-five positive cases were sequenced in this period. Fifty-five of them were from an outbreak in Almeria province (SE Spain) in 2003, five from an outbreak in Benidorm (Alicante, E Spain) in 2003, six from another outbreak occurred in Ibiza Island (Mediterranean sea) in 2001, two from Valencia in 2003 and the remaining 17 were sporadic cases throughout Spain. Genotype D7 was detected in 13 cases from Valencia, Canary Islands, Cordoba and Ibiza outbreak. B3 was detected in 58 cases from the Almeria outbreak, Murcia, Granada and one sporadic case coming from Equatorial Guinea. Genotype C2 was seen in Madrid and Granada. Genotype D8 was detected in the Benidorm outbreak. Genotype D4 was seen in a sporadic case from Valencia and H1 in an imported one originally from China coexistent with the Ibiza outbreak. Vaccine genotype A was demonstrated in one sporadic case from Badajoz (W Spain), three previously included in the Almeria outbreak and one initially assigned to the Ibiza outbreak. Three cases were confirmed by sequencing the strain obtained by culture in B95a cells in a different gene.
Conclusion: Most cases showed imported genotypes as H1, described in China; B3, found in different West and Central Africa countries; D4, detected in East and Southern Africa, Russia, Iran, India and Pakistan; and D8 circulating in India, Nepal and Ethiopia. Only genotype D7 is known to circulate nowadays in Europe, however its presence seems to be caused by importation from other countries in Europe more than local circulation, according to the high vaccine coverage and the broad variety of genotypes detected. Post-vaccine cases can be detected in the context of outbreaks of wild type strains. In summary, this technique is useful not only to classify cases as imported or native but also to trace virus circulation during outbreaks.

P824 Molecular epidemiology of rotavirus in Korea, July 2002 through June 2003: emergence of G4P6 and G9P8 strains in two surveillance systems

J.O. Kang, P. Kilgore, J.S. Kim, B. Nyambat, J. Kim, H.S. Suh, Y. Yoon, S. Jang, C. Chang, S. Choi, M. Kim, J. Gentsch, J. Bresee, R. Glass
Guri, Seoul, Jeonju, Gangnung, Daegu, Cheju, Gwangju, Busan, Daejun, KOR; Atlanta, USA

Objectives: This study was conducted to characterise the G (VP7) and P (VP4) genotypes of group A human rotavirus strains collected from eight nationally representative hospitals (Korean Rotavirus Strain Surveillance Network; KRSSN) and a community-based rotavirus surveillance site in Jeongeub, Korea.
Methods: From July 2002 through June 2003, rotavirus antigen-positive faecal specimens were collected from the eight KRSSN hospitals. Each hospital laboratory stored the first 10 rotavirus-positive faecal specimens each month from hospitalised children. Other rotavirus antigen-positive faecal specimens were obtained from a community-based disease burden study region. G and P genotypes were characterised by reverse transcription-polymerase chain reaction. A total of 488 strains were obtained from the KRSSN and 196 strains from Jeongeub.
Results: A total of 5612 faecal specimens were tested for rotavirus antigen at the eight hospitals of the KRSSN and, of these, 1105

(19.7%) were rotavirus-antigen positive. For community-based rotavirus surveillance in Jeongeub, a total of 1081 specimens were tested of which 199 (18.4%) were rotavirus-antigen positive. In the KRSSN, the most common strain detected was G4P6 (26.7%), of which 88.8% (111/125) were detected in specimens from neonates, but this strain was not detected at all in Jeongeub, community-based surveillance site. For the first time in Korea, we report G9P8 strains; these accounted for 41.4% of rotavirus strains in Jeongeub and 10.5% of strains in the KRSSN. Globally important strains (G1P8, G2P4, G3P8, and G4P8) accounted for 54.5% in KRSSN and 52.0% in Jeongeub.
Conclusion: Globally unusual strain, G4P6, can be an important cause of rotavirus diarrhoea in some countries, and emergent strains (e.g. G9P8) can be detected in nationwide laboratory-based surveillance. Given variations observed in circulating rotavirus strains, continued monitoring of genotypes is essential to identify emerging strains, track common strains, anticipate impact of rotavirus vaccines, and detect nosocomial outbreaks among vulnerable groups including neonates.

P825 CMV DNA in plasma and peripheral blood leukocytes of healthy CMV-seropositive individuals

K. Midilli, S. Ergin, M. Ozdamar, M. Kuskucu, K. Altas
Istanbul, TR

Objective: There are conflicting reports regarding CMV-DNA positivity among healthy CMV-seropositive individuals. We aimed to determine the frequency of CMV-DNA positivity among healthy subjects and to evaluate its association with physical and mental stress in a longitudinal study. Subjects and methods: Weekly peripheral blood samples were drawn into from 17 healthy CMV seropositive subjects aged between 24 and 53 years during a 8-week study period. Each subject rated their physical and mental stress and they also recorded their alcohol consumption and any change in their health status. CMV DNA was screened in plasma and peripheral blood leukocyte samples with a nested PCR using primers targeting MIE gene of CMV.
Results: In total, 272 samples (136 plasma and peripheral blood leukocytes, each) were screened and only one peripheral blood sample obtained during the second week of the study gave positive result. This sample belonged to the oldest subject of the study. According to our results, CMV-DNA positivity among healthy CMV seropositive individuals seems to be a rare event.

P826 Acute cytomegalovirus meningoencephalitis in immunocompetent patient

R. Mauri, A. Tocchetti, E. Rinaldi, E. Sala, E. Longoni, D. Santoro
Como, I

Objectives: Cytomegalovirus infection in immunocompetent subjects is generally mild and self-limiting. We describe a case of severe CMV meningoencephalitis in which the CNS is the only one apparent site of disease, referred to a 61-year-old man with hypertension history treated with amlodipine, admitted after 2 days of intermittent fever (39–40°C) to our unit.
Methods: The patient showed headache and confusional state; objective neurological signs were represented by nuchal rigidity and Kernig signs positivity. We performed: CT brain scan, routine haematological and chemical tests, lumbar puncture, CSF culture and Gram stain, bacterial antigen latex test and PCR investigation for neurotrophic viruses. HIV antibody test was also performed with immunoelectrophoresis and limphocytes immunophenotyping and antibody research for CMV. Empirical therapy with ceftriaxone, ampicilline and acyclovir started.
Results: CT brain scan gave negative results. Routine haematological and chemical tests were normal. Lumbar puncture revealed clear cerebrospinal fluid with 250 WBC/mm^3 (lymphocites), slight increase in protein level (102 mg/dL) and normal glucose level. Gram stain, culture and CSF latex bacterial antigen were negative. HIV test, serum immunoelectrophoresis, lymphocytes immunophenotyping gave negative results. PCR for EBV, HSV1 and HSV2 on CSF were negative, while PCR for CMV resulted positive on CSF and negative on peripheral blood cells. CMV antibodies were positive for IgG and negative for IgM. Empirical therapy was interrupted and lumbar puncture performed 10 days after revealed 32 WBC/mm^3 and normal protein level. The patient was discharged after 12 days in good clinical condition.
Conclusions: Our case shows that CMV's replication in CNS is able to cause a meningoencephalitis in the absence of systemic infection's clinical signs.

P827 Epstein–Barr virus and gastric carcinoma: the frequency of EBV association and quantification of viral DNA

C. Nogueira, M. Correia Leitão, P. Figueiredo, J. Ganho, F. Martinho, C. Gregòrio, M. Martins, T. Lindo, P. Santos, F. Regateiro, A. Magalhães-Sant'Ana
Coimbra, P

Objectives: Determination of the Epstein–Barr virus (EBV) association with gastric carcinomas and quantification of the viral DNA in the biological samples.
Methods: In this work, 39 individuals with gastric carcinoma have been studied. The detection and quantification of EBV DNA was carried out by real-time polymerase chain reaction (PCR) in serum, in saliva, in gastric tumour and in normal gastric mucous samples. The PCR reaction was performed using the Taqman PCR kit as previously described in Kimura et al. (1999). The primers were selected for the BALF5 gene that encodes the viral DNA polymerase. The immunological status was established by the detection of EBV-specific antibodies – VCA IgM, EBNA IgM, VCA IgG, EBNA IgG and EA-D IgG using Enzyme-Linked Immunosorbent Assay (ELISA).
Results: The DNA of the EBV was detected in 2.56% of serums, in 33.3% of the saliva, in 25.6% of the gastric carcinomas and in 23.1% of the normal gastric mucous. In 41% of the patients the search of the viral genoma was negative. The mean EBV-DNA copy number in the serums was 24 copies/μg of DNA, in the saliva 6089 copies/μg of DNA, in the gastric tumour 1728 copies/μg of DNA and in normal gastric mucous 20 copies/μg of DNA. Serologic results indicated that 93.1% of the CG (gastric carcinoma) EBV negative, 90% of positive GC EBV and 100% of the control group show a past infection. A reactivation of the infection was confirmed in 10% of CG EBV positive; and 6.9% of CG EBV negative were seronegatives.
Conclusion: Gastric carcinoma is strongly associated with EBV infection (25.6%). The survival was significantly better in the EBV-negative carcinomas than EBV-positive carcinomas; indeed 66.7% of the deaths were verified in EBV-positive gastric carcinomas ($P = 0.012$). These findings suggest an association of the gastric tumours with EBV and this variable emerges as a poorer prognostic factor.

P828 External quality assessment for molecular detection and quantification of human cytomegalovirus (HCMV) DNA in the Belgian centres for molecular diagnosis

F. Brancart, B. Vincart, C. Liesnard and the Belgian Centres for Molecular Diagnosis
Brussels, B

Objective: A national external quality assessment was conducted to evaluate CMDs' performances in molecular detection and quantification of HCMV DNA.
Methods: The panel consisted of amniotic fluid or Hank's balanced salt solution (HBSS) spiked with dilutions of AD169 virus stock. Three different concentrations of human lymphocytes were added to the samples. A questionnaire allowed gathering information on laboratory methodology and use of sample internal control to detect PCR inhibition.

Results: All 16 centres reported qualitative results; four centres also reported quantitative results. All samples were correctly identified by all centres. Various extraction and amplification methods were used. Fourteen centres reported results of internal controls. Most of the centres controlled only the amplification step and did not adjust the detection sensitivity of the internal control to the detection limit of the target. Three centres failed to detect one internal control in two positive samples and one negative sample. For quantification of HCMV DNA all centres used real-time quantitative PCR. CV of HCMV DNA load between centres were low (3.6–7%) except for one sample (13%), but this could be attributed to a heterogeneous preparation of this sample by the organisers. Using Student's *t*-test, no statistically significant difference was observed between HCMV load whatever the medium or the number of added cells.
Conclusion: Results of this external quality assessment for molecular detection and quantification of HCMV DNA were excellent. Almost all centres used internal control of PCR inhibition; however, control of the whole PCR process, including extraction and better adjustment of the detection sensitivity of the internal control to the sensitivity limit of the PCR target is desirable.

P829 A simple technique for internal control of real-time amplification assays

S. Burggraf, K. Appel, N. Malik, J. Neumann, E. Schuhmacher, B. Olgemöller
Munich, D

Objectives: The most accurate way to identify false negative results, e.g. those caused by PCR inhibitors, in real-time PCR assays is to spike samples with an internal control that will be co-amplified with the target (pathogen) DNA. However, current internal control procedures, which usually involve the introduction of a DNA fragment, are complex, time consuming and expensive. We present a novel technique for simple internal control of real-time amplification assays.
Methods: Single-stranded oligonucleotides, which contain little more than primer and probe binding sites, were used as internal controls in real-time PCR assays. Mismatches were included in the probe-binding region of the internal control oligonucleotide (ICO) to prevent probe-control hybridisation during the fluorescence acquisition step of the PCR. ICOs could be added directly to the sample material prior to DNA extraction.
Results: To demonstrate the feasibility of the new approach, we designed ICOs for the following LightCycler hybridisation probe assays: Mycobacterium tuberculosis complex, hepatitis B virus, herpes simplex virus and varicella zoster virus. In each case, the controls did not interfere with detection of the pathogen, but were clearly detectable during a subsequent melting point analysis of the PCR products.
Conclusion: A single-stranded oligonucleotide, which mimics the target region of the pathogen yet is clearly distinguishable from the target during analysis, can serve as a simple, cost-effective internal control for real-time amplification assays. Such control oligonucleotides are easy to design and cheap. A costly second probe system is not necessary. Moreover, the internally controlled assay uses only one fluorescence detection channel of the instrument, leaving the second channel free for multiplex applications.

P830 External quality assessment for the molecular detection of viruses in simulated cerebral spinal fluid

V.J. Chalker, Z. Mee, V. James
London, UK

Background: Viral infections of the central nervous system (CNS) can result in severe clinical signs including meningitis or encephalitis and may have lasting side affects or be fatal. Several viruses are known to infect the CNS including herpes and enteroviruses. Molecular based technologies are being used in clinical diagnostics to detect several viruses in cerebral spinal fluid (CSF). External quality assessment (EQA) provides valuable information regarding methods, standardisation and the success in detecting and reporting infections in clinical diagnostic laboratories.
Objective: To provide an up to date summary of performance for the molecular detection of HSV-1, HSV-2, VZV and enteroviruses in a simulated CSF.
Method: UK NEQAS for Microbiology provides a scheme for molecular detection of viruses in CSF in which participants are asked to determine the presence and genotype (if relevant) in freeze-dried simulated CSF spiked with live viruses. Some participants also provide quantitative data. This scheme was introduced in 2001 and currently there are 71 registered participants in 15 countries. Reported results were analysed to determine the percentage of participants correctly detecting the virus type and species in specimens and when provided, viral load. Performance was analysed over time and by viral load for each virus.
Results: Standards of detection varied depending upon the type of virus and quantity. For HSV DNA detection 46.5–100.0% of participants detected HSV DNA depending upon viral load, whereas 100% of those participants typing the virus correctly identified type HSV-1 or HSV-2. Although standards for VZV DNA detection were of a similar level, with 56.0–95.9% of participants detecting VZV DNA depending upon viral load, considerable improvement has occurred with time. Enterovirus RNA was detected by 48.0–97.4% of participants depending upon viral load and for the two negative specimens distributed false positives were reported by 2.4 and 5.4% of participants, respectively. Quantitative results were variable with differences in the log 10 copies/mL as high as 4.4 and improvements were seen with time and increasing viral load.
Conclusions: This data provides an interesting insight into the performance for the molecular detection of viruses in simulated CSF. Variation in performance for the detection of HSV1/2, VZV and enteroviruses is dependant upon viral load.

P831 EQA programmes in clinical virology and microbiology

C. Forde, C. Scott, C. Steel, P. Wallace, A.M. van Loon
Glasgow, UK

Objectives: Quality Control for Molecular Diagnostics (QCMD) is an independent 'not for profit' organisation whose primary aim is to establish and develop external quality assessment (EQA) programmes for the evaluation of nucleic acid amplification techniques (NATs) in the diagnostic laboratory. Since its establishment in 2001, QCMD has evolved into the leading EQA provider in the area of molecular diagnostics in infectious diseases.
Methods: Proficiency programme panels were distributed to over 1000 participants in more than 40 countries worldwide in 2003. Twelve different programmes were distributed covering a range of infectious diseases and including molecular diagnosis of respiratory, blood-borne, STD and CNS pathogens as well as molecular genotyping. In addition, a Human Immunodeficiency Virus (HIV) DNA pilot programme was carried out by participants in South Africa.
Results: In 2003, 1041 laboratories participated in QCMD proficiency programmes. This represented an overall increase of 24.2% in the total number of laboratories participating in comparison to the total number participating in programmes in 2002. Of these, 220 laboratories participated in proficiency programmes for *Neisseria gonorrhoae*, Varicella-Zoster virus and Hepatitis C Virus (HCV) genotyping which were offered for the first time in 2003.
Conclusion: At present, over 1600 individuals from around 70 countries worldwide have registered an interest in QCMD programmes. The programme portfolio continues to grow and, in 2004, three new pilot programmes will be launched for Respiratory Viruses, *Toxoplasma gondii* and *Legionella pneumophila*. In addition to expanding its portfolio of EQA programmes, in 2004 QCMD will launch an on-line information management and knowledge retrieval system which will allow information

exchange and act as a valuable QC information resource for participants.

P832 Nucleic acid isolation using the NucliSens mini MAG instrument

P. van Deursen, A. Verhoeven, P. de Bie, A. van Grinsven, M. Jacobs, P. van de Wiel
Boxtel, 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 facilitating the washing and collection of the silica particles in a user friendly and efficient way. In principle the extraction method is generic and can be applied to a broad range of different sample types. The objective of this study was to measure the performance of this new extraction platform in terms of RNA and DNA recovery, purity and integrity. In addition, user aspects were also addressed in the study.
Methods: RNA recovery was measured by spiking *E. coli* RNA to human normal EDTA plasma, extracted RNA was quantified by using a fluorescence dye for RNA detection (SYBR Green II). DNA recovery was measured by spiking plasmid DNA (pBR322); extracted DNA was determined by A260 measurement. An indication of RNA and DNA purity was obtained by measuring A260/A280 ratios. The integrity/intactness of the extracted nucleic acid was determined by gel analysis or by using the bioAnalyzer (Agilent Technologies) for RNA and DNA, respectively. The extraction method was tested on three external test sites in order to score relevant user aspects.
Results: The average recovery rates were 83 and 85% for RNA and DNA, respectively. For RNA extracts an average A260/A280 ratio of 2.13 was measured, whereas for DNA this value was 1.82. These values indicate that the purity of both preparations is high since for pure preparation the expected values are 2.0 and 1.8 for RNA and DNA, respectively. In addition, it was found that both RNA and DNA were intact recovered since no degradation products were detected. In addition, all users scored the method as labour friendly. The total amount of time needed to process 12 samples was <60 min, the throughput time was improved further by using two instruments in parallel, in this way 24 samples can be completed within 90 min. In addition the method was also verified for a broad range of different sample types including plasma, serum, CSF, sputum and stool.
Conclusion: The NucliSens Magnetic Extraction Reagents in combination with the NucliSens mini MAG instrument efficiently extracted RNA and DNA, in terms of recovery, purity and integrity. Moreover, the method is scored as labour-friendly, 24 samples can be processed within 90 min and was successfully used for various sample types.

P833 Pyrosequencing technology: a novel DNA sequencing technique for rapid and reliable typing of human papillomaviruses

B. Gharizadeh, M. Oggionni, B. Zheng, N. Pourmand, E. Akom, K. Wallin, P. Nyren
Stockholm, S; Milan, I; Palo Alto, USA; Quebec, CAN

DNA sequencing is the gold standard method for accurate genotyping of human papillomaviruses (HPV) and provides nucleic acid sequence information, which is the core of every organism. Pyrosequencing method has been successfully used for HPV genotyping with sequencing of only 14–21 bases. Multiple HPV infections are a common phenomenon in clinical samples with a varying rate depending on the group investigated. DNA sequencing techniques cannot differentiate between different genotypes as uninterruptible sequence results are obtained when multiples infections and unspecific amplification products are present in the amplicon. To address these problems, a type-specific multiple-sequencing primer DNA sequencing strategy, suitable for genotyping and detection of HPV-6, -11, -16, -18, -31, -33 and -45 has been developed. In the new method seven type-specific sequencing primers, combined in a pool, are added to the DNA sample. The oligonucleotide hybridising to the DNA sample will function as a primer during the subsequent DNA sequencing procedure. The new method is especially suited for detection and typing of samples harbouring different HPV genotypes (multiple infections) and unspecific amplifications, which eliminates the need for nested PCR, stringent PCR conditions and cloning. Furthermore, the method has proved to be useful for samples containing subdominant types/species, and samples with low PCR yield, which avoids re-performing 'failed' PCRs. We also introduce the sequence pattern recognition when there is a plurality of genotypes in the sample, which facilitates typing of more than one target DNA in the sample. Moreover, target specific sequencing primers could be easily tailored and adapted according to the desired applications or clinical settings based on regional prevalence of HPV as well as other microorganisms and viruses. As the cost for DNA sequencing is dropping, a sample could be sequenced in parallel with two or three different target specific primer pools covering a broader range of genotypes. The Pyrosequencing HPV detection assay is fully automated and could be used for detection and identification of different microorganisms and viruses.

P834 Comparison of the NucliSens Magnetic Extraction Reagents to reference extraction methods for the isolation of RNA and DNA from various sample types

G. Onland, L. Bartels, P. van Deursen, M. Jacobs, C.H.E. Boel, A.J.C. van den Brule
Veldhoven, Boxtel, NL

Objectives: A new nucleic acid extraction methodology that uses Boom chemistry in combination with magnetic silica particles is developed (NucliSens Magnetic Extraction Reagents, bioMérieux). This method is generic and can be used for the simultaneous nucleic acid extraction of 12 samples in combination with the NucliSense miniMAG instrument to facilitate the extraction procedure. The objective of this study was to investigate the suitability of this method for the isolation of Enterovirus RNA and *Mycobacterium tuberculosis* DNA from a broad spectrum of clinical specimens.
Methods: The NucliSens Magnetic Extraction procedure was compared with reference extraction procedures, i.e. QIAamp (Qiagen) and Boom, followed by Enterovirus RT-PCR and *M. tuberculosis* PCR, respectively. Several clinical specimens ($n = 100$) were used, a.o. Cerebrospinal Fluid (CSF), faeces, throat swabs, etc.
Results: By performing several extractions (up to 12) of a dilution series of strain Coxacksie B5 in CSF, it was shown that the analytical sensitivity of the Enterovirus RT-PCR was found to be independent of the extraction method used, whereas in very low frequency higher sensitivities were obtained in combination with magnetic extraction. As expected the higher input samples gave better reproducible results than lower input samples. After evaluation of the Enterovirus PCR using CSF and stool samples a 100% correlation between the two extraction methods was found. In addition, using a broad panel of clinical specimens for *M. tuberculosis* PCR, the same samples were identified as positive using the Boom extraction method and magnetic extraction. However, the latter method resulted in less samples having inhibition in PCR, but this needs to be confirmed in a larger study group.
Conclusion: This new magnetic silica based nucleic acid extraction method was successfully used to isolate Enterovirus RNA and *M. tuberculosis* DNA from clinical samples. In comparison to the reference methods (Qiagen and Boom), this procedure performed at least equally well regarding the functional performance and the throughput, but was considered to be more user convenient. For high throughput sample processing this chemistry can be transferred to an automated magnetic extraction device.

P835 Rapid detection of adenovirus DNA in conjunctival scrapings by real-time PCR using SYBR Green I as the detection dye

L. Folgueira, C. Prieto, S. Maldonado, M.J. Babiano, J.R. Otero
Madrid, E

Objectives: We have used a real-time PCR strategy, based on the Light-Cycler technology, to detect Adenovirus DNA in conjunctival scraping specimens collected from nursing home residents that presented clinical signs of conjunctivitis, and the results were compared with those obtained by conventional cell culture.
Methods: 23 conjunctival scrapings were sent to our laboratory in 2 mL of viral transport medium and were inoculated to monolayers of A-549 and MRC-5 cells in tubes, incubated at 37°C in stationary phase, and scored daily for cytophathic effect (CPE) for 7 days or until CPE developed. When a characteristic Adenovirus CPE was observed (usually after 5 days of culture), a passage was done to two homologous monolayers in shell vials that were incubated 24 h at 37°C and stained with specific fluorescent reagents to Adenovirus. DNA from 0.2 mL of the remaining transport medium was purified by a commercial procedure and resuspended to a final volume of 50 μL. Five microlitres of this purified DNA was used for real-time amplification in a final 20 μL reaction volume, using 1× Fast Start SYBR Green I master mix (Roche), $MgCl_2$ (3 mM), and 0.5 μM of each primer. The region amplified belongs to the hexon gene. Total processing time was less than 3 h.
Results: Adenovirus was isolated in 10 of 23 samples processed by conventional cell culture and all theses culture-positive samples were positive by real-time PCR; of 13 samples testing negative with conventional cell culture, real-time PCR detected eight as negative and five as positive. Gel electrophoresis analysis showed amplification bands of the expected molecular weight in all these real-time PCR positive, cell culture negative samples. A control group of 20 samples from patients with bacterial conjunctivitis was tested and all of them were negative by PCR.
Conclusions: Real-time PCR using the SYBR Green I fluorescent dye as the detection signal is simple, rapid and sensitive tool for detection of Adenovirus DNA from conjunctival specimens.

P836 Enterovirus detection by real-time RT-PCR in the study of aseptic meningitis

L. Santos, J. Simões, S. Martins, A. Silvestre, R. Costa, J. Freitas Da Fonseca, H. Lecour
Porto, P

Objectives: The aim of the study was the diagnosis of meningitis by Enterovirus in an urban area using real time reverse transcription polymerase chain reaction (RT-PCR) in cerebrospinal fluid (CSF) viral detection.
Methods: Aseptic meningitis was defined when CSF cytosis exceeded 6 leukocytes/mL with negative culture or negative PCR for usual bacterial aetiology. CSF samples were kept frozen at −70°C until used. Primers and probes were manufactured by Epoch Biosciences (MGB Eclipse Probe system) and RT-PCR conditions adapted to Rotor Gene real time cycler (Corbett Research).
Results: From 2001 to 2003, 185 patients were included. Ages ranged from 11 days to 71 years with 116 (63%) less than 15 years and eight newborns. On admission CSF cytosis vary from 10 to 1800 cells/mm^3 (138 ± 213), glucose from 15 to 143 mg/dL (64 ± 54), and proteins from 8 to 245 (52 ± 72) mg/dL. Forty-six per cent have blood leukocytosis and 35% high values of serum protein C reactive. Antibiotics have been prescribed to 57 (31%) of the patients on admission. Enterovirus RT-PCR assay was positive in 65 (35%) of the samples. Fifty-four (83%) of these were from children younger than 15 years with four infants and two newborns. No outbreak of acute meningitis was registered in that 2-year period. Enterovirus meningitis was observed all around the year with a summer and fall peak incidence.
Conclusions: Although meningitis by Enterovirus is usually a benign clinical condition, sometimes clinical and analytical data overlap those of bacterial meningitis. Real time RT-PCR is then a rapid and useful technique in clarifying the aetiology of acute meningitis.

P837 Lack of detection of human retrovirus-5 (HRV-5) proviral DNA in synovial tissue and blood specimens from individuals with rheumatoid arthritis or osteoarthritis

K. Piper, A. Hanssen, D. Lewallen, E. Matteson, R. Hagan, J. Steckelberg, R. Patel
Rochester, USA

Objectives: It had been suggested that there is an association between HRV-5, a recently identified retrovirus, and rheumatoid arthritis (RA). The purpose of this study was to determine if HRV-5 proviral DNA is present in synovial tissue and blood specimens from patients with RA or osteoarthritis (OA).
Methods: Synovial tissue and whole blood from 75 patients with RA, 75 patients with OA, and 50 patients without joint disease were collected in sterile containers. Whole blood was separated into mononuclear cells and granulocytes using Histopaque 1119 and 1077 (Sigma, St Louis, MO, USA). DNA was extracted using the QIAamp DNA mini kit (Qiagen, Valencia, CA, USA) and assayed in duplicate by LightCycler (Roche Molecular Biochemical, Indianapolis, IN, USA) real-time quantitative PCR using primers that amplify a 186-bp fragment of HRV-5 proviral DNA. A plasmid containing the HRV-5 sequence spiked into a negative synovial tissue or blood specimen was used as a positive control. Extracted DNA from a negative synovial tissue or blood specimen was included between every two specimens as a negative control. Suitability of DNA for PCR was verified using a PCR assay for beta-globin. Positive specimens were subjected to bidirectional sequencing. Fisher exact test (two-tailed) was used for statistical analysis.
Results: All 600 specimens were positive for beta-globin (extraction control). Cloned HRV-5 proviral DNA spiked into tissue, mononuclear cells and granulocytes followed by extraction yielded an amplified product in all cases. The limit of detection of the assay was 166.6 copies/mL blood and 6.6 copies/mg tissue. Two hundred tissue specimens, 200 mononuclear cells, and 196 of 200 granulocyte specimens tested negative for HRV-5 proviral DNA. Two RA and two OA granulocyte specimens, however, yielded a positive signal for HRV-5 proviral DNA. All were detected at a low copy number (quantitated by comparison to a known quantity of cloned HRV-5 proviral DNA spiked into blood), range 83–1365 copies of HRV-5 proviral DNA/mL blood. All four showed 95–98% identity to GenBank sequence AF480924 by NCBI BLAST search.
Conclusion: We did not find an association between HRV-5 and RA or OA ($P = 0.516$) using a real time PCR assay. Recently it has been shown that HRV-5 is actually rabbit endogenous retrovirus H (*J Virol* 2002: 76; 7094–7102). We hypothesise that experimental rabbit studies ongoing in our laboratory while the granulocyte specimens were being prepared account for the low level of 'HRV-5' proviral DNA detected in 0.7% of the specimens tested.

P838 Diagnosis of viral infections of central nervous system by polymerase chain reaction

A. Domingos, G. Marrão, C. Nogueira, A. Pinto de Oliveira, G. Rocha, A. Magalhães-Sant'Ana
Coimbra, P

Objectives: Establish an aetiological diagnosis of viral infections of the Central Nervous System (CNS) with the determination of the most frequently virus implicated in those infections. It was also our aim to improve the diagnosis efficiency through molecular techniques.
Methods: We analysed a total of 31 cerebrospinal fluid samples (CSF) (12 paediatric and 19 non-paediatric) between September

2002 and June 2003 from 30 patients with clinically suspicion of viral encephalitis, meningitis or other CNS disease, from several hospitals of Portugal's Central region. The mean age of patients was 26 years (range from 1 month to 65 years). We use the PCR method in CSF samples to identify the following virus: HSV, VZV, EBV, CMV, Parvovirus B19, Adenovirus and Enterovirus, using specific primers for genome's regions that are highly conserved and that identify several serotypes of the same group, such as the primers for the 5′ non-coding region of Enterovirus and the adenoviral hexon gene that allows the detection of 51 human adenovirus prototype strains and different genome variants, for the others virus we amplify the following gene fragments, BamHIW, MCP, gene for DNA polymerase, gene 28 and gene for VP1-VP2 of EBV, CMV, HSV, VZV and Parvovirus B19, respectively. The viral nucleic acids were extracted from CSF specimen using the commercials kits, QIAampÒDNA Mini Kit and QIAampÒViral RNA Mini Kit, for DNA and RNA extractions, respectively. The products of nucleic acid amplification were detected by visualisation of DNA bands of the expected size in agarose gel electrophoresis after staining with ethidium-bromide.
Results: Of the 30 patients evaluated in this study, four (three >19 years old) were enterovirus-positive (12.9%), four (one >19 years old) were adenovirus-positive (12.9%), four (all with age less than 19 years) were HSV-positive and eight (five >19 years) were CMV-positive (25.8%), with three of them were HIV seropositive.
Conclusion: CMV was the most frequent virus detected in our study. Our study explains the growing interest of clinicians in new technology, as offer a rapid, sensibility and specific response to diagnosis.

P839 Infection of human cytomegalovirus (HCMV) DNA in placenta using PCR

E. Paradowska, M. Przepiorkiewicz, D. Nowakowska, M. Studzinska, J. Wilczynski, Z.J. Lesnikowski
Lodz, PL

Objectives: Placentas from pregnant women with suspected human cytomegalovirus (HCMV) infection and diagnosed on the basis of clinical symptoms, elevated HCMV IgM and IgA titres were examined for the presence of HCMV DNA. Searching of HCMV was focused on trophoblasts and Hofbauer cells from fetal part of placenta as well as decidual macrophages. For the detection of viral genome three qualitative PCR assays, targeting the US14, US13/US14 or UL55 gene, and a real-time quantitative PCR were applied.
Methods: Twenty human placentas after delivery from pregnancies complicated with HCMV infection were examined by PCR method and indirect immunofluorescent assay.
Results: HCMV DNA was found in 7/20 placentas (35%) either in decidual and/or chorionic villi using different PCR tests. In one placenta we observed inhibition of the virus transmission from decidua to fetal tissues. Placental HCMV infection was also characterised by immunofluorescence staining with monoclonal antibodies to HCMV antigens and specific cell markers.
Conclusions: Our results demonstrate that the detection of HCMV DNA in placental tissues by amplification methods provides a rapid, specific and sensitive method for diagnostics of HCMV infection. However, perinatal diagnosis should include PCR in addition to viral culture and immunofluoresence assay. Rapid detection of HCMV in placenta may play an important role in diagnostic process of congenital HCMV infection leading to identification of newborns at risk of severe complications.
Acknowledgement: This work was supported by the State Committee for Scientific Research, grant no 3 P05E 001 25.

P840 Quantitative real-time PCR for diagnosis of VZV infection in cerebrospinal fluid

E. Zampachova, N. Piskunova
Ceske Budejovice, CZ

For the detection of Varicella zoster virus (VZV) in CSF, PCR is the method of choice. Relation between the severity of illness and quantity of virus in CSF is unknown.
Method: Quantitative real-time PCR was performed on Light Cycler instrument (Roche, UK) using Real Art VZV LC kit (Artus, Germany). Standards for quantitative curve assessment were a part of the kit. DNA was isolated by High Pure Viral Nucleic Acid Kit (Roche) according to the manufacturer protocol. In limited number of samples the interassay variability of the method and variability in yield of isolation were tested. Interassay difference was maximum 163 copies per millilitre of sample. The difference in DNA isolation yield was higher, 188–881 copies per millilitre of sample.
Patients: Samples for quantitative testing were selected by qualitative PCR. VZV DNA was detected in 17 CSF samples from 16 patients with Herpes zoster (age 21–88 years) and in one sample from a child with Varicella, complicated by meningitis. All patients had clinical signs of meningeal irritation and biochemical markers of neuroinfection in CSF. One additional sample of vesicular fluid and blood sample from an adult patient with acute severe varicella without meningitis was tested.
Results: CSF virus load ranged from 10^2 to 5×10^4 copies per millilitre. In comparison the virus load in vesicular fluid was 3×10^6 copies per millilitre. The highest virus loads (5×10^4 and 2×10^4) were detected in a patient with paresis of facial nerve and a young patient with relatively mild disease. The lowest virus load (10×10^2 copies per millilitre) had a child with varicella meningitis and an old patient with severe Herpes zoster of the trunk. Quantitative PCR has good reproducibility and is useful for assessment of viral load in CSF samples. However, the correlation between virus load and severity of illness remains uncertain.

P841 Detection of herpes simplex virus and enteroviruses in cerebrospinal fluid by real-time PCR

J. Garcia-Martinez, L. Folgueira, C. Prieto, S. Maldonado, M.J. Babiano, J. Otero
Madrid, E

Objectives: We evaluate real-time PCR as a laboratory tool for Herpes simplex virus (HSV) DNA and Enterovirus (ETV) RNA detection in cerebrospinal fluid (CSF).
Methods: Over a 15-month period viral nucleic acids were extracted from 200 μL of CSF specimens from patients with clinical signs of meningitis and/or encephalitis with the High Pure Viral Nucleic Acid Kit (Roche Diagnostic GmbH). Glicoprotein D target gene was amplified in a total volume of 20 μL in a LightCycler Instrument (Roche Diagnostic GmbH) with the FastStart DNA Polymerase amplification kit (Roche Diagnostic GmbH). The temperature of melting differentiated HSV-1 (89.9°C) and HSV-2 (90.8°C) viral types. ETV 5′ non-translated region was reverse transcribed and nested amplified in a LightCycler Instrument. CSF specimens were also destined to viral culture on MRC-5 and A-549 cellular lines.
Results: 325 CSF samples from 295 patients, mean age of 31.3 years (0–91) were investigated. HSV DNA was detected in eight samples belonging to seven patients (2.37%) with a mean age of 43.1 years (0–84). All but one were HSV-1; none of them grew on cell culture. Fifty-five samples from unique patients were tested for ETV RNA; six samples (10.9%) yielded a positive result from patients with a mean age of 4 years (0–10); three of them demonstrated a specific cytopathic effect on cell culture. Leukocyte counts, glucose and protein levels in CSF ranged from 0–205 μL^{-1}, 40–100 mg/dL and 0.31–1.79 g/L, respectively, for HSV DNA positive samples and 2–100 μL^{-1}, 40.9–60 mg/dL and 0.28–0.7 g/L, respectively, for ETV RNA positive samples. There were no other microbiological data that accounted for the clinical

signs. Although all seven patients with HSV DNA positive CSF samples received different aciclovir iv doses based on their age, three of them (42.8%) died.
Conclusion: Real-time PCR is an important tool in the diagnosis of viral CNS infections because of its rapidity, sensitivity, specificity and reliability compared with traditional viral diagnostic methods. It is necessary to test systematically all CSF samples from patients with clinical suspicions of meningitis and/or encephalitis despite biochemical findings. A positive real-time PCR result could anticipate antiviral treatment, diminish antibiotic administration, avoid unnecessary procedures and allow earlier discharges, which would improve patient outcome.

P842 Comparison of three commercial glycoprotein G-based enzyme immunoassays for detection of HSV-1/HSV-2 IgG antibodies in pregnant women and sex workers

A. Aksözek, M. Özdamar, S. Ergin, K. Midilli, S. Serdaroglu, S. Türkoglu, G. Yilmaz
Istanbul, TR

Objectives: Genital herpes is one of the most prevalent sexually transmitted diseases, usually caused by herpes simplex virus type 2 (HSV-2). The high rate of undetected carriers is important in the spread of HSV-2 as a sexually transmitted disease. Since direct virus isolation methods are effective mostly in active phases of infection, serological screening is necessary to able to detect past HSV-2 infection which makes the choice of commercial assay differentiating HSV-1/HSV-2 critical. In this study we aimed to compare three commercial glycoprotein G-based ELISAs in screening pregnant women and sex workers.
Materials and Methods: A total of 88 sera were tested (44 sex workers, 44 pregnant women). The three HSV 1 and HSV 2 type specific assays used were IgG-ELISA (Euroimmun, Lübeck, Germany), HerpeSelect ELISA (Focus Technologies) and IBL (Hamburg, Germany).
Results: Results are shown in Tables 1 and 2.
Conclusion: HSV-1 IgG results were concordant in all three assays in both groups. In HSV-2 IgG detection, Euroimmun and HerpeSelect assays gave identical results but with IBL assay more positivity rates especially in pregnant women were obtained. Selection of commercial ELISA kits may have a critical role in follow up of pregnant women and screening of high risk groups of HSV infections.

Table 1

HSV-1 IgG	Negative	Positive
Pregnants		
Euroimmun	1	43
HerpeSelect	1	43
IBL	1	43
Sex-Workers		
Euroimmun	1	43
HerpeSelect	3	41
IBL	1	43

Table 2

HSV-2 IgG	Negative	Positive
Pregnants		
Euroimmun	43	1
HerpeSelect	41	3
IBL	22	22
Sex-Workers		
Euroimmun	11	33
HerpeSelect	9	35
IBL	2	42

P843 A novel enzyme immunoassay for the detection of anti-herpesvirus-8 lytic IgG antibodies: evaluation of clinical performance characteristics

F. Loftus, S. O'Keeffe, D. O'Leary, G. Elliott, S. Reid
Dublin, IRL

Objective: Human Herpesvirus-8 (HHV-8) is also known as Kaposi's Sarcoma-associated Herpesvirus (KSHV). The virus is classified as a gamma herpesvirus and resembles EBV in its tropism for B cells and ability to exist in a latent state. The Biotrin HHV-8 IgG EIA was developed as a screening tool for HIV and organ transplant associated populations.
Method: The EIA format incorporates synthetic peptides immobilised on a solid phase. The peptides are derived from lytic antigens K8.1 and KS65. A panel of 91 sera from HHV-8 seropositive men with AIDS associated Kaposi's sarcoma were analysed using the Biotrin HHV-8 IgG EIA to determine the clinical sensitivity of the assay kit. A HHV-8 negative panel consisting of 56 normal human sera and 54 potential-cross-reactives (including HIV, EBV, CMV and other Herpes sub-groups) were tested to evaluate the specificity of the new assay.
Results: Of the 91 AIDS associated KS samples 82 were found to be positive for anti-HHV-8 IgG antibodies, one specimen was negative, while eight specimens were within the equivocal zone (greater than or equal to 0.8 and less than or equal to 1.2 index value); these figures translate to a clinical sensitivity value of 90.1%. Specificity of the assay, including the cross-reactive specimens, was measured at 94.65%.
Conclusion: These results demonstrate that rational selection of peptide antigens and careful optimisation of assay conditions have given high assay specificity while maintaining assay sensitivity.

P844 A new enzyme immunoassay for the detection herpes simplex virus type 2 – specific antibodies

A. Obriadina, S. Klimashevskaya, M. Gladisheva, V. Puzyrev, A. Burkov, T. Ulanova
Nizhniy Novgorod, RUS

Objectives: The purpose of this study was to develop enzyme immunoassay (EIA) for the detection of IgG anti-HSV-2 activity using two new recombinant proteins as antigenic targets, and to evaluate these EIA with the aid of statistical methods.
Methods: Fragments of glycoprotein G (gG-2), comprising residues 525 to 578aa of herpes simplex virus type 2 (HSV-2) and glycoprotein D of HSV-2 (gD-2(266-394aa)), were expressed in the *E. coli* as GST fusion proteins to develop an assay for the detection and HSV-2 type-specific antibodies.
Results: A new enzyme immunoassay for the detection of IgG anti-HSV-2 (IgG-EIA) in sera was developed using two new recombinant proteins. The IgG-EIA was evaluated using serum specimens obtained from patients with culture-proven HSV-2 infection (CP) ($n = 13$) and from normal blood donors (BD) ($n = 629$). All specimens were additionally tested for IgG anti-HSV-2 activity by two commercially available EIAs. This new IgG-EIA detected anti-HSV2 activity in all specimens from HSV2 infected patients. When BD were tested the overall concordance between these three assays varied between 39 and 63.6%, concordance between positive samples ranged from 18.4 to 46.7%. In the absence of a gold standard the accuracy of these EIAs was assessed by the computer program based on a maximum likelihood approach using a 'latent class' model. This analysis estimated the IgG-EIA sensitivity and specificity to be within the range 98–100% and 95–100%, respectively.

Conclusion: The results show that the new two proteins-based enzyme immunoassays may be useful tools for the detection of type-specific HSV-2 antibodies. However, if only one assay is performed, careful interpretation of the results is indicated and for determination of the definitive HSV-2 serostatus, statistical models may be necessary.

P845 Detection of the six commonest human herpesviruses in clinical specimens by a single PCR (Herpes Consensus methodology)

S. Levidiotou, G. Vrioni, C. Gartzonika, T. Priavali, C. Christaki
Ioannina, GR

Objectives: A wide spectrum of clinical disease is associated with the human herpesviruses. The six most frequent aetiologic agents of herpetic infections are herpes simplex virus 1 and 2 (HSV1, HSV2), varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein–Barr virus (EBV) and human herpesvirus 6 (HHV-6). The aim of this study was the simultaneous detection and identification of the six major human herpesviruses in various clinical specimens by a single PCR.
Methods: A total of 136 clinical samples obtained from 135 patients, including 78 specimens of cerebrospinal fluid (CSF) from patients with suspected encephalitis or meningitis, six swabs from patients with vesicular skin lesions, 41 aqueous humor specimens from patients with uveitis or iridocyclitis, nine conjunctival swabs from patients with conjunctivitis, and one vesicle aspirate and one bronchoalveolar lavage from the same patient with suspected varicella pneumonitis, were studied for the presence of herpesvirus DNA. These specimens were collected from March 2001 to November 2003. DNAs were amplified using a new type of primer system, 'stair primer', in a single tube, bringing simultaneous amplification of the six Herpesviruses (Herpes Consensus Hybridowell, Argene Biosoft, France). The amplified product was detected by hybridisation in a microtitre plate with six different biotinylated probes, specific for the six viruses. The method was successfully tested with positive and negative controls from the European Union Concerted Action for Quality Control of Nucleic Acid Amplification in Diagnostic Virology (Herpes-2001 and VZV-2003 Proficiency Panels). According to our results: (a) HSV1 DNA was detected in one CSF specimen from a patient with aseptic meningitis, in three aqueous humor specimens from patients with uveitis, in one swab from a patient with herpetic vesicular skin lesions and in three conjunctival swabs from patients with conjunctivitis, and (b) VZV DNA was detected in two aqueous humor specimens from patients with iridocyclitis, in two swabs from patients with vesicular skin lesions, and in the vesicle aspirate and bronchoalveolar lavage from the patient with varicella pneumonitis. The precise diagnosis of herpetic infection was available within 24–48 h, which allowed for an early initiation of adapted antiviral therapy.
Conclusion: The detection of the six commonest human herpesviruses in clinical specimens by the Herpesvirus Consensus PCR methodology allowed rapid, sensitive and specific results.

P846 Questioning the programmed cell death in bovine herpesvirus 1 infected MDBK cells and the possible role of nitric oxide in this process

Z. Yazici, Y. Baskin, H. Baskin, O. Gecer, A. Ozkul, I.H. Bahar
Izmir, TR

Objectives: Bovine herpesvirus-1 (BHV-1) is the aetiological agent of many infections and may predispose infected animals, possibly through immunosupression, to secondary bacterial infections. Immunosupression may directly be associated with the induction of programmed cell death (PCD) in some virus infected cells. Nitric oxide (NO) has an important mediating role against fungal, bacterial, protozoal, viral pathogens and tumours. In this study, role of NO was questioned in the PCD process.
Methods: This study was planned in two consecutive stages. In the first stage, the morphological (with and without staurosporin) and biochemical changes caused by virus-induced PCD in MDBK cells were investigated. Morphological assessment of PCD was performed using Hoechst 33342 nuclear staining and fluorescence microscopy technique. In the second phase of the study, the induction of PCD with staurosporin (SS) (alone or with BHV-1 addition) and apoptotic route of BHV-1 infections (with/without staurosporin) were analysed by applying 1, 3, 8, 9 caspase inhibitors (R&D, Germany).
Results: It was interesting to see that BHV-1 inhibited PCD following 1 h of poi instead of being induced by staurosporin and induced apoptosis alone between 0.5 and 3 h of poi in MDBK cells, however, between 3 and 6 h of poi, PCD response has found to be decreased. These results showed similarities with those obtained from herpes simplex type-1 infections in human epithelial cells. Following caspase 1, 3, 8, and 9 inhibitors applications PCD responses decreased after 1 h whereas NO responses increased following 3 h of infections with caspase 1, 3, 8, and 9 inhibitory peptides.
Conclusion: In conclusion, BHV-1 inhibited the apoptotic response in a caspase-independent way and BHV-1 may modulate the NO response through the apoptotic pathways.

P847 Inversely proportional relation between programmed cell death and nitric oxide responses: latency behaviour of bovine herpesvirus-1 in an epithelial like microenvironment

Y. Baskin, Z. Yazici, H. Baskin, O. Gecer, N. Olgun, A. Ozkul, I. Hakki Bahar
Izmir, TR

Objectives: The aim of this study is the questioning the programmed cell death (PCD) process in acute phase of BHV-1 infection in cultured epithelial like cells' microenvironments and to investigate its relation with possible nitric oxide responses in HEp-2 cells infected with BHV-1 with and without staurosporin induction.
Methods: This study was planned in two consecutive stages. In the first stage, the morphological (with and without staurosporin) and biochemical changes caused by virus-induced PCD in HEp-2 cells were investigated. Morphological assessment of PCD was performed using Hoechst 33342 nuclear staining and fluorescence microscopy technique. In the second phase of the study, the induction of PCD with staurosporin (SS) (alone or with BHV-1 addition) and apoptotic route of BHV-1 infections (with/without staurosporin) were analysed by applying 1, 3, 8, 9, and total caspase inhibitors (R&D, Germany).
Results: It is known that following HSV-1 infection of 3–6 h of poi anti-apoptotic activity is triggered in human cells. And this activity is through caspase 3. It is interesting to see that in these experiments following 1 h of BHV-1 infection the number of apoptotic cells reduced whereas NO response continuously increased following 1-h poi.
Conclusion: Anti-apoptotic activity of BHV-1 seems to be activated through caspase 3 like HSV-1, and this inversely proportional relation between NO and PCD responses seem to be related with the triggering effect of NO on PCD response.

P848 *In vitro* inhibition of herpes simplex virus type 1 replication caused by various probiotics

A. Voeikova, E. Ermolenko, V. Furaeva, A. Suvorov
Saint-Petersburg, RUS

Objectives: Anti-viral effect of various probiotics as alternative to traditional anti-viral drug therapy was recently demonstrated. This effect was explained as non-specific stimulation of the host immune system. However, direct anti-viral effect cannot be excluded. The goal of present study was to evaluate the effect of probi-

otics strains derivative metabolites on the reproduction of herpes simplex virus type 1 (HSV-1).
Materials and methods: Probiotic strains used were: *Lactobacillus plantarum* 8A-P3, *Enterococcus faecium*-L3, *Escherichia coli* M17. One hundred and six Vero cells were infected with 103-104 ID of HSV-1 and then incubated with supernatants from bacteria or bacteriocin preparations applied in serial dilutions. Acyclovir 20% (Lek, Slovenia) was used as anti-viral drug control. Cytopathic effect of the virus was determined by light or immunoflourescence microscopy after 72 h.
Results: HSV-1 alone or in the presence of the *E. coli* M17 extracts caused the most profound cytopathic effect. Addition of acyclovir completely inactivated the effect of the virus that was taken for 100%. Supernatants obtained from *L. plantarum*, and *E. faecium* generated dose dependant effect from 90 to 35% of viral inhibition. *E. faecium* strain L-3 extract was 10–20% more active than *L. plantarum*. Extract from the strain L-3 was analysed for the presence of bacteriocins. Two types of peptides were determined – enterocin A and enterocin B (5.5–5.6 kD). Bacteriocin preparation demonstrated similar anti-viral effect (65–85% of inhibition) which allows to consider enterococcal bacteriocins as major anti-viral agents in present model.
Conclusions: Extracts of several probiotic bacterial strains express a specific activity against reproduction of HSV-1 *in vitro*. Anti-viral effect of *E. faecium* strain L-3 was the strongest due to the presence of enterocins A and B in the supernatant.
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Clostridium difficile

P849 Prevalence of *Clostridium difficile* in hospitalised patients

M. Lemeshevskay, I. Malov, S. Balakhonov, K. Aitov, Y. Plotnikhova
Irkutsk, RUS

Objectives: Antimicrobial therapy renders the bowel susceptible to colonisation and/or overgrowth of *Clostridium difficile*. *Cl. difficile* is responsible for more than 90% cases of pseudomembranous colitis and *c.* 25% cases of diarrhoea observed during or after antibiotic therapy. The goal of this innovative study is to discover main epidemiological aspects and clinical examples of *Cl. difficile*-associated diseases (CDAD) on the basis of comprehensive use of the most recent bacteriological, immunological, and molecular-genetic methods.
Methods: Investigated population included 479 individuals ranging in age from 13 to 79 years divided into four clinical and one control sample. Stool specimens were examined using standard bacteriological methods, PCR, and enzyme immunoassay. Identification of *Cl. difficile* was done using the anaerobic technique on cycloserine-cefoxitine-fructose agar. PCR with two specific primer pairs of *Cl. difficile* different genes was performed. The determined genes (cytotoxins A and B) are localised on chromosome of *Cl. difficile*.
Results: The frequency of *Cl. difficile* in hospitalised individuals varied from 3.5 to 15.8% and depended on the hospital type and the treatment administered. The *Cl. difficile* infection was most frequently detected in patients with HIV (15.8%) and patients after surgical treatment (12.2%). A history of antibacterial therapy and immunodeficiency conditions are risk factors increasing the frequency of CDAD. To identify various microorganisms in clinical material, we have developed a variant PCR-based technique involving amplification of the cytotoxine B gene fragment. The method shows high degree of specificity and sensitivity measuring not less than 103 xx/ml. *Cl. difficile*-associated diseases are characterised by a number of clinical symptoms varying from asymptomatic carriage (60%) to various forms of colitis (40%). The most frequent clinical symptoms include the following: increased body temperature, continuous stomach aches, most frequently localised in the mezogastric and left abdominal area, and loose stool with variable blood content.
Conclusions: This research revealed wide prevalence of toxigenic strains of *Cl. difficile* among the sample population. This supports the importance and necessity of implementing the most recent methods to diagnose and treat CDAD.

P850 The first report of the occurrence of Cardiff-PCR-ribotypes among clinical *Clostridium difficile* strains isolated from patients with AAD in a university hospital, Warsaw

H. Pituch, J.S. Brazier, P. Obuch-Woszczatynski, D. Wultanska, F. Meisel-Mikolajczyk, M. Luczak
Warsaw, PL; Cardiff, UK

Clostridium difficile is the main aetiological agent of nosocomial diarrhoea. The pathogenicity of this bacterium is determined by the production of two major toxins: enterotoxin A (TcdA) and cytotoxin B (TcdB). However, *C. difficile* strains producing only toxin B (TcdA–TcdB+) can be isolated from AAD. The application of different typing methods has revealed that *C. difficile* is a heterogeneous species. O'Neill and colleagues developed a modification of PCR ribotyping method based on polymorphisms in the 16S–23S rRNA intergenic region spacer for the routine typing of *C. difficile*. In this study we used PCR-ribotyping methods to discriminate Polish *C. difficile* strains. For this purpose 78 Polish *C. difficile* strains isolated between 2002 and 2003 including our collection were studied. We have compared 49 Polish *C. difficile* strains randomly chosen with PCR-ribotypes of ARU Cardiff collection. Among these strains 43 were toxigenic: 23 TcdA+TcdB+, 20 TcdA−TcdB+ but six strains were non-toxigenic TcdA−TcdB−. A total of 18 different ribotypes were detected among 49 Polish isolates. Among toxigenic strains (TcdA+TcdB+) we detected 13 ribotypes, among toxin B producing strains (TcdA−TcdB+) only one ribotype was detected. Among non-toxigenic strains four ribotypes were detected. It seemed to be interesting to observe the dominating ribotypes. Between toxigenic (TcdA+TcdB+) five belonged to ribotype 014 and four to 046. All strains ($n = 20$) (TcdA−TcdB+) belonged to one ribotype - 017. In summary, PCR-ribotyping is a good method to discriminate *C. difficile* strains. We decided to continue further epidemiological study in Poland.

P851 Risk factors of *Clostridium difficile*-associated diarrhoea due to actin-specific ADP-ribosyl transferase producing strains

F. Barbut, D. Decré, V. Lalande, L. Noussair, B. Burghoffer, A. Gigandon, F. Espinasse, L. Raskine, J. Robert, A. Mangeol, C. Branger, J. Petit
Paris, Clichy, Boulogne, Montfermeil, F

Clostridium difficile (CD) is responsible for intestinal disorders including post-antibiotic mild diarrhoea to fulminant pseudomembranous (PMC) colitis. The two large toxins A and B are thought to be the primary virulence factors of *C. difficile*. In 1988, Popoff *et al.* (*Infect Immun* 1988; 56; 2299–2306) described a strain which produces an actin-specific ADP-ribosyl transferase (CDT or binary toxin) isolated from a patient with a severe PMC.
Objectives: The aim of the study was to identify risk factors of *C. difficile*-associated diarrhoea due to ADP-ribosyl transferase producing strains.
Materials and Method: A retrospective case control study was performed. Each case (patient with a diarrhoea due to an actin-specific ADP-ribosyl transferase producing strain) was compared with two controls (patient with diarrhoea due to a *C. difficile* strain which does not produce an actin-specific ADP-ribosyl transferase) matched on ward and on date of hospitalisation. CdtA and cdtB genes were screened by PCR (Stubbs *et al.*, *FEMS Microbiol Letters* 2000; 186; 307–312). Production of CDT was studied by Western blot using an antiserum anti Ia and Ib from *C. perfringens* and the activity of the toxin was assessed using an ADP-ribosyl transferase assay.
Results: Twenty-six cases (14 males and 12 females) were identified in 1999 and 2000. They were hospitalised in six different hospitals of Paris and its surrounding area. All the CDT positive strains were also positive for toxins A and B. Cases were compared with 42 controls. Cases and controls did not differ significantly for sex, age, previous administration of antibiotics, of chemotherapy or immunosuppressive treatment. Endoscopic examination was performed in 30.5% of cases and in 23.8% of controls ($P = 0.52$) and frequency of mucosal abnormalities was similar. Diarrhoea was more often community-acquired in cases than in controls (65.4 vs. 35.7%, $P = 0.017$) and represented more often the cause of hospitalisation (61.5 vs. 26.2%, $P = 0.003$). Moreover, diarrhoea from cases was more frequently associated to abdominal pain (63.6 vs. 39.4%, $P = 0.007$) and to liquid stools (76.9 vs. 59.5%, $P = 0.14$).
Conclusions: These results suggest that there could be a correlation between the production of binary toxin and the severity of diarrhoea. The binary toxin could induce intestinal lesion independently of toxins A and B or it may act in synergy with these toxins.

P852 Control of an outbreak of *Clostridium difficile* infection among geriatric patients

S. Cherifi, J. Robberecht, V. Ledeganck, B. Byl, M. Delmee, G. Mascart
Brussels, B

Objectives: We investigated a nosocomial outbreak of *Clostridium difficile*-diarrhoea which occurred in March 2003 in the 127-bed geriatrics department of the Brugmann University Hospital (21 March 2003 to 29 April 2003).
Methods: Outbreak was detected by the *C. difficile* surveillance programme survey of the Infection Control Unit. *C. difficile* infection was diagnosed by stool culture and by detection of toxin A with a qualitative rapid immunoassay. Isolates of *C. difficile* were genotyped using pulsed-field gel electrophoresis.
Results: Incidence of *C. difficile*-associated diarrhoea increased from 16 cases per 100 000 patient-days before to 90 cases per 100 000 patient-days during the outbreak. This outbreak involved 21 patients of four geriatrics wards, located on two geographically distinct sites (with the same medical team). Mean age was 83 (range 71–100) years; sex-ratio (F/M) = 1.1; 90% (19/21) of cases had received one or more antibiotics before onset of diarrhoea. About 24% (5/21) of cases were long-term care facilities-acquired diarrhoea, and secondary hospital transmission resulted in three clusters involving 16 cases. Serotyping and genotyping were performed on isolates from 19 different stools; 16 of these strains belonged to the same type A1 whereas three displayed profiles different from the outbreak strain. Management of this outbreak consisted in reinforcement of contact isolation precautions for patients with diarrhoea, cohortage of infected patients in the same ward and in promotion of hand disinfection with an alcoholic solution. Environmental disinfection with hypochlorite was introduced during the outbreak. The ward where most transmission occurred was closed during 10 days for a completed disinfection after last patient discharge. After resolution of the outbreak, incidence for acquisition was 12 cases per 100 000 patient-days. Ninety per cent (19/21) of patients were treated by metronidazole or vancomycin. Relapses occurred in 29% (6/21) of patients. Two patients died with severe colitis. Mean hospital stay was 39 (range 11–97) days (annual mean of length of stay in the department = 21 days).
Conclusion: Rapid control of this nosocomial outbreak of *C. difficile* among geriatric patients was obtained with early implementation of cohortage and ward closure associated to reinforcement of environmental disinfection, hand hygiene and enteric isolation.

P853 Toxigenic status of *C. difficile* in a large hospital: classical and molecular approaches

A. Martín, R. Alonso, T. Peláez, M. Marín, M. Rodríguez-Creixems, E. Bouza
Madrid, E

Introduction: Toxigenic *Clostridium difficile* is the main cause of nosocomial diarrhoea and has recently been described as involved in community acquired infections. Two main toxins have been classically described as the main virulence factors although strains that lack one of them are emerging with increasing frequency.
Objective: We aimed to characterise toxigenic phenotypes in an institution with high prevalence of *C. difficile*-associated diarrhoea (CDAD).
Materials and methods: *C. difficile* isolates were obtained and collected over a 6-month period from diarrheic stools submitted to our laboratory. Specimens were cultured in CCFA plates with blood and presumptive colonies identified by standard procedures. Toxin B was detected with a standard cytotoxicity assay on human fibroblast culture using both diluted samples and pure broth cultures of the microorganism. Toxin A was detected by a commercial enzyme-immunoassay (CdTOX A OIA, BioStar, Finland) using colony suspensions in order to increase the sensitivity of the test. All negative results for any of both toxins were checked by PCR using previously published primers and conditions.
Results: A total of 220 *C. difficile* isolates were obtained during the study period. One hundred and ninety-nine isolates (90.5%) produced both toxins (A+B+); 10 isolates (4.5%) were classified as non-toxigenic (A−B−) by phenotypic procedures; in 11 isolates (5%) only toxin B was detected (A−B+), while no isolates were classified as producers of toxin A exclusively (A+B−). All non-toxigenic strains showed PCR positive results for gene B and four of them also for gene A (six isolates were A−B+ and four were A+B+). From all A−B+ isolates, only five were confirmed by PCR, while in six of them, toxin A gene was also detected.
Conclusion: The vast majority of *C. difficile* isolates obtained in our laboratory were toxigenic (A+B+) by traditional approaches. We have detected, using classical methods and confirmation by PCR, the presence of A−B+ isolates in our collection. All isolates considered as non-toxigenic by phenotypical methods were PCR-positive for one or both toxins. Disagreements between results of phenotypical and genetic methods can be justified as the presence of incomplete or unexpressed genes or a lack of sensitivity of the former methods.

P854 An investigation into the carriage of *Clostridium difficile* on the hands of health care workers

S. Maxwell, F. Hudson
Stockport, UK

Background: It has been estimated that the extra cost to the NHS for every patient that contracts *C. difficile* in hospital is £4000. In light of this it seemed imperative that the possible components involved in the mode of transmission of this nosocomial infection be investigated with a view to control the spread.
Objective: To look at the level of contamination of health care workers' hands with *C. difficile* after dealing with a known positive patient.
Methods: Hands were sampled using the finger streak method on a *C. difficile* moxalactam norfloxacin (CDMN) agar plate. Plates were incubated for 48 h under anaerobic conditions and then examined for any possible colonies of *C. difficile*. These were identified using the Gram stain and RapID ANA II system. Hands were sampled directly after patient contact and the type of contact was also noted. Hands were also sampled after the removal of gloves and after hand washing. In all, 54 duplicate samples were taken after various contacts with 14 colonised patients.
Results: 21% of samples taken immediately after patient contact were positive. Nine per cent of samples taken after the removal of gloves were positive. No samples taken after hand washing were positive.
Conclusion: This study showed that hands do readily and regularly become contaminated after contact with a known positive patient and that this contamination can follow fairly minimal contact with the patient.

P855 Tolemaver (GT160-246) binds *Clostridium* cytotoxins A/B and is associated with restoration of components of the anaerobic intestinal microflora during treatment of *C. difficile*-associated diarrhoea

T. Louie, B. Byrne, J. Emery, W. Krulicki, L. Ward, D.R. MacCannell, M. Montgomery, D. Gregson, M. Mah
Calgary, CAN

Objectives: During the conduct of a Phase 2 clinical trial on the efficacy of tolevamer 1 or 2 g TID for 14 days compared with vancomycin 125 mg QID for 10 days, we collected serial faecal samples on study entry, days 4, 7, 10, 14, 21, 28 and 42 to determine if non-antibiotic therapy can neutralise *C. difficile* toxin B in faecal filtrates, promote restoration of the normal microbiota and achieve clinical response.
Methods: 33 patients were randomised into the study at Calgary study sites (out of 289 patients/58 centres). Faecal filtrate concentrations of *C. difficile* cytotoxin B, quantitative counts of *C. difficile* vegetative organisms, *C. difficile* spore counts were determined. Quantitative aerobic/anaerobic cultures using serial dilutions of faeces 10E-3,5,7,9,11 g^{-1} wet weight were performed using criteria as outlined in the Wadsworth Anaerobe Laboratory Manual. Stools from healthy donors served as normal microflora controls.
Results: Thirty of 33 patients provided one or more samples, and 22/30 provided serial samples beyond 7 days and up to 42 days. Normal flora controls showed an average of four different Bacteroides species in counts of 10^{10-12} g^{-1} faeces wet weight, plus other anaerobic genera in a more inconsistent manner. Using Bacteroides species as a marker genus for the anaerobic microflora, 15, 8, and 7 patients had bacteroides counts below the limit of detection, between 10^{3-8}, or $>10^8$ CFU/g faeces, respectively, at study entry. Vancomycin treatment eliminated vegetative *C. difficile* with variable spore persistence, and the Bacteroides genera remained suppressed in the majority of patients during and after the course of therapy. On the other hand, response to tolemaver therapy appears to be accounted for by the inter-relationship between toxin neutralisation, *C. difficile* growth/persistence, and the pattern of recovery of the microflora. In general, patients who responded to toxin binding therapy exhibited non-emergence of toxin combined with increase in the numbers of anaerobic organisms. Recovery of the anaerobic microflora appeared not to be complete at 14 days in the majority of patients.
Conclusion: Patients with *C. difficile* diarrhoea have markedly depleted components of the normal intestinal microbiota. Non-antibiotic therapy of *C. difficile* associated diarrhoea should be further explored to optimise dosage and duration of toxin binding therapy.

Nosocomial infections: nonfermentative Gram-negative bacilli - I

P856 Empirical antimicrobial treatment established according to the temporal evolution of hospital outbreaks on patients with bloodstream *A. baumannii* infections

J.J. Camarena, J.C. Navarro, R. González, R. Zaragoza, A. Artero, A. Gea, J.M. Nogueira
Valencia, E

Objectives: The treatment of choice for *A. baumannii* bacteraemia has not been established. There are few data to guide the selection of agents for treating these infections. Carbapenems are generally considered the drugs of choice, but an increasing of the resistant strains has been described. Several alternatives guide lines have been proposed: ampicillin–sulbactam (SAM) alone or associated with an aminoglycoside, piperacillin–tazobactam (TZP) or tetracyclines. The aim of this study is to know the best alternative in the empirical treatment of these infections according to the temporal evolution of the nosocomial outbreaks or endemic infections in our hospital.
Methods: From June 1995 to December 2001 we collected all *A. baumannii* strains from bacteraemia infections and their related focus. All the isolates were characterised by molecular methods in order to obtain different clones using PFGE and REP-PCR. Susceptibility study was performed by disk diffusion to 23 antibiotics and MIC-E-Test in the mainly treatment alternatives (imipenem, meropenem, SAM, TZP, tobramicine (TM), amikacine (AN), and ceftazidime) and interpreted according to NCCLS criteria.
Results: In 1995–1996 the empirical antimicrobial treatment (EAT) of choice was imipenem because all 64 isolates were carbapenem sensible (S), with two mainly molecular clones (34 isolates C1-aminoglycosides resistant (R), and 30 C2-gentamicin-R, but AN, netilmicin and TM-S). According to detection of an outbreak carbapenem-R in 1997 (153 of the 163 isolates) clone C3 multiresistant (only some strains SAM or AN-TN-sensible) the EAT changed to SAM and AN or TM. This clone was persistent until 1999 and replaced with another multiresistant outbreak (C3B – 94% SAM-R and 25–75% aminoglycosides-R). Then the EAT was chosen as monotherapy with AN or TN (the only ones sensible of the 23 antimicrobial tested). In the last period (2000–2001) emerges a new clone (C5-carbapenems, SAM, aminoglycosides and doxycycline-S) and imipenem returns like the actual EAT in our hospital to control bloodstream *A. baumannii* infections.
Conclusions: Empirical antimicrobial treatment on patients with bloodstream *A. baumannii* infections in a hospital, with changes in the temporal evolution of the clones associated to outbreaks or endemic infections must be established according to the susceptibility test and molecular characterisation of the strains in different clones.

P857 Outbreak of ampicillin-resistant and high-level gentamicin-resistant enterococci in paediatric blood-stream infections in a tertiary hospital in Tanzania

B. Blomberg, S.C. Mohn, K.P. Manji, N. Langeland on behalf of the joint study group on antimicrobial resistance at Muhimbili National Hospital, Dar es Salaam, Tanzania and the University of Bergen, Norway

Objectives: Enterococci have emerged as major pathogens causing urinary tract, wound and blood stream infections (BSI). Nosocomial spread of enterococci resistant to multiple antimicrobials is a great therapeutic challenge. Little is known about the role of these pathogens in BSI in East Africa. The objective of the study was to assess the prevalence and resistance patterns of enterococcal isolates causing BSI in children at Muhimbili National Hospital, Dar es Salaam, Tanzania.
Methods: Blood cultures were obtained from 1789 children (age 0–7 years) with fever or signs of serious infection admitted to the hospital during the period August 2001 to August 2002. Isolates were identified by standard methods. The identities of *Enterococcus fecalis* and *E. faecium* isolates were confirmed by polymerase chain reaction (PCR), the isolates were susceptibility tested by E-test and assessed for genetic relatedness by pulsed field gel electrophoresis (PFGE). Twelve *E. faecium* isolates were also investigated by MLST.
Results: Thirty-two of 1789 children (1.8%) had growth of enterococcal isolates in blood culture. Nine of 17 *E. faecium* isolates showed combined resistance to ampicillin (ARE), ciprofloxacin and high-level gentamicin resistance (HLGRE). Six of 15 *E. fecalis* isolates were HLGRE, but none of these were resistant to ampicillin or ciprofloxacin. All except one of the HLGRE were also resistant to chloramphenicol. The resistant strains were recovered from several geographically separated wards, including the neonatal ward. The majority of the *E. faecium* and *E. fecalis* were closely related when investigated by PFGE. MLST conducted on 12 *E. faecium* strains also confirmed this result.
Conclusion: This is the first study to identify outbreaks of blood-stream infections caused by combined ARE/HLGRE *E. faecium* and HLGRE *E. fecalis* in Tanzania. *E. faecium* was more frequent than *E. fecalis*. The commonly used treatment regimens at the hospital (ampicillin and gentamicin or penicillin and chloramphenicol) are insufficient for infections caused by ARE/HLGRE enterococci.

P858 Molecular characterisation of an outbreak caused by cefoxitin-resistant *Klebsiella pneumoniae*

G. Kartali, E. Tzelepi, M. Tsivitanidou, D. Sofianou
Thessalonica, Athens, GR

Objectives: To describe the epidemiology, antimicrobial susceptibility and genomic profile of an outbreak due to cefoxitin (FOX) – resistant *Klebsiella pneumoniae*.
Methods: During 7 months, from November 2000 to June 2001, 23 nonrepetitive (one per patient) resistant to FOX *K. pneumoniae* strains were isolated from clinical specimens (10 from blood, two from bronchial secretions, four from urine, two from wound and five from catheter tips). Patients were cared in different wards including intensive care unit (ICU) and neonatal intensive care unit (NICU). Species identification was done by using the Vitek system (bioMerieux, France). MICs were determined with Vitek automated microdilution system and by disk diffusion method. The criteria of the NCCLS were used to define susceptibility or resistance to antimicrobial agents. Expanded spectrum α-lactamase (ESBL) production was assessed by the double disk synergy test. The isolates were typed by enterobacterial repetitive intergenic consensus (ERIC) PCR with the ERIC-2 primer. Isoelectric focusing (IEF) of β-lactamases was performed to representative group isolates.
Results: Antimicrobial profiles demonstrated that all isolates were resistant to third-generation cephalosporins, to aztreonam, cefoxitin, amoxicillin/clavulanate, ticarcillin/clavulanate and piperacillin/tazobactam. Four isolates were also resistant to cefepime and cefpirome. All isolates were susceptible to imipenem. IEF showed that all isolates expressed two β-lactamases, one with pI of 8.2 which correlated with the SHV-5 and one with pI of 9.1 which corresponded to LAT-2. ERIC-PCR analysis demonstrated three strain types. Type I, consisting of two subtypes, was common to 15 strains, indicating that the clonal spread was mainly responsible for the outbreak. Type II comprised two isolates and type III was unique. Five isolates were not identified with ERIC-PCR.
Conclusions: *K. pneumoniae* strains, harbouring plasmid-coding for AmpC-type β-lactamase, have been established in our hospital. Nosocomial infection surveillance, such as restriction of particular antibiotics and adjustment of the infection control measures, has been recommended.

P859 Intensive care unit outbreak caused by a strain of *Acinetobacter baumannii* producing the PER-1 extended-spectrum β-lactamase

S.H. Jeong
Busan, KOR

Objectives: Recently PER-1 extended-spectrum β-lactamase (ESBL) was discovered in a *Peudomonas aeruginosa* strain in France and was subsequently detected in *Acinetobacter* spp. and *Pseudomonas aeruginosa* in other countries including Turkey. The purpose of this study was to clarify the molecular epidemiology of infection caused by a strain of cefepime-resistant *A. baumannii* and also to determine the mechanism of drug resistance.
Methods: Cefepime-resistant *A. baumannii* strains were isolated from clinical specimens of nine patients hospitalised in an intensive care unit in Busan, Korea. Antimicrobial susceptibilities were determined by the disk diffusion and agar dilution methods. The double disk synergy (DDS) test was performed for screening of ESBL production. Isoelectric focusing and conjugation experiments were performed. blaPER-1 and blaPER-2 alleles were detected by PCR, and sequences of amplified products were determined by using the dideoxy-chain termination method. Pulsed-field gel electrophoresis (PFGE) was performed for molecular typing of isolates.
Results: The isolates showed same antimicrobial susceptibility pattern, positive DDS results and PFGE patterns. The isolates contained three β-lactamase bands: pI 5.3, 7.9, and 9.4. PCR-based experiments detected blaPER-1 genes. MICs of ampicillin, piperacillin, cephalothin, cefoxitin, cefoperazone, ceftazidime, cefotaxime, cefepime, and aztreonam to these isolates were $\geq$256 mg/L, respectively, and those of imipenem were 8–16 mg/L. Despite repeated attempts, the resistance to cefepime of *A. baumannii* isolates was not transferred to the recipient.
Conclusions: *A. baumannii* isolates from clinical specimens of nine patients hospitalised in a same intensive care unit were shown to be of the same clone. All these isolates contained blaPER-1 gene which caused resistance to cefepime. To the best of our knowledge, outbreaks caused by PER-1 ESBL-producing *A. baumannii* have not previously been described.

P860 Nosocomial outbreak of *Salmonella enteritidis* in a neonatal unit

G. Gonzalez Mediero, H. Bello, M. Alvarez, I. Otero, I. Iglesias, T. Del Blanco, M. Usera
Vigo, Madrid, E

Objectives: Hospital outbreaks of *Salmonella* spp. infections are not uncommon not only in Europe but also in the United States, but in neonatal Units it is rarer. The maternity unit has approximately 4000 deliveries each year. We described the results as well as infection control that stopped the outbreak.
Methods: From October 2001 to January 2002 six neonates were infected in the neonatal Unit of our Hospital. The index case corresponding to a newborn delivered in our Hospital, she was born by normal vaginal delivery. The 15-day-old patient was admitted again by neurological deficits. Seven days at the Hospital she developed diarrhoea. The group included five prematures, only

case index was not premature. All stool specimens from family case index were negative. Stool samples were request for culture from asymtomatic staff and all babies from the neonatal Unit ($n = 66$). The isolates were identified by standard methods and serotyped by agglutination with monospecific antisera. The antibiotics (AB) taken in the study were: ampicillin (A), ticarcillin (T), amoxicillin/clavulanate (A/C), cefalothin (CE), ciprofloxacin (CP), co-trimoxazole (CO), nalidixic acid (NA), gentamicin (G), third-generation cephalosporins (3GC). Its was evaluated by a microdilution method and confirmation by E-test.
Results: Eight strains of *Salmonella enteritidis* serotype O 9,12:H g,m were identified. The phage type (PT) involved was same in all cases, PT 6a. Seven were isolated from faeces and one from blood culture. All isolates demonstrated same antibiotic susceptibility pattern with resistance to ampicillin and ticarcillin. Fortunately no babies died. No salmonella from stools of nurses, staff personnel and mothers was isolated.
Conclusions: A hand washing was not sufficiently frequent the infection was probably transmitted by hand contact to prepared milk, infusion and other equipment from the index case. This hypothesis was subsequently confirmed as the outbreak was terminated after eradication of the presumed contamination sources by changing the mattresses, disinfecting the unit and ensuring strict observance of hand washing before and after every manipulation. *Salmonella enteritidis* PT6 is third commonest phage type in Spain.

P861 Pseudo-outbreak of *Pseudomonas aeruginosa* infections associated with bronchoscopic procedures

F.J. Vasallo, S. Pérez, M.A. Domínguez, A. González-Escalada, V. del Campo, J.L. Guerra, J. Torres
Vigo, Barcelona, E

Objectives: To investigate the cause of an outbreak of *Pseudomonas aeruginosa* isolations following bronchoscopic procedures.
Methods: From 16 to 31 January 2003, we detected a cluster of *P. aeruginosa* isolates associated with bronchoscopy (nine samples from eight patients). Laboratory culture, bronchoscope and medical records of all cases were reviewed. All of them were related with one bronchoscope and environmental samples were obtained from it. Microbiological identification and susceptibility testing were performed with the MicroScan Walkaway 40 System (Dade Behring). Random Amplified Polymorphic DNA analysis (RAPD) and Pulsed Field Gel Electrophoresis (PFGE) were performed on all available isolates of *P. aeruginosa* (eight clinical isolates from seven patients and six bronchoscope related isolates).
Results: Two of the eight patients showed clinical evidence of infection and required specific antimicrobial therapy (the index case and other patient with two isolates separated by 8 days). All isolates were ceftazidime, aminoglycosides and ciprofloxacin susceptible and imipenem resistant. RAPD and PFGE patterns revealed that all the clinical and bronchoscope isolates (eight and six, respectively) were indistinguishable. The bronchoscope was replaced and no further cases appeared.
Conclusions: We documented contamination of a bronchoscope with *P. aeruginosa* and possible secondary infection of at least one patient. Microbiologists have an essential role in the detection of medical devices contamination, especially by surveillance of the emergence of infrequent bacterial recovery.

P862 Outbreak of *Klebsiella pneumoniae* causing fulminant sepsis in a neonatal unit

R. Alonso, B. Padilla, C. Sánchez-Carrillo, E. Cercenado, M. Sánchez-Luna, E. Bouza
Madrid, E

Introduction: Three cases of fulminant sepsis were detected in premature newborns, with no underlying diseases, admitted to the neonatology unit of our hospital during a 14-day period. Blood samples were taken and submitted to the microbiology laboratory. In all three cases *Klebsiella pneumoniae* was isolated from the blood cultures. The aim of this study was to investigate the epidemiological relation among the isolates and to try to find a common source for the infection.
Methods: Environmental samples, including different i.v. fluids and drugs, and skin samples were obtained in order to detect the source of the infection. Microbial identification and *in vitro* susceptibility tests were carried out automatically with the MicroScan System (DADE). Clinical isolates were molecular typed by Random Amplification of Polymorphic DNA (RAPD) using one 10-mer oligonucleotide, PCR-Ribotyping with oligonucleotide of the intergenic 16S/23S region and PFGE using XbaI as a restriction enzyme. An unrelated strain was also included in all the experiments, as a control, in order to check the discrimination power of the techniques.
Results: All three clinical isolates of *K. pneumoniae* obtained from blood cultures shared the same biotype and antibiotype, and were all resistant to ampicillin, gentamycin and tobramycin. Molecular typing methods proved clonal identities among the clinical strains. Patterns generated were different from those of the control strain. The source of the infection could not be demonstrated in any of the environmental or newborn skin samples.
Conclusions: A single *K. pneumoniae* strain was the cause of the fulminant sepsis in the three newborns. All three molecular typing methods, RAPD, PCR-Ribotyping and PFGE accurately demonstrated clonal identities of the isolates. The common source of the infection could not be detected due, probably, to the logical delay in culture growth and identification.

P863 Epidemic ceratoconjunctivitis outbreak at a university eye clinic

A. Willke, L. Kocabas, A. Kubar, S. Erdogan, N. Yuksel, S. Gündes, M. Celik, S. Ustacelebi, Y. Caglar
Kocaeli, Ankara, TR

Objectives: An outbreak of epidemic keratokonjunktivitis (EKC) occurred at the eye clinic of Kocaeli University (KOU) Hospital between early December 2001 and late January 2002. The objectives of this presentation are to describe the outbreak and the infection control measures implemented, and to constitute an example for handling possible future outbreaks with limited resources. This is the first EKC outbreak reported from Turkey.
Methods: Eye clinic of KOU Hospital is equipped with modern devices; however it has limited physical conditions (e.g. insufficient hand-washing facilities) because of temporary settlement of the hospital after the earthquake of 1999.On 12 December 2001, the Infection Control Team (ICT) was alerted to EKC cases. An investigation began and infection control measures (ICM) were implemented. Conjunctival swabs of patients with EKC and environmental swabs were obtained and studied in GATA and Hacettepe University microbiology laboratories. Infection control protocol (ICP) was implemented as recommended by APIC guidelines with some modifications. In addition, terminal disinfection (TD) was applied two times and after TDs clinic was closed for first 2 and than 4 days (Figure 1).
Results: A total of 116 EKC cases were diagnosed among the 1033 patients who visited the eye clinic during the outbreak (General attack rate: 11.2%). Seventy-five of the EKC cases were male and average age was 41.39 ± 21.98 (range: 6 months to 85 years). Primary and secondary attack rates were found to be 26 and 119%, respectively. Adenovirus type D was isolated from patient samples, biomicroscope and device solution. With the implementation of ICP and TD, EKC cases decreased by time and outbreak disappeared about 14 days after the second TD and closing the clinic for four days (Figure 1).
Conclusion: This is the first outbreak reported from Turkey. Isolation of virus from biomicroscope and device solutions which are used for more than one patient is an evidence of transmission from environment. Although several reports have described ICM that terminated outbreaks of nosocomial EKC, this study demonstrates that implementing TD and/or closing the clinic for four days in addition to ICM, may control nosocomial EKC outbreaks.

Catheter-related infections

P864 Health and economic outcomes of nosocomial catheter-related candidaemia in critically ill patients

S. Blot, K. Vandewoude, J. De Waele, E. Hoste, J. Deschuijmer, J.Decruyenaere, F. Colardyn
Ghent, B

Background: Because of severity of underlying disease, multiple venous accesses, parenteral nutrition and often increased length of stay, intensive care unit (ICU) patients are at increased risk for catheter-related candidemia (CRC). We investigated health and economic outcomes in ICU patients with CRC.
Methods: In a retrospective matched cohort study (1992–2002) attributable mortality and excess length of stay for CRC was investigated. Matching was (1:2 ratio) based on severity of underlying disease and acute illness (APACHE II score and admission diagnosis) and length of ICU stay prior to the onset of the candidemia. As expected mortality can be derived from APACHE II; this matching procedure results in an equal prognosis for cases and control subjects. Attributable mortality is determined by subtracting the hospital mortality rate of the controls from this of the candidemic cases. Excesses in length of ICU stay and hospitalisation were determined by subtracting the median length of stay of the controls from this of the cases.
Results: During the study period 21 ICU patients developed a microbiologically documented CRC (out of a total of 83 candidemic patients). Nineteen catheters were removed within 24 h. Cases ($n = 21$) and controls ($n = 42$) had an equal age (resp. 49 ± 20 vs. 53 ± 19 year; $P = 0.470$), APACHE II score (resp. 23 ± 8 vs. 23 ± 8; $P = 0.754$) and incidence of respiratory failure (95 vs. 86%; $P = 0.479$), acute renal failure (33 vs. 14%; $P = 0.153$) and haemodynamic instability (76 vs. 69%; $P = 0.767$). The excess length of ICU stay was 11 days (median 31 vs. 20 days; $P = 0.002$). Although patients with CRC had a longer length of hospital stay this difference was not significant (52 vs. 30 days; $P = 0.508$). The attributable mortality of CRC was 19.1% (95% CI: −6 to 44%) as hospital mortality rates in cases and controls were 42.9 and 23.8%, respectively ($P = 0.207$).
Conclusion: Our data revealed that, after careful adjustment for severity of underlying disease and acute illness, CRC is not associated with a significantly higher mortality in ICU patients. It is, however, associated with a significant excess in length of ICU stay, thereby representing an important economic burden.

P865 Investigation of central intravenous catheter-related bacteraemia

A. Tarpatzi, I. Stefanou, A. Katsandri, J. Papaparaskevas, G. Petrikkos, G.L. Daikos, A. Avlamis
Athens, GR

Aim: To determine the cause of Central Catheter Bloodstream Infection (CCBRI) with respect to the colonisation of the different parts and connections of the intravenous catheter device.
Material and methods: A total of 187 central i.v. catheters from patients of 'Laikon' General Hospital in Athens were studied. Patients were hospitalised in two Internal Medicine departments, two Surgical departments, the Nephrology department and the Intensive Care Unit, during the period July 2001 to November 2003. The catheter tips were cultured using the following methods: (a) Semi-quantitative Maki's and co. and (b) quantitative Cleri's and co. Samples for culture were taken also from the site of catheter insertion into the skin and from the hub. Blood culture samples were taken from a peripheral vein in cases of clinical suspicion of bacteraemia or sepsis and they were incubated using the BactAlert (Organon Teknika) automated system for 6 days.
Results: 33 cases of CCBRI were recorded. The incidence of CCBRI was 9.3 per 1000 catheter days. In 29 of the 33 cases of CCBRI the origin of colonisation was determined. In 10 cases which all had positive catheter tip and hub cultures with the same strain, the Gram-negative bacteria prevailed (6/10, analytically *E. aerogenes* three, *K. pneumoniae* two and *P. aeruginosa* one) while in four cases *Candida* spp. (three cases) and coagulase negative staphylococcus (CoNS) (one case) were isolated. In contrast, in 19 cases of CCBRI with positive catheter tip and skin point entry cultures with the same strain, the Gram-positive bacteria prevailed (15/19, analytically *S. aureus* eight, CoNS six and *Corynobacterium* spp. one).
Conclusions: (1) The incidence of CCBRI was 9.3 per 1000 catheter days. (2) CCBRI caused from Gram-positive bacteria was mainly derived from the catheter site entry, whilst colonisation of hub caused mainly Gram-negative CCBRI. (3) The preventive measures should be focused on better aseptic techniques and hand hygiene, care of the catheter's entry site and better training of the medical staff.

P866 Differences in development of catheter-related infections in critically ill adults

M. Sredkova, V. Edreva, V. Todorov
Pleven, BG

Objectives: To study and compare some characteristics of infections associated with central venous catheters in patients in units with high risk – Intensive Care Unit (ICU) and Haemodialysis unit (HDU).
Methods: Semiquantitative method of Maki *et al*. for study of external surface of 5 cm catheter tip, quantitative method of Linares et al. for study of catheter lumen, semiquantitative analysis of the skin in insertion site and blood cultures obtained from peripheral vein at the moment of removing of the catheter.
Results: We studied 104 patients, suspect for catheter-related infections (CRI) – 52 from ICU and 52 from HDU with central venous catheters used for parenteral nutrition, drug administration or haemodialysis. The preferred vein in ICU was v.subclavia, 48, and in HDU, v. femoralis, 50 catheters. Mean duration of catheterisation – 10 days in ICU and 22.8 days in HDU. Signs for colonisation of the catheters were found in 57 cases – 32 in ICU and 25 in HDU. The most common microorganisms in ICU were Gram-negative rods (KESgroup, *B. cepacia*, *Pseudomonas* spp.) – 21 (65.6%) followed by Coagulase-negative staphylococci (CNS) –10 (31.2%). In HDU in most of cases were isolated CNS – 19 (73.0%) and *S. aureus* – 4 (15.3%) ($P < 0.01$). As catheter-related bacteraemias (CRB) were considered in 16 cases, 11 of them in ICU and 5 in HDU. Causative microorganisms of CRB in ICU were most Gram-negative rods – 9(81.8%) and *Syaphylococcus* spp. 3 (60.0%) in HDU ($P < 0.01$).
Conclusions: The frequency of CRB in ICU is significantly higher, 21.1–9.6%, in HDU ($P < 0.01$).They developed earlier and were caused by Gram-negative rods. More probable way to development of CRB in ICU is the catheter hub and HDU is the skin of the patients.

P867 Catheter-related sepsis in critically ill and associated mortality

I. Leonard, A. Vandenberghe, P. Laterre
Brussels, B

Catheter-related infection (CRI) is considered as a cause of increased Hospital morbidity but its influence on Hospital mortality remains a matter of debate. In critically ill patients, baseline severity, underlying conditions and various confounding factors may explain the observed increased mortality rather than CRI itself. In order to determine the influence of CRI on Hospital

mortality in ICU, all episodes of nosocomial septicaemia were reviewed.
Material and methods: Retrospective analysis of all nosocomial septicaemia occurring over a 7-year period in a teaching Hospital. Septicaemia episodes were separated in secondary, primary and proven catheter-related bloodstream infections. Baseline severity (SAPS score), delay between admission and infection, and Hospital mortality were determined.
Results: Over this 7-year period, 195 853 patients were admitted to the Hospital and 2720 episodes of CRI were recorded (1.38%, 1.5/1000 Catheter-day (KTD)). Hospital mortality for all septicaemia was 21.7% while mortality related to secondary septicaemia was 28.5% ($P < 0.05$). During the same period, 22 313 patients were admitted to the ICU, corresponding to 81 740 KTD. Four hundred twenty-four episodes of septicaemia occurred in these patients (5/1000 KTD), of which 166 were primary septicaemia and 87 were proven CRI (1.06/1000 KTD). Mean SAPS score for all ICU patients was 30 and Hospital mortality 6.9%. ICU patients developing infection had a mean baseline SAPS score >40. CRI occurred more than 2 weeks after ICU admission (median : 14 days, mean 20.5 days). Pathogen-associated CRI were SCN 31%, *S. Aureus* 18%, *E. faecalis* 12%, *Candida* spp. 10%, Other 29%. Hospital mortality in patients developing CRI was 42/87 (48.2%).
Conclusions: In this study, Hospital mortality in Critically Ill patients developing CRI was high but seemed to be primarily determined by baseline severity and underlying conditions as reflected by SAPS score and prolonged delay between ICU admission and septicaemia.

P868 Relationship of catheter-related infections in patients with skin and nasal colonisation by endemic hospital-acquired oxacillin-resistant *Staphylococcus epidermidis* strains

D. Blum-Menezes, O.J. Bratfich, M.C. Padoveze, M.L. Moretti
Sao Paulo, BR

Background: Central vascular catheters (CVC) represent an important risk for nosocomial bloodstream infections and *Staphylococcus epidermidis* is the most important pathogen of these systemic infections.
Objectives: To study the genomic DNA profiles of *S. epidermidis* isolated from catheter-related infections and bloodstream infections comparing with the strains isolated from skin and nasal swab in patients hospitalised in a tertiary care university hospital.
Methods: Catheter-related infections were defined according to the CDC definitions. Patients with a culture for *S. epidermidis* from blood and catheter tip (>15 CFU) were selected to have swabs from skin and nasal for *S. epidermidis*. The *S. epidermidis* were typed using PFGE, antibiotic susceptibility testing and biofilm detection, by Congo red method, were performed.
Results: Twelve patients with 17 episodes of catheter-related infections were included in this study and 255 strains were analysed. In 10 episodes, the same DNA profile was detected in CVC/blood and in the skin/nasal and in seven episodes the clone causing CVC/blood infections were not found in skin/nasal. The mean time of isolation of *S. epidermidis* with clonal relation between CVC/blood and skin/ nasal colonisation from the first day of hospitalisation until the detection in CVC/blood was 25.3 days. In episodes without *S. epidermidis* clonal relation, the mean time was 13.7 days. PFGE identified three hospital endemic profiles that were present in 46.6% (119/225) of all strains from 10 episodes, including the strains from CVC/blood infections and in skin/nasal colonisation. In the strains from skin/nasal colonisation, the endemic profiles were present in 47.9% (93/194) of the strains. The endemic DNA profiles were biofilm producers and were resistant to penicillin G, oxacillin and ciprofloxacin, variable susceptibility to aminoglycosides and were susceptible to vancomycin.
Conclusion: Patients with long term hospitalisation were previously colonised by hospital endemic *S. epidermidis* strains that were responsible for catheter-related infections.

P869 Incidence and risk factors of central venous catheter-related bacteraemia in gastrointestinal surgical units

J.-M. Januel, P. Vanhems, C. Chambrier, C. Chemorin, E. Tissot, X. Barth, J. Fabry, P. Bouletreau, R. Ecochard
Lyon, F

Objectives: To calculate the incidence and the risk factors of Central Venous Catheter-related infections (CVC-RI) in digestive surgery units.
Methods: All patients admitted in two emergency gastrointestinal surgery units (A and B) of Edouard Herriot University hospital (Lyon, France) between 1 July 2002 and 31 December 2002, and requiring a CVC were included in this retrospective cohort. The following data were analysed: patient and CVC characteristics, risk factors and microbiological results. The diagnosis of CVC-RI was based on Brun-Buisson methodology (1987). The comparisons were done using the Chi-square and Student's *t*-tests. A multiple logistic-regression model was used to identify risk factors of CVC-RI according to their adjusted odds ratio (aOR; 95% CI) and completed by a survival analysis adjusted on duration of hospitalisation and CVC, the unit and the timing of CVC implementation (before or after admission in the unit).
Results: A total of 89 patients were included who required 102 CVC (60 CVC were implanted as per the hospitalisation in this study units [Gr#1] and 42 before the patient admission in this same units [Gr#2]). The total of catheter days was 1086 (respectively 581 for Gr#1 and 505 for Gr#2). The number of CVC-RI was 21 that is 20.6% of CVC and the incidence rate per patient was 21%. The part number of CVC-RI were respectively 9 (15%) for Gr#1and 12 (28.6%) for Gr#2. The incidence density of CVC-RI was 1.93/100 catheter days for the totally cohort, 1.55/100 for Gr#1 and 2.38/100 for Gr#2. In the totally cohort CVC-RI was monomicrobial in 16 cases (76.2%). In that case the most prevalent bacteria were: Coagulase-negative *Staphylococcus* spp. (43.8%) and *Staphylococcus aureus* (37.5%) and in case of plurimicrobial infection the most prevalent agents were: *Staphylococcus aureus* (60%), Coagulase-negative *Staphylococcus* spp. and *Enterococcus faecalis* (40% for each). Intestinum somatoplasty was not was a risk factor for CVC-RI in this study. The crude mortality rate was 5.3% (1/19) and 10% (7/70) for CVC-RI and non-CVC-RI, respectively ($P = 0.5$).
Conclusion: In these surgical units, the incidence of CVC-RI is high and was related to the frequency of manipulations of the line such as infusion, parenteral nutrition, injections and dressing even after adjustment on the duration of CVC and timing of CVC implantation. An intervention focused on these risk factors is planned to reduced CVC-RI and improve the quality of care.

Table 1. Risk factors associated with CVC-RI in a multiple logistic-regression ($n = 102$ CVC)

Included data	aOR*	CI 95%**	*P****
Male gender	21.1	[3.6–123]	0.001
Unit B	13.1	[2.4–72.2]	0.003
Daily use of CVC for intravenous antibiotic[1]	0.6	[0.3–0.9]	0.024
Nb. of daily access to CVC for dressing removal	1.4[2]	[1.0–1.9]	0.048
Nb. of daily use of CVC for infusion[1]	2.0	[1–4.02]	0.053
Nb. of daily use of CVC for parenteral nutrition[1]	4.0	[0.9–16.2]	0.06

*Adjusted odds ratios. ** 95% Confidence interval. ***P-value for stastic significant.
[1]Daily mean of total number on the duration of CVC in the units.
[2]Per one dressing-removal increase.

P870 Usefulness of a disinfectable needleless connector in the prevention of arterial catheter colonisation

J.C. Yébenes, G. Sauca, G. Miró, J. Almirall, R. Martínez, M. Serra-Prat, X. Buquet, X. Balanzó
Mataró, E

Objective: Novel technologies have been reported to aid in the prevention of endovascular catheter colonisation in experimental studies. The aim of this study is to evaluate the usefulness of a disinfectable needleless connector in the prevention of radial arterial catheter related infection in critically ill patients.
Methods: In a medical–surgical intensive care unit (ICU), radial artery catheters (RAC) inserted in critically ill patients were prospectively randomised and assigned to control group (catheter covered with conventional cap) or study group (catheter covered with a disinfectable needleless connector, Smartsite-plus®). Patients and RAC inserted during more than 48 h characteristics were registered. At their removal, a hub brush culture, subcutaneous catheter segment culture and a catheter tip culture (Cleri's method) were performed. Two peripheral blood cultures were also performed if bacteraemia was suspected.
Results: One hundred consecutive RACs were inserted in 77 critically ill patients (85% medical/15% surgical, mean age 69.1 ± 15.9, SAPS II 44.2 ± 18.7, length of ICU stay 7.8 ± 14.5). Ninety-nine were correctly randomised (53 in control and 46 in study group). Twenty-four RACs were removed in less than 48 h (10 in control and 14 in study group) and 11 were lost (six in control and five in study group) and not included in the analysis. Mean length of insertion of studied catheters was 6.2 ± 2.9. No catheter related bacteraemia was observed. In the control group hub colonisation was higher than in the study group, 22.2% (8/36) vs. 3.5% (1/28) (Fisher exact test *P* value 0.034). No differences were observed in catheter, 0% (0/36) vs. 7% (2/28), or skin colonisation, 2.8% (1/36) vs. 3.5% (1/28), in control or study group respectively. Coagulase-negative staphylococci were the predominant microorganisms involved. No differences were observed in ICU stay or mortality.
Conclusion: Despite the needleless disinfectable connector is useful to prevent hub colonisation, a higher number of catheters must be analysed to evaluate its impact in the prevention of arterial catheter related bacteraemia. Coagulase-negative staphylococci were the predominant microorganisms involved.

P871 The 'antibiotic-lock technique' for eradication of 'difficult' or multiple micro-organisms in infections of long-term central venous catheters

A. Scalzini, G. Gattuso, D. Tomasoni, L. Palvarini
Mantua, I

Background: Catheter-related bacteraemia is a major complication in patients with long-term surgically implanted central venous catheters (CVC) and their removal for infection is difficult and expensive; now many catheter infections can be handled with so-called 'antibiotic lock-in technique' combined or not with a systemic antibiotic treatment. Results of several open studies indicate that this method may be regarded as an alternative to the removal of the device infected with coagulase-negative staphylococci; for 'difficult' microorganism reports are rare. The Aa refer case-reports of long-term CVC infections with multi-drug-R or multiple microogranisms treated combining a local antibiotic lock with a systemic antibiotic treatment.
Case 1: a schizophrenic 41-year-old man was admitted to the hospital because of fever of 2 weeks duration; he was affected by diabetes type II and NH lymphoma diagnosed 6 months earlier and treated with chemotherapy through a Groshong CVC and, subsequently, with chronic steroid. Multiple blood cultures, performed from CVC and peripheric veins, were positive for *E. faecalis* and *E. coli*; the patient was treated with ceftriaxone 2 g ev qid ×2w + lock-in therapy with teicoplanin 60 mg (in 3 mL) and ciprofloxacin 6 mg (in 3 mL) for 6 h a day for 10 days. It was obtained a clinical and microbiological resolution without removal of CVC.
Case 2: a 49-year-old man was admitted to the hospital for septic fever; 3 months earlier a Groshong CVC had been placed to treat with chemotherapy a rhinopharyngeal carcinoma. Multiple blood cultures (from CVC and peripheric veins) were positive for a multi-drug-resistant *Stenotrophomonas maltophilia* (S only to chloramphenicol, trimethoprim-sulfamethoxazole and levofloxacin). The patient was successfully treated, without removal CVC, with systemic trimethoprim–sulfamethoxazole + levofloxacin combined to antibiotic lock (ciprofloxacin 8 mg in 4 mL for 12 h a day for 7 days).
Conclusion: The cases reported by the Aa confirm that many catheter infections can be maintained in place and sterilised with lock-in therapy avoiding to replace expensive intravascular lines with unnecessary and risky insertions. One of the questions to resolve will be whether or not concomitant systemic antibiotic therapy is necessary.

P872 Determination of disinfectants' effectiveness and their activity to adherent catheter bacterial strains isolated in the hospital environment

K.W. Pancer, E. Mikulak, A.E. Laudy, B. Sawicka, D. Rabczenko, T. Wernik, A. Gliniewicz, H. Stypulkowska-Misiurewicz
Warsaw, PL

Background: Nosocomial infections influence upon the mortality, quality of patients' life, costs and length of hospitalisation. The source of those infections might be staff members, contaminated water system, air-conditioning or pests. Disinfectants are helpful in reducing or eradicating harmful pathogens existing in hospital environment. Some bacteria are able to grow on a surface as a biofilm. This form is more resistant to external harmful conditions such as antibiotics, disinfectants or host defence. Bacterial adhesion was recognised as the important virulence factor for colonisation of patient or biofilm formation. In our study the susceptibility of 65 bacterial strains isolated in hospital environment (colonising or infecting patients or carried by German cockroaches) to antibiotics and chemical disinfectants was determined. Moreover the efficacy of the disinfectant working solution (active ingredients: sodium dichloroisocyanorate 1795.2 mg/L; glucoprotamine 5200 mg/L; potassium persulphate 4300 mg/L) on selected bacterial strains adherent to catheter (after growing for 5 days on it) by treating then for 15 min was determined.
Results: Susceptibility profile to antibiotics varied; among Gram-positive bacteria the MLSb, MRCNS strains were found; among Gram-negative bacteria the ESBL, AmpC phenotype were described. Determined MIC values or disinfectants were in range: sodium dichloroisocyanorate 7.8125–2000 mg/L; glucoprotamine 1.453–500 mg/L; potassium persulphate 7.8125–1000 mg/L. The results indicate that the working solution of the disinfectant might be ineffective to some strains of well-known pathogens: *Serratia marcescens*, *Citrobacter freundii*, *Enterobacter cloacae* and *Staphylococcus epidermidis*. The examination of disinfectants efficacy on selected strains showed that some bacterial strains were more resistant when they were grown on catheter for 5 days. The MIC value was lower than working solution of that chemical even more than 300 times. Moreover it was found that all tested disinfectants were ineffective to some strains adherent to catheter ex. *S. marcescens* and *E. cloacae* strains isolated from the body surface of German cockroaches.
Conclusions: The possibility of biofilm formation could explain the increase of resistance to disinfectants of some strains. German cockroaches carrying them in hospital should be considered not only as nuisance insects, but also as a real source of resistant to antibiotics and disinfectants bacteria.

P873 *Propionibacterium acnes* is a common coloniser of intravascular catheter tips

P. Martín-Rabadán, L. Alcalá, M. Rodriguez, E. Bouza
Madrid, E

Background: Indwelling catheters are commonly colonised by skin flora. *Propionibacterium* spp. are among the commonest bacteria of normal human skin but currently recommended catheter-culture procedures would not detect its presence. Furthermore, *Propionibacterium* is nearly always regarded as a blood culture contaminant and automated blood culture methods may not detect a proportion of them. Our objective was to determine the rate of catheter colonisation by *Propionibacterium* spp. in unselected intravascular catheters submitted for culture.
Methods: 368 intravascular catheters were processed by the roll-plate technique and incubated in air at 37°C for at least 2 days. Organisms that were present in significant counts were subcultured for identification and susceptibility testing. When the conventional aerobic processing was finished, all primary culture plates were reincubated in an anaerobic jar. After 7 days of anaerobic incubation the plates were read looking for bacterial colonies that were not initially present. Control plates were inoculated with a suspension of *P. acnes* to assess the influence of aerobic preincubation on the final number of colony forming units (CFU). Conventional processing detected significant growth of bacteria in 24.8% of all catheters and no significant number of colonies (<15) in an additional 17.6% samples. Anaerobic reincubation yielded *P. acnes* in significant counts in 3.5% of all catheters (15% of all positive catheters) and no significant number of colonies in an additional 14.1% of samples. Three samples yielded significant growth of both aerobic and anaerobic bacteria. Of all the organisms recovered in significant counts, Coagulase-Negative staphylococci represented 55.5%, *P. acnes* 11.1%, *S. aureus* 7.7% and *Corynebacterium* spp. 6%, *Enterococcus* spp. 5.1% and other bacteria and yeast 14.5%. Anaerobic bacteria other than *P. acnes* were rarely recovered in non-significant counts. Aerobic preincubation for 5 days did not substantially affect the final number of CFU.
Conclusion: *P. acnes* is the second most frequent coloniser of intravascular catheters. Anaerobic reincubation of plates used in standard routine is a simple method that could be useful for catheter-related research projects. The potential of *P. acnes* as a cause of catheter-related bacteraemia merits further studies.

P874 Vascular access site infections in haemodialysis patients in Italy: a surveillance study

L. Scudeller, G. Brunori, N. Petrosillo, G. Cancarini, G. Carosi, P. Viale
Udine, Brescia, Rome, I

Objective: To estimate the incidence of vascular access-related infections (VARI) in chronic haemodialysis patients in Italy, overall and by access type.
Methods: All vascular accesses (fistula, graft, temporary or permanent vascular access) in haemodialysis patients in 10 centres (10–50 beds) in Italy were surveyed from June 2002 to July 2003. Data were collected on a standardised form. VARI were detected using two key events: antibiotics prescription or hospital admission. Rates were calculated per 1000 access-days (AD). Variables associated to VARI were assessed by Cox regression.
Results: 810 patients were recruited, with mean age 64.8 years (SD 15.2), median dialysis duration 31 months (IQR 8–76). Males were 56%, the median Karnovsky index 80 (IQR 60–90). Prevalence of HCV infection was 16%, HBsAg carriage 5%, HIV infection 2%, diabetes 20%. Four per cent were taking steroid therapy, 3% were on immunosuppressants, 3% had autoimmune diseases, 4% a malignancy and 2% other causes of immunodepression. Most patients (90%) had three dialysis sessions per week. Most (89%) had only one access during the study, 7% two, the rest up to 7. Data on 949 accesses were collected: 53 were grafts, 629 fistulas, 140 permanent CVC, 127 temporary CVC. Of permanent CVC, 74.4% were tunnelled. Eighty-four VARI were observed, for an overall rate of 0.41/1000 AD (95% CI 0.33–0.51). Local infections were 41%, systemic 53%, both 6%. Isolates were 94; the most common were *S. aureus* ($n = 40$; 72% methicillin resistant), Coagulase Negative staphylococci ($n = 32$), *E. coli* ($n = 9$). Rates per access type were: fistulas 0.07/1000 AD, grafts 0.63, permanent CVC 2.39, temporary CVC 1.21, for a HR (vs. fistulas) of 8.8, 30.2, 16.4, respectively. Rates were lower when the access was in the subclavian site (1.45/1000 AD), than in the jugular (1.78) or in the femoral site (2.8).
Conclusions: In Italy, rates of VARI appear comparable to those reported in the medical literature. Rates were lowest for fistulas, and highest for permanent CVC. Surprisingly, rates for temporary CVC were somewhat lower than for permanent CVC, possibly indicating poor CVC management in the long-term. The femoral site of insertion had the highest rate of infection. The most common isolate was, by far, *S. aureus*, confirming the growing relevance of this pathogen in all access site infections. Ongoing surveillance of VARI in Italy appears feasible and reproducible.

Nosocomial infections: I

P875 Catheter-associated urinary tract infections in hospitalised elderly women

M. Kompoti, A. Mousouli, K. Kontos, A. Georgopali, O. Zarkotou, C. Nikolopoulou, S. Drimis
Athens, GR

Objectives: To detect the incidence of bacteriuria and/or funguria following indwelling urethral catheterisation among hospitalised elderly women.
Methods: We studied prospectively all elderly female patients >70 years old who were admitted to our medical department during a two-month period, provided that they were newly catheterised upon admission and that the first urine culture obtained within the first 24 h of hospitalisation was sterile. A urine specimen was obtained everyday for culture and microscopic examination.
Results: Thirty-four consecutive patients were enrolled in the study. Mean age was 77.0 ± 6.2 years. Main comorbidity included arterial hypertension (54%), diabetes mellitus (37.5%), coronary heart disease (20%), Parkinson's disease (11.8%), chronic obstructive pulmonary disease (8.8%), cerebral vascular disease (26.4%). Twenty-one patients (61.7%) had 22 episodes of bacteriuria. Median duration of hospitalisation before detection of catheter colonisation was 4.0 days (range 3–22 days). Mean duration of hospitalisation was 12.8 ± 8.9 days in patients with bacteriuria and 10.0 ± 6.5 days in patients without bacteriuria (P = NS). Isolated microorganisms were *Escherichia coli* (15/22), *Candida albicans* (3/22), *Enterococcus faecalis* (1/22), *Citrobacter freundii* (1/22), *Providencia rettgeri* (1/22) and *Klebsiella* sp. (1/22). Antimicrobial resistance rates of the isolated strains were low. In 13 patients (61.9%), the microorganism isolation accounted for cystitis, while in the rest it accounted for asymptomatic bacteriuria. Cystitis was treated with the appropriate antimicrobials according to susceptibility tests. In patients with candiduria, the urinary catheter was removed or replaced.
Conclusions: Urinary bladder catheterisation in our series of hospitalised elderly women was frequently followed by bacterial and/or fungal catheter colonisation, while in most cases the latter accounted for cystitis.

P876 Antimicrobial resistance and causative agents of nosocomial urinary tract infection in the medical wards in a tertiary hospital

E. Vagiakou-Voudris, A. Kapogli, G. Zorbas, A. Strouza, E. Malamou-Lada
Athens, GR

Objectives: To establish the frequency and antibiotic resistance of uropathogens of nosocomial urinary tract infection in three medicals wards in a tertiary hospital of Athens.
Methods: During a 1-year period (1/11/2002 to 1/11/2003) 2300 urine samples were obtained from inpatients, from three medical wards 48 h after their admission. Urine cultures were performed according to standard techniques. The identification of urinary isolates was performed using API and Crystal ID systems, while the susceptibility of them was tested by Kirby-Bauer and M.I.C methods.
Results: 803 out of 2300 urine samples (35%) fulfilled the criteria for significant bacteriuria (Pure growth of >100 000 CFU/mL urine for bacteria and >1000 CFU/mL for *Candida* sp.). The nosocomial isolated uropathogens were as following: *E. coli* 276 (34.37%), *Candida* sp. 116 (20.6%), *Proteus* sp. 87 (10.83%), *Ps. aeruginosa* 86 (10.7%), *Klebsiella* sp. 75 (9.33%), *Enterobacter* sp. 18 (2.24%), *A. baumannii* 19 (2.36%), *Serratia* sp. 3 (0.37%), *E. faecalis* 36 (4.48%), *E. faecium* 10 (1.24%), *S. aureus* 6 (0.74%), CoNS 19 (2.36%). The resistance of isolated strains to antibiotics was as following: *E. coli* and *Klebsiella* sp. were resistant to ampicillin (41.3–100%), amoxicillin–clavulanic acid (2.53–2.66%), cephalothin (8.69–33.3%), cefuroxime (3.6–30.6%), nitrofurantoin (1.44–18.66%), cotrimoxazole (10.86–25.3%), quinolones (3.26–9.33%) retrospectively. The resistance of *P. aeruginosa* and *A. baumannii* to imipenem was (11.6–31.5%), ceftazidime (12.7–10.5%), quinolones (31.3–15%) and aminoglycosides (36–26%) retrospectively. The resistance of CoNS to oxacillin was 47.3%, gentamycin 31.5%, cotrimoxazole 31.5%, quinolones 31.5% and nitrofurantoin 21%. *E. faecalis* was resistant to gentamycin high level 5.55% and *E. faecium* 30%. There was no resistance to glycopeptides for *Staphylococci* as well as *Enterococcus* sp. About 5.2, 3.5 and 2.5% of isolates *E. coli*, *Proteus* sp. and *Klebsiella* sp. expressed ESBL production.
Conclusions: *E. coli*, *Candida* sp., *Proteus* sp., *Klebsiella* sp., *Pseudomonas* sp. and *Enterococcus* sp. were the most common uropathogens isolated from inpatients in the three medical wards in our hospital. About 41% of isolates of *E. coli* isolates were resistant to ampicillin and 11.2% of Enterobacteriaceae were ESBL producers. Additionally, 4% of *P. aeruginosa* and 12.5% of *A. baumannii* produced carbapenemases.

P877 Epidemiology of ventriculostomy-related infections in neurosurgical patients

B. Tiodorovic, M. Babic, M. Dinic
Nis, CS

Objective: To assess the epidemiology of meningitis related to external ventricular CSF drainage in neurosurgical patients.
Methods: From 01/01/2001 to 01/01/2002, 121 patients undergoing ventricular catheterisation (VC) were surveyed in the 12-bed ICU of the neurosurgery ward. All patients were managed in a standardised way for nursing, daily bacteriological and chemical CSF examination with removal of VC in case of infection.
Results: Meningitis was diagnosis in 14 patients (12.31%). Five cases were not documented (15.3%) and other cases were due principally to Gram-positive cocci (76%).The median delay of onset was 7 days. No significant difference was found between infected and non-infected patients in terms of age. SAPS II, presence of comorbity and mortality (duration of VC-19 vs. 10 days).
Conclusion: The proportion of VC-related meningitis without bacteriological documentation appears not negligible and these nosocomial infections seem to have an impact on morbidity but not on mortality rates.

P878 Cerebrospinal fluid shunt infections in adults: a 15-year experience

X. Santesmases, A. Pallarés, N. Sopena, M. Giménez, M.L. Pedro-Botet, J.M. Cladellas, S. Colet, C. Rey-Joly, M. Sabrià
Badalona, E

Background: Cerebrospinal fluid (CSF) shunt infection is a common cause of shunt failure.
Objectives: To study CSF shunt infections occurring in Hospital Germans Trias i Pujol.
Methods: Retrospective study of adult patients with CSF shunt infections between April 1987 and December 2002 in a tertiary 600-bed hospital in Badalona (Barcelona). Epidemiological and clinical characteristics and outcome of these patients were recorded in a data base and analysed by SPSS.
Results: Eighteen patients with CSF shunt infections, eight (44.4%) males, with a mean age of 56.22 ± 20.4 years and seven (38.9%) with comorbity, were included in the study. The most common indication of shunt was normal-pressure communicating hydrocephalus in six (33.3%), hydrocephalus due to intraventricular haemorrhage in six (33.3%) and secondary to tumour in four (22.2%). The shunt was ventriculoperitoneal (VP) in 16 (88.9%) cases and lumboperitoneal (LP) in 2 (11.1%). The clinical presentation was fever in 16 (88.9%), symptoms related to shunt malfunction in 12 (66.6%) (lethargy in 55.6%, nausea-vomiting in 22.2%, headache in 16.7%, convulsions in 5.6%), meningeal signs in 5 (27.8%), symptoms related to distal end (abdominal pain) in 4 (22.2%) and wound infection in 2 (11.1%). Mean duration of symptoms before diagnosis was 3.28 ± 5.67 days. The mean peripheral white blood count was $11\,384 \pm 6716.4$ mm^{-3}. The findings of CSF (mean, SD) were: leucocyte count 408.8 ± 1034.6, glucose 3.48 ± 2.2 mmol/mm^3, proteins 0.59 ± 0.74 g/L. The CSF Gram stain was positive in six (33.3%) of the cases. The aetiologic agents of shunt infections were: coagulase-negative staphylococci (nine cases, 56.2%), *Sthaphycoccus aureus* (one), *Streptococcus sanguis* (one), *Pseudomonas aeruginosa* (two), *Escherichia coli* (one), *Citrobacter diversus* (one), *Propionibacterium acnes* (one), Polimicrobial infection (two). Blood cultures were positive in only one case. Antibiotic treatment was indicated in all the cases; moreover, prosthetic shunt was removed in 14 (77.8%) with reshunt in six (33.3%) cases. The outcome was known in 16 cases: resolution of infection in 11 cases, relapse in one case and death in four cases (related to shunt infection in two).
Conclusions: CSF shunt infections in adults occurred mainly in patients with ventriculoperitoneal shunts, presenting with symptoms related to shunt malfunction. These infections were mainly caused by coagulase-negative staphylococci and require removal of the prosthetic shunt.

P879 Evaluation of postneurosurgical nosocomial meningitis in adults

C. Bulut, A. Tekiner, M.A. Yetkin, C.A. Hatipoglu, Z. Buharali, N. Tulek
Ankara, TR

Objectives: To assess the clinical and therapeutic outcomes of nosocomial Gram-negative bacillary meningitis (GNBM) in adult postneurosurgical patients.
Methods: Between 1 January 2000 and 31 December 2002, 19 adult patient with GNBM were included in this study. Nosocomial meningitis was defined according to the CDC criteria. Their clinical features, laboratory data, predisposing factors and therapeutic outcome were analysed.
Results: Nineteen patients included 10 males and nine females, whose ages ranged from 15 to 67 years (mean 41 years). All of the patients were hospitalised in the neurosurgical department. The most common underlying conditions were intracranial haemorrhage (8/19 cases), followed by hydrocephalus (4/19 cases) and cranial injury secondary to trauma (3/19). All patients underwent surgical procedures prior to infection, which included 15 craniotomies and four ventriculostomies. All patients were receiving

antibiotic therapy at the onset of infection. Mean time between surgical procedure and diagnosis of meningitis was 15 days (6–27 days). Fever and neck stiffness was found in eight and seven patients, respectively. In 12 patients serum leukocyte count was higher than 10×1000/cu mm. Mean leukocyte count in serum and cerebrospinal fluid was 16×1000/cu mm (min 10×1000/cu mm, max; 35×1000/cu mm) and 19×1000/cu mm (min 10/cu mm, max; 5620/cu mm) respectively. Mean CSF protein concentration was 210 mg/dL and mean CSF glucose concentration was 44 mg/dL. Only in 14 of 19 cases the microorganism was isolated from cerebrospinal fluid. *Acinetobacter* spp. (11 cases), *K. pneumoniae* (two cases) and *E. cloacae* were the isolated microorganisms. Most of the *Acinetobacter* isolates were susceptible to carbapenems but all of them were resistant to third-generation cephalosporins. A combination of carbapenem plus an aminoglycoside and/or vancomycin therapy was applied most of the patients. An additional intrathecal aminoglycoside dosage was needed for seven patients who responded poorly. The overall mortality rate in these patients was 43%.

Conclusion: There has been an increase of post neurosurgery meningitis cases. In addition, the emergence of strains resistant to third-generation cephalosporins in this group has also been noted in recent years, and has become a great therapeutic challenge. Early diagnosis and initiation of appropriate antibiotic therapy is needed in this potentially fatal disease.

P880 The influence of antimicrobial prophylaxis on upper respiratory tract microflora in hospitalised patients with resectable lung cancer

U. Kosikowska, R. Los, A. Biernasiuk, M. Juda, A. Malm, P. Rybojad, F. Furmanik
Lublin, PL

Objectives: Pulmonary resection is associated with considerable risk of infection, so antimicrobial prophylaxis has become routine practice in thoracic surgery. The aim of this study was to assess changes in microflora of upper respiratory tract in hospitalised patients with non-small cell lung cancer (NSCLC) before and after preoperative antimicrobial prophylaxis.

Methods: 51 patients with NSCLC aged 37–73 years were subdivided into two groups: (A) control group (21 patients without antimicrobial prophylaxis and surgery), (B) 'prophylaxis' group (30 patients undergoing pulmonary operation with preoperative antimicrobial prophylaxis, including piperacillin, cefuroxime or ceftriaxone alone or in combination with amikacin). Throat and nasal specimens were taken up two times: examination I – on the day of hospital admission and examination II – on the third or fourth day of hospitalisation in group A and on the third or fourth day after the surgery in group B. The routine microbiological methods were used for isolation and identification of bacteria and fungi. Statistical analyses were performed by nonparametric tests.

Results: The colonisation of nasal mucous membranes by pathogenic microflora did not differ significantly during hospitalisation between group A and B. Similar situation was observed in the case of pathogenic microflora on throat mucous membranes in group A. Different results were obtained in group B. The increased prevalence of pathogenic microflora on throat mucous membranes was observed – from 46.67% in examination I to 80% in examination II. This difference was statistically significant ($P = 0.007$). In group B colonisation of throat mucous membranes by Enterobacteriaceae family and *Candida* spp. was increased significantly during hospitalisation (from 10 to 26.67% and from 36.67 to 70%, respectively).

Conclusion: Our results indicate that antimicrobial prophylaxis can be regarded as an important predisposing factor for changes of upper respiratory tract microflora and for colonisation of mucous membrane of throat with enteric Gram-negative rods and yeast-like fungi – *Candida* spp. These microorganisms are potential causative agents of endogenous infections in immunocompromised patients with lung cancer.

P881 Aerobic and anaerobic flora isolated from pleural drains in patients with lung cancer after thoracic surgery

R. Los, I. Korona-Glowniak, A. Malm, P. Rybojad, F. Furmanik
Lublin, PL

Objectives: The purpose of this study was to determine aerobic and anaerobic bacteria colonising pleural drains in patients with non-small cell lung cancer (NSCLC) undergoing thoracic surgery and to define antimicrobial agents susceptibility of isolated strains. Routine antimicrobial prophylaxis included piperacillin or cefuroxime. In some cases beta-lactam was used in combination with amikacin.

Methods: Material for research was fluid from pleural drains collected from 34 patients aged 38–72 years two times – on the day of pulmonary resection and on the fourth day after operation. Samples were routinely cultured under aerobic and anaerobic conditions and determined using Api system (bioMerieux). Antimicrobial resistance was estimated by the disc diffusion method according NCCLS recommendations.

Results: Aerobic (49 strains) and anaerobic (23 strains) bacteria were found in 30 (44%) and 15 (22%) samples, respectively. Among aerobic bacteria, Gram-negative rods (22 strains; 18 – belonging to non-fermenting rods) and coagulase negative staphylococci (CNS; 14 strains) were most often cultured. Fifteen strains of non-fermenting rods and 11 isolates of CNS were classified as multidrug resistant (MDR) organisms. Two isolates of *S. marcescens* were producers of extended spectrum beta-lactamases (ESBLs) and inducible beta-lactamases (IBLs). All staphylococci were susceptible to vancomycin and teicoplanin. CNS strains resistant to penicillin and oxacillin but sensitive to amoxicillin/clavulanate were most frequently isolated. Only two methicillin-resistant strains, belonging to *S. haemolyticus* were found. The most common anaerobic bacteria were from the genera *Eubacterium* (nine strains) and *Actinomyces* (six strains). All of them were highly susceptible to antimicrobial agents except metronidazol (69.6% resistant strains) and chloramphenicol (52.2% resistant isolates).

Conclusion: Colonisation of pleural drains does not mean infection, however knowledge about bacterial species found in drain fluid in a local population and antimicrobial resistance (especially MDR strains) has a major impact on the success of prophylaxis and therapy of potential postoperative infections.

P882 Severe bacteraemic infections in patients with diabetes mellitus

A. Artero, J.J. Camarena, R. Zaragoza, S. Sancho, J. Tamarit, R. González, J. Nogueira
Valencia, E

Objectives: To know the clinical and microbiological characteristics of diabetic patients with severe bacteraemia. To identify the differential features of severe bacteraemia between patients with and without diabetes mellitus (DM).

Materials and methods: During a 7-year period (1996–2003) we have evaluated all bacteraemias with severe sepsis or septic shock in an intensive care unit of a teaching hospital. Clinical and microbiological features were recorded from clinical charts. The SPSS package (9.0) was used to identify significant differences between DM and no-DM cases, and to determine if the presence of DM was associated with mortality by a multivariate analysis.

Results: The prevalence of DM in patients with severe bacteremic infections was 23.8% ($n = 60$). In the group of DM the mean age of patients was 69.2 ± 7.9 years, the relation between men/women was 1.06, the origin of the bacteraemias was nosocomial in 88.3%, severe sepsis was present in 61.6% and septic shock in 31.6%. The focus of infection in diabetic patients was: unknown ($n = 29$), catheter ($n = 11$), respiratory ($n = 9$), urinary ($n = 5$), abdominal ($n = 3$), vascular ($n = 2$) and cutaneous ($n = 1$). The main microorganisms causing of bacteraemias in patients with DM were: CNS (21.6%), *Acinetobacter baumannii* (15%), *Staphylococcus aureus* (11.6%), *Escherichia coli* (8.3%) and

Enterococcus spp. (8.3%). A higher proportion of nosocomial cases in DM was the only differential feature between patients with and without DM ($P = 0.041$). The global mortality in patients with and without DM were 43.3 and 56.7% ($P = 0.068$), respectively, and the related mortality were 21.6 and 27.6% ($P = 0.361$), respectively. DM was related neither to global (OR = 0.523, 95% IC 0.269–1.015) nor related mortality (OR = 0.730, 95% IC 0.337–1.583) by multivariate analysis.
Conclusion: DM is prevalent between critically ill patients with severe bacteraemic sepsis and bacteraemic septic shock. Diabetic patients had a higher proportion of nosocomial origin of bacteraemia. We did not find that DM was related to mortality in severe bacteraemic infections.

P883 A rare case of *Brucella* infection of an obstetrician during the delivery of an infected infant

A. Poulou, F. Markou, X. Efthimiou, F. Mountaki
Serres, GR

Objectives: Brucellosis is a zoonotic disease whose prevalence in Northern Greece is high and constitutes a significant problem for the local health authorities. The aim of this study is to report a rare case of transmission of *Brucella melitensis*.
Patients: A female infant showed signs of respiratory distress during delivery. The obstetrician in charge tried to clear the respiratory tract of saliva and amniotic fluid. In his attempt he swallowed some secretions. A blood culture from the infant was incubated in the Bactec 9120. After 3 days *B. melitansis* was isolated. The case was proved to be a rare case of congenital brucellosis. The family of the infant was checked and the mother was found to be positive at 1/80 titre by *Brucella* agglutination test though her blood culture was negative. Neither her husband nor her other two children were positive on the Wright agglutination test. Both parents were involved in animal husbandry. Two months after the delivery of the infected infant the obstetrician reported pains in the back of his neck and low fever. A blood test revealed leucopenia and neutropenia (white cell count 2800/mm^3). The Wright agglutination test was positive at titre 1/80. A blood culture was taken and *B. melitensis* was isolated. Transaminases were normal. The obstetrician reported that he had not consumed unpasteurised milk or dairy products. He was treated with vibramycin and rifadin for 40 days. Two months later the Wright agglutination test was found negative and the white cell count was normal.
Conclusions: *B. melitansis* is usually transmitted through consumption of unpasteurised diary products. In these cases we had transplacental transmission and transmission through infectious secretions via the gastro-intestinal tract. Therefore it is essential that detailed medical case histories should be taken from pregnant women in order to avoid congenital infections and that medical personnel should be aware of the possibility of such transmission.

P884 Characterisation and sensitivity of coryneforme bacteria isolated in clinically significant pictures: a 3-year study

J.L. Gómez-Garcés, A. Alhambra Mosquera, J.-I. Alós
Madrid, E

Objectives: Coryneforme bacteria have gradually acquired greater importance in infectious pathologies, especially as opportunistic nosocomial pathogens, some of them displaying resistance to various antibiotics. The aim of this report is to describe some of these bacteria with significant implication in different clinical pictures.
Methods: Over a 3-year period we characterised the coryneforme isolates with presumable clinical significance. Clinical significance of the isolates was evaluated according to clinical information received (fever, intravascular devices, underlying disease, prolonged antibiotic therapy, etc.) as well as microbiological criteria (more than one isolation from habitually sterile anatomical areas and/or repeated isolations as predominant flora in sites contaminated with comensal flora).
Results: In 35 patients the isolations were clinically significant. The most frequent isolations (18) were found in blood culture: seven *Corynebacterium amycolatum*, five *Corynebacterium jeikeium*, two *Corynebacterium minutissimum*, two *Dermabacter hominis*, one *Corynebacterium* group G, one *Brevibacterium* sp. In another eight cases bacteraemia was accompanied by isolation of the same species in intravenous catheters (two *C. amycolatum*, one *C. striatum*, one *C. jeikeium*, one C. group G), a pace-maker cable (*C. minutissimum*) or soft tissue wound (one *C. urealyticum*, one *Brevibacterium* sp.). In addition, four *C. striatum* were isolated (three in respiratory secretions and one in a lower limb abcess), two *C. amycolatum* in mammary abscesses, one *C. jeikeium* in articular fluid and two *C. urealyticum* in urine. All the isolates were sensitive to vancomycin (MICs <0.5 mg/L), while sensitivity to beta-lactamics, macrolides and fluorquinolones was variable.
Conclusions: (1) *C. amycolatum* and *C. jeikeium* were the most frequently found corynebacteria with presumable clinical significance. *Dermabacter* and *Brevibacterium* were the genera identified among the non-corynebacterias. (2) In 26 of the 35 patients the isolates were obtained from bacteraemias with or without identifiable origin. (3) Vancomycin and linezolid were the only antibiotics with efficacy against all isolates. (4) More attention should be paid to the isolation of coryneforme bacteria in clinical samples from hospitalised patients since their role as opportunistic infections agents has been clearly demonstrated.

P885 Enterotoxigenic *Bacteroides fragilis* (ETBF) strains isolated from clinical specimens of human and animal origin

P. Obuch-Woszczatynski, A. Rokosz, A. Sawicka-Grzelak, H. Pituch, M. Luczak
Warsaw, PL

Objectives: To detect the presence of *B. fragilis* enterotoxin (fragilysin) gene in clinical *B. fragilis* strains by polymerase chain reaction (PCR) and to define types of infections in which ETBF strains are involved.
Methods: Clinical *B. fragilis* strains of human origin isolated in different European countries and of animal origin cultured in the United States of America were examined. PCR was performed with primers 404/407 detecting *B. fragilis* enterotoxin (fragilysin) gene. Susceptibility of strains to antimicrobial agents was determined with Etest. Two reference enterotoxigenic *B. fragilis* (ETBF) strains: ATCC 43858, ATCC 43859 and two reference non-enterotoxigenic (NTBF) strains ATCC 25285, IPL E 323 served as control strains in all experiments.
Results: In 18 strains isolated from human clinical samples in England, France, The Netherlands and Poland the presence of *B. fragilis* enterotoxin-fragilysin gene was found by PCR. Also, four strains cultured from animal clinical samples in the United States were positive for fragilysin gene. All examined reference and clinical strains were susceptible to amoxicillin/clavulanic acid, imipenem and metronidazole.
Conclusions: Enterotoxigenic *B. fragilis* strains cause different types of infections (intestinal and extra-intestinal) in hospitalised patients. ETBF strains are also involved in intestinal and extra-intestinal infections in animals. Susceptibility of ETBF strains to antimicrobial agents is typical of *B. fragilis* rods. The most active *in vitro* antimicrobials are beta-lactams combined with beta-lactamase inhibitors, carbapenems and metronidazole.

P886 Impetigo bullosa at the neonatological and obstetrician department – molecular analysis

J. Jursa, A. Kordek, S. Giedrys-Kalemba
Szczecin, PL

Introduction: Staphylococcal scalded syndrome (SSSS) and localised SSSS such as impetigo bullosa are caused by an epidermolytic toxin od *S. aureus* (S.a.) that can be either chromosomally

encoded as exfoliative toxin A (ETA) or plasmid-encoded as toxin B. Outbreaks of nosocomial SSSS and impetigo bullosa in infants have been well-described to be associated with the well baby nursery. The source of infection has been traced to health care workers in the delivery room or the newborn nursery. The initial site of S.a. colonisation/infection may be the anterior nares, nasopharynx, conjuctiva, umbilicus and/or the blood rather than the skin. Often the personnel are asymptomatic carriers of the epidemic strain of S.a.

Objectives: The aim was determine the genetic relatedness of S.a. isolated from patients and staff and investigation of the potential source of the infection.

Material and methods: In November 2001 27 strains of S.a. were isolated from various materials from newborns hospitalised at neonatological and obstetrician departments as well as from the staff. Biochemical test Api Staph was used for the species identification. To molecular typing of isolates was used pulsed field gel electrophoresis (PFGE). Interpretation criteria for the gels followed manufacturer's guidelines: isolates with identical restriction profiles were assigned the same type, isolates that differed by one genetic event (one to three bands) were considered closely related, isolates with a four- to five-band difference were considered possibly related, and isolates that differed by more than six bands were different strains.

Results: Comparative analysis of the banding pattern for the isolates can be divided into several categories: Genetic: type A – six strains from newborns with impetigo bullosa and one from staff (baby nursery); type B – two strains from the staff; type C – seven strains from the staff; type D – two strains from the staff; nine other types – each one from one person from the staff.

Conclusions: All the cases of impetigo bullosa were caused by one genetic type of *S. aureus* which allows to characterise the infection as a hospital infection. Strains isolated from the staff (except one person) belonged to different genetic types (unrelated strains). Isolation of the same genetic type from infected newborns and a person from the stuff may suggest that this person was the source of the infection, but we can not exclude that she was accidentally colonised during the hospital outbreaks.

P887 The potential role of the German cockroach (*Blatella germanica*) in carrying pathogens in two general hospitals, Tehran

K. Holakouie Naieni, H. Ladoni, H. Soliemani, S. Afhami, B. Haghighat
Tehran, IR

Background: Cockroaches are capable of carrying many human pathogens and they may have a role in transmitting some serious nosocomial infections.

Objectives: To determine the potential role of the German cockroach in carrying pathogens in general hospitals belonging to Tehran University of Medical Sciences.

Methods: Using adhesive traps and manual methods to catch the cockroaches. Hospitals were located in different areas. A total of 30 cockroaches were caught and subjected to microbial investigation at the Research and Diagnostic Laboratories, Institute of Public Health.

Results: The rate of bacterial contamination was 100%. Isolated organisms included *Klebsiella*, *Pseudomonas*, *Proteus*, *Acinetobacter*, *Entrobacter* and *Seratia*. Single and multiple antibiotic resistances were detected in the isolates. The most frequently recovered fungal pathogens were *Aspergillus flavus* and *Aspergillus fumigatus*.

Conclusions: The German cockroach could play an important role as a mechanical carrier of pathogens in the hospital setting. Hence a thorough knowledge of the biological characteristics of this insect would be essential in designing effective control programmes.

P888 Role of *Coxiella burnetii* in hospitalised patients with atypical pneumonia

P. Douramani, S. Mavrea, E. Anastasakou
Athens, GR

Introduction: *Coxiella burnetii* is a small Gram-negative coccobacillus that is an obligate intacellular parasite. The most common animal reservoirs are goats, cattle, sheep, cats, and occasionally dogs. Aerosolisation occurs at the time of parturition and infection follows inhalation of this aerosol. The pneumonic form of the illness can range from very mild to severe pneumonia requiring assisted ventilation. The aim of this study is to evaluate the proportion of cases of atypical pneumonia caused by *Coxiella burnetii* in adults at a Chest Diseases Greek hospital.

Materials and methods: The study population consisted of 280 patients (160 males and 120 females) with ages ranging from 20 to 65 years. The diagnosis of atypical pneumonia was based on the clinical symptoms, the chest X-ray and the serological study. An immunofluorescene assay (IFA) was used to detect the presence of antibodies of the IgG isotope against epitopes from phase II of *Coxiella burnetii*, which are the first antibodies to appear in infected people. The criteria for acute infection from *C. burnetii* were: phase II IgG titres 1/5122, phase II IgM 1/20.

Results: Our serological study showed that the positive rate of IgG antibodies (titre 1:512) against phase II of *C. burnetii* was 12.1% (34/280). IgM antibodies were detected in 23 out of the 34 patients with positive IgG antibodies (65%). Furthermore two of the infected patients had fourfold increase in *C. burnetii*-specific IgG antibody titre. There was no significant difference in age and gender in these patients.

Conclusion: *C. burnetii* seems to play significant role in hospitalised patients with atypical pneumonia. *C. burnetii* pneumonia should be considered when there is a suitable exposure history and when outbreaks of a pneumonic illness are being investigated. A wider use of the serology in cases of atypical pneumonia should lead to an increase of diagnosed cases.

P889 Prediction of post-cardiosurgery infectious complications by means of procalcitonin monitoring

N. Beloborodova, D. Popov
Moscow, RUS

Objectives: To study the predictive value of the procalcitonin (PCT) as an early marker of postoperative infectious complications in adult high-risk patients (pts) with acquired heart diseases after cardiac surgery with cardiopulmonary bypass.

Methods: Consecutive immunoluminometric tests (LUMItest© PCT, B.R.A.H.M.S Aktiengesellschaft, Germany) taken in 60 patents before and at first, second, third and sixth days after cardiac valve(s) replacement. Following established correlations all cases were retrospectively distributed into four groups (A–D) according to the first post-operative reading and consecutive dynamic change of PCT serum concentration: Group A – no increase of PCT concentration, Group B – 0.5–2 ng/mL, Group C – >2 ng/mL with subsequent decrease and Group D – >2 ng/mL, persisting. Also the post-op infection rates were analysed in these groups.

Results: None of the pts had exhibited any signs of infection before surgery. Initial PCT concentrations did not exceed the normal values (<0.5 ng/mL). Postoperatively 14 (23.3%) out of 60 pts developed infectious complications: pneumonia – ten, surgical site infection (SSI) – one, pneumonia and SSI – one, sepsis – one, pneumonia and sepsis – one. Correlation between the incidence of postoperative infectious complications and PCT levels after surgery is presented in the table.

Table.

Group	n	PCT concentrations, ng/mL [*M* (min max)] Day 1	Day 2	Day 3	Day 6	Infectious complications
A	6	0.40 (0.22–0.46)	0.26 (0.09–0.47)	0.26 (0.08–0.42)	0.17 (0–0.38)	0
B	23	1.15 (0.51–1.82)	0.92 (0.26–1.90)	0.60 (0.26–1.30)	0.40 (0–2.94)	4 (17.4 %)
C	26	6.57 (2.05–23.96)*	5.13 (0.39–17.59)*	3.39 (0.19–16.65*)	0.44 (0.05–1.29)	7 (26.9 %)
D	5	13.57 (6.51–33.42)*	18.41 (5.51–58.40)*	12.52 (4.95–38.46)*	8.17 (1.33–18.64)*	3 (60 %)

*p[C,D vs. B] < 0.01.

Conclusions: PCT monitoring is useful to early diagnosis and prediction of severe post-op infections in cardiac surgery patients. PCT serum concentration more than 2 ng/mL on the first day after surgery, especially with a tendency to persist till third day postoperatively is associated with high prevalence of infectious complications.

Animal models

P890 Is multiple trauma accompanied by bacterial translocation? An experimental model

E. Bathrelos, E.J. Giamarellos-Bourboulis, N. Efstathopoulos, J. Lazarettos, V. Nikolaou, A. Papalois, P. Grecka
Athens, GR

Objectives: To define whether bacterial translocation is a process involved in the series of events following multiple trauma.
Methods: Crushing fracture of the middle of the right femor was performed in 12 New Zealand rabbits. Blood sampling was performed before and 4 h after fracture for the determination of tumour necrosis factor-alpha (TNF-alpha) and of nitric oxide (NO). TNF-alpha was estimated by a bioassay on L929 fibrosarcoma cell line and NO by a colorimetric assay. Survival was recorded and after death segments of liver, spleen and lower lobe of the right lung were cut for quantitative culture.
Results: Mean ± SE survival was 0.80 ± 0.15 days. Mean ± SE of serum TNF-alpha was 6.06 ± 1.34 and 44.65 ± 25.77 pg/mL (P: 0.05) before and after fracture, respectively. Mean ± SE of serum NO after fracture was 195.8 ± 24.9 μM. In tissue segments derived from all animals viable counts of *Serratia marscecens* and of *Pseudomonas aeruginosa* were identified. Mean ± SD of log 10 of viable cell counts of *S. marscecens* of liver, spleen, and lower lobe of the right lung after death were 7.30 ± 0.44, 7.16 ± 0.32 and 6.14 ± 2.56 CFU/g, respectively. Those of *P. aeruginosa* were 6.11 ± 1.52, 4.41 ± 2.31 and 5.69 ± 1.84 CFU/g, respectively.
Conclusions: In an experimental model of multiple trauma with an absolute mortality rate and elevated serum levels of TNF-alpha and NO, bacterial translocation was found in deep organs. That process might be correlated to the elevated serum levels of NO.

P891 Pharmacodynamic conditions of emergence and extinction of mutant resistant *Streptococcus pneumoniae* in experimental pneumonia treated with moxifloxacin: interest of the Mutant Selection Window Concept

M. Etienne, D. Croisier, P. Charles, L. Piroth, H. Portier, Chavanet
Dijon, F

Background: We showed that a MFX human-like treatment (400 mg qd) was associated with the emergence of MRSp, when PNP in immunocompetent rabbits was induced by parC mutated strains (Abstract B-708, 42nd ICAAC). The MSW was delineated by PKPD parameters using the Selection Index (SI: Mutant Prevention Concentration-MPC-/MIC).
Objectives: To confirm the MSW concept, we enforced to: (a) make mutants emerge with PKPD parameters within the MSW boundaries; (b) extinct resistant mutants with PKPD parameters exceeding the upper MSW boundary.
Methods: To reach the target PKPD values corresponding to the boundaries of the MSW, we simulated a 48-h-long MFX underexposure in PNP induced by WT (S1) and efflux (S2) strains, and an overexposure for PNP induced by parC-79 mutated strain (S3). Susceptibilities to MFX [MIC/MPC (mg/L)] were: S1 = 0.125/0.125; S2 = 0.25/0.25; S3 = 0.25/4.
Results: We could not open the window since no MRSp emerged with PKPD parameters within the MSW, when PNP was due to WT and efflux strains. When parC strains was used mutant appeared for very low drug exposition (6–10% of the standard 400 mg regimen). We could close the window, extinction MRSp with PKPD parameters exceeding the MSW upper boundary, when PNP was due to parC mutated strains.
Conclusion: In our *in vivo* model of experimental PNP we could not confirm both parts of the MSW concept, but we validated a PKPD threshold of mutant extinction. Par C mutation was strongly predictive of the emergence of MRSp with MFX treatment.

	Underexposure of S1	Underexposure of S2	S3: Standard dose (400 mg qd)	Overexposure of S3
AUC_{n-24} (mg h/L)*	6.3 ± 1.5	8.5 ± 2	27.6 ± 1.9	137 ± 31
Cmax (mg h/L)*	0.9 ± 0.2	0.9 ± 0.2	4.1 ± 0.2	20 ± 0.5
Pulmonary bacterial reduction (log_{10}CFU/g)**	0.7 ± 1.6	1.8 ± 0.5	1 ± 1.7	6.62 ± 0.46†
Rabbits with MRSp/Rabbits	0/4	0/6	11/11	0/17

*Protein-free fraction; **as compared to untreated rabbits;
†P < 0.001.

P892 Pharmacodynamic evaluation of the efficacy of a human-like treatment with gatifloxacin on experimental pneumococcal pneumonia: impact of low levels of resistance

D. Croisier, M. Etienne, P. Charles, L. Piroth, H. Portier, P. Chavanet
Dijon, F

Background: The efficacy of last generation fluoroquinolones (FQ) was recently questioned by the emergence of *Streptococcus pneumoniae* (Sp) strains having low levels of resistance. These strains exhibit low MICs, thus remaining susceptible, but high Mutant Prevention Concentrations (MPCs); the difference between MIC and MPC was used to determine PKPD parameters delineating the Mutant Selection Window (MSW).
Objectives: To investigate the *in vivo* efficacy of GAT in a model of PP due to Sp exhibiting various levels of susceptibility to FQ, by determining both the pulmonary bacterial reduction and the PKPD conditions associated with the emergence of resistant mutants (RM).

Methods: To induce PP in immunocompetent rabbits, five penicillin-resistant strains were used, defined for GAT as [MIC/MPC (mg/L)/genotype]: A = 0.25/0.25/Wild Type; B = 0.5/1/efflux; C = 1/8/parC S79F; D = 1/8/parC S79T; E = 4/4/gyrA S81F. A 48-h standard GAT treatment was simulated. Pharmacokinetic (PK) data (C_{max}, AUC0-24 h) and residual pulmonary bacterial concentration were obtained for each animal. *$P < 0.05$.
Results: 7–11 animals per group were tested. Mean unbound PK parameters were AUC0-24 (mg h/L) = 28.5 ± 8.1 and C_{max} (mg/L) = 2.6 ± 0.5. Lung bacterial reductions (log 10 CFU/g) as compared with controls (percentage % of animals exhibiting at least one mutant) were: A = −6.95 ± 0.88* (0); B = −5.67 ± 1.65* (27); C = 0.49 ± 0.45 (100); D = 0.03 ± 0.96 (100), E = 0.54 ± 0.96 (0), respectively. RM appeared for following PKPD parameters (unbound fraction): $2.5 < C_{max}/MIC < 3.4$, $27.2 < AUC/MIC < 40.6$, $0.28 < C_{max}/MPC < 0.96$, $2.7 < AUC/MPC < 11.5$.
Conclusion: In this *in vivo* model of PP (1) GAT was strongly effective on the fully susceptible strain and its efflux derivative, despite the emergence of RM for this later one. (2) GAT was ineffective, as expected on resistant gyrA strain, but more surprisingly on parC mutated strains, mainly due to the presence of RM. (3) these mutants were selected *in vivo* in a MSW more precisely defined by PKPD parameters using MPC. (4) Low levels of resistance to FQ should be detected by simple tests to guide the therapeutic options.

P893 Hearing loss in experimental pneumococcal meningitis increases after pretreatment with G-CSF

C. Brandt, S.P. Lund, C. stergaard, N. Frimodt-Møller, F. Espersen, P. Caye-Thomassen, J.D. Lundgren
Copenhagen, DK

Background: Mechanisms leading to hearing loss in pneumococcal meningitis is poorly understood.

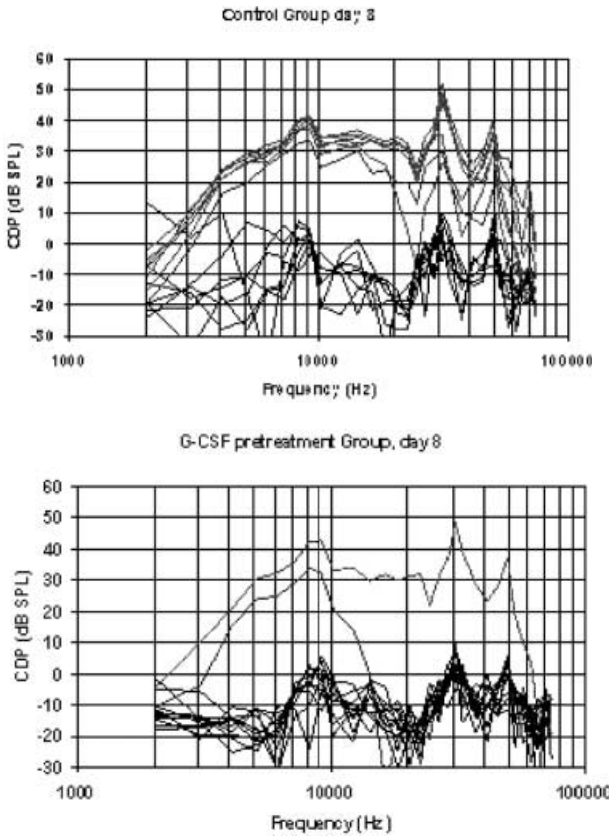

Figure 1. DP-grams from control and G-CSF pretreatment groups 7 days after infection.

Objective: Boost of systemic neutrophil count by G-CSF prior to infection leads to diminished growth of pneumococci in experimental meningitis and improves survival. Whether this protective effect also includes attenuation of hearing loss is reported here.
Materials and methods: Rats – infected intracisternally with ~1 × 105 *S. pneumoniae* serotype 3 – were randomly allocated to receive G-CSF (10 μg/kg s.c. TD) 48 h prior to infection ($n = 16$), late treatment (28 h postinfection, $n = 16$) or no G-CSF ($n = 22$). All animals also received ceftriaxone started 28 h postinfection. Infection was documented by blood and CSF tap 24 h post infection. Just before, 24 h and 7 days after infection, assessments of hearing was made by measurements of distortion product otoacoustic emissions (DPOAE) at f_2 = 4–70 kHz and by assessment of hearing thresholds by auditory brain stem responses (ABR) at 52 kHz in levels from 20 to 100 dB SPL.
Results: 24-h postinfection hearing loss was significantly increased in G-CSF treated animals compared with untreated (hearing loss in 37.5 vs. 14.3% of animals from f_2 = 10–50 000 Hz and 75 vs. 42.9% f_2 > 50 000 Hz, respectively, Mann–Whitney, $P = 0.026$). On day 8 postinfection among surviving animals, severity of hearing loss in G-CSF pretreated animals was furthermore increased compared with the control group (severe hearing loss in 92.3 vs. 50% from f_2 = 10–65 000 Hz, respectively, Mann–Whitney, $P = 0.013$). Late G-CSF treatment did not affect hearing loss significantly compared with the control group.
Conclusion: Increased systemic neutrophil level at time of infection increased risk of hearing loss and development of deafness in a rat model of experimental meningitis despite better control of bacterial growth and better survival. Neutrophils contribute to the sensorineural hearing loss seen in experimental meningitis.

P894 In pneumococcal meningitis adjuvant doxycycline improves survival, and reduces neuronal injury

D.N. Meli, D.G. Erhardt, J. Kummer, S.L. Leib
Berne, CH

Objective: Bacterial meningitis is characterised by an intense inflammatory host response that contributes to the high mortality and morbidity of the disease. Doxycycline is a clinically used antibiotic which has anti-inflammatory effects that are separate and distinct from its antimicrobial action, including the reduction of cytokine release and the inhibition of matrix metalloproteases. The present study assessed the effect of doxycycline, when given as adjuvant therapy in experimental pneumococcal meningitis.
Methods: Eleven-day-old rats were infected intracisternally with 10 μL of saline containing 2.5–1.5 × 10^6 CFU/mL *Streptococcus pneumoniae*. At 18 h after infection all animals received ceftriaxone (100 mg/kg i.p., q12 h) and were randomised for administration of a single dose of doxycycline (30 mg/kg s.c.; $n = 67$) or an equal volume of saline (500 μL; $n = 65$). At 40 h after infection, surviving animals were sacrificed. Albumin concentration in the brain was assessed as an index for blood–brain barrier (BBB) leakage. Brain damage was quantified by histomorphometry.
Results: A single dose of doxycycline (30 mg/kg) vs. saline improved survival (survival rate: 80 vs. 52%, $P < 0.001$), protected the BBB (cortical albumin/total protein: 7.9 vs. 12.7 μg/mg, $P < 0.04$) and reduced injury in the cerebral cortex (damage in percent of cortex; median [range] 0 [0–2.8] vs. 0 [0–26.9], $P < 0.05$).
Conclusion: Adjuvant treatment with doxycycline may be a promising approach to prevent death or neuronal injury as a consequence bacterial meningitis.

P895 *In vivo* synergism of amoxicillin sub-inhibitory concentrations and specific antibodies against *Streptococcus pneumoniae* in a mice sepsis model

J. Casal, M. Giménez, D. Tarragó, A. Fenoll, L. Aguilar
Majadahonda Madrid, E

Objective: To explore if the *in vivo* combined effect of specific antibodies and amoxicillin is a synergistic or additive effect by

establishing conditions resulting in null survival by antibodies protection or antibiotic treatment.
Methods: A fully amoxicillin-resistant (MIC of 8 mg/L) serotype 6B *Streptococcus pneumoniae* was used as infecting strain. Amoxicillin was administered at a dose (3.12 mg/kg) producing serum concentration lower than the MIC of the infecting strain all over the treatment period (C_{max}: 6.1 mg/L). Passive immunisation was performed with hyperimmune serum (HS; obtained from mice weekly inoculated with whole cell heat-inactivated inoculum for 5 weeks) diluted in PBS up to dilution 1/6 that had shown null protection (0% survival) in preliminary experiments. Groups of 10 BALB/c mice weighing 19–22 g were passively immunised with one-single intraperitoneal (ip) injection of the 1/6 dilution of HS, 1 h prior to infection with the 6B pneumococcus. Amoxicillin treatment was started 1 h after inoculation and continued t.i.d for 48 h. Groups of animals receiving placebo (PBS), non-immune serum, non-diluted HS, 1/6 dilution of HS or amoxicillin 3.12 mg/kg alone were included as control groups. Mortality was recorded over the 7-day follow-up period.
Results: Survival rates in all control groups were lower than 10% except in the non-diluted HS that was 100%. Antibiotic treatment in passively immunised animals produced survival rates of 100%, with significant differences vs. controls (except the non-diluted HS).
Conclusion: Since amoxicillin concentrations were below the MIC (8 mg/L) of the infecting organisms all over the treatment period (C_{max} of 6.1 mg/L), the presence of specific antibodies produced *in vivo* efficacy of sub-inhibitory concentrations. The *in vivo* combined effect antibodies/amoxicillin is synergistic and not only additive considering the survival rates obtained by the antibodies (0% survival) and amoxicillin sub-inhibitory concentrations (10% survival) alone and those obtained when acting together (100% survival).

P896 Daptomycin is highly efficacious against penicillin-resistant and penicillin- and quinolone-resistant pneumococci in experimental meningitis by sterilising the CSF within 4 h

P. Cottagnoud, M. Pfister, M. Cottagnoud, F. Acosta, L. Flatz, F. Kühn, A. Stucki
Berne, CH; San Francisco, USA

Objective: Daptomycin (D) was tested against a penicillin-resistant (PenR) and against a penicillin- and quinolone-resistant (PenR + -QuR) pneumococcal strain in the rabbit meningitis model.
Methods: Standard regimen was ceftriaxone(CRO) combined with vancomycin (V). MICs of the PenR strain in mg/L: PenG: 4, D: 0.06, CRO: 0.5, V: 0.12–0.25. MICs of the PenR + QuR strain: PenG: 4, D: 0.06, CRO: 0.5, V: 0.12–0.25, trovafloxacin: 4, ciprofloxacin: 32. Meningitis was induced by intracisternal inoculation of 10 000 CFU. Ten hours later treatment was started for 8 h. D (15 mg/kg) and CRO (100 mg/kg) were injected at hour 0 and V (20 mg/kg) were injected at hours 0 and 4. CRO and V were standard doses. D corresponded to high doses in humans. CSF samples were repeatedly collected during therapy in order to determine antibiotic levels and killing rates. D serum levels peaked at 200 mg/L decreasing slowly to 36 mg/L 8 h later. D CSF levels ranges between 5 and 3 mg/L. D penetration into inflamed meninges was 5%. Results of bactericidal activity of the different regimens are expressed in Delta log 10 CFU/mL h and Delta log 10 CFU/mL over 8 h. Results are presented in Table 1.
Conclusions: (1) D is highly efficacious against PenR and PenR+ QurR pneumococci in experimental meningitis, sterilising the CSF of rabbits within 4 h (9 out of 10 in both D treatment groups). (2) D as monotherapy is significantly superior to the standard regimen based on a combination of CRO with V against both strains. (3) The efficacy of D was also confirmed in time-killing assays over 8 h.

Table.

Groups (*n*) [Strain]	Inoculam (log_{10} CFU/mL)	Killing rates/h (log_{10} CFU/mL h)	Killing rates/8 h (log_{10} CFU/mL 8 h)
Controls (10) [PenR]	6.75 ± 0.30	+0.04 ± 0.06	+0.04 ± 0.42[1]
D (10) [PenR]	6.95 ± 0.85	−1.20 ± 0.32[1]	−6.95 ± 0.39[1]
CRO + V (10) [PenR]	6.25 ± 0.45	−0.57 ± 0.24[1]	−4.50 ± 0.47[1]
Controls (10) [PenR + QuR]	5.39 ± 1.0	+0.20 ± 0.19	+1.31 ± 0.87
D (10) [PenR + QuR]	6.22 ± 0.23	−0.91 ± 0.08[2]	−6.22 ± 0.23[3]
CRO+V (10) [PenR + QuR]	5.79 ± 0.64	−0.63 ± 0.13[2]	−5.02 ± 1.11[3]

1: $P < 0.001$ D vs. CRO + D; 2: $P < 0.001$ D vs. CRO + D; 3: $P < 0.03$ D vs. CRO + V.

P897 Moxifloxacin and levofloxacin treatment outcomes against pneumococcal pneumonia in a novel skin-temperature murine model

D. Bast, D. Bell, X. Chen, L. Dresser, L. Mandell, D. Low, J. Azavedo de
Toronto, Hamilton, CAN

Objectives: Skin-temperature is an effective measure of the severity of pneumococcal pneumonia in mice and can be used to predict lung bacterial counts and imminent death. Skin-temperatures vary considerably in groups of infected mice and thus, drug intervention at a particular skin-temperature more closely resembles that which is used in humans. In this study, we compared the efficacy of moxifloxacin (MFX) with levofloxacin (LVX) in the treatment of pneumococcal pneumonia using our novel skin-temperature model.
Methods: Swiss Webster mice were inoculated endotracheally with 5-log 10 CFU of the *Streptococcus pneumoniae* A66 strain (MICs: MFX, 0.12 μg/mL; LVX, 0.5 μg/mL). Skin temperature at 35 h was used to assess disease severity prior to drug treatment. A skin temperature of ≥32°C is indicative of a moderate infection with a pulmonary bacterial count of 6-log 10 CFU whereas temperatures <32°C but >30°C are suggestive of a severe infection with a count of 7-log 10 CFU. All mice with a temperature of ≤30°C were excluded from the study, as death is imminent within 24 h. A 50 mg/kg subcutaneous dose of MFX or LVX was given twice daily for 5 days. Skin temperature was measured daily to monitor clinical improvement or failure (≤30°C for at least 48 h). All mice deemed to have failed therapy were euthanised immediately. Viable counts in the lungs were determined for all mice.
Results: Of the mice classed as moderate, 24/29 (83%) mice treated with MFX and 10/20 (50%) mice treated with LVX survived. Complete eradication was obtained in 93 and 20% of mice treated with MFX and LVX, respectively, in this group. Of the mice classed as severe, 24/31 (77%) and 5/20 (20%) mice treated with MFX and LVX, respectively, survived. Complete eradication was obtained in 84 and 15% of mice treated with MFX and LVX in this group.
Conclusions: MFX showed significantly enhanced activity over LVX at both an early and late stage pneumococcal lung infection.

P898 Evaluation of fosfomycin in the treatment of experimental meningitis caused by a high-level cephalosporin-resistant *Streptococcus pneumoniae*

S. Ribes, A. Domenech, F. Taberner, C. Cabellos, F. Tubau, J. Liñares, P. Fernández Viladrich, F. Gudiol
Hospitalet de Llobregat, Barcelona, E

Objective: Fosfomycin, a broad-spectrum antibiotic with good penetration into the cerebrospinal fluid (CSF), could be useful in the treatment of cephalosporin-resistant (CR) pneumococcal meningitis.

Table.

Therapy (*n*)	Log CFU 0 h	KR 6 h	KR 24 h	KR 26 h
FOS (9)	5.16 (±0.89)	−2.95(±1.31)[a]	−4.38(±0.86)[a]	−5.15(±0.96)[a]
CRO (9)	5.23 (±0.88)	−0.76(±1.7)	−0.75(±1.7)	−1.01(±1.8)
RIF (8)	5.45 (±0.76)	−2.42(±0.48)	−4.33(±0.93)[a]	−5.14(±1.09)[a]
TEI (8)	5.21 (±0.83)	−3.21(±0.80)[a]	−4.39(±0.86)[a]	−5.19(±0.89)[a]
Control (11)	4.91 (±0.73)	+1.1(±0.91)	+1.41(±1.18)	+0.65(±2.45)

[a]$P < 0.01$ vs. CRO (ANOVA).

Methods: We tested the efficacy of fosfomycin (FOS) 300 mg/kg/6 h compared with those of ceftriaxone (CRO) 100 mg/kg/day, rifampicin (RIF) 15 mg/kg/day and teicoplanin (TEI) 15 mg/kg/day. These dosage regimens were selected after pharmacokinetic studies in order to achieve serum concentrations comparable to those observed in humans. Meningitis was induced by intracisternal inoculation of 10×10^6 CFU/mL of a high CR pneumococcus strain (ATCC 51916). MICs (mg/L) were: PEN 0.12, CRO 32, FOS 2, RIF 0.06, TEI 0.03. After 40 h inoculation a baseline CSF sample was taken (hour 0) and therapy was started. Antibiotics were given intravenously for 26 h. Control animals received saline solution. CSF samples were collected during therapy to determine bacterial killing rates, antibiotic concentrations and inflammatory parameters.
Results: FOS serum levels ranged between 324.5 and 1.02 mg/L and CSF levels between 35.06 and 1.09 mg/L. CSF penetration of FOS was 49% based on comparison of areas under the curve (serum AUC/CSF AUC). FOS was as effective as RIF and TEI. Bacterial counts at 0 h expressed as mean log 10 CFU/mL (±SD), and killing rates (KR) in CSF calculated as mean delta - log 10 CFU/mL (±SD), are shown in the table.
Conclusion: FOS penetrated to a high extent into the CSF and showed good bactericidal activity against this particular pneumococcal strain.

P899 Combination of linezolid and rifampin is highly synergistic in experimental *Staphylococcus aureus* joint prosthesis infection

A. Saleh-Mghir, C. Muller-Serieys, L. Massias, A.C. Crémieux
Paris, F

Background: Prosthesis infections are difficult to cure. Methicillin-resistant *Staphylococcus aureus* (MRSA) are becoming more common and optimal therapy (Rx) remains to be defined. Linezolid (LZD), the first oxazolidinone antibiotic, is active on Gram-positive pathogens as MRSA strains.
Methods: In a MRSA knee prosthesis infection in rabbits, we compared the efficacies of LZD (70 mg/kg t.i.d.) or vancomycin (VAN) (60 mg/kg b.i.d.) alone or combined with rifampin (RIF) (10 mg/kg b.i.d.). A partial knee replacement was performed with a silicone implant fitting into the intramedullary canal of the tibia, and 107 CFU of MRSA, were injected into the knees. Rx was started 7 days after inoculation and continued for 7 days intramuscularly.
Results: MICs (mg/L) of LZD, Van and RIF were 1.5, 1.5 and 0.008, respectively. *In vivo*, LZD reduced significantly the mean log 10 CFU/g of bone (2.58 ± 0.85, $n = 9$) vs. controls and VAN (6.22 ± 0.43, $n = 7$; 4.87 ± 0.61, $n = 8$), respectively ($P < 0.01$). Both Rx were not sufficient to sterilise animals (1/9 and 0/6 respectively). The combination of RIF with LZD (1.6 ± 0.01, CFU/g of bone, 6/6 sterile animals) or with VAN (1.6 ± 0.08 CFU/g of bone, 6/6 sterile animals), was significantly more effective than monotherapy ($P < 0.01$). Emergence of resistance to Rif was not detected *in vivo*.
Conclusion: In this MRSA joint prosthesis infection, LZD combined with Rif was highly effective *in vivo* and prevented the selection of mutant resistant of rifampin. LZD should be of interest for treating MRSA joint prothesis infection.

P900 Evaluation of lauric acid monoester compounds in a *Staphylococcus aureus* nasal decolonisation model

M. Rouse, M. Scholz, J. Steckelberg, R. Patel
Rochester, St. Paul, USA

Objectives: Topical mupirocin is used to eradicate *S. aureus* from the nares of *S. aureus* colonised patients. Mupirocin resistance in *S. aureus* has been reported and associated with failure of nasal eradication of *S. aureus*. Lauric acid monoester compounds are active *in vitro* against methicillin-resistant *S. aureus* (MRSA) and may be effective MRSA nasal decontaminants. We used a murine model of MRSA nasopharangeal colonisation to compare *in vivo* activity of mupirocin with 3 lauric acid monoester formulations for nasal *S. aureus* decolonisation.
Methods: 239 Hsd:ICR mice were challenged intranasally with 10^8 colony forming units of MRSA. Five days later, *S. aureus* colonisation was documented by culture in 189 mice. Colonised mice were treated intranasally with bland ointment, mupirocin or one of three lauric acid monoester ointments (49D, 49E or 53A), three times daily for 2 days. Three days after treatment was complete, both anterior nares were cultured for *S. aureus*.
Results: Results of treatment are listed as number (percent) success (MRSA negative nasopharynx cultures). Twelve of 38 (32%) mice were successfully treated with bland ointment. Nineteen of 38 (50%) mice were successfully treated with mupirocin. Eighteen of 39 (46%) mice were successfully treated with 49-D. Twenty-four of 34 (71%) mice were successfully treated with 49E. Thirty-three of 40 (83%) mice were successfully treated with 53A. Treatment with 49E or 53A was more effective than bland ointment ($P < 0.05$) and 53A was more active than mupirocin ($P < 0.002$) in this model. MRSA recovered from mice after treatment were tested for susceptibility to the antimicrobial used for treatment to detect emergence of resistance. We recovered mupirocin resistant (MIC <4 μg/mL) MRSA in four of 19 mice that failed mupirocin treatment. MRSA recovered from mice failing lauric acid monoester treatment had MIC values within three dilutions of pre-treatment MRSA.
Conclusions: In a murine model of MRSA nasal decolonisation, 53A was significantly more active than mupirocin and 49D or 49E was as active as mupirocin as measured by eradication of MRSA from the nasopharynx and emergence of resistance to mupirocin after treatment was observed.

P901 TolC but not AcrB plays an essential role in colonisation of chicks with multi-resistant *Salmonella typhimurium* DT104

S. Baucheron, C. Mouline, K. Praud, E. Chaslus-Dancla, A. Cloeckaert
Nouzilly, F

Objectives: To study the role of the multidrug efflux system AcrAB–TolC in resistance of *Salmonella typhimurium* DT104 to detergents and bile salts. To evaluate the importance of the components AcrB and TolC of this efflux system in the colonisation of a multidrug-resistant *S. typhimurium* DT104 strain in chicks.
Methods: acrB and tolC mutants of a multidrug-resistant *S. typhimurium* DT104 strain were constructed by deletion or insertional inactivation of the genes. MICs of detergents and bile salts were determined for the acrB and the tolC mutants, comparatively to the wild type mutidrug-resistant strain. The effect of sodium choleate on the *in vitro* growth of these three strains was evaluated. The LD50s of the strains were measured in a one day old chicken model, inoculated with several doses (3–9 log CFU) by the oral route, during 7 days post-inoculation. The colonisation levels were assessed at the subletal dose 7 days post-inoculation by determining the number of CFU of *Salmonella* in the faeces, caeca, spleen, and liver.

Results: The decrease of resistance to detergents and bile salts was much more important for the tolC mutant than for the acrB mutant. For example, MICs of SDS decreased of 1024 and 128 times, MICs of sodium deoxycholate decreased of 64 and 8 times, for the tolC and acrB mutants, respectively. Addition of choleate in culture medium had no effect on the growth of the wild type strains and of the acrB mutant but inhibited the growth of the tolC mutant. The LD50s in the 1-day old chicken model, were 6 log CFU and 7 log CFU for the wild type strain and the acrB mutant, respectively, and not calculable for the tolC mutant because of a too small number of dead chicks. Furthermore, in contrast to the acrB mutant, the tolC mutant was unable to colonise the caeca, spleen, and liver after 1 week of infection. Moreover, in most chicks no intestinal excretion was detected for the tolC mutant. The colonisation levels of the acrB mutant were the same as those of the parental strain.
Conclusion: TolC but not AcrB appears to be essential in multidrug-resistant *S. typhimurium* DT104 colonisation of chicks, which is in accordance with their respective roles in resistance to detergents and bile salts. Therefore, TolC could be a better target than AcrB for the development of efflux system inhibitors.

P902 Efficacy of cefepime and imipenem in experimental pneumonia caused by porin-deficient *Klebsiella pneumoniae* producing CMY-2 -lactamase

C. Pichardo, J.M. Rodriguez-Martinez, M. Herrero, M.-C. Conejo, M. Aguilar, L. Martinez-Martinez, J. Pachón, A. Pascual
Seville, E

Objectives: To compare the activity of CFP and IMP in the experimental pneumonia caused by Kp.
Methods: Strains: Porin deficient Kp (Kp17) and its derivative producing the plasmid-mediated AmpC-type β-lactamase CMY-2 (Kp27). *In vitro* studies: MIC/MBC: microdilution method (NCCLS), inoculum: 105, 106 and 107 CFU/mL. The *in vitro* postantibiotic effect (PAE) was investigated by exposing the bacteria to IMP and CEP at concentration equal to two and six times the MICs for 1.5 h. The PAE was quantitated calculating the difference between the times required for the numbers of drug-exposed and untreated organism to increase 10-fold above the numbers present immediately after removal of the antibiotic. PK/PD parameters (C_{max} and time above the MIC) were determined after a single dose of antimicrobials. *In vivo* studies: Experimental pneumonia in C57BL/6 mice, with intratracheal inoculum of 108 CFU/mL. The animals were grouped in: CON (no treatment), CFP (360 mg/kg/day) and IMP (240 mg/kg/day), during 72 h. Variables: mortality rates and bacterial clearance from lungs. Statistical analysis: Chi-squared and Fisher tests, ANOVA, and post-hoc tests.
Results: *In vitro*: MIC/MBC (105, 106, and 107): CFP (0.125/0.125, 0.125/0.125, 4/8) for Kp17 and (8/16, 16/16, 32/32) for Kp27; IMP (0.25/0.25, 0.5/0.5, 1/1) for Kp17 and (16/16, 32/32, 32/32) for Kp27. PAE: The study demonstrate a significant PAE of IMP (2 h at least) on Kp27 at 2 × MIC and 6 × MIC. No *in vitro* PAE of IMP was observed on Kp17, and no *in vitro* PAE of CEP was observed on Kp17 or Kp27. PK/PD parameters: C_{max} (mg/L), time above the MIC (h) for Kp17 and for Kp27, respectively: CFP (124.06, 1.88, 1.79), IMP (16.9, 1.32, 0.23). *In vivo*: For Kp17, CFP and IMP decreased the mortality respect to CON (0 vs. 73%, $P < 0.003$) and (33.3 vs. 60%, $P < 0.05$); for Kp27, IMP was the only therapy that decreased the mortality compared with CON and CFP (13 vs. 60% and 60%, $P < 0.01$). Bacterial clearance from lungs: For Kp17, CFP and IMP cleared the lungs respect to CON (1.74 and 3.38 vs. 9.16 log CFU/mL, $P < 0.01$), CFP being better than IMP ($P < 0.001$); for Kp27, CFP and IMP cleared the lung respect to CON (4.33 and 4.06 vs. 9.07 log CFU/mL, $P < 0.000$).
Conclusions: The presence of plasmid-mediated AmpC-type β-lactamase CMY-2 in *K. pneumoniae* diminished the *in vivo* efficacy of cefepime and not that of imipenem. The inoculum effect for cefepime and the PAE of imipenem partially explain these results.

P903 *Mycobacterium abscessus* causes a granulomatous infection controlled by IFNγ in C57BL/6 mice

P. Hochedez, M. Rottman, C. Soudais, F. Ripoll, J.-F. Emile, J.-L. Gaillard
Garches, Paris, Boulogne Billancourt, F

M. abscessus is a rapidly growing mycobacterium (RGM) that is emerging as a significant pathogen in humans, both as a respiratory pathogen in patients with or without recognised comorbidities, and as the agent of inoculation infections. The histopathologic features of the human infection suggest that *M. abscessus* causes a tuberculosis-like infection. We investigated the systemic challenge of C57BL/6 mice with the type strain of *M. abscessus* through intravenous and intraperitoneal routes. With both high (107 CFU) and low (105 CFU) doses, the initial bacterial load remained stable for 5 days in liver and spleen until the establishment of a granulomatous response. The differentiation of the granuloma (central F4/80+ epithelioid cells with a peripheral CD4+ and CD8+ lymphocytic crown) was contemporary to a drastic decrease of the bacterial load in the organs studied. However, 90 days following the challenge some mice still harboured bacteria capable of *in vitro* growth in their livers and spleens despite an overall effective control of the infection, and all mice infected presented granulomas of various differentiation stages in their livers. This response is highly reminiscent of the IFNγ dependent response to *M. tuberculosis*. Mice deleted for the gene encoding IFNγ were challenged intraperitoneally with *M. abscessus* and significantly failed to reduce the bacterial load by day 14. We show for the first time that the rapidly growing *M. abscessus* can cause a long lasting, tuberculosis-like, IFNγ dependent infection in C57BL/6 mice. These results show promise for the elucidation of *M. abscessus* disease since data from *M. tuberculosis* might be relevant. Reciprocally, *M. abscessus* faithfully models key features of mycobacterial infection.

P904 Animal model of Guillain–Barré syndrome by sensitisation with a *Campylobacter jejuni* lipopolysaccharide

P. Gandolfi, M. Luciani, C.M. Caporale, G. Armillotta, A. Uncini
Teramo, Chieti, I

Objectives: *Campylobacter jejuni* infection is the most common antecedent in the axonal variant of Guillain–Barré syndrome (GBS). Antibodies against nerve gangliosides found in GBS patients recognise cross reactive epitopes in the lipopolysaccharide (LPS) of *C. jejuni*. This led to the molecular mimicry hypothesis of GBS. To investigate the connection among *C. jejuni*, antibodies anti gangliosides and GBS we designed an animal model employing a LPS isolated from a GBS patient.
Methods: We immunised eleven rabbits with a LPS extracted from Penner serotype 0:19 *C. jejuni* strain isolated from patient with GBS and Freund's adjuvant (CFA) (group I). In a second experiment we immunised seven rabbits with LPS, CFA and keyhole limpet hemocyanin (KLH) (group II).
Results: All rabbits of groups I and II developed a strong humoral response to LPS. Elevated IgM and IgG antibodies to LPS could be detected as early as 2 weeks after the first immunisation. IgG raised during the immunisation period up to 25 600 in group I and 6400 in group II. Anti-GM1 IgM antibodies were detectable at low titres 2 weeks after the first immunisation in both groups and raised up to 3200 in group I and to 6400 in group II. IgG anti-GM1 could already be detected at low titres in both groups 2 weeks after the first immunisation and increased up to 51 200 in group I and up to 25 600 in group II. Titre of anti-GM1 IgG showed a steep rise during the 6 weeks following the first immunisation. In Western immunoblotting of *C. jejuni* LPS, the serum of immunised rabbits reacted strongly with a band that co-migrated at 10 kD at the same level of CT, PNA and serum of the patient with anti-GM1 antibodies. The kinetics of IgM and IgG anti-GD1b was similar to that of antibody anti-GM1 but the maximal titres were lower as IgG raised up to 3200 in group I and

12 800 in group II. IgM anti-GD1a were at low titre in both groups throughout the experiment whereas IgG anti-GD1a raised up to 3200 in group I and to 800 in group II. IgM and IgG anti-GQ1b were not detectable in group I and II sera.
Conclusion: *C. jejuni* LPS is a potent B-cell stimulator capable to induce a strong antiganglioside response in rabbits. However to induce the neuropathy is crucial to employ KLH a glycoprotein known to stimulate both humoral and cellular responses. This is the first animal model reproducing the pathogenetic process hypothesised in axonal GBS with antiganglioside antibodies post-*C. jejuni* infection.

P905 *Campylobacter concisus* infection in mice

R. Aabenhus, Å. Ljungh, U. Stenram, H. Permin, L. Andersen
Copenhagen, DK; Lund, S

Objective: To establish a model of *Campylobacter concisus* infection in mice.
Methods: Three separate experiments were conducted in order to screen the ability of five clinical *C. concisus* isolates and the ATCC 33237 type strain of oral origin to infect BALB/cA mice. All mice were pre-treated with vancomycin, and half of the animals received cyclophosphamide to disturb immune functions, prior to *C. concisus* challenge by direct intragastrical inoculation with 0.3 mL 10^9 CFU, controls received 0.3 mL of PBS. Measured parameters were bacterial isolation from stool and internal organs, loss of body weight and histological examinations of tissue samples. Mice were sacrificed on days 7, 21 and 56 of the studies. Isolation of *C. concisus* was performed by the selective filter method and PCR.
Results: Isolation and identification: *C. concisus* was isolated on day 7 from the cyclophosphamide treated group infected with the clinical isolate 10776 (study 1). Liver (3/3), ileum (3/3) and jejunum (1/3) were culture positive. PCR results from tissue samples were only positive in one mouse from the same group (liver, ileum and jejunum). Faecal pellets were consistently negative. During the two following studies, no isolation of *C. concisus* was possible. Histological examination: Microabscesses (1/3) were found in the liver in two untreated groups. Oedema of villi in the ileum was occasionally noted in infected groups, but not in controls (study 2). Two mice in the untreated group infected with the ATCC 33237 type strain, presented leukocyte infiltration of colon. Loss of body weight: Compared with controls, the *C. concisus* infected mice had a significant weight loss ($P < 0.05$) (study 3). Loose stools: On days 2 and 3, *C. concisus* inoculated groups had loose and slimy stools compared with control groups (study 3). One mouse inoculated with the clinical isolate 10776 died on day 5 (study 3).
Discussion: The present model mimics a relevant intragastrical exposure to *C. concisus* infection of imunocompetent BALB/cA mice upon cyclophosphamide treatment and results indicate a possible transient colonisation of liver and ileum, with clinical signs of illness as loss of bodyweight and loose stools. Histological examination was inconclusive. Isolation of *C. concisus* was not reproducible in two subsequent studies, which severely hampers the present model. Future studies should concentrate on the first days of infection, as the organism is rapidly cleared from the GI tract.

P906 *n*–6 Polyunsaturated fatty acids enhance the activity of antimicrobials in experimental sepsis by multiresistant *Pseudomonas aeruginosa*

M. Mouktaroudi, P. Koutoukas, T. Adamis, I. Alexandrou, H. Giamarellou, E.J. Giamarellos-Bourboulis
Athens, GR

Objectives: $n-6$ polyunsaturated fatty acids (PUFAs) like gamma-linolenate (GLA) and arachidonate (AA) have been shown to enhance the activity of ceftazidime and amikacin both *in vitro* and *ex vivo* on multidrug-resistant (MDR) *P. aeruginosa* (Giamarellos-Bourboulis et al. AAC 2000; JAC 2003). They were co-administered with antimicrobials in an experimental model of sepsis by an MDR isolate.
Methods: Sepsis was induced in 30 rabbits after the iv infusion of an 8 log 10 inoculum of a *P. aeruginosa* isolate resistant to ceftazidime (CZ), imipenem, ciprofloxacin and amikacin (AM) by a catheter inserted into the right jugular vein. Animals were assigned into five groups of treatment of six animals each: A controls; B iv CZ and AM; C iv CZ, AM and alcohol 99%; D iv CZ, AM and an alcoholic solution of GLA; and E iv CZ, AM and an alcoholic solution of AA. Therapy was administered 30 min after bacterial challenge. CZ was given at a 50 mg/kg dose, AM at 15 mg/kg and both $n-6$ PUFAs at 25 mg/kg. $n-6$ PUFAs were infused within 10 min. All agents were administered by a catheter inserted into the left jugular vein. Survival was recorded; after death segments of various organs were cut for quantitative cultures.
Results: Mean (±SE) survival of groups A, B, C, D and E were 2.58 ± 0.74, 9.52 ± 2.59 (pNS compared with A), 9.67 ± 2.50 (pNS compared with A), 9.53 ± 2.58 (pNS compared with A) and12.25 ± 1.60 days (P: 0.017 compared with A) respectively. Mean log 10 of viable cell counts of *P. aeruginosa* form liver of groups A, B, C, D and E were 3.27, 1.82, 2.78, 2.34 and 1.26 CFU/g, respectively. Respective values of spleen counts were 4.63, 1.74, 3.00, 2.30 and 1.32 CFU/g. Respective values of the counts from the lower lobe of the right lung were 2.85, 3.17, 5.95, 1.36 and 1.20 CFU/g. Respective values of counts of mesenterial lymph nodes were 2.87, 1.25, 2.23, 1.35 and 1.20 CFU/g.
Conclusions: Co-administration of $n-6$ PUFAs with antimicrobials in an experimental model of sepsis by MDR *P. aeruginosa* enhances their effect by prolonging survival and decreasing the number of viable cell counts in organs.

P907 Successful management of experimental sepsis by *Acinetobacter baumannii* with the co-administration of colistin and rifampin

A. Pantopoulou, M. Raftogiannis, M. Mouktaroudi, I. Dontas, D. Perrea, E. J. Giamarellos-Bourboulis
Athens, GR

Objectives: *In vitro* synergy between colistin (CL) and rifampin (RF) has been reported on multidrug-resistant (MDR) isolates of *A. baumannii* (Giamarellos-Bourboulis et al. DMID 2001). This synergy is tested in an experimental model.
Methods: Thirty-five Wistar rats became neutropenic by the intraperitoneal injection of 100 mg/kg of cyclophosphamide on day 1 and 150 mg/kg on day 3. On day 5 an 8 log 10 inoculum of one MDR isolate was intramuscularly injected into the right femor of animals. Rats were assigned into four groups of treatment: A ($n = 6$) controls; B ($n = 8$) RF treated; C ($n = 11$) CL treated; and D ($n = 10$) treated with both agents. Therapy was given four hours after bacterial challenge. CL was administered im 3 mg/kg into the left femor and RF iv from a catheter inserted into the right jugular vein at 5 mg/kg. Survival was recorded.
Results: Mean ± SE survival of animals of groups A, B, C and D were 1.75 ± 0.17 days, 3.13 ± 0.52 days (P: 0.031 compared with A), 3.86 ± 0.50 (P: 0.004 compared with A) and 5.50 ± 0.11 (P: 0.011 compared with A) respectively.
Conclusions: Co-administration of CL and RF is beneficiary accompanied by prolonged survival in an experimental model of sepsis by MDR *A. baumannii*.

P908 Beta-lactams and aminoglycosides in combination for pneumonia by carbapenem-resistant *Acinetobacter baumannii* in a mice model

A. Montero, J. Ariza, X. Corbella, A. Domenech, S. Ribes, J. Ayats, F. Tubau, F. Gudiol
L'Hospitalet, Barcelona, E

Infections by *Acinetobacter baumannii* (Ab) with high-degree resistance (HDR) to carbapenems have recently increased. Only colistin

seems to keep its *in vitro* efficacy, but clinical practice is scarce. To our knowledge, no clinical data are currently available to evaluate the systematic use of the beta-lactam (BL)–aminoglycoside (AG) combination to treat serious Ab infections in a way similar to that in other infections by Gram-negative bacteria.
Objective: To analyse the efficacy of the combination of two BL (imipenem [I] or sulbactam [S]) and one AG (tobramycin [T]) in an experimental model of pneumonia by Ab in mice.
Methods: We used immunocompetent C57BL/6 mice and three strains of Ab with susceptibility, moderate-degree resistance and HDR to carbapenems (A, D and E respectively). MICs (mg/L) were (strains A, D, E): I: 1, 8, 512; S: 2, 4, 128; and T: 128, 8, 8. The *in vivo* activity was examined by quantitative evaluation of the lung homogenate cultures after 48 h of induction of pneumonia.
Results: In control (CON) animals ($n = 45$), the bacterial counts in lungs at 48 h were (mean ± SD): 10.66 ± 0.37, 10.83 ± 0.32 and 10.77 ± 0.35 log 10 CFU/g of tissue for strains A, D and E, respectively (P = NS between strains). Results of antibiotic activity were expressed as differences between treated ($n = 4$ in each therapy) and CON groups (delta log 10 CFU/g) (see Table).

Table.

Therapy (dose)	Strain A	Strain D	Strain E
I (200 mg/kg/day)	−5.38[a]	−4.48[a]	0.24[a]
S (120 mg/kg/day)	−4.64[a]	−3.67[a]	−0.04[a]
T (60mg/kg/day)	0.02	−3.45[a]	−4.16[a]
I+T	−4.54[a]	−5.37[a,b]	−5.59[a,b]
S+T	−3.83[a]	−4.62[a,b]	−4.95[a,b]

[a]$P < 0.05$ vs. CON (*t*-student).
[b]$P < 0.05$ vs. monotherapies (ANOVA).

Conclusions: In this mice pneumonia model, I or S kept his efficacy for Ab with moderate resistance to carbapenems. In infections caused by this strain D, T in combination conferred a possible greater efficacy on these BLs. In infections by Ab with HDR to carbapenems, T alone was also effective. Interestingly the combination BL + AG also showed a higher effect on the infection by this HDR strain E, against which monotherapy with I or S were totally ineffective. Although the pharmacodynamics of T in this model may have been overestimated, because of the peak levels achieved are not usually found in humans at the recommended doses (C_{max} 32.87 ± 5.45 mg/L), these results are promising to treat multiresistant Ab infections.

P909 Cranberry juice and its organic acids inhibits *Escherichia coli* colonisation of the bladder in a mice model of urinary tract infection

H.D. Jensen, S.B. Christensen, K.A. Krogfelt
Copenhagen, DK

Objectives: To investigate the effect of orally administered cranberry juice and its organic acids on *Escherichia coli* in an experimental mouse model of ascending urinary tract infection.
Methods: *E. coli* C175-94, a clinical isolate from a patient with UTI was used. It expresses type 1 fimbriae but not P or S fimbriae. The transurethrally infected mice were at all times were allowed free access to chow and water (control group) or treatments. The control group and the treated groups all consisted of six mice in every trial; after 1 week, the mice were sacrificed and urine, bladders and kidneys collected for determination of bacterial counts. Most of the treatments were repeated two or more times in independent trials and these data were pooled. Treatments were commercially available cranberry juice cocktail, freshly prepared cranberry juice, the hydrophilic fraction of cranberry juice (contains sugars and organic acids) and organic acids (quinic, malic, shikimic and citric acid in concentrations corresponding to cranberry juice).
Results: A reduced number of organisms could be recovered from the bladder ($P < 0.01$) and urine ($P < 0.05$) of mice orally treated with unsweetened cranberry juice. Commercially available cranberry juice cocktail also reduced the CFU in the bladder ($P < 0.01$), as did the hydrophilic fraction of cranberry juice ($P < 0.05$). Quinic, malic, shikimic and citric acid were administered in combination and one by one. The four organic acids decreased the CFU in the bladder when administered together ($P < 0.001$), and so did the combination of malic plus citric acid ($P < 0.01$) and malic plus quinic acid ($P < 0.05$). These data indicate that the beneficial effect of the organic acids from cranberry juice during urinary tract infection is obtained when the acids are administered together.
Conclusion: For the fist time the effect of cranberry juice and its dominating organic acids has been tested in an experimental mouse model of long-term ascending urinary tract infection under controlled conditions. Cranberry juice inhibited *E. coli* colonisation of the bladder, and the organic acids were the active component involved. The active treatments reduced the bacterial load in the bladder to sub therapeutic concentrations, which indicates that cranberry juice is no final treatment but a remedy that could help the patient to clear the infection, before it eventually becomes a final cystitis.

P910 Correlation between growth rates and murine LD90 for a variety of *Aspergillus fumigatus* isolates

D. Paisley, G. Robson, D. Denning
Manchester, UK

Objectives: Determinants of pathogenicity have been elusive in *Aspergillus fumigatus*, despite the overwhelmingly high frequency of this species of *Aspergillus* compared with others. Growth arrest *in vivo* (as in PABA synthase mutants) abolished infection (Brown et al., Mol Microbiol 2000; 36: 1371–1380) and deletions of different chitin synthase genes had an effect on virulence only if radial growth rate was reduced (Mellado et al., Mol Microbiol 1996; 20: 667–679). We describe a kinetic microbroth method of measuring the growth rates of *Aspergillus fumigatus* spectrophotometrically. Using this method, growth rates (as defined by V_{max} values) were determined for nine *Aspergillus fumigatus* isolates for which an LD90 value in temporarily neutropenic CD-1 mice, infected intravenously, had previously been obtained.
Methods: An inoculum of 10^4 spores in 50 μL Sab medium gave us uniformly shaped growth curves and allowed the measurement of V_{max} values with greater sensitivity. Soft max pro software was used to determine the V_{max} value for each growth curve by performing linear regression on as many five data point line segments as possible, calculating the slope for each line segment and reporting the steepest slope as the V_{max} (mOD/min). Growth rate was determined in quadruplicate in three separate experiments and the average V_{max} measurement across these experiments calculated.
Results: Mean growth rate varied from 1.558 (AF10) to 2.411 (AF71). LD90 varied from 3×10^5 to 5×10^6. Comparison of the growth rates and LD90 values of these isolates suggests a correlation exists between the two parameters, omitting the one significant outlier (AF65, which is amphotericin B resistant), $r^2 = 0.6687$.
Conclusion: These data are important in describing a simple method for measuring the growth rate of the common filamentous fungus *A. fumigatus*, and proving a direct link between pathogenicity *in vivo* and growth rate *in vitro*.

P911 Histopathological follow-up of the pancreas and small intestines of mice, experimentally infected by the oral and intraperitoneal routes

J. Kovacova, S. Bopegamage, A. Vargova, J. Motusova, M. Borsanyiova, A. Petrovicova, M. Benkovicova, P. Gomolcak, J. Galama
Bratislava, SK; Nijmegen, NL

Background: Coxsackieviruses belong to genus Human Enterovirus of the family Picornaviridae. The natural infection is acquired by the faecal–oral route, whereas in mouse studies the intraperitoneal route is used. We have established a new mouse model with infection by the oral route.

Objective: To compare the histological changes, viral persistence and localisation of the virus in the pancreas and the small intestines of mice, experimentally infected by oral or intraperitoneal route.
Method: Mice were infected with CVB 3 (Nancy) by the oral or intraperitoneal route. Doses ranged from 5×10^3 to 5×10^9 TCID50. Selected organs from each mouse were embedded into paraffin and sections were attached on silanised slides. For histological observation the sections were stained by Mayer's haematoxylin eosin method. For localisation of the antigen by immunohistochemical staining, the VP1 protein served as an indicator for the presence of the virus. The method was standardised. The tissue sections were processed and stained by the avidin–biotin method, using the monoclonal mouse anti-enterovirus antibody against VP1 protein.
Results: The histological observations reveal that the tissue of exocrine pancreas showed inflammatory changes on the 3rd, 7th, 10th, 14th and 21st day post-infection in exocrine pancreas of the intraperitoneally infected mice. After oral infection no destruction of the exocrine pancreas was observed, but on day 35th post-peroral infection liposis was seen. VP1 was detected mainly on the third and seventh days after infection in the small intestine. We found differences in VP1 localisation between oral and intraperitoneal infection. In small intestine of orally infected mice positive staining was localised in smooth intestinal muscles whereas after intraperitoneal infection. VP1 was detected within the villi. There was no correlation between the virus concentration and tissue damage.
Conclusions: The pathogenesis of CVB3 infection is influenced by the route of virus administration, which has direct implications for the use of mouse models to study the pathogenesis of Coxsackieviruses.

P912 Mouse models of Coxsackie B virus infection: comparison of oral and intraperitoneal route of infection with follow-up of virus shedding in stool and virus replication in the small intestine

A. Vargova, M. Borsanyiova, A. Petrovicova, S. Bopegamage
Bratislava, SK

Objectives: The portal of entry of coxsackieviruses may influence the pathogenesis of infections caused by these viruses. In this study an outbred murine model (Swiss albino mice) was used for experimental infection with coxsackie B3 virus (CVB3), strain Nancy to follow-up the virus shedding in the stool and the presence of replicating virus in the small intestine of mice after oral and intraperitoneal route of infection.
Methods: For infection of mice different concentrations of the virus (10^4, 10^6, 10^8 and 10^{10}) were used. The stool and small intestine specimens of dissected mice were collected on days 3, 7, 10 post-infection (p.i.) and from day 14 in weekly intervals up to a day 147 p.i. The suspensions made from the collected specimens were studied for presence of replicating virus in Hep-2 cell cultures. The virus titre was determined in Hep-2 monolayers on microtitre plates and calculated by Reed and Muench method.
Results: The replicating virus in the stool pellets was detectable from day 3 p.i. to day 14 p.i. in both orally and intraperitoneally infected mice with a virus titre reaching the level $10^{1.5}$ TCID50/mL. In the small intestine of orally infected mice the presence of replicating virus was detected up to day 35 p.i. In the small intestine of intraperitoneally infected mice the replicating virus was present for a shorter time, up to day 21 p.i., irrespective of the dose of infection.
Conclusion: There was no difference in the length of virus shedding in stool specimens of mice infected by oral or intraperitoneal route. However a longer presence of replicating virus in the small intestine of orally infected mice in contrast to intraperitoneally infected mice was observed. This was confirmed by the immunohistopathological studies, these observations support the suggestion that the pathogenesis of coxsackieviral infections is influenced by the route of virus administration.

P913 Identification of porcine endogenous retroviruses in severe combined immunodeficient mice and Lewis rats xenotransplanted with porcine neonatal pancreatic cell clusters

S. Kim, Y. Kim, J. Sohn, K. Yoon, W. Shin, M. Kang
Seoul, KOR

Object: In xenotransplantation with porcine neonatal pancreatic cell clusters (NPCCs), the risk of cross-species porcine endogenous retrovirus (PERV) infection remained as problem. We used the severe combined immunodeficient (SCID) mouse and the Lewis rat model to identify the PERV transmission with the time course and the differences between the models.
Methods: NPCCs were transplanted to SCID mice and Lewis rats and left for 1–70 days before being sacrificed. DNA and RNA were extracted from the liver, spleen, pancreas, lung, kidney and testis. To examine the PERV transmission, nested-PCR and RT-PCR were used upon pol/env/gag regions of PERV. The pig mitochondrial cytochrome oxidase II subunit gene (COII) was amplified simultaneously to monitor the microchimerism.
Results: Total 264 samples from seven mice and five rats were tested. Ten weeks after xenotransplantation, two mice and four rats were identified to have permissive PERV infection. In the SCID mice, 92.9% of tested organs were positive for PERV-pol gene and 85.7% were positive for COII gene with DNA examination. In the Lewis rats, 86.7% of organs were positive for PERV-pol gene and 14.1% for COII gene with DNA examination. Examinations of organs of mice showed that 35 (83.3%) organs were positive for the PERV-pol gene and COII gene simultaneously that presumed as microchimerism, but 15 (50%) organs of rats were presumed as microchimerism. Results of PERV-pol positive and COII negative that presumed as permissive PERV infection were observed in 9.5% of organs in the SCID mice and 36.7% in the Lewis rats. Organs presumed as permissive PERV infection were the spleen (day 3), liver (day 70), lung (day 70), and testis (day 70) in the SCID mice by DNA examinations. In the Lewis rats, the spleen and testis of day 1; the liver, spleen, and kidney of day 5; the testis and kidney of day 7; the liver, spleen, lung, and testis of day 14 were identified to have permissive PERV infection.
Conclusion: The cross-species PERV infection was identified from these animal models. Expression of PERV depends on the immunity of the recipients, because the xenotransplanted SCID mice had more PERV microchimerism but less permissive infection than that of the Lewis rats. Detection rate was increased with the time course, accordingly in the early period after transplantation, PERV considered to exist as an inactive form.

New drugs

P914 Antimicrobial spectrum and activity of NVP PDF-713 (PDF7), a novel peptide deformylase inhibitor, tested against 1837 recent Gram-positive clinical isolates

R. Jones, T. Fritsche, H. Sader
North Liberty, USA

Background: The continued development and expansion of resistance (R) among Gram-positive pathogens (GPP) has threatened the therapy of numerous antimicrobial classes including the recently introduced quinupristin/dalfopristin, telithromycin and the oxazolidinones. Clearly the need for antimicrobial discovery persists, and this should be a continued priority for the pharmaceutical industry. This report addresses the spectrum of activity for PDF7 tested against a collection of recent (2002) clinical isolates cultured from patients infected with pathogens within the spectrum for peptide deformylase inhibitors.

Methods: PDF7 was acquired from Novartis. The compound was dispensed into reference broth microdilution trays in appropriate media over the range of 0.06–8 mg/L. Mueller–Hinton broth was supplemented with 2–5% lysed horse blood when testing fastidious streptococci, and the Corynebacteria. NCCLS QC strains were used concurrently and all PDF7 MIC results were within proposed ranges.
Results: 1837 Gram-positive strains were tested with a species rank order of *S. aureus* (875 strains) > CoNS (381) > streptococci (285) > enterococci (273) > *Listeria* spp. (11). The PDF7 MIC results against *S. aureus* and the CoNS strains ranged from ≈0.06 to 4 mg/L, but the vast majority of staphylococci (87.0%) had MICs of 0.25–2 mg/L. Staphylococcal MIC90 results for PDF7 were 1 or 2 mg/L, regardless of oxacillin R pattern. Also tested were *Corynebacterium* spp. (eight strains; PDF7 MIC50, 0.25 mg/L), *Bacillus* spp. (three; MIC50, 0.12 mg/L) and one *Micrococcus* spp. (MIC, 0.25 mg/L). PDF7 MICs for enterococci ranged to >8 mg/L, but 99.3% of strains were inhibited at ≈8 mg/L, the proposed susceptible breakpoint. R to other classes of agents did not influence the PDF7 MIC values among enterococci. All streptococci were inhibited at ≈4 mg/L, and the *f*Ò-haemolytic and *S. bovis* strains were susceptible to PDF7 at ≈1 mg/L. Previous reports have shown that >90% of *H. influenzae* (MIC90, 8 mg/L) and all *M. catarrhalis* (MIC90, 0.5 mg/L) are inhibited by PDF7.
Conclusions: To address the established decline in potency or coverage of GPP by existing antimicrobials, PDF7 appears to be a promising new agent applicable by oral and parenteral routes. The combined spectrum of activity against MRSA, VRE, DRSP and key Gram-negative community-acquired respiratory tract pathogens warrants further investigations of PDF7 in human trials.

P915 Antistaphylococcal activity of NVP-PDF713, a new peptide deformylase inhibitor compared with other agents

P. Appelbaum, L. Ednie, M. Jacobs
Hershey, Cleveland, USA

Background: Resistance of staphylococci to *β*-lactams, quinolones and other agents is increasing and recently VISA as well as VRSA strains have been reported.
Objective: NVP-PDF713 is a new peptide deformylase inhibitor active against a wide variety of Gram-positive and -negative bacteria. The current study examines the activity of NVP-PDF713 compared with those of ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, vancomycin, teicoplanin, linezolid, ranbezolid, daptomycin, oritavancin and quinupristin/dalfopristin against 131 *S. aureus* (62 methicillin resistant) and 127 coagulase-negative staphylococci (60 methicillin resistant).
Methods: Microdilution using frozen trays containing cation-adjusted Mueller–Hinton broth and inocula of 1 × 105 CFU/mL with trays incubated in air.
Results: MIC_{50} and MIC_{90} values (μg/mL) were as seen in the following table. NVP-PDF713 was equally active against all staphylococcal strains (MICs <0.06–4 μg/mL), irrespective of susceptibility to other agents. Quinolone resistance was mainly seen in methicillin R strains. Vancomycin, linezolid, ranbezolid, daptomycin, oritavancin and quinupristin/dalfopristin were all active at MICs <4.0 μg/mL and teicoplanin was less active against coagulase-negative strains.
Conclusions: NVP-PDF713, a new peptide deformylase inhibitor, was active *in vitro* against staphylococci.

	S. aureus				Coagulase-negative staphylococci			
	Methicillin R (62)		Methicillin S (69)		Methicillin R (60)		Methicillin S (67)	
Drug	MIC_{30}	MIC_{90}	MIC_{30}	MIC_{90}	MIC_{30}	MIC_{90}	MIC_{30}	MIC_{90}
NVP PDF-713	2	4	1	2	1	2	1	2
Ciprofloxacin	>16	>16	0.25	>16	8	>16	0.125	4
Levofloxacin	8	>16	0.125	8	4	8	0.125	4
Gatifloxacin	4	8	0.06	4	1	2	0.06	1
Moxifloxacin	2	4	0.03	2	1	2	0.03	0.5
Vanomycin	1	1	0.5	1	2	2	1	2
Teicoplanin	0.5	1	0.5	1	4	8	1	8
Linezolid	2	4	2	4	1	2	1	2
Ranbezolid	2	4	1	2	0.25	1	0.125	0.5
Deptomycin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Oritavancin	2	2	2	2	2	2	2	2
Quin/delfo	0.5	0.5	0.25	0.5	0.125	0.5	0.125	0.5

P916 Antipneumococcal activity of NVP-PDF713 compared with 18 other agents

P. Appelbaum, L. Ednie, M. Jacobs
Hershey, Cleveland, USA

Background: Drug resistance in pneumococci is found worldwide.
Objective: NVP-PDF713 is a new peptide deformylase inhibitor active against Gram-positive and -negative bacteria. This study tests activities of NVP-PDF713, amoxicillin ± clavulanate, imipenem, meropenem, ceftriaxone, cefuroxime, cefpodoxime, cefdinir, ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, azithromycin, clarithromycin, linezolid, quinupristin/dalfopristin, vancomycin and teicoplanin against 80 pen S, 88 pen I, 132 pen R pneumococci (154 macrolide R and 30 quinolone R strains with defined R genotypes).
Methods: Agar dilution using cation-adjusted Mueller–Hinton agar + 5% sheep blood and inocula of 1 × 104 CFU/spot; plates incubated in air.
Results: MIC50 and MIC90 values (μg/mL) are shown in Table 1. NVP-PDF713 was equally active against all pneumococci, irrespective of activity of other drugs. Beta-Lactam MICs rose with those of pen G. Moxi was the most potent quinolone followed by gati, levo cipro. Vanco, teico, linez, quin/dalf were all active at MICs <4.0 μg/mL.
Conclusions: NVP-PDF713 was active *in vitro* against Beta-lactam, macrolide and quinolone S and R pneumococci.

Drug	Pen S	Pen I	Pen R	Macrolide R	Quin R
NVP	1.0/2.0	0.5/1.0	0.5/1.0	0.5/2.0	0.5/2.0
Amox	0.03/0.03	0.25/1.0	2.0/8.0	0.25/8.0	0.06/4.0
Amox/clav	0.03	0.25/1.0	2.0/4.0	0.25/4.0	0.06/2.0
Imip	0.008/0.008	0.03/0.12	0.25/0.25	0.06/0.25	0.016/0.25
Merop	0.016/0.016	0.06/0.25	0.5/0.5	0.12/0.5	0.03/0.5
Ceftriax	0.03/0.06	0.12/1.0	1.0/2.0	0.25/2.0	0.12/2.0
Cefurox	0.03/0.12	0.25/4.0	4.0/16.0	0.5/8.0	0.25/8.0
Cefpodox	0.03/0.06	0.25/2.0	4.0/8.0	0.5/4.0	0.25/8.0
Cefdinir	0.06/0.12	0.5/4.0	8.0/8.0	0.5/8.0	0.25/8.0
Cipro	2.0/32.0	1.0/2.0	2.0/4.0	1.0/2.0	32.0/>32.0
Levo	1.0/6.0	1.0/2.0	1.0/2.0	1.0/2.0	16.0/32.0
Gati	0.5/4.0	0.25/0.5	0.5/1.0	0.5/0.5	4.0/8.0
Moxi	0.25/4.0	0.12/0.25	0.25/0.5	0.12/0.25	2.0/4.0
Azithro	2.0/>64.0	2.0/>64.0	0.12/>64.0	>64.0/ >64.0	0.12/>64.0
Clarithro	0.5/>64.0	1.0/>64.0	0.06/>64.0	64.0/ >64.0	0.03/32.0
Linez	1.0/2.0	1.0/2.0	1.0/2.0	1.0/2.0	1.0/2.0
Quin/Dalf	0.5/0.5	0.25/0.5	0.5/1.0	0.5/1.0	0.5/0.5
Vanco	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5
Teico	0.06/0.06	0.06/0.06	0.06/0.06	0.06/0.06	0.06/0.12

P917 Evaluation of the *in vitro* activity of NVP PDF-713 against clinical anaerobic isolates with emphasis on the *Bacteroides fragilis* group

D.R. Snydman, N.V. Jacobus, L.A. McDermott
Boston, USA

Objective: NVP PDF-713 is a novel peptide deformylase (PDF) that targets bacterial metalloproteases. Recent reports have demonstrated its potent *in vitro* activity against respiratory and skin pathogens such as MRSA, *S. pneumoniae*, *S. pyogenes*, *S. agalactiae* and enterococci. We undertook this study to compare the *in vitro* activity NVP PDF-713 to that of agents with antianaerobic activity against clinical anaerobic isolates emphasising on the *B. fragilis* group.
Method: The MICs of 400 *B. fragilis* group and 90 Gram-positive anaerobic isolates were determined using NCCLS recommended procedures. The activity of NVP PDF-713 was compared with that of cefoxitin, clindamycin, imipenem, garenoxacin, linezolid, moxifloxacin and tigecycline. Vancomycin was included in the evaluation of the Gram-positive organisms. All *B. fragilis* group isolates were referred during 2002–2003 by various medical centres throughout the USA and represented the various species within the group.
Results: Against the *B. fragilis* group isolates, NVP PDF-713 showed excellent *in vitro* activity against all the species. The new agent showed the lowest MICs among all comparators (MIC range of 0.03–0.5 mg/L and an MIC90 of 0.5 mg/L. Of the comparators, only imipenem showed such low MICs. NVP PDF-713 was active against strains resistant to beta-lactams, quinolones or clindamycin and the MICs were much lower than those of newer agents such as linezolid, tigecycline or garenoxacin. Next to imipenem, NVP PDF-713 was the most active agent against anaerobic positive cocci, MIC range of 0.03–1 mg/L. The activity of NVP PDF-713 was somewhat decreased against *Clostridium* species and MICs greater than 4 mg/L were observed.
Conclusion: Given the frequency of isolation of anaerobic bacteria and their increasing resistance, a novel agent effective against a new bacterial target such as NVP PDF-713 (with activity against a broad range of bacteria) is an ideal agent for potential use against mixed infections. PK and PD parameters, safety and usage in clinical trials will determine the clinical efficacy of the new agent.

P918 Potential utility of a peptide deformylase inhibitor, NVP PDF-713 (PDF7) against oxazolidinone-resistant or streptogramin-resistant Gram-positive isolates

R. Jones, G. Moet, H. Sader, T. Fritsche
North Liberty, USA

Background: New and novel antimicrobials have been introduced into infectious disease practice in the last decade to address emerging resistances (R) among Gram-positive cocci. R to oxazolidinones, streptogramin combinations and various glycopeptides require expanded development of agents with alternative modes of action. In this investigation, PDF7, a new peptide deformylase inhibitor, was tested against clinical isolates having documented R to linezolid (LZD) or quinupristin/dalfopristin (Q/D) using reference susceptibility test methods.
Methods: A total of 45 organisms were collected from our recent (2001–2002) stock culture collection that were originally isolated at R surveillance sites in the United States, Canada, Brazil and Europe. These organisms included *E. faecalis* (LZD-R, three strains; Q/D-R was intrinsic), *E. faecium* (LZD-R, 10 strains; Q/D-R, six strains), *S. aureus* (LZD-R, five strains; Q/D-R, 10 strains), CoNS (LZD-R, one strain; Q/D-R, nine strains) and *S. oralis* (LZD-R, one strain). The mechanisms of R for all LZD-R strains (MIC $\leq$8 mg/L) were confirmed by gene sequencing with the detection of a G2576U mutation. PCR tests for vatD and vatE were negative. QC of the reference PDF7 MIC results was performed using acceptable MIC ranges reported by Anderegg et al. The proposed or tentative susceptible breakpoint for PDF7 was $\approx$8 mg/mL based on pharmacokinetic/pharmacodynamic characteristics.
Results: Among the 13 LZD-R strains, three were *E. faecalis* and 10 were *E. faecium*. Also 10 enterococci were R to vancomycin (VANCO) and all *E. faecalis* strains had the intrinsic Q/D R (MIC, 8 mg/L). These enterococci had PDF7 MIC results between 0.5 and 4 mg/L (MIC90, 4 mg/L). The six Q/D-R *E. faecium* were susceptible to VANCO and were inhibited by 1 or 2 mg/L of PDF7. PDF7 was highly active against LZD-R *S. aureus* (MICs, 0.25–0.5 mg/L), *S. epidermidis* (MIC, 2 mg/L) and the viridans group streptococcus (MIC, 0.5 mg/L). Q/D and vancomycin were active against these LZD-R organisms. The Q/D-R staphylococci (19) were inhibited by PDF7 (MIC range, 0.12–2 mg/L), LZD (1 or 2 mg/L) and VANCO (1 or 2 mg/L).
Conclusions: These results indicate that PDF7 among the candidate peptide deformylase inhibitors, demonstrated excellent activity (all MICs at $\leq$4 mg/L) against emerging Gram-positive clinical isolates that have become R to oxazolidinones or streptogramin combinations.

P919 Time-kill study of antistaphylococcal activity of NVP PDF-713 compared with other agents

P. Appelbaum, K. Credito, G. Lin, M. Jacobs
Hershey, Cleveland, USA

Background: Methicillin resistance is increasingly seen in *S. aureus* and coagulase-negative staph (CONS).
Objective: NVP PDF-713 is a new peptide deformylase inhibitor active against Gram-positive and Gram-negative strains. This study tested activity of NVP PDF-713, ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, vancomycin, teicoplanin, linezolid, ranbezolid, daptomycin, tigecycline, oritavancin and quinupristin/dalfopristin against six *S. aureus* (3 methi R) and six CONS (3 methi R).
Methods: NCCLS macrodilution MIC was used. For time-kills, 5×10^5–5×10^6 CFU/mL inocula in cation-adjusted Mueller–Hinton broth were incubated aerobically in a shaking water bath at 1×, 2×, 4× MIC. Viabilities were done after 3, 6, 12, 24 h. Ca^{2+} was added for dapto.
Results: MIC ranges (μg/mL) were: NVP PDF-713, 0.25–2; cipro, 0.25 to >32; levo, 0.25–32; gati, 0.125–16; moxi, 0.03–8; vanco, 1–4; teico, 0.5–16; linez, 1–4; ranbez, 0.125–4; dapto, 0.125–2; tige,

Table.

	3 h			6 h			12 h			24 h		
Drug	−1	−2	−3	−1	−2	−3	−1	−2	−3	−1	−2	−3
NVP	0/0	0/0	0/0	1/1	1/1	0/0	1/2	1/1	0/0	0/0	0/0	0/0
Cipro	3/5	1/2	0/0	6/7	2/3	2/2	6/7	2/5	2/3	6/7	2/7	2/4
Levo	4/5	1/2	0/0	8/8	3/5	1/1	9/9	4/8	1/5	9/9	7/8	3/7
Gati	6/10	2/2	0/0	10/12	5/9	3/4	11/12	9/12	2/7	10/12	7/12	3/8
Moxi	8/10	2/5	0/0	9/11	4/9	1/3	10/12	7/9	2/7	8/12	3/9	2/7
Vanco	33	0/0	0/0	9/10	1/2	0/0	11/12	4/9	3/3	8/12	5/10	3/9
Teico	02	0/0	0/0	4/8	0/0	0/0	10/10	4/6	0/0	9/12	6/11	3/6
Linezolid	00	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0
Ranbez	00	0/0	0/0	1/1	0/0	0/0	1/3	0/0	0/0	2/10	0/1	0/0
Depto	911	6/9	0/5	12/12	9/11	6/9	12/12	11/12	5/10	11/12	10/12	8/11
Tigecyt	00	0/0	0/0	0/0	0/0	0/0	0/2	0/1	0/0	0/6	0/0	0/0
Oritavan	1212	10/12	2/7	12/12	12/12	8/12	11/12	9/11	9/10	8/12	6/12	6/12
Quin/Dalf	00	0/0	0/0	4/5	0/0	0/0	5/7	1/2	0/0	5/8	1/3	0/1

0.25–1; orita, 0.25–1; quinu/dalfo, 0.125–1. No. of strains at MIC/2 × MIC with delta-1 log 10 CFU/mL (90%), delta-2 log 10 CFU/mL (99%) and delta-3 log 10 CFU/mL (99.9%) Killing at the various time periods are shown in Table 1. NVP PDF-713 was not cidal at 1 × MIC and 2 × MIC, but was static against all 12 strains at MIC after 24 h. Cipro and moxi were cidal against four to seven strains at 2 × MIC after 24 h. Vanco was cidal at 2 × MIC for nine strains after 24 h. Oxazolidinones, tigec and quinu/dalfo were mainly bacteriostatic and dapto and orita rapidly cidal.
Conclusions: NVP PDF-713 gave low MICs and static activity against all strains, irrespective of methicillin susceptibility status.

P920 Time-kill study of the antipneumococcal activity of NVP PDF-713, a new peptide deformylase inhibitor, compared with 13 other agents

P. Appelbaum, G. Pankuch, M. Jacobs
Hershey, Cleveland, USA

Background: Drug-resistant pneumococci are an increasing worldwide problem.
Objective: NVP PDF-713 is a new peptide deformylase inhibitor. This study used time-kill analysis to examine the antipneumococcal activity of NVP PDF-713 compared with imipenem, meropenem, ceftriaxone, moxifloxacin, levofloxacin, gatifloxacin, azithromycin, clarithromycin, vancomycin, teicoplanin, linezolid, daptomycin, and quinupristin/dalfopristin. Twelve strains were tested: Three penicillin sensitive, two intermediate, and seven resistant pneumococci. Of the 12 strains tested; 10 were macrolide resistant [4 erm (B), 4 mef, 2 L4], and two quinolone resistant. Methodology: NCCLS macrodilution MIC methodology was used. Time-kill analyses were in cation-adjusted Mueller–Hinton broth with 5% lysed horse blood, and final inocula of 5×10^5–5×10^6 CFU/mL. Mueller–Hinton broth was supplemented to a final concentration of 50 mg Ca^{2+}/L for testing daptomycin. Viability counts were done after 0, 3, 6, 12, and 24 h.
Results: MICs (μg/mL) were as follows: NVP PDF-713, 0.125–2.0; imipen, 0.004–0.25; meropen, 0.008–1.0; ceftriax, 0.016–4.0; moxi, 0.125–4.0; levo, 1.0–16; gati, 0.25–8.0; azithro, 0.06 to >64; clarithro, 0.016 to >64; vanco 0.25–0.5; teico, 0.06–0.125; linez, 0.5–2.0; dapto, 0.125–0.5; quin/dal, 0.25–1.0. The number of strains at MIC/2 × MIC with log 10 CFU/mL values of −1 (90% killing), −2 (99% killing) and −3 (99.9% killing) at the various time periods are shown in Table 1.
Conclusions: NVP PDF-713 had kill kinetics similar to those of linezolid. NVP PDF-713 at 2 × MIC was bactericidal (99.9% killing) against six strains after 24 h. Linezolid at 2 × MIC was bactericidal against seven strains at the same period. Daptomycin and quinupristin/dalfopristin showed rapid killing. Imipenem, meropenem, vancomycin, and quinupristin/dalfopristin were bactericidal against all 12 strains at 2 × MIC after 24 h.

Table.

	3 h			6 h			12 h			24 h		
Drug	−1	−2	−3	−1	−2	−3	−1	−2	−3	−1	−2	−3
NVP	0/0	0/0	0/0	2/3	0/1	0/0	8/9	1/2	1/2	7/10	4/8	2/6
Imipen	11/12	4/5	0/0	11/12	6/10	3/3	12/12	11/12	8/11	12/12	11/12	10/12
Meropen	8/9	2/3	1/1	11/12	7/7	3/3	11/12	7/11	4/9	8/12	7/12	5/12
Ceftriax	4/6	1/2	0/0	8/12	5/7	3/3	9/12	9/12	6/10	11/12	8/12	4/10
Moxi[1]	5/8	0/1	0/0	8/10	4/6	1/2	9/10	8/10	5/9	10/10	8/10	7/10
Levo[1]	5/5	0/0	0/0	8/10	4/6	1/1	10/10	8/10	3/6	9/10	9/10	5/10
Gati[1]	4/6	0/1	0/0	7/10	2/7	1/1	10/10	6/10	3/7	9/10	8/10	4/9
Azithro[2]	1/1	0/0	0/0	1/1	1/1	1/1	2/2	1/2	1/2	2/2	2/2	2/2
Clarithro[2]	0/0	0/0	0/0	2/2	1/2	0/0	2/2	2/2	2/2	2/2	2/2	2/2
Vanco	4/4	0/1	0/0	12/12	5/6	2/3	12/12	11/11	9/9	11/12	10/12	9/12
Teico	0/0	0/0	0/0	5/6	1/1	0/0	9/10	6/7	3/4	9/12	8/12	7/10
Linez	0/0	0/0	0/0	2/4	0/1	0/1	6/11	1/4	0/1	8/12	4/11	2/7
Depto	7/10	2/4	2/2	12/12	11/11	4/8	11/12	10/11	7/11	8/12	5/11	3/11
Quin/Dal	10/11	7/9	4/5	12/12	11/11	6/9	11/12	10/11	7/11	7/12	5/12	3/12

[1]Ten strains cipro MIC <8.0. [2]Two macrolide sensitive strains.

P921 *In vivo* pharmacodynamics of NVP-PDF-713, a new peptide deformylase inhibitor

W. Craig, D. Andes
Madison, USA

Objectives: NVP-PDF-713 is a new peptide deformylase inhibitor antimicrobial with excellent activity against Gram-positive cocci, including methicillin-resistant *Staphylococcus aureus* (MRSA) and penicillin-resistant *Streptococcus pneumoniae* (PRSP). We used the neutropenic murine thigh-infection model to measure *in vivo* postantibiotic effects (PAEs) and determine which PK/PD parameter best correlated with *in vivo* efficacy.
Methods: Mice had 106.6–7.4 CFU/thigh of *Staphylococcus aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC 10813 when treated for 24 h with 40–1280 mg/kg/day of NVP-PDF-713 fractionated for 3-, 6-, 12-, and 24-h dosing. Mice were sacrificed at the end of therapy. Ten per cent thigh homogenates were prepared, and serial dilutions were plated for CFU determinations. Serum levels after both oral and subcutaneous injection of doses of 20, 80 and 320 mg/kg were measured by microbiologic assay. Non-linear regression analysis was used to determine which PK/PD parameter (24-h AUC/MIC, peak/MIC or time above MIC) best correlated with CFU/thigh at 24 h. *In vivo* PAEs were measured from serial 2–6 h CFU/thigh values after doses of 80 and 320 mg/kg.
Results: Pharmacokinetic studies exhibited linear kinetics with doses from 20 to 320 mg/kg, with peak/dose values of 0.10–0.12, AUC/dose values of 0.09–0.11 and half-lives of 26–30 min. Oral bioavailability was 67–88%. Protein binding in mouse serum was low at 25%. NAV-PDF-713 produced *in vivo* PAEs of 3–5 h with *S. aureus* and 10–13.5 h with *S. pneumonia*e. The 24-h AUC/MIC was highly correlated with efficacy ($R^2 = 84$–87% for 24-h AUC/MIC compared with 34–76% for peak/MIC and 40–60% for time above MIC for *S. pneumonia*e and *S. aureus*, respectively). Because of the rapid half-life in mice, once-daily dosing was slightly less effective than the more frequent dosing regimens.
Conclusions: The 24-h AUC/MIC is the parameter that best correlates with *in vivo* activity of NVP-PDF-713. The prolonged *in vivo* PAEs would support at least twice daily dosing.

P922 *In vivo* pharmacodynamic activity of NVP-PDF-713 against multiple bacterial pathogens

W. Craig, D. Andes
Madison, Wisconsin, USA

Objectives: The 24-h AUC/MIC is the PK/PD parameter that best correlates with *in vivo* activity of NVP-PDF-713, a new peptide deformylase inhibitor. We used the murine thigh-infection model

in normal and neutropenic mice to determine (1) the magnitude of the 24-h AUC/MIC needed for efficacy of NVP-PDF-713 with various pathogens (including MRSA and penicillin-, macrolide- and tetracycline-resistant strains of *S. pneumoniae*) and (2) the impact of neutrophils on the drug's *in vivo* activity.
Methods: Mice had 106.7–7.9 CFU/thigh of five isolates of *Staphylococcus aureus* (two MRSA) and six isolates of *Streptococcus pneumoniae* (five penicillin-resistant, four macrolide-resistant, three tetracycline-resistant strains) when treated for 24 h with 20–320 mg/kg of NVP-PDF-713 subcutantously every 6 h. *Streptococcus pneumoniae* ATCC10813 and *Staphylococcus aureus* ATCC 29213 were studied simultaneously in normal and neutropenic mice. Mice were sacrificed at the start and end of therapy. Ten per cent thigh homogenates were prepared and serial dilutions were plated for CFU determinations. Serum levels were determined by microbiologic assay after subcutaneous doses of 20, 80 and 320 mg/kg. A sigmoid dose–response model was used to estimate the dose (mg/kg/24 h) required to achieve a net bacteriostatic effect over 24 h.
Results: PK studies exhibited linear kinetics with AUC/dose values 0.09–0.11 and half-lives of 26–30 min. Protein binding was 25%. MICs ranged from 0.5 to 2.0 mg/L. Static doses for the various organisms ranged from 48 to 124 mg/kg/day. Mean 24-h AUC(free)/MIC values (±SD) were 33.3 ± 8.9 for *S. aureus* and 34.9 ± 10.0 for *S. pneumoniae*. The differences were not significant. Methicillin and penicillin resistance did not alter the magnitude of the AUC/MIC required for efficacy. The presence of neutrophils reduced the 24-AUC(free)/MIC required for efficacy by about fourfold.
Conclusion: The 24-h AUC/MIC of NVP-PDF-713 required for *in vivo* efficacy was relatively similar among various pathogens, was not altered by drug resistance, and was reduced fourfold by the presence of neutrophils.

P923 Determination of quality control guidelines for MIC dilution and disk diffusion methods when testing NVP-PDF713, a novel peptide deformylase inhibitor

T. Fritsche, T. Anderegg, R. Jones
North Liberty, USA

Background: Quality control (QC) guidelines remain necessary for accurate determination of antimicrobial susceptibility testing and should be established early in the development of new antimicrobial classes. NVP-PDF713 is a PDF inhibitor rapidly progressing into Phase II and III human clinical trials, thus QC guidelines appear necessary for NCCLS methods.
Methods: Multi-laboratory (seven or eight sites) trials were initiated using the NCCLS M23-A2 guideline for QC determinations. Key technical details were: MIC phase – four Mueller–Hinton (MH) broth lots, eight participant sites and 10 replicates of four appropriate QC strains; and disk diffusion phase – three MH agar lots, seven sites and 10 replicates of three QC strains. Results were analysed by statistical methods found in M23-A2. Control drugs included vancomycin, clarithromycin, linezolid and levofloxacin; 99.9–100.0% of control results were within published NCCLS ranges (640 and 1050 results for MIC and zone tests, respectively). Inoculum concentration controls averaged 3.5×10^5 (MIC trial only).
Results: Seven or eight participants provided qualifying results in the two separate QC studies, and the calculated (proposed) ranges were (range; % results in range): *E. faecalis* ATCC 29212 (2–8 mg/L; 95.6), *S. aureus* ATCC 29213 (0.5–2 mg/L; 99.4), *S. pneumoniae* ATCC 49619 (0.25–1 mg/L; 97.5 and 30–37 mm; 97.6), *H. influenzae* ATCC 49247 (1–4 mg/L; 97.5 and 24–32 mm; 99.8), and *S. aureus* ATCC 25923 (25–35 mm; 97.8). All QC ranges were maximised to contain ≈95% of reported results and zone sise variation was elevated due to the bacteriostatic character of this PDF inhibitor, creating non-discreet zone edges.
Conclusions: QC ranges for NCCLS methods when testing NVP-PDF713 have been established. Results from these NCCLS M23-A2-conforming trials can be utilised to control the accuracy of the susceptibility testing of this PDF inhibitor projected to be among the 'first' to reach human clinical studies.

P924 Determination of dry-form commercial reagent reproducibility and MIC validations for NVP-PDF713, a novel peptide deformylase inhibitor

G. Moet, R. Jones, P. Rhomberg, T. Fritsche
North Liberty, USA

Background: NVP-PDF713 is a new PDF inhibitor rapidly being advanced to human clinical trials. Commercial reagent broth microdilution MIC panels will be required for investigator laboratory use, especially those products with extended shelf-lives (dry-form). This study reports the results of reagent qualifying tests.
Methods: The experiment was performed by NCCLS M23-A2 guidelines to assess dry-form MIC reproducibility (10 organisms $\times$ 3 tests/day $\times$ 3 days $=$ 90 tests) and comparative MIC accuracy to the reference MIC (REF; M7-A6, 2003) using ≈100 strains representing the following organism groups: staphylococci, enterococci, *S. pneumoniae*, other streptococci, *H. influenzae*, and selected species refractory to PDF inhibitor action. All trays were manufactured by Sensititre (TREK Diagnostics, Cleveland, OH).
Results: Reproducibility results showed 80% of MICs were identical and 97.8% of MICs were within one log 2 dilution step. Validation test results comparing dry-form to REF MICs were (% identical/twofold/fourfold): for staphylococci (71/99/100%), for enterococci (55/99/100%), for *S. pneumoniae* (33/91/97%), for other streptococci (69/100/100%) and for *H. influenzae* (36/97/100%). Consistent variations were detected with SPN (49% of dry-form panel results being one dilution higher than REF) and HI (60% of results being one dilution lower than REF). NVP-PDF713 MICs were off-scale (MIC values, >32 mg/L) for Enterobacteriaceae and non-fermentative Gram-negative bacilli (40 strains). Overall, 97% of Sensititre MIC results for were within one log 2 dilution of REF MIC values.
Conclusions: NVP-PDF713 dry-form diagnostic MIC panels have been validated for accuracy and reproducibility using 520 recent clinical isolates from five major pathogen groups. The spectrum of activity for this PDF inhibitor compound appears focused toward Gram-positive cocci and specific fastidious respiratory tract pathogens.

P925 Activity of BAY 73-7388, a novel aminomethylcycline, and other novel antibiotic classes against resistant bacteria *in vitro*

B. Bhatia, T. Bowser, J. Chen, M. Ismail, L. Honeyman, R. Mechiche, M. Nelson, K. Ohemeng, A. Verma, K. Tanaka, A. Macone
Boston, USA

Objectives: The emergence of antibiotic resistance among Gram-positive pathogens has impacted the clinical management of these infections. Paratek Pharmaceuticals initiated a programme to apply medicinal chemistry to the core structure of tetracycline (TET) with the goal of creating novel classes of proprietary antibiotics that would (a) be unaffected by the known TET resistance mechanisms and (b) retain the safety and tolerability profile of the TET family. Since there is no cross-resistance between the TETs and other antibiotics, such new agents would be expected to be active against isolates resistant to all other currently available classes. The aim of the programme was to synthesise new agents

active against Gram-positive, common Gram-negative, atypical and anaerobic bacteria.
Methods: A series of 7-position and 7,9-position derivatives of sancycline were synthesised and tested for activity *in vitro* against MRSA, VRE, *Enterococcus faecalis* and *Streptococcus pneumoniae* by microdilution. The presence of TET-resistance determinants was assessed by PCR and confirmed by resistance to currently available TETs.
Results: A number of 7-dimethylamino-9-aminomethylcyclines (AMC) and 7-aryl or heteroaryl sancyclines with potent activity *in vitro* (MIC range less than or equal to 0.06–2.0 mg/L) were identified. Both novel series were more potent against one or more of the resistant strains than currently available antibiotics tested (MIC range 16–64 mg/L). The AMC derivatives were active against bacteria resistant to TET by both efflux and ribosome-protection mechanisms.
Conclusions: This study identified the AMCs as a novel class of antibiotics evolved from TET that exhibit potent activity *in vitro* against TET-resistant bacteria, including Gram-positive bacteria resistant to currently available antibiotics. One agent of this class, BAY 73-7388 (discovered by Paratek Pharmaceuticals, Inc., Boston, MA, and designated PTK 0796) has been chosen for development.

P926 Potent activity of BAY 73-7388, a novel aminomethylcycline, against susceptible and resistant Gram-positive and Gram-negative organisms

A. Macone, J. Donatelli, T. Dumont, S. Weir, S.B. Levy, K. Tanaka
Boston, USA

Objectives: BAY 73-7388 is the first of a new class of antibiotics, the aminomethylcyclines, which evolved from the tetracycline (TET) family. BAY 73-7388 has potent activity against antibiotic susceptible and resistant Gram-positive and Gram-negative pathogens. The present study compared the activity *in vitro* of BAY 73-7388 and 10 other agents including vancomycin (VAN), linezolid (LIN), levofloxacin (LVX) and TET against recent clinical isolates including MRSA, VAN-resistant *Enterococcus faecium* (Efa VRE), *Enterococcus faecalis* (Ef), penicillin-resistant *Streptococcus pneumoniae* (Spn PENR), Groups A and B beta-haemolytic streptococci (BHS), *Escherichia coli* (Ec), and other pathogens. Potential microbiological interactions between BAY 73-7388 and other antibiotics were also assessed.
Methods: Microdilution MIC tests were performed according to NCCLS guidelines. The activity of BAY 73-7388 in the presence of other antibacterial agents was assessed using standard chequerboard MIC methods. TET-resistance determinants were identified using multiplex PCR.
Results: Susceptibility *in vitro* (MIC90 mg/L) for selected agents is shown in the table below.
Conclusions: BAY 73-7388 has potent activity *in vitro* against a range of common pathogens, including those resistant to currently available antibiotics: most notably MRSA, VRE and penicillin-resistant *S. pneumonia*e. Chequerboard studies *in vitro* demonstrate BAY 73-7388 does not affect, and is not affected by, the activity of other antibiotics. (BAY 73-7388 was discovered by Paratek Pharmaceuticals Inc., Boston, MA, and designated PTK 0796.)

Table. 1

	MIC 90 (mg/L)			
Strains	BAY 73-7388	VAN	LIN	LVX
MRSA ($n = 39$)	0.5	1.0	2.0	32.0
Efa VRE ($n = 19$)	0.5	>64.0	2.0	>64.0
Ef ($n = 31$)	0.5	2.0	2.0	32.0
Spn PENR ($n = 23$)	0.06	0.25	1.0	1.0
BHS ($n = 48$)	0.25	0.5	1.0	0.5
Ec ($n = 23$)	2.0	NA	NA	4.0

P927 BAY 73-7388, a novel aminomethylcycline, exhibits potent efficacy in pulmonary murine models of infection

D. McKenney, J.M. Quinn, C.L. Jackson, J.L. Guilmet, J.A. Landry, K. Tanaka, E.P. Cannon
Boston, USA

Objective: With the emergence of resistance to currently available antibiotics in the treatment of infectious diseases, the development of novel antibiotic classes has become of major importance. BAY 73-7388 is the first aminomethylcycline antibacterial agent and is characterised by potent activity *in vitro* against sensitive and multi-antibiotic resistant Gram-positive, Gram-negative and atypical bacteria. We have evaluated BAY 73-7388 in several murine pulmonary infection models with a range of pathogens in both neutropenic (Neut) and immunocompetent (IC) mice.
Methods: BAY 73-7388 and reference antibiotics were evaluated in acute, systemic lethal infections caused by multi-resistant (res) and susceptible (sus) *Streptococcus pneumoniae* (Spn); acute, lethal pulmonary Spn infection in Neut mice; chronic, Spn lung model in IC mice; and chronic *Haemophilus influenzae* (Hflu) infection model in IC mice. In each infection BAY 73-7388 and other antibiotics were administered i.v.
Results: PD50 (survival) and ED50 (bacterial burden) results for BAY 73-7388 and comparators, vancomycin (VAN), linezolid (LIN), ciprofloxacin (CIP), azithromycin (AZI) and doxycycline (DOX) against sus and res strains of Spn and Hflu (sus), are detailed in the table below.
Conclusions: Overall, BAY 73-7388 performed as well or better than the currently available therapeutic agents in all the models investigated in this study. (BAY 73-7388 was discovered by Paratek Pharmaceuticals Inc., Boston, MA, and designated PTK 0796.)

Table. 1

	Efficacy (mg/kg)					
In vivo model	BAY 73-7388	AZI	CIP	LIN	VAN	DOX
Acute, systemic Spn, IC (sus) (PD50)	0.09	2.21	>50	3.5	1.4	1.8
Acute, systemic Spn, Neut (res) (PD50)	0.14	18.9	21.3	7.1	0.14	>50
Chronic Spn,IC (ED50)	7.4	5.15	>50	>40	>40	31.6
Chronic Hflu,IC (ED50)	4.7	31.6	1.0	NA	NA	18.6
Acute, pulmonary Spn, Neut (PD50)	11.0	7.5	31.6	>40	7.2	>50
Acute, pulmonary Spn, Neut (res) (PD50)	8.5	>50	>50	>40	5.4	>50

P928 BAY 73-7388 is highly efficacious in animal models of intra-abdominal infections caused by a range of aerobic and anaerobic organisms, including VRE

R. Endermann, C.H. Ladel, H. Broetz-Oesterhelt, H. Labischinski
Wuppertal, D

Objective: BAY 73-7388, a novel antibiotic compound from the aminomethylcycline class, has an antibacterial spectrum encompassing Gram-positive, Gram-negative and anaerobic bacteria, including those resistant to currently available antibiotics. The

efficacy of BAY 73-7388 in four different mouse infection models with pathogens causing intra-abdominal infections was compared with that of vancomycin (VAN), linezolid (LIN), imipenem (IMI) and metronidazole (MTN).
Methods: For systemic infections (sepsis), enterococci (tetracycline (TET)-resistant *Enterococcus faecalis* or *Enterococcus faecium* VRE) were administered intraperitoneally, and i.v. treatment was started 30 min post-infection; survival of the infected mice until day 5 was used as read-out. In the pouch model using *Bacteroides fragilis* as infecting pathogen, therapeutic efficacy of BAY 73-7388 compared with MTN was determined as reduction of CFU. The mouse model of caecal ligation was used as a model for polymicrobial peritonitis after surgical intervention and 10 days survival used as read-out.
Results: In systemic infections with TET-resistant *E. faecalis* or *E. faecium* VRE, efficacy of BAY 73-7388 was superior to VAN or LIN: 100% survival was observed at 1 mg/kg BAY 73-7388, 10 mg/kg VAN and 3 mg/kg LIN. For the *E. faecium* septicaemia model, 100% survival was found at 15 mg/kg BAY 73-7388, while neither VAN nor LIN treatment resulted in 100% survival, even at 50 mg/kg, the highest dose tested. In the pouch model with *B. fragilis*, the CFU reduction caused by BAY 73-7388 was superior to MTN (CFU reduction >6 log compared with 4 log at 25 mg/kg, respectively). Therapy (2×10 mg/kg i.v. on day 1) of intra-abdominal infections and post-operative polymicrobial peritonitis with BAY 73-7388 showed increased survival compared with IMI or LIN (80 vs. 70 vs. 30%, respectively).
Conclusions: Against pathogens causing intra-abdominal infections (including VRE and TET-resistant strains), BAY 73-7388 demonstrated superior therapeutic efficacy compared with VAN, LIN, MTN or IMI. (BAY 73-7388 was discovered by Paratek Pharmaceuticals, Inc., Boston, MA, and designated PTK 0796.)

P929 BAY 73-7388 demonstrates greater activity than linezolid in a range of murine models of skin and soft tissue infection

C.H. Ladel, R. Endermann, H. Broetz-Oesterhelt, H. Labischinski
Wuppertal, D

BAY 73-7388 is a novel antibiotic compound being developed for the treatment of severe bacterial infections. It is the first compound selected from the novel class of aminomethylcyclines and was designed to meet an increasingly significant need for additional therapies for treatment of infections, including those resistant to currently available antibiotics. The efficacy of BAY 73-7388 in different mouse models of skin and soft tissue infection (SSTI) was compared with that of vancomycin (VAN) and linezolid (LIN).
Methods: Two mouse models were employed to determine the efficacy of BAY 73-7388: (1) infected abscess model (induced by implantation and subsequent infection of Gelfoam (TM)) and (2) infected thigh muscle model in neutropenic mice. *Staphylococcus aureus* strain DSM11823 (MSSA) was used to infect the respective structures in the skin and soft tissues. Infected abscess bearing mice were treated i.v. bid for 2 days, while thigh muscle infection model mice were treated s.c. 30 min post-infection. CFU reduction of infected tissues and bacterial load in different organs (spread from the infection site) were used as read-out for therapeutic efficacy.
Results: As measured by reduction of bacterial load, therapy of infected abscesses with BAY 73-7388 (CFU reduction >4 log units at 10 mg/kg) was superior to VAN and LIN (no reduction in bacterial load). Furthermore, BAY 73-7388 reduced the overall bacterial load in spleen, liver, lung and heart. In the reduction of organ load, BAY 73-7388 was as efficacious as VAN or LIN. In the mouse thigh muscle infection model, BAY 73-7388 proved to be as least as effective as VAN (2 log CFU reduction) and superior to LIN, which demonstrated no efficacy at the same dose (0.5 log CFU increase).
Conclusions: BAY 73-7388 was clearly more potent than LIN and VAN in the infected abscess model, and more potent than LIN and at least as effective as VAN in the thigh wound model. (BAY 73-7388 was discovered by Paratek Pharmaceuticals, Inc., Boston, MA, and designated PTK 0796.)

P930 Superior efficacy of BAY 73-7388, a novel aminomethylcycline, compared with linezolid and vancomycin in murine sepsis caused by susceptible or multiresistant staphylococci

H. Broetz-Oesterhelt, R. Endermann, C.H. Ladel, H. Labischinski
Wuppertal, D

Objective: BAY 73-7388 is the first compound selected from the novel class of aminomethylcyclines and was designed to meet an increasingly significant need for additional therapies for treatment of bacterial infections, including those resistant to currently available antibiotics. The increasing frequency of multi-resistant staphylococci is of particular concern in the clinical setting. Therefore, the efficacy of BAY 73-7388 was compared with that of vancomycin (VAN) and linezolid (LIN) in different mouse models of staphylococcal infection.
Methods: Murine sepsis was used to determine the efficacy of BAY 73-7388 compared with VAN and LIN. For systemic infections, *Staphylococcus aureus* MSSA/quinolone-resistant MRSA and *Staphylococcus epidermidis* (MRSE, TET-resistant) were administered intraperitoneally, and i.v. treatment was started 30 min post-infection. Survival of the infected mice was monitored until day 5. For the investigation in immunocompromised animals, mice were rendered neutropenic by two injections of 150 and 100 mg/kg cyclophosphamide at days 4 and 1 prior to infection.
Results: In systemic infections with MSSA, BAY 73-7388 was more effective than VAN or LIN resulting in 100% survival at 0.3 mg/kg compared with 10 and 10 mg/kg, respectively. The efficacy of BAY 73-7388 therapy on systemic quinolone-resistant MRSA and MRSE infection was also pronounced (100% survival for quinolone-resistant MRSA at 3 mg/kg compared with >10 and >10 mg/kg, respectively; 100% survival for MRSE at 1 mg/kg compared with 10 and >10 mg/kg, respectively). Moreover, in neutropenic mice, BAY 73-7388 was the only curative agent (100% survival for BAY 73-7388 at 50 mg/kg). In contrast there was zero survival in the VAN and the LIN groups at the highest dosage tested, 50 mg/kg.
Conclusions: Treatment with BAY 73-7388 is highly effective in systemic staphylococcal infections (susceptible and multi-resistant strains) in mice, and is superior compared with LIN and VAN. (BAY 73-7388 was discovered by Paratek Pharmaceuticals, Inc., Boston, MA, and designated PTK 0796.)

P931 BAY 73-7388, a novel aminomethylcycline, is highly active *in vivo* in a murine model of pneumococcal pneumonia

R. Endermann, C.H. Ladel, H. Broetz-Oesterhelt, H. Labischinski
Wuppertal, D

Objectives: BAY 73-7388 is the first antibiotic compound from the novel class, the aminomethylcyclines. BAY 73-7388 is being investigated for the treatment of severe bacterial infections including those caused by strains resistant to current classes of antibiotics. The efficacy of BAY 73-7388 was compared with that of vancomycin (VAN) and linezolid (LIN) in a mouse model of pneumococcal pneumonia.

Methods: For lung infections, *Streptococcus pneumoniae* (strain L3 TV, Serotype 3) was administered intranasally to 5 mice per group, followed by i.v. antibiotic treatment bid over 2 days. The bacterial counts in the lungs on day 4 were used as read-out. Survival was monitored until day 4 post-infection.
Results: (a) CFU: Bacterial CFU in the lung at day 4 were lower with BAY 73-7388 and VAN in comparison with LIN therapy (reduction in CFU >6 log units at 1 mg/kg BAY 73-7388 vs. no reduction in CFU at the same doses of LIN). A 4.5 log unit CFU reduction was only obtained at a 10 mg/kg dose of LIN. (b) Survival assays: For BAY 73-7388 and VAN all mice survived at the three tested doses (1, 3 and 10 mg/kg). In contrast for LIN 0, 2, and 4 animals survived when tested at the same dose range.
Conclusions: Treatment with BAY 73-7388 is highly effective in an animal model of pneumococcal pneumonia. (BAY 73-7388 was discovered by Paratek Pharmaceuticals, Inc., Boston, MA, and designated PTK 0796.)

P932 Activity of dalbavancin against European clinical isolates of Staphylococci and Streptococci

R. Flamm, D. Draghi, M. Jones, C. Thornsberry, J. Karlowsky, D. Sahm
Herndon, USA

Objectives: Dalbavancin (DAL) is a semi-synthetic derivative of the glycopeptide A40926 that has shown potent *in vitro* activity against *Staphylococcus aureus* (SA) and coagulase-negative staphylococci (CNS). DAL has a long half-life (9–12 days), which offers promise for a convenient weekly dosing regimen. The current study surveyed DAL activity against isolates from Europe and provides a baseline to monitor the activity of DAL against common Gram-positive pathogens.
Methods: DAL and comparator agents were tested against 152 SA, 153 CNS, 99 *S. pyogenes* (SPY), 57 *S. pneumonia*e (SP) and 25 *S. agalactiae* (SAG) by the NCCLS broth microdilution method. Clinical isolates collected through 2003 from patients in Europe were chosen to include phenotypes both susceptible (S) and resistant (R) to clinically available antibiotics.
Results: The DAL MIC90 was 0.06 mg/L for all SA and 0.12 mg/L for all CNS. DAL had similar activity for both oxacillin (OX) S (MIC90, 0.06 mg/L) and OX R (MIC90, 0.12 mg/L) SA and CNS isolates. DAL MICs ranged from 0.25 to 0.5 mg/L against four SA that had vancomycin (VAN) MICs $\geq$4 mg/L and 0.03 mg/L against one SA that was non-susceptible to linezolid. Potent activity was demonstrated against 97 SA and 65 CNS multi-drug resistant (MDR) isolates as defined by resistance to $\geq$3 antimicrobial classes. The MIC90s (mg/L) for DAL against MDR SA and CNS were 0.12 and 0.25, respectively and against non-MDR SA and CNS were 0.06 for both. DAL had potent activity against streptococci with a MIC90 of 0.015 mg/L for all SPY including erythromycin (ERY) R isolates, 0.03 mg/L for all SP including penicillin (PEN) and ERY R isolates, and 0.015 mg/L for all SAG. The MIC90s for DAL against both non-MDR ($n = 39$) and MDR ($n = 18$) SP were 0.03 mg/L.
Conclusions: DAL showed potent *in vitro* activity against staphylococci and streptococci including resistant and MDR phenotypes. DAL, currently in Phase 3 clinical trials for use in complicated and uncomplicated skin and skin structure infections, may serve as a potent therapeutic choice against Gram-positive infections.

P933 *In vitro* antistaphylococcal and antistreptococcal activity of dalbavancin, assessed by British & NCCLS methods

S. Mushtaq, M. Warner, D.M. Livermore
London, UK

Objectives: Dalbavancin is a new long-half-life (*c.* 10 days) glycopeptide, now in Phase III development. Its pharmacokinetics support weekly dosing, and may facilitate home i.v. therapy and/or early hospital discharge. It was tested, *in vitro*, versus species that are major agents of Gram-positive infections in neutropenic patients and of endocarditis.
Methods: MICs were determined by the British Society for Antimicrobial Chemotherapy (BSAC) and the National Committee for Clinical Laboratory Standards (NCCLS) agar dilution methods for 92–94 isolates each of *S. aureus*, coagulase-negative staphylococci (CNS) and viridans streptococci. The test panels over-represented strains with resistance to conventional agents, including, for staphylococci, teicoplanin.
Results: By BSAC methodology, the MIC ranges of dalbavancin for *S. aureus*, CNS and viridans streptococci were 0.06–1, 0.12–1 and <0.03–0.5 mg/L, respectively, with unimodal distributions and with mode values of 0.25, 0.25 and 0.12 mg/L, respectively. Corresponding MIC ranges by NCCLS methodology were 0.016–2, 0.06–1 and <0.03–0.5 mg/L, respectively, with modes of 0.25, 0.25 and 0.12 mg/L. In general, the MICs of dalbavancin by the BSAC and NCCLS methods were identical, or else those by the BSAC method were twofold lower than by the NCCLS method; in either case the MICs for each study species mostly were 2- to 16-fold below those of vancomycin. Against teicoplanin-non-susceptible *S. aureus* strains (MIC 8–16 mg/L) dalbavancin was still highly active although its MICs were slightly raised (0.5–2 mg/L, varying with the particular strain and method). For CNS isolates, dalbavancin MICs were tightly clustered and – as with vancomycin – there was no consistent increase even for strains with teicoplanin MICs of 16–64 mg/L. MICs of dalbavancin (and other glycopeptides) were independent of those of non-glycopeptide agents, including beta-lactams, gentamicin, rifampicin and linezolid.
Conclusion: Dalbavancin has good activity versus the streptococcal and staphylococcal species that are common agents of neutropenic fevers and endocarditis. Full activity was retained against teicoplanin-resistant CNS. MIC determination by the BSAC and NCCLS agar dilution methods gave similar results, though slightly lower MICs have been recorded elsewhere by broth microdilution.

P934 Efficacy and safety of once-weekly dalbavancin vs. vancomycin in catheter-related bloodstream infections

E. Seltzer, M. Wible, B.P. Goldstein, M.B. Dorr, T.J. Henkel, R. Darouiche, J. Vazquez, I. Raad
King of Prussia, Houston, Detroit, USA

Background: DAL is a novel semisynthetic glycopeptide in phase 3 clinical development, more active *in vitro* and in animal models than vancomycin or teicoplanin. The PK and *in vitro* and *in vivo* activity of DAL against Gram-positive bacteria, including most drug resistant strains, predict that weekly dosing will be effective.
Objectives: To compare the safety and efficacy of DAL vs. VAN in patients (pts) with CRBSI.
Methods: Pts were required to have a central venous catheter at the onset of signs and symptoms of CRBSI. Causative pathogens required at least one positive peripheral blood culture for *S. aureus* and at least two for other Gram-positive bacteria; coagulase negative staphylococci (CoNS) were verified as identical pathogens by pulsed field gel electrophoresis. Pts were randomised to DAL 1 g IV on day 1, 500 mg IV on day 8 vs. VAN 1 g IV BID for 7–14 days. A single dose of DAL or 7 days of VAN was allowed in cases of CoNS CRBSI with catheter removal. Adverse events (AEs) and lab safety were assessed. The primary endpoint was overall [clinical and microbiological (micro)] response at follow up (FU), 21 days after end of therapy, in the micro-ITT population. The micro-ITT population included patients who had a baseline causative pathogen and took at least one dose of study drug.
Results: 67 pts were enrolled to receive DAL (33) or VAN (34). The most common pathogen was CoNS (46%), followed by *S. aureus* [39% (59% MRSA)] and *Enterococcus* sp. (14%). Outcomes in the primary population (micro ITT) were: DAL was well tolerated and the proportion of pts with AEs was similar across study arms. The majority of AEs were reported as mild to

Table. 1 Successful response of Fu (%, n/N)

Endpoint	DAL	VAN
Overall	87 (20/23)	50(14/18)
(95% CI)	(73, 100)	(31, 68.5)
Clinical	87 (20/23)	50(14/28)
Micro	96(22/23)	79 (22/28)

moderate. There were no related serious AEs in the DAL treatment group. Few deaths were reported in either arm DAL ($n = 1$) and VAN ($n = 2$). There were no trends in any lab abnormalities.
Conclusion: Weekly dosing of DAL was highly effective in CRBSI and superior to VAN. Overall, DAL was well tolerated. Further studies evaluating DAL in BSIs are warranted.

P935 Once-weekly dalbavancin in catheter-related bloodstream infections: microbiological findings

B.P. Goldstein, R.N. Jones, D.J. Biedenbach, E. Seltzer, M.B. Dorr, D. Krause, T.J. Henkel
King of Prussia, North Liberty, USA

Objectives: Dalbavancin (DAL), a novel semisynthetic glycopeptide in Phase 3 clinical development, is more active than vancomycin (VAN) or teicoplanin against Gram-positive bacteria, including antibiotic-resistant strains, *in vitro* and in animal models. The PK and activity of DAL predict that weekly dosing will be effective. The main objective of this Phase 2 randomised, controlled study was to compare the efficacy of DAL vs. VAN in patients (pt) with catheter-related bloodstream infections (CRBSI).
Methods: Pt had at least 1 central venous catheter and a positive peripheral blood culture for *S. aureus* or at least two cultures for other Gram-positive bacteria. Isolates were sent to a reference laboratory (RL) for definitive identification and MIC determinations. Duplicate isolates of coagulase negative staphylococci (CoNS) were verified as identical by pulsed field gel electrophoresis (PFGE). Treatment arms were: DAL 1 g IV on day 1 and 500 mg on day 8; VAN IV for 7–14 days.
Results: Among 67 pt enrolled (DAL 33, VAN 34) the most common baseline pathogens (BLP) were PFGE-confirmed CoNS (46%), *S. aureus* (39%) and *Enterococcus* sp. (14%). Of 54 BLP sent to the RL, 63% of *S. aureus* and 85% of CoNS were methicillin-resistant (MR) (Table). Per pathogen microbiological success rates in the microbiological ITT population at follow up were 92% (DAL) and 79% (VAN). DAL and VAN MICs are in the Table.
Conclusion: DAL had a higher response rate than VAN. As expected for CRBSI, CoNS were the predominant BLP and the majority of staphylococci were MR. DAL MICs were tightly clustered and lower than those of VAN, as previously observed in the *in vitro* surveys and in another clinical trial.

Table. 1 BLPs tested at the RL

	No. of isolates		MIC range [mg/L,both arms]	
Organism	DAL arm	VAN arm	DAL	VAN
CoNS	14 (11)*	13 (12)	0.03–0.25	0.25-1
S. aureus	8 (4)	11 (8)	0.03–0.12	0.5-2
E. faecalis	4	4	0.06	0.5–2

*In parenthesis for staphylococci, no. MRSA.

P936 Evaluations of dalbavancin activity and spectrum tested against European isolates

R. Jones, T. Fritsche, H. Sader
North Liberty, USA

Background: Ongoing development of novel resistances (R) and the increased frequency of currently characterised R requires the search for alternative antimicrobials. DAL (formerly BI397)) is an amide glycopeptide derivative of A40926 in late stage clinical development with an extended elimination half-life enabling once weekly dosing. A large collection of clinical strains were tested to establish DAL spectrum and potency against European isolates.
Methods: DAL and over 20 comparators were tested against 2881 recent (year 2002) clinical isolates from Europe using NCCLS (M7-A6) susceptibility (S) testing methods. The characteristics of this collection were: oxacillin (OXA)-R *S. aureus* = 31.1% and CoNS = 79.0%; VRE = 2.6–8.8% and penicillin (PEN)-non-susceptible pneumococci = 27.6%. Some fastidious Gram-negative species, *H. influenzae* (HI; 725), *M. catarrhalis* (MCAT; 238) were also tested.
Results: Species distribution and MIC data for highly represented Gram-positive species. OXA-, PEN- or macrolide-R had no effect on DAL potency among staphylococci or streptococci. DAL results for VRE were most similar to the teicoplanin-S rates overall (active against VanB strains). Twenty-four tested strains (*Bacillus* spp., *Corynebacterium* spp., *L. monocytogenes*, *Micrococcus* spp., *S. bovis*) not cited in the Table had a combined DAL MIC90 of 0.12 mg/L; highest MIC at 0.25 mg/L. DAL MICs were lower than those of available glycopeptides by 4- to >16-fold. DAL also had some activity against fastidious Gram-negative species, but MICs were higher (HI MIC90 at 32 mg/L and MCAT MIC90 4 mg/L).

Table. 1

	DAL MIC (mg/L):			
Organism (no. tested)	50%	90%	Range	% 1 mg/L
S. aureus (599)	0.06	0.06	0.015–0.25	100.0
Coag-neg staphylococci(247)	0.03	0.06	0.015–0.25	100.0
Enterococci(195)	0.03	0.06	0.015–>32	96.9
S. pneumoniae(726)	0.015	0.03	0.015–0.25	100.0
β-Streptococci(67)	0.015	0.03	0.015–0.12	100.0
Viridans group streptococci(65)	0.015	0.03	0.015–0.06	100.0

Conclusions: This DAL activity survey indicates that this new glycopeptide has significant Gram-positive activity (96.9–100.0% inhibited at ≤1 mg/L), superior to available agents in the class, and the potency was similar for European isolates when compared with prior experience in other geographic areas.

P937 *In vitro* activity of the glycylcycline tigecycline tested against a worldwide collection of 10 127 contemporary Staphylococci, Streptococci and Enterococci

T. Fritsche, H. Sader, J. Kirby, R. Jones
North Liberty, USA

Background: Tigecycline (TIG) is a novel glycylcycline with broad spectrum activity. Increasing reports of resistance (R) among commonly occurring Gram-positive cocci (GPC) that produce respiratory tract and skin and soft tissue infections has created a need for development of new antimicrobial agents. In this study the activity and potency of TIG, tetracycline (TC) and other comparator agents was evaluated using contemporary isolates of commonly occurring species of GPC, including the presence of R organism subsets.

Methods: The activity of TIG and nine comparators was challenged with a collection of GPC including oxacillin (OXA)-susceptible (S; 3196 strains) and -R (1881 strains) *S. aureus* (SA); OXA-S (321 strains) and -R (1111 strains) coagulase-negative staphylococci (CoNS); penicillin (PEN)-S (1126 strains) and non-susceptible (NS; 459 strains) *S. pneumoniae* (SPN); penicillin-S (161 strains) and -NS (51 strains) viridans-group streptococci (VGS); beta-haemolytic streptococci (BHS; 405 strains); and vancomycin-S (1294 strains) and -R (122 strains) enterococci (ENT). Broth microdilution susceptibility tests were performed and analysed using NCCLs reference methods and interpretive criteria.
Results: Whereas OXA-R subsets of both SA and CoNS displayed cross-resistance to TC, macrolides, clindamycin and quinolones, no differences were seen with TIG (MIC50/90 being 0.25 and 0.5 mg/L, respectively). Among streptococci, all SPN and VGS (regardless of PEN-S), and BHS demonstrated TIG MIC50/90s of $\leq$0.12 mg/L (one exception being PEN-intermediate VGS with the MIC90 at 0.25 mg/L). TIG was also uniformly active against enterococcal isolates, with MIC50/90s of vancomycin-S and -R subsets being 0.25 and 0.5 mg/L, and 0.12 and 0.25 mg/L, respectively. When using the NCCLS TC S breakpoint of $\leq$4 mg/L, all 10 127 staphylococci, streptococci and enterococci tested would be classified as S to TIG.
Conclusions: TIG displays a remarkable spectrum of activity and potency against S and R subsets of GPC with the highest MIC90 being 0.5 mg/L. In addition to for use in treating community-acquired respiratory tract infections, TIG may also be a candidate for treatment of complicated skin and soft tissue infections and, possibly, urinary tract infections caused by GPC.

P938 Endemic, highly resistant *Acinetobacter* in the intensive care unit – is tigecycline the answer?

P. Wilson
London, UK

Objective: To find satisfactory antibiotic treatment against an organism, *Acinetobacter baumanii*, that became endemic on the intensive care unit of a busy District General Hospital. This organism is resistant to many antibiotics and in one case was ultimately resistant to all currently marketed antibiotics.
Methods: (1) Surveillance of patients in the intensive care unit for the presence of *Acinetobacter baumanii*. (2) Clinical assessment of patients with the organism to establish those needing antibiotic therapy. (3) Patients requiring treatment were given an antibiotic combination using colistin (usually combined with oral minocycline) or tigecycline monotherapy, a first-in-class glycylcycline agent. (4) Treatment and outcome were monitored. The study was observational. Allocation to treatment categories was not randomised or blinded. The tigecycline was used on a compassionate basis.
Results: The intensive care unit was free of *Acinetobacter* until the beginning of 2001. By the end of 2001, 5–10 new isolates of *Acinetobacter baumanii* were isolated per quarter. Initially these pathogens were sensitive to imipenem, meropenem, tobramycin, amikacin, colistin, and minocycline. This sensitivity began to wane and, by the end of 2002, one patient had died with *Acinetobacter baumanii* in his bloodstream that was resistant to everything available. After this death, we tested further isolates of *Acinetobacter* against tigecycline, a new broad spectrum agent currently in Phase 3 development, and found it to be active against the endemic strain. Two patients with ventilator-associated pneumonia caused by this organism were treated with tigecycline and made a full recovery. There were no adverse effects related to tigecycline treatment. Conversely, five patients with ventilator-associated pneumonias caused by the same organism were treated with colistin and failed to respond. *Acinetobacter* finds the respiratory system a favourable environment, and this, combined with the fact that the vast majority of the patients were ventilated resulted in ventilator-associated pneumonia being the commonest infection.
Conclusion: Tigecycline is likely to be a useful agent in clinical practice on intensive care units when dealing with this difficult organism. Further evaluation is warranted. It may well be the antibiotic of choice.

P939 Antimicrobial activity of tigecycline (GAR-936) tested against enterobacteriaceae, and selected non-fermentative Gram-negative bacilli, a worldwide sample

R. Jones, T. Fritsche, H. Sader, M. Beach
North Liberty, USA

Background: As resistances (R) among Gram-negative bacilli (GNBs) expand, few antimicrobial agents have been developed to address this clinical problem. Tigecycline (TIG), a novel glycylcycline, has an expanded spectrum of activity and potency, tigecycline covers many routine Gram-negative resistant strains and additionally possesses activity versus some uncommonly isolated non-fermentative GNBs. This study compares TIG with contemporary broad-spectrum agents using recent clinical isolates from Europe and other continents.
Methods: All strains (2420) were centrally processed by reference, broth microdilution methods against more than 20 antimicrobials. All concurrent QC results were within NCCLS published ranges, with identifications performed by traditional methods and/or the Vitek System. Over 2400 isolates were tested from the Enterobacteriaceae (ENT) and non-fermentative GNBs categories. Susceptibility (S) for TIG was defined as $\leq$4 mg/L, that breakpoint used for all tetracyclines by the NCCLS.
Results: The ENT were divided into three groups for analysis: ESBL-producing isolates (154 strains), Proteae group (131 strains; includes *P. mirabilis* and indole-positive species) and all enteric bacilli. TIG was very active against all ESBL-producing isolates (MIC90, 0.25–2 mg/L; highest among TC-R subsets), and all ENT (MIC50/90, 0.25/1 mg/L). Proteae had a MIC90 at 4 mg/L and all but one of TIG-R or intermediate strains (MICs, 8 and 16 mg/L) were *M. morganii* or *P. mirabilis*. *P. aeruginosa* was marginally inhibited by TIG (MIC90, 32 mg/L). In contrast, *Acinetobacter* spp. (MIC90, 2 mg/L; 96.1% S) and *S. maltophilia* (MIC90, 2 mg/L; 100.0% S) were readily inhibited by TIG. Among all ENT studied, 31.0% were TC-R, but only one strain (*P. mirabilis*) was TIG-R (MIC at 16 mg/L).
Conclusions: Remarkable potency and breadth of spectrum was observed for TIG against ENT (99.4% at $\leq$4 mg/L vs. 66.8% for TC), *S. maltophilia* and *Acinetobacter* spp. Limited activity was noted versus *P. aeruginosa* (16.0% at $\leq$4 mg/L) and some Proteae (MIC90, 4 mg/L). TIG should be of value for the treatment of infections caused by several commonly R GNB groups.

P940 Activity of tigecycline tested against clinical isolates of *Haemophilus influenzae*, *Moraxella catarrhalis* and *Neisseria menigitidis*, a worldwide perspective

H. Sader, T. Fritsche, J. Kirby, R. Jones
North Liberty, USA

Background: Tigecycline (TIG, formerly GAR-936), is a novel glycylcycline which is currently in phase 3 clinical trials. The *in vitro* activity of the TIG was evaluated in comparison with tetracycline (TET) and other antimicrobial agents against recent (2000–2002) clinical isolates collected worldwide from patients with respiratory infections and meningitis.
Methods: A total of 1727 isolates were tested against TIG and more than 20 comparator agents by broth microdilution according to the NCCLS reference methods and interpretative criteria. The collection included, *H. influenzae* (HI; 1215 strains, 20% beta-lactamase-producing), *M. catarrhalis* (MCAT; 495 strains, 96% beta-lactamase-producing), and *N. meningitidis* (NM; 17 strains).
Results: TIG demonstrated excellent activity against these organisms with all isolates being inhibited at $\leq$4 mg/L (TET susceptibility breakpoint). TIG was highly active against HI (MIC90, 1 mg/L) and MCAT (MIC90, 0.25 mg/L), and its potency against these pathogens was not affected by beta-lactamase production. TIG was fourfold more potent than TET against HI and TET-resistant isolates showed low ($\leq$1 mg/L) TIG MICs. NM isolates were highly susceptible to TIG (MIC90, $\leq$0.12 mg/L) and to the vast majority of antimicrobial agents evaluated.

Conclusions: These results indicate that tigecycline has potent *in vitro* activity against clinically important Gram-negative bacteria that cause community-acquired respiratory infections and meningitis, including TET-R isolates. Further evaluations of TIG activity, as well as, clinical studies are necessary to assess the role of this compound in the treatment of both community- and hospital-acquired infections.

Molecular bacteriology: antibiotic susceptibility

P941 DNA microarray technology for detection of beta-lactamase genes in pathogenic bacteria

M.M. D'Andrea, M. Cassone, T. Giani, G. Coratza, G. Pozzi, G.M. Rossolini
Siena, I

Background and objectives: Beta-lactamase production is the major mechanism of bacterial resistance to beta-lactam antibiotics in Gram-negative pathogens, and surveillance of beta-lactamase determinants is an important issue of microbial drug resistance. Given the great diversity of beta-lactamases and their overlapping substrate specificities, molecular analysis is necessary to identify the nature of beta-lactamase genes in clinical isolates. In this work we investigated the potential of the DNA microarray technology for a rapid and comprehensive detection of beta-lactamase genes in drug-resistant bacteria.
Methods: A total of 104 oligonucleotide probes were designed for specific recognition of beta-lactamase genes of 65 different lineages (18 of molecular class A, 19 of class B, 13 of class C and 15 of class D). A DNA chip was designed including a triplicate set of probes, as well as positive hybridisation controls. The microarray was printed on epoxy-modified glass slides using an Affymetrix GMS 417 robotic spotter. Genomic DNA was labelled with Cy3 or Cy5 by random priming. Hybridisation signals were then detected using an Affymetrix 418 laser scanner and images were analysed by the GenePix Pro (version 5.0) software.
Results: The DNA chip was tested with 23 Gram-negative strains (including both reference strains and clinical isolates) in which the repertoire of beta-lactamase genes was partially known or unknown. All the predicted beta-lactamase genes (among which there were members of the blaTEM, blaSHV, blaCTX-M, blaPER, blaVIM, blaIMP, blaCMY-LAT groups of acquired genes) were correctly detected by microarray hybridisation. In clinical isolates of unknown beta-lactamase content, the microarray detected genes whose presence was subsequently confirmed by conventional PCR assays. False-positives were observed with a subset of probes, which had to be redesigned to overcome the problem.
Conclusions: Successful detection of several different beta-lactamase genes of clinical importance was achieved by using a DNA microchip. The DNA microarray technology appears to be a sensitive and specific tool for rapid detection and characterisation of beta-lactamase genes in clinical isolates.
Acknowledgement: This work was funded in part by grants from the University of Siena (P.A.R. 2001), and MURST (COFIN 2003).

P942 Multiplex PCR detection of CTX-M type gene

C.J. Munday, V.M. Ensor, W.E. Liu, P.M. Hawkey
Birmingham, UK

Objective: The CTX-M family of Extended Spectrum beta-lactamase (ESBL) can be divided into four groups of enzymes based on amino acid homology, CTX-M-1, CTX-M-2, CTX-M-8 and CTX-M-9. The molecular detection of genes from each of these CTX-M groups has previously relied on the use of multiple PCR reactions due to nucleic acid heterogeneity between groups, and previous publications have been fraught with inaccuracies or incomplete CTX-M group detection. We aimed to design a multiplex PCR for the detection and subsequent sequencing of blaCTX-M from all family groups found in Enterobacteriaceae spp.
Methods: Primers were designed based on a central homologous region found in all blaCTX-M and the upstream (five primers) and downstream (four primers) consensus regions of each respective CTX-M group giving a universal forward or reverse primer and further primers correlating to each CTX-M group. PCR reactions were conducted in 25 μL reaction volume containing equal amounts (25 pM) of each primer, suitable for screening to a range of known CTX-M producing Enterobacteriaceae spp.
Results: Previously characterised isolates producing blaCTX-M were included for PCR. This included isolates of *K. pneumoniae* producing CTX-M-26 and *E. coli* producing one of CTX-M-3, CTX-M-9, CTX-M-14 or CTX-M-15. After multiplex PCR, for the upstream region (*c.* 300 bp) and downstream region (*c.* 650 bp) products of the indicted sizes were found for all isolates. Furthermore, this method was used to characterise ESBL producing clinical isolates of *E. coli* and *K. pneumoniae* to detect the presence of CTX-M genes, with subsequent sequencing confirming the presence of CTX-M-15.
Conclusion: We have designed a multiplex PCR for the detection of CTX-M type genes using a single reaction tube allowing the screening of ESBL producing isolates for blaCTX-M genes and their direct DNA sequencing, without the need for multiple PCR reactions. Recent data from the UK shows that CTX-M ESBLs are rapidly increasing in importance.

P943 Differentiation of *Citrobacter koseri* and *Citrobacter amalonaticus* isolates using beta-lactamase gene sequences

M. Avison, S. Underwood
Bristol, UK

Objectives: Some members of the genus *Citrobacter* are potential pathogens of debilitated hospital patients. They can become resistant to beta-lactamases, including third generation cephalosporins due to over-expression of a chromosomal beta-lactamase. Eleven species are currently known, but speciation is often difficult using biochemical tests. Isolates previously typed as *Citrobacter diversus* are now known as *Citrobacter koseri*. Here we measured sequence variation at the beta-lactamase structural gene amongst a group of clinical isolates, originally identified as *C. diversus* by API 20E profiling.
Methods: Nine *C. diversus* isolates were collected from faecal samples of children being treated in the oncology department of Bristol Children's hospital in the early 1980s. Beta-lactamase and 16S rRNA genes were amplified by PCR and sequenced by standard methods. Beta-lactamase induction was attempted in liquid-grown cultures using cefoxitin (10 mg/L for 2 h). Nitrocefin hydrolysis assays were performed using a spectrophotometer.
Results: Analysis of 16S rRNA gene sequences confirm that, of the nine clinical isolates, Five, which all have an inducible beta-lactamase gene whose sequence is closely related to *C. diversus* NF85 and ULA27, are actually *Citrobacter amalonaticus*. Given that *C. diversus* isolates have all been renamed *C. koseri*, this error in nomenclature must be addressed. The reason for the error is that *C. diversus* was known to have variability in its ability to utilise malonate, the only differentiation between *C. koseri* and *C. diversus*. Four of the test isolates do type as *C. koseri* using 16S rRNA sequencing. These true *C. koseri* isolates produce a novel, acidic, class A beta-lactamase, named CkoA, constitutively. The sequence of this beta lactamase gene was determined, and is only 40% identical to the *C. diversus* (now *C. amalonaticus* cdiA).

Conclusions: We present a new beta-lactamase sequence, from *C. koseri* and shows that *C. koseri* NF85 and ULA27 should be retyped as *C. amalonaticus*. Beta-lactamase-specific PCR may provide a valuable tool for typing *Citrobacter* spp. isolates, and is very suitable for separating *C. amalonaticus* and *C. koseri*, which are very closely related biochemically. The knowledge that clinical *C. koseri* isolates produce a beta-lactamase constitutively at low levels may be useful clinically.

P944 A single-tube PCR with MGB Eclipse probes for detection of SHV-type extended-spectrum beta-lactamases (ESBLs)

A. Ekimov, M. Edelstein, E. Belousov
Smolensk, RUS; Bothell, USA

Objectives: ESBLs of the SHV-type are one of the most common and clinically significant beta-lactamases. The number of SHV variants is continuously growing; however ESBL activity of SHV enzymes has been associated with mutations at relatively few amino acid positions (aa-s) as compared with the TEM enzymes. Here we propose a simple and rapid method that allows detection of all the known SHV ESBLs in a single real-time PCR reaction.
Methods: The proposed method is based on amplification of blaSHV genes in the presence of short (13–14 nt) fluorogenic probes capable of hybridisation-triggered fluorescence. These probes commercially known as MGB Eclipse probes contain a dark quencher with a conjugated minor groove binder at the 5′-end and a fluorescent dye at the 3′-end. This structure allows detection and differentiation of nucleotide polymorphisms at targeted sites by post-PCR melting curve analysis. Four probes were designed to perfectly match the wild-type (WT) sequences at mutation sites corresponding to aa-s 146, 149, 156, 179 and 238. Thus, mutations conferring ESBL activity were expected to specifically lower the melting temperatures (Tm-s) of the probe-template duplexes. Each probe was labelled with a unique dye permitting analysis of mutations at multiple sites in a single reaction.
Results: The method was validated using laboratory strains producing the SHV-1 (WT, non-ESBL control), SHV-2, 3, 4, 5 (G238S), SHV-18 (G238A), SHV-6 (D179A), SHV-8 (D179N) and strains carrying cloned blaSHV fragments to which the naturally occurring mutations D179G, G156D, T149S and A146V were introduced by site-directed mutagenesis. Following careful design of the probes and optimisation of PCR conditions, all the above mutations were successfully detected and discriminated from the WT sequence and each other according to specific Tm-s. The detection was precise and highly reproducible in repeated experiments. Furthermore, when applied to the analysis of 10 clinical isolates of *Klebsiella pneumoniae* expressing ESBL phenotype, the method was able to detect multiple SHV alleles (WT and G238S or D179A) in the same isolates. This observation is particularly important considering the high frequency of co-production of the SHV-1 and ESBLs in klebsiellae.
Conclusions: A PCR with MGB Eclipse probes has a great potential for studying the epidemiology of SHV ESBLs and possibly for analysis of other antimicrobial resistance mechanisms associated with mutations at defined loci.

P945 Evaluation of gentamicin disk diffusion method for detecting high-level Gentamicin-resistant Enterococci by comparison with a polymerase chain reaction technique detecting the aac(6′)-Ie-aph(2″)-Ia aminoglycoside modifying gene

B. Saeedi, A. Hällgren, B. Isaksson, J. Jonasson, L.E. Nilsson, H. Hanberger
Linköping, S

Objectives: To evaluate the efficiency of a gentamicin disk diffusion method for detection of high-level gentamicin resistance (HLGR) in clinical isolates of enterococci by comparison with a polymerase chain reaction (PCR) technique detecting the aac(6′)-Ie-aph(2″)-Ia aminoglycoside modifying gene.
Methods: A total of 356 non-repeat enterococcal blood isolates (250 *E. faecalis* and 106 *E. faecium*) were collected during 1994 to 2001 from hospitals located in south east of Sweden. The bacterial isolates were identified by standard microbiological methods and susceptibility testing was performed with a 30-μg gentamicin disk on PDM-agar (AB Biodisk) to detect HLGR isolates. All isolates were tested for the presence of the aac(6′)Ie-aph(2″)Ia gene using the polymerase chain reaction (PCR) technique.
Results: There was complete correlation between the gentamicin disk diffusion test and the PCR results. All 40 HLGR isolates, as defined by disk diffusion, and the positive control (*E. faecalis* ATCC 51299) carried the aac(6′)Ie-aph(2″)Ia gene as judged from the PCR results. The resistant gene was not found in the negative control ATCC 29212 or any of the 316 non-HLGR enterococci.
Conclusion: This study shows that in our setting the sensitivity and specificity of the disk diffusion method for the detection of HLGR enterococci is very high and there is a total agreement with the results obtained by using a PCR technique for detection of the aac(6′)Ie-aph(2″)Ia aminoglycoside modifying gene.

P946 Identification of *Enterococcus* spp. of the human normal microbiota by pyrosequencing

M. Lindgren, P. Huovinen, C. Edlund, J. Jalava
Turku, FIN; Stockholm, S

Objectives: The main objective was to develop a pyrosequencing method for identification of *Enterococcus* spp. species with Pyrosequencing method. Also, development of antibiotic resistance with special reference to macrolide resistance will be studied by susceptibility testing in samples isolated serially from subject exposed to clindamycin.
Methods: Biochemical identification of the enterococcal strains from faecal samples was done by growth at 45°C, catalase and hydrolyse of 1-pyrridonyl-beta-naphtylamide (PYR). Species identification was done with Pyrosequencing method. PSQ 96MA pyrosequencing technique enabled identification of different *Enterococcus* species based on their 16S rRNA V2-regions signature-sequences. Antibiotic susceptibility testing was done by agar dilution method on Müller–Hinton II medium, according to NCCLS. MIC values were tested against erythromycin, clindamycin, ciprofloxacin, ampicillin, gentamicin, vancomycin and tetracycline. Macrolide resistance genes; erm(B), erm(TR) and mef(A) was studied by Multiplex-PCR.
Results: With Pyrosequencing method, we identified 46 *Enterococcus faecium*, 22 *E. faecalis*, 11 *E. avium* and 33 *E. casseliflavus* species, and 54 non-enterococci species. The antibiotic susceptibility testing showed that 26.5% of the *Enterococcus* strains were resistant to erythromycin, 14.8% to ciprofloxacin and 17.4% to tetracycline. About 31.6% of the Enterococcae had erm(B)-gene.
Conclusion: Pyrosequencing was rapid and easy method for identification of bacterial strains even to the species level. Antibiotic resistance varied a lot between different bacterial strains, as *E. faecium* and *E. casseliflavus* species being the most resistant ones. Pyrosequencing results correlated well with species phenotype and antibiotic resistance.

P947 Detection of resistance genes of vancomycin-resistant enterococci by sandwich hybridisation method

V. Skandami, A. Tsiringa, A. Xanthaki, M. Toutouza, C. Kontou-Castellanou
Athens, GR

Objectives: To determine the species distribution of vancomycin resistant enterococci (VRE) isolated from hospitalised patients and detect genes encoding resistance to vancomycin and teicoplanin, by sandwich hybridisation method.

Methods: The study was performed on 57 strains of enterococci all from patients with severe underlying diseases. Strains were isolated from urine (37.9%), blood cultures (18.9%), pus (5.4%), peritoneal fluid (5.4%), intravenous catheter (21.6%), infection of the drainage site (10.8%). Identification to the species level was performed by VITEK 2 (Bio-Merieux, France). Antibiotic susceptibility testing was done by Kirby-Bauer and MIC by E-test and VITEK 2. The sandwich hybridisation method was performed in all strains using the commercially available EVIGENETM VRE Detection kit (Statens Serum Institute), for the presence of *vanA* and *vanB* genes.
Results: From the stains tested, 37 were vancomycin and teicoplanin resistant (*vanA* phenotype) and 20 susceptible to these antibiotics, as determined by Kirby–Bauer and MICs by VITEK 2 and E-test methods. Of them, 10 were *E. faecalis*, 22 *E. faecium*, three *E. casseliflavus* and two *E. hirae*. All the VREs strains, which were suggesting the presence of *vanA* phenotype by Kirby–Bauer and MIC, were identified to be *vanA* positive by the sandwich hybridisation method. The 20 susceptible strains were negative for the detection of the genes *vanA* and *vanB*.
Conclusions: Identification of VRE to the species level and knowledge of the type and the profile of resistance is critical for infection control purposes in the hospital environment. The sandwich hybridisation is a rapid (3.5 h) and easy to use commercially available molecular method to detect the *vanA* and *vanB* genes, while the phenotypic resistance determination requires incubation for at least 24 h and other molecular methods require specific instruments and experienced technicians. The sensitivity and specificity of the method is 100%.

P948 Evaluation of the Evigene™ VRE detection kit for detecting of enterococci including vancomycin resistance genes

A. Kilic, M. Baysallar, G. Bahar, A. Kucukkaraaslan, L. Doganci
Ankara, TR

Objectives: Evaluating the correlation of the EVIGENETM VRE Detection kit using PCR, which is the golden standard for gene detection and correlating the minimum inhibitory concentration (MIC) for vancomycin and teicoplanin are the aim of this study.
Methods: The vancomycin-resistant enterococci (VRE) Detection Kit is based on microwell plates where to DNA probes specific for the bacterial targets DNA are bound. Test wells include: a positive (16S rRNA) and a negative control, a vanA microwell and a vanB microwell. The PCR detects the *vanA*, *vanB*, and *vanC-2* genes. The MIC determination was performed by E-test according to the NCCLS guidelines.
Results: We tested a total of 64 diverse vancomycin resistant enterococci: *Enterococcus casseliflavus* ($n = 50$) and *Enterococcus faecium* ($n = 14$). All strains were vanA positive (OD: all strains >1.192). All results obtained with the VRE kit were confirmed by the PCR. The MIC determination correlated with the PCR and kit results for all *vanA* positive strains with high MIC for vancomycin.
Conclusion: As a result, the EVIGENE VRE Detection Kit can clearly distinguish VRE with the *vanA* and *vanB* genotypes among a large collection of enterococci and with the same specificity as PCR.

P949 Development of antibiotic resistance in enterobacteria

S.D. Nyberg, A. Hakanen, M. Österblad, P. Huovinen, C. Edlund, J. Jalava
Turku, FIN; Stockholm, S

Objectives: The main objective is to get a better knowledge of the human microflora in gastro-intestinal organ by following variations among intestinal enterobacteria in four healthy subjects receiving oral clindamycin. The microflora in the chosen subjects will be monitored for a 2-year period. The presence and stability of specific resistance genes will be studied in samples collected serially from selected antibiotic exposed subjects. *blaTEM* and *blaSHV* that code for an extended spectrum beta-lactamase in Enterobacteriaceae will be studied. The study will be done by using identification, susceptibility testing, PCR and molecular fingerprinting methods.
Methods: Serially collected faecal samples from four healthy subjects who had received clindamycin perorally for 7 days were cultured and screened for Enterobacteriaceae. Sampling was performed pretreatment, day 7, 3 weeks, 3, 6, 9, 12 and 18 months after clindamycin administration. Between 20 and 50 colonies of suspected Enterobacteriaceae were picked from each sample. Biochemical identification of the bacterial isolates was done by oxidase, indole production and activity of beta-glucoronidase. MICs were determined according to NCCLS by standard agar dilution method on Müller–Hinton II medium. The following antimicrobials were tested: ampicillin, cephalothin, cefuroxime, piperacillin/tazobactam, amoxicillin–clavulanic acid, ceftazidime, cefotaxime, imipenem, aztreonam, gentamicin, streptomycin, chloramphenicol, tetracycline, nalidixic acid, trimethoprim, sulfamethoxazole and ciprofloxacin.
Results: A total of 521 isolates were identified as oxidase negative, Gram-negative rods and thus belonged to the Enterobacteriaceae. The isolates were then screened for indole and betaglucoronidase activity. These results showed that 81% of all strains were *E. coli*. Of all strains, 60% were resistant to ampicillin, 46% against sulfamethoxazole, 11.9% against cephalothin and 7.7% against nalidixic acid. The variation of antibiotic resistance between subjects is broad.
Conclusion: Enterobacteriaceae are naturally resistant to clindamycin. However, after clindamycin treatment alterations in the susceptibility to other antimicrobial agents still occur in the microflora. Additional research needs to be done to clarify if these alterations in antibiotic resistance are caused by variation of strains/species or exchange of resistant elements.

P950 Prevalence and implication of the *cfiA* and *cphA* genes in imipenem resistance among *Bacteroides* spp.

M. Theron, M.N. Janse van Rensburg, C. Roussouw
Bloemfontein, ZA

Objectives: Bacteroides is a major cause of intra-abdominal and female genital tract infections as well as subcutaneous abscesses. Beta-lactam agents and carbapenems are currently used in monotherapy against anaerobic infections. The study was done to: (1) investigate the susceptibility of *Bacteroides* strains isolated from Bloemfontein Academic Hospitals; (2) compare results with a previous study; (3) determine the prevalence of carbapenemases/metallo-beta-lactamases in *Bacteroides* spp.
Methods: Fifty-one *Bacteroides* spp. strains were isolated from patients in the Universitas and Pelonomi Hospitals in Bloemfontein. MICs of 12 antimicrobial agents were determined by the NCCLS agar dilution method. A bioassay was used to screen for carbapenemase or metallo-beta-lactamase production. PCR amplification was performed for the detection of *cfiA* and *cphA* genes. Plasmids were extracted using a High Pure Plasmid Isolation Kit.
Results: Susceptibility levels were relatively high for imipenem (95%), meropenem (90%) and metronidazole (88%). Comparing the results with a previous study (isolates from 1996/1997), showed a reduction in susceptibility to imipenem (100–95%), meropenem (100–90%) and metronidazole (100–88%). The bioassay results gave no indication of the presence of significant concentrations of a carbapenemase or metallo-beta-lactamase. PCR amplification showed the cfiA gene (747 bp) in 4/18 strains (imipenem MIC 1 to >128 μg/mL) and the *cphA* gene (769 bp) in 3/18 of the isolates (imipenem MIC 1–4 μg/mL). No plasmids were detected.
Conclusions: Although >90% of the isolates were susceptible to the carbapenems, it is evident that resistance has increased over the last decade. Fortunately the production of metallo-beta-lactamases has been found to give rise to MICs that only range from 2 to 4 μg/mL. This study supports these findings with the exception of one isolate with a MIC >128 μg/mL. Demonstration of the *cfiA*

and *cphA* genes by PCR, but not actual enzyme production, may be attributed to so-called 'silent' genes. Susceptible strains are known to be able to convert to high-level beta-lactam/carbapenem resistance by increasing the expression of 'nearly' silent metallo-beta-lactamase genes. Metallo-beta-lactamases have been found to be carried on a small plasmid (13.6 kb) that appears to be self-transmissible, posing a potential threat of rapid spread of resistance. Therefore early recognition of metallo-beta-lactamase producing strains is imperative.

P951 Identification of *Nocardia* clinical isolates by PCR and restriction analysis and susceptibility patterns

M. Marín, E. Cercenado, P. Martín-Rabadán, P. Muñoz, C. Radice, E. Bouza
Madrid, E

Objective: To describe the distribution of species in our *Nocardia* isolates and to evaluate the usefulness of an easy and rapid method based on a short battery of susceptibility tests to identify clinical *Nocardia* isolates compared with PCR and restriction analysis of hsp65 routinely used in our laboratory.
Methods: *Nocardia* sp. isolated from 1995 to 2003 were selected to study. Molecular identification was performed by hsp65 PCR-RFLP. Identification by susceptibility testing was by disk diffusion with gentamicin (CN), tobramycin (TOB), amikacin (AK) and erythromycin (E) and by broth microdilution and E-test with ampicillin (AMP), ciprofloxacin (C), cefotaxime (CTX) and amoxicillin-clavulanate (AUG).
Results: 34 isolates of *Nocardia* sp. were studied. Distribution of species according to results from PCR-RFLP was: *N. asteroides* I (4), *N. asteroides* VI (15), *N. farcinica* (10), *N. nova* (3), *N. otitidis-caviarum* (2). *N. asteroides* I isolates had two different susceptibility patterns, two isolates were CN-S, TOB-S, AK-S, E-R and the other two were CN-R, TOB-S, AK-S, E-R. All *N. asteroides* I isolates were AMP > 8 μg/mL and C > 4 μg/mL and CTX < 2 μg/mL. Eighty-seven per cent of *N. asteroides* VI were CN-S, TOB-S, AK-S, E-R, AMP > 8 μg/mL, CTX < 8 μg/mL whereas C was variable. Hundred per cent of isolates of *N. farcinica* were CN-R, TOB-R, AK-S, E-R, AMP > 8 μg/mL, C < 4 μg/mL and CTX > 32 μg/mL. *N. nova* isolates were CN-S, TOB-S, AK-S, E-S, AMP < 4 μg/mL, CTX < 2 μg/mL and C < 4 μg/mL. *N. otitidis-caviarum* isolates were CN-S, TOB-S, AK-S, E-R, AMP > 8 μg/mL, CTX > 32 μg/mL and C < 4 μg/mL. Medium time to obtain results by both methods was 48 h.
Conclusions: 94% of isolates belonged to the former *N. asteroides* complex. *N. farcinica* and *N. nova* were easily distinguished from other *Nocardia* species by its susceptibility patterns. The main group *N. asteroides* VI was more difficult to distinguish from *N. asteroides* I and *N. otitidis-caviarum*. A short battery of susceptibility tests permits rapid differentiation of our most frequent *Nocardia* isolates, although genotypic tests are more discriminatory.

Molecular bacteriology: borrelia

P952 Isolation and identification of *Borrelia* strains from ticks collected in the Czech Republic

H. Drevová, D. Hulínská, J. Plch, Z. Kurzova
Prague, CZ

Objectives: The *Ixodes ricinus* tick, common ectoparasite of animals and humans, is the main vector of Lyme disease in the Czech republic. Detection of *Borrelia* under microscope, isolation in BSK-H medium and PCR identification was the aim of this work.
Methods: A tick was crushed in drop of sterile phosphate buffer saline and admired under microscope in dark-field. Samples, in which spirochetes had been detected, were incubated in liquid BSK-H medium (Sigma) at 33°C and admired weekly for 6 weeks. Each strais was passaged twice and was frozen in 1.8-mL aliquots at −70°C. Direct fluorescence assay (DFA) with fluorescein labelled polyclonal antibody to *Borrelia burgdorferi* was used for screening. Deoxyribonucleic acid of borrelial strains was isolated with Invisorb Genomic DNA Kit III (Invitec). Three sets of primers (for *B. burgdorferi* sensu lato, *B. garinii* and *B. afzelii*) derived from 16SrRNA gene (Rosa and Schwan) were used for elementary identification of strains. Detailed analysis of strains was made by Light cycler real-time PCR (RT-PCR). Primers and probe derived from recA gene were used in this method.
Results: There was a collection of 6283 ticks in urban and suburban localities of the Czech republic from 1998 to 2002 years. Incidence of spirochetes in tick population differed from 1 to 23.5% in different localities. Spirochetes were cultured from at least one of six ticks (27 out of 156) that were tested positive by dark-field microscopy. All strains reacted positively by DFA and gave positive response with primers specific for *B. burgdorferi* sensu lato complex. Nineteen strains belonged to *B. garinii*, four to *B. afzelii* and two to *B. burgdorferi* sensu stricto genospecies. One strain did not react with 16SrRNA primers for *B. garinii* but had melting temperature of recA gene product identical with *B. garinii* type strain. We identified the genotype of two strains determined as *B. burgdorferi* sensu lato neither by PCR, nor by RT-PCR.
Conclusion: Majority of *Borrelia* strains cultured in our laboratory was identified as *B. garinii*. This might be caused by supposed higher presence of *B. garinii* in ticks or possible better adaptation of this strain to culture medium. The results clearly show the obvious risk of borreliosis in urban and suburban areas of the Czech republic.

P953 Identification of Lyme disease spirochetes in humans and animals

D. Hulínská, J. Votpka, H. Drevová, K. Langrova, M. Bojar, M. Valesová
Prague, CZ

Background: To unveil the prevalence of different *Borrelia* genotypes and their OspA, OspC variants in the Czech Republic where Lyme disease is common (3000 cases annually reported). To analyse sequence types of *Borrelia* sp. in patients with different symptoms, in tick vectors and in animal reservoirs.
Methods: We applied LightCycler real-time PCR assays using SYBR green I dye and fluorescent probes. Positive PCR products from liq-

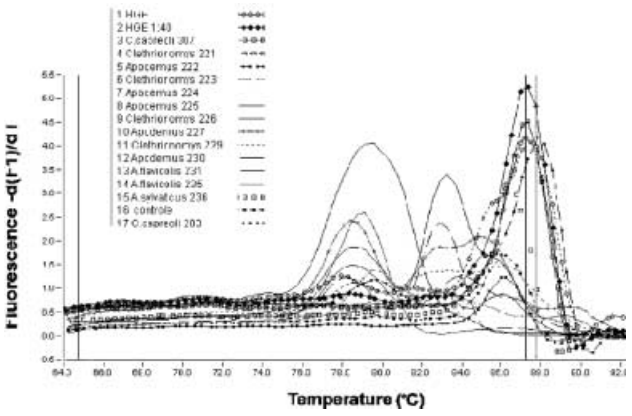

Figure. Lightcycler real-time PCR with primers p521–790 shows temperature value Tri 87.20-87-70°C for specific ehrlichial DNA from different animals and positive-GE controls in 10-fold dilution.

uors, blood and tissue were subjected to sequencing with the dideoxy chain termination technique using CEQ 2000CX sequencer. Cultivation, immunocytochemistry and Western blots were used for confirmation.
Results: We cultured four blood, six skin, six CSF isolates, numerous tick and two animal isolates. Real-time PCR targeted recA, 16S and OspA genes showed that involvement of the nervous system, joints and skin in Czech patients was predominantly caused by *B. garinii*, serotypes 3,5,6 (54%), then *B. burgdorferi* ss (26%) and *B. afzelii* (10–16%). The remaining 4–10% comprise coinfection with *Anaplasma phagocytophila* or mixed borrelial infections. Similar results were found in 424 animals. Among game animals 22% tested positive with *B. garinii* and *B. burgdorferi*. Wild boars and murids hosted *Borrelia* sp. in 7 and 12% with prevalence of *B. afzelii*. No significant differences were noticed between the infection of adult and nymphal ticks, both reaching 20 and 12% in June and September, respectively. Diferences were also between regions, in east Bohemia with *B. garinii* prevailing and in Moravia with prevalence of *B. afzelii* and human cases of *Erythema migrans* and *Acrodermatitis atrophicans*. Infection prevalence data for patients were in agreement with data for the tick and animals.

P954 Diversity of *Borrelia burgdorferi* sensu lato in the Russian Far East

O. Mediannikov, L. Ivanov, N. Zdanovskaya, R. Vorobyova, P.-E. Fournier, I. Tarasevich, D. Raoult
Moscow, Khabarovsk, RUS; Marseille, F

Objectives: The aim of our study was to identify the strains of *Borrelia* isolated from ticks and Lyme disease patients in the Russian Far East and to analyse their taxonomic positions based on ospA gene phylogeny.
Methods: We have analysed 30 strains of *Borrelia burgdorferi* sensu lato isolated from *Ixodes persulcatus* ticks (25) and skin biopsies of *Erythema migrans* from Lyme disease patients (5) isolated by standard methods during last 6 years in the Russian Far East. After amplification with newly designed primers, we obtained full-length ospA gene sequence of each of the 30 strains.
Results: We identified four strains as *B. afzelii* completely identical to the strain XJ23, isolated in Japan. All of them were isolated from ticks. The other 26 strains were found to be genetically variable, but the closest homology found was with *B. garinii*. After phylogenetic analysis of ospA gene we found that these strains form three distinct and well-defined clades at the phylogenetic tree. Genogroups 1 and 2 represent only species isolated in the Far Easter regions of the Russian Federation and in Japan only, whereas genogroup 3 represents mostly European isolates, including sero- and genogroups defined in the works of B. Wilsske *et al*. and G. Will *et al*. and four isolates from the Russian Far East. European serogroups 3 and 7 form the clade localised between genogroups 2 and 3. Human strains were found within genogroups 1 and 2.
Conclusion: *B. garinii* was found to dominate among other *B. burgdorferi* sensu lato strains isolated from ticks and Lyme disease patients form the Russian Far East. Phylogenetic analysis showed that the species identified as *B. garinii* have significant variability in the ospA gene and form three major groups. Two groups consisting only of strains isolated in the Far East are significantly remote from all other *B. burgdorferi* sensu lato species. Bootstrap values and distances among these groups suggest their solidity, especially genogroup 1. This, probably, indicates the distinct origin of defined genogroups 1 and 2 of *B. garinii* and may suggest another taxonomic status.

P955 A new recombinant IgG and IgM line immunoblot improves serodiagnosis of early Lyme borreliosis

G. Goettner, U. Schulte-Spechtel, V. Fingerle, G. Liegl, B. Wilske
Munich, D

Objectives: For diagnosis of Lyme borreliosis (LB) a two-step approach is recommended by CDC and DGHM (screening ELISA followed by immunoblot (IB) in case of reactive ELISA). Though *Borrelia* IBs are widely used, they are still poorly defined regarding sensitivity, specificity and standardisation. A recently described recombinant Western immunoblot (WIB) complemented with *Borrelia* antigens produced *in vivo* but not in culture (i.e. VlsE) could improve previous tests (1). Here a recombinant *Borrelia* line IB (LIB) was developed where each recombinant antigen is separately detectable, even those antigens with identical molecular weight.
Methods: The following recombinant IgG and IgM IBs were compared: (a) The WIB described in (1) with p83/p100 (strain PKo, *B. afzelii*), p58 (strain PBi, *B. garinii* OspA-type 4), BmpA (strains PKa2, *B. burgdorferi* sensu stricto, PKo, and PBi), VlsE (strain PKa2), OspC (strains PKa2, PKo, PBi, and *B. garinii* strain 20047), and DbpA (strains PKo and PBr, *B. garinii* OspA-type 3). (b) The LIB with all antigens of the WIB and in addition VlsE (strains PKo and PBi), OspC (strain PLe, *B. afzelii*) and DbpA (strains B31 and PBi). To verify sensitivity and specificity, 65 sera of patients with early LB (50 early neuroborreliosis, 15 *Erythema migrans*) and 110 control sera (60 blood donors, 10 rheumatoid factor positive, 10 syphilis patients and 30 patients with fever of unknown origin) were studied.
Results: IB interpretation criteria defining a serum as positive with at least two reactive bands or in case of IgM at least one strong OspC band were used (2). Sensitivity significantly increased from 63% (WIB) to 80% (LIB) for IgG and from 46% (WIB) to 69% (LIB) for IgM while specificity remained unchanged (99% for IgG tests and 98% for IgM tests). The increase of sensitivity was mainly due to the line blot technique, which allows detection and identification of antibodies differently reactive with homologues of the same protein.
Conclusion: The LIB is more sensitive than the WIB for both IgG and IgM antibody detection in acute LB while specificity remains unchanged. The LIB is better to standardise and results are easier to interpret.
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P956 The first study of identification of *Borrelia, Ehrlichia* and *Babesia* in *Ixodes ricinus* ticks from Lithuania using molecular methods

D. Ambrasiene, I. Vascilo, A. Jenkins, A.-G. Allum, L. Strand, B.-E. Kristiansen
Kaunas, Vilnius, LIT; Bo i Telemark, Skien, N

Background: Tick of the *Ixodes ricinus* group are well known as major vectors of the causative agents of Lyme borreliosis, granulocytic anaplasmosis, ehrlichiosis and babesiosis in European countries. The humans infected with these agents can experience a wide range of clinical manifestations. *I. ricinus* is a widely distributed tick in Lithuania and may transmit pathogens to mammalian hosts, including human beings. A single tick may contain several different pathogens so double-infection with borreliosis and ehrlichiosis may be seen.
Objectives: The aim of this study was to determine whether *I. ricinus* ticks collected in different regions of Lithuania were infected with the causative agents of Lyme borreliosis, anaplasmosis, ehrlichiosis and babesiosis agents and to estimate the prevalence of mixed infections in them by PCR. No investigations have been carried out to assess the prevalence of *Borrelia*, *Anaplasma*, *Ehrlichia* and *Babesia* infection in *I. ricinus* in Lithuania using the PCR method before.
Methods: Altogether, 243 *I. ricinus* ticks collected from 10 different regions of Lithuania, were included in this study. All ticks were analysed individually. The presence *Ehrlichia/Anaplasma* group pathogen was determined by using PCR with *Ehrlichia/Anaplasma*-specific primers HR521/EHR747, multiplex PCRs using species-specific *Borrelia* primers GI-R/GI-L (*Borrelia burgdorferi* s.s.), GII-R/GII-L (*B. garinii*), GIII-R/GIII-L (*B. afzelii*). Real-time PCR method with the ABI Prism 7000 system was used to detect

Babesia divergens. *Ehrlichia/Anaplasma* species were determined using the reverse line blot hybridisation.
Results: Of the 243 individually processed ticks, 12 (5%) were positive for *Ehrlichia/Anaplasma* (HGE – 3, HGE variant – 1, E Schotii – 2 and 6 were not identified), 38 (16%) for *Borrelia* (*B. burgdorferi* s.s – one (0.4%), *B. garinii* – 12 (5%), *B. afzelii* – 25 (10%) and 5 (2%) were positive for *Babesia* divergens. One tick contained both *Ehrlichia/Anaplasma* and *Babesia*, two contained both *Babesia* and *B. afzelii* and one *Ehrlichia/Anaplasma* and *B. garinii*.
Conclusions: Our results represent the first study in Lithuania in which *Borrelia*, *Ehrlichia*, *Anaplasma* and *Babesia* parasites were directly identified in *I. ricinus* ticks by PCR, multiplex PCR, reverse line blot hybridisation and real-time PCR. It was detected that *B. afzelii* was the dominant genospecies in Lithuanian ticks (10%) and *Ehrlichia/Anaplasma* and *Babesia* were found in ticks too and might cause human diseases.

Molecular bacteriology: characterisation of agents

P957 Improved automated ribotyping using HindIII to discriminate previously uniform *Listeria monocytogenes* serotype 4b strains

I. Heller, K. Grif, M. Dierich, R. Würzner
Innsbruck, A

Objectives: To develop improved automated subtyping approaches for *Listeria monocytogenes*, we characterised the discriminatory power of different restriction enzymes for ribotyping. PvuII and HindIII were evaluated for their ability to differentiate among isolates representing one of the two major serotype 4b epidemic clones, having ribotype reference pattern DUP-1038 (which differs from the other clone DUP-1042 in the EcoRI pattern only). This is of utmost importance, as the presence of only two major patterns within the serotype 4b does not allow sufficient epidemiology of *Listeria* infections.
Methods and results: The eight selected *L. monocytogenes* isolates (serotype 4b) with the ribotype reference pattern DUP-1038 were responsible for human listeriosis outbreaks in France, Canada, Switzerland and Turkey from 1978 to 2002, and for sporadic foodborne cases in Austria (2002), England (1987 and 1989) and the USA. Ribotyping was performed using the RiboPrinter microbial characterisation system according to the manufacturer's instructions using EcoRI, PvuII and HindIII as restriction enzymes. We found that the eight isolates belonging to DUP-1038 (i.e. indistinguishable by EcoRI) were also indistinguishable by PvuII but yielded two clearly different patterns when using HindIII.
Conclusions: We conclude that automated ribotyping using HindIII allows discriminating previously uniform *L. monocytogenes* 4b isolates. This discrimination may facilitate the tracing of outbreaks and may also improve epidemiological surveys.

P958 Detection of BFT, the isoforms of the enterotoxin gene and cfiA gene in *Bacteroides fragilis* isolates of different origins

G. Terhes, J. Soki, K. Ago, E. Urban, E. Nagy
Szeged, HUN

Objectives: *Bacteroides fragilis* is an obligate anaerobic, Gram-negative rod constituting 1% of the normal intestinal flora of humans, and is the Gram-negative anaerobic rod most frequently isolated from human clinical samples. Some of the *B. fragilis* isolates produce a zinc-dependent metallo-protease, enterotoxin coded by the bft gene. This protein has enterotoxic activity; it causes fluid accumulation in a lamb ligated ileal loop model. To date, three different isoforms, designated bft-1, bft-2 and bft-3, have been identified. The literature regards the enterotoxin-producing property of *B. fragilis* as a virulence factor since these strains can be isolated more often from severe infections such as sepsis, or abdominal and deep soft-tissue abscesses. It is also thought to be involved in diarrhoea in 1–5-year-old children.
Aims and methods: The aim of the present study was to examine the prevalence of enterotoxin production among *B. fragilis* strains isolated between 2001 and 2003 from specimens originating in clinical wards of our university or in other hospitals by HT-29 cytotoxicity testing or PCR detection of the bft gene. The results obtained with the two methods were compared. The frequencies of three alleles of bft genes in enterotoxigenic strains from different sources were determined by using PCR-restriction fragment length polymorphism analysis. The *B. fragilis* strains can be divided into two major groups by molecular typing methods and most importantly according to the carriage of the cfiA gene. We therefore also examined the occurrence of the cfiA gene by PCR and the co-incidence of bft and cfiA among the above collection of strains.
Results: The average occurrence of toxigenic *B. fragilis* strains in the different groups of clinical samples was 10% and in deep-tissue infections was 15% by both the PCR method and the cytotoxicity assay. bft genes were found only in the cfiA-negative group. The prevalence of the cfiA gene corresponded to our earlier findings and data from the literature and we did not observe co-incidence of the bft and cfiA genes in this study.

P959 Prevalence and characterisation of binary toxin (actin specific ADP-ribosyltransferase) from *Clostridium difficile*

D. Decré, F. Barbut, C. Goncalves, B. Burghoffer, J.C. Petit
Paris, F

Introduction: In addition to the two large clostridial cytotoxins (LCT – toxins A and B) some strains of *Clostridium difficile* also produce an actin-specific ADP-ribosyltransferase (binary toxin CDT). CDT may serve as an additional virulence factor.
Methods: We used PCR and Southern blotting methods for detection of genes encoding the enzymatic (CDTa) and binding (CDTb) components of binary toxin in 369 strains isolated from patients with suspected *C. difficile*-associated diarrhoea or colitis. Binary toxin production was assessed by Western blotting using antisera against the iota toxin of *C. perfringens* (anti-Ia and Ib). Toxin activity was detected with an ADP-ribosyltransferase assay. PCR amplification was performed to detect the gene encoding for toxin B. Binary positive strains were subjected to toxinotyping and were characterised by phenotypic (serogrouping) and genotypic markers (PCR-ribotyping, arbitrarily primed PCR (AP-PCR) and pulsed-field gel electrophoresis (PFGE)).
Results: Twenty-two strains (prevalence 6%) harboured both genes cdtA and cdtB; 19 out of the 22 strains reacted with antisera against the iota toxin of *C. perfringens*; the binary toxin activity was positive in only 17 of the 22 strains. All strains also produced toxins B. However, they had significant changes in tcdA and tcdB genes and belonged to variant toxinotypes III, IV, V, VII, IX and XIII. With typing methods used we could differentiate 16 profiles, indicating that most of binary toxin positive strains were unrelated.
Conclusion: Binary toxin-producing isolates of *C. difficile* are widespread but prevalence varies from one country to another. More studies are needed to define the role of binary toxin in pathogenesis.

P960 Molecular characterisation and prevalence of *Clostridium difficile* in Singapore

W.Y. Leong, R. Das Ramadas, T.H. Koh, K.P. Song
Singapore, SGP

Objective: Occurrence of nosocomial *Clostridium difficile*-associated diarrhoea and pseudomembranous colitis is related to the production of toxins A and B (encoded by tcdA and tcdB, respectively) from the pathogen. tcdA and tcdB, together with their accessory genes, tcdC-E are arranged within a well-defined chromosomal region termed Pathogenicity Locus (PaLoc). Another virulence factor, ADP-ribosyltransferase binary toxin (encoded by cdt genes) was reported to be found in approximately 12% of pathogenic strains of *C. difficile*. Despite the availability of a number of detection methods, the identification methods commonly used are not designed to detect all the virulence factors known. We present here an alternative characterisation of the toxigenic and the related genes of *C. difficile* based on genotyping. The correlation between PaLoc and cdt genes was also examined.
Methods: All 110 clinical isolates from Singapore General Hospital (SGH) were screened with PCR and multiplex PCR for the presence of tcdA-E and cdtA-B in the PaLoc region and cdt operon, respectively. The production and activity of toxins A and B were analysed by commercial kit and cytotoxicity testing.
Results: The isolates could be classified into 16 groups based on the genotypic analysis of the PaLoc and cdt genes. Approximately 21% of them shared a common profile with the reference strain VPI 10463, and about 36% were completely devoid of the genes tested. Variations demonstrated in tcdC-E were complicated and no specific profile could be attributed to a particular genotype. An atypical toxigenic variant was discovered which contains only tcdB. In contrast to data reported elsewhere, none of the pathogenic strains was found to contain complete cdt genes. When tested for TcdA and TcdB production, six strains were identified to be toxins A-negative, B-positive.
Conclusion: The great genetic polymorphisms displayed by the *C. difficile* isolates here confirm that these strains were highly heterogeneous and could originate from endogenous source. There is no significant correlation between presence of the structural genes (tcdA-B), accessory genes (tcdC-E) and cdt genes. Pathogenic strains do not necessarily contain all the genes in the PaLoc. In conclusion, our results using this toxino-genotyping method for the studies of genetic distribution of toxinogenic genes correlates well with the phenotype of the bacteria i.e. toxin expression.

P961 Characterisation of *Clostridium difficile* strains isolated in different time periods and belonging to different ribotypes

P. Spigaglia, V. Carucci, P. Mastrantonio
Rome, I

Objectives: Seventy-four *Clostridium difficile* clinical isolates, collected in different time periods, were typed by PCR-ribotyping. Strains belonging to the two main PCR-ribotypes were characterised for virulence determinants and for antibiotics resistance.
Methods: PaLoc genes analysis, detection of binary toxin gene and antibiotic resistance determinants (ermB, tetM and catD) were performed by PCR assays. erm(B) sequence type was identified by a RFLP-PCR. MICs for erythromycin, clindamycin, tetracycline and chloramphenicol were determined by E-test.
Results: Two main PCR-ribotypes named A and R, respectively, were identified. PCR-ribotype A collected 20 strains whereas 15 strains belonged to PCR-ribotype R. Old strains (from 1985 to 1990) belonged to PCR-ribotype A, whereas recent strains (from 2000 to 2001) belonged to PCR-ribotype R. All strains with PCR-ribotype A had classical PaLoc genes and did not have the binary toxin gene. Ninety percent of these strains were multi-resistant and the sequence type of the ermB genes was similar to that of *C. difficile* 630. All strains belonging to PCR-ribotype R had the binary toxin gene, four of them showed major variations in the toxin A gene and 87% had a mutated toxin negative regulator. None of these strains was multi-resistant although one showed all three antibiotic resistance determinants. Fifty three percent had a tetM gene, 13% tetM and ermB genes and 7% only an ermB gene with a sequence similar to that of *C. perfringens* CP592. Interestingly, as far as resistance is concerned, there was no correspondence between phenotype and genotype in 75% of these strains. In particular, all strains with a tetM or a catD gene were susceptible to tetracycline and chloramphenicol *in vitro*, whereas five strains, resistant to erythromycin but not to clindamycin, did not have an ermB gene. All these strains showed, after induction with erythromycin, some clindamycin resistant colonies.
Conclusions: The results seem to indicate a recent spread of *C. difficile* clones that add together a potential increase of virulence by acquisition of the binary toxin, variations in genes belonging to the PaLoc and acquisition of different mechanisms of antibiotic resistance.

P962 Virulence in *Enterococci*: chromosomal versus plasmidic location of virulence genes

T.M. Leitão Semedo, M.F. Silva Lopes, J.J. Figueiredo Marques, M.T. Barreto Crespo, R. Tenreiro
Lisbon, P

Enterococci are natural inhabitants of the gastrointestinal flora of humans and animals and are widely distributed in the environment. Members of this genus are recognised as important opportunistic pathogens responsible for serious infections but the molecular mechanisms of enterococcal virulence are not yet completely understood. In this study 42 *Enterococci* from different sources, including clinical isolates (from human and veterinarian origin), non-clinical isolates and reference strains from 19 enterococcal species, were typified and their virulence potential characterised. The relationships among these *Enterococci* were first analysed using SmaI pulsed-field gel electrophoresis and M13 PCR-fingerprinting, in order to evaluate the genomic heterogeneity of the isolates. *Enterococci* were also screened for several virulence traits such as cytolysin (cyl genes), adhesins (agg, esp, EfaAfs and EfaAfm genes) and gelatinase (gelE), revealing distinct virulence potentials. In *Enterococcus faecalis*, it was recently described that some virulence determinants can be clustered on large pathogenicity islands and not only in pheromone-responsive plasmids. Dot–blot DNA–DNA hybridisation was used to locate virulence determinants in the bacterial genome of the *Enterococci* under study. No conclusive results were obtained for esp and gelE, whereas EfaAfs and EfaAfm were found on the chromosome as expected. Although cyl genes and agg are plasmidic, in most isolates, they were detected on the chromosome of five strains, suggesting that these *Enterococci* may harbour a pathogenicity island. Beyond the widespread nature of virulence traits, chromosomal integration of virulence genes seems to occur in different enterococcal species and isolates from non-clinical sources.

P963 Identification of *Salmonella* serotypes in sheep by PCR

T. Zahraei-Salehi
Tehran, IR

Introduction: *Salmonella abortusovis*, *S. dublin*, *S. montevideo* and *S. typhimurium* are more common serotypes in sheep. One way of transferring of contamination is from visceral organs specially gallbladder, intestine and liver, which can be transferred from meat to human. Because of this, this research was essential to consider about it.
Objectives: (1) Isolation of *Salmonella* serotypes from visceral organs of sheep and goats. (2) Detection of invA gene in isolated serotypes by PCR.
Materials and methods: For these goals, samples from 96 livers, 86 gallbladders, 110 mesenteric lymph nodes and 10 faeces (totally 302 samples) were taken, and then cultured in enrichment and selective media. Doubtful colonies were selected and transferred

to TSI agar, urea agar, SIM, MR-VP broth and nitrate broth. PCR reaction was carried out in Master cycle (Eppendorf). For DNA extraction isolated *Salmonella* serotypes was cultured in LB broth for 24 h at 37°C. LB broth (200 μL) was boiled for 10 min and centrifuged at 6000×*g* for 3 min. A total of 1.5 μL of the supernatant was used for amplification by PCR with *Salmonella*-specific (139 and 141) primers.

Results: Three *Salmonella* serotypes were isolated from mesenteric lymph nodes (two cases) and gallbladder (one case). Serotyping test showed that two of them belong to group B and one of them to group D of *Salmonella*. When subjected to *Salmonella*-specific primer invA, all isolates, including positive control, generated a single 284-bp amplified DNA fragment, on 1.5% agarose gel.

Conclusion: *Salmonella*-specific PCR with primer set invA is rapid, Sensitive, and reliable for detection of *Salmonella* in many clinical samples. The present research supports the ability of this specific primer set to confirm the isolates as *Salmonella*. All isolates, including positive controls (*S. typhimurium* and *S. dublin*), screened by PCR resulted in 284-bp amplified product. No amplified products were obtained from negative controls (water and O2K12 *Escherichia coli* serotype).

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P964 Molecular typing of *Salmonella enterica* serotype *Typhimurium* strains from Slovak Republic

H. Drahovská, E. Mikasová, E. Kaclíková, R. Karpísková, M. Sásik, J. Turna
Bratislava, SK; Brno, CZ

Objectives: The variability of *Salmonella typhimurium* strains was studied by PCR-based methods.

Methods: 28 strains of *S. typhimurium* were isolated from food or animal sources in the course of surveillance programmes. Strains were phagotyped and their antibiotic resistance was determined by disk diffusion method. Fluorescent AFLP was done using EcoRI and MseI enzymes and AFLP products were separated by capillary electrophoresis.

Results: The presence of integrons was analysed in all 28 strains of *S. typhimurium* and three different integron profiles (IPs) were detected by amplification of variable region of the integrons. The IP-1 profile, characterised by two PCR products of 1.0 and 1.2 kb, was present in six strains. All these strains were multiresistant with resistance ACSSuT or ACSSuTNa. The IP-2 profile contained single 1.6 kb PCR product and was present in six strains resistant to ASuTmp or ASSuTmp. The dhfrA1 gene was confirmed to be an integral part of IP-2 integron. A total of 0.2 kb PCR product (IP-3) was amplified in two strains sensitive to all antimicrobials. As lysogenic bacteriopahges could frequently transfer their DNA into the bacterial cell and thus change chromosomal composition, phage-related sequences were probed in *S. typhimurium* strains by PCR with primers complementary to four genes of phage P22 (g8, g13, eae, eac). Three different types of PCR products were detected in multiplex reaction: the presence of g8 sequence only, the simultaneous occurrence of g8 and eac or presence of g8 and g13. Nine strains did not contain any from the tested phage-related genes. The relatedness between strains was further monitored by AFLP. We observed high strain-to-strain similarity as Dice coefficients fell in the range of 94–100%. According to the presence of several DNA fragments, strains were separated into eight AFLP clusters.

Conclusions: By comparison of all methods we obtained corresponding results in strain clustering. All methods can be used for subtyping of *S. typhimurium* strains.

P965 Detection of fimbrial adhesin genes in ESBL-producing *Klebsiella* strains isolated from nosocomial infections

K. Matusiewicz, B. Maczynska, D. Olejniczak, A. Przondo-Mordarska, R. Franiczek
Wroclaw, PL

Objectives: *Klebsiella bacilli* present many pathogenic properties, which determine their ability to survive and rapid spreading in hospital environment. The adhesive properties of *Klebsiella bacilli* associated with the presence of fimbrial and non-fimbrial adhesins play a very important role in pathogenicity of these bacteria. Rapid spread of patogenical factors is often connected with presence of their plasmid-mediated genes. The aim of our study was to detect plasmid and chromosomally born fimH and mrkD genes encoding main adhesins: MS and MR, respectively.

Methods: A total of 55 *Klebsiella* clinical isolates obtained from patients hospitalised in different hospital wards were studied. The phenotypic activity of fimbriae was characterised by haemagglutination method. The genomic and plasmid DNA were isolated using manual method as well as Qiagen DNA kits. The presence of genes encoding main adhesins were detected using PCR-method with primers detected fimH and mrkD genes

Results: 40% of strains displayed phenotypic activity of both type 1 and type 3 fimbriae, 25.5% showed only activity of type 1 fimbriae, 30.9% only of 3 type fimbriae and 3.6% strains showed the lack of hemagglutination activity. The percentage of detected genes using PCR, was higher then showed results of phenotypic activity. The presence of mrkD genes was detected in 100% investigated strains in chromosomal DNA and 85.4% showed both mrkD and fimH genes. A total of 5.5% strains demonstrated only fimH genes in chromosomal DNA and 3.6% strains showed no genes. In plasmid DNA, the presence of main adhesin genes confirmed in 33% *Klebsiella* strains (mrkD genes in 20% strains, both fimH and mrkD in 9% and only fimH in 4% strains).

Conclusions: The presence of fimH and mrkD genes in genomic and plasmid DNA not always leads to phenotypic expression of fimbrial adhesins. The activity of type 3 fimbriae is connected with chromosomal variant of mrkD gene. In case of fimH genes, the plasmid variant is enough for haemagglutination activity of type 1 fimbriae. The percentage of detected fimH and mrkD plasmid genes depended on hospital units from which these strains were isolated. This suggests the spread of plasmid-encoded adhesins among *Klebsiella* strains.

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P966 Development and validation of amplified fragment length polymorphism (AFLP) for molecular epidemiology and identification of *Klebsiella pneumoniae* and *Klebsiella oxytoca* phylogenetic groups

D. Jonas, B. Spitzmüller, F.D. Daschner, J. Verhoef, S. Brisse
Freiburg, D; Utrecht, NL; Paris, F

Objectives: Bacteria of the genus *Klebsiella* are opportunistic pathogens responsible for an increasing number of multiresistant infections in hospitals. The two clinically and epidemiologically most important species, *Klebsiella pneumoniae* and *K. oxytoca*, have recently been shown to be subdivided into three and two respective phylogenetic groups. The aim of this study was in-depth evaluation of the amplified fragment length polymorphism (AFLP) genetic characterisation method.

Methods: First, we investigated the variability of AFLP patterns for *Klebsiella* strains within and between different outbreaks. Second, by use of carefully characterised, phylogenetically representative strains, we examined whether different *Klebsiella* species and phylogenetic groups can be discriminated using AFLP.

Twenty-four strains originating from seven presumed outbreaks and 31 non-associated strains were investigated.
Results: The AFLP fingerprints of all epidemiologically associated strains showed three or fewer fragment differences, whereas unrelated strains differed by at least four fragments. Cluster analysis of the AFLP data revealed a very high concordance with the phylogenetic assignation of strains based on gyrA sequence and ribotyping data. The species *K. pneumoniae*, *K. oxytoca*, *K. terrigena* and the possibly synonymous pair *K. planticola*/*K. ornithinolytica* each formed a separate cluster. Similarly, strains of the phylogenetic groups of *K. pneumoniae* and *K. oxytoca* fell into their corresponding cluster, with only two exceptions.
Conclusion: This study provides a preliminary cut-off value for distinguishing epidemiologically non-related *Klebsiella* isolates based on AFLP data, confirms the sharp delineation of the recently identified phylogenetic groups and demonstrates that AFLP is suitable for identification of *Klebsiella* species and phylogenetic groups.

P967 Molecular serotyping of *Klebsiella* by restriction of the amplified capsular antigen gene cluster

S. Brisse, S. Issenhuth-Jeanjean, P.A.D. Grimont
Paris, F

Objectives: K-serotyping, i.e. determination of the capsular antigen, has been the preferred typing method for *Klebsiella* isolates, as it is highly discriminatory (77-types are known) and as K-types are known to differ in their pathogenic potential. Unfortunately, K-serotyping requires a large collection of sera and is restricted to a few reference centres. Moreover, K-serotyping suffers from cross-reactions and is not applicable to non-capsulated strains. The objective of this work was to develop a molecular method that would enable to determine the K-serotype without using antiserum.
Methods: We amplified by PCR the capsular antigen gene cluster (cps) and the PCR product (10–18 kb long) was digested with HincII, followed by agarose gel electrophoresis (cps PCR–RFLP).
Results: The profiles (called C-patterns) obtained for 228 strains representing the 77 known K-serotypes showed four to 13 bands in the size range 0.2–4.361 kb. A total of 128 distinct C-patterns were obtained. The following important observations were made: (i) The C-patterns obtained for strains of any K-serotype were distinct from the C-pattern of all other K-serotypes, with the only exception of serotypes K22 and K37, which are known to cross-react. (ii) For 12 K-types, C-pattern variation was found among strains with the same K-serotype; in most cases, the strains with variant C-patterns belonged to other *Klebsiella* species than the reference strain. Thus, cps PCR–RFLP has a higher discriminatory power than classical K-serotyping. (iii) Within *K. pneumoniae*, we observed C-pattern identity among strains of a given K-type, for example K1 or K3, that were collected many years apart and from distinct sources. This stability of the C-pattern indicates that cps PCR–RFLP is suitable for long-term epidemiology of capsular types. (iv) Only 2.8% (compared with 8–23% for classical K-serotyping) of the strains analysed by cps PCR–RFLP were non-typable, because PCR amplification failed. (v) The value of cps PCR-RFLP for K-serotype determination was tested on 21 recent *K. pneumoniae* clinical isolates. The K-serotype of 18 (86%) of them could be deduced from the comparison of their C-pattern with the database. (vi) Four of five non-capsulated strains analysed showed a recognisable C-pattern.
Conclusions: cps PCR–RFLP allows determination of the K-serotype, while being easier to perform and more discriminatory than classical serotyping, and allowing the characterisation of non-capsulated strains.

P968 Cloning of 16S rRNA genes amplified from normal and disturbed vaginal microflora points to a strong association between *Atopobium vaginae*, *Gardnerella vaginalis* and bacterial vaginosis

R. Verhelst, H. Verstraelen, G. Claeys, G. Verschraegen, J. Delanghe, L. Van Simaey, C. De Ganck, M. Temmerman, M. Vaneechoutte
Ghent, B

Objective: Genotypic analysis of the microbiological differences between the bacterial vaginosis (BV) microflora and the healthy vaginal microflora.
Methods: A total of 150 vaginal swab samples from healthy women were categorised on the basis of Gram stain of direct smear as grade I (only *Lactobacillus* morphotypes, $n = 112$), grade II (intermediate, 26), grade III (bacterial vaginosis, 9) or grade IV (only Gram-positive cocci, 3). (1) The composition of the vaginal microbial community of eight of these vaginal swabs (three grade I, two grade II and three grade III), were studied by culture and by cloning of the 16S rRNA genes obtained after direct amplification. (2) Species-specific PCR for *Atopobium vaginae* and *Gardnerella vaginalis* was carried out for all 150 vaginal swab samples. (3) Forty-six cultured isolates were identified by tDNA-PCR and 854 cloned 16S rRNA gene fragments were sequenced, yielding a total of 38 species.
Results: Cloning revealed that *A. vaginae* was abundant in four out of the five non-grade I specimens and that *Lactobacillus iners* was the only *Lactobacillus* species that was present in non-grade I specimens, while it was absent from grade I samples. Respectively 1.8% (grade I), 15.7% (grade II) and 77.7% (grade III) of the vaginal swab samples were positive for both *A. vaginae* and *G. vaginalis* species-specific PCR ($P < 0{\cdot}001$, chi square).
Discussion: Culture independent, molecular analysis revealed a higher microbial diversity in non-grade I specimens than did culture. Together, culture, 16S rRNA gene cloning and species-specific PCR point to the presence of nine presumptively novel bacterial species and to a strong association between *A. vaginae*, *G. vaginalis* and bacterial vaginosis and to an ambiguous role for *L. iners*. It appears as if *A. vaginae* may be a constituent – in low numbers – of the human vagina, possibly attaining replicative dominance in association with decreasing lactobacillary grading. The presence of *A. vaginae* in bacterial vaginosis(-like) microflora may shed new light on the aetiology of this condition.

P969 Molecular typing of *Vibrio cholerae* strains having different sets of 'pathogenicity islands'

E.A. Kostromitina, G.A. Yeroshenko, N.I. Smirnova
Saratov, RUS

Vibrio cholerae El Tor biovariant is characterised by the presence of a number of different mobile chromosomal genetical elements. Using multilocus PCR tests with various primers in the genomes of 186 strains isolated in the territories of Russia and Turkmenistan we were able to detect three housekeeping genes (hapA, toxR, rtxA) and nine virulence genes located in prophages and 'pathogenicity' and 'persistence' islands: CTXphi (ctxA, zot, ace), RS1phi (rstC), VPI (tcpA, aldA, toxT), VPI-2 (nanH), EPI (mshQ). Besides, we used the methods of ribotyping and PCR typing which involved the 'random' primer, 1281, to elucidate genetical relationship between the strains of varying epidemic significance. The genome of clinical isolates obtained from patients during several epidemic outbreaks, was shown to be stable and to contain all the genes tested. *C. vibrios* isolated during the interepidemic period from natural ecosystems, formed a heterogenous population represented by single virulent clones that had retained the complete set of the genes under study, by non-toxinogenic strains, that had lost only individual genes and (or) pathogenicity blocks of genes, i.e. either CTXphi and RS1phi, or CTXphi, VPI and RS1phi, or by those carrying deficient prophages CTXphi (ctxA− zot+ ace+) and VPI (ctpA− aldA+ toxT+), as well as by clones containing only

housekeeping chromosomal genes and sometimes a gene from the 'persistence island'. As soon as virulent clones get into water environment, they lose their virulence blocks in the following order: CTXphi and RS1phi, then VPI, gene VPI-2 being the last one to be lost. In conformity with the results of the above three genotyping methods, epidemically hazardous strains, represented a homogenous group, suggesting a single clonal origin. Close genetical relationship between these strains and non-toxinogenic vibrios, that partly retained their virulence genes, was also established. At the same time, as shown by ribotyping and PCR typing studies, avirulent 'water' vibrios formed an independent group, because their genotypes manifested quite distinct features, in contrast to the first two vibrio groups. Thus, the observed genotype heterogeneity of El Tor cholera vibrios living in water ecosystems was likely to be a result of the loss of DNA fragments varying in their length and functions. The genotyping procedures used in the work made it possible to discover evolutional relationships among the bacterial strains under study.

P970 Comparative study of enterotoxigenic *Bacteroides fragilis* (ETBF) strains isolated in France and in Poland

P. Obuch-Woszczatynski, A. Belkum van, L. Dubreuil, D. Dzierzanowska, H. Pituch, M. Luczak, F. Meisel-Mikolajczyk
Warsaw, PL; Rotterdam, NL; Lille, F

Bacteroides fragilis Gram-negative anaerobic rods, 132 strains isolated in Poland and 53 in France (from intestinal and extraintestinal sources) were compared in this study. The identification of bacterial strains was done on the basis of Gram staining, growth on selective BBE (Bacteroides Bile Esculine) medium, and biochemical characteristics determined by the API 20 A test (bioMérieux, France). For assessment of the presence of enterotoxin (fragilysin) gene in analysed strains, the PCR method was used. DNA for PCR was isolated using Genomic DNA PREP PLUS (A&A Biotechnology, Poland) and amplification was performed in a Techne thermocycler with primers 404 (5'-GAG CCG AAG ACG GTG TAT GTG ATT TGT-3'-TGC TCA GCG CCC AGT ATA TGA CCT AGT-3'). The PCR program consisted of the following steps: 94°C for 4 min, 40 cycles of 94°C (1 min), 52°C (1 min) and 74°C (1 min). Among the Polish 132 strains, 16 contained the fragilysin gene. Of the 53 French strains 10 contained the fragilysin gene. For all these strains, pulsed field gel electrophoresis (PFGE) was performed. Bacteria were suspended in SE buffer (75 mM NaCl, 2.5 EDTA pH 8.0), embedded in 0.5% agarose plugs and lysed overnight at 55°C. Plugs were washed five times in SE at room temperature afterwards. DNA in the plugs was digested using Not I (Boehringer Mannheim, Germany). Electrophoresis was performed in a CHEF Mapper (BioRad, Venendaal, The Netherlands). The voltage was 10 V/cm for 18 h with linear ramping from 5 to 35 s at ±60° angles. In conclusion, 19% strains isolated in France and 15% of those isolated in Poland contained the fragilysin gene. The PFGE analysis revealed that strains isolated in Poland and in France show genetically differentiation (these strains are genetically not homogenous).

P971 Rapid detection of mutations in *Candida albicans* by pyrosequencing technique

G. Orr, D. Concas, V. Piras, S. Cosentino, M.B. Pisano, M.E. Fadda
Cagliari, Monserrato, I

Objectives: Different molecular mechanisms of resistance to azole antifungal agents, that can exist simultaneously, have been described in *Candida albicans* strains. One of these mechanisms includes alterations in the gene encoding the target enzyme ERG11. In the present study we used Pyrosequencing method to conduct an epidemiologic survey in Ketoconazole-susceptible and -resistant strains of clinical *C. albicans* strains isolated in our region, to determine differences in the gene encoding lanosterol-demethylase (ERG11).

Methods: The strains of *C. albicans* were obtained by swabbing the oral mucosa of subjects with oropharyngeal candidiasis. Susceptibility to Ketoconazole was tested using the broth microdilution method recommended by the NCCLS document M27-A. Concentrations of Ketoconazole tested were in the range 0.03–16 mg/mL. The MIC endpoint was defined as the lowest concentration at which 80% of growth was inhibited, compared with the drug-free control. Yeasts were grown in Sabouraud agar and DNA was extracted by using QIAamp DNA Mini Kit (Quiagen). PCR primers matched an ERG11 gene region of 178 bp. One of the primers of PCR fragment was biotinylated, a single strand of PCR products was obtained with streptavidin-coated beads method. Samples were analysed using a PSQ 96 System with SQA Software and SQA reagent.
Results: A total of 31.2% of strains exhibited DDS or resistance to ketoconazole (MIC >0.25 μg/mL). The sequence analysis was designed to cover a region of the ERG11 gene including codons 464–483. Previous studies showed that in this region, the mutations G464S, G465S, R467K and I471T are associated with azole resistance in *C. albicans*. In our study the sensitive strains have shown no mutations. Among DDS and resistant strains, only the mutation G464S was found in two strains, while no mutations were demonstrated in the remaining isolates.
Conclusion: This study is the first to use the Pyrosequencing system to characterise changes in nucleotide sequence of the ERG11 gene fragment involved in azole resistance of *C. albicans* strains. The observation of one point mutation in only two resistant strains tested suggests a limited role of the region of the ERG11 gene analysed in the azole resistance among *C. albicans* strains present in our region. However, the Pyrosequencing system has shown to be a fast and specific technique for detection of point mutations in the region of ERG11 gene of *C. albicans* strains.

P972 Development of a novel typing method for the *Escherichia coli* verocytotoxin 2 variants. Correlations to the clinical manifestations

S. Persson, F. Scheutz, K.E.P. Olsen
Copenhagen, DK

Background: Verocytotoxin 2 (VT2) of verocytotoxin producing *Escherichia coli* (VTEC) is a potent toxin, capable of producing serious complication, when excreted from the bacteria colonising the intestinal tracts. The mature toxin is composed of one A-subunit and five identical B-subunits, and is encoded by the approximately 1240 bp vtx2AB operon. Based on the variable nucleic acid sequence of both subunits, several toxin variants have been identified.
Objectives: The subtype designation, important sequence motifs and clinical significance of the vtx2 variants, are not consistent throughout the literature. To shed more light on these features, a novel typing method was developed for the investigation of subtype-specific correlations to the clinical outcome.
Methods: The subtyping method relies on PCR and sequencing. By use of vtx2 universal primers, a 630-bp fragment covering the most variable regions of subunit A and B was amplified by PCR, and subsequently sequenced.
Results and conclusion: The present method was used for the analysis of vtx2-positive strains from our strain collection, counting 274 strains, isolated from patients with known clinical manifestations (HUS, HC, bloody diarrhoea, diarrhoea, fever, etc.). Compared with traditional subtyping, our preliminary results indicate that most strains in our strain collection harbour the vtx2 or vtx2c subtype, in addition to a few strains containing the activatable carboxy-terminus of subunit A, referred to as vtx2d. Correlations between these subtypes and the clinical complications will be presented. Additionally, the novel sequences from our strain collection will be investigated for other sequence motifs connected to the clinical outcome. As sequencing has become more accessible and less expensive, we believe that this method, offers a good and reliable alternative for diagnostic subtyping of VTEC strains from these infections.

P973 Application of molecular biological techniques to the study of alterations in hamster gut microflora and assessment of treatment with *Saccharomyces boulardii*

L. Coroler, G. Philippe-Taine, E. Bayart, T. Cécile, J.-M. Gillardin, H. Goïot
Compiègne, F

Objectives: Studies of the intestinal microbial ecosystem by classical culture techniques suggest that only 30% of the microflora can be cultured. PCR procedures based on 16S rRNA gene specific for bacteria were developed to detect bacterial populations in hamster faeces.
Methods: A total of 30 populations of bacteria were characterised by their genomic DNA sequences and targeted by PCR probes: *Actinomyces* group, *Bacteroides distasonis*, *Bacteroides fragilis*, *Bifidobacterium* group, *B. adolescentis*, *B. angulatum*, *B. catenulatum*, *B. infantis*, *B. longum*, *Clostridium* group, *C. clostridiiforme*, *C. coccoides*, *C. difficile*, *C. leptum*, *C. perfringens*, *Fusobacterium prausnitzii*, *Lactobacillus* group, *Peptosteptococcus productus*, *Propionibacterium* group, *Pseudomonas aeruginosa*, *Ruminococcus obeum*, *Citrobacter* group, *C. freundii*, *Escherichia* group, *Enterobacteria* group, *Enterobacter cloacae*, *Morganella morganii*, *Proteus mirabilis*, *Staphylococcus* group, *Salmonella* group.
Results: Sensitivity was measured by extraction of total genomic DNA and PCR amplification and a significant detection level of 10^3 bacteria/faecal sample was obtained. Qualitative variations of bacteria population were observed during the first 2 weeks of acclimatisation, suggesting a stabilisation period for hamster microflora in new environmental conditions. After oral antibiotherapy, with one dose of 30 mg/kg amoxicillin-clavulanic acid, some groups were eradicated from hamster faeces: *Propionibacterium*, *Staphylococcus* and *C. leptum*, *C. clostridiiforme*. As reported in the literature, no antibiotic effect was observed on levels of dominant faecal groups: *Bifidobacterium*, *Peptostreptococcus*. Antibiotic-associated perturbations are linked with the disruption of the normal intestinal flora leading to a colonisation of pathogen bacteria species. In order to understand the role of *Saccharomyces boulardii* (S.b.) in prevention of antibiotic-associated diarrhoea, 4×10^{10} CFU/kg/day of S.b. were administered to hamsters during oral antibiotic treatment. The results showed that populations that were eradicated by antibiotic administration remained expressed and stabilised with concomitant S.b. treatment, suggesting an effective protection by S.b. on the intestinal flora.
Conclusions: These PCR results should be used to quantify the intestinal microflora by DNA microarray analysis.

P974 Identification of *Acinetobacter calcoaceticus* – *A. baumannii* complex by sequence analysis of the 16S–23S rDNA spacer region

Y.F. Wei, H.C. Chang, M. Vaneechoutte, A.H. Huang, T.C. Chang
Tainan, TW; Ghent, B

Objectives: The *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex (Acb complex) includes *A. calcoaceticus* (genospecies 1), *A. baumannii* (genospecies 2), unnamed genospecies 3 and 13TU. These species are difficult to differentiate by phenotype. In this study, the feasibility of using sequences of the 16S–23S rDNA spacer region (ITS) for identification of the Acb complex was evaluated.
Methods: The bacteria-specific universal primers 13 BF (GTGAA TACGT TCCCG GGCCT) and 6R (GGGTT YCCCC RTTCR GAAAT) (Y = C or T, and R = A or G) were used to amplify a DNA fragment that encompassed a small portion of the 16S rDNA region, the ITS, and a small portion of the 23S rDNA region. The ITS regions from 108 reference strains (42 species) of nonfermenters including strains of Acb complex were amplified by PCR and sequenced; the sequence data in combination with those available in GenBank were used to construct an ITS sequence database for the identification of Acb complex. For reference strains of each species of the Acb complex, the sequence similarities of the ITS regions were obtained by comparing their ITS sequences with that of the type strain of the same species. The database was used to test 82 clinical isolates of Acb complex, including 63 isolates of *A. baumannii* and 19 isolates of *A. calcoaceticus*, as identified by API 20NE.
Results: *A. baumannii* had the shortest ITS fragment (607–609 bp) followed by genospecies 13TU (608–615 bp), genospecies 3 (619–621 bp) and *A. calcoaceticus* (627–638 bp). The intraspecies ITS similarity of the Acb complex was very high, ranging from 0.98 to 1.0, whereas the interspecies ITS similarity was relatively low (range: 0.86–0.91). Among the 63 clinical isolates of *A. baumannii*, two isolates were genospecies 3 and 14 isolates were ungroupable, as revealed by ITS sequence analysis. Therefore, about 25% of clinical isolates of *A. baumannii* was misidentified. Furthermore, among the 19 clinical isolates of *A. calcoaceticus*, 16 isolates were genospecies 3 and three isolates were ungroupable. Therefore, the designation of *A. calcoaceticus* to clinical isolates is, under most conditions, not correct. These results were confirmed by amplified rDNA restriction analysis (ARDRA).
Conclusions: ITS sequence analysis provides a simple and useful alternative for species delineation of the Acb complex.

P975 Utility of the CVD432 probe for identification of enteroaggregative *Escherichia coli* amongst isolates from traveller's diarrhoea

L.R. Macfarlane, J.N. Fletcher, R. Ashton, P.A. Chapman, A.M. Snelling, I.N. Okeke
Bradford, Sheffield, UK; Pennsylvania, USA

Objectives: Enteroaggregative *Escherichia coli* (EAEC) are increasingly implicated in acute and persistent diarrhoea around the world. Phenotypically, EAEC have a defining 'stacked brick' pattern of aggregative adherence (AA) to epithelial cell lines *in vitro*. Genotypically, they are diverse, and while a range of EAEC pathogenicity factors are known, their distribution amongst strains varies. The most widely used DNA probe for EAEC is CVD432, which has been reported to have limited sensitivity in some studies, but it is presumed to be specific for EAEC. The aim of this study was to determine whether the CVD432 probe is a specific tool for identifying EAEC strains imported into the UK.
Methods: A total of 520 *E. coli* isolates (four per patient) were obtained from consecutive stool samples of 130 diarrhoeal patients with a recent history of foreign travel (33 different countries). All were screened for hybridisation with the CVD432 probe, as well as EAEC plasmid encoded virulence factors aggR (aggregative adherence regulator), aap (dispersin) and the chromosomal pathogenicity-island-encoded mucinase pic. Other pathogenic *E. coli* were identified using standard probes. CVD432 probe positive strains were then examined for adherence to HEp 2 cells after co-incubation for 3 h.
Results: The prevalence of EAEC-associated genes amongst the 520 isolates was: CVD432 8.3%; aggR 6.3%; aap 11%; pic 13.3%. Adherence assays on the 43 isolates that were CVD432 positive revealed a mixture of aggregative (24 isolates) and non-adherent strains (nine isolates) plus 10 isolates that gave an unusual pattern of loose, highly localised aggregation, present on <5% of the HEp-2 cells. Of the CVD432 positive strains, 65% were aap, aggR, and pic positive as well, but this group also contained strains of all three adherence types. None of the other EAEC-associated probes was unequivocally predictive of actual AA among CVD432 positive strains. Unexpectedly, CVD432 positive isolates that hybridised with the enteropathogenic *E. coli* probe eae were isolated from one patient (returning from Turkey).
Conclusions: This study suggests that the CVD432 probe may not be specific for true EAEC, even when combined with the other probes used here. The significance of the newly described adherence pattern in relation to diarrhoeal disease remains to be elucidated, as does the finding of *E. coli* with both EAEC and EPEC properties.

P976 Shiga toxin-producing *Escherichia coli* O157 in Slovenia

P. Zabukovnik, A. Andlovic, A. Zore
Ljubljana, SI

Objectives: In the Institute of Microbiology and Immunology (Department for bacterial diagnostics of diarrhoeal infections), Medical Faculty in Ljubljana, we wanted to introduce multiplex PCR test for detection of Shiga toxin-producing *Escherichia coli* (STEC). Until recently we used only enzyme immunoassay (EIA) to detect production of Shiga toxin (STX) in specimens. Institute of Microbiology and Immunology has extensive collection of *E. coli* isolates from human faeces (mostly from hospitals in Ljubljana). We decided to test isolates in our collection from 1993 to 2002, with serogroup O157. We used multiplex PCR assay that amplified sequences in four virulence genes (Shiga toxin 1 (stx1), Shiga toxin 2 (stx2), intimin (eaeA), enterohemolysin (ehxA)).
Methods: All isolates were serotyped with rabbit O antisera. We used multiplex PCR to detect presence of Shiga toxin 1, Shiga toxin 2 (and sub variants, but did not discriminate between them), intimin and enterohemolysin genes. We also tested those strains for production of STX with EIA.
Results: We tested 20 *E. coli* isolates with serogroup O157 and found 10 STEC. stx2 and ehxA genes were present in almost all STEC O157 isolates. The most common PCR profile (five of 20) of O157 isolates had stx2, eaeA and ehxA genes. One isolate had stx2 gene but did not produce Shiga toxin (or possibly EIA did not detect produced Shiga toxin). Most of those 10 STEC O157 were isolated in summer months of July and August. Two O157 STEC were isolated in the year 1997 shortly one after another. They had identical multiplex PCR profile. The same happened in the year 2002.
Conclusion: We notice increase in the number of STEC O157 isolates per year in years after 1997. This may be because of use of better diagnostic methods. In last years STEC O157 with PCR profile stx2, eaeA and ehxA is dominant. In years 1993 to 1998 the dominant PCR profile had stx1, stx2, eaeA and ehxA genes.

Urinary tract infections

P977 Prevalence and aetiology of chronic bacterial prostatitis

M. Sukhorukova, B. Gvasalia, I. Tascherev, L. Stratchounski
Smolensk, RUS

Background: Chronic prostatitis is recognised to be caused by infectious and non-infectious prostatic inflammation as well as non-inflammatory diseases, but the separation of various prostatitis syndromes is difficult to perform. Bacterial prostatitis is a common diagnosis and a frequent indication for antimicrobial therapy. However, confirmation of aetiology of inflammation is exceedingly uncommon.
Objectives: The aim of this study was to determine the prevalence and aetiology of chronic bacterial prostatitis among the patients with clinically confirmed diagnosis.
Methods: Between October 2002 and October 2003 the patients with suspected prostatitis were examined. The clinical diagnosis was confirmed in patients within 3 months or greater duration of the following signs and symptoms: perineal discomfort, pain following ejaculation, urinary frequency, urgency, dysuria, low back pain, suprapubic pain, palpation of a tender prostate on physical examination. The bacteriological diagnosis was determined in patients, who had not been taking antibiotics in the previous month, by Meares and Stamey technique. Prostatitis was categorised according to NIH classification.
Results: A total of 129 patients were examined. Chronic bacterial prostatitis (NIH category II) was found in nine patients (7.0%), inflammatory chronic pelvic pain syndrome (NIH category IIIa) – in 59 (45.7%), non-inflammatory chronic pelvic pain syndrome (NIH category IIIb) – in 61 (47.3%). The following pathogens were isolated in NIH Category II: *Staphylococcus* spp. – in three (33.3%), anaerobic bacteria (*Prevotella* spp., *Prevotella* spp. and *Peptostreptococcus* spp.) – in three (33.3%), *Escherichia coli* – in two patients (22.2%), *Acinetobacter lwoffii* – in one (11.1%).
Conclusions: Chronic bacterial prostatitis is an important but rare clinical entity. Careful examination using quantitative segmented bacteriologic cultures leads to proper categorisation into the recognised forms of the prostatic syndrome. The most common pathogens of chronic bacterial prostatitis were *Staphylococcus* spp., anaerobic bacteria (*Prevotella* spp. and *Peptostreptococcus* spp.) and *E. coli*.

P978 Comparison of prevalence and clinical management of prostatitis in the Italian and North American experience

F. Marchetti, M. Rizzo, F. Travaglini, A. Trinchieri, C. Nickel
Verona, Florence, Milan, I; Kingston, CAN

Objectives: A prospective multicenter urology outpatient survey, undertaken to examine prostatitis in Italy, is used to compare the prevalence, characterisation, diagnosis and treatment of prostatitis patient with the North American (NA) prostatitis patient.
Methods and materials: Seventy urologists, representing a cross-section of urologic centres in Italy, counted and recorded the overall total male patients reported in the clinic and the overall total patients diagnosed with prostatitis over a 5-week period. Results were compared with published practice prevalence and cohort data (in particular the NIH Chronic Prostatitis Cohort Study – CPC and Seattle Prostatitis cohorts) examining similar data in NA.
Results: A total of 1148 patients were identified with prostatitis (12.8%). The mean age of the prostatitis patients was 47.1 (range 16–83). The most common urinary diseases were benign prostatic hyperplasia (17.4%), recurrent urinary tract infections (11.2%) and urinary calculogenesis (11.1%), while the most common concurrent diseases were diabetes (7.2%) and depression (6.8%). The most frequently reported and most severe symptoms at time of evaluation were irritative voiding symptoms, perineal and suprapubic pain and discomfort. Over three quarters of the patients were dissatisfied with their quality of life. Bacteria were cultured in 15.6, 17.7 and 14.0% of EPS, VB3 and semen specimens, respectively. Comparison to NA data suggests that the European prostatitis patient and the European urologists' approach to the diagnosis and treatment of prostatitis are not that dissimilar to prevalence and management of prostatitis in NA.
Conclusion: Prostatitis is a common worldwide outpatient diagnosis, comprising a significant percentage of male outpatient visits to urologists in both Europe and NA. The similarities in prevalence, characterisation and management of the typical prostatitis suggests that an international collaborative research effort is indicated in this important urological condition.

P979 Aetiology and resistance of community urinary tract infections in São Paulo, Brazil: a three-year survey with 27 437 positive cultures

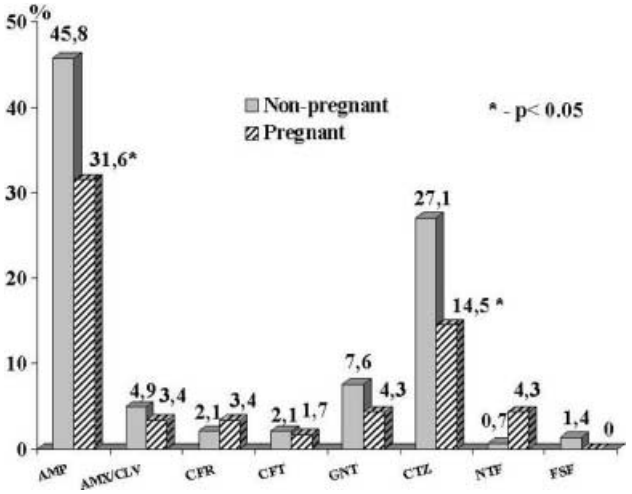
C. Kiffer, C. Oplustil, J. Sampaio, L. Kudo, C. Mendes
Sao Paulo, BR

Objectives: Assess the prevalence of pathogens responsible for urinary tract infections (UTI) in community patients and their antimicrobial resistance pattern in São Paulo, Brazil.
Methods: From January 2000 to December 2002, 199 334 urine cultures from community patients were collected. Positive cultures with one single pathogen and a colony count $\geq$ 100 000 CFU/mL were selected. Based on these criteria, 27 437 (13.8%) positive cultures were analysed in this survey. Chi-square test for trend (Altman, 1999) was performed to evaluate the resistance prevalence ordering in the years surveyed ($P < 0.05$ was considered significant).
Results: Among the 27 437 positive cultures, 88.8% were from female and 11.2% from male patients. Among the positive cultures 89.3% presented growth of Enterobacteriaceae followed by 6.7% of Gram-positive cocci. *Escherichia coli* (72%) presented the highest prevalence, followed by *K. pneumoniae* (6.3%) and *P. mirabilis* (5.9%). Among Gram-positive isolates, *E. faecalis* was the most prevalent (4.6%). Susceptibility tests were performed in 23 240 cultures. *E. coli* presented a resistance rate to ampicillin of 42.8%, to trimeth/sulfa of 33.6% and to tetracycline of 31.5%. For the quinolones, ciprofloxacin and norfloxacin, *E. coli* presented a higher resistance rate among the age group of $\geq$60 years old as compared with the age group of $\geq$13 and <60 years old (22.8 vs. 6.8% and 22.9 vs. 6.9%, respectively). Ciprofloxacin presented a Chi-square trend = 50 388 ($P < 0.05$), with ascending resistance in the years studied.
Conclusions: An important difference in the resistance pattern was observed among pathogens and age groups. The difference in age groups suggests the possibility of selective pressure due to previous antimicrobial use in the community setting. Ciprofloxacin could be used for empiric therapy in community UTI. However, its apparent ascending resistance should raise awareness as to possible usage restriction in this setting. Surveillance studies are useful for guiding therapy and helping curbing resistance.

P980 Resistance of *Escherichia coli* isolates from pregnant and non-pregnant women with community-acquired urinary tract infections in Russia: results of multicentre studies UTIAP-2002 and ARIMB

V. Rafalski, A. Shevelev, L. Stratchounski
Smolensk, RUS

Objective: To compare the resistance of *Escherichia coli* isolates from non-pregnant women with uncomplicated community-acquired urinary tract infections (CA-UTI) and pregnant women in different cities of the Russian Federation.
Methods: One hundred and forty-four non-pregnant and 117 pregnant women with signs of upper or lower community-acquired uncomplicated UTIs were enrolled in two multicentre prospective epidemiological studies (eight medical centres), UTIAP-2002 and ARIMB, respectively. The strains isolated from the patients who had significant bacteriuria (>105 CFU/mL) were included in the microbiological analysis. The MICs of antibiotics (ampicillin – AMP, amoxicillin-clavulanate – AMX-CLV, cefuroxime –CFR, cefotaxime – CFT, gentamicin – GNT, co-trimoxazole – CTZ, nitrofurantoin – NTF, fosfomycin – FSF) were determined by the agar dilution, as described in the NCCLS (2003) guidelines. Quality control was performed using reference strains including *E. coli* ATCC 25922, *E. coli* ATCC 35218.
Results: Resistance rates of *E. coli* from pregnant and non-pregnant women with CA-UTI in Russia are shown in Figure. There are some statistically significant differences in antimicrobial resistance between studied groups. Ampicillin resistance was higher among UTI isolates of *E. coli* in non-pregnant women (45.8%) than in pregnant women (31.6%), $P < 0.05$ (Chi-square statistic). Similar distinction in co-trimoxazole resistance was found – 27.1 and 14.5%, $P < 0.05$ (Chi-square statistic).
Conclusions: The resistance rates of *E. coli* from non-pregnant women with CA-UTIs in Russia to ampicillin and co-trimoxazole are significantly higher than in pregnant ones. There are no significant differences in resistance to other antimicrobials tested.



P981 Community-acquired urinary tract infections. A large scale prospective study of medical practices in French emergency departments

D. Elkharrat, F. Benhamou, P. Briquet-Brochard, C. Ginzburg, C. Leroux for the APPUI Study Group

Background: Urinary tract infections (UTI) are the second most frequent infections in French emergency departments (ED). However, their management in terms of antimicrobials (AM) and disposition of patients, according to anatomical site and severity of UTI, is unclear.
Objectives: Estimate prevalence and identify management of patients with UTI presenting in 78 EDs in France.
Methods: Consecutive patients with presumed UTI were included during 14 days if they were older than 15 years and had a positive urine dipstick. Subsequently, urine culture (UC) was prescribed and patients classified according to nine UTI categories. Centres were also required to notify all visits motivated by infectious diseases (ID) during the study period.
Results: Of 109 potential participants, 78 included 1054 UTI patients (13 $\pm$ 9/centre [2–58]). Based on a total of 47.2 $\pm$ 25.1 non-trauma patients and 6.2 $\pm$ 3.9 ID patients /centre/day during the same period, prevalence of ID is estimated at 13.4% of non-trauma visits and prevalence of UTI at 15.9% of all ID. The main UTI categories were acute cystitis (AC = 43.3%), acute pyelonephritis (AP = 38.0%), bacterial prostatitis (BP = 9.0%). Mean age of patients was 46.0 $\pm$ 23.8 years and sex ratio F in 71%. However, both differ significantly according to UTI category, from 36.5 $\pm$ 19.0 years and 92.5% F in uncomplicated AC to 65.0 $\pm$ 25.0 years and 72.4% F in complicated AC to 38.9 $\pm$ 19.6 years and 85.0% F in AUP. UC was ordered in 820 cases (77.8%) and data collected in 463. The latter were considered positive in 95% of cases and *Escherichia coli* was present in 81.6%. AM drugs were 990/846 patients, in combination in 17% of cases. Fluoroquinolones (FQ), whether in single therapy or in combination, accounted for 78.2% of all AM. From the ED, patients (n = 1037) were discharged (55.7%), maintained in the

observation unit for <24 h. before discharge (7.0%) or admitted (38.3%). The two factors that significantly correlate to hospital admission were the severity of UTI (53% of complicated AC, 34% of AUP, 80% of complicated AP and 57.3% of acute prostatitis) and patients' age (76.2 ± 17.9 years in those admitted with complicated AC vs. 48.8 ± 24.9 years in the non-admitted, 44.5 ± 22.0 vs. 33.3 ± 15.0 years in AUP and 61.8 ± 24.3 vs. 48.5 ± 24.1 years in complicated AP).
Conclusions: (1) Prevalence of UTI in French ED is very high. Based on seven million non-trauma visits nationwide in 2002, the number of expected UTI patients in the ED would amount to 147 000 annually. (2) Specifically designed guidelines for European ED may be helpful to ED physicians.

P982 Prostatitis syndromes and Stamey–Meares test results. Recording of 6-year experience in the department of internal medicine of a Greek tertiary hospital

P. Panagopoulos, I. Katsarolis, G. Koratzanis, K. Kanellakopoulou, H. Giamarellou
Athens, GR

Objectives: To record the results of four-glass test in a group of patients who have visited our Infectious Diseases Department due to Chronic Prostatitis symptomatology.
Methods: Two hundred and eighteen (218) males [mean age 32 years (18–70)] were studied retrospectively. The majority of patients (85%) received antimicrobial treatment during the past year. A four-glass test was performed for all patients and Gram (+), Gram (−), chlamydia, mycoplasma and ureoplasma were investigated accordingly.
Results: In a total of 218 patients, prostatic secretion specimens were collected in 168 (77%). In the remaining 50 (23%), voided bladder VB1, VB2 and VB3 specimens were collected and examined. Chronic Bacterial Prostatitis (CBP) was documented in 64 patients (29.35%), Chronic Pelvic Pain Syndrome (CPPS) was diagnosed in 131 (60.1%), whereas 23 (10.55%) had urethritis. Cultures of specimens revealed the following microorganisms: *Enterococcus* spp. 24, *Escherichia coli*, 6, *Pseudomonas* spp. 4, *Enterobacter* spp. 5, *Klebsiella* spp. 4, *Morganella* spp. 1, *Citrobacter* spp. 1, *Mycoplasma* spp. 1, *Chlamydia* spp. 3, *Ureoplasma* spp. 5.
Conclusion: The four-glass test is absolutely necessary for the diagnosis of chronic prostatitis syndrome and should be performed correctly in order to elevate the prostatic secretion specimen collection. The percentage of CPPS and CBP and the frequency of *Enterococcus* spp. as the responsible factor for CBP in this study are different considered to the English literature.

P983 Simultaneous urinary and respiratory infection or respiratory manifestations of urinary infection?

J. Losa, M. Tejero, L. Moreno, M. Velasco, R. Barba, A. Espinosa, C. Guijarro, A. Delgado-Iribarren, J. Valverde, V. Castilla, A. Zapatero
Madrid, E

Objectives: We often attend disabled old patients with presumed diagnosis of simultaneous urinary and respiratory infection (SUARI). Data concerning this problem are scant. We thus performed a study to describe it.
Methods: Descriptive analysis of all 162 patients diagnosed as SUARI within 24 h after hospital admission (June 2000 to June 2002). Demographic factors, underlying conditions, symptoms and signs, laboratory, radiological and microbiological data, antimicrobial therapy, outcome and final diagnosis were evaluated. Results are expressed by percentages or median as appropriate.
Results: Median age was 86 years, 50% were female and 56% were nursing home residents. Seventy-four per cent were dependent for activities of daily living, 22% had a permanent urinary catheter and 58% had cognitive impairment. The most frequent symptoms were fever (72%), decline in function (54%) and dyspnoea (50%); only 7% referred dysuria. Stupor (46%), crackles (40%) and ronchi (34%) were the commonest signs. Leucocytosis (14065/uL), elevated urea (64 mg/dL), respiratory failure (59%) and high C-reactive protein (142 mg/L) were the main laboratory abnormalities. Pyuria was observed in 71%, chest X-ray showed a pulmonary infiltrate in 48%, and 52% of cases fulfilled criteria of severe sepsis. Blood and urine cultures were positive in 18 and 52% of patients, respectively; gram-negative bacilli (GNB) were found in 82% of positive cultures, *Escherichia coli* being the most common agent. No pneumococci were isolated either in blood or sputum. Amoxicillin-clavulanate was the antimicrobial therapy most frequently administered (51%). Median hospital stay and mortality were 6 days and 27% respectively. Urinary tract infection was the commonest final diagnosis (61%).
Conclusion: Respiratory manifestations predominate in disabled old patients with GNB severe urinary sepsis initially diagnosed as SUARI. Respiratory distress may underlie this presentation. Further studies are required to support this contention.

P984 Risk factors for bacteriuria due to *Pseudomonas* or *Enterococcus* in patients hospitalised through the emergency department

D. Raveh, I. Rosenzweig, B. Rudensky, A.M. Yinnon
Jerusalem, IL

Objectives: To determine the incidence of, and risk factors for, isolation of *Pseudomonas aeruginosa* or *Enterococcus* from urine cultures obtained from patients in the emergency department (ED).
Methods: One year prospective, non-interventional study of all urine specimens collected in the ED, out of which one organism was isolated at a concentration of >100 000 CFU/mL. In this study were included all patients with *P. aeruginosa* or *Enterococcus* bacteriuria (study patients), and control patients with *Escherichia coli* bacteriuria subsequently hospitalised, at a ratio of two controls for each study case. Patients were interviewed with a structured questionnaire and charts were reviewed for demographic, clinical and laboratory indicators of *Enterococcus* or *Pseudomonas* bacteriuria as compared with *E. coli* bacteriuria.
Results: Over the 1-year study period, 744 positive urine samples were obtained from ED patients: 610 (82%) *Enterobacteriaceae* (including 476 isolates of *E. coli*) and 134 (18%) other organisms, of which 39 (5%) were *P. aeruginosa* and 28 *Enterococcus* (4%). Comparison with a randomly chosen control cohort of 80 patients with *E. coli* bacteriuria revealed several indicators for *Pseudomonas* bacteriuria, including male gender (odds ratio 3.3, 95%CI 1.5–7.2, $P < 0.005$), presence of a permanent urinary catheter (OR 15.4, 95%CI 2.2–140, $P < 0.005$), past prostatectomy (OR 13.4, 95% CI 1.5–315, $P < 0.01$), hospitalisation in the previous 2 months (OR 4.2, 95% CI 1.5–4.9, $P < 0.005$), and pregnancy (OR 2.8, 95%CI 2.1–3.6, $P < 0.05$). In addition, both *Enterococcus* and *Pseudomonas*, as compared with *E. coli*, significantly more often indicated asymptomatic bacteriuria in patients with other diagnoses, as opposed to clinically manifest bacteriuria, than isolation of *E. coli* (OR 4.2, 95%CI 1.2–14.4, $P < 0.05$).
Conclusions: *Pseudomonas* (5%) and *Enterococcus* (4%) are isolated from a significant minority of urine samples obtained from ED patients with clinically suspected bacterial infection. Isolation of these organisms, as compared with *E. coli*, more often indicates asymptomatic bacteriuria in patients with other infectious disease diagnoses. In addition, several independent clinical indicators for *Pseudomonas* bacteriuria were identified. These data may assist in selecting optimal antibiotic treatment for patients admitted with suspected urinary tract infection.

P985 **Influence of *Escherichia coli* virulence factors in the levels of C reactive protein in febrile urinary tract infections**

A. Smithson, J. Ruiz, S. Soto, J.P. Horcajada, J.A. Martinez, A. Moreno-Martinez, J. Vila, J. Mensa
Barcelona, E

Objectives: Certain virulence factors (VF), particularly pap fimbriae, are able to trigger production of cytokines, especially through activation of Toll-like receptor 4 (TLR-4), and therefore produce inflammation. The aim of this study was to assess the influence of certain VF in the degree of inflammation in febrile urinary tract infections (FUTI).
Methods: From 2002 to 2003, 117 adult patients with febrile community acquired FUTI (81 female with acute pyelonephritis (mean age 50 (SD = 22)) and 36 acute prostatitis (mean age 60 (SD = 16)) caused by *Escherichia coli* were prospectively included. Levels of C reactive proteins (CRP), white blood cell count (WBCC) and days until apirexia after beginning antibiotic treatment were recorded in all patients and considered as indirect markers of inflammation. Genes encoding haemolysin, type 1 fimbriae, pap G fimbriae, cytotoxic necrotising factor, aerobactin and autotransporter toxin were detected by a PCR. Additionally expression of type 1 fimbriae and haemolysin were detected by agglutination and growth on blood agar.
Results: Strains carrying pap G fimbriae were involved in FUTI with higher CRP levels than pap G fimbriae negative strains (14.95 vs. 10.51; $P = 0.028$). The relation between the rest of VF and CRP levels did not reach statistical significance. No differences were found regarding the WBCC and the duration of the fever.
Conclusions: These data indirectly suggest that the degree of inflammation in FUTI caused by *E. coli* is associated with the presence of pap G fimbriae, which is coherent with the fact that pap G fimbriae are coreceptors of TLR4.

Mycology: candida and aspergillosis

P986 **Initiation of an active surveillance programme on yeast-related bloodstream infections in France (ASPYRIF)**

E. Dannaoui on behalf of the French Mycoses Study Group

An active surveillance program has been implemented in France to prospectively analyse yeast-related blood stream infections. A pilot study was conducted from 1 October 2002 through 30 September 2003 in 23 medical centres in Paris and suburbs. For each patient, one isolate of each identified species was sent to the NRCM together with clinical data filled on a standard form. Identification was confirmed using phenotyping tests and a PCR assay was performed on all *Candida albicans* isolates to identify *C. dubliniensis*. Antifungal susceptibility testing to amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole and caspofungin was performed according to EUCAST recommendations. The median age of the 282 patients was 57 years [0–94 years], with a male predominance (59%). Underlying factors for yeast-related blood stream infections were often multiple for a given patient dominated by recent surgery (69%), central venous catheter (66%), hospitalisation in intensive care unit (53%), malignancy (42%), immunosuppressive therapy (28%), HIV infection (10%), solid organ (6%) or bone marrow (3%) transplantation and prosthetic devices (6%). Overall, the mortality rate was high with 43% of deaths within 30 days after the first positive blood culture. *Candida* spp. was the most frequent genus (96%) with *C. albicans* (49%), *C. glabrata* (14%), *C. parapsilosis* (13%) and *C. tropicalis* (10%) being the most frequent species isolated. Other *Candida* were recovered below 3% (*C. krusei*, *C. kefyr*, *C. lusitaniae*). Non- *Candida* spp. were *Trichosporon asahii*, *T. mucoides*, *Geotrichum capitatum* and *Cryptococcus neoformans*. Our data show that the percentage of non- *albicans* species equal that of *C. albicans* among the yeasts recovered during fungaemia. The proportion of the four major species differed significantly according to the presence of central venous catheter ($P = 0.02$). Analysis of the antifungal susceptibility testing results revealed that most of the isolates had usual antifungal susceptibility profiles. In conclusion, ASPYRIF is a powerful tool that should allow us to accurately describe the epidemiology of yeast-related blood stream infections in France without restriction to any underlying disease or species.

P987 **Nosocomial candidaemia: emerging resistance, newer antifungals and cost implications**

A. Guleri, G.D. Corcoran, S.R. Alcock, A.B.J. Speekenbrink, G.S. Shankland
Glasgow, UK

Background: Nosocomial candidaemia is associated with significant morbidity and mortality in the critically ill. Emergence of fluconazole resistance raises further problems, but the newer antifungal drugs [voriconazole, caspofungin, ambisome and abelcet] offer alternative therapeutic options. They also raise the issue of treatment-associated costs. An 8-year [1996 to November 2003] clinical audit was conducted across two tertiary care hospitals [Western Infirmary and Gartnavel General Hospital, Glasgow]. The distribution of *Candida* species and fluconazole/itraconazole resistance, with emphasis on high-risk areas was studied. It also addresses the newer antifungal options, cost implications and patient risk-stratification approach.
Methods: Eight-year data on blood culture yeast isolates with *in vitro* [NCCLS] antifungal susceptibility [AFST] profiles.
Results: Of 154 yeast isolates *Candida albicans* [55.8%] was commonest, *C. parapsilosis* [18.8%], *C. glabrata* [18.2%], *C. krusei* and *C. lusitaniae* [1.9% each]. All isolates were sensitive [*in vitro*] to amphotericin B. Sensitivity to fluconazole and itraconazole, respectively: 70.1 and 78.5% *Candida* species overall, *C. albicans* [84.8 and 90.7%], *C. parapsilosis* [82.7 and 86.2%], *C. glabrata* [21.4 and 35.7%] and *C. krusei* [0% each].
Conclusion: Candidaemia with *C. albicans* predominates. The likelihood of isolating *Candida* species other than *C. albicans* from blood culture is nearly 50% in newly diagnosed candidaemia. Pending identification and AFST, treatment with amphotericin B deoxycholate, voriconazole, caspofungin, abelcet or ambisome are options with costs varying from $\leq$18 to $\leq$4017 per week. Patient risk-stratification, local epidemiology and faster diagnostic options [FISH, E-tests, automated technology] are also discussed.

P988 Candidaemia: epidemiological data, complications and outcome of patients from the VAMC during 7 years of evaluation

G. Gonzalez-Claudio, M. Colon, E. Arroyo, C. Rivera-Vazquez, S. Saavedra, C. Ramirez-Ronda
San Juan, PR

Objectives: To evaluate the outcome and complications in patients with candidaemia treated with antifungals. To identify the most common *Candida* species isolated in the VAMC patients with *Candida* and evaluate the risk factors and epidemiological data of the patients.
Methods: All patients admitted in the VAMC from August 1995 to August 2002 with blood cultures positive for *Candida* were included in this study. Epidemiological data, medical history, risk factors, co-morbid diseases and laboratory results were evaluated in record review. *Candida* species were identified to determine the prevalence of *Candida* species in the VAMC. The patients were assigned to three different groups according to the therapeutic regime provided to the patient by the primary physician. Outcome and complications including nephrotoxicity, electrolytes disturbances and hepatotoxicity were evaluated in each therapeutic group. Statistical analysis was performed using the SPSS (Statistical Package or Social Science). A regression model was used for the analysis of risk factors associated with mortality in patients with candidaemia.
Results: One hundred and seven patients were randomised in the study. *C. tropicalis* was the most commonly isolated *Candida* species 45%, followed by *C. albicans* 31%. Mortality rate is high 73%, especially in those patients infected with *C. tropicalis* and *C. glabrata* 88% ($P = 0.01$). The mortality rate increased to 81.4% if no treatment was given ($P < 0.0001$) and was worse if *C. tropicalis* was isolated and not treated 92%. The patients treated had a similar mortality rate irrespective of the administered agent, Amphotericin (64%), Abelcet (60%) and Diflucan (60%), but was worse in those patients admitted to an ICU, Amphotericin (86.6%), Abelcet (60%) and Diflucan 71.4% ($P < 0.0001$). Response rate in the patients infected with *C. albicans* was 38.7 vs. 9% in patients with *C. tropicalis*. Nephrotoxicity developed in 54% of patients and no difference was found in those patients treated with Amphotericin B vs. Abelcet.
Conclusion: Candidaemia has been increasing in frequency. *C. tropicalis* is the most commonly isolated *Candida* species in our institution. Candidaemia has a high mortality rate and is worse if *C. tropicalis* is isolated and the patient is admitted to an ICU and no treatment is given. There is no difference in response rate within the different therapeutic options. Nephrotoxicity is higher in patients treated with Amphotericin irrespective of the formulations administered.

P989 The risk factors of candidaemia in cancer patients after surgical operation and patients receiving chemotherapy

M. Koziol-Montewka, A. Miroslaw, K. Buczkowski, A. Chudnicka, A. Magrys, A. Kolodziejek, B. Gabczynska, E. Staroslawska, M. Montewka, M. Wojtowicz
Lublin, PL

Background: Invasive candidaemia is a life-threatening complication occurring especially in hospitalised cancer patients due to surgical operation and application of aggravating chemotherapy. *Candida* colonisation, dysfunction of humoral and cellular immune system and prolonged periods of hospitalisation are considered to be the risk factors of invasive candidaemia development. Early diagnosis and evaluation of the risk factors are still a major challenge.
Objectives: The aim of our study was to evaluate the relationship between the rate of *Candida* colonisation, disorders in immune responses (associated with adverse changes in concentration of TNF-alpha, IL-12, and myeloperoxidase) and development of invasive candidaemia in hospitalised cancer patients.
Methods: Study group included 78 patients with lung cancer admitted for surgical operation and 31 women with carcinoma ovariorum after the third course of treatment with taxol and cisplatin. Patients were examined for fungal colonisation of mucosal membranes with culture methods. Presence of *Candida* antigens and DNA of the pathogen in the bloodstream was determined with ELISA and PCR assay, respectively. Cytokine and myeloperoxidase concentration in serum of the patients was specified with ELISA commercial kits.
Results: The study revealed that 43 (39%) lung cancer patients were colonised with *Candida* in nosepharynx before the operation. Pneumonia and wound infections were observed in 15 patients of this group, *Candida albicans* was isolated as the only pathogen from three patients colonised previously with *Candida*. In case of patient group with ovariorum carcinoma, colonisation with *Candida* of two or three sites was demonstrated in five (15%) of 31 women. The *Candida* antigen was present in blood in four of them; positive PCR result was found in blood sample collected from one of them. Significant relationships between *Candida* colonisation or infection and myeloperoxidase concentration were found (5.9–13.1 vs. 170 ng/mL in healthy persons).
Conclusions: High rate of *Candida* colonisation and drastic decrease in myeloperoxidase serum concentration in patients with lung and ovariorum cancer are predisposing risk factors for invasive *Candida* infection. Detection of *Candida* antigens and DNA of the pathogen may improve early diagnosis of the candidosis.

P990 Evaluation of BacT/ALERT 3D system to diagnose bloodstream infections due to yeasts

M.A. Miguel, M. Cuervo, M. Lecuona, B. Castro, M.J. Ramos, M.I. Montesinos, A. Sierra
St. Cruz de Tenerife, E

Objective: Evaluation of Bact/ALERT 3D system in recovering yeasts from blood cultures (BC).
Methods: Over a period of 17 months (1/6/02 to 31/10/03), all BC received in the Microbiology Service were included in our study. All the specimens were performed with Bact/ALERT 3D (bioMerieux) initially during 5 days or 30 days in special cases. Yeast identification was performed with ID-YST VITEK TWO and/or API 20C AUX (bioMerieux).
Results: Of 15 008 analysed BC, 4150 gave a positive result and 142 (3.42%) of them proved to be yeast strains. In 68 cases (47.89%) both bottles (aerobic and anaerobic) were positive; in 69 cases (48.59%) only the aerobic bottles was positive and in five cases (3.52%) only the anaerobic bottles was positive. Related to the detection time in aerobic bottles, 36.62% gave a positive result in the first 12 h of incubation (average 6.8 h) cumulative percentage of 50% at 24 h. In the second day, 80.28% were positive. In anaerobic bottles 50.68% gave a positive result in the first 12 h of incubation (average 7.8 h) cumulative percentage of 67.11% at 24 h. In the second day 93.14% were positive. *Candida albicans* was isolated in 47.37% cases. We recovered a positive result in the first 12 h of incubation from 50% aerobic bottles (average 5.82 h) cumulative percentage of 63% at 24 h. For the anaerobic bottles 48.78% were positive in the first 12 h (average 8 h) cumulative percentage of 68.29% at 24 h. *C. parapsilosis* was isolated in 26.32% cases, always recovered from aerobic bottles. The average of growth was 3.32 days in the 83% of cases. Anaerobic bottles gave a negative result in 88%. *C. glabrata* was isolated in 10.53% cases. The average of growth was 2.7 days in the 90.9% of cases. Anaerobic bottles gave a negative result in 63.64%. *C. tropicalis* was isolated in 10.53% cases. Aerobic bottles were positive in 91.67%, and 92.31% of them had 9.55 h average of growth. Anaerobic bottles were positive in 46.15% (average 15.6 h).
Conclusion: Bact/ALERT 3D system has demonstrated to be an effective method in recovering yeasts from BC. In our hospital *C. albicans* was the yeast most frequently isolated from BC followed by *C. parapsilosis*, *C. glabrata* and *C. tropicalis*. Strain recovered fastest was *C. albicans* followed by *C. tropicalis*.

P991 *Candida* spp. in a large Italian paediatric hospital: species distribution and comparison of the susceptibilities to fluconazole and voriconazole

G. Manno, A. Scaramuccia, L. Pescetto, C. Peri, M. Mentasti, R. Bandettini
Genoa, I

Objective: The large use of azoles in paediatric setting raise concern about the development of resistance on yeasts and the emergence of some non-susceptible species. We conducted a prospective surveillance study to determine distribution of species and azoles antifungal susceptibilities of *Candida* spp. from patients (pts) hospitalised in Gaslini Children's Hospital Genova, Italy.
Methods: From 1/03/2003 to 31/11/2003 we studied all yeasts considered pathogens from all body sites, from paediatric pts in all in-hospital locations. Isolation and yeasts species identification were carried out by conventional methods. On isolates, FLU and VOR susceptibilities were assessed by the NCCLS M44-P method, with disks tested in Mueller–Hinton medium with glucose and methylen blue, 0.5 MacFarland inoculum. All susceptibility test results were read by BIOMIC Plate Reader System (Giles Scientific). *C. albicans* (Ca) ATCC 90028 was included. NCCLS FLU Breakpoints (mcg/ml) were S < 8, S–DD 16–32, R > 64 with corresponding zone interpretative criteria (mm) S > 19, S–DD 15–18, R < 14. Breakpoints for VOR have not yet been established.
Results: In the study period we recovered 65 Ca, 21 *C. parapsilosis* (Cp), 16 *C. tropicalis* (Ct), two *C. krusei*, two *C. glabrata* (Cg), two *C. lusitaniae* (Cl) and one *Tricosporon beigelii*. Species were isolated: 20% from urinary tract, 25%, upper respiratory tract, 19% miscellaneous fluids, 13% lower respiratory tract, 10% blood, 9% CVC, 4% various. Patients with yeasts infections were hospitalised: 49% in PICU/NICU, 14% haematology–oncology, 11% surgery, 6% infectious diseases, 6% nephrology, 5% pneumology, 5% medicine, 2% orthopedics, 2% dermathology. Distribution of bloodstream isolates were: four Cp, three Ct, one Ca and one Cl. Seventy percent of Cp strains were recovered from PICU/NICU pts. The average zone diameter (mm) – MIC50/MIC90 (mcg/mL) (agar disk gradients) were: Ca FLU 34 – 0.53/1.8, VOR 36 – 0.04/0.18; Cp FLU 35 – 0.3/0.87, VOR 39 – 0.01/0.05; Ct FLU 31 – 1.1/14, VOR 33 – 0.15/4.8. Ca (4/65) were resistant to FLU with VOR MICs >48 mcg/mL, one Ct was FLU SDD, one Gg was R to FLU and inhibited with 1.1 mcg/mL of VOR.
Conclusions: Our results show that Ca is still the predominant species recovered from paediatric pts; Cp and Ct appear to be recovered with increased frequency in serious infections of critically ill pts. FLU showed a still good '*in vitro*' susceptibility on our strains; VOR appeared more active than FLU against Cp and Ct.

P992 Effect of voriconazole on ergosterol content of *Candida krusei*

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

Voriconazole (Vor) is a new azole antifungal agent with a similar structure to fluconazole (Flu). As with other azoles, its primary mechanism of action is through disrupting the normal sterol biosynthetic pathway, leading to a reduction in ergosterol content (1). Nevertheless Vor is more potent against most *Candida* spp., and shows a wide spectrum of activity. Thus *Candida krusei*, which is intrinsically resistant to fluconazole (by unknown mechanism), shows low MIC values to Vor. This lack of cross-resistance and the fact of being fungicidal to some fungi suggest a distinct mechanism of action.
Objective: To study the effect of Vor on the amount of ergosterol of *C. krusei* strains, in comparison with Flu.
Methods: The MIC to Vor was determined according to the NCCLS protocol M27-A on 24 strains of *C. krusei*, all resistant to Flu (MIC $\geq$ 64 μg/mL). Ergosterol was isolated from *C. krusei* cells by saponification and the non-saponifiable lipids were extracted with heptane. Ergosterol was identified by its spectrophotometric absorbance profile (240–300 nm) (2). A quantification of ergosterol was determined after incubation with and without both azoles at MIC and sub-inhibitory concentrations.
Results: In all the strains, MIC to Vor ranged between 0.125 and 0.5 ug/mL. All *C. krusei* have a significant amount of ergosterol, with no significant differences among the strains. After incubation with MIC concentrations of Vor an 80–100% reduction of the ergosterol content was observed. A similar effect was obtained with fluconazole but only with highest concentrations (64 ug/mL).
Conclusion: The Vor induces a considerable impairment on the biosynthesis of ergosterol by *C. krusei* strains. It is much more potent inhibitor of ergosterol biosynthesis than Flu.
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P993 Trends in species distribution and antifungal susceptibility in *Candida* wound infections: an overview of a 6-year period

J. Martínez-Alarcón, T. Peláez, J.V. Guinea, O. Cuevas, P. Muñoz, M. Rodríguez-Créixems, E. Bouza
Madrid, E

Background: Mycotic infections of hospitalised patients are emerging as a significant public health issue. Numerous studies have shown that candidaemia is associated with a significant attributable mortality and prolonged hospital stay, but only a few reports analyse the incidence of *Candida* spp. in wounds.
Objective: To analyse the species distribution and antifungal susceptibility of *Candida* infection in wounds in our hospital during a 6-year period (1997–2002).
Methods: The *in vitro* activities of amphotericin B (AB), fluconazole (FZ), itraconazole (IZ), ketoconazole (KZ) and flucytosine were determined by the broth microdilution method following NCCLS criteria. MICs were visually determined after 24 and 48 h incubation at 35°C
Results: From 1997 to 2002 we processed 18 573 wound samples in our laboratory. Of these, 14 060 (75.7%) were positive, 13 413 (95.4%) showed bacterial growth without *Candida* and 647 (4.6%) with *Candida*. The rate of isolation of *Candida* in wounds/year was as follows: 1997 (2.7%), 1998 (3.7%), 1999 (4.8%), 2000 (4.3%), 2001 (6.3%) and 2002 (5.7%). Globally, *Candida albicans* was the most frequently isolated species per patient (296; 57.9%), followed by *C. parapsilosis* (71; 13.9%), *C. glabrata* (40; 7.8%) and *C. tropicalis* (37; 7.2%). The trends in species distribution were similar in both the adult and paediatric population. The evolution in the successive years of wounds with more than one species of *Candida* was as follows: 2/59, 8/94, 8/97, 4/92, 16/163 and 16/142. Overall, the percentages of resistance of *Candida* spp. isolated were: AB (4.6%), FZ (10.2%), IZ (17%), KZ (12.5%) and FC (2.3%).
Conclusion: Our study shows an increasing presence of *Candida* spp. among the wound isolates in the microbiology laboratory. A high proportion is due to species other than *C. albicans* and it can be probably attributed to the increase in antibiotic burden in our hospital.

P994 UK enhanced surveillance of invasive fungal infections in very low birth weight infants

T. Lamagni, L. Clerihew, W. McGuire
London, Dundee, UK

Objectives: Preliminary analyses from enhanced surveillance of invasive fungal infections in very low birth weight (VLBW)

infants were performed to estimate disease burden, short-term outcome and microbiological characteristics of causative organisms.
Methods: Prospective enhanced surveillance of invasive fungal infections in VLBW (<1500 g) infants began in February 2003, with cases defined as meeting of one or more of the following diagnostic criteria: (1) culture from a sterile site – CSF, blood (peripheral sample), urine (supra-pubic aspirate or in–out catheter sample), bone/joint, peritoneal or pleural space; (2) pathognomonic findings on ophthalmological examination; (3) pathognomonic findings on renal ultrasound examination; and (4) autopsy diagnosis of invasive fungal infection. Cases were identified through three separate surveillance schemes: monthly notifications from paediatricians to the British Paediatric Surveillance Unit; continuous reports from microbiology laboratories to the Communicable Disease Surveillance Centre (England) and Scottish Centre for Infection and Environmental Health (Scotland). Reports from the three systems were reconciled and analysed. Rates were calculated using Office for National Statistics total live birth estimates.
Results: Between February and July, 38 confirmed cases of invasive fungal infection in VLBW infants were reported, 10.02/1000 births of VLBW. Median age at diagnosis was 11 days (range 1–126) and birth weight 800 (520–1200) g. Thirty-four of the 38 infants were of extremely low birth weight (<1000 g). *Candida albicans* was the most common pathogen, found in 55% of cases, and *C. parapsilosis* in 23%. Organisms were most commonly isolated from blood (73%), followed by urine (23%), CSF (8%) and central line tips (53%). Just over a third of cases (36%) had received prophylactic antifungal therapy. One case of drug resistance was identified during this period (fluconazole resistance in a non-albicans *Candida* spp.). Of the 32 infants for whom outcome data were available, 22 were alive at 37 weeks post-conceptional age.
Conclusion: Preliminary findings from enhanced surveillance suggest an incidence of invasive mycoses in VLBW infants of one in 100. As per adult cases, *C. albicans* was the most common fungal pathogen involved, although *C. parapsilosis* was relatively more common than in adults. The majority of cases occurred in extremely low birth weight infants, and mortality was found to be high.

P995 Surveillance cultures of throat and rectum combined with enteral polyenes control fungal infections in prolonged paediatric illness

S. Rajan, N. Taylor, P.B. Baines, H.K.F. van Saene
Liverpool, UK

Objectives: To evaluate six endpoints [i] carriage rate; [ii] rate of carriers with overgrowth; [iii] fungal infection rate; [iv] correlation between overgrowth and infection; [v] impact of oral polyenes on carriage and infection; [vi] mortality.
Methods: Surveillance swabs of throat and rectum were taken on admission and twice weekly afterwards. Diagnostic samples were obtained on clinical indication. All samples were processed using standard mycological techniques. Overgrowth was defined as $\geq$3+ or $\geq$1 000 000 yeast cells/mL of saliva and/or gram of faeces. Carriage index is the ratio of the sum of all semi-quantitative growth densities of positive surveillance swabs divided by the total number of swabs; on a particular sampling day. Oral polyenes were started following the identification of the carrier state.
Results: A total of 1241 children requiring minimally 4 days of ventilation were enrolled in this 4-year observational, prospective study [01/03/99 to 28/02/03]. The median paediatric index of mortality was 0.06 [IQR 0.02–0.13], and the actual mortality was 9.6%. Enteral polyenes as part of selective digestive decontamination [SDD] were administered to half of the study population [53%]. The median length of stay was 8 days [IQR 5–13 days]. Carriage rate was 45%. Eighty percent of carriers had overgrowth concentrations. Forty-four patients [3.5%] developed a total of 59 infections. The median onset of infection was 7 days [IQR 3–12.5]. Eighty-five percent of all infections were primary endogenous, due to yeasts present in the patients admission flora. Infections of wounds [32%], vagina [19%], blood [15%] and bladder [12%] were predominant. Thirty-nine patients [90%] had overgrowth at the time of the infection. Only three patients developed an infection without overgrowth. Oral polyenes reduced the carriage index below the threshold of overgrowth after 1 week of treatment. Fifty-six children who were yeast carriers died [4.5%]. The mortality rate in the carriers with overgrowth was 3.9% [48 patients]. Of the 44 infected children 13 died [30%].
Conclusion: Patients develop primary endogenous fungal infections in the presence of overgrowth within the first week of PICU admission. Oral polyenes reduce the level of carriage and prevent subsequent secondary endogenous infections.

P996 *Candida dubliniensis* in Kuwait

Z. Khan, S. Ahmad, E. Mokaddas, N. Al-Sweih, R. Chandy
Kuwait, KWT

Objective: *Candida dubliniensis* is a newly described pathogenic species, first isolated from HIV-infected patients with oropharyngeal candidiasis. It shares many phenotypic features with *C. albicans*, including the ability to form germ tubes and chlamydospores. These similarities have caused significant problems in its differentiation from *C. albicans* in routine clinical microbiology laboratories. This study reports isolation and identification of *C. dubliniensis* for the first time from Kuwait and presents data on antifungal susceptibility profile.
Methods: Over a period of 21 months, 800 germ-tube positive yeasts identified as *C. albicans* and recovered from different clinical specimens were screened for their ability to grow at 45°C on Sabouraud dextrose agar. Isolates which failed to grow at 45°C were presumptively identified as *C. dubliniensis*. The identity of *C. dubliniensis* isolates was further confirmed by formation of rough colonies and chlamydospores on sunflower seed agar, by Vitek 2 system, and by semi-nested PCR using species-specific primers corresponding to unique sequences within the internally transcribed spacer 2 (ITS2) of *C. dubliniensis* and by direct sequencing of ITS2. The antifungal susceptibility testing was performed on RPMI 1640 medium as recommended in NCCLS, M27A document.
Results: Of the 800 germ tube positive yeast isolates, 27 (3.3%) were identified as *C. dubliniensis*. They were isolated from sputum ($n = 12$), vaginal swabs ($n = 5$), endotracheal secretion ($n = 3$), throat swabs ($n = 2$), urine ($n = 2$) and one each from bronchoalveolar lavage, catheter tip and peritoneal fluid. None of the isolates originated from HIV-positive patients. All the *C. dubliniensis* isolates were susceptible to amphotericin B, fluconazole, itraconazole and voriconazole. However, 19% of the isolates were resistant to 5-flucytosine (>32 μg/mL) without any known previous exposure.
Conclusion: Identification of *C. dubliniensis* from 3.3% of the yeast isolates in our study suggests that this species is not uncommon in Kuwait. There is a need to carry out a systematic study in high-risk patient groups to know its epidemiologic significance.
Acknowledgement: The work is supported by Kuwait University Research grant MPI-118.

P997 *Candida glabrata* fungaemia: an uncommon entity affecting the elderly

M. Sánchez-Somolinos, P. Muñoz, L. Alcalá, T. Peláez, M. Rodríguez, J. Guinea, E. Bouza
Madrid, E

Background: Fungaemia remains a severe nosocomial complication and the emergence of non-albicans species is posing new challenges both to clinicians and to microbiologists.
Objective: To assess the incidence and clinical presentation of *C. glabrata* fungaemia, its susceptibility and its clinical outcome.
Methods: From 1987 to 2001, we had 479 episodes of fungaemias and 37 cases corresponded to *C. glabrata* (7.7%). Thirty cases were

compared with *C. albicans* controls. Susceptibility testing was performed with the modified microdilution method (M-27A, NCCLS), with RPMI-2% glucose medium.
Results: The distribution of *C. glabrata* fungaemias was: 1987–1993, two cases; 1994–2001, 28 cases. Underlying conditions included: cancer (23%), AIDS (16%), surgery (50%), TPN (37%) and IV lines (90%). The most common origins were: unknown (27%) and IV catheters (23%). Clinical presentation included: fever (93%), shock (17%) and chorioretinitis (10%). When compared with *C. albicans*, patients with *C. glabrata* fungaemia were older (58 vs. 42), had received more previous antifungals (37 vs. 10%, $P = 0.01$) and antimicrobial agents (97 vs. 73%, $P = 0.01$), had more indwelling bladder catheters (90 vs. 50%, $P < 0.001$) and had more septic metastasis (23 vs. 6%, $P = 0.07$). IV catheters were more commonly withdrawn in patients with *C. glabrata* fungaemia (60 vs. 33%, $P = 0.03$), whereas these patients received fewer antifungals (57 vs. 70%, NS). MIC90 of *C. glabrata* were fluconazole (FLU) 16 mg/L, itraconazole 1 mg/L, amphotericin B (AMB) 1 mg/L and voriconazole 0.5 mg/L. Surprisingly, FLU was more frequently selected to treat patients with *C. glabrata* (57 vs. 24%). Mortality was similar (50 vs. 53%). Six of the 10 patients treated with FLU died, as well as four of the seven treated with AMB. Two patients had persistent fungaemia despite catheter withdrawal and FLU therapy.
Conclusion: *C. glabrata* fungaemia is an increasingly frequent and very severe disease. It is clinically indistinguishable from *C. albicans* and microbiology departments must assure that proper messages regarding identity and antimicrobial susceptibility of this yeast reach clinicians in due time.

P998 *Candida* spp. in patients with otitis externa

I. Radonjic, A. Dzamic, L. Petkovic, A. Trpkovic, N. Arsovic, V. Arsic Arsenijevic
Belgrade, CS

Objectives: Otomycosis represents a significant percentage of clinical external otitis and is usually caused by *Candida*, *Aspergillus*, *Penicillium* and *Malassezia*. Clinical symptoms such as otorrea, erythema and stenosis of the external auditory canal are commonly present and create appropriate conditions for fungal growth. The objectives of this study were to determine the prevalence of *Candida* otomycoses and to evaluate the relationship between albicans and non-albicans species.
Methods: From April 2002 to November 2003, a total number of 67 patients were found to be suffering from symptoms indicating otitis externa. The specimens were taken by cotton swab from bony portion of external ear. All specimens were inoculated on Sabouraud dextrose agar, incubated at 26 and 37°C for 7 days and examined macroscopically every day. Suspected cultures were examined microscopically in order to confirm finding of *Candida* spp. The identification of isolated *Candida* strains was carried out by germ tube test and API 20C AUX assimilation test (BioMerieux, France).
Results: In a base of microbiological findings 12 (17.9%) patients considered to be negative, 39 (58.2%) confirm bacterial or mould results and in 16 (23.8%) patients *Candida* spp. was found. Out of 16 patients with diagnosed *Candida* otomycosis, in 11 patients only *Candida* spp. was isolated and in five patients otitis externa was caused by *Candida* associated with bacterial or mould infection. *C. albicans* was identified in three (3/16) cases, while all other was non-albicans strains as three cases of *C. guilliermondii* (3/16), four of *C. famata* (4/16) and six of *C. parapsilosis* (6/16).
Conclusion: In clinical finding of otitis externa mycological examination could be very important in setting the accurate diagnosis and appropriate therapy. These results suggest that *C. albicans* is not the predominant causative agent of otitis externa. Isolation of non-albicans species has particular interest in therapy of otitis externa because of their reduced susceptibility to antifungal agents.

P999 Recurrent vulvovaginal candidiasis: prevalence, antifungal susceptibility patterns and genotyping of different species

S. Asticcioli, R. Migliavacca, E. Nucleo, M. Spalla, L. Sacco, L. Pagani
Pavia, I

Objectives: *Candida albicans* is the main species involved in vulvovaginal infections, even if *Candida* non-albicans species, with broad resistance to antifungal agents, are emerging. This finding emphasises the pathogenic role of non-albicans species in vulvovaginal candidiasis with important implications for therapeutic management and the possibility of recurrent infections. Thirteen women with recurrent vulvovaginal candidiasis (RVVC) were observed out of 157 women attending the Sexual Transmitted Diseases ambulatory of the S. Matteo hospital of Pavia, during the period 2000–2003. The study was focused on the species involved and their *in vitro* antifungal susceptibility. Molecular typing of the isolates involved in subsequent episodes of RVVC allowed establishing if the strains showed the same DNA type.
Methods: Isolates were identified by standard morphological and biochemical methods. MICs of amphotericin-B, itraconazole, fluconazole, ketoconazole, 5-fluorocytosine, voriconazole were determined by Sensititre YeastOne colorimetric antifungal panel plates according to NCCLS document M27-A. The strains were typed using pulsed-field gel electrophoresis (PFGE) and repetitive extragenic palindromic-PCR DNA fingerprints.
Results: *C. glabrata* was isolated in 53.8%, *C. albicans* in 30.7%, *C. krusei* in 15.5% of cases. The yeasts involved in each recurrence were characterised by identical biochemical profiles and drug resistance phenotypes. *C. albicans* strains isolated from one RVVC resulted in *in vitro* resistant to azoles. The genotyping by PFGE revealed that *C. albicans* and *C. glabrata* obtained from different patients were clinically unrelated to each other while an identical profile, indicating clonal relatedness, was observed with yeasts recovered from the same patient.
Conclusion: Our data underline the persistence of strains, with the same antifungal susceptibility profile and clinically related genotypes in patient with recurrent infections, suggesting a colonisation with the same strain over different periods of time despite therapy. These results stress the need for molecular tools for strain typing in order to clarify the epidemiology of the RVVC and to control drug-resistant fungal agent spread.

P1000 Invasive aspergillosis: an emerging pathogen with a huge mortality in non-haemato-oncological patients

W. Meersseman, S.J. Vandecasteele, E. Van Wijngaerden, J. Maertens, A. Wilmer, W.E. Peetermans
Leuven, B

Objectives: Using criteria designed for invasive aspergillosis (IA) in neutropenic patients, the present study aimed to determine the impact of invasive aspergillosis in different groups of non-haemato-oncological ICU patients.
Methods: This study is a retrospective analysis of all patients that were hospitalised in the 17-bed medical intensive care unit (MICU) between 1 January 2000 and 1 January 2003. Any admitted patient fulfilling one or more of the following criteria was included in the study: (a) histopathological evidence of Aspergillosis (including autopsy) or (b) microbiological evidence of aspergillosis during stay in the MICU (positive culture or positive circulating galactomannan). IA was classified as proven, probable or possible, according to the EORTC/MSG definitions. *Aspergillus* isolation from a non-sterile site in patients without appropriate clinical setting was considered as 'colonisation'.
Results: Between 2000 and 2003, 127 of 1850 patients (6.9%) fulfilled the inclusion criteria. Thirty-eight patients (29%) had haematological malignancies and were not further analysed. Eighty-nine (71%) were non-haemato-oncological patients (37 COPD, nine solid organ transplant recipients, 17 autoimmune diseases,

six cirrhosis patients and 20 miscellaneous). Following the EORTC/MSG criteria, these patients were classified as proven IA ($n = 30$), probable IA ($n = 37$), possible IA ($n = 2$) and 'colonisation' ($n = 20$). Mean SAPS II score was 52 with a predicted mortality of 48.6%. Overall mortality was 80% ($n = 71$). Mortality of the proven and probable group was 96.7 and 86.5%, respectively. Among the 18 patients who survived, 10 just had 'colonisation' with *Aspergillus*. Post-mortem examination in the non-haemato-oncological group was done in 46 out of the 71 patients who died (70%) and 29/46 autopsies (63%) showed hyphael invasion with *Aspergillus* (mainly the lung as target organ). There were five proven cases in patients without compromising host factors according to the EORTC/MSG definitions (three liver cirrhosis, one pneumonia in a 95-year-old man, one *Klebsiella* sepsis with MOF).
Conclusion: IA is an emerging infectious disease in non-haemato-oncological ICU patients. There seems to be a broad group of patients at risk of IA. IA was diagnosed in patients without characteristics described in the EORTC/MSG definitions. It seems worthwhile to investigate the validity of the available diagnostic tools in non-haemato-oncological patients at risk for IA in a prospective manner.

P1001 Epidemiology of invasive aspergillosis in a teaching hospital, France: a 6-year survey (1998–2003)

A. Cornillet, C. Camus, S. Nimubona, V. Gandemer, P. Tattevin, C. Belleguic, S. Chevrier, C. Meunier, C. Lebert, M. Aupée, B. Lelong, C. Guiguen, J.-P. Gangneux for the Aspergillosis Study Group

Objectives and methods: The aim of this survey was to characterise a file of patients who developed an invasive aspergillosis (IA) in our institution, their risk factors and management. We analysed retrospectively the cases of IA, which occurred between 1998 and 2001, then prospectively all new cases until the end of 2003. The overall survey covered a 6-year period. Cases were classified as suspected, probable or proven IA, using criteria derived from the EORTC/MSG classification.
Results and discussion: Until 11/2003, out of the 80 cases of IA analysed, nine were histologically proven, 47 were probable IA and 24 were suspected IA. The sex ratio was 1.5 male:one female with a mean age of 53 years (ranging from 4 to 89 years). Fifty percent of cases were diagnosed in the Intensive Care Units, and 36% in Haematology units (28% in adults and 8% in paediatrics). Neutropenia was the major risk factor in 55% of the patients (during haematological malignancies and solid cancers). However, we also noted an increasing number of IA in patients under corticosteroid therapy for COBP, asthma, rheumatoid arthritis, Horton and microvascular diseases, in comparison to available data in the literature. Other cases occurred in solid organ transplant recipients and only one out of the 80 patients was infected by HIV. Prognosis factors will be discussed. Regarding biological diagnosis, good sensitivities of the mycologic examination (microscopy + culture) and the galactomannan antigen detection by enzyme immunoassay (Platelia Aspergillus, Biorad) were noted: 75 and 71%, respectively. The sensitivity reached 80% when both tests were combined. Pulmonary imagery was less efficient, probably due to the fact that, in our institution, CT scans are performed later than proposed in the literature. During this survey, we observed great modifications in therapeutic approaches. First line treatment progressively switched from deoxycholate Amphotericin B (AmB) to voriconazole and second line treatments now include lipid formulations of AmB and caspofungin acetate. AmB deoxycholate and voriconazole were the two drugs used for empirical therapy. The overall mortality was >70%.
Conclusion: IA remains a major life-threatening infection among immunosuppressed patients, although protective measures such as air filtration significantly reduced its incidence in neutropenic patients. However, this 6-year-survey points out the increasing number of cases in non-neutropenic patients hospitalised in wards without air filtration. This emerging population of patients must be taken into account and imposes to reinforce surveillance for high-risk groups and to rethink our preventive measures.

P1002 Invasive aspergillosis in patients with COPD

G. Serrate, B. Font, D. Mariscal, M. Sala, E. Antón, F. Segura
Sabadell, E

Objective: To determine if news definitions of the Invasive Fungal Infections (IFI) (1) with the terms: 'proven', 'probable' and 'possible' could be applied in patients with COPD and *Aspergillus* spp. in respiratory samples to express disease certainty.
Methods: Prospective study, between January 1997 and December 2000, of patients with COPD and *Aspergillus* spp. in respiratory samples to determine risk factors and outcome.
Results: We identified 219 patients with COPD and *Aspergillus* spp. in respiratory samples. Median age was 72 ± 9.3 years. Eighty-three percent were men. Forty-one patients had criteria for 'probable' IFI and in seven cases of them that was 'proven' (17%). Moreover, three were treated and cured and eight were treated too but died. In all these cases, the host factor criterion was prolonged (>3 weeks) use of corticosteroids in previous 60 days. Seventy-one cases had criteria for 'possible' IFI. Of them, one case treated had a negative necropsy, 14 died for other causes and the remainder were well at the pursuit (colonisation). None of 107 cases had criteria to suspect an IFI, however, nine were treated and all but one died. The remainder were colonisations.
Conclusions: A progressive increase of COPD patients with *Aspergillus* spp. has been observed but frequently, this is a colonisation. However, we observed that patients in 'probable' category have a high rate (17%) of 'proven' IFI, similar to other known risk groups. We think that these categories could help in clinical practice and to identify homogeneous groups for clinical research in diagnostic methods and therapeutic interventions. (1) Cooperative Group of the European Organisation for Research and Treatment of Cancer and Mycoses Study Group of the National Institute of Allergy and Infectios.

P1003 Evaluation of serum galactomannan ELISA during caspofungin therapy: results from the Caspofungin Salvage Invasive Aspergillosis Study

J. Maertens, A. Glasmacher, D. Selleslag, M. Layton, A. Taylor, C. Sable, N. Kartsonis
Leuven, B; Bonn, D; Brugge, B; West Point, USA

Objectives: A sandwich ELISA assay, which detects circulating *Aspergillus galactomannan* antigen using a rat monoclonal antibody has recently been licensed (Platelia®, BIORAD). Yet, animal models of IA have shown that treatment (Rx) with an echinocandin may result in a paradoxical increase in antigenemia despite clinical/radiographic improvement. Concern also remains that using ELISA as the sole means of IA diagnosis may result in exaggerated favourable outcomes. To address these concerns, we reviewed the ELISA experience from the caspofungin (CAS) salvage invasive aspergillosis (IA) study.
Methods: Patients (pts) with proven/probable IA were eligible for enrolment. Probable IA was limited to pulmonary sites. Probable pulmonary IA could be diagnosed serologically provided the pt had an appropriate chest CT appearance (halo sign, air-crescent sign) and positive ELISA on more than two consecutive tests. All pts were refractory (>7 days) or intolerant of prior antifungal Rx. CAS, with doses ranging from 50–100 mg/day, was administered as monoRx. Efficacy was assessed at the end of CAS Rx. Favourable responses were limited to complete or partial responses.
Results: Of the 127 pts enrolled, 20 (16%) had consecutively positive serum ELISA at the onset of CAS. Positive ELISA (and appro-

priate chest CT appearance) was the sole basis of the diagnosis in 13 (65%) pts. In the remaining seven (35%) pts, positive ELISA accompanied either histopathological or microbiological evidence of IA. Five (38%) of these 13 pts were later upgraded to definite IA. Nineteen of the 20 pts were assessed for efficacy at the end of CAS Rx. The favourable response rate was 26% (5/19). Pts whose only evidence of IA at CAS onset was ELISA (and characteristic chest CT findings) had a 38% (5/13) success rate. Follow-up ELISA data was available in 17 pts. Four of five pts with a favourable response to CAS had negative ELISA by the end of Rx. The one other pt with a favourable response had quantitative ELISA improvement that was temporally associated with clinical and radiographic response. Of the 12 pts with unfavourable responses and follow-up ELISA data, 10 had no ELISA improvement and two had normalisation of ELISA while on CAS.
Conclusions: In this study, the use of ELISA did not result in an exaggerated favourable response rate. In general, the ELISA was associated with clinical/radiographic response. Paradoxical ELISA increases in pts clinically/radiographically responding to CAS were not noted.

P1004 Screening for *Aspergillus galactomannan* antigenemia in a haematology unit: how useful is it in daily practice?

A. Sonet, Y. DeGheldre, B. Delaere, C. Doyen, A. Bosly, Y. Glupczynski
Yvoir, B

Objectives: To assess the role of *Aspergillus galactomannan* (GM) antigen detection in a general hematology unit.
Methods: Between 06/02 and 12/03, there were 667 admissions corresponding to 244 patients (pts) in the hematology unit. Overall 151 pts were tested at least twice by the *Aspergillus* GM ELISA. A total of 1813 serum samples were analysed (mean no. of tests/pt: 11.85; range: 2–51; SD: 10.52). The mean period of follow-up (FU) was 100.7 days (range: 3–487, SD: 91.6). GM antigen detection was made in the setting of: routine surveillance after chemotherapy (105 pts), after haematopoietic stem cells transplantation (HSCT) (2 allo- and 3 'mini' allo-HSCT; 27 auto-HSCT) or in the FU of infection (14 pts). Underlying diseases were: lymphoid: 55%; myeloid: 40%; non-malignant: 5%. Clinical efficiency of the test was tested at three different cut-off values 1.5, 1.0 and 0.7.
Results: Results are summarised in the table. The overall incidence of invasive aspergillosis (IA) was 6.4% (43/667 admissions). Following EORTC definition criteria, the repartition was: two Definite IA, 16 Probable IA and 25 Possible IA. The definition of 'probable' IA was substantiated by positive GM antigen tests (eight cases); both by microbiological (positive cultures) and positive GM antigen tests (four cases) or only by microbiological criteria (four cases). GM antigen was detected at all different cut-off values in 30 cases corresponding to: 1/2 Definite IA, 12/16 Probable IA. Results were considered as false-positives in 17 patients: four cases without clinical context; 15 cases with a negative chest CT-scan; two pts were even treated as possible IA, one because of a positive chest CT-scan (CMV pneumonia), the other because of past proven IA. All 'false-positives' results were in patients who received antibiotics at time of antigen testing (Pipera/tazobactam (seven pts); Amoxi/clavulanate (nine pts); Cefepime (one pt)).
Conclusion: Detection of circulating GM antigen may be helpful for the diagnosis of IA, particularly in the absence of microbiological data, but a substantiated number of false-positive results do occur among patients undergoing antibiotic therapy with Pipera/tazobactam or Amoxi/clavulanate. Considering different cut-off values did not improve the sensitivity or the specificity of the assay.

Table 1.

	Episodes Total	Antigen negative	Antigen Positive	Antigen No AB	Concomitant antibiotherapy(AB) Pipera/ Tazobact	Amoxi/ Clavulanate	Cefepime	Others
Proven	2	1	1	0	1	0	0	0
Probable	16	4	12	2	3	0	3	4
Possible	25	–	–	–	–	–	–	–
False Positive		-	17	0	7	9	1	0

P1005 Diagnosing of invasive Aspergillosis during antifungal therapy by polymerase chain reaction on blood samples

C. Lass-Flörl, A. Petzer, M. Dierich
Innsbruck, A

Objectives: We evaluated the value of *Aspergillus* PCR as a tool for diagnosing invasive aspergillosis during antifungal therapy from whole blood samples.
Methods: In a 3-year study, 36 patients receiving antifungal therapy due to chest radiographic findings highly suggestive for fungal pneumonia were evaluated. The PCR results of whole blood samples were compared with those obtained from bronchoalveolar lavage fluids and/or tissue specimens.
Results: A total of 205 whole blood samples, 15 fine needle aspirations or tissue biopsy specimens, 21 bronchoalveolar lavage fluids and tracheal secrets were analysed using PCR. Fifteen patients had proven, nine probable and 12 possible invasive *Aspergillus* infections according to European Organization for Research and Treatment of Cancer/Mycosis Study Group definitions. In patients with proven infections, the sensitivities of PCR of lung and blood samples were 100 and 40%, respectively. The specificities were 100%. The negative predictive value of blood monitoring under antifungal treatment was 44%. In patients with probable infections, the sensitivities of PCR of lung fluids and blood were 66 and 44%, respectively. The specificities were 100%. The negative predictive value of blood monitoring under antifungal therapy was 58%.
Conclusions: The benefits of PCR diagnosing of whole blood are limited if sampling takes place once treatment has started. The performance of *Aspergillus* PCR should be recommended in addition to microscopic examination and culture technique for sensitive detection of fungal infection.

P1006 Air surveillance of *Aspergillus*. Are two different growth media required?

J. Guinea, T. Peláez, J. Blanco, O. Cuevas, J. Martínez-Alarcón, L. Alcalá, P. Muñoz, E. Bouza
Madrid, E

Objective: Air is considered the main vehicle of *Aspergillus* spores causing community or nosocomially-acquired invasive aspergillosis (IA). Air surveillance is nowadays performed in protected air environments in many institutions. Sabouraud Dextrose Agar Irradiated (SD) is used for the control of air in our institution but Czapeck Agar is also recommended for this purpose. The aim of our study was to compare the efficiency of both media for *Aspergillus* isolation in air samples.
Methods: We collected 332 samples using the Merck Air Sampler MAS 100® with a volume of air per culture of 200 L. Every sample was cultured in both media (pair of samples), and agar plates were incubated at 35°C for 5 days. *Aspergillus* spp. was identified by conventional methods. The pairs were checked daily to observe the growth of fungi and after the incubation period the

results were reported as the number of isolates of each species per plate of the pair.
Results: A total of 332 pairs of samples were evaluated. Of these, 42 showed growth of *Mucor* spp. (40 in SD and two in Czapeck) and could not be studied for *Aspergillus*. Of the remaining 290 pairs, 157 pairs (54.1%) were positive for *Aspergillus* spp. [49 (31.2%) on both plates, 59 (37.6%) only in SD and 49 (31.2%) only in Czapeck]. *A. fumigatus* was the most frequently isolated species, 98 pairs (33.8%) were positive [28 (28.6%) on both plates, 39 (39.8%) only in SD and 31 (31.6%) only in Czapeck]. Both media were equally suitable for *Aspergillus* spp. isolation. Overall, 581 *Aspergillus* (289 on Czapeck and 292 on SD) and 326 *A. fumigatus* (164 on Czapeck and 162 on SD) were isolated in both media.
Conclusions: Our data supports the recommendation that both media (Czapeck and SD) should be used for correct air sampling. The use of the standard media alone (Czapeck) led to nearly 40% false negatives and the use of only SD to nearly 30%. The use of both media for environmental control of *Aspergillus* spp. in air should be mandatory, as this allows more efficient detection of conidia and improves the interpretation of Czapeck plates when mucorals are present. (This study was financed by Comunidad de Madrid grant number 08.2/0026/2001 1).

P1007 *In vivo* interaction between caspofungin and flucytosine in a murine model of disseminated aspergillosis

E. Dannaoui, O. Lortholary, F. Dromer
Paris, F

Objective: Antifungal combination of caspofungin with flucytosine has been shown to be additive to synergistic *in vitro* against *Aspergillus fumigatus*. The aim of the present study was to evaluate the interaction between these two drugs *in vivo* in an animal model of disseminated aspergillosis.
Methods: For *in vivo* experiments, non-immunocompromised outbred mice were infected intravenously with a spore suspension calibrated at the lethal dose 90%. Treatment was started 24 h after infection and continued for 7 days. Serum level of flucytosine were determined by HPLC in preliminary experiments for different regimen. Flucytosine was given in drinking water at 500 or 1000 mg/kg/day and caspofungin was given by once-daily intraperitoneal injection at 0.25 or 0.5 mg/kg/day either alone or in combination with flucytosine (four combinations were tested). Control groups received either NaCl intraperitoneally or water per os. There were 14 mice in each group. Mortality was recorded daily until the end of experiment at day 14 post-infection. Survival curves were compared by a log–rank test.
Results: An 82% mortality was obtained for control mice receiving no active treatment. Survival was 50% for mice treated with flucytosine alone at 500 mg/kg/day and 36% for the higher dose of 1000 mg/kg/day. Survival of mice treated with caspofungin alone at 0.25 and 0.5 mg/kg/day were 50 and 64%, respectively. Survival rates of mice treated with the combination of caspofungin at 0.25 mg/kg/day with flucytosine at 500 and 1000 mg/kg/day were 79 and 86%, respectively. Mice treated with caspofungin at 0.5 mg/kg/day combined with flucytosine at 500 and 1000 mg/kg/day had a 92 and 79% survival, respectively.
Conclusions: The combination of caspofungin with flucytosine showed a positive effect *in vivo* in this animal model of *Aspergillosis*. Antagonism was not observed. Further evaluation of the potential treatment efficacy of this combination is warranted.

P1008 Activation of the complement system in cerebral aspergillosis

G. Rambach, I. Mohsenipour, H. Maier, J. Rainer, G. Vago, M. Dierich, C. Speth
Innsbruck, A; Milan, I

Objectives: The spreading of *Aspergillus* hyphae into the brain of immunocompromised patients is a complication of invasive aspergillosis that leads to death in nearly 100% of the cases. The most frequent species for induction of cerebral aspergillosis is *Aspergillus fumigatus*. Our aim was to study the interaction of *A. fumigatus* with the complement system to determine the reason for the failure of the cerebral immune system. Furthermore, these experiments might give first approaches for a putative immune therapy to support current antimycotical treatment.
Methods: Different pools of cerebrospinal fluid (CSF) were tested for their ability to opsonise fungal hyphae with different complement factors. Germinated conidia were fixated, incubated in CSF, and the deposition of complement was shown via indirect immunofluorescense (IF) by suitable specific antibodies. The extent of surface labelling on *Aspergillus* was compared with *Pseudallescheria boydii*, another neurotropic fungus. Immunohistochemical (IHC) staining of paraffin-embedded tissue sections derived from patients with cerebral aspergillosis allowed the comparison with the complement deposition *in vivo*.
Results: The levels of the complement factors C1q, C4, C3, C5, C6 and C7 in the CSF of normal persons were sufficient for opsonisation of the fungal hyphae, although the deposition was much weaker than in human serum. However, the recognition of *Aspergillus* surface was not optimal in comparison to *P. boydii* that showed a clearly stronger deposition. Concentrations of different complement proteins and complement activation products were highly elevated in CSF derived from a patient with cerebral aspergillosis. This CSF showed a significantly stronger complement deposition on the fungal surface than the non-inflammatory CSF. However, IHC-analyses in tissue sections of patients with cerebral aspergillosis showed only limited opsonisation on the fungus.
Conclusion: CSF harbours the ability of complement deposition on the surface of neurotropic fungi. Frequent pathogens like *Aspergillus fumigatus* have adopted their surface to minimise recognition by the complement cascade. Cerebral complement production is upregulated as a consequence of fungal infection, which might contribute to antifungal immune defence but also to inflammation and tissue damage. The amount of deposited factors on the fungal hyphae *in vivo* is low, indicating the expression of complement inhibitory factor(s) by *A. fumigatus*.

P1009 Genotypic characterisation of *Aspergillus* spp. isolates from patients with cystic fibrosis by Random Amplified Polymorphic DNA PCR

M. Serrano, A. Perkins, T. Alarcón, B. Buendía, D. Monclús, M. López-Brea
Madrid, E

Objective: To evaluate the use of RAPD-PCR for typing of *Aspergillus* spp. in Cystic Fibrosis (CF) patients.
Methods: Thirty *Aspergillus* spp. strains (28 *A. fumigatus* and two *A. flavus*) from 12 CF patients were studied between June 1998 and April 2003. The patients were aged 18–44 years. The isolates were obtained from sputum samples and were identified by their culture characteristics and on the basis of conidiophores and conidia morphologies. RAPD-PCR fingerprinting was performed by using primer R-108 and AP12h.
Results: Fifteen out of 34 patients (44.11%) had colonisation or infection by *Aspergillus* spp. The most frequent isolate was *A. fumigatus* (86.66%). By using primer R-108, 17 strains were typed and three types were identified (A, B, C) with the subtypes A1, A2 and A3. Using primer AP12h, 19 strains were typed and nine different types were obtained (A-I), with some subtypes (A1, A2, A3), (B1–B4) and (F1, F2). Primer AP12h distinguished some strains that could not be differentiated by primer R-108. Twenty-two strains were typed when the results of the two primers used were combined. The patients with more than one isolate showed different patterns.
Conclusion: RAPD-PCR is a quick and easy procedure to genotype *Aspergillus* spp. in CF patients. Primer AP12h yielded the

best RAPD patterns with respect to number, spreading and intensity of the bands, but the highest level of discrimination was achieved by a combination of data generated by both of them. Therefore, we emphasise the convenience of using at least two primers for RAPD typing.

Molecular bacteriology: staphylococcus

P1010 Molecular epidemiology of bacteraemia due to methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in a tertiary hospital in Madrid

J. Garcia-Martinez, S. De Miguel, F. Sanz, F. Chaves, J. Otero
Madrid, E

Objectives: To gain insight into the molecular epidemiology of *Staphylococcus aureus* at a tertiary hospital.
Methods: All *S. aureus* isolates recovered from blood samples over a 1-year period were analysed. Demographic, clinical and microbiological data from these patients were collected. Antimicrobial susceptibility tests were performed by the Wider System and the disk diffusion method; all methicillin-resistant *S. aureus* (MRSA) isolates underwent confirmatory PCR analysis for the mecA gene. Molecular characterisation was performed by pulsed-field gel electrophoresis (PFGE) following DNA extraction and SmaI digestion. Patterns differing by less than seven DNA fragments and with a Dice coefficient of correlation >80% were considered a common bacterial type while subtypes included isolates with indistinguishable PFGE patterns. Univariate and multivariate analyses were performed with Epi-Info 2002 and SPSS 10.0 softwares.
Results: One hundred and sixty-two episodes of *S. aureus* bacteraemia, whether methicillin-resistant or methicillin-susceptible (MSSA), were nosocomial in origin (77.2%) or were cases associated with the healthcare system (15.4%). Only a total of 12 cases of bacteraemia (7.4%), one MRSA and 11 MSSA, were strictly considered to be community-acquired. Thirty-five unique *S. aureus* PFGE types were identified among 154 DNA macrorestriction patterns. Within the isolates of MRSA, four major genotypes were identified, with 36 isolates (85.7%) represented by a single PFGE type. In contrast, the 112 isolates of MSSA comprised 31 different PFGE types, of which 16 represented more than one isolate. Three PFGE types were found to represent 42% of all MSSA isolates. These common strains were found with equal frequency among adults and paediatric patients, and were evenly distributed between nosocomial and community-acquired cases.
Conclusion: Our results provide indirect evidence of ongoing transmission of MRSA and MSSA in our hospital. In the case of MRSA, the spread is predominantly due to a single clone, with transmission favoured by increased length of stay in hospital and the administration of beta-lactam antibiotics. In contrast, the spread of MSSA bacteraemia in this population is associated with multiple, genetically distinct strains.

P1011 Real-time PCR for *Staphylococcus aureus*: identification at species level and detection of mecA gene

T. Alarcón, J.A. García -Campos, M.S. Abanades, D. Monclus, D. Domingo, M. Lopez-Brea
Madrid, E

Objetives: (1) To use a real-time PCR (LightCycler, Roche Diagnostics, Spain) to differentiate *Staphylococcus aureus* from coagulase negative Staphylococcus (CNS) and (2) to use a real-time PCR to detect the presence of mecA gene in *S. aureus* clinical isolates.
Methods: Seventy-three strains obtained from clinical specimens were identified by MicroScan (Dade Behring) and coagulase test (28 *S. aureus*, 15 *S. epidermidis* and 30 other coagulase negative staphylococci). *In vitro* susceptibility was determined by MicroScan and disc diffusion. A total of 39 *S. aureus* strains were classified according to methicillin susceptibility: 21 resistant and 18 susceptible to methicillin. DNA was obtained by incubation at 100°C in lysis buffer. Real-time PCR was performed in a LightCycler instrument (Roche Diagnostics, Spain) using two commercially available kits: (1) LightCycler Staphylococcus kit Mgrade: PCR was positive in all staphylococci and they were differentiated according to melting temperature (62.1 ± 2°C for *S. aureus*, and 43.4–59°C for CNS) and (2) LightCycler MRSA detection kit: PCR was positive in mecA positive *S. aureus*. An internal control excludes the presence of inhibition. Once the DNA was extracted the whole process takes 1 h.
Results: Twenty-seven out of 28 *S. aureus* strains were clearly identified by real-time PCR due to the melting temperature (range from 60.2 to 63.4°C). One *S. aureus* showed melting temperature of 59.8°C. All *S. epidermidis* strains showed melting temperature from 50.1 to 53.4°C. *S. lugdunensis* showed melting temperature of 58.6, 54.6 and 53.1°C. Other CNS showed melting temperature from 51 to 57.1°C. Twenty-five out of 39 (64.1%) strains tested were mecA positive by using this LightCycler MRSA kit and real-time PCR. Among the 25 mecA positive, 21 were fenotipically methicillin resistant (84%) whilst four were methicillin susceptible (16%). All 14 mecA negative strains were susceptible to methicillin by phenotypic methods.
Conclusions: Real-time PCR (LightCycler) seems to be an accurate method to identify *S. aureus* and differentiate it from different CNS and to detect resistance to methicillin in *S. aureus*. Both reactions could be done simultaneously and the whole process takes less than 2 h (DNA extraction plus real-time PCR).

P1012 A comparative genomic hybridisation study of epidemic strains of methicillin-resistant *Staphylococcus aureus* in Canada

S. Christianson, M.R. Mulvey
Winnipeg, CAN

Objectives: Surveillance of methicillin resistant *Staphylococcus aureus* (MRSA) in Canada began in 1995. From this surveillance, six epidemic strains of MRSA have been identified and named CMRSA1–6. In order to better understand the relatedness of these strains, as well as their genetic content, we have used microarrays to compare their genomes to that of the fully characterised genome of the MRSA strain Col.
Methods: Genomic DNA from representatives of the six epidemic strains, as well as Col was fragmented and labelled using random primers with Cy5 or Cy3 labelled dCTP. Col and each of the epidemic strains (labelled with different dyes) were hybridised to arrays containing PCR products or 70 bp oligomers representing 2740 of the open reading frames (ORFs) in the Col genome. Data

were processed with the ArrayPro software package, positive/negative cut-off values were determined using Genomotyping Analysis by Charles Kim and then analysed using the GeneMaths program. Macrorestriction digest patters were generated using SmaI.

Results: Results indicate that all Canadian epidemic strains have six common regions of deletion, a portion of the type I SCCmec region, bacteriophage L54a, and four smaller areas composed of two to four ORFs. The only gene of known function in these smaller areas was the *Staphylococcal enterotoxin B*. Apart from these major deletions, many sporadic, single deletions are seen throughout the strains. Larger regions of deletion that are not present in all strains also occur. The only obvious ORF duplication is of IS431 in CMRSA3, 5 and 6, which is found in multiple copies in the typeIII SCCmec region in these strains. Macrorestriction digest data were used to approximate the sizes of the CMRSA genomes. CMRSA4 shows the smallest genome (~1862 kb), and the least genetic content in common with COL (79%). Though CMRSA1 and 2 appear to have larger genomes (~2378 and ~2706 kb, respectively), they show fewer ORFs in common with Col than other strains (81 and 86%, respectively), suggesting a substantial portion of the genome may be novel.

Conclusions: This is the first study of epidemic MRSA using the comparative genomic hybridisation approach. While the CMRSA strains show a high degree of relatedness to Col, there are considerable differences in genetic content. This study also indicates that there may be genetic content which is unaccounted for in the Col genome. Other studies are being devised to identify and characterise the novel genetic content.

P1013 Recent trends in molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in Finland

A.-M. Santala, O. Lyytikäinen, M. Kardén-Lilja, S. Ibrahem, S. Salmenlinna, A. Virolainen-Julkunen, J. Vuopio-Varkila
Helsinki, FIN

Objectives: In Finland, the annual number of MRSA isolates notified to the National Infectious Disease Register (NIDR) has constantly increased, especially outside Helsinki metropolitan area. Molecular typing has revealed numerous outbreak strains of MRSA, and some of them have been associated with community acquisition. We analysed strain types identified by pulsed-field gel electrophoresis (PFGE) of MRSA isolates sent to the National Reference Laboratory (NRL) during 1997–2003.

Methods: All isolates of MRSA notified by the Finnish clinical microbiology laboratories were sent to NRL for further verification and characterisation, including PFGE analysis. PFGE profiles differing by fewer than six bands were interpreted as identical or closely related. One isolate per person were included in the analysis. Strain types were categorised as sporadic (strain type only found from one person), domestic outbreak or international epidemic (strain type found from more than one person) as well as community-acquired (strain type associated with community acquisition in our previous study). The proportions of MRSA isolates included in each category were assessed.

Results: A total of 2496 MRSA isolates were studied. The number of MRSA isolates increased from 138 in 1997 to 804 in 2003. PFGE identified more than 200 different strain types. Of the MRSA isolates, 9% were sporadic, 66% domestic outbreak and 25% international epidemic. One strain type disappeared compared with years before 1997, and 11 new strain types appeared during 1997–2003. The proportion of sporadic strains varied between 3 and 18% during the study period. Of the international epidemic strains, Bel EC-3 increased from <1% in 1997 to 27% in 2003, mainly outside Helsinki metropolitan area. UK EMRSA-16 decreased from 13% in 1997 to <1% in 2003, and Helsinki I (a representative of MLST ST-5 strains) from 18 to 6%, respectively. UK EMRSA-15 varied between 1 and 6%. The three main strains with community-acquisition fluctuated during the study period (range, 10–30%).

Conclusions: Intensive national surveillance with molecular typing revealed that the predominant MRSA strains change over time. The internationally spread epidemic strains of MRSA have also been found in Finland. However, most of them show a decreasing trend or have disappeared. These results encourage us to continue aggressive interventions with each new MRSA case.

P1014 Existence of PVL-genes and SCCmec IV amongst Finnish CA-MRSA strains

M. Kardén-Lilja, J. Vuopio-Varkila, S. Ibrahem, S. Salmenlinna, O. Lyytikäinen, A. Virolainen-Julkunen
Helsinki, FIN

Introduction: According to recent studies, community-acquired (CA)-methicillin-resistant *Staphylococcus aureus* (MRSA) strains often contain a type IV SCCmec cassette and Panton-Valentine leukocidin (PVL) locus. It has also been shown that certain multilocus sequence types (ST) seem to be connected to CA-MRSA strains from different continents.

Materials and methods: We studied 108 Finnish CA-MRSA strains for their genotype by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST), the methicillin resistance genes by SCCmec PCR and the presence of PVL gene locus by PCR. MRSA was defined as community acquired if the MRSA specimen was obtained outside hospital settings or within 2 days of hospital admission from a person who had not been hospitalised within 2 years before the date of MRSA isolation. To confirm the functionality of the PVL-PCR reaction and the quality of the DNA, nuc gene was amplified at the same time.

Results: The majority of CA-MRSA strains studied (91/108, 84%) possessed SCCmec cassette type IV but only 13 (12%) were PVL positive. All but two PVL positive strains contained SCCmec type IV. One strain had SCCmec cassette III subtype, and for one strain the type was not determined. The PVL positive strains were mostly (9/13, 69%) of multilocus ST80. The four remaining PVL-positive strains were of ST1 (two strains) and ST8 and 96. The sequence types correlated well with the PFGE results: All strains with ST80 were analysed as PFGE profile HkiVIII and strains with ST1 as PFGE profile Nurmes. ST96 (SCCmec cassette -III subtype) and ST8 (SCCmec not determined) strains were considered as sporadic. The 95 PVL negative CA-MRSA strains belonged to 15 different shared and four sporadic PFGE profile types. The MLST analysis of PVL negative strains is currently underway.

Conclusions: Most of the Finnish CA-MRSA strains have SCCmec cassette type IV but only a minority contain PVL gene locus, which is in contrast to previous reports. Majority of the PVL gene positive strains possessed ST80. In spite of the strict definition for community-acquisition we used, majority (88%) of Finnish CA-MRSA were PVL negative and showed heterogeneous PFGE profiles.

P1015 Evaluation of susceptibility methods for detection of methicillin resistance in *Staphylococcus aureus*

M. Kantzanou, E. Alepopoulou, M. Panopoulou, E. Kantzanos, A. Grapsa, I. Dimopoulou, S. Kartali
Alexandroupolis, GR

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is among the major pathogens. The most common methods currently used for identifying methicillin (oxacillin) resistance in many clinical laboratories are susceptibility tests. The performance of these tests has been erratic because the expression of resistance is variable and commonly heterogeneous within strains.

Methods: A retrospective laboratory-based study was carried out with clinical isolates of *S. aureus* in a tertiary care providing Uni-

versity hospital in Thrace, Greece. Methicillin (oxacillin) susceptibility of 118 *S. aureus* isolates, which were recovered from various clinical specimens (blood cultures, tracheal aspirates, wound swabs and central venous catheters) were studied by four different methods: (1) agar screening test [MH-oxacillin (6 μg/mL) agar supplemented with 4% NaCl], (2) susceptibility determination by the Vitek 2 (BioMerieux), (3) MIC was determined by E-test (AB Biodisk), (4) mec-A gene detection by PCR, using specific primers. The strains were evaluated by using the presence of mecA gene detected by PCR, as definitive criteria for MRSA and non-MRSA. The susceptibility tests were carried out as recommended by the NCCLS.

Results: Among all the isolates, 44 were identified as mecA-positive and the remaining 74 as mecA-negative. The percentages of correct results (% sensitivity/% specificity) were: oxacillin agar screen, 98/100; E-test, 93/100; and Vitek-2, 100/89. Ten isolates, negative for the mec-A gene by PCR, were recognised by at least one phenotyping method as oxacillin resistant. Only one strain mecA-positive was incorrectly identified as oxacillin-negative by the oxacillin agar screen.

Conclusions: As shown in this and other studies, no phenotypic method is completely reliable for the detection of oxacillin resistance in *S. aureus*. The specificity was generally high, especially with the agar screening and E-test methods, while the sensitivity varied between the different methods. In particular, the oxacillin screen test is the most accurate test and approaches the accuracy of PCR. Although, the presence of the mecA gene, as detected by PCR, still remains the 'gold standard', agar-screening test should be considered in association with other susceptibility methods to maximise the ability to correctly detect oxacillin-susceptibility in *S. aureus*.

P1016 Amplification of DNA fragments surrounding rare restriction sites (ADSRRS-fingerprinting) for typing *Staphylococcus aureus* isolated from patients with recurrent furunculosis

W. Baranska-Rybak, R. Nowicki, E. Scieburako, J. Kur, E. Arlukowicz, A. Samet
Gdansk, PL

Introduction: In the present study we report data on phenotypic and genotypic characteristics of *Staphylococcus aureus* strains. The aim of our research was to identify SA genotypes in the patients suffering from recurrent furunculosis.

Materials: We obtained 70 isolates from 45 patients with recurrent furunculosis. Purulent discharge from furuncle, nasal and throat swabs were taken for culture.

Methods: The identity strain of SA was confirmed by novel DNA-typing technique. Amplification of DNA fragments surrounding rare restriction sites (ADSRRS-fingerprinting) is an effective and rapid method for molecular typing of isolates of bacteria. This method is based on suppression of PCR (polymerase chain reaction) reaction. SA DNA was digested with two restriction enzymes: BamHI (10 U/mL) (Sigma) and XbaI (10 U/mL) (Sigma). Cohesive ends of DNA were ligated with adapters (XbaI short adapter and BamHI long adapter) and amplified. PCR products were electrophoresed on polyacrylamide gels, stained by ethidium bromide and photographed under UV.

Results: ADSRRS-fingerprinting of 70 SA isolates revealed 10 unique patterns. In most cases the strains isolated from the same patient (nose, throat and furuncle) gave identical pattern. The reverse situation was found in five patients.

Conclusions: (1) In most cases we confirmed the identity between nasal/throat and furuncle SA isolates. (2) We found no specific genotype, which is responsible for recurrent furunculosis. (3) ADSRRS-fingerprinting seems to be a very useful method for epidemiological studies of SA.

P1017 Comparison of well-established methods with new genotyping methods for typing *Staphylococcus aureus*

N. Malachowa, A. Sabat, K. Kosowska, J. Krzyszton-Russjan, J. Empel, J. Miedzobrodzki, P.C. Appelbaum, W. Hryniewicz
Cracow, Warsaw, PL; Hershey, USA

Objectives: Rapid and efficient epidemiologic typing systems may be useful to investigate dissemination of the lineages of *Staphylococcus aureus*. We have compared the usefulness of well-established methods to those of newly developed rapid typing methods as epidemiological tools.

Methods: A total of 59 *S. aureus* isolates were analysed by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), repetitive-element PCR technique (rep-PCR) based on the presence of DNA sequence that are homologous to MP3 repeat in *Mycoplasma pneumoniae*, multiple-locus variable-number tandem repeat analysis (MLVA), and multiplex PCR-based method with primer mix of the spa gene, the coa gene, and the hypervariable region adjacent to mecA gene.

Results: Fifty-nine *S. aureus* isolates clustered by PFGE in 50 different genotypes. MLVA, which had the highest compatibility with PFGE of all testing methods in this study, clustered into 38 different genotypes, multiplex PCR-based method clustered into 23, and rep-PCR clustered into 16 different genotypes. Rep-PCR differentiated *S. aureus* isolates in a way similar to MLST that clustered these isolates in 19 groups.

Conclusion: Although PFGE is still the gold standard, owing to its high discriminatory power amongst molecular typing methods, genotyping methods based on PCR may be useful in respect of speed and ease of performance. MLVA, multiplex PCR-based methodology and rep-PCR are rapid, reproducible, and easy to perform. However, MLVA and multiplex PCR-based method generate more unambiguous results than those of rep-PCR.

P1018 Coagulase-negative *Staphylococci* strain types affect visual outcome in patients with endophthalmitis

S. Lightman, A. Khan, N. Okhravi, W. Lynn, T. Elliott, P. Lambert, A. Worthington
London, UK

Objectives: To determine whether the variable visual outcome in endophthalmitis secondary to coagulase-negative *Staphylococci* spp. are due to different strains causing intraocular infection, with a possible difference in virulence of each strain or resistance to the antibiotics given.

Methods: Twenty-eight intraocular samples infected with coagulase-negative *Staphylococci* spp. were analysed using both biotyping and pulsed-field gel electrophoresis for strain identification. The results were correlated with the visual outcome after 6 months post-treatment.

Results: Four different strains of coagulase-negative *Staphylococci* spp. were found to cause endophthalmitis; *S. epidermidis*, *S. haemolyticus*, *S. equorum* and *S. warneri*. Twenty-one out of the 28 isolates were identified as *S. epidermidis* and the others were grouped as non- *S. epidermidis* for correlation with the clinical data. Comparing the *S. epidermidis* with the non- *S. epidermidis* infected cases, it was found that the mean visual gain was significantly better for the non- *S. epidermidis* infected cases [(mean visual gain of 38.1 vs. 83.3 LogMAR letters, respectively) ($P = 0.029$)].The visual outcome was significantly worse for patients infected with *S. epidermidis* and antibiotic resistance was more common among these isolates although all were sensitive to at least one of the three/four antibiotics given. Comparing the non- *S. epidermidis* infected cases to the *S. epidermidis* infected cases that were sensitive to all four antibiotics used, the visual outcome was still significantly better in the non- *S. epidermidis* group [mean visual gain 83.3 vs. 25.75 LogMAR letters, respectively) ($P = 0.022$)].

Conclusion: SE carries a significantly worse visual outcome than non-SE strains of CNS and is likely to be due to increased virulence within the eye rather than antibiotic resistance.

P1019 Evaluation of Roche LightCycler kits for the detection of methicillin-resistant *Staphylococcus aureus* in patient screening broths

K. Levi, K.J. Towner
Nottingham, UK

Objectives: To evaluate two LightCycler kits for the detection of methicillin-resistant *Staphylococcus aureus* from patient screening swabs in comparison with conventional culture methods.
Methods: Sets of patient screening swabs ($n = 100$) were incubated in an overnight enrichment broth containing oxacillin to eliminate methicillin-sensitive *S. aureus*. Samples were lysed with lysozyme/lysostaphin before amplification. Broths were also cultured on Oxacillin Resistance Screening Agar. Samples were initially tested with the LightCycler Staphylococcus kit. Differentiation of *S. aureus* from coagulase-negative staphylococci (CNS) was based on melting curve analysis of the amplified product. Samples positive for *S. aureus* were also tested for the methicillin resistance gene mecA with the LightCycler MRSA Detection kit.
Results: The LightCycler Staphylococcus and MRSA Detection kits identified 29 MRSA-positive samples from overnight screening broths; MRSA colonies were isolated from 22 of these samples. All seven samples positive only by the LightCycler assays remained positive on repeat testing, and negative controls were consistently negative. Two of these seven samples were obtained from patients who had been MRSA-positive in the previous 6 months. One sample, which was MRSA-positive by culture and LightCycler-negative for *S. aureus* DNA, was LightCycler-positive on repeat testing. No *S. aureus*-positive, mecA-negative samples were detected, indicating that the selective broth inhibited the growth of methicillin-sensitive *S. aureus*. Two melting peaks, characteristic of *S. aureus* and CNS, were identified in 12 samples; MRSA was isolated from eight of these.
Conclusions: The LightCycler Staphylococcus and MRSA Detection kits were highly sensitive and may be particularly useful for rapidly investigating the possibility of MRSA carriage by health care workers or patients in a potential outbreak situation.

P1020 Sigma B dependent expression of oxacillin resistance in *Staphylococcus epidermidis*: transcriptional analysis of mecA, and other factors required for resistance expression

J.K.-M. Knobloch, J. Huck, S. Jäger, M.A. Horstkotte, D. Mack
Hamburg, D

Objectives: *Staphylococcus epidermidis* is a major pathogen in nosocomial infections, and infectious isolates display a high prevalence of oxacillin resistance (oxaR). Tn917 mutagenesis of rsbU, encoding a positive regulator of the alternative sigma factor sigma B lead to a reduced oxaR in *S. epidermidis* 1057. However, the mechanism of this regulatory pathway is still unknown. The role of sigma B in the regulation of oxaR in *S. epidermidis* was investigated in this study.
Methods: Two mutants with inactivation of the entire sigma B operon (1057rsbUVWsigB) or the regulatory cascade rsbUVW (1057rsbUVW) were generated by allelic gene replacement in *S. epidermidis* 1057, which displays a heterogeneous oxacillin resistance phenotype. RNA was extracted at 9 and 17 h from cultures in Mueller Hinton + 2% NaCl (MHNaCl) and MHNaCl supplemented with 1 μg/mL Oxacillin (MHoxa). Quantitative transcriptional analysis of mecA, femABCDF, fmtA, mrp (fmtB), and mprF (fmtC) were performed by real-time RT-PCR. At least a 2.5-fold difference compared with the wild type in the average of three independent experiments was defined as cut-off for differentially expressed genes.
Results: Population analysis of the mutants and the wild type strain revealed that mutant 1057rsbUVWsigB displayed a more heterogeneous phenotype with a smaller subpopulation expressing methicillin resistance compared with the wild type. Mutant 1057rsbUVW with constitutive expression of sigma B displayed a strong increase of methicillin resistance and a homogeneous resistance phenotype compared with the wild type. Transcriptional analysis revealed that the homogeneously resistant mutant 1057rsbUVW displayed no differences compared with the wild type under all conditions investigated, except of the gene fmtA, which was downregulated in MHoxa at 9 h. Interestingly, in the less resistant mutant 1057rsbUVWsigB the genes mecA, femB, femD, fmtA, and mprF were upregulated in MHNaCl compared with the wild type at both time points, whereas in MHoxa only the genes femD, fmtA, and mprF were upregulated at 9 or 17 h.
Conclusions: None of the investigated genes including mecA is responsible for the homogeneous expression of oxaR in mutant 1057rsbUVW. Mutant 1057rsbUVWsigB displayed a less resistant phenotype compared with the wild type strain, despite the upregulation of several genes required for oxaR. Therefore, an additional sigma B dependent factor must be required for homogeneous expression of oxaR in *S. epidermidis*.

P1021 Detecting the response of *Staphylococcus aureus* to antimicrobial agents by measuring changes in mRNA levels using a flow through microarray

R.M. Anthony, A.R.J. Schuitema, L. Oskam, P.R. Klatser
Amsterdam, NL

Objectives: To develop methods to measure the initial response of *S. aureus* after exposure to antimicrobial agents. Such an approach has the potential to allow both the sensitivity and mechanism of resistance to be rapidly determined from isolated bacterial strains.
Methods: mRNA was extracted from a selection of *S. aureus* isolates either with or without 30 min exposure to antimicrobial agents (including oxacillin and mupirocin). The mRNA extracted was then used to produce labelled nucleic acid suitable for hybridisation to a low-density flow through oligonucleotide array targeting specific genes. These arrays are suitable for high throughput screening and provide very rapid hybridisation kinetics.
Results: Distinctive changes in mRNA levels were detected for each agent tested and for isolates with different phenotypic susceptibilities. Oxacillin resulted in a significant increase in the levels of penicillin binding protein 2 (PBP2) mRNA in both sensitive and resistant isolates and an increase in the levels of PBP2prime mRNA in resistant isolates only. In contrast mupirocin resulted in very high levels of ile-tRNA synthetase in both strains with high- or low-level mupirocin resistance but not in sensitive strains.
Conclusion: Future developments in RNA extraction and labelling as well as the increased availability of DNA array technology will allow this approach to be more widely used. This and similar methods have the potential to provide information on both the resistance phenotype of the isolate and the mechanism of resistance, in contrast to 'classical' molecular tests for drug resistance which generally target known genotypes.