Metallo-beta-lactamases and resistance to carbapenems

P408

First isolated of Vim-1 in Enterobacteriaceae in Spain

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Objective: During the year 2003 we have investigated the carbapenem resistance in enterobacteriaceae from fecal carriers and other clinical samples.
Methods: All stool specimens submitted for bacterial culture were inoculated onto MacConkey agar supplemented with 2 mg/L of imipenem (IMAC). These IMAC plates were incubated overnight. The enterobacteriaceae strains suspected to be carrier of MBLs were identified by API 20E system (bioMérieux, Marcy l'Etoile, France). In vitro susceptibility testing were determined by the standard disk diffusion method and it was confirmed by Etest. To detect MBLs production a synergy test using disks or an Etest strip containing imipenem plus EDTA were employed. The detection of the gene blaVIM-1 and class 1 integrons was performed by PCR amplification. Nucleotide sequences of the PCR products were determined with a Beckmann 8000 sequencer. Conjugation and transformation experiments were conducted by the filter mating method and following Bio-Rad recommendations (Bio-Rad, Laboratories) respectively.
Results: Of 1043 stools studied and 4345 susceptibility tests determined in clinical samples during the year 2003 we obtained two strains, *Klebsiella penumoniae* from stool and *Escherichia coli* from urine sample, suspected to be carrier of MBLs. These strains were obtained from two patients. The in vitro susceptibility testing showed that both strains were resistant to all b-lactamics but were sensitive to aztreonam. Although imipenem was sensitive its diameter was low (22 mm). MIC to imipenem was 4–6 mg/L and 0.75 mg/L for *K. pneumoniae* and *E. coli* respectively. The synergy test using disks showed a positive result. The PCR amplification of blaVIM-1 was positive and direct sequencing of this amplification product showed 100% homology for the gen blaVIM-1 in both strains. PCR of class 1 integron yielded a 4 kb amplification product from *K. pneumoniae* and *E. coli*. The conjugal transfer of the resistance was managed with *K. pneumoniae* and *E. coli*, but in *E. coli* a previous transformation was needed.
Conclusion: The prevalence of carbapenemases in enterobacteriaceae strains is low. To our knowledge, these isolates of *K. pneumoniae* and *E. coli* are the first enterobacteriaceae species producing VIM-1 in Spain. The MBLs production should be studied when the strain showed a profile of resistance to cefotaxime, ceftazidime and cefepime but sensitive to aztreonam and imipenem.

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Metallo-beta-lactamase in clinical *Pseudomonas aeruginosa* isolate in a Portuguese hospital and identification of a new VIM-2 like enzyme

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Objectives: Metallo-beta-lactamases are responsible for carbapenems-resistant *Pseudomonas aeruginosa* . The spread of genes enconding for these enzymes among gram-negative bacteria has been described in different countries as well as in different species and is a threat to public health.The aim of this work was to identify metallo-beta-lactamases in *Pseudomonas aeruginosa* clinical isolates.
Methods: A total of 27 clinical isolates of *Pseudomonas aeruginosa* collected from February to October 2004 at an hospital in north of Portugal were found to be resistant to Imipenem. Antibiotic susceptibilities were determined by the agar disk diffusion according to the protocol recommended by NCCLS. Detection of metallo-beta-lactamases was performed by using the Imipenem-EDTA disk method and confirmed by the MBL E-test. The isolates were subjected to PCR assays with specific primers for blaIMP and blaVIM and sequence analysis were made to identify the metallo-beta-lactamase. Epidemiological typing was performed by M13 fingerprinting.
Results: Antibiotic susceptibility tests revealed high percentage of resistance to most antibiotics tested: Imipenem (96.3%), Meropenem (84%), Ciprofloxacin (74.1%), Piperacillin-tazobactam (34.6%), Ceftazidime (34.6%), Gentamicin (25.9%), Amikacin (14.8%) and Tobramicin (7.7%). In the Imipenem-EDTA test only one strain (Ps10VR) demonstrated a growth-inhibitory zone with 16 mm of diameter, suggesting production of metallo-beta-lactamases, while no remarkable distinct change was noticed in the others isolates. The MBL E-test was realized for this strain and Imipenem MIC has decreased from 16 to <1 mg/l.In PCR experiments using specific primers for blaIMP the results were negative among all strains, while in those using specific primers for blaVIM one strain generated a positive result, the same that was positive to Imipenem-EDTA test and MBL E-test (Ps10VR). The sequence analysis revealed that this strain was carrying a variant blaVIM-2 that encodes a VIM-2 like enzyme. The novel gene differs from blaVIM-2 by replacement of a G with a A at nucleotide 443 of structural gene, which results in Ser-to- Asp change at amino acid positions 136.M13 fingerprinting revealed that six clinical isolates were closely or possibly genetically related and that one VIM-2 like producing *Ps.aeruginosa* Ps10VR isolate was genetically indistinguishable.
Conclusion: The results showed a new VIM-2 like enzyme in *Pseudomonas aeruginosa*.

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Detection of a new VIM-type metallo-beta-lactamase (VIM-11) in a *Pseudomonas aeruginosa* clinical isolate from Italy

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Objectives: A one-year prospective study of multidrug-resistant Gram-negative microorganisms was carried out in a neonatal intensive care unit (NICU) in Palermo, Italy, within the framework of an infection control programme. All the newborns that were admitted to the NICU for at least 24 h – namely 211 cases – were enrolled in the study. All the imipenem-resistant *Pseudomonas aeruginosa* isolates from all kinds of specimens were tested for metallo-beta-lactamase (MBL) production.
Methods: Antimicrobial susceptibility testing was performed by both diffusion and microdilution and interpreted according

to the latest NCCLS documents. The presence of carbapenemase was screened by means of the MBL-etest, and further investigated by following carbapenem hydrolysis and its inhibition by EDTA. PCR and sequencing were carried out by standard procedures. An enzyme endowed with carbapenemase activity was cloned on a phagemid vector pPCR script Cam SK+ and transformed in *Escherichia coli* XL10 ultracompetent cells using the ligation kit polishing protocol.

Results and Conclusions: 54.5% (115/211) of newborns were colonised by MDR Gram-negatives. 16.1% (34/211) were colonised by an imipenem-resistant strain of *P. aeruginosa*. Twenty-one of these latter strains shared the same PFGE profile and all showed clear positivity with the MBL etest. Imipenem hydrolysis by spectrophotometric analysis of crude sonic extracts confirmed that imipenem was hydrolysed at a rate of 5×10^{-8} mol/min/mg, whereas on adding EDTA 2 mM the kinetics was 7.2×10^{-10} mol/min/mg.A polymerase chain reaction (PCR) performed with either bla(VIM) or bla(IMP) primers yielded positive results only with the bla(VIM) primers; a specific PCR yielded a product of about 800 bp that - after sequencing - was found to code for a polypeptide differing from VIM-4 in one amino acid, namely G31S. The new enzyme was named VIM-11 at the www.lahey.org site and received the accession number AY635904.

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JAP-1, a new member of subclass B3 metallo-beta-lactamase from *Bradyrhizobium japonicum*

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Objectives: The most common cause of bacterial resistance to beta-lactam antibiotics is the production of beta-lactamase. Among these, metallo-beta-lactamases (MBLs) are zinc-dependent enzymes that have been the focus of increasing attention because of their ability to hydrolyze nearly all known beta-lactams and of the lack of clinically-useful inhibitors. However, several MBLs have been reported also from bacterial species that are primarily members of the environmental microbiota (e.g. CAU-1 from *Caulobacter crescentus* and THIN-B from *Janthinobacterium lividum*). In the genome of *Bradyrhizobium japonicum* USDA 110, a nitrogen-fixing symbiont of legumes among the most important in agriculture and plant biotechnology, an open reading frame was found (ORF blr 6230) that encodes a protein sharing 28–40% sequence identity with subclass B3 MBLs. The functional properties of the enzyme, named JAP-1, were investigated.

Methods: ORF blr 6230 was amplified from *Bradyrhizobium japonicum* USDA110 by PCR using custom primers, and the amplification product was cloned into the T7-based expression vector pET-9a to yield the recombinant plasmid pET-JAP-1. *E. coli* BL21(DE3)[pET-JAP-1] strain was used for protein production. The enzyme was subjected to biochemical characterization and the kinetic properties investigated by spectrophotometry.

Results: ORF blr 6230 encodes a putative protein of 294 residues and a predicted molecular mass of 32 kDa. JAP-1 exhibits the highest sequence identity with CAU-1 and FEZ-1 (from *Legionella gormanii*) enzymes (40 and 34% respectively). *E. coli* BL21(DE3)[pET-JAP-1] produced a imipenemase activity (sp. act. 155 nmol/min.mg of protein) that was inhibited >95% after incubation in presence of 5 mM EDTA. The purified JAP-1 enzyme efficiently hydrolyzed penicillins, cephalosporins and carbapenems.

Conclusion: The MBL homologue encoded by the *B. japonicum* USDA 110 chromosome is a functional MBL of subclass B3 that exhibits a broad substrate profile. This is the first example of a MBL found in the bacterial species belonging to the Rhizobiales order.

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The diverse integron structures disseminating VIM genes in Poland

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Objectives: Recently we have characterized a novel blaVIM-4 metallo-beta-lactamase (MBL) gene cassette from clinical strains isolated from hospitalized children in Warsaw, Poland. This novel gene cassette was harboured on class 1 integron and found in 11 strains of *Pseudomonas aeruginosa* with various PFGE patterns. The aim of this study was to genetically examine other carbapenem-resistant *P. aeruginosa* and *P. putida* clinical strains for similar integrons.

Methods: Phenotypic screening involved the MBL Etest strip (AB BIODISK). Biochemical analysis used crude extrats examining imipenem (IMP) and meropenem (MEM) hydrolysis preincubated with 20 mM EDTA. PCR analysis was performed using primers specific for blaIMP and blaVIM genes and class 1 integrons. Sequencing was carried out using Perkin-Elmer Biosystems 377 DNA sequencer and analysed using DNAstar.

Results: The Etest produced for the majority of isolates a positive phenotypic test for MBL: IMP MIC > 256 mg/l; IMP/EDTA 1–8 mg/L. Isolates were resistant to all beta-lactams, and some additionally to aminoglycosides. The crude extracts of isolates showed hydrolysis of IMP and MEM, which were inhibited over 90% with EDTA. Screening for MBL using PCR analysis gave a positive result for the presence of the blaVIM gene. Two isolates of *P. putida* had an integron containing MBL gene blaVIM-4/blaVIM-4 partial repeat, then aacA4 (or aadB), OXA-2, orfD followed by qacEdelta1. *P. aeruginosa* isolate 266/03 had restriction pattern of blaVIM-4 as previously isolated in our hospital. Two *P. aeruginosa* isolates: 414/03 from our hospital and 303/03 from other hospital in Warsaw, had MBL gene blaVIM-2 as isolated previously in Warsaw. However, they had different additional genes within the class 1 integron.

Conclusions: Since 1998, *P. aeruginosa* strains with blaVIM-4 MBL genes have become endemic in children hospitalized in our institute. The source of blaVIM-4 gene cassette are probably the *P. putida* strains. Diverse integrons found in *P. putida* and *P. aeruginosa* isolates are responsible for the spread blaVIM genes in Poland.

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Diverse transposons are responsible for the dissemination of Japanese metallo-beta-lactamase alleles: Report from the SENTRY Surveillance Programme

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Objectives: MBL's were initially characterised in Japan, usually of the IMP type and found in *Pseudomonas aeruginosa* , *Acinetobacter* or *Serratia marcescens* and have been endemic in Japan for the last decade. However, little work has been done to characterize the integron structures or classify the mobile

genetic elements associated with them. Here we have sequenced upstream of the integrons harbouring MBL genes in five isolates to identify genes responsible for dissemination.
Methods: Integron structures were determined by sequencing PCR products amplified with 5′ and 3′ conserved sequence primers. Adjacent sequences to the Class 1 integron were amplified by PCR with nested primers anchored to the 5′ end of the Class 1 integron and degenerate primers designed to randomly hybridize to upstream sequences. Sequencing was performed on both strands.
Results: *S. marcescens* isolates 206-3105 and 205-3106 were of an identical integron structure with blaIMP-1 in the first gene cassette position followed by an OXA-1 gene cassette, both integrons were inserted into a Tn21 transposon in an identical position to the integron previously found in Tn21. The *S. marcescens* isolate 206-825 harboured a blaIMP-1 containing integron which was inserted into a resolvase gene of a Tn5044-like transposon. Isolate 205-1297 harboured a novel blaIMP-11 integron which was inserted into a sequence which displayed high identities to a Tn5053 transposon recently identified from Russian permafrost. Isolate 205-5353 harboured an blaIMP-1 containing integron which was inserted into a sequence of plasmid origin not linked to any transposon genes.
Conclusions: Analysis of the genetic elements flanking the blaIMP containing integrons revealed a surprising degree of diversity of genetic elements associated with these structures. The integrons harboured by Tn21 would be expected to be very mobile. However integrons inserted into functional transposase genes such as in isolates 206-825 and 205-1297 would be expected to be less mobile.

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Genetic characterisation of the IMP-1 metallo-beta-lactamase gene in *Enterobacter cloacae* strains from Turkey

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Objectives: The occurrence of metallo-beta-lactamase (MBL) genes in clinical strains of Enterobacteriaceae is worrisome since carbapenems have been used clinically to counteract AmpC producing pathogens. Herein, we describe the genetic context of MBL genes from clinical isolates of *E. cloacae* isolated from two medical centres in Turkey.
Methods: 6 strains of *E. cloacae* had imipenem MICs of 2–4mg/l. Genetic screening for blaIMP, blaVIM, blaSPM, blaGIM MBL genes and the NmcA gene was performed by PCR. The genetic context of MBL genes was also investigated by PCR with primers designed to amplify Class 1 and Class 3 integrons which have been implicated in the transfer of antimicrobial resistant genes and also primers designed to common regions known to be associated with numerous lactamase genes, such as the blaSPM. MBL genes were also examined to ascertain whether they were plasmid or chromosomally mediated. Plasmids were electroporated into *Escherichia coli*. The isolates were molecular typed by automated ribotyping and PFGE.
Results: The MBL gene blaIMP-1 was detected in all six strains of *E. cloacae* but PCR was negative for all other beta-lactamase genes. Plasmids were detected in all strains of *E. cloacae* with three different agarose gel profiles. Two different plasmid profiles were detected at site 69 and a third profile at site 68. blaIMP-1 PCR of plasmids indicated that blaIMP-1 was plasmid located in the *E. cloacae* strains from site 69 but probably chromosomally located in the strain from site 68. These results were further confirmed by electroporation of *E. coli* with plasmids isolated from each strain, followed by blaIMP-1 PCR using the *E. coli* host as template. No Class 1 integrons were detected by PCR. Although products were produced by PCR using Class 3 integron primers, subsequent PCR did not detect blaIMP-1 on this product. Common region (CR) PCR detected putative CRs in 5 of 6 isolates. 4 isolates from site 69 showed identical ribotype and PFGE pattern, while the other 2 strains showed unique ribotypes.
Conclusions: blaIMP-1, was detected in *E. cloacae* isolated at two different institutions within Turkey. The blaIMP-1 gene was found on different plasmid backgrounds and also on the chromosome in one isolate demonstrating mobility. However, in all cases the MBL gene did not appear to be associated with an integron. Putative common regions were detected in these isolates but any genetic linkage with blaIMP-1 has yet to be confirmed.

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In vitro activity of beta-lactam antimicrobial agent combinations with aztreonam when testing metallo-beta-lactamase-producing *Pseudomonas aeruginosa* and *Acinetobacter* spp.

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Objectives: To evaluate the interactions between aztreonam (AZT) and selected beta-lactams (BL) when tested against metallo-beta-lactamase (MBL)-producing *P. aeruginosa* (PSA) and *Acinetobacter* spp. (ASP) strains. AZT often displays on-scale MIC results tested against MBL-producing strains.
Methods: Ten PSA strains, including 9 well-characterized MBL-producers (IMP-1, -2, -13, -16, VIM-1, -2, -7, SPM-1 and GIM-1) and 5 ASP strains, including 3 MBL-producers (IMP-1 and -2) were tested using time kill/bactericidal activity methods. AZT at 4, 8 and 16 mg/L was combined with 4 BLs (cefepime [CPM], ceftazidime [CAZ], meropenem [MEM] and piperacillin/tazobactam [P/T] for PSA or ampicillin/sulbactam [A/S] for ASP), which were tested at the NCCLS susceptible (S) breakpoint concentration. Bacterial counts were determined at time 0, 4, 8 and 24 hours. Enhanced activity was defined as a ≥ 1 and synergy (SYN) as ≥ 2 $\log_{10}$ reduction in the CFU/ml compared to the result of the most active antimicrobial tested alone. Antagonism (ANT) was defined as a ≥ 2 $\log_{10}$ increase in CFU/ml compared to the most active drug.
Results: All MBL-producing PSA were resistant (R) to tested BLs, except for AZT on IMP-16 (MIC, 1 mg/L), SPM-1 (8 mg/L) and GIM-1 (16 mg/L), and for P/T on IMP-16 (4/4 mg/L), VIM-2 (16/4 mg/L) and IMP-2 (32/4 mg/L). Enhanced activity was observed with 4 PSA strains (IMP-16, VIM-2, SPM-1 and GIM-1) and 4 ASP, while ANT was observed with 1 PSA (IMP-16) with MEM and 1 ASP (non-MBL-producing). All other strains showed indifferent interactions (CFU/ml variation of ± 1 $\log_{10}$) with any combination evaluated. Results of CFU/ml variation observed when AZT at 8 mg/L was combined with the BLs are listed in the Table: Enhanced activity was also observed when AZT at 4 mg/L was combined with P/T (PSA IMP16, VIM-2 and SPM-1), MEM (PSA SPM-1), CPM (PSA GIM-

Organism	CFU/ml variation (log_{10}) +AZT				
	CPM (8 mg/L)	CAZ (8 mg/L)	MEM (4 mg/L)	P/T (64/4 mg/L)	A/S (8/4 mg/L)
PSA IMP-16	-3.8[a]	-2.6	+2.5	-3.8	-
PSA VIM-2	+0.3	-1.0	-0.6	-2.7	-
PSA SPM-1	-0.4	+1.4	+0.8	-1.2	-
PSA GIM-1	-1.6	+0.1	0.0	+0.1	-
ACB IMP-2	+0.1	+0.2	0.0	-	-2.2
ACB 4-575 (non-MBL)	+0.3	+0.4	0.0	-	-2.7

a. underline values indicate SYN.

1) or A/S (ASP IMP-1 and non-MBL) and when AZT at 16 mg/L was combined with CPM, MEM and P/T (PSA GIM-1), CAZ (ASP IMP-1) or A/S (ASP non-MBL). Among PSA strains, ANT was observed only with the IMP-16 strain when AZT at 4 and 8 mg/L was combined with MEM or when AZT at 4 mg/L was combined with CAZ, while among ASP ANT was observed when AZT at 4 mg/L was combined with CPM.
Conclusions: MBL-producing PSA and ASP strains are usually R to most BLs except AZT. AZT can favorably interact with other BL agents against some multi-drug resistant isolates for possible chemotherapy.

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Transfer and carriage of naturally occurring *Pseudomonas aeruginosa* plasmids harbouring metallo-beta-lactamase to Enterobacteriaceae strains

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Background: The presence of metallo-beta-lactamase (MBL) genes in clinical strains of some Enterobacteriaceae is worrisome since carbapenems have been used clinically to counteract extended-spectrum beta-lactamase producing pathogens. Herein, we assessed the frequency and the viability of plasmids carrying MBL genes transferred into Enterobacteriaceae.
Methods: Twenty-one carbapenem-susceptible and intermediate resistant Enterobacteriaceae (*Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Citrobacter freundii*) clinical isolates from different genotypes were screened for absence of plasmids and activity against carbapenems. The isolates were transformed with wild-type plasmids (24 kb–400 kb) harbouring the MBL genes blaIMP-1, blaVIM-7, blaSPM-1 and blaGIM-1 by electroporation and conjugation. Plasmids were isolated using Qiagen commercial kits. Selection was performed with a range of ceftazidime (20–100 μg/ml) or imipenem (10–50 μg/ml) incorporated into the medium. The plasmids RSF1010 and pK18 were used as controls during the transformation experiments. PCR reactions for the internal regions of the MBL genes and activity assays against imipenem were performed to confirm the transfer of the gene.
Results: Twelve isolates containing no plasmids and showing high-level of sensibility to carbapenems were transformed with the four MBL harbouring plasmids. Transformants could not be recovered in 11 from the 12 isolates, although the controls demonstrated positive transfer at high frequencies. Evidence of transfer was obtained in plates containing 20, 30 and 40 μg/ml of imipenem with an imipenem intermediate-resistant *C. freundii* isolate (MIC 8 μg/ml) transformed with blaVIM-7 plasmid. The presence of the MBL gene in the transformants was confirmed by PCR and sequencing; however, no activity against imipenem could be observed.
Conclusions: The spread of the MBL genes in Enterobacteriaceae is a great concern. However, data from this pilot study indicates that in-vitro transfer does not happen at a high frequency and may be strain dependent.

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Silencing of a *Pseudomonas aeruginosa* metallo-beta-lactamase gene in Enterobacteriaceae isolate: evidence of post-transcriptional modifications

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Background: Gene silencing represents a cellular defense system for controlling foreign gene expression. As metallo-beta-lactamase genes are becoming more spread, it is important to evaluate the ability of Enterobacteriaceae to express Pseudomonas metallo-beta-lactamase genes carried on native plasmids.
Methods: Plasmids harbouring metallo-beta-lactamase genes were transformed in K2-23, a *Citrobacter freundii* strain with intermediate resistance to imipenem and meropenem due to decreased expression of 42 kDa outer membrane protein. Selection was performed with imipenem (10–50 mg/L). PCR reactions and sequencing of the metallo-beta-lactamases gene and surrounding regions were carried out to confirm integrity of the structural gene and promoter regions. Activity assays against imipenem, meropenem and ceftazidime were performed to the original strain and transformants. Gene expression was analysed by RT-PCR with blaVIM-7 primers using housekeeping genes as controls.
Results: Colonies were recovered from transformation with blaVIM-7 in imipenem concentrations of 20, 30 and 40 mg/L. PCR reactions showed the presence of blaVIM-7 in different colonies, showing increased imipenem MICs (>32 mg/L). Gene sequencing results confirmed the presence of blaVIM-7 in the transformants. However, detectable activity against imipenem and meropenem could not be demonstrated in any of the transformant colonies. Sequencing results confirmed the integrity of the blaVIM-7 gene and no alterations could be observed in the upstream (including the integron promoter) and downstream regions of the gene cassette. RT-PCR demonstrated the presence of mRNA of blaVIM-7, showing that the gene has been transcribed.
Conclusions: Silencing of genes occurs at the transcriptional level, whereas in other cases, silencing is due to a postranscriptional process. The results of this study indicate the occurrence of postranscriptional modifications.

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Phenotypic detection of *Pseudomonas aeruginosa* producing metallo-beta-lactamases

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Objectives: The purpose of our study was to determinate the proportion of *Pseudomonas aeruginosa* producing metallo-beta-lactamases.
Methods: We have studied a random sample of 44 *Pseudomonas aeruginosa* strains. 34 of these strains were Imipenem resistance and 10 were Imipenem sensitive. Strains were isolated by clinical sample cultures. The samples were collected during 2003 from Intensive Care Units (52%), Surgical wings (25%) and Pathological wings (23%) of the university hospital AHEPA. The cultures became in common nutrient substrates. The identification and determination of antibiotic sensitivity was performed by Vitek 2 system (BioMérieux). The identification was confirmed by API 20NE system. The Imipenem resistance was

confirmed by E-test (AB Biodisk). The phenotypic detection of producing of metallo-beta-lactamases was performed by E-test MBL (Imipenem (IP) / Imipenem+EDTA (IPI)) of AB Biodisk.
Results: We found 8 negative and 2 undetermined results for producing metallo-beta-lactamases during phenotypic control of Imipenem sensitive strains. Strains with undetermined results had borderline Imipenem MIC (4 μg/ml). Also, we found 23 positive (67%), 8 negative (24%) and 3 undetermined (9%) results during phenotypic control of Imipenem resistance strains. Strains produced metallo-beta-lactamases had resistance phenotype as follows: 100% resistance to Ampicillin, Ampicillin/Sulbactam, Cefixime, Cefotaxime and Meropenem, 90% to Ceftazidime, Cefepime and Ciprofloxacine, 86% to Gentamicin and Tobramycin, 78% to Piperacillin and 26% to Piperacillin/Tazobactam. The positive strains were isolated from bronchial excretions (11), pus (3), blood (2), urine (2) venous catheters (2) ear excretions (2) and CSF (1). Also, positive strains were collected from Intensive Cure Units (61%), Surgical wings (26%) and Pathological wings (13%).
Conclusions: a)A high proportion of strains produced metallo-beta-lactamases was found. b) The biggest proportion of positive strains was collected from Intensive care Units. c) The detection of producing metallo-beta-lactamases was observed in multi-resistant *Pseudomonas aeruginosa*. d) All positive strains were Imipenem-resistant. e) It was remarkable that while Tazobactam does not inhibit metallo-beta-lactamases, it was found a particularly low resistance proportion to Piperacillin/Tazobactam.

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Metallo-beta-lactamase production and susceptibility to antibiotics of *Pseudomonas aeruginosa* isolates from Zagreb, Croatia

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Objectives: The aim of this study was to determine the susceptibilities to wide range of antibiotics of *P. aeruginosa* isolates with reduced susceptibility to either imipenem or meropenem and the prevalence of metallo-b-lactamases (MBLs) produced by these isolates.
Methods: 100 *P. aeruginosa* isolates with reduced susceptibility to carbapenems were collected in three hospitals in Zagreb during 2002–2004. The antibiotic susceptibilities were determined by broth microdilution method. MBLs were detected with three different phenotypic methods: EDTA double-disk potentiation test using ceftazidime disk, combined disk test and E test. The sensitivity and specificity of these methods were compared. Positive and negative control strains were included in the tests.
Results: **Antibiotic susceptibilities** The concentration of antibiotic necessary to inhibit 90% of the strains was 16 mg/L for meropenem, 32 mg/L for imipenem and ciprofloxacin, 128 mg/L for ceftazidime alone and combined with clavulanate and cefepime, and 256 mg/L for piperacillin alone and combined with tazobactam, cefoperazone, cefotaxime and gentamicin. 33% of the strains were resistant to piperacillin/tazobactam, 36% to imipenem, 40% to ceftazidime, 41% to piperacillin and ceftazidime/clavulanate, 42% to meropenem, 49% to cefoperazone, 55% to cefepime, 56% to cefotaxime, 70% to ciprofloxacin and 75% to gentamicin. **Detection of MBLs** Combined disk test detected MBLs in 51 strains, double-disk test in 40 and E test in 7 strains. Detection of MBLs was not determinable by E test in 8 strain because of the off-scale MIC values.
Conclusions: None of the antibiotics tested in this study should be recommended as the antibiotic of choice for the treatment of *P. aeruginosa* strains with reduced susceptibility to carbapenems due to high percentage of resistance.Combined-disk test has shown to be the most sensitive in detection of MBLs, followed double-disk test. E-test gave the lowest rate of positive results. Specificity of all three test was 100%. As previously observed by other authors E test detects MBLs only in strains with high level enzyme production and this can be an explanation for imperfect correlation between phenotypic tests for MBLs.With increasing incidences of MBLs among *P. aeruginosa* isolates, it would be important that screening to detect them is incorporated in routine diagnostic testing.

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Spread of highly carbapenem-resistant *Acinetobacter baumannii* in an intensive care unit of a tertiary hospital: the role of AmpC overproduction in this resistance

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Objectives: To investigate the resistance mechanisms of highly imipenem- and meropenem-resistant *P. aeruginosa* in an intensive-care unit (ICU) of a large tertiary hospital.
Methods: A total of 17 non-repetitive *Acinetobacter baumannii* strains were collected from hospital infection samples as well as environmental sources in the ICU of Red Cross General Hospital, Athens, Greece. Identification and susceptibility testing were performed by using the Vitek 2 Automated System (Biomerieux, Marcy l' Etoile, France). Imipenem and meropenem MICs were determined using E-test. The isolates were tested by E-test MBL for a possible metallo-beta-lactamase production, PCR for the presence of carbapenemase-encoding genes blaIMP, blaVIM and blaOXA, and synergy experiments using meropenem and the efflux pump-inhibitor carbonyl-cyanide-chloro-phenylhydrazone (CCCP). Also, RT-PCR for ampC gene was performed to test for a possible contribution of AmpC overexpression to the carbapenem resistance.
Results: During the study period, 17 carbapenem-resistant *A. baumannii* isolates were recovered from clinical infections and environmental samples. MICs of imipenem and meropenem ranged from 8 to >256 mg/L. All the isolates except two were negative by the E-test MBL, while all were negative by PCR for genes encoding known carbapenemases. No isolate exhibited significant synergy between meropenem and CCCP, indicating that over-expression of proton-gradient dependent efflux pumps did not contribute to the meropenem resistance. AmpC overexpression was demonstrated in seven isolates. In these isolates, inhibition of AmpC by cloxacillin, reduced MICs for several cephalosporins and carbapenems.
Conclusions: The aetiology of resistance phenotype of these isolates is still unclear. The overexpressed AmpC only in part contributes to the carbapenem resistance. The possible presence of an unknown carbapenemase needs further investigation.

P421

Development of phenotypic and genotypic methods for the detection of carbapenemases in clinical isolates of *A. baumannii* resistant to imipenem

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Objectives: The aim of this study was to develop different techniques to detect and identify the presence of

carbapenemases in clinical isolates of *Acinetobacter baumannii* resistant to imipenem.

Methods: The study included 58 resistant isolates obtained at Hospital de Santa Marina (Bilbao,Northern Spain) during 2002 belonging to previously identified clone I (19 isolates), II (13), III (3) and IV (1). Phenotypic detection was done using the following methods: Hodge test, Hodge test plus zinc sulphate, EDTA test, and two variants of DDST test (one disk containing EDTA+SMA and double disk EDTA/SMA). Genetic experiments to detect bla-OXA 40, bla-IMP and bla-VIM genes were performed by DNA amplification with the primers P1/OXA-24: 5′-GTACTAATCAAAGTTGTGAA-3′ and P2/OXA-24: 5′-TTCCCCTAACATGAATTTGT-3′), BLAIMPF: 5′-CTACCG-CAGCAGAGTCTTTG-3′ and BLAIMPR 5′-AACCAGTTTTGCC-TTACCAT-3′); VIM-DIA/r 5′-AGGTGGGCCATTCAGCCAGA-3′; VIM1-upv 5′-GTCGCAAGTCCGTTAGCCCAT-3′ and VIM 2-upv 5′-GATTCTAGCGGTGAGTATCCG-3′.

Results: The results obtained are sumarized in the following table:

	HODGE			DDST				PCR			
CLON	H1	H2	TOTAL	EDTA	e+s	e/s	TOTAL	OXA	VIM1	VIM2	IMP
I	+	+	27	–	–	–	19	1+/18*	0	0	1
				+	–	–	2	2*	0	0	0
				+	+	–	1	1*	0	0	0
				–	–	+	1	1*	0	0	0
				–	–	+	3	3*	0	0	0
				+	–	+	1	0	0	0	0
	+	–	9	+			3	3*	0	0	0
				–			5	5*	0	0	0
				–	–	–	1	1*	0	0	0
	–	–	3	–			3	1+/1*	0	1+	0
	–	+	2	–	–	–	2	2*	0	0	0
II	+	+	12	–	–	–	10	10+	0	3+	0
				+	–	–	1	1+	0	0	0
				–	–	+	1	1+	0	0	0
	+	–	1	–	–	–	1	1+	0	0	0
III	+	+	1	–	+	+	1	1*	0	0	0
	–	–	2	–			2	0	0	0	0
IV	–	–	1	–			1	0	0	0	0

H1: Hodge test H2: Hodge test plus SO,Zn
e+s: single disk (EDTA plus SMA: **e/s:** double disk (EDTA and SMA)
* :faint band

Conclusions: Phenotypic tests were easy to perform but, specially with metallobetalactamase tests the interpretation was rather difficult. The majority of isolates showed carbapenemase activity which was correspondent with the detection of the OXA-40 gene. Some metallobetalactamase tests were positive but no genetic correspondence was observed; moreover, the VIM-2 and IMP positive isolates found were all negative in the phenotypic tests. This is the first time we detect VIM and IMP enzymes in our envirorment.

P422

Genetic analysis of imipenem-resistant *Pseudomonas aeruginosa* oprD gene from clinical isolates in Korea

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Objectives: The emergence of multidrug-resistant strains in *Pseudomonas aeruginosa* isolates has increased worldwide. Imipenem (IPM) is the most potent agent for treatment of multidrug resistant *P. aeruginosa* infection but a number of clinical *P. aeruginosa* isolates have shown reduced susceptibility or resistance to IPM. IPM-resistant strains have either lost OprD or have strongly reduced OprD levels, and loss of OprD has been reported to occur in isolates with mutation, base transitions or deletions, in the oprD structural gene. In recent years, IPM-resistant *P. aeruginosa* isolates have emerged rapidly in South Korea. The objective of this study was to evaluate the association between mutations in oprD gene and IPM resistance.

Methods: Fifteen IPM-resistant and 3 IPM-susceptible *P. aeruginosa* evaluated in this study were isolated from non-tertiary hospitals in Korea during 2003. MICs of imipenem and meropenem were determined according to the criteria of NCCLS. The oprD gene was amplified by PCR using specific primers and sequencing was performed by the dideoxy-chain termination method. The amino acid alterations in the oprD gene of IPM-resistant isolates were compared with the corresponding sequence of *P. aeruginosa* PAO1 and IPM-susceptible isolates. The mRNA levels from oprD gene of *P. aeruginosa* isolates obtained by real-time quantitative PCR.

Results: All of IPM-resistant isolates produced metallo-beta-lactamase, VIM-2, and the MICs of IPM for these isolates were 16 to 512 mg/L. All oprD sequences from *P. aeruginosa* isolates were divided into three distinct groups (A, B and C), with identities ranging from 91.1% to 100% for amino acids. In these isolates, amino acid variations among oprD groups were observed mainly at the external loops. We did not find the direct correlation between the oprD group and imipenem resistance. However, IPM-resistant isolates in each oprD group had amino acid substitutions in the external loops (loop 2, 3, 5, 6, 7 or 8). The level of oprD mRNA in IPM-resistant isolates was down-regulated, compared with IPM-susceptible isolates.

Conclusion: These results suggested that amino acid alterations in external loops of oprD gene from *P. aeruginosa* isolates was associated with the down-regulation of oprD transcription and IPM resistance.

P423

Resistance to carbapenems in *Pseudomonas aeruginosa* clinical isolates

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Objective: Study of sensitivity to antibiotics of clinical isolates of carbapenem-resistant *P. aeruginosa*,and determination of the presence of metallo-betalactamases(MBL).

Methods: We selected 133 strains of *P. aeruginosa* with MIC ≥ 4 to imipenem and/or meropenem from August-02 to August-03. Sensitivity to antibiotics was determined by automated systems for piperacillin (PIP), piperacillin-tazobactam (PT), imipenem (IP), meropenem (MER), ceftazidime (CTZ), gentamicin(GE), tobramycin(TO), ciprofloxacin (CIP) and levofloxacin(LEV, and E-test for aztreonam(AZ) and colistin(CO). To determine the presence of MBL, we used the E-test with imipenem (IP) / imipenem plus EDTA (IPI). With strains which proved positive with this screening test, we carried out a PCR with the primers described by Nordmann and Poirel for VIM type genes.

Results: Of the 133 strains selected, 109 (82%) had MIC ≥ 4 to both carbapenems, 23 (17.3 %) only to IP, and only 1 strain (0.75%) had MIC ≥ 4 for MER, and was sensitive to IM. The most active antibiotics against these carbapenem-resistant strains were the aminoglycosides, especially TO(73.7% sensitive strains), and COL(82.7%). The least active antibiotics were CIP(33.8%), CTZ(41.35%) and AZ(52.6%). The most active betalactam antibiotic in vitro was the combination PT. Of the 67 strains resistant to PIP(1.2%), they become sensitive with the introduction of tazobactam. As a result of the screening test to evaluate the presence of MBL,we obtained 4/133 strains as probable producers of metalloenzyme. We selected strains with MIC IP/MIC IPI ≥ 5 due to previous experience in our hospital. The MIC of these 4 strains to IP and MER were, respectively: 4–4, 4–4, 8–8 and>16–2. Of these 4 strains, two were resistant to GE, and one to TO (sensitive to GE); all were sensitive to the betalactams, quinolones and to AZ. The MIC for colistin were

0.5,6,6 and 12. With these 4 strains a PCR was carried out with primers for VIM-type genes, and we obtained a 676 bp band for each of the strains.
Conclusions: The most active antibiotics in vitro against *P. aeruginosa* with an MIC ≥ 4 to carbapenems are TO and COL.AZ is active against 52.6% of the betalactams. The four strains carrying VIM-like MBL were sensitive in vitro to betalactams, and two of these had an MIC = 4 for IP and MER. Carbapenemases are enzymes capable of hydrolyzing all the betalactam antibiotics. Therefore,laboratory detection of these enzymes is important, since it can predict therapeutic failure.

P424

Spread of OXA-58-producing carbapenem-resistant *Acinetobacter baumannii* isolates in Iasi, Romania

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Purpose: To characterize the clonal relationship and the beta-lactamase content of carbapenem-resistant *Acinetobacter baumannii* isolates recovered in a Pediatric Hospital in Iasi, Romania.
Material and methods: Fifteen carbapenem-resistant *Acinetobacter* sp. isolates were recovered during the 1999–2003 period at the St.-Maria Pediatric Hospital of Iasi. Species identification was done by the biochemical API32 GN test (bio-Mérieux, France) and by 16S rRNA sequencing. Sensitivity testing was done by disk diffusion method and Minimum Inhibitory Concentration (MICs) were determined by agar dilution. The presence of oxacillinase or metallo beta-lactamase genes was performed by PCR. In particular, genes coding for the carbapenem-hydrolyzing OXA-23, OXA-40 and OXA-58 subgroups were searched. Genotyping was done by pulsed field gel electrophoresis (PFGE) after digestion by ApaI.
Results: All isolates but one were identified as *A. baumannii*. A single isolate was *A. junii*. The majority of strains (n = 11), including the *A. junii* strain, possessed the blaOXA-58 gene, whereas three isolates were blaOXA-23 positive and one was blaOXA-40 positive. Genotyping revealed that eight out of ten OXA-58-positive *A. baumannii* isolates corresponded to a single clone. The blaOXA-58 gene was plasmid-located in all cases.
Conclusion: As observed in other European countries, increasing prevalence of carbapenem resistance in *Acinetobacter* sp. may be explained by the presence of carbapenem-hydrolyzing oxacillinases.

P425

Analysis of the genetic structures at the origin of acquisition and expression of the blaOXA-58 carbapenem-hydrolysing oxacillinase gene of *Acinetobacter baumannii*

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Objectives: Oxacillinase-mediated resistance to carbapenems in *Acinetobacter baumannii* is increasingly reported worldwide. Three clusters of carbapenem-hydrolyzing Ambler class D beta-lactamases (OXA-23, OXA-40 and OXA-58) are known in that species. The blaOXA-23 gene was plasmid-located in several isolates and associated to the ISAba1 insertion sequence (IS). The blaOXA-40 gene was chromosomally-located in different clones and not associated to integron or transposon structures. The aim of this work was to characterize the genetic structure surrounding the blaOXA-58 gene and involved in its expression.
Methods: *A. baumannii* MAD strain isolated in Toulouse, France, was used as OXA-58-positive reference strain. Cloning and PCR experiments were performed to identify the upstream- and downstream-located regions of blaOXA-58 gene in isolates from different geographical origins. Primer extension experiments identified the promoter sequences responsible for its expression in several genetic structures.
Results: The blaOXA-58 gene was located on a 30-kb plasmid in *A. baumannii* MAD. An ISAba3 element was identified downstream of blaOXA-58 and an isoform of ISAba3 was present upstream of it. ISAba2 (weakly related) was inserted in the upstream-located ISAba3 sequence. This structure was conserved in blaOXA-58-positive isolates from various European origins even if ISAba2 was not always detected. This genetic structure was bracketed by two 27-bp long repeated sequences that were likely the signature of an illegitimate recombination event at the origin of the blaOXA-58 gene acquisition. This hypothesis was reinforced by the analysis of a plasmid present in a clonally-related but carbapenem-susceptible *A. baumannii* isolate recovered in the environment from the same hospital in Toulouse. In that isolate, the ISAba2/ISAba3/blaOXA-58-containing structure was lost and only one out of the two 27 bp sequences was conserved.The sequences involved in the blaOXA-58 expression were made of a -35 promoter region located in ISAba2 and a -10 promoter region located in ISAba3, thus constituting an hybrid promoter. Analysis of several isolates in which ISAba2 was absent revealed that ISAba3 may provide both −35 and −10 promoter sequences.
Conclusion: This works described novel structures at the origin of acquisition of a carbapenem-hydrolyzing beta-lactamase identified in *A. baumannii* isolates from several European countries.

P426

Occurrence of carbapenem-resistant *Bacteroides fragilis* strains in Sweden during a relatively long period (1990–2004) and analysis of the resistance mechanisms

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Objectives: The aim was to study the resistance mechanisms of identified carbapenem-resistant *B. fragilis* isolates from different Swedish hospitals and the trend in their appearance frequency was also followed.
Methods: Agar dilution and Etests were applied for antimicrobial susceptibility measurements; PCR was used to detect the cfiA genes and activating insertion sequence (IS) elements, and finally the IS elements were sequenced.
Results: The investigated strains were isolated during the period from 1990 to 2004. Eight *B. fragilis* strains were studied, all of which had previously been identified as imipenem-resistant at various Swedish hospitals. Six of these isolates originated from clinical samples and the remaining two were from diarrhoeal faeces. The occurrence of these strains could be regarded as sporadic as no significant increase was observed during time. All were cfiA-positive and the insertion of IS1186 (three strains), IS614B (two), IS1187 (one) and IS942 (one) elements were detected in the upstream regions of the resistance genes. In one isolate, *B. fragilis* 1619, the upstream region was altered in such a way that no IS element could be found by the method employed in the present study. The three IS1186-bearing strains also carried IS4351 in the genome and were nimB gene-positive. The insertion sequence IS1186 was inserted at

different positions in the conserved upstream region of the cfiA genes. Such a configuration or coincidence of these genetic elements implies a common origin. IS elements similar to those that activated the cfiA genes in the present study have been detected in *B. fragilis* isolates from the USA and the UK.
Conclusion: The presence of IS elements upstream of cfiA might activate the expression of carbapenem resistance. However, further investigations are needed to elucidate the resistance mechanisms in *B. fragilis*. For some carbapenem-resistant isolates, a prevailing and prevalent pre-carbapenem-resistant strain is probably the predecessor for such strains, before IS insertion occurs.

P427

Novel class A carbapenemase, KPC-4, in an *Enterobacter* isolate from Scotland

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Objectives: The BSAC Bacteraemia Resistance Surveillance Programme began in 2001. It aims to allow long-term monitoring of resistance trends and the emergence of new resistance mechanisms. A carbapenem-resistant isolate of *Enterobacter* sp. (E624) was identified in 2003. Two further *Enterobacter* isolates from the same patient varied in their degrees of carbapenem susceptibility. We sought to determine their mechanism(s) of carbapenem resistance.
Methods: Isolates were typed by PFGE of XbaI-digested genomic DNA and analysed with BioNumerics software. MICs were determined and interpreted using to BSAC guidelines. Isolates were screened by PCR for genes encoding known carbapenemases; amplicons were sequenced using dye-terminator chemistry. Imipenem (IPM) hydrolysis was investigated by spectrophotometry. Iso-electric focusing (IEF) was performed to visualise beta-lactamases. Outer membrane profiles were examined by SDS-PAGE analysis.
Results: The three *Enterobacter* isolates were highly-related by PFGE and represented a single strain. Despite this, one isolate was susceptible to carbapenems (MICs of ertapenem [ETP], IPM and meropenem [MEM] 2 mg/L, 0.5 mg/L and 0.25 mg/L, respectively), whereas E624 was highly-resistant (MICs >16 mg/L). Carbapenemase activity was detected in these 2 isolates by spectrophotometry, and a blaKPC allele was identified. This allele encoded a novel KPC variant, designated KPC-4 (GenBank AY700571), with 3 amino acid substitutions, Pro(103)Arg, Ser(174)Gly, and Val(239)Gly, in comparison with KPC-1. Two beta-lactamases were apparent by IEF, and were consistent with KPC and an AmpC enzyme. The third isolate was resistant to ETP (MIC > 16 mg/L), but less so to MEM (MIC 8 mg/L) and IPM (MIC 4 mg/L); it lacked a blaKPC allele, carbapenemase activity was not detected by spectrophotometry, and its susceptibility to carbapenems was restored in the presence of 100 mg/L cloxacillin. The two carbapenem-resistant isolates lacked a major outer membrane protein (OMP) present in the susceptible isolate.
Conclusions: *Enterobacter* E624 produced a novel carbapenemase, KPC-4. To our knowledge this is the first KPC enzyme detected outside of the United States. Production of KPC-4 alone did not confer high-level resistance to IPM, MEM and ETP; rather resistance needed concomitant OMP loss. In the absence of KPC-4, high-level resistance to ETP and moderate levels of resistance to IPM and MEM was associated with a combination of AmpC activity plus OMP loss.

Epidemiology of ESBL and MBL – I

P428

Enterobacter aerogenes and extended-spectrum beta-lactamases: prevalence and development of a routine ESBL testing protocol in a Belgian hospital

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Objectives: Extended-spectrum beta-lactamase (ESBL) producing strains of Enterobacteriaceae other than *E. coli* and *Klebsiella* spp. are a cause of increasing concern in Belgium. No NCCLS guidelines are available for ESBL detection in these species. The presence of membrane permeability alterations, high-level AmpC beta-lactamases and other beta-lactamases, often in combination, makes testing of beta-lactam antibiotics a complicated matter. Aim of our study was to establish the prevalence of ESBL producing strains of *Enterobacter aerogenes* in our hospital and to develop an optimal routine ESBL testing protocol.
Methods: A total of 97 nonrepetitive isolates of *Enterobacter aerogenes* were obtained from clinical specimens between November 2003 and July 2004. Identification and antimicrobial susceptibility testing were performed by Phoenix (BD). All strains were tested for ESBL production using a modified double-disk synergy test (MDDST) with three 3rd generation cefalosporines, aztreonam and cefepime.
Results: Of the 97 isolates, 67 (=69%) were flagged for ESBL by BDXpert. Only 26 of them could be confirmed as ESBL producer by MDDST. In the subgroup without BDXpert flagging (n = 30), 11 strains were also ESBL positive. In order to develop a reliable, cost-effective screening protocol, MIC values of cefotaxime, ceftazidime, aztreonam, cefepime, ciprofloxacin, co-trimoxazol and amikacin/gentamicin ratio were compared between ESBL positive and negative group.None of the isolates with a MIC for cefotaxime, ceftazidime and aztreonam ≤2 (group A, n = 12) harboured an ESBL. Isolates with a MIC for cefotaxime, ceftazidime or aztreonam >2 and cefepime >2 (group B1, n = 26), were all ESBL positive except four. In the subgroup with a MIC for cefepime ≤2 (group B2, n = 59), 44 strains were ESBL negative and 15 positive. The following protocol was worked out: group A = ESBL negative, group B1 = ESBL positive and group B2 = ESBL confirmation with MDDST. After application of this protocol, 59 strains (61%) remained to be tested.
Conclusion: EBSL flagging by BDXpert proved to be not very sensitive nor specific for *Enterobacter aerogenes*. By developing a screening protocol, the number strains requiring confirmation for ESBL could be safely reduced.

P429

Community-associated emergence of CTX-M and TEM extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in Belgium

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Objectives: Recent reports indicate that CTX-M-producing Enterobacteriaceae are emerging in Europe and threaten current treatment strategies. We study the molecular epidemiology of ESBL-producing *E. coli* (ESBL-EC) over the last 4 years in a University hospital in Brussels, Belgium.
Methods: In 2000–2003, 10,281 *E. coli* clinical isolates were tested by disk diffusion and screened for ESBL production by double-disk synergy test. ESBL were characterized by Iso-electric focusing, multiplex PCR for bla genes of the SHV, TEM and CTX-M family and DNA sequencing. ESBL-EC strains were tested for MIC of 12 antimicrobials by agar dilution, genotyped by pulsed-field gel electrophoresis (PFGE) and screened for class I and II integrase by PCR.
Results: The proportion of ESBL-EC increased from 21(0.92%) in 2000, 33 (1.25%) in 2001, 48 (1.85%) in 2002 to 64 (2.34%) in 2003 ($p < 0.001$). Strains were isolated from 79 male and 86 female with a mean age of 63 years (0–94). Cases were community-associated (i.e. <48 hours after admission) in 41%, of whom 11% had previous contact with our institution. ESBL-EC isolates harboured CTX-M+TEM (35%), TEM alone (44%), CTX-M alone (6%), CTX-M+SHV (2%) or other ESBL combination (10%). DNA sequencing revealed CTX-M1 or CTX-M2. Class I integrase was detected in 71% of strains. Isolates included screening isolates from rectal swabs (37%), clinical isolates from urinary tract (38%), respiratory tract (10%), blood (2%) or other sites (13%). Nosocomial isolates (59%) originated from all hospital wards. PFGE typing showed polyclonality with a majority of sporadic cases with unique genotypes and a few clusters of 2 to 6 patients sharing the same genotype without direct contact during hospital stay. ESBL-EC showed high frequency of co-resistance to ciprofloxacin (61%), cotrimoxazole (62%), gentamicin (37%) and tobramycin (55%). Only meropenem and amikacin remained active on >95% of these isolates. Strains harbouring CTX-M enzymes showed significantly higher MICs to ceftriaxone, cefepime, amikacin and tobramicin, compared to those with other enzymes.
Conclusion: Although not previously reported in Belgium, multiresistant CTX-M and/or TEM ESBL-EC were detected with increasing frequency at this tertiary care centre. ESBL-EC appeared present on admission in a third of cases and was typically associated with urinary tract infection in elderly patients. Further analysis of clinical presentation and risk factors for infections with ESBL-EC is needed.

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Dissemination of CTX-M-type beta-lactamases among clinical isolates of *E. coli* from geriatric long-term care and rehabilitation facilities in northern Italy

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Objectives: To investigate the presence of CTX-M type enzymes in extended-spectrum beta-lactamases (ESBLs) producing *E.coli* isolates from patients at different geriatric long-term care and rehabilitation facilities (LTCFs) in Northern Italy, where CTX-M producers were first detected in 2001.
Methods: 77 consecutive nonreplicate isolates of *E. coli* with an ESBL-positive phenotype were obtained from inpatients at different LCTFs in Northern Italy. ESBL production was screened by VITEK system (BioMérieux) and was confirmed by double-disk synergy test between tazobactam and oxyimino cephalosporins or aztreonam (ATM). Beta-lactamase production was investigated by analytical isoelectric focusing (IEF) coupled with a bioassay. The presence of beta-lactamase genes was investigated by colony blot hybridization. PCR amplification of blaTEM, blaSHV, blaCTX-M alleles was carried out with primers designed on highly conserved gene regions. Direct sequencing of PCR products was carried out. Conjugation experiments were performed in liquid medium. Pulsed-field gel electrophoresis (PFGE) profiles of genomic DNA digested with NotI were analysed by using the Bio-Rad Gene Path procedure.
Results: During the period March 2003–May 2004 at the Laboratory of Clinical Microbiology of 'Redaelli' LCTF in Milan, Italy, a total of 529 samples positive for *E.coli* were processed and 77 nonreplicate ESBL producers were identified by Vitek System. 61/77 isolates were characterized by higher levels of resistance to cefotaxime (CTX) than to ceftazidime (CAZ). IEF revealed multiple beta-lactamase bands including one enzyme with pI 8.4 that, in a bioassay, was more active on CTX, ATM than on CAZ. 43 isolates produced both TEM-1 and CTX-M-type enzymes, 14 strains expressed only CTX-M-type beta-lactamase while in 4 cases were found blaCTX-M with blaTEM and blaSHV genes.The remainders (n = 16), characterized by high levels of resistance to both CTX and CAZ, produced TEM-1 and SHV-5 enzymes (n = 1) and TEM type ESBLs (n = 15).All ESBLs producers retained susceptibility to piperacillin-tazobactam. The ESBL determinants were transferable by conjugation. Comparison of PFGE profiles of ESBL producers revealed clonal heterogeneity.
Conclusions: Our work confirms the emergence of CTX-M-type enzymes and their spread in Northern Italy also in long-term care and rehabilitation facilities that may be an important reservoir of ESBL producing *E.coli*.

P431

Occurrence of metallo-beta-lactamase VIM-2 in *Pseudomonas aeruginosa* clinical isolates resistant to carbapenems in a hospital in central Portugal

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Objectives: The worldwide spread of acquired metallo-beta-lactamases (MBLs) in Gram negative bacilli has become a great concern. Based on amino acid sequence homology, these MBLs have been classified into three major types: IMP, VIM and SPM enzymes. The two first subtypes have been identified in clinical isolates of *Pseudomonas aeruginosa* (PA) in many parts of the world, especially Asia and Europe, whereas SPM has been reported only in Brazil.The aim of this study was to determine the occurrence of these enzymes in Portuguese clinical isolates of PA imipenem (IP) resistant.
Methods: Nonduplicate isolates of PA (n = 273) were collected from hospitalized patients in Centro Hospitalar de Coimbra, a central hospital from the Centre of Portugal during one year (April 2003–April 2004). Bacterial identification and susceptibility were performed by MicroScan WalkAway (Dadebehering) system. In selected isolates, susceptibilities were also determined by disk diffusion method on Mueller–Hinton agar, and susceptibilities to Piperacillin, Piperacillin plus Tazobactam, Aztreonam (AZT), Ceftazidime (CAZ), IP, Meropenem (MP), Amikacin, Tobramicin, Gentamicin, and Ciprofloxacin were

guideline by NCCLS. Double-disk synergy test was used for screening MBLs, and they were analysed by PCR for blaIMP, blaVIM and blaSPM using primers specifics for IMP, VIM, and SPM-1 enzymes, and DNA sequencing.
Results: Among the 273 nosocomial isolates, fifty three were resistant to IP (19.4%) and thirty two to MP (11.7%). These isolates resistant to carbapenems were selected for this study. Twenty eight carbapenem resistant were double disk synergy test positive (52.8%). Among these, PCR experiments and DNA sequencing revealed that the VIM-2 determinant was present in seventeen strains, but none of them presented blaIMP or blaSPM. The majority of these isolates were susceptible to AZT (94.1%), resistance to CAZ was 35.3%, and the aminoglycosides and CIP presented the same resistance (52.9%).
Conclusions: The emergence of MBLs producing bacilli that are resistant to carbapenems is becoming a severe therapeutic problem. In general, they have a common multidrug-resistant phenotype that not only included carbapenems, but also last generation cephalosporins, aminoglycosides and fluoroquinolones. Appropriate therapeutic protocols and a regular screening/monitoring system should be established to prevent the wider spread of this worrisome resistance determinant.

P432

Prevalence and characterisation of extended-spectrum beta-lactamases produced in Enterobacteriaceae isolated from patients hospitalised in a Belgian hospital

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Objectives: To determine the prevalence and charcterize ESBLs produced in Enterobacteriaceae isolated from patients hospitalized in our institution.
Methods: All Enterobacteriaceae strains were screened prospectively between January 2003 and September 2004 for ESBL production, on the basis of a positive double-disk synergy test or positive ceftazidime and cefotaxime clavulanic combination discs tests. Only one strain of each species was evaluated per patient. Isoelectrofocusing (IEF) and PCR for blaTEM, blaSHV and blaCTX-M genes were used to confirm ESBL-production. When IEF and PCR indicated concordant results, final identification of ESBLs was obtained by sequence analysis of PCR products. Pulsed Field Gel Electrophoresis (PFGE) was used to delineate clonal relationships between strains of the same species recovered from different patients and showing identical ESBL profiles.
Results: Among the 1737 Enterobacteriaceae isolated during the study period, 80 (7%) were detected as ESBL-producers [*Enterobacter aerogenes* (n = 51), *Escherichia coli* (n = 16), *Citrobacter freundii* (n = 5), *Klebsiella pneumoniae* (n = 4), *Klebsiella oxytoca* (n = 2), *Enterobacter cloacae*, *Proteus mirabilis* (n = 1 each)]. *E. aerogenes* and *E. coli* represented 64% and 20% of ESBL-producing strains, respectively. Thirty-nine *Enterobacter aerogenes* strains which had been previously characterized as TEM-24 producers during a national surveillance study performed in 2003 were not analysed. IEF and PCR confirmed the presence of ESBLs in 37 (90%) of the remaining 41 strains, which were identified based on their pI as TEM-24 (n = 21), TEM-3 (n = 5), CTX-M-9 group (n = 4), TEM-4 (n = 3), SHV-4, CTX-M-1 and CTX-M-2 groups (n = 2 each), TEM-12 and TEM-21 (n = 1 each). PCR-sequencing analysis is ongoing and already confirmed CTX-M-15, -2 and -9 ESBLs. Four strains produced more than one ESBL. PFGE analysis revealed the dissemination of 2 *K. oxytoca*, *K. pneumoniae* and *E. cloacae* strains between 5 patients and the absence of any clonal relationship between strains of other species.
Conclusions: This study illustrates the diversity of type of ESBLs produced by a wide range of species among Enterobacteriaceae in our institution, including the emergence of CTX-M group beta-lactamases and the rising proportion of ESBL-producing *E. coli* strains. The large clonal variability between the majority of strains was also delineated.

P433

The early phase epidemiology of CTX-M type extended spectrum beta lactamase-producing *Escherichia coli* among hospitalised patients in Belfast

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Objective: To describe the demographics, prevalence of risk factors and outcomes among the first adult hospitalised patients who had colonisation or infection and with ESBL-producing *E. coli* in a university hospital in Belfast.
Methods: Retrospective casenote review of patients who were culture-positive between 1st January 2004 and 31st May 2004.
Results: Of 53 patients identified, 45 casenotes were available for review. The mean age was 73.1 (+14.6) years and 67% of patients were female. Most (56%) patients were in a medicine or elderly care unit at the time of the positive culture. The most frequently recorded comorbidity was haemodialysis-dependence. Many (51%) patients had an indwelling urinary catheter and prior to isolation of the ESBL-producing *E. coli*, 24.4% of patients had a positive culture for MRSA.The majority (57.8%) of patients had an isolate from urine only; 17.8% had positive blood cultures. The organism had been acquired in hospital in 69% of instances; of these, the mean duration of inpatient stay prior to onset of infection was 45.2 (+52.9) days. Overall, 81% of evaluable patients had received antibiotic therapy within 30 days prior to the first isolate; the mean number of antibiotic-days per patient in this time was 13.9 (range 0–48). The most frequently consumed class of antibiotic was β-lactam/β-lactamase inhibitor combinations.Of the 45 episodes, 35 (77.8%) were associated with clinically determined infections; of these, 74.2% were successfully treated. Overall, the crude 30-day mortality among patients with such infection was 34.3%; the attributable mortality was estimated at 20%. Among patients with bacteraemia, the attributable mortality was 25%.

P434

Extended spectrum beta-lactamase production of enteroaggregative *Escherichia coli* strains isolated in the United Arab Emirates

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Objectives: Extended spectrum beta-lactamase (ESBL) production was investigated among enteroaggregative *Escherichia coli* (EAEC) strains isolated in the United Arab Emirates.
Methods: Thirty six strains randomly selected from isolates identified as EAEC by a pCVD432 specific PCR in diarrhoeal stool samples of children (24 isolates), and those of adults (12 strains) were studied. The isolates were serotyped and their antibiotic sensitivity was tested by disc diffusion. Strains non-susceptible to ceftazidime and/or ceftriaxone were selected for MIC determination of ceftriaxone, ceftazidime, cefotaxime, cefepime and aztreonam by E-test. ESBL-production was

proven by ceftazidime and cefotaxime E-test with and without clavulanic acid. The type of beta-lactamase gene present was tested with TEM-, CTX-M-, and SHV-specific PCRs. The cefotaxim resistant phenotype was transferred to *E. coli* J53 by conjugation. The similarity between ESBL producing strains was determined by pulsed field gel electrophoresis (PFGE) following XbaI digestion of the genomic DNA.

Results: Of the 36 isolates tested 5 exhibited non-susceptibility to 3rd generation cephalosporins and were shown to produce ESBL, indeed by having an over 8 times higher MIC value against ceftazidime and cefotaxime without, than with clavulanic acid. From one isolate the resistance to cefotaxime was successfully transferred by conjugation to *E. coli* J53. In all the five wild type isolates, as well as in the transconjugant the MIC of cefotaxime and ceftriaxone exceeded that of ceftazidime. All the five isolates and the transconjugant gave a positive PCR reaction with the CTX-M -, as well as with the TEM -specific primers. Beyond beta-lactams, all the 5 isolates were non-susceptible to chloramphenicol, 4 to tetracycline, trimethoprim-sulphamethoxasole, and nalidixic acid, 2 to ciprofloxacin and one to gentamicin, respectively. The conjugal transfer of cefotaxime resistance was not accompanied by the co-transfer of resistance to any other antibiotics tested but the beta-lactams. Two of the 5 ESBL producing strains exhibited the same serotype (O153:HNT), while the others were ONT:H21, O90:HNT, O25:H4. Macrorestriction analysis by PFGE revealed that the similarity coefficient never exceeded 75% when comparing the isolates.

Conclusion: Multiresistant EAEC producing plasmid coded ESBL, most likely cefotaximase, are common among strains isolated in the Middle East.

P435

Diverse extended spectrum beta-lactamases in minor serotypes of foodborne *Salmonella enterica* subsp. enterica from Greece

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Objectives: Sporadic foodborne salmonellae with extended-spectrum beta-lactamases (ESBLs), causing resistance to 'third generation' cephalosporins, were first isolated in Greece from human isolates in 1998–99. The present study aimed to examine the extent of the phenomenon amongst both human and animal isolates during 2000–02, and investigate the plasmid vectors as well as the chromosomal background of the isolates harbouring ESBL-encoding genes.

Methods: The antimicrobial susceptibility of 727 (23% of the total 3142) randomly selected human isolates from 2000–02 and 94 (8% of the total 1148) food, animal, and animal feed isolates from 2000–01 was assessed by disk diffusion and interpreted according to NCLLS recommendations. Chromosomal fingerprints of representative isolates were obtained by pulsed field gel electrophoresis (PFGE) after XbaI digestion of genomic DNA and compared using the GelCompar software; patterns differing by up to four DNA fragments were assigned to (subtypes of) the same type. Plasmid transfer was tested by conjugation. ESBLs were characterised by isoelectric focusing, and the corresponding genes identified by PCR and nucleotide sequencing.

Results: Three ESBL-producing non-typhoid salmonella strains of serotypes Brandenburg, Blockley, and Hadar were isolated from humans in Greece during 2000–2002. Salmonella Brandenburg harboured an SHV-5-encoding plasmid similar to those found in nosocomial enterobacteria. *S. Blockley* and *S. Hadar* both produced TEM-52 encoded by an identical plasmid. An additional *S. Virchow* strain producing a plasmid-mediated CTX-M-32 was isolated from two chicken meat isolates from a production unit. All ESBL-encoding plasmids were multidrug-resistant and self-transferable. With the exception of Blockley, PFGE typing showed that these ESBL genes had been acquired by *Salmonella enterica* clones currently common in Greece.

Conclusions: While still sporadic, ESBL enzymes are nevertheless found amongst foodborne salmonellae from both humans and animals in Greece. The identity of responsible genes and plasmids highlights the intricate interplay between gene 'abduction' from the environment (for example, by food animals), gene incorporation into multidrug-resistant 'nosocomial' plasmids, and transmission via dominant bacterial clones.

P436

Evaluation of antibiotic containing agars for screening patients for ESBL carriage in an ITU unit of a Scottish hospital

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Objectives: The detection of ESBL producing organisms is important because conventional antimicrobial susceptibility testing can often miss them. This is important in ITU units where it is essential that patients receive the correct antimicrobial regime. This study evaluated the use of two screening agars to facilitate the detection of ESBL producing organisms in the ITU unit of our hospital.

Methods: The antibiotics Cefotaxime (CTX) and Ceftazidine (CAZ) were separately added to MacConkey agar (Oxoid, England) at concentrations of 0.5 and 1.0 mg/l. A total of 39 known positive ESBL producers and 50 known negative organisms were used as positive and negative controls. Over a 6-month period 630 patient samples were tested. The samples consisted of 200 groin swabs, 192 urines, 122 endotracheal aspirates, 106 wound swabs and 10 faeces. These were tested against the different concentrations of CTX and CAZ along with a MacConkey containing no antibiotics to act as a control plate. Any growth was identified and antimicrobial sensitivities were carried out using the Vitek 2 automated system (bioMerieux, France). The detection of ESBL production was carried out by double disc synergy (DDS) and both CTX and CAZ combination disc methods (Oxoid, England). AMP C production was also looked for by Cefoxitin/CTX disc antagonism and the modified Hodge test.

Results: Negative controls did not grow on the screening agars. For the positive controls 100% were confirmed by DDS and 72% by combination disc methods. A total of 14 ESBLs were detected from the clinical samples with 12 confirmed by DDS and 14 by CAZ combination discs, 2 were negative with CTX combination discs. There was no difference between the two antibiotics or the two concentrations tested. The screening agars had a sensitivity of 100% (CI 78.5–100) and a specificity of 82.8% (CI 79.6–85.6) for the isolation of ESBL producers. 50% of the clinical ESBLs were isolated from groin swabs. In addition 3 AMP C isolates were detected by the modified Hodge test but only two by Cefoxitin/CTX disc antagonism.

Conclusions: In order to detect ESBL producing organisms the combined use of CTX and CAZ screening agars are suggested. This would also allow the detection of CTX-M enzyme producers. MRSA screens are a natural regime in ICUs and usually include a groin swab, which could easily be used with the 2 ESBL screening plates. This could form an adjunct to hospital infection control measures.

P437

Impact of extended-spectrum beta-lactamases produced by Gram-negative bacteria on efficacy of cephalosporins and quinolones

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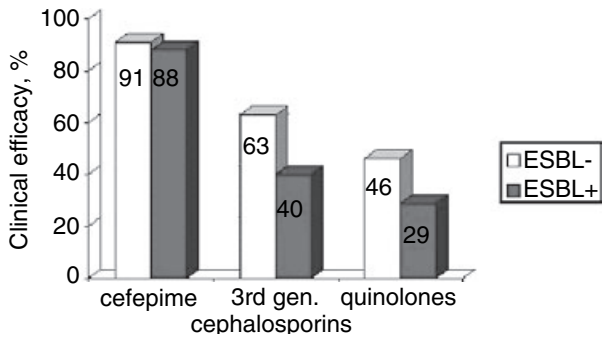
Objective: Assessment of efficacy of 3rd and 4th generation cephalosporins and quinolones in nosocomial pneumonia caused by gram-negative bacteria resistant to multiple antibiotics depending on their capacity to produce extended-spectrum beta-lactamases (ESBL).
Methods: We studied the cases of nosocomial pneumonia caused by gram-negative micro-organisms at an emergency hospital. The patients included in the study were on 3rd or 4th generation cephalosporins or quinolones therapy as a initial empiric therapy for nosocomial pneumonia. The strains which supposedly produced ESBL were tested for ESBL production in compliance of NCCLS criteria. To identify the type of ESBL, PCR and sequencing techniques were used.
Results: During the period of 2001–2003, sixty-two cases of nosocomial pneumonia were diag-nosed, resulting from infection with Enterobacteriaceae strains or non-fermenting microorganisms suspicious of producing ESBL. As the initial monotherapy, 25 patients were taking cefepime, 13 individuals – 3rd generation cephalosporins, while 20 patients were on quinolones. The etiologic factors of pneumonia were *Klebsiella* spp. (22 strains), *E.coli* (14), *P. aeruginosa* (15), *Acinetobacter* spp. (11). The ESBL-producing capacity has been confirmed in twenty-five Enterobacteriaceae strains by eighteen *Klebsiella* spp. strains and seven *E. coli* strains that produced ESBL. In contrast, none of non-fermenting microorganisms demonstrated the capacity to produce ESBL. The testing of strains that produced ESBL helped find the prevalence of ESBL types as follows (totally 30 types): TEM-3, SHV-15, CTX-UNI-6, CTX-A-3, CTX-C-3. The clinical efficacy of the antibiotics under investigation against the microorganisms, both with and without the ESBL-producing capacity, is shown in the Figure. The eradication rate of ESBL-producers was twice as higher in patients who were on cefepime as compared with those in patients taking 3rd generation cephalosporins or quinolones, 88.4 and 43.0%, respectively.



Conclusions: The efficacy of cefepime in nosocomial pneumonia is high and does not depend on the microorganism's ESBL-producing capacity. The effectiveness of 3rd generation cephalosporins proved satisfactory in case of lack of the bacterial ESBL-producing capacity, but it tended to be 1.5-fold lower if it was due to a ESBL-producer. The same was true in individuals on quinolones. Thus, the empiric therapy of nosocomial pneumonia was the most effective with cefepime.

P438

Prevalence of extend-spectrum beta-lactamase producing enterobacteria in different samples of patients hospitalised in a Serbian clinical centre

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Objective: It has been observed that Enterobacteriaceae producing ESBL are becoming increasingly widespread as the result of use of cefalosorins. ESBL producers provide a therapeutic chelange. The objective of the study was to establish the prevalence as well as in vitro sensitivity of ESBL-producing Enterobacteriaceae isolated from different clinical samples to antimicrobial drugs.
Methods: In the period January–June 2004, 1974 strains of gram negative bacilles were isolated from different clinical samples (wound swabs, blood cultures, urine, liquor) obtained from patients hospitalized in several CCS institutes. Identification and sensitivity to antibiotics were carried out by standard microbiological methods (Kirby–Bauer disc-diffusion). ESBL positive strains were detected by double disc synergy test by Jarlier and all.
Results: During the observation period, 1026 strains of enterobacteria (51.9%) and 948 (48.1%) of non-fermentative bacilles were isolated. A total of 428 ESBL positive strains (21.6%) were detected, out of which: *E. coli* – 98 (22.9%), *Klebsiella* spp. – 90 (21%), *Enterobacter* spp. – 78 (18.2%), *Proteus mirabilis* – 88 (20.6%), *Serratia* spp. – 53 (12.4%), *Rettgerela rettgeri* – 7 (1.6%), *Proteus vulgaris* - 3 (0.7%), *Citrobacter freundii* – 2 (0.5%), as well as 9 (2.1%) strains of non-fermentative ESBL positive bacilles. These strains were isolated from wounds – soft tissues (77.8%), urine (17.2%), blood cultures (2.6%), liquor (0.2%) and respiratory tract (2.2%). All tested ESBL positive strains of enterobacteria were sensitive to meropenem, while 0.7% of strains were resistant to imipenem (3 strains of *Serratia* spp.). Co-resistance of isolated ESBL positive strains to aminoglycosides and fluoroquinolones was frequent (89% of them were resistant to gentamicin, 58% to amikacin and 56.8% to ciprofloxacin).
Conclusion: All strains were sensitive to meropenem and exhibited varying degree of resistance to aminoglycosides and fluoroquinolones. High percentage of ESBL-producing strains of enterobacteria suggests the need of introduction of ESBL screening on regular basis. Further studies related to typing and epidemiology of ESBL strains are necessary.

P439

Epidemiology and infection control of ESBL-producing *Klebsiella pneumoniae* strains caused 5 outbreaks in a Hungarian neonatal intensive care unit during the years 2001–2004

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Objectives: The aim of this study was to perform a prospective survey on prevalence and epidemiological relatedness among ESBL KP strains isolated in a Hungarian NICU during a four-year period.
Methods: All patients admitted to NICU during the four-year period were screened for colonisation with ESBL-producing *K. pneumoniae*. Confirmation of ESBL production was done by Etest, double-disc diffusion and agar dilution methods according to NCCLS. We performed PCR analysis for beta-lactamase detection using specific primers, phage typing, resistance transfer, plasmid profile analysis and plasmid REA, genomic

fingerprinting by ERIC-PCR and PFGE to determine the genetic relatedness of strains.
Results: Among 210 *K. pneumoniae* isolates recovered from NICU between 2001–2004, 119 were ESBL-producers, but none of these were isolated in 2004. 107 ESBL isolates were recovered from five outbreak periods. The outbreak strains showed multidrug-resistance with similar antibiotypes. 87 of 107 isolates belonged to six different phage types, but 20 were non typable. PFGE analysis revealed six different genetic clones at 90% similarity level. All isolates harboured plasmids ranging from 2,6 to 203 kb: 14 different plasmid profiles were identified. The same plasmid of 94 kb was obtained from the transconjugants of six outbreak clones by plasmid restriction analysis. The sequence analysis of the SHV PCR products showed that SHV-5 was presented in all outbreak clones and their transconjugants.
Conclusions: This prospective study confirms that the same ESBL coding plasmid persisted from 2001–2003 in this hospital ward, while six different outbreak clones were revealed during the given period. After the change of the antibiotic treatment regiment at the beginning of 2004 there were no more ESBL-producers isolated.

P440

A nosocomial outbreak of CTX-M-15 producing *E. coli* in Norway

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Objective: The first nosocomial outbreak of ESBL-producing *E. coli* in Norway involving 8 elderly patients is described.
Material and methods: Four ESBL-producing *E. coli* strains were isolated from different patients at the Department of Pulmonary Medicine, Regional Hospital of Rogaland august 2004. Additional 4 urinary tract *E. coli* isolates with an ESBL production phenotype were detected during the following month from out-patients that had been hospitalised in the same department in august 2004. The patients were between 66 and 93 years; 6 men and 2 women. The 8 isolates were recovered from urine samples (N = 5), blood, sputum, lung and kidney tissue at autopsy. Antimicrobial susceptibilities were tested by Etest and agar disk diffusion and interpretation was according to the Norwegian Working Group for Antibiotics. Molecular typing was performed by TEM-, SHV, CTX-M, and ISEcp1 PCRs, sequence analysis of PCR amplicons and XbaI-PFGE.
Results: All strains expressed a typical ESBL-cefotaximase profile (cefotaxime MIC > ceftazidime MIC) and clavulanic acid synergy. Seven strains displayed high-level resistance towards all penicillins and cephalosporines, while one strain were susceptible to ceftazidime. Multiple resistances to other antimicrobial agents including gentamicin, nitrofurantoin, trimethoprim-sulfamethoxazole, ciprofloxacin and piperacillin-tazobactam were detected. All strains were susceptible to carbapenems. Molecular characterization revealed a CTX-M-15 in 6 strains, CTX-M-3 in one strain, and one strain to be analysed. The six CTX-M-15 strains had indistinguishable XbaI-PFGE patterns and positive ISEcp1-element PCRs consistent with a clonal relationship. Three of the patients died during this period and the potential impact of the actual infection is currently being assessed. Infection control measurements were implemented and additional ESBL-producing *E. coli* has not been recovered. Preliminary environmental samples and additional investigations have not discerned any apparent source for this outbreak
Conclusions: (i) The outbreak illustrates the epidemic potential of this particular multiple-antibiotic resistant CTX-M-15 producing *E. coli* strain also in a country with a low prevalence of antimicrobial resistance. (ii) The clonal relationship to epidemic CTX-M-15 producing *E. coli* strains in other countries has to be investigated to identify potential common reservoirs and lines of resistance transmission as a basis for intervention.

P441

Escherichia coli and *Klebsiella pneumoniae* clinical isolates from intensive care unit bearing CTX-M-15 extended-spectrum beta-lactamase

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Objectives: Five strains of *Escherichia coli* and one of *Klebsiella pneumoniae* with a phenotype suggestive of an ESBL and more resistant to cefotaxime than to ceftazidime were isolated from bronchial aspirates of six different patients admitted to the intensive care unit of the Verona University Hospital between 13 April and 15 July 2004. The strains were investigated for the presence of CTX-M-type enzymes.
Methods: Antimicrobial susceptibility testing was performed by both diffusion and microdilution and interpreted according to the latest NCCLS documents. The presence of an ESBL was preliminarily investigated by means of a double-disk synergy test. Isoelectrofocusing (IEF), PCR and sequencing were carried out according to standard procedures. Plasmid extraction was performed by alkaline lysis. Conjugation was used to ascertain whether the CTX-M enzyme was transferable.
Results: MICs were >128 mg/L for both aztreonam and cefotaxime and 64 mg/L for ceftazidime. The presence of an ESBL was suggested by the synergies detected between clavulanic acid and third-generation cephalosporins (3-GCs) in the disk-diffusion assay. Spectrophotometric analysis of crude sonic extracts confirmed that 3-GCs were hydrolysed with fast kinetics. IEF visualised two beta-lactamases in all *E. coli* strains with isoelectric points (pI) of 7.4 and 8.9, respectively. The latter value matched that previously reported for CTX-M-15. Two bands (namely 7.6 and 8.9) were also visualised in the *K. pneumoniae* strain. The 8.9 band of *E.coli* and both bands of *K.pneumoniae* were inhibited by clavulanic acid.A polymerase chain reaction (PCR) was performed using primers specific for the bla(SHV), bla (TEM) and bla (CTX-M) genes. For the *E. coli* strains we obtained PCR products of about 950 bp only with the bla (CTX-M) primers, which - after sequencing - were found to code for CTX-M-15. CTX-M-15 was indeed the only enzyme transferred to *E. coli* J53 by conjugation. Besides amplifying for CTX-M-15, the *K.pneumoniae* strain also amplified for an SHV enzyme that – after sequencing – was found to be SHV-1. Only CTX-M-15 was transferable by conjugation to *E. coli* J53.
Conclusions: is responsible for 3-GC-resistant Enterobacteriaceae outbreaks throughout Europe and also made its appearance in Italy. The finding of CTX-M-15 in the ICU of the Verona Hospital is a disquieting addition to this picture.

P442

Detection of extended-spectrum beta-lactamases, including the CTX-M-type enzymes, in *Escherichia coli* and *Klebsiella pneumoniae* using VITEK 2 System and the Advanced Expert System

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From November 2003 until February 2004, 42 isolates of *Escherichia coli* and *Klebsiella pneumoniae* were recovered from 45 inpatients of different wards of the University Hospitals of

Coimbra, Portugal, identified as extended-spectrum beta-lactamases (ESBL) producers by the automatic VITEK 2 System and the Advanced Expert System (VITEK 2 AES) (BioMérieux, Marcy L'Étoile, France).
Objective: The main objective of this study was to investigate if the VITEK 2 ASE could accurately detect ESBL in those species, comparing the final data report with the results of phenotypic and genotypic based methods. A special approach was given to the new emergent CTX-M-type ESBL.
Methods: The antibiotic susceptibilities were determined by VITEK 2 and the susceptibility reports were edited after interpretation by AES of the inferred resistance phenotype. ESBL were also detected by Etest® ESBL with Ceftazidime/Ceftazidime + Clavulanic ac. and Cefotaxime/Cefotaxime + Clavulanic ac. strips, according manufacturer's instructions, and by using the double-disc diffusion method (DDM) with amoxicillin/clavulunate, aztreonam, cefepime, CAZ and CTX. PCR was used to screen for CTX-M-type enzymes with specific primers in isolates resistant to CTX.
Results: VITEK 2 determined that: for CAZ, 35 isolates were resistant, 5 were intermediate and 2 isolates were susceptible; while for CTX, 23 isolates were resistant, 2 were classified as intermediate and 17 isolates were susceptible. AES edited these data as resistance phenotypes, suggesting the production of ESBL. Using the Etest, 39 isolates produced ESBL. The results for 3 isolates were indeterminate, according the manufacturer's guidelines. However, two of them showed synergism by the DDM suggestive of the presence of an ESBL. The other isolate showed a band of ca550 bp with specific primers for CTX-M-type enzyme, after PCR. CTX-M-type enzymes were detected in 24 *E. coli* and 6 *K. pneumoniae*. It is noteworthy that VITEK 2 classified 6 *K. pneumonia* and 2 *E. coli* isolates as susceptible to CTX, but AES interpreted as being resistant, which is in accordance with the PCR results.
Conclusion: This study demonstrated the capacity of VITEK 2 AES to detected and interpreted resistance mechanisms, inferring with good accuracy the production of ESBL in *E. coli* and *K. pneumoniae*, and including the most recent emergent CTX-M-enzymes, even when the VITEK 2 shows a susceptible MIC for CTX.

P443

Outbreak of *Klebsiella pneumoniae* producing SHV-12 and CMY-2 in NICU of a Korean tertiary care hospital

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Objectives: Extended-spectrum beta-lactamases (ESBLs)-producing Gram-negative bacilli are usually resistant to the 3rd and 4th cephalosporins, but susceptible to cephamycins. Recently, simultaneous ESBL- and plasmid-mediated AmpC beta-lactamase (PABL)-producing strains, which were also resistant to cefoxitin, were reported. We report the outbreak of SHV-12- and CMY-2-producing *K. pneumoniae* in neurological intensive care unit (NICU) patients.
Methods: *K. pneumoniae* were isolated from patients at a Korean tertiary-care hospital between March to June, 2003. Detection for ESBL-producing strains was performed using double disk synergy test (DDST) with cefotaxime, ceftazidime, aztreonam, cefepime and amoxicillin-clavulanic disk. The PABL genes were detected by multiplex PCR designed by Perez–Perez and Hanson. The transferability of resistance was tested by the agar mating method using a sodium azide-resistant *E. coli* J53 recipient. The isoelectric points of beta-lactamases were determined. Nucleotide sequencings were carried out by the dideoxy-chain termination method with an automatic DNA sequencer (ABI 3700, Perkin-Elmer, Foster City, Ca., USA). Xba I-digested genomic DNA bands of the isolates were separated by PFGE.
Results: Among the 114 ESBL-producing *K. pneumoniae*, 9 isolates showed indistinct results of DDST. However, blaCMY-2 and blaSHV-12 alleles were detected simultaneously by PCR. The resistance was transferred in all 9 strains. The pIs of beta-lactamase were 5.4 and >8.0. The PFGE band patterns of 9 isolates were identical. All of them were isolated from patients, who were admitted in NICU.
Conclusion: It is worrisome that the emergence and spread of simultaneous ESBL- and PABL-producing *K. pneumoniae* isolates. Further modifications of method to detect them would be required, because of the false-negative results in DDST. More effective infection control measures would be required to control the spread of the resistant isolates.

P444

An outbreak of ESBL producing *Klebsiella pneumoniae* in neonatal intensive care unit of a university hospital: the long-term gut carriage among infection-free patients

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Objectives: An outbreak of ESBL producing *K. pneumoniae* was investigated.
Methods: An outbreak of ESBL producing *Klebsiella* was detected in a neonatal intensive care unit between 12th of September and 6th of October. Surveillance was carried out by obtaining twice a week of stool cultures from the neonates hospitalized in the ICU up to 7 weeks after the first case. The DNA relatedness of the strains was investigated by ERIC-PCR patterns. ESBL was detected by double-disk synergy test. Later the existence of TEM, CTXM and SHV-type beta-lactamases were investigated by PCR tests.
Results: During the first 25 days period of the investigation, five neonates were infected with an ESBL producing *K. pneumoniae*. Among these two were died. The last isolate from an infected patient was on the 6th of October. However, during the following 22 days neonates those were hospitalized in the same unit were continued to excrete an ESBL producing *K. pneumoniae* by their stools. A total of 24 *K. pneumonia* have been isolated from clinical materials and the stool samples of five infected and five non-infected patients. ERIC-PCR confirmed the clonal relatedness of these strains. SHV 5 gene was detected by PCR.
Conclusion: This study showed that outbreak strains may continue to be excreted by the stool of non-infected neonates even after the outbreak itself. This data reminds the significance of surveillance for resistant bacteria in stool samples in ICUs.

P445

Persistent import and transient nosocomial establishment of ESBL-producing *Klebsiella pneumoniae* causing bloodstream infection in a neonatal intensive care unit

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Methods: We conducted a 6-year prospective study of bacteremic episodes from ESBL-KP in neonates hospitalized in NICU

(30 beds) from Jan 1999 through Nov 2004, as part of a hospital infection control programme. An epidemiological questionnaire was completed for each patient with ESBL-KP bacteraemia. Blood specimens were incubated in the automated system BacT/Alert (bioMerieux), identification of microorganisms was performed by the API system, susceptibility to antimicrobials by a disc-diffusion method and extended-spectrum β-lactamase expression were detected according to NCCLS criteria. Molecular typing of isolates was performed using PFGE.

Results: A total of 17 episodes of ESBL-KP bacteraemia occurred in 16 patients (male/female ratio:1). The distribution of cases for the years 1999 through 2004 was: 0, 2, 3, 9, 3, 0, respectively. Mean gestational age was 34 weeks (range: 25–40) and mean birth weight was 2063 g (range: 600–4030). The following risk factors were identified: total parenteral nutrition 94%, mechanical ventilation 69% and central venus catheter 37%. All babies had severe underlying disease. Seven out of 17 bacteremic episodes were due to ESBL-KP isolates which had colonized the neonates during their stay in the referring maternity hospitals (MH-1, MH-2), while the remaining ten were NICU-acquired. Mean hospital stay before the bacteremic episode was 40 days (range: 6–218) for NICU-acquired cases. All cases were treated with imipenem. Two patients died of septic shock, while in four, death was probably due to the underlying disease. PFGE analysis revealed three clones: A from MH-I, B from MH-II and C responsible for two NICU-acquired bloodstream infections. Five NICU-acquired infections that occurred from Sep 2001 to Dec 2002 were due to the imported clone A. This clone has persisted in MH-I since 1997 (previous published data). No case was referred from general or maternity hospitals outside of Athens.

Conclusions: We detected sporadic cases as well as an outbreak of ESBL-KP bacteraemia in our NICU. Most cases occurred in neonates with severe underlying disease and risk factors. Maternity hospitals MH-1 and MH-2 were the sources of two ESBL-KP clones, one of which was transiently established as a nosocomial pathogen in our NICU. Reinforcement of infection control measures, especially handwashing stopped the outbreak. No case was detected during 2004.

P446

A new outbreak of VIM-1-producing *Pseudomonas aeruginosa* in the same site as the first VIM description

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Objectives: *Pseudomonas aeruginosa* strain PSE VR143/97 was responsible for an outbreak in the Verona University Hospital in 1997, this being the first world occurrence of a VIM-type enzyme. Between February 2003 and July 2004, 14 strains of *P. aeruginosa* highly resistant to carbapenems, 12 from the ICU and two from the hematology unit, were isolated from the same hospital. All strains were investigated for their possible production of metallo-beta-lactamase.

Methods: Antimicrobial susceptibility testing was performed by both diffusion and microdilution and interpreted according to the latest NCCLS documents. The presence of carbapenemase was screened by means of the MBL-etest, and further investigated spectrophotometrically by following the crude sonic extract hydrolysis of imipenem and its inhibition by EDTA. PCRs were performed with either bla(VIM) or bla(IMP) primers. PFGE, isoelectrofocusing, and sequencing were carried out according to standard procedures. The integron carrying the bla gene was also studied by both PCR and sequencing.

Results: All strains proved resistant to all beta-lactams tested, with the sole exception of aztreonam, as well as to ciprofloxacin, gentamicin, amikacin, and tobramycin. The MBL-etest was suggestive of an MBL in all cases. Imipenem hydrolysis confirmed that imipenem was hydrolysed at a rate of 1×10^{-8} mol/min/mg, whereas in the presence of EDTA 2 mM the kinetics was 1×10^{-9} mol/min/mg. The isoelectrofocusing showed a band at 5.3, i.e. the same level as VIM-1 from the index strain PSE VR143/97. PCRs performed with either bla(VIM) or bla(IMP) primers yielded positive results only with the bla(VIM) primers; a specific PCR performed with the bla(VIM-1) primers yielded a product of about 800 bp which, after sequencing, was found to code for VIM-1. The strains showed PFGE patterns that were either identical or correlated to one another but were different from the pattern of index strain PSE VR143/97 which was responsible for the outbreak in 1997 in the same hospital.

Conclusions: All strains carried a VIM-1 enzyme, and seemed to be derived from the same clone. They sharply differed from the index strain responsible for the 1997 outbreak. These results enabled us to exclude the persistence of PSE VR143/97 in the two wards of the Verona University Hospital, but showed that the existing infection control procedures were not sufficient to avoid the diffusion and long-term persistence of a new VIM-1-bearing *P. aeruginosa*.

P447

The antimicrobial susceptibility pattern of extended-spectrum beta-lactamase producing *Escherichia coli* in Surabaya, Indonesia

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Objectives: To study the susceptibility pattern of ESBL (Extended spectrum beta lactamase) producing *Escherichia coli* from clinical isolates against commonly used antimicrobial agents.

Methods: The study was conducted in Dr. Soetomo Hospital with 1500 beds capacity in Surabaya, Indonesia. All clinical isolates *Escherichia coli* resistant to third generation cephalosporine in the routine examination were included in this study. The susceptibility test was performed by disk diffusion test (NCCLS, 2002). The phenotypic confirmatory test for ESBL was performed by disk diffusion test using ceftazidime disk (CAZ) and combination ceftazidime plus clavulanic acid (CD). A 5 millimeter in zone around CD compare to CAZ, was used to identify ESBL producer. These isolates were further tested against Ceftazidime (CAZ), Cefotaxim (CTX), Ceftriaxone (CRO), Ciprofloxacin (CIP), Cefixim (CFM), Cefepim (FEP), Meropenem (MEM) and Cefoperazone-sulbactam (SCF).

Results: During January to September 2004, 435 clinical strains of *E. coli*, including 358 strains (82.3%) from urine specimens were included. 57 strains (10.8%) were confirmed as ESBL producers. The antimicrobial susceptibility pattern (in % of S = Susceptible, I = Intermediate, R = Resistance) against the tested antimicrobials were as follow: S, I, R (%) against CAZ 19.3, 28.1, 52.6; CTX 0, 7, 93; CRO 8.8, 17.5, 73.7; CIP 19.3, 3.5, 77.2; CFM 5.3, 5.3, 89.5; FEP 43.9, 26.3, 29.8; MEM 94.7, 0, 5.3; SCF 78.9, 17.5, 3.5.

Conclusions: ESBL producing *Escherichia coli* were frequently isolated in this region of the world, they mostly resistant to ceftazidime (52.6%), cefotaxime (93%), ceftriaxone (73.7%) and ciprofloxacine (77.2%), and on the lesser extend to cefepime (29.8%). The resistance rate to the second tier agents meropenem and the combination of cefoperazone-sulbactam, was very low, 5.5% and 3.3% respectively.

P448

The use of cefadroxil in primary susceptibility testing to screen for cephalosporin resistance in Enterobacteriaceae, including ESBLs

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Objectives: Bacterial resistance to second and third generation cephalosporins, including ESBLs, is an increasing problem even in the community. Within Enterobacteriaceae a number of different betalactam resistance mechanisms have evolved over recent years. TEM and SHV mutants, and CTX-M types, collectively known as ESBLs, have emerged. A low strength cefpodoxime disc is probably the best method for sensitive (but non-specific) detection of strains with these mechanisms. The alternative would be to use both cefotaxime and ceftazidime. However, either strategy would for most laboratories require the use of an extra plate in Enterobacteriaceae from community acquired urinary tract infections (CA-UTI) and most laboratories have not considered this to be worthwhile. Recent epidemics with CTX-M:s in the community emphasise the need for this procedure. We describe the use of a first generation cephalosporin as a surrogate sensitive but non-specific screen method for ESBLs. This has the advantage of being a valid alternative in the treatment of uncomplicated CA-UTI.
Methods: Disc diffusion with antibiotic discs from Oxoid UK, semi-confluent inoculum and overnight incubation at 35–37 C on IsoSensitest Agar (Oxoid, UK). Antibiotics, zone breakpoints and disc strengths are given in the Table. 52 strains (*E. coli*, *Klebsiella*e, *C. freundii* and *E. cloacae*), each showing one or more resistance mechanism, were tested. 33 strains were ESBL producers of which 21 of type CTX-M, nine of SHV type, two of TEM type and one with an unidentified ESBL. The remaining strains consisted of ten with AmpC, three K1 hyperproducers, three showing cefuroxime resistance and three of Imp+ type.
Results:

		Number of strains correctly identified by			
	n	CDR 30 mcg <14 mm	CAZ 10 mcg <23 mm	CTX 5 mcg <24 mm	CPO 10 mcg <21 mm
All strains	52	45	35	48	49
ESBL strains	33	29	23	32	33
ESBL of CTX-M type	21	21	11	21	21

Conclusion: Cefpodoxime identified 49 of 52 Enterobacteriaceae with cephalosporin resistance, including all ESBL strains. Cefadroxil detected 45 of 52 Enterobacteriaceae with cephalosporin resistance including all 21 of the ESBL producers of CTX-M type. A low content cefotaxime disc detected 92.3% of all resistant strains including 96.9% of ESBL producers. Ceftazidime 10 mcg disc detected only 67.3% overall and was not helpful in detection of CTX-M resistance. However, a combination of cefadroxil and ceftazidime would have identified all 33 ESBL producers in this collection.

P449

Detection of ESBL in *Enterobacter cloacae*

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Objectives: Extended-spectrum β-lactamases (ESBL) are increasingly prevalent in *E. coli* and *Klebsiella* spp. Consequently, detection of ESBL focuses on these two species. However, ESBL have been described in various Enterobacteriaceae, including *Citrobacter* and *Enterobacter*. Detection is difficult in these two genera because constitutive expression of their chromosomal AmpC β-lactamase interferes with or obscures the actions of concomitant ESBL. We wanted to assess the frequency of ESBL in clinical strains of *Enterobacter cloacae*.
Methods: To detect ESBL in species producing a chromosomal AmpC β-lactamase a modified double disk diffusion test (MDDT) described by Pitout et al. was used. 240 non-copy strains of *Enterobacter cloacae* were tested with the MDDT and equivocal results were resolved by E-test, or by testing combinations of clavulanic acid and cephalosporins.
Result: We that 20% showed a deformation of inhibition zones typically observed in Enterobacteriaceae expressing ESBL. A plasmid preparation of one of these isolates was transformed into *E. coli* and the transformand expressed a β-lactamase that was active against third generation cephalosporins but not against cefoxitin.
Conclusions: The results show that strains of *Enterobacacter cloacae* may express ESBL. While this observation is probably of little therapeutic importance, it may be epidemiologically relevant since *Enterobacter* and *Citrobacter* may function an a reservoir for plasmids carrying ESBL-encoding genes.

Extended-spectrum beta-lactamase and other beta-lactamases

P450

Cloning and characterisation of chromosomal class C β-lactamase and its regulatory gene in *Laribacter hongkongensis*

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Objective: To clone and characterize a chromosomal class C β-lactamase and its regulatory gene in *Laribacter hongkongensis*, a newly discovered bacterium associated with gastroenteritis.
Methods: The β-lactam susceptibility profile of 7 strains of *L. hongkongensis* was studied. A genomic DNA library of strain HLHK5 was constructed and antibiotic-resistant clones were selected on LB plates containing cefoperazone with their inserts sequenced. The identified β-lactamase gene sequenced was analysed and the prevalence of the gene in *L. hongkongensis* studied. Southern hybridization of the identified β-lactamase gene was performed on strain HLHK5. The cloned β-lactamase was also studied by β-lactamase assays and isoelectric focusing analysis.
Results: Among seven clinical isolates of *L. hongkongensis* tested, the MICs of ampicillin were 32 to 256 μg/ml and those of third generation cephalosporins, were 64 to >256 μg/ml. Clavulanic acid and sulbactam were ineffective in restoring susceptibility to ampicillin and/or cefoperazone. Two genes, ampC and ampR, were cloned by inserting restriction fragments of genomic DNA from *L. hongkongensis* strain HLHK5 into pBK-CMV to give the recombinant plasmid pBK-LHK-5. The ampR and ampC genes and their promoters were divergently oriented, with the ampR gene immediately upstream to the ampC gene

and an intercistronic Lys-R motif, typical of inducible ampC–ampR regulatory systems. The deduced amino acid sequence of the cloned AmpC β-lactamase (pI, 8.1) contained consensus motifs characteristic of class C β-lactamases, but had no greater than 48% identities to known class C β-lactamases. When expressed in *E. coli*, the AmpC conferred resistance to ampicillin, ampicillin/clavulanic acid, ampicillin/sulbactam, cefuroxime, ceftazidime, cefoperazone and cefoperazone/sulbactam. The kinetic properties of this AmpC were also compatible with class C β-lactamase. The expression of the ampC gene was inducible, which required the presence of ampR. PCR of 20 clinical isolates of *L. hongkongensis*, including HLHK5, showed the presence of both ampC and ampR genes in all isolates. Southern hybridization suggested that the ampC gene of HLHK5 was chromosomally encoded.
Conclusion: A novel chromosomal class C β-lactamase was identified in *L. hognkongensis*. The β-lactamase characterized from strain HLHK5 was named LHK-5 (gene, blaLHK-5) and represented the first example of AmpC β-lactamase in the β-subdivision of proteobacteria.

P451

Prevalence and characteristics of plasmid-mediated AmpC beta-lactamase in *Escherichia coli* and *Klebsiella pneumoniae* isolates in a Korean hospital

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Objectives: Various plasmid-mediated AmpC beta-lactamases (PABL) have been found, particularly in *Klebsiella* spp. and *Escherichia coli*. AmpC beta-lactamase provided resistance to 3rd generation cephalosporins and cephamycins Because of the transferability of their resistance gene to other strains, PABL have been considered to be clinically important. The aims of this study were to determine the prevalence of PABL-producing *E. coli* and *K. pneumoniae* isolates in a Korean University Hospital, and the genetic characteristics of their PABL.
Methods: Cefoxitin-nonsusceptible *E. coli* and *K. pneumoniae* were isolated in patients at a Korean University hospital between March to June, 2003. PABL-producing isolates were screened by the Modified Hodge test, and the genes were detected by multiplex PCR designed by Perez–Perez and Hanson. Some of the PCR-generated amplicons were purified by agarose gel electrophoresis and using a DNA extraction kit (Quiagen, Hiden, Germany). Nucleotide sequencing were carried out by the dideoxy-chain termination method with an automatic DNA sequencer (ABI 3700, Perkin–Elmer, Foster City, Ca., USA). To determine the genetic characteristics of blaDHA, PCR using allele-specific primers were performed additionally.
Results: Among the 56 *E. coli* and 71 *K. pneumoniae*, PABL genes were detected by multiplex PCR in 41 (73%) and 55 (77%) isolates, respectively. PABL genes of CIT, DHA, and MOX group were detected in 32, 7, and 2 *E. coli* isolates, and 14, 38, and 3 *K. pneumoniae* isolates, respectively. All were found to be identical to blaCMY-1 and blaCMY-2. All of the isolates with blaDHA alleles showed positive PCR reaction with blaDHA-1 allele-specific primers, but did not with blaDHA-2 allele-specific primers.
Conclusion: Among the cefoxitin-nonsusceptible *E. coli* and *K. pneumoniae* isolates, PABL-producing strains were very prevalent. AmpC beta-lactamases of CIT group were most prevalent in *E. coli*, DHA group in *K. pneumoniae* isolates.

P452

Characterisation of clinical isolates of *Klebsiella oxytoca* susceptible to ampicillin

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Objective: *Klebsiella oxytoca* expresses class A chromosomal beta-lactamases (K1 or KOXY) and is generally resistant to Ampicillin and Piperacilin at high inoculum size. Betalactamase-negative strains are extremely uncommon. The aim of this study was to analyse two clinical isolates of *K. oxytoca* fully susceptible to Ampicillin.
Material and Methods: The two isolates (HUS-837 and HUS-610) belonged to a collection of 154 *K. oxytoca* isolates recovered from clinical samples during a 4-year period. Identification was performed by reference biochemical tests, VITEK 2 system, API 20 E and then by sequence analysis of the 387-bp fragment of the 16S and the 512-bp fragments of the rpoB genes respectively. Susceptibility to Ampicillin (range 0.5–64 mg/L) and Ampicillin plus Clavulanic acid (fixed concentration of 4 mg/L) was determined by microdilution using three different inocula of 5×10^5, 5×10^6 and 5×10^7 CFU/ml. Sonicated crude beta-lactamase extracts were studied using cephaloridine. The bla-OXY gene was amplified and sequenced. Southern hybridizations, using bla-OXY-1 and bla-OXY-2 genes as probes, were also carried out.
Results: The two clinical *K. oxytoca* strains showed Ampicillin MIC 0.5 mg/L irrespective of inoculum size tested and this value did not change with clavulanic acid. No beta-lactamase activity was detected in any strain. Analysis of the 16S and rpoB sequences placed both strains within the oxy-1 genetic group and DNA from both of them strongly hybridized with the bla-OXY-1 probe. The sequence of bla-OXY of HUS-837 strain revealed the presence of three amino-acid substitutions within the coding region and the promoter region was 100% identical to that of bla-OXY1 gene. Bla-OXY gene of HUS-610 strain could not be amplified by standard PCR. Southern hybridisation patterns suggest that bla-OXY genes from both mutant strains are located in a different genetic environment to that bla-OXY-1 and bla-OXY-2.
Conclusion: The occurrence of Ampicillin susceptible *K. oxytoca* is rare (1.3%) and could be caused by different genetic events. The presence of three amino-acid substitution in the coding region of the bla-OXY gene of HUS-837 might be sufficient to modify the conformation and in turn the functional properties of this mutant beta-lactamase. Further analyses are currently carried out to characterize the HUS-610 strain and also the transcription of both bla-OXY genes.

P453

Genetic analysis of CMY-2 beta-lactamase genes and class 1 integrons in *Escherichia coli* and *Enterobacter cloacae* from Norway

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Objectives: As part of the Norwegian reference laboratory screening programme *E. coli* and *E. cloacae* exhibiting high-level resistance to extended spectrum cephalosporins were forwarded for molecular analysis. Therefore, we molecularly investigated the mechanism of this broad-spectrum beta-lactam resistance in five strains: two *E. coli* (K4-14, K5-63) and three *E. cloacae* (K2-38, K2-41 and K2-49).
Methods: Genetically screening for Eextended-Spectrum beta-lactamase genes (TEM, SHV, CTX-M and OXA-type) and

CMY-type beta-lactamase (BL) genes involved PCR. Degenerate primers used were designed on the conserved regions of the genes and often more than one primer set was required. To examine the genetic context of BL genes, primers were designed to amplify class 1 integrons and common regions implicated in the transfer of antibiotic resistant genes. BL genes were also examined to ascertain whether they were plasmid or chromosomally mediated. PCR amplicons of the desired size were sequenced by an AB sequencer.
Results: CMY-2 was found in all strains except for K2-41 and CTX-M-1 was found in K2-38 and K2-41. Plasmids were isolated from all strains and CMY-2 was found on plasmids from strain K4-14. Class 1 integrons were amplified only from *E. coli* K4-14 and K5-63. Sequence analysis determined both integrons were identical and contained the gene cassettes dhfrVII and aadA4 in the variable region. Positive amplicons for common regions were found in both *E. coli* strains but none of the *E. cloacae* strains. Flanking PCR approach to determine the genetic context of CMY-2 gave a product of 300 bp which is being analysed.
Conclusion: The data arising from these studies would indicate that the CMY-2 BL has arisen from different sources in *E. coli* and *E. cloacae*. The *E. coli* CMY-2 gene is probably associated with common regions. The *E. cloacae* CMY-2 gene is neither associated with common regions, integrons or plasmid mediated.

P454

Study of beta-lactamases and outer membrane proteins in clinical isolates of the genus *Klebsiella*

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Objectives: To study occurrence of extended-spectrum beta-lactamases (ESBLs) and porin expression (OmpK35, OmpK36) in clinical isolates of the genus *Klebsiella* obtained from Hospital Ruzinov, Bratislava (Slovakia) during the years 2002–2003.
Methods: Clinical isolates were selected as ESBL-producers and identified by ENTEROtest24 (Pliva–Lachema, Czech Republic). Minimal inhibitory concentrations (MICs) of beta-lactam antibiotics were determined by standard agar dilution method according to the NCCLS guidelines. The following antibiotics were tested: ampicillin (AMPI), cefoxitin (CFOX), cefotaxime (CTAX), ceftazidime (CTAZ), ceftriaxone (CIAX), cefepime (CEPI), azthreonam (AZTR), meropenem (MERP). Transferability of resistance to beta-lactam antibiotics from clinical isolates to *Escherichia coli* K-12 3110 was carried out by bacterial conjugation. Transconjugants were selected as resistant to ampicillin. ESBL production was detected by combination disk method (NCCLS). PCR was used to determine the presence of blaTEM, blaSHV and blaCTX-M genes. Outer membrane protein (OMP) profiles were determined by SDS-PAGE.
Results: In the set of 43 clinical isolates 42 were identifiend as *Klebsiella pneumoniae* and one as *Klebsiella oxytoca*. All clinical isolates tested were resistant to AMPI and none of them was resistant to MERP. 54% of clinical isolates were resistant to CFOX, 84% to CTAX, 95% to CTAZ, 86% to CIAX, 43% to CEPI and 86% to AZTR. ESBL production was detected by combination disk method in all clinical isolates and in 71% of transconjugants. The presence of blaTEM , blaSHV and blaCTX-M genes was detected in 44%, 95% and 5% of clinical isolates, respectively. The same genes were detected in 5%, 54% and 0% of transconjugants, respectively. In the selected clinical isolates the presence of 32 kDa OmpA-like protein and 35–37 kDa OMPs (OmpK35, OmpK36) was observed. In *Klebsiella oxytoca* clinical isolate, which was resistant to CFOX (MIC 64 mg/l) the loss of 35–37 kDa OMPs was detected.
Conclusions: Nosocomial outbreaks due to ESBL-producing Enterobacteriaceae have become a serious problem worldwide. Treatment of infections caused by these microorganisms is a difficult task because beta-lactamase production inactivates most of the beta-lactam antibiotics. Cephamycins such as cefoxitin are active in vitro against these strains, but this agent can select porin-deficient mutants with increased levels of resistance to cefoxitin and other cephalosporins.

P455

In vitro activity of beta-lactams against clinical isolates of *Escherichia coli* with AmpC-hyperproduction phenotype

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Objective: To study the in-vitro activity of beta-lactams against clinical isolates of *Escherichia coli* with a phenotype compatible with AmpC-hyperproduction, to characterize beta-lactamases (BLs) produced in these organisms and to determine the clonal relationship of the isolates.
Methods: *E. coli* from clinical samples obtained at the Service of Microbiology, Univ. H. Virgen Macarena, Seville, Spain during 1999–2001 were evaluated. Identification and preliminary susceptibility testing were performed with Vitek-2. Reference susceptibility testing was performed by microdilution (NCCLS guidelines). Eighty organisms resistant to ampicillin, amoxicillin plus clavulanate (CLV), cephalothin and cefoxitin and lacking extended-spectrum BLs [no synergy of cefotaxime (CTX) or ceftazidime (CAZ) with CLV] were selected. The following agents were additionally tested: ampicillin-sulbactam (ASB), piperacillin (PIP), PIP-tazobactam (PTZ), carbenicillin (CAR), temocillin (TEM), cefotetan (CTT), cefpodoxime (POD), cefepime (FEP), imipenem (IMP) and meropenem (MPM). Clonal relationship was determined by REP-PCR, with primer 5′-III GCG CCG ICA TCA GGC-3′. Isoelectric focusing (PhastGel, IEF range 3–9) was performed to identify the numbers and isoelectric points (pIs) of the BLs present. In-situ inhibition of BL with cloxacillin (CLX) or CLV was also assessed.
Results: Sixty REP-PCR profiles were observed. Forty-two (52.5%) isolates produced two BL with a pI of ≥9 (inhibited by CLX but not by CLV, compatible with AmpC) and 5.4 (inhibited by CLV but not by CLX, compatible with TEM-1), respectively, 35 strains (43.8%) only produced the BLs of pI ≥9, and 3 (3.7%) strains a BLs of pI 8.0 All 80 isolates were susceptible to IMP, MPM and FEP. The percentages of strains susceptible to the tested agents were: TEM: 95, CTX: 91, PTZ: 84, CTT: 74, CAZ: 48, PIP: 29, POD: 7, CAR: 4 and AMS: 1. Resistance to CAR and to PIP but not to other agents was associated with production of the pI 5.4 BL.
Conclusions: There is considerable clonal variability among *Escherichia coli* isolates with an AmpC-hyperproduction phenotype. Carbapenems and cefepime showed an excellent activity against these isolates. Marked differences in the activities of cefoxitin and cefotetan against the isolates were observed.

P456

Beta-lactamases and antibiotic susceptibilities of *Moraxella catarrhalis* isolates from Zagreb, Croatia

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Background and objectives: The β-lactamase production in *M. catarrhalis* first described in 1977 has been reported with

increasing frequency in many countries of the world. However there were no reports from Croatia so far.The aim of this study was to investigate antibiotic susceptibilities and β-lactamase production in *M. catarrhalis* isolates from Croatia.
Methods: Fifty *M. catarrhalis* strains were collected in University Children's Hospital in Zagreb. Suceptibilities to wide range of antibiotics were determined by broth microdilution method. β-lactamases were detected with commercially available chromogenic cephalosporin disk β-lactamase test containing nitrocefin. The substrate profile of the enzymes was determined by biological assay.
Results: β-lactamases were detected by disk chromogenic substrate (nitrocephin) test in all strains. Prevalence of β-lactamase positive strains in the study period was 100%. Eighty% of the strains were resistant to amoxycillin, 4% to cefadroxil and 2% to cefuroxime and cefprozil. No resistance to cephalexin, ceftibuten, tetracycline, erythromycine, azithromycine and chloramphenicol was observed. The activity of amoxycillin was strongly enhanced in the presence of clavulanic acid in all strains. Older cephalosporins were equally active but third generation cephalosporin ceftibuten displayed significantly lower MICs compared to older compounds. Among non β-lactam antibiotics tetracycline and erythromycine showed similar activity. Azithromycine had markedly stronger inhibitory activity in comparison with erythromycine and tetracycline. Crude β-lactamases extracts from *M. catarrhalis* strains antagonized the activities of disks containing ampicillin. Enzymes did not affect the inhibition zones around cephalosporin disks.
Conclusions: Prevalence of β-lactamase producing *M. catarrhalis* strains is higher in Croatia than in other countries of the world except of Japan.The results of the susceptibility tests were in concordance with the results reported by other investigators. Wide range of MICs for amoxycillin alone and combined with clavulante could be attributed to various level of enzyme production by *M. catarrhalis* strains According to our results amoxycillin combined with clavulanic acid should be the antibiotic of choice for the treatment of infections caused with β-lactamase positive isolates of *M. catarrhalis*. Oral cephalosporins, tetracycline, macrolides or azithromycine could be an option too.

P457

Detection of extended-spectrum beta-lactamase producers with four variants of the Oxoid combination disc method

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Objectives: To evaluate the usefulness of four variants of the Oxoid combination disc method to detect ESBLs in nosocomial strains of Gram-negative rods. To compare the Oxoid test with the double disc synergy test (DDST) for ESBLs detection.
Methods: A total number of 110 ESBL-positive (DDST-positive) nosocomial isolates cultured in 2004 were investigated. Ninety nine strains (90%) of enteric rods and eleven strains (10%) of nonfermentative rods were examined. Two control strains were included in the study. Four variants of the Oxoid combination disc method: CPD/CD 01, CAZ/CD 02, CTX/CD 03 and CPO/CD 04 were applied for ESBLs detection.
Results: All examined strains were DDST-positive. Positive results in the Oxoid combination disc method were as follows: CPD/CD 01–59 strains (53.6%),CAZ/CD 02–79 strains (71.8%), CTX/CD 03–91 strains (82.7%) and CPO/CD 04–109 strains (99.1%).
Conclusions: CPO/CD 04 discs were the best combination for detection of ESBLs in clinical strains of Gram-negative rods collected in our laboratory. Application of several methods to detect ESBL producers increases the probability of their proper identification.

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Epidemiology of CTX-M beta-lactamases in Spain

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Objectives: Extended spectrum beta-lactamases (ESBL) are one of the main resistance problems affecting beta-lactams now. In Spain, recent study suggest CTX-M group ESBLs are becoming the most frequent ESBL group, and their frequency is increasing in the community setting. We have identified the ESBLs-producing isolates and characterized the enzymes found in the Hospital Universitario de Salamanca during 2004.
Methods: Presumptive detection was done by double disc diffusion method or MIC by the microdilution method. ESBLs were studied by isoelectric focusing and PCR and sequencing.
Results: We have detected phenotypically 125 isolates suspicious of being ESBL-producers. ESBLs belonging to the CTX-M group were detected in 80 isolates (64%). The most frequent CTX-9 enzyme was CTX-M 14 (55 isolates, 68.8%), followed by CTX-M 9 and CTX-M 15(11 isolates, 13.8% each). SHV and TEM ESBLs were isolated in 41 (32.8%) and 39 (31.2%) isolates respectively. CTX-M were mainly found in *E. coli*, *K. pneumoniae* and *K. oxytoca* isolates, excepting 4 CTX-M which were found in *Salmonella*. 19 CTX-M-producing isolates (23.8%) produced two or more different ESBL enzymes. The presence of two or more ESBLs was found in 14 *E. coli* and 5 *K. pneumoniae* isolates producing CTX-M 9, CTX-M 14 or CTX-m 15 enzymes. No *K. oxytoca* isolates producing more than one ESBL were found. The most frequent ESBL combination was CTX-M 14 + TEM-116, found in 8 *E. coli* and 1 *K. pneumoniae* isolates, followed by CTX-M 14 + TEM-116 + SHV-5, found in 3 *K. pneumoniae* isolates. Most CTX-M-producing isolates were obtained from UTIs and from community patients.
Comments: CTX-M are becoming the most frequent ESBLs group in our area. As in other studies in Spain CTX-M 14 is, by far, the most frequent ESBL. The finding of isolates producing more than one ESBL, formerly very infrequent, is becoming much more usual. Most ESBL-producing isolates are obtained from UTIs, and frequently in community patients.

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An ESBL-producing *Klebsiella pneumoniae* strain isolated from a nosocomial outbreak becomes resistant to all clinically available antibiotics by acquisition of a plasmid-mediated AmpC beta-lactamase

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Objectives: Outbreaks caused by multiresistant *Klebsiella pneumoniae* strains, especially extended-spectrum beta-lactamase (ESBL)-producing strains, are an increasing serious problem. Carbapenems are valuable therapeutic options against those strains, as, even with a porin loss, ESBL-producing *K. pneumoniae* remain susceptible to carbapenems. But they may become carbapenem-resistant because of porin loss in combination with plasmid-mediated AmpC beta-lactamase production.In this work, we analysed the molecular background of a multiresistant clinical ESBL-producing *K. pneumoniae* isolate. We wanted to

examine its ability to become carbapenem-resistant by acquisition of a plasmid-mediated AmpC enzyme.
Methods: *K. pneumoniae* 5111 was isolated during an outbreak of multiresistant ESBL-producing *K. pneumoniae* in the surgical ICU of a german university hospital in April 2002. MIC values were determined by broth microdilution according to NCCLS. Resistance genes were detected by PCR and sequencing. Conjugation experiments were performed by filtermating using two different clinical *E. coli* and one *K. pneumoniae* isolate harbouring the AmpC beta-lactamase CMY-2 on different plasmids as donor strains and *K. pneumoniae* 5111 as the recipient.
Results: Strain 5111 was resistant to all tested beta-lactams except carbapenems and also resistant to aminoglycosides, quinolones, tetracyclines, cotrimoxazol and chloramphenicol. The beta-lactam resistance is caused by production of the ESBL SHV-12. Cefoxitin resistance indicates a porin loss. Two chromosomal mutations in gyrA and one in parC are responsible for the quinolone resistance. The strain carries at least six genes for aminoglycoside-modifying enzymes and four additional resistance genes.Conjugation experiments were successful with one *E. coli* donor strain with a conjugation frequency of 1.7×10^{-8}. The transconjugant had acquired the AmpC CMY-2 and was resistant to carbapenems.
Conclusion: The examined strain becomes completely resistant to all clinically available antibiotics by acquisition of a plasmid-mediated AmpC beta-lactamase by conjugation. Conjugation events can also occur in vivo in the gastrointestinal tract of colonized patients. Appropriate surveillance is therefore necessary for early identification of such strains and prevention of their spread.

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Detection of ESBL in Enterobacteriaceae in the Polish National External Quality Assesment scheme – POLMICRO 2004

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Objectives: The objective was to analyse the results of proficiency testing obtained by Polish microbiology laboratories participating in POLMICRO.
Materials and methods: Gram-negative rods belonging to the family Enterobacteriaceae were distributed among 480 laboratories all over the country. The following organisms were included in the edition; four ESBL - positive strains: *Escherichia coli*, *Proteus mirabilis*, *Serratia marcescens*, *Citrobacter freundii* and two ESBL – negative strains: *Klebsiella pneumoniae*, *Enterobacter cloacae* (AmpC) however each laboratory obtained three organisms selected from the set. The laboratories were asked to provide identification to the species level and susceptibility results and interpretation. One of the main tasks was to detect ESBL production and correctness of clinical interpretation of the susceptibility test results.
Results: Most of the laboratories reported correct identification of the species, however, 10.2% of them misidentified the *E. cloacae* strain. Approximately 93% laboratories correctly identified ESBL production. Thus, 31 laboratories (12.6%) did not detect ESBL in the *E. coli* strain. In the case of *P. mirabillis* 26 laboratories (11.1%) did not identify ESBL activity. More than 90% of the laboratories which tested *K. pneumoniae* obtained acceptable antimicrobial susceptibility results. About 60% laboratories were not able to detect correctly susceptibility of *S. marcescens* and *E. coli* to piperacillin/tazobactam. No single very major error was produced by 336 laboratories (70%). However, one very major error was made by 48 (10%) laboratories in susceptibility testing. Total of 326 laboratories reached accurate results of the proficiency testing performance.
Conclusions: Over 90% laboratories correctly identified and interpreted ESBL production in the strains of the family Enterobacteriaceae.

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ESBL in *Escherichia coli*: increase in prevalence and diversity

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Objectives: To evaluate the prevalence and diversity of extended-spectrum beta-lactamases (ESBLs) in *Escherichia coli* infections from 1994 to 2003.
Methods: All non-duplicate isolates of *E. coli* resistant or with reduced susceptibility to third generation cephalosporins and with synergistic effect to clavulanic acid were collected. ESBLs isoelectric points were determined. bla genes for TEM-type, SHV-type, CTX-M-1, CTX-M-5 and CTX-M-9 related enzymes were amplified by PCR and most were sequenced. To differentiate CTX-M-9 from other CTX-M-9-related enzymes, the presence of the orf513 and orf1005 surrounding sequences described in integron In60 were verified by PCR.
Results: The prevalence of ESBLs increased from 0.08% in 1994 to 2.6% in 2003. A total of 209 ESBLs-producing strains from 23,818 *E. coli* strains were collected.The presence of TEM-type ESBLs was sporadic, with 7 strains (3 blaTEM-12, 1 blaTEM-10,1 blaTEM-19 and 1 blaTEM-104), representing 3.3% of all ESBLs isolates. SHV-type ESBLs were present throughout the study period (20.6% of ESBLs isolates; 46 strains), with a steady increase in the last three years. Of 39 sequenced amplicons 12 were blaSHV-2 and 27 blaSHV-12 genes. SHV-2 was present from 1994 to 2001. SHV-12 appeared in 2000 and was the only enzyme detected in 2002. All strains with SHV-12 isolated in 2001 and 2002 also produced CTX-M-9. The CTX-M-types were the most frequent ESBLs found (76.2% of ESBLs isolates; 170 strains). Among them, 55.9% (95 strains) were CTX-M-9 and their prevalence increased from 1996 to 2002. The first CTX-M-9-related enzymes appeared in 1999 and is now the most frequent (58 strains; 34.1%). The presence of CTX-M-14 enzymes was documented in all thirty sequenced strains. After 2001, we also observed an increase in the CTX-M-1-group (16 strains; 9.4%). There were four CTX-M-1, two CTX-M-15 and one CTX-M-32. Only one CTX-M-5 type (CTX-M-2) was observed.
Conclusions: Although the incidence of ESBLs in our hospital remains low, we observed an increase over the study period from 0.08% to 2.6%. The prevalence of SHV and CTX-M-related enzymes rose from 0.04% to 0.6% and from 0% to 2%, respectively. A major range of diversity of the ESBLs involved accompanied this increase, mainly due to CTX-M-type enzymes (CTX-M-1, CTX-M-2, CTX-M-9, CTX-M-14, CTX-M-15 and CTX-M-32).

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Molecular genetics of extended-spectrum beta-lactamase among *Escherichia coli* and *Klebsiella* spp. isolates in Finland

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Objectives: Extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella* spp. isolates are spreading and

becoming an increasing problem, especially, concerning treatment, diagnostics and hospital hygiene. The prevalence of ESBL-producers in Finland was studied in the 1990s, but only a few strains were found. This work was done to find out the molecular genetics of ESBL-enzymes among cefuroxime resistant *E. coli* and *Klebsiella* spp isolates in Finland.

Methods: From January 2002 until October 2004 cefuroxime resistant *E. coli* and *Klebsiella* spp. isolates were collected from 26 laboratories included in the Finnish Study Group for Antimicrobial Resistance (FiRe). MICs were determined by the agar dilution method on Mueller–Hinton II medium for 22 antimicrobial agents representing penicillin, carbapenem and cephalosporin derivatives. Part of the isolates representing different phenotypes and laboratories were chosen for further studies. These isolates were screened for resistance genes by PCR with specific TEM, SHV and CTX-M primers and then sequenced with an Applied Biosystems 3730 DNA Analyzer.

Results: A total of 460 isolates were collected. Screening for blaTEM, blaSHV and blaCTX-M were done for 160 isolates (54% *E. coli* and 46% *Klebsiella* spp.). The presence of a gene coding for ESBL-enzymes was confirmed in 79% of the strains. TEM-1 was represented in 62.5% of the isolates. Only two isolates had an ESBL variant of TEM (TEM-2 and TEM-52). Different kinds of SHV-enzymes were present in 37% and CTX-M in 73% of the isolates. The most common mutations in SHV were the ones representing SHV-12 which was seen among 6% of the isolates. 4% of the isolates produced two different ESBL-enzymes (TEM + SHV or SHV + CTX-M).

Conclusion: blaCTX-M alone was the most common ESBL-gene. Isolates with TEM-1 and an ESBL-gene were very common in the studied Finnish material.

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Molecular characterisation of TEM-123 and TEM-124 extended-spectrum beta-lactamases from clinical isolates of Enterobacteriaceae

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Objectives: Enterobacteriaceae are the major recipients of extended-spectrum beta-lactamases (ESBLs). A number of these ESBLs are variants of TEM-1 or TEM-2 which have modified substrate specificity due to the presence of one or more amino acid substitutions. Diffusion of these enzymes constitute a relevant emerging problem for antimicrobial chemotherapy. The aim of this work was that to characterize the TEM-123 and TEM-124 TEM-type ESBLs produced by *Proteus mirabilis* and *Morganella morganii* respectively.

Methods: The nature of blaTEM gene was determined by PCR and direct sequencing. Sequence was determined on two independent amplification products for each isolate. The deduced amino acid sequences were compared to those of known TEM variants. The amplicons were cloned in pBC-SK and transformation of recombinants plasmids was performed using *E. coli* HB-101 competent cells. TEM-123 and TEM-124 were purified from *E. coli* HB-101 (pBC-SK-TEM-123) and *E. coli* HB-101 (pBC-SK-TEM-124), respectively, by two chromatographic steps using Sepharose S and Superdex G-75.

Results: Sequence analysis of amplicons revealed the presence of two novel TEM-type variants: TEM-123 and TEM-124. TEM-123 was detected in *Proteus mirabilis* and carried the following mutations compared to TEM-1: Q6K, E104K and G238S; TEM-124 was found in *Morganella morganii* and carried the following mutations compared to TEM-1: Q6K, E104K and M182T. The kinetic parameters of the purified β-lactamases showed that TEM-123 and TEM-124 were able to hydrolyze several β-lactams including oxyiminocephalosporins. The highest hydrolytic efficiencies were observed with cefotaxime (kcat/Km, 1.05 μM^{-1} s^{-1}) for TEM-123 and with penicillin G (kcat/Km, 1.2 μM^{-1} s^{-1}) for TEM-124. The kcat/Km value of cefotaxime for TEM-124 was 0.15 μM^{-1} s^{-1}. No hydrolysis of aztreonam was observed for TEM-123 and TEM-124 enzymes. Both clavulanic acid and tazobactam behaved as good competitive inhibitors: Ki value of 2.8 μM and 0.065 μM, respectively for TEM-123; Ki value of 0.63 μM and 0.003 μM, respectively for TEM-124.

Conclusions: TEM-123 and TEM-124 are natural TEM-type ESBLs that differ from TEM-15 and TEM-106, respectively, in one amino acid substitution in the signal peptide sequence (Q6K). This position apparently plays an important role in efficient protein secretion across the membrane.

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Characterisation of *Escherichia coli* producing CTX-M-9-like beta-lactamases in the UK

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Objectives: Approximately 5% of CTX-M beta-lactamase-producing *E. coli* isolates from the UK produce group 9 CTX-M enzymes and, between August 2003 and October 2004, the national reference laboratory received 30 such isolates from 22 centres. The aim of this study was to characterise these organisms.

Methods: Isolates were compared by PFGE of XbaI-digested genomic DNA; data were analysed with BioNumerics software. MICs were determined and interpreted according to BSAC guidelines. The genes for CTX-M group 9 enzymes were detected by PCR with primers specific for blaCTX-M-9; selected alleles were sequenced directly.

Results: The PFGE profiles for 18 of 30 isolates were each unique; those of 11 isolates separated into five clusters with >85% similarity (one cluster comprised 3 isolates from the same centre with 100% similarity, whilst another comprised 2 identical isolates from a different centre); DNA from one isolate autodigested. The isolates were resistant to cefotaxime (MIC 16–256 mg/L), whilst the majority (n = 24) lacked obvious resistance to ceftazidime (MIC ≤ 2 mg/L). MICs of CTZ-R isolates (n = 6) were 8–16 mg/L. All the isolates (n = 30) were susceptible to carbapenems. Alleles from ten isolates from different hospitals were sequenced: 6 isolates produced CTX-M-14-like enzymes; 4 produced CTX-M-9-like enzymes. One of the latter had a novel variant, which differed from CTX-M-9 by an Ala234Val substitution.

Conclusions: We found multiple different strains of *E. coli* with group 9 CTX-M enzymes (CTX-M-9 or 14-like) at 22 centres across the UK. There appeared to be local spread of some strains, but there was no evidence of 'epidemic' strains, in contrast to the situation with UK producers of group 1 enzymes, where multiple serotype O25 strains have spread among sites and where one such strain is widespread in geographically remote parts of the UK.

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Association of plasmid-mediated QnrA-like determinant and gene coding for extended-spectrum beta-lactamase VEB-1

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Objectives: A plasmid mediated QnrA determinant had been identified in *Escherichia coli* at the Bicetre hospital (France) in

2003. It was associated with a gene coding for the expanded-spectrum beta-lactamase (ESBL) VEB-1. Thus, an investigation was performed for analyzing the prevalence of qnrA-like genes among nalidixic-acid resistant enterobacterial isolates (excluding *E. coli*) from the same French hospital and in a series of blaVEB-1-positive Gram negative rods.
Methods: One hundred thirty nalidixic-acid resistant enterobacterial clinical isolates that were recovered at the Bicetre hospital in 2003 were screened for qnrA-like gene by PCR. A second collection of 92 strains isolated in Thailand during a previous study and including 37 blaVEB-1-positive enterobacterial isolates, 22 blaVEB-1-negative enterobacterial isolates, and 33 blaVEB-1-positive *Pseudomonas aeruginosa*, was also tested. Mapping and sequencing of the sul1-type integrons were also performed.
Results: Among the nalidixic-acid resistant enterobacterial strains isolated at the Bicetre hospital, a single *Enterobacter cloacae* that was blaVEB-1-positive harboured a qnrA-like gene whereas eleven blaVEB-1-positive enterobacterial isolates from Thailand were positive. However, qnr-like gene was not identified from the blaVEB-1-negative enterobacterial isolates from Thailand and not from blaVEB-1-positive *Pseudomonas aeruginosa* isolates. In all isolates, the qnrA-like gene was identical and differed from the original qnrA gene reported by an asparagine to aspartate substitution at position 178 that did not lead to change in the quinolone resistance profile. The qnrA-like genes were located in sul1-type integrons that differed in structure.
Conclusion: This study indicated a further dissemination of Qnr-like determinants among European and South-East Asian isolates and identified a frequent association between this resistance determinant and the gene coding for the ESBL VEB-1.

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Prevalence of Ambler class A extended-spectrum *ß*-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Korean hospitals

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Objectives: Prevalences of TEM-, SHV-, and CTX-M-type extended-spectrum *β*-lactamases(ESBLs) in Korea are investigated repeatedly, but those of PER-, VEB-, GES-, IBC-, and TLS-type ESBLs are rarely studied. The aim of this study was to determine the nationwide prevalence of Ambler class A ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* and to characterize genotypes of ESBLs including rarely studied ones.
Methods: During the period of February to July, 2003, *E. coli* and *K. pneumoniae* isolates were collected from 12 hospitals in Korea. Antimicrobial susceptibilities were tested by disk diffusion method, and ESBL-production was determined by the double disk synergy test. MICs of *β*-lactam antibiotics were tested by agar dilution method. Transferabilities of ceftazidime-resistance of ESBL-producers were tested by conjugation. Isoelectric focusing was performed to determine the pIs of ESBLs. Searches for blaTEM, blaSHV, blaCTX-M, blaPER-1, blaVEB, blaIBC, blaGES and blaTLA genes were performed by PCR amplification, and the genotypes of ESBLs were determined by direct nucleotide sequence analysis of amplification products.
Results: Resistance ratios of *E. coli* (n = 246) and *K. pneumoniae* (n = 239) isolates to ceftazidime were 8.5% and 20.1%, respectively. Twenty-three isolates (9.3%) of *E. coli* and 55 (23.0%) *K. pneumoniae* isolates showed positive results in the double disk synergy test. Most prevalent Ambler class A ESBL genotypes in *E. coli* isolates were blaCTX-M-15 (n = 4) and blaCTX-M-3 (n = 3), and each of blaCTX-M-14, blaSHV-12, and blaTEM-52 gene was also found in one isolate. One *E. coli* isolate contained blaCTX-M-15 and blaSHV-12 genes, simultaneously. Most prevalent ESBL genotypes in *K. pneumoniae* were blaSHV-12 (n = 30) and blaCTX-M-3 (n = 13), and blaCTX-M-14 (n = 5), blaSHV-2a (n = 3), blaSHV-5 (n = 2), blaTEM-52 (n = 1), blaGES (n = 2) genes were also found in *K. pneumoniae* isolates. Some of *K. pneumoniae* isolates contained more than 2 ESBL genes, simultaneously; blaSHV-12 plus blaCTX-M-3 (n = 1), blaSHV-12 plus blaCTX-M-14 (n = 2), blaSHV-12 plus blaGES (n = 2), and blaTEM-52 plus blaSHV-12 plus blaCTX-M-3 genes (n = 1). Nucleotide sequence of blaGES genes from 2 *K. pneumoniae* isolates were different from those of other ESBL genes.
Conclusion: It is concluded that CTX-M-type ESBL-producing *E. coli* and *K. pneumoniae* isolates are spreading, and a new GES-type ESBL has emerged in Korea. Periodical survey for inspecting spread of ESBLs are needed.

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CTX-m type A-lactamases among ESBL producing *Escherichia coli* in a tertiary care Greek hospital

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Objectives: The CTX-M- type enzymes are a group of molecular class A extended-spectrum *β*-lactamases (ESBLs) that exhibit an overall preference for cefotaxime rather than ceftazidime and higher susceptibility to tazobactam than to clavulanate. The aim of this study was to investigate the presence of CTX M type enzymes among ESBL producing *Escherichia coli*.
Methods: A total of 150 non duplicate *E.coli* were recovered as ESBL producers All strains were isolated from clinical specimens. Identification and susceptibility testing was performed using VITEK 2 automated system(bioMerieux, France). All isolates were screened for ESBL production by the double–disk–synergy–test(DDST). The presence of bla CTX-M alleles was evaluated by PCR, initially with universal primers and then with primers specific for CTX-M-3. The PCR products were separated in 1.5% agarose gels and were visualized under UV light after staining with ethidium bromide.
Results: Among 150 ESBL positive strains studied 68 (44%) were CTX-M producers. Most isolates showed high MIC values for Cefotaxime (8–64 mg/l) and bordline values for ceftazidime. Most of isolates were sensitive to cefoxitin (except six isolates that were resistant to cefoxitin) and all were sensitive to piperacillin/tazobactam. The majority of CTX-M positive isolates belongs to CTX-M-3. The CTX-M positive isolates were recovered from hospitalized patients and from outpatients though at lower rates. Epidemiological analysis showed that forty eight (73%) CTX-M positive isolates were identified in urine sample, followed by those recovered from wounds (12 isolates; 18%), blood (3isolates; 4.5%) and bronchial exudates (3 isolates; 4.5%).
Conclusion: CTX-M type enzymes appear to be emerging among *E.coli* isolates in both the hospital and community environment. This study provides further evidence of the global dissemination of CTX-M type ESBLs and emphasizes the need for their epidemiological monitoring.

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Functional characterisation of IS1999, an IS4-family member involved in beta-lactam resistance gene mobilisation

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Objectives: The insertion sequence IS1999 is widespread among *Pseudomonas aeruginosa* isolates where it is inserted

inside a class 1 integron upstream of the veb1 gene cassette encoding an extended-spectrum beta-lactamase. Recently, an iso-IS1999 (five silent nucleotide substitutions as compared to IS1999) has been detected in *Klebsiella pneumoniae* upstream of another beta-lactam resistance gene, blaOXA-48, which possesses a significant carbapenemase activity. An outward directed promoter, pOUT located close to the left inverted repeat of IS1999 was shown to be responsible for enhancing the blaVEB-1 gene expression in *P. aeruginosa*. The aim of this work was to study the role of IS1999 in expression and mobilization of medically relevant antibiotic resistance genes.

Methods: Transposition experiments combined with a mating-out assay system have been undertaken using single IS1999 elements or composite transposons tagged with a kanamycin resistance gene. 5′RACE was used to map the promoter of blaOXA-48 gene expression.

Results: The upstream located iso-IS1999 element provided blaOXA-48 with the outward-directed promoter, pOUT, responsible for its expression. Further analysis of the genetic environment of blaOXA-48 identified a second identical copy of iso-IS1999, thus defining a novel composite transposon, named Tn1999. On both sides of Tn1999 a 9-bp target site was found which is the signature of a transposition event.This insertion sequence was capable of transposing onto the conjugative plasmid pOX38-Gen either as a single element or as various composite transposons. Sequence analysis of the insertion sites revealed that IS1999 inserted preferentially into DNA targets containing the consensus sequence 5′-NGCNNNGCN-3′. The transposition frequencies of the iso-IS1999 element was 10 fold lower as compared to IS1999, thus suggesting that these five nucleotide substitutions, even though silent, may play a role in the transposition process.

Conclusions: This study characterized an actively transposing insertion sequence, IS1999 capable of mobilization and activation of another expanded spectrum β-lactamase gene.

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Integrase-mediated veb1 gene cassette mobilization

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Objectives: The veb1 gene cassette that encodes the extended spectrum beta-lactamase, VEB-1 is increasingly isolated from Gram-negative rods, where it is inserted into the variable region of class 1 integrons varying in size and composition. However in all these integrons, the veb1 gene cassette was always associated with the aadB gene cassette encoding an aminoglycoside adenylyltransferase and in *Pseudomonas aeruginosa*, in addition to aadB, an insertion sequence, IS1999, was inserted upstream of the veb1 gene cassette within the integron specific recombination site, attI1.

Methods: Naturally encountered integron-structures either with or without aadB and/or IS1999 were investigated in respect to cassette excision and excision/integration into the recipient integron In3 located onto the conjugative plasmid R388 and in presence of over-expressed integrase gene.

Results: The co-excision of both veb1/aadB gene cassettes was more efficient than excision of veb1 alone and the frequency of veb1/aadB excision/integration frequency was 50 fold higher than veb1 alone. Replacement of attC site of veb1 with that of aadB gene cassette, restored a recombination frequency for veb1 gene cassette similar to that of aadB. Furthermore, the insertion of IS1999 within the attI1 recombination site reduced significantly the recombination frequency and only co-excised veb1/aadB gene cassettes could be detected.

Conclusions: Besides gene activation and mobilization, ISs are usually capable of inactivating functional DNA sequences. This is the case for IS1999 which insertion into attI1 site, reduced significantly recombinational properties of attI1, thus maintaining the veb1 gene cassette in the first position of the integron and thus ensuring optimal gene expression. The higher excision rates of veb1/aadB gene cassettes together may explain the frequent association between these two gene cassettes and suggests that the attC site of veb1 gene cassette is not efficient for specific recombination.

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Development of a multiplex PCR assay for genes encoding CTX-M extended-spectrum beta-lactamases

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Objectives: CTX-M extended-spectrum beta-lactamases (ESBLs) are increasingly prevalent worldwide, including in the UK where nearly one half of all microbiology laboratories have encountered producers. More than 30 CTX-M beta-lactamases have been described and are divided into five phylogenetic groups. Characterisation of the blaCTX-M alleles present in clinical isolates is time-consuming, typically requiring multiple PCRs with universal and then group-specific primers. We sought to develop a multiplex assay that could differentiate alleles encoding CTX-M enzymes belonging to the five major phylogenetic groups.

Methods: The sequences of reference blaCTX-M alleles were aligned and group-specific regions were identified. Primers specific for alleles encoding enzymes belonging to groups 1, 2 and 9 were designed in silico; those for blaCTX-M-25/-26 and for blaCTX-M-8 were developed and optimised empirically. Predicted amplicon sizes for blaCTX-M alleles were: group 1, 415 bp; group 2, 552 bp; group 9, 205 bp; group 25/26, 327 bp; and CTX-M-8, 677 bp. The primers were evaluated separately and in a multiplex. One hundred and forty-two referred isolates of Enterobacteriaceae were used as test samples. As controls, we included 6 isolates known to produce CTX-M-15 enzyme, and one producer each of CTX-M-2, -9, -14, -25 and -26. No CTX-M-8 producer was available.

Results: A multiplex assay was designed that incorporated 9 primers. The phylogenetic groups assigned by this assay to the 11 control isolates were consistent with the blaCTX-M allele present, and amplicons matched the predicted sizes. Among the referred isolates, 113 (79.5%; 88 *E. coli*, 24 *Klebsiella* spp., 1 *Morganella* sp.) contained alleles encoding group 1 enzymes, 28 (19.7%; 26 *E. coli*, 2 *Klebsiella* spp.) produced group 9 enzymes, and 1 isolate of *E. coli* produced CTX-M-2. The inferred producers of group 1 enzymes included 17 prospectively-tested members of a UK epidemic CTX-M-15-producing *E. coli* strain.

Conclusions: We have developed a multiplex PCR that can detect and distinguish alleles encoding CTX-M enzymes of groups 1, 2, 9 and 25/26. Its ability to detect blaCTX-M-8 has not been proven, but it includes primers that should detect this rare variant. CTX-M ESBLs are recognized worldwide as a increasingly-serious public health concern. This simple assay will assist in monitoring their emergence and dissemination.

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Molecular characterisation of plasmids from CTX-M-15-producing *Escherichia coli* strains responsible for outbreaks in the United Kingdom

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Objectives: Since 2003, *E. coli* with CTX-M ESBLs have spread widely in the UK. The epidemiology is partly clonal, including

five major strains (A-E), as well as many diverse producers. Strains A (the most prevalent type) and D are the most clearly-distinct of the major lineages, and this study aimed to characterise the plasmids responsible for their multi-drug resistance.
Methods: Plasmids were extracted by alkaline lysis and blotted onto nylon membranes for hybridisation. Antibiotic resistance was transferred into *E. coli* DH5-alpha by electroporation. MICs for clinical isolates and transformants were determined and interpreted according to BSAC guidelines. Isoelectric focusing (IEF) was used to detect beta-lactamases; bla genes were identified by PCR and sequencing.
Results: Eleven isolates of strain A and 3 of strain D showed various combinations of beta-lactamase expression, with pI values of 5.4, 7.5 and 8.6, corresponding respectively to the blaTEM-1, blaOXA-1-like and blaCTX-M-15 genes detected by PCR. Strain D isolates consistently expressed all three enzymes. All strain A isolates expressed OXA-1-like and CTX-M-15, though the latter was difficult to detect by IEF, apparently owing to low expression associated with an IS26 insertion element between blaCTX-M-15 and its normal promoter. Only 6 of the 11 strain A isolates produced TEM-1. Hybridization studies showed blaCTX-M was located on plasmids of 150 kb and 70 kb in strains A and D, respectively. blaTEM-1 and/or blaOXA-1-like genes were also located on these plasmids. MICs for transformants revealed these plasmids also mediated resistance to tetracyclines, tobramycin, amikacin (low level), gentamicin (plasmid from strain D only), and trimethoprim (plasmid from strain A only). The original clinical isolates were also resistant to ciprofloxacin.
Conclusions: blaCTX-M-15 was plasmid-mediated in both major *E. coli* strains and was linked with blaTEM-1 and/or blaOXA-1-like on large plasmids, which also conferred resistance to several aminoglycosides, tetracyclines and trimethoprim. This multi-resistance left only carbapenems as possible treatments for severe infections.

P472

Salmonella enterica serovar Virchow with TEM-52 ESBL in Greece

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Introduction: *Salmonella enterica serovar Virchow*, is one of the non-typhoidal *Salmonella* species (NTS) that infect humans. Due to it's potency to cause invasive diseases, isolation of this serovar and specially isolation of strains with acquired resistance mechanisms is of great concern. TEM-52 is an extended spectrum beta-lactamase (ESBL), well established in many countries. Here we report on the first detection of TEM-52 in *S. enterica serovar Virchow*.
Case summary – ESBL analysis: The strain was isolated from the stools of an adult patient with a 7-day history of gastroenteritis. On admission he was afebrile and dehydrated. Physical examination was otherwise normal. Biochemistry revealed mildly elevated urea levels. Identification was made with the API 20E system and serotyping was performed by the slide agglutination method with commercial antisera. Disk diffusion and E-test method were used to determine susceptibilities and MICs to antimicrobials. Among others the strain was susceptible to aminoglycosides and newer quinolones, but resistant to ampicillin, ticarcillin, piperacillin and intermediately resistant to third generation cephalosporins.A positive double-disk synergy test, indicative of an ESBL production led us to further in-depth investigation with analytical isoelectric focusing (IEF), PCR with specific SHV- and TEM-type primers and sequencing. Only TEM-specific primers gave a positive signal, which upon sequencing, revealed to match totally with the TEM-52 sequence determinant. This is in accordance with the result of the IEF that showed one beta-lactamase band of isoelectric point (pI) 6.
Conclusion: Extended-spectrum beta-lactamases (ESBLs) are rarely found among *Salmonella* strains, compared with other members of Enterobacteriaceae. Sporadic cases or even outbreaks caused by ESBL-producing *Salmonella* strains have already been described. To our knowledge, this is the first time that TEM-52 is being detected in *S. enterica serovar Virchow*. The emergence of ESBLs in NTS represents a serious problem, as treatment alternatives are limited especially in children.

P473

Effect of inoculum size on the activity of betalactams and betalactam-inhibitors against SHV-, TEM- and CTX-M-producing Enterobacteriaceae

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Objective: To investigate the effect of inoculum size on the activity of imipenem (IMP), amoxicillin-clavulanate (AMC) and piperacillin-tazobactam (PTZ) against well-characterized and clinically isolated ESBL-producers strains and their respective transconjugants.
Material and methods: A total of 16 *E. coli* and 6 *K. pneumoniae* isolates producing only one ESBL were selected. The characterization of beta-lactamases was realized by Isoelectric focusing (IEF) and then by amplification and sequencing of the respective bla genes. Thirteen expressed CTX-M-14, one CTX-M-9, five SHV-12, two SHV-4 and one TEM-4. MICs were determined by NCCLS broth microdilution method using as inocula: 5×10^5 (NCCLS standard inoculum), 5×10^6 and 5×10^7 CFU/ml.
Results: When the standard inoculum was used, all the strains were found to be susceptible to IMP, 20 (91%) to AMC and 20 (91%) to PTZ. When the inoculum size was increased to 5×10^6 CFU/ml, the MICs values increased 1–2 dilutions with the three antibiotics in all the cases. At an inoculum of 5×10^7 CFU/ml, a 2–4-fold increase in the MICs values were observed with IMP, but all the strains showed low MICs values ≤0.5 mg/L. For AMC, the increase observed with 5×10^7 CFU/ml were of 1–2 dilutions (21 strains) and 3 dilutions in one strain. Forteen (64%) strains remained susceptible to AMC with the highest inoculum, 5 (23%) strains became intermediate and 3 (14%) became resistants. The change to resistant category in AMC was only observed in CTX-M-producing isolates. At an inoculum of 5×10^7 CFU/ml, the PTZ MICs values increased dramatically to 256 mg/L (5–9 dilutions). With this inoculum, all the strains became resistants to PTZ regardless the ESBL-type produced.
Conclusions: At a high inoculum of 5×10^7 CFU/ml, significant differences were found between AMC and PTZ against the ESBL-producing strains. While 64% of strains remained susceptible to AMC when the inoculum rose, all the strains became resistant to PTZ with very high MICs values. All the strains remained susceptible to IMP irrespective of the inoculum tested. These data suggest that AMC should be evaluated in clinical studies as an alternative to IMP for the treatment of susceptible strains even when causing infections with a high bacterial charge.

P474

Elevated frequency of second-step ceftazidime resistance in hypermutable mutS negative *Escherichia coli* harbouring an extended spectrum beta-lactamase

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Objectives: Hypermutability in *E. coli* can result from lesions in the DNA-repair pathway gene mutS. The rate of ESBL development has been shown to be elevated in a mutS-negative strain carrying the pUC19-TEM or TEM-1 beta-lactamases. To examine the effect of hypermutability on the emergence of second-step ceftazidime (CTZ) resistance, we tested the rate of mutation to resistance at up to 16 × CTZ MIC in wild-type and mutS-negative *E. coli* each harbouring a first-step TEM extended-spectrum beta-lactamase (ESBL) mutant encoded on pUC19.
Methods: Etest was used to determine the CTZ MICs for wild-type and mutS negative *E. coli* harbouring an Arg164His substituted first-step TEM mutant on pUC19. Mutants resistant to 32, 64 and 128 ug/ml CTZ were selected in triplicate experiments on agar, and the frequency of mutation to each level of resistance was calculated. MICs were determined, and beta-lactamase gene sequencing was performed.
Results: MICs of CTZ for both mutS-negative *E. coli* and the corresponding wild-type parent with the first-step ceftazidimase mutant of the TEM-pUC19 beta-lactamase were 8 ug/ml. The emergence of mutants resistant to 32, 64 and 128 ug/ml CTZ was 10–15 fold higher for the mutS-negative strain (e.g. 1.82×10^{-5}, SE 9.89×10^{-6} at 32 ug/ml CTZ) than the wild-type (e.g. 1.33×10^{-6}, SE 9.15×10^{-7} at 32 ug/ml CTZ). MICs of CTZ for mutants from the mutS-negative and wild-type host ranged from 8–64 μg/ml. MICs of non-ESBL substrates such as cefoxitin and ciprofloxacin were also raised. DNA sequencing of 8 selected mutants from each parent showed no additional changes beyond the first-step Arg164His substitution. Thus, the selection of second-step resistance was not contingent upon additional mutations in the beta-lactamase gene.
Conclusion: Second-step CTZ resistance was readily selected in both wild-type and mutS hosts harbouring a TEM-ESBL, but the mutS host gave rise to second-step resistance more readily. The mechanism of these additional resistances may entail permeability and/or efflux changes; the beta-lactamase gene was unaltered.

P475

Accelerated development of TEM-29 extended-spectrum beta-lactamase from TEM-1 in hypermutable, mutS, *Escherichia coli*

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Objectives: Lesions in the DNA-repair pathway gene mutS can result in a hypermutable phenotype in *E. coli*, and this may expedite the diversification of beta-lactamases. To gain significant activity against ceftazidime (CTZ), at least one amino acid substitution must occur in the TEM-1 beta-lactamase. In order to examine the effect of hypermutability on extended-spectrum beta-lactamase (ESBL) development from TEM-1, we compared the rate of CTZ resistance development from blaTEM-1 in wild-type and mutS-negative *E. coli*.
Methods: The XmnI and BsrFI fragment of pBR322 replaced the corresponding fragment of pUC19, creating a pUC19-based vector encoding classical TEM-1; designated pTEM-1. CTZ MICs for isogenic wild-type and mutS-negative *E. coli* harbouring pTEM-1 were determined by Etest. Selection of mutants resistant to 4 × MIC CTZ was performed in triplicate experiments from 3 independent cultures on agar, and the frequency of CTZ resistance was calculated. MICs for randomly-selected colonies were determined. Mutations in blaTEM-1 were identified by sequencing.
Results: MICs of CTZ for the wild-type and mutS-negative parent strains harbouring pTEM-1 were 0.25 μg/ml. The emergence rate of CTZ-resistant mutants was >300-fold higher for the mutS-negative strain (1.58×10^{-6}, SE 8.55×10^{-7}) than for the wild-type (4.14×10^{-9}, SE 8.43×10^{-10}). Thirteen of 19 mutants from the mutS-negative host were CTZ resistant at the British Society for Antimicrobial Chemotherapy breakpoint of >2 μg/ml (range 4–16 μg/ml). DNA sequencing showed an Arg164His substitution in all of 4 sequenced mutants. This mutation changes TEM-1 into the ESBL TEM-29. All of 20 mutants selected from the wild-type host were susceptible to <2 μg/ml CTZ, and, none of these had mutations in the blaTEM-1 gene.
Conclusions: Resistance-conferring point mutations occurred readily within blaTEM-1 in a mutS background but did not occur at a detectable frequency in a wild-type background. Whilst the role of hypermutators in ESBL evolution is uncertain, these experiments indicate its potential.

Various antimicrobial agents including disinfectants

P476

Antimicrobial effect of allicin on intramacrophages *Brucella* spp.

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Objectives: Investigation the effect of Allicin as a antimicrobial agent on intramacrophage brucellae.
Methods: In this study, garlic choloroformic extract prepared and choloroform separated from garlic extract in vacuum pump. The purity and concentration of the allicin in the extract was determined by high performance liquid chromatography (HPLC) based on a previously published method. Then effect of extract on mouse peritoneal macrophages (6–8 weeks old female BALB/c mice) and on intramacrophage survival of *B.melitensis* Rev.1 & *B.abortus* S19 on cell culture of mouse peritoneal macrophages studied.
Results: Results indicated that dilution 1:20 of extract (1071 microgr/ml allicin) shown inhibitory effect on peritoneal macrophages while this effect were not seen by other dilutions. At the second stage number of CFU of Rev.1 & S19 in lysates for each treatment group shown significance differences with control group. We have shown, dilutions 1:40 (439 microgr/ml allicin), 1:80 (218 microgr/ml allicin)and 1:160 (128 microgr/ml allicin)causes complete elimination of intracellular brucellae (5,00,000 organism) at 24 hr.
Conclusion: Data clearly show that garlic extract was effective on intramacrophages *Brucella* in vitro. Also other investigators shown the immune enhancing effects of garlic extract such as

activation of macrophages and T-cells. We suggest using of garlic and its compounds in treatment of brucellosis, especially for animal brucellosis, there is no effective antibiotic therapy.

P477

Bactericidal and anti-adhesive properties of culinary and medicinal plants against *Helicobacter pylori*

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Objectives: *H. pylori* colonises the stomach of over half the human population and in 5–20% of those infected, causes severe gastroduodenal diseases, including adenocarcinomas. Significant resistance of *H. pylori* to current antibiotic treatment is being reported, resulting in failure to eradicate the organism. Alternative approaches are needed and we therefore investigated both the bactericidal and anti-adhesive properties of a number of plants that are frequently used in cooking as well as plants which are used medicinally in Sri Lanka, Iran and the Middle East.
Methods: We have used *H. pylori* NCTC Type I (11637) as well as 6 fresh clinical isolates of *H. pylori*. 18 plants were tested, of which 14 have never before been tested against *H. pylori*: *Aegle marmelos*, *Coscinium fenestratum*, *Solanum xanthocarpum*, *Mollugo cerviana*, *Solanum surattense*, *Piper longum*, *Glycrrhiza glabra*, *Allium sativum*, *Petroselinum crispum*, *Anethum graveolens*, *Salvia officinalis*, *Oreganum vulgare*, *Artemisia dracunculus*, *Myristica fragrans*, *Piper nigrum*, *Cinnamomum verum*, *Nigella sativa* and *Borago officinalis*. Plants were boiled in water to make extracts and simulate effects of cooking. The bactericidal activity of the extracts (0.1 g/ml) was assessed by a standard kill-curve with a final inoculum of 1×10^7 organisms per ml over a time course of 60 minutes. The anti-adhesive property was assessed by inhibition of binding of FITC-labelled *H. pylori* to stomach sections using confocal microscopy and quantified with the aid of a software package (Metamorph).
Results: 5 of the plants had no bactericidal effect on any of the isolates: *Aegle*, *Piper nigrum*, *Anethum*, *Solanum surattense* and *Allium*. A weak effect (approx. 30% of bacteria killed) was seen with *Solanum xanthocarpum*, *Myristica*, *Petroselinum*, *Mollugo*, *Piper longum* and *Coscinium*. Successful killing of *H. pylori* was demonstrated with *Borago*, *Nigella*, *Cinnamomum*, *Origanum*, *Salvia*, *Artemisia* and *Glycrrhiza*, (100% of bacteria killed within 15–60 mins. *Borago* and *Petroselinum* also inhibited the adhesion of *H. pylori* to stomach sections. Mean inhibition of the 4 strains to stomach sections expressing the Lewis a antigen was 61% and 34% (for *Borago* and *Petroselinium* respectively) and for sections expressing the Lewis b antigen, inhibition was 60% and 49%.
Conclusion: We have demonstrated both bactericidal and anti-adhesive properties of a range of plants against *H. pylori*, which could represent an alternative to failed antibiotic therapy.

P478

The investigation of antimicrobial impact of Thai medicinal plant extracts against *Escherichia coli* strains

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Objectives: The effect of medicinal plant extracts on cell surface hydrophobicity has been claimed to be one important antimicrobial mechanism. The aim of our study was to closely investigate the effect of aqueous extracts of medicinal plants, widely used in Thai traditional medicine for the treatment of diarrhoea, on *Escherichia coli* surface properties and to correlate this mechanism with their antimicrobial activity.
Methods: The effects of aqueous and ethanolic extracts of ten medicinal plant species including *Punica granatum Acacia catechu*, *Andrographis paniculata*, *Holarrhena antidysenterica*, *Peltophorum pterocarpum*, *Piper sarmentosum*, *Pluchea indica*, *Psidium guajava*, *Punica granatum*, *Tamarindus indica*, and *Walsura robusta* were used against 15 different strains of *E. coli*. Salt aggregation test to study their cell surface hydrophobicity, agar diffusion assay, and agar microdilution method for detection of antimicrobial activity were studied.
Results: *Acacia catechu* and *Punica granatum* increased hydrophobicity of all bacterial strains tested 3 strains of *E. coli* O157: H7 isolated from outbreak in Japan 1997, *E. coli* O26: H11, *E. coli* O111: NM, *E. coli* O22, 3 strains of *E. coli* O157 O157: H7 isolated from food, 5 strains of *E. coli* isolated from bovine, and *E. coli* ATCC 25922. The hydrophobicity of bacterial strains increased depending on the concentration of medicinal plants tested. There was no correlation between aggregation property and antimicrobial activity.
Conclusions: The activity of medicinal plants on hydrophobicity of the *E. coli* cells showed no correlaton with the antibacterial activity.
Acknowledgement: This work was supported by a fund from Thailand-Tropical Diseases Research Programme. National Center for Genetic Engineering and Biotechnology, Fiscal year 2004.

P479

Antimicrobial activity of chemical constituents from *Inula pseudolimonella* (Asteraceae) growing in Greece

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Objectives: The aerial parts of *Inula pseudolimonella* yielded 9â-hydroxyparthenolide 1, a new sesquiterpenic lactone 2, together with the known sesquiterpenic lactones inusoniolide 3, tomentosine 4, as well as the triterpenoid dammara-20,24-dien-3â-yl-acetate 5. All isolated compounds were identified by means of spectral data (IR, 1H NMR 13C NMR, HRFABMS and CIDMS).
Methods: The in vitro antimicrobial activities of the extract as well as from the pure isolated compounds were determined, using the dilution technique, by measuring the MIC of them against two Gram positive bacteria: *S. aureus* (25923) and *S. epidermidis* (2228), and four Gram negative: *P. aeruginosa* (27853), *E. coli* (25922), *E. cloacae* (13047), *K. pneumoniae* (13883), as well as against three pathogenic fungi, *Candida albicans* (10231), *C. tropicalis* (13801) and *C. glabrata* (28838). Standard antibiotics were used in order to control the sensitivity of the tested bacteria and fungi. The assayed strains were standards, from ATCC.
Results: Through the antimicrobial screening, the extract proved to be active against all six tested bacteria as well as the tested fungi, while the pure isolated compounds 1–5, which were also assayed against the same microorganisms, showed an interesting profile mostly against Gram (+) bacteria. Compounds 1, 2 and 4 exhibited the strongest antibacterial activity (MIC values 0.03–0.5 mg/ml) and 2,4 the strongest antifungal one (MIC values 0.025–0.07 mg/ml). All the rest of the tested compounds, showed moderate to strong activities against all the above microorganisms.
Conclusions: The results of the assays suggest that *Inula pseudolimonella*, possesses strong antimicrobial activities. These activities can be attributed, mostly, to the existence of sesquiterpenes (such as 2–4), which appeared to possess strong activities against all tested microbia.

P480

Antimicrobial activity of Greek royal jelly and chemical constituents

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Objectives: As a part of a systematic research on the chemical composition and antimicrobial activities of Greek Bee-hive products, we report in this study the antimicrobial activities of Royal Jelly (extracts and pure isolated compounds), which, to our knowledge, have never been studied before. Royal Jelly, so called because it is the exclusive food of the Queen-bees.
Methods: The in vitro antimicrobial activities of Greek Royal Jelly and of pure isolated compounds were determined, using the disk diffusion technique, by measuring the zones of inhibition of them against two Gram positive bacteria: *S. aureus* and *S. epidermidis*, and four Gram negative: *P. aeruginosa*, *E. coli*, *E. cloacae*, *K. pneumoniae*, as well as against three pathogenic fungi, *Candida albicans*, *C. tropicalis* and *C. glabrata*. The assayed strains were standards from ATCC. Besides, the antimicrobial activities of all the above referred compounds were tested against 11 clinical isolated microbial strains (of *S. aureus*, *S. epidermides*, *E. faecalis*, *Streptococcus haemoliticus*, *P. aeruginosa*, *K. pneumonia*, *Salmonella enteritides*, *E. coli*, *C. albicans*, *C. crusei*, and *C. tropicalis*).
Results: Through the antimicrobial screening, the extracts proved to be active against all six tested bacteria as well as the tested fungi (zones of inhibition 12–16 mm). The chemical composition of the extracts has been further studied and from the 10 identified constituents, the major compounds (sembacic acid, paraben, decanoic acid, 10-hydroxy-2-decenoic acid, 3,10 dihydroxy decanoic acid, 3,9 dihydroxy decanoic acid and 8,9 dihydroxy-decanoic acid) have been isolated and were also tested, showing moderate to strong activities against all the above referred microorganisms.
Conclusions: The results of the assays suggest that, Greek Royal Jelly, possess strong antimicrobial activities. These activities can be attributed, to a considerable degree, to the existence of decanoic acids derivatives (compounds with unique chemistry existing exclusively in Royal Jelly, among which two are new natural products) which appeared to possess strong activities against all tested microbia.

P481

In vitro hexetidine effect on bacterial adherence to restorative materials used in dentistry

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Adhesion of oral bacteria to restorative materials plays an important role in the pathogenesis of oral diseases especially in secondary caries onset. This study investigated the effect of a hexetidine commercially available oral rinse on initial adhesion of *Streptococcus mutans* to restorative materials in vitro. Three types of biomaterials: glass ionomer, composite resin and amalgam were included in this study. For standardization, from each of these materials were prepared 1 cm^2 square shape pieces, with the surfaces polished as in routine dental office practice. The biomaterials were incubated 2 hours at 37°C, together with an inoculum of *Streptococcus mutans* in Biofilm Study Broth in two different conditions with and without hexetidine. The Hexetidine was added to the medium in a subminimal inhibitory concentration. The bacterial adherence was evaluated by fingerprinting a solid medium (Columbia agar supplemented with 5% sheep blood) with the pieces of biomaterials after washing with phosphate buffer saline solution and the colony counting per 1 cm^2 was carried out after 24 h incubation at 37°C. In hexetidine free conditions, glass ionomer was highly colonised and the lowest colonised was the amalgam square piece. The presence of hexetidine in subminimal concentration decrease the adherence with almost 30% for all three biomaterials tested, with no relationship with the material type.

P482

Antimicrobial effects of nonantibiotics – reversal of resistance in Gram-positive bacteria

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Objectives: The project investigates the antimicrobial activity of defined non-antibiotics against clinically relevant gram positive bacteria and the interaction of these compounds with antibiotics.Growing problems with antibiotic resistance have increased the need for alternative antimicrobial treatment strategies. Psychotropic therapeutics, especially the phenothiazines,have antimicrobial activities and may reverse antibiotic resistance in vitro (1). Such agents could be used as helper-compounds to antibiotics.
Methods: The Minimum Inhibitory Concentration (MIC) of each antibiotic against various strains employed in this study was determined by the E-test system and checkerboard microdilution techniques. MICs for all non-antibiotic test substances were determined by agar- dilution. The interaction of each antibiotic with the test substances was determined by the checkerboard method and interpretation of synergy, additive or interference effects was recorded.
Results: We found that all Gram-positive bacterial strains, regardless of their susceptibility to regularly used antibiotics, were inhibited by the test substances at concentrations of 8–128 mg/ ml. In case of sertraline, racemic and stero-isomeric forms of thioridazine MIC levels at 8–32 mg/ ml were defined. Furthermore, racemic and stereo-isomeric forms of thioridazine are characterized by resistance modifying abilities at subinhibitory concentrations. The study showed up to 256- fold reduction of primarily defined MIC values of different antibiotics such as oxacillin, erytromycin, vancomycin and ampicillin for different Gram positive species (2). The concentrations used in synergy studies are comparable to those found in body tissues after therapeutically application of chlorpromazine.
Conclusion: Since many of the classical narcoleptics are known to be mixtures of stereoisomers, the challenge for the future is screening the pure isomers for reduced central nervous system activity and maintained antibiotic potential and especially use the CNS inactive stereoisomeres as ''helper-compounds'' utilizing their potentials as antimicrobial resistant reversal agents of Gram positive organisms.
Literature 1. Kristiansen JE. The antimicrobial activity of psychotropic drugs and stereo isomeric-analogues (disp)Dan Med Bull 1990; 37:165–82.
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P483

The antibacterial properties of EMLA-cream. An in vitro study on common pathogenic bacteria

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Objective: The purpose of this study was to investigate the antibacterial properties of EMLA-cream on common wound

pathogenic bacteria compared to lidocaine (lignocaine). Many local anesthetic drugs have antibacterial properties. EMLA-cream contain two anaesthetic drugs, lignocaine (25 mg/g) and prilocaine (25 mg/g). Little is known of the antimicrobial activity of this combination of compounds.
Method: An in vitro study was performed on 5 ATCC strains and 5 clinical isolates of each of the following species: *Staphylococcus aureus*, mehticillin resistant *Staphylococcus aureus* (MRSA), *Eschericia coli*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. The test organisms were exposed to a pure solution of EMLA-cream and a commercially available pure solution of lidocaine 1% over a 24-hour period and time-kill-curves were recorded.
Results: The anaesthetic cream was forund to have a substantial antibacterial effect compared to growth controls. Lidocaine had antibacterial effects to a lesser extent. With the anaesthetic cream there was a rapid and significant kill of all the tested strains within two hours. *Pseudomonas aeruginosa* and *Eschericia coli* were almost immediately killed.
Conclusion: EMLA-cream is an easily applicable and non-painful way of topical anaesthesia. It can be used for example prior to bedside debridement of a chronic wound. The cream has significant antibacterial properties on common wound pathogenic bacteria in vitro. In the clinical setting, as a positive side effect to the anaesthetic effect, EMLA-cream may minimise the risk of iatrogenic bacterial spread and transient bacteraemia during wound debridement.

P484

Comparative assessment of chloramine T trihydrate against suspension and attachment status of *Legionella pneumophila* strains

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Objectives: *Legionella pneumophila* has frequently been isolated from man-made recirculating water systems, such as humidifiers, hot water systems and cooling towers. Several outbreaks of Legionnaires' Disease have been directly linked with contaminated recirculating water systems. In order to prevent these outbreaks, before practical usage, activity of a water treatment biocide needs to be evaluated in laboratory conditions for both attachment and suspension bacteria.The aim of this study was to investigate the fatal activity of various concentrations of monochloramine compound (Chloramine T trihydrate), which is potential alternative to chlorine, at different contact times against different *Legionella* strains [C1 isolated from a cooling tower, C2 isolated from water and standard strain *L. pneumophila* serogrup1 ATCC 33152, which was obtained from Hertfordshire University Biodeterioration Center]. The susceptibility of environmental isolates and standard strain,in different physiological status (suspension and attachment) against biocide sensitivity was investigated.
Methods: In this study, Skaliy and other's qualitative suspension test method which was modified based on ASTM (The American Society for Testing Materials) E645-91 standard test method for efficacy of biocides prepared in cooling systems was used.
Results: In suspension status, it was found that in 100 mg/L biocide killed all strains at zero time. 10 mg/L biocide killed C1 and standard strain in 3 hours contact time, and C2 strain in 6 hours contact time. 1 mg/L biocide killed all strains in 24 hours contact time. It was found that C2 strain is the most resistant to biocides. The susceptibility of standard and C1 strain was found to be similar against Chloramine.In attachment status, it was determined that 15000 mg/L Chloramine killed C1 strain in 24 hours contact time, there was >1 log reduction for C2 strain and >2 log reduction for standard strain. 10000 mg/L biocide dosage was found to have no effect within 24 hours contact time for all strains. In attachment status, the sensivity of strains was in order of C1, standard and C2 strain.
Conclusions: Attachment bacteria was found to be more resistant than in suspension status bacteria. When a sample is undertaken for evaulation of bacterial counts or the efficacy of biocide, samples must be taken from the both bulk water and attachment and according to these results the effective biocide dosage must be detected.

P485

Efficacy of various biocides against *L. pneumophila* depending on the contact time

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Background: Cooling towers have been related to community-acquired legionellosis outbreaks. Thus, the proper maintenance and choice of biocides is important to maintain *Legionella* counts at undetectable levels.
Aims: The aim of this study was to compare the antimicrobial activity of four commercial cooling tower microbicides against *Legionella pneumophila*, and to determine whether the contact time was an important factor in the application of the correct biocidal concentration.
Material and methods: Four cooling tower biocides were tested against *Legionella pneumophila* (ATCC 33152): 2,2-dibromo-3-nitrilepropionamide (biocide 1), a dilution of biocide 1 (biocide 2), bromo-chloro-5,5-dimethylhydantoin (biodide 3) and 1,3,5-trichloro-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (biocide 4). And in vitro test was performed following the prEN 13623 test method (Chemical desinfectants and antiseptics- Bactericidal activity of products against *L.pneumophila*- Test method requirements (phase2/step1)).
Results: Biocide 1 was found to be the best disinfectant (effective at lower concentrations, 0.005 ppm). Biocide 2 was active at concentrations 20-fold higher than biocide 1 (0.1 ppm), followed by biocide 3, which was active at 5–10 ppm. The data obtained indicated that biocide 4 also had bactericidal activity at concentrations higher than 5ppm. No differences were found when *L. pneumophila* was exposed to biocide 1, 2 and 4, at different contact times (60 minutes or 24 hours). However, biocide 3 presented a slow bactericidal activity; at least twice the concentration of biocide 3 was needed at short contact times (60 minutes), in contrast to prolonged contact times (24 hours).
Conclusions: Of the biocides tested we found 2,2-dibromo-3-nitrilepropionamide (biocide 1) to be the most effective, because it is active at very low concentrations, and its activity is long-lasting.

P486

Effect of *Thymbra capitata* on *Aspergillus*: a cytometric approach

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Essential oils have been empirically used as antimicrobial agents, but its mechanisms of action are still unknown. The increased incidence of aspergilosis, particularly in patients with impaired immunity, justifies the search of alternatives to classical antifungal therapy. *Thymbra capitata* is a widespread plant in southern Portugal (Algarve), traditionally used due to its anticrobial proprieties, with great activity against Aspergillus (1).

Objective: To clarify the mechanism of action of *T. capitata* and of their main active components (carvacrol and p-cymene) on clinical species of *Aspergillus*.
Methods: Four strains of *A. fumigatus* (3 clinical isolates and 1 American Type Culture Collection strain), 2 clinical isolates of *A. niger* and 1 of *A. flavus* were studied. The Minimal Inhibitory Concentration (MIC) and Minimal Lethal Concentration (MLC) were determined using NCCLS M38-A protocol. To clarify the mechanism of action a flow cytometry assay using propidium iodide (PI; Sigma) was used (2). 106 conidia/ml were incubated with MIC and MLC of *T. capitata* and their main components during 1, 3, 5 and 7 hours at 37°C, following staining with 1 μg/ml of PI during 15 minutes, and the percentage of stained conidia evaluated by flow cytometry (Beckman Coulter XL-MCL) . PI only penetrates cells with a severe impairment of the cell membrane, i.e. dead cells.
Results: The MIC and MLC values were similar, ranging between different strains from 0.16 to 0.64 μl/ml(V/V), according the different strains. The percentage of dead conidia increased with the incubation time. Seven hours after incubation with MLC values over than 90% of the cells dead.
Conclusions: This study clearly shows that *T. capitata* and its main components are fungicidal by producing severe lesion on fungal membrane, an effect that deserves evaluation in future clinical trials.
(1) Salgueiro LR, et al. Planta Med 2004; 70(6):572–5.
(2) Pina-Vaz C, et al. J Med Microb 2000; 49: 831–840.
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P487

Aspects of the mode of action of the novel biocide Akacid Forte on *E. coli*

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Objective: Akacid Forte (Poly-oxyalkylenebiguanide) is a novel biguanidic biocide intended for use as a disinfectant. The aim of this work was to investigate the mechanism of action of this biocide on *E. coli* using a variety of techniques.
Methods: Initially the rapid bactericidal effect of the substance was demonstrated and quantified by MIC and MBC testing as well as in killing curve experiments. Potassium efflux from *E. coli* was evaluated using atomic absorption spectroscopy. The uptake of the hydrophobic probe NPN by *E. coli* was measured. Membrane disruption was assessed with carboxyfluorescein containing phospholipid vesicles and with *E. coli* spheroplasts. Photomicrographies of bacteria and vesicles were taken.
Results: The MIC and MBC of Akacid Forte for *E. coli* was 1 μg/ml. Akacid Forte induced rapid efflux of potassium ions from *E. coli* cells. Also, NPN uptake was enhanced by the substance. Vesicle and spheroplast membranes were disrupted by Akacid Forte.
Conclusion: The experiments show that Akacid Forte has distinct membrane damaging properties. Given the fact that an intact membrane function is essential for the survival of any cell, these results provide evidence that damage to the membrane is an important contribution to the mode of action of Akacid Forte against *E. coli*.

P488

The role of iodine in ampicillin and tetracycline antibiotic resistance transfer

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Disinfectants or chemical biocides are widely used to remove infectious agents, but resistance to the agents is observed among some bacteria. A number of research reports have expressed concern that the use of biocides may contribute to the development of antibiotic resistance. Therefor we studied the effects of different concentration of iodine on antibiotic resistance transfer to *Salmonella typhimurium*. *Salmonella typhimurium* was considered as recipient species and *E.coli* strains was selected as donor of antibiotic resistance. The bacterial antibiotic susceptibility was accomplished by Disk diffusion method, and MIC was also determined. Then the *Salmonella* strains (recipient) was treated with sub MIC of iodine, and *Salmonella* and *E. coli*(donor) were mixed in TSB broth. Antibiotic resistance transfer was studied in the Bismuth sulfate agar containing antibiotics. The results indicated that the treatment of *Salmonella* strains with %0.1–%0.3 iodine, causes an increase in resistance transfer rate to Ampicillin and Tetracycline, but it was decreased when the concentration increased. This finding was significant, and emphasizes the important role of the special concentration of disinfectant substances like iodine on antibiotic resistance transfer.

Urinary tract infections

P489

Pathogenicity and susceptibility to antibiotics of *Proteus mirabilis* strains isolated from urinary catheters

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Objectives: *Proteus mirabilis* is common microorganism isolated from catheterized patients. *P. mirabilis* may persist in the urinary tract despite many changes of catheter and antibiotic therapy. It blocks urinary catheters through the formation of extensive crystalline biofilms. The obstruction of urine flow can induce episodes of pyelonephritis, septicemia and urinary calculi. The goal of this study was to determine important virulence factors of *P. mirabilis* strains recovered from catheter of long-term catheterized patients, as well as their resistance to antibiotics.
Methods: Collection of 22 *P. mirabilis* strains was obtained from 88 Foley catheters. We analysed the following virulence factors: type of fimbriae by hemagglutination test, activity of urease using method of Weatherburn and ability of these bacteria to swarm over the surface of agar media. Invasiveness and adherence of selected strains to Hu 609 line (normal human bladder urothelium) were also determined. Urifast Twin 1C xp test was used to study the susceptibility of the *P. mirabilis*. The antibiotics included in this test were tested at one concentration, the low breakpoint according to the NCCLS standard.
Results: It was found that all of tested *P. mirabilis* strains indicated urease activity and it was ranged from 217 to 95 U (1 U is a number of micrograms ammonium released per min per milligram protein of bacteria). Among of all strains 13 (49%) were able to swarm over solid surface. Fimbriae were observed in most strains, both MR/P and MR/K (72%, 59% strains,

respectively). It was also found that *P.mirabilis* isolated from urinary catheter demonstrated a significant ability to adhere and penetrate normal urothelium. Resistance rate of *P.mirabilis* was as follows: ciprofloxacin, 91%, gentamicin, 86%, amoxicillin 76%, trimethoprim/sulfamethoxazole, 67%, norfloxacin, 52%, amoxicillin/clavulanic acid, 33% and cephalothin, 33%. Ceftriaxone and amikacin were the most active antimicrobials.
Conclusion: Our results showed that *P.mirabilis* colonizing urinary catheter were drug resistant as well as had virulence factors enable them to invade the kidney and induce infection, usually chronic commonly results in the formation of bladder and kidney stones.

P490

Comparison of two closed urinary catheter system bags with single and double non-return valve using a laboratory model

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Objectives: Comparison in the laboratory of two urinary closed system bags (CSB), one with single the other with double non-return valve (NRV) with regard to bacterial ascension from the drainage bag to the bladder.
Methods: The test was performed in a microbiological laboratory using a urinary tract model: 10 drainage bags with a single NRV, (CSB A) and another 10 with the double NRV (CSB B) were inoculated with *E. coli* (ATCC 25922). Daily samples were taken from two drainage ports of each catheter system - one at the connection between the catheter and the drainage tube (Port I), and one above the top of the bladder (between the peristaltic pump and the bladder, Port II) -Time till the *E. coli* reached the drainage ports (time to positivity) was measured.
Results: No great differences were observed between the two systems in time to positivity at Port I (mean: 9.2 (CSB A) vs 9.5 days (CSB B), range: 6–12 days each). However, substantial differences were observed between the two systems in time to positivity at Port II. Port II in the bladder model using CSB A became positive after a mean of 13.9 days (range: 12–17 days), whereas Port II of the model using CSB B became positive after 19.2 days (range: 15–20 days). This amounts to a difference of 5.3 days.
Conclusions: Under laboratory conditions, colonization of the bladder (time to positivity at Port II) was clearly delayed using the double NRV in comparison to using the single NRV-system. Clinical trials should be conducted to investigate whether the CSB with the double NRV-system really has the ability to delay infection in vivo.

P491

Antimicrobial resistance rates in nosocomial catheter-associated urinary tract infections

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Object: To assess the incidence of urinary tract infections (UTI) and antimicrobial resistance rates of the isolated microorganisms following indwelling urethral catheterization among hospitalized patients.
Methods: We studied prospectively all patients admitted to our medical department during a one-year period, provided that they had been newly catheterized upon admission and that the urine culture obtained within the first 24 h of hospitalization was sterile. A urine specimen was obtained everyday for culture and microscopic examination.
Results: One hundred consecutive patients were enrolled in the study, 24 males (24%) and 76 females (74%). Mean age was 73.0 ± 15.9 years for males and 76.3 ± 10.6 years for females. Bacteriuria was detected in 44 patients, 39 females and 5 males. Median duration of hospitalization before detection of catheter colonization was 5.6 ± 3.5 days. Mean duration of hospitalization was 11.1 ± 6.7 days. Risk factors for catheter-related bacteriuria were female gender [odds ratio (OR) = 4.0, 95% confidence intervals (CI) 1.3–11.8] and previous UTI history (OR=5.7, 95% CI 1.1–29.4). Isolated microorganisms were *Eschericia coli* (54.5%), other Enterobacteriacae (11.3%), *Candida albicans* (11.3%), *Enterococcus* sp. (9.0%), *Pseudomonas* sp. (6.8%), *Acinetobacter* sp. (4.5%) and coagulase-negative *staphylococci* (2.2%). Antimicrobial resistance rates of the isolated Enterobacteriacae strains were: ampicillin 61.5%, coamoxiclav 7.7%, cefalothin 34.6%, cefuroxime 3.8%, cefotaxime 16%, cotrimoxazole 34.6%, fluoroquinolones 7.6%, piperacillin 32.0%, piperacillin/tazobactam 4.0%, aminoglycosides 3.8%. In 16 patients (36.3%), the isolated microorganism accounted for cystitis, while in the rest 28 (63.6%) it accounted for asymptomatic bacteriuria. Cystitis was treated with the appropriate antimicrobials according to susceptibility tests.
Conclusions: Urinary bladder catheterization is frequently followed by bacterial and/or fungal catheter colonization. In our series, female gender and previous UTI history were the main risk factors for urinary catheter-associated UTI. In most cases, *E. coli* was the isolated microorganism. Antimicrobial resistance rates of the isolated Enterobacteriacae were high for ampicillin but low or intermediate for other antimicrobial agents.

P492

Comparison of flow cytometry with urine culture and Gram stain in a routine microbiology laboratory

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Objectives: In an attempt to lower the workload in a bacteriology laboratory providing services for seven general hospitals we wanted to find out if we could eliminate unnecessary urine cultures using flow cytometry as screening method and also to replace microscopy.
Methods: Over a period of two weeks 673 urine specimens send in with a request for urine culture were processed using the Sysmex UF-100 automated urine cell analyzer in parallel with Gram stain and culturing overnight of 0.01 ml concentrated urine and 0.01 ml of a 1:500 dilution onto Columbia sheep blood agar and C.L.E.D. agar using calibrated loops. Specimens were also tested for inhibitory activity using spore plates.
Results: 659 specimens were left for evaluation as the UF-100 rejected 14 (2%) of the specimens for various reasons. UF-100 results were considered to be in full agreement with culture results when urinary tract infection (UTI) flags were either positive (on the basis of the cut-offs suggested by the manufacturer) and culture yielded relevant organisms at 100,000 colony forming units (CFU)/ml or higher or UTI flags were negative and culture yielded no growth or low counts or contaminants. On this basis 31% of the UF-100 results were true positive, 30% true negative, 11% showed partial agreement, 25% were false positive and 3% were false negative. 66% of the specimens with false positive results showed inhibitory activity vs. 41% of the other specimens. Out of the 18 false negative results a closer look revealed that only three of them, i.e. 0.5% of all specimens evaluated, came from same day specimens and yielded a single relevant organism (two with *E. coli* and one with *enterococci*). UF-100 results were clearly superior to Gram stain regarding leukocytes and comparable regarding quantification of bacteria.

Conclusions: Using the UF-100 we would only have to culture about 70% of the urine specimens. The UF-100 could also replace time-consuming microscopy for our purposes. The low rate of truly false negative results around 0.5% appears to be tolerable. Specimens from certain patients (e.g. haematology patients with cytopenia) or certain materials (e.g. suprapubic bladder aspirates) should be cultured in any case.

P493

Escherichia coli causing recurrent urinary tract infections belong mainly to the phylogenetic group B2

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Objectives: *Escherichia coli* (*E. coli*) is divided into four main phylogenetic groups (PGs), designated A, B1, B2 and D. Most *E. coli* strains causing urinary tract infections (UTI) and other extraintestinal infections have been shown to belong to group B2 or, to a lesser extent, to group D. Overall strains belonging to B2 and D have virulence factors (VF) lacking in group A and B1. The aim of this study was to determine the PG in relation to the ability to cause recurrent urinary tract infections (RUTI).
Methods: A multicentre, double-blind, placebo-controlled comparative study of different dosing regimens of pivmecillinam of 1143 women with community acquired lower UTI. Clinical/bacteriological evaluation was done at day 1, day 8–10 and day 35–49. 156 women with *E. coli* at the index episode and at follow-up and 90 women with *E. coli* at the index episode and negative urine culture at follow-up were randomly selected and the *E. coli* strain studied with respect to phylogenetic grouping in relation to the ability to cause recurrence. Phylogenetic grouping was done by the triplex PCR of the genes chuA, yjaA and the DNA fragment TSPE4.C2 (Clermont O. et al, 2000). PFGE with XbaI was performed and interpreted with BioNumerics.
Results: *E. coli* index strains followed by bacterial persistence or reinfection with the same strain (100% identical PFGE profiles) at follow-up showed the following distribution of the PGs: A: 10%, B1: 4%, B2: 71%, D: 15%. *E. coli* index strains followed by persistence/reinfection with a new strain (PFGE profiles not 100% identical) or a negative urine culture at follow-up showed following distribution of the PGs: A: 22%, B1: 4%, B2: 54%, D: 20%. The differences between the two groups for the PG B2 ($p = 0.008$) and for PG A ($p = 0.01$) were highly significant. The difference between the two groups for the PG B1 and D was not significant.
Conclusion: *E. coli* strains causing RUTI with the same strain belong mainly to the PG B2 (71%), which is significantly higher than for *E. coli* not capable of causing RUTI. This is in accordance with studies showing B2 to be associated with at higher number of VF and warrant further research into VF related to strains causing RUTI.

P494

Comparison of intravenous ceftriaxone and switch therapy to oral ofloxacin versus the oral ofloxacin alone in patients with UTIs

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Introduction: Urinary tract infections (UTIs) are one of the most important causes of hospitalization patients in Iran. An intravenous antibiotic therapy is associated with side effects, toxicity, high cost, and long period of hospitalization in treatment of UTIs, "switch" therapy (intravenous-to-oral antibiotic) has been considered. The aim of this study was to compare the efficacy of intravenous Ceftriaxone and switch therapy to oral Ofloxacin versus the oral Ofloxacin alone in patients with UTIs.
Materials and Methods: In this randomized clinical trial, 53 patients (30% aged <25 years) with complicated urinary tract infections, who were determined by positive urine analysis and culture, were enrolled and divided in two groups of A and B. Patients in group A (n = 24) treated with intravenous Ceftriaxone (1 g/daily) for the first 2 days and then switch to Ofloxacin (400 mg/daily) for 8 days. Patients in group B (n = 29) treated with oral Ofloxacin (400 mg/daily) for 10 days. One week after treatment, patients were evaluated clinically and microbiologically
Results: Causative agents were isolated: *E. coli* (79%), *Klebsiella* sp (11%), *Proteus* sp (6%) and others microorganisms (4%). Ninety per cent of strains were susceptible to Amikacin, Cefotaxime, Ceftazidime, Amoxi-clave, Cefixime, Ciprofloxacin and Ofloxacin. Rate of response (clinically and microbiologically) in patients of A & B groups were 95%.
Conclusion: Switch therapy shortens duration of hospitalization, in which decreases costs and risk of nosocomial infections. The data indicates a good possibility of using ofloxacin as a drug of the treatment of UTIs.

P495

Community-acquired urinary tract infections by extended-spectrum beta-lactamase-producing Enterobacteriaceae in the Zenica-Doboj canton, Bosnia and Herzegovina

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Objectives: The presence of ESBL producing strains in outpatient samples are at least underreported. The aim of this study was to determine the incidence and antimicrobial resistance of ESBL-producing strains in the community-acquired urinary tract infections (CAUTIs), which is necessary for antimicrobial therapy selection.
Methods: From January 2003 to September 2004, 4224 consecutive, non-duplicate coliform isolates from CAUTIs analysed. Antimicrobial susceptibility testing was performed by disc-diffusion method to twenty three antimicrobials. Double-disk synergy test with amoxicillin-clavulanat, cefotaxime, ceftazidime, ceftriaxone and aztreonam (by NCCLS recommendation) and Etest strips with PM/PML (AB Biodisk) were performed to detect the ESBL producers.
Results: The overall incidence of ESBL producing strains was 2.6% (108 of 4224), significantly higher in male, 7.9% (79 of 1006) than in female, 1.5% (29 of 3218). The highest number ESBL producers noted in oldest and youngest age group: 4.7% (52 of 114) and 2.6% (27 of 1051), respectively. There observed the increase from 2.1% (52 of 2463) to 3.2% (56 of 1761), and shift ESBL producers toward younger age group (1.6% and 3.8%, respectively) in this period. The incidence of ESBL producing strains among isolated *Klebsiella* spp. were 7.8% (83 of 1060), *E. coli* 0.7% (18 of 2561), *Citrobacter* spp. 0.6% (1 of 156), *Enterobacter* spp. 7.7% (3 of 39) and *Proteus* spp. 1.0% (3 of 297). Among ESBL producing isolates *Klebsiella* predominated, 76.9% (83 of 108), *E. coli* 16.7% (18), *Enterobacter* and *Proteus* 2.8% each (3), and *Citrobacter* 0.9% (1). ESBL producing strains showed significantly higher resistance rates for all tested antibiotics comparing to non-ESBL-producers, and they were in the range 60% to 90% (98% for ampicillin), except for imipenem, 1%. In non-ESBL-producing isolates resistance rates were about 20% for the most antibiotics tested, for cefotaxime and aztreonam less than 10%, for imipenem, 0.1%, and for ampicillin, tetracycline, co-trimoxasole and carbenicillin were in the range 40–70%.
Conclusions: It is worrying the increase and the shift toward youngest age group of the ESBL producer incidences in the

CAUTIs in this region. ESBL producers associated with multi-drug resistance, thus, its surveillance appeared to be important in the community-acquired infections. Further studies are needed to detect ESBL types in the term of highly different geographical dissemination of these isolates.

P496

Crystalline bacterial biofilm formation on urinary catheters by urease producing urinary tract pathogens

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Objective: Long-term bladder catheterisation inevitably results in bacteriuria and the formation of biofilms on the catheters. Crystalline biofilms form when urease-producing bacteria infect the catheterised urinary tract, generate alkaline urine and cause the crystallization of calcium and magnesium phosphates. The objective of this study was to compare the abilities of various urease-positive species to encrust and block catheters with crystalline biofilm.
Methods: Laboratory models of the catheterised bladder were infected with clinical isolates of various urease-positive species and supplied with urine for up to four days. The pH of the urine in the bladder chamber and the numbers of viable cells in the urine were measured at 24 h intervals. Catheters were removed from the models after four days or at the time they blocked. The catheter biofilms were examined by scanning electron microscopy.
Results: Catheters in models infected with urease positive strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Providencia stuartii* drained freely for the four day experimental period. In each case the urine remained acidic (pH < 6.5) and electron microscopy showed no evidence of crystal formation in the catheter biofilms. In models infected with *Morganella morganii* the urine remained acidic for 72 h but by 96 h the pH had reached 7.4. The catheters did not block but electron microscopy revealed the presence of crystals in the biofilm. *Proteus mirabilis*, *Proteus vulgaris*, and *Providencia rettgeri* all blocked the catheters within 48 h. The pH of the urine became alkaline (pH 8–9) and extensive crystalline biofilm was observed on the catheters.
Conclusions: Not all urease-positive urinary tract pathogens are capable of forming crystalline biofilms on urinary catheters. Of those species that can induce rapid catheter encrustation, *Pr. mirabilis* is by far the most commonly found in the catheterised urinary tract and therefore should be the main target for strategies to control the clinical problem.

P497

Parenteral human albumin leads to quicker recovery in patients with acute pyelonephritis and hypoalbuminaemia

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Objectives: Patients admitted to a hospital due to microbial infection such as pyelonephritis or community-acquired pneumonia, have increased mortality and morbidity. Hypoalbuminaemia, which develops in the early stage of the disease, and it is probably due to a decreased protein production by the liver, seems to be a critical factor influencing the course of the infection. The aim of this study was to investigate the role of parenteral human albumin, given in the early stage of infection, in improving the outcome of the disease. The clinical outcome was determined using the temperature and the CRP levels.
Patients/Methods: From May 2003 to October 2004, 60 immunocompetent adults were admitted to our hospital with acute pyelonephritis and hypoalbuminaemia (admission albumin < 3.1 mg/dl). These patients were randomly separated in two groups of 30. All 60 patients had a positive urine culture, while 20 patients from first and 21 from second group had also a positive blood culture. Both groups had similar mean values of age (67.97 ± 15.011 vs 71.17 ± 15.746), admission temperature (38.487 ± 0.685 vs 38.49 ± 0.6838) , admission serum CRP (288.17 ± 95.824 vs 311.57 ± 103.545) and admission serum albumin (2.543 ± 0.4289 vs 2.413 ± 0.4425). Patients in the first group received antibiotics and additionally 100 ml/day parenteral solution of human albumin (inject. solution infusion 20% 250 mg/ml), starting within 24 hours from admission. Patients in the second group received only antibiotics, During the 4th day of hospitalization the patient's temperature, serum CRP and albumin levels were measured.
Results: During the 4th day of hospitalization the temperature of the first group was significantly lower than that of the second group (36.75 ± 0.601 vs 37.323 ± 0.715 p = 0.0001). Likewise there was a significant reduction of serum CRP in the first group compared to the second group (73.30 ± 35.226 vs 140.83 ± 56.64 p = 0.009). The mean value of serum albumin during the 4th day was significantly higher in patients who received human albumin (3.42 ± 0.2285 vs 2.45 ± 0.4315 p = 0.003). The hospitalisation time was significantly shorter in patients who received albumin.
Conclusions: The level of serum albumin represents a nutritional marker for patients with an infectious disease. Administration of human albumin in the early stage of infection increases the albumin levels, improves the clinical outcome and leads to shorter hospitalization time in patients with acute pyelonephritis.

P498

Four-year survey and resistance trend analysis in 37,261 isolates causing community urinary tract infections in Brazil

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Objectives: Assess the antimicrobial resistance pattern and trends in pathogens responsible for urinary tract infections (UTI) in community-based patients in São Paulo, Brazil between the years 2000 and 2003.
Methods: From January 2000 to December 2003, 266,692 urine cultures from community-based patients were collected. All positive cultures with one single pathogen and a colony count ≥ 100,000 CFU/mL were selected for the analysis. Based on these criteria, 37,261 (14%) positive cultures were analysed in this survey. Chi-square for trend test (Altman, 1999) was performed to evaluate resistance prevalence ordering in the years surveyed (p value below 0.05 was considered significant).
Results: Among the 37,261 positive cultures, 88.8% belonged to female and 11.2% to male patients. Among the Gram-negative, *E. coli* (71.6%) presented the highest prevalence, followed by *K. pneumoniae* (6.4%) and *P. mirabilis* (6.1%). Among Gram positive isolates, *E. faecalis* was the most prevalent (4.8%) followed by *S. saprophyticus* (1.6%). Susceptibility tests were performed in 31,716 cultures. Resistance rates to *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *E. aerogenes*, *E. cloacae*, *E. faecalis*, and *S. saprophyticus* are presented. For *E. coli*, ascending resistance trend in 4 years was observed for nalidixic acid, norfloxacin and ciprofloxacin (p < 0.0001); descending resistance for cephalothin and tetracycline (p < 0.0001); elevated and stable resistance to ampicillin and trimeth-sulfa (p > 0.5); low and stable resistance to nitrofurantoin (p > 0.5).
Conclusions: Elevated resistance rates to trimeth-sulfa (>15%) among most prevalent pathogens preclude the use of this drug in the population studied. Quinolones may be used as first line agents for community UTIs in São Paulo; however, a trend for

Resistance rates to most commonly isolated pathogens in community urinary tract infections 2000 to 2003-São Paulo, Brazil

	% Resistant										
	N	Nalidix acid	Ampicillin	Cephalothin	Ceftriaxone	Ciprofloxacin	Gentamicin	Nitrofurantoin	Norfloxacin	Trimeth-Sulfa	Tetracycline
E. coli	22682	15.5	43.4	13.9	0.3	11.9	3	2.9	12	33.7	30.5
K. pneumoniae	2059	15.2	99.9	7.6	1.7	6	3.3	21.2	8.9	17.7	19.8
P. miratillis	1944	8.9	18.9	3.1	0.4	4.2	2.3	97.6	4	21.5	99.8
P. aeroginosa	605	40.3	40.7	39.8	85.1	62.8	47.9	42	57.4	98.3	41.3
E. aerogenes	510	9.6	99.2	98.6	2	5.1	2.2	21.2	5.5	7.8	6.9
E. cloacae	331	44.4	99.4	99.4	32.9	38.7	24.2	39.6	41.1	38.4	42.6
E. faecalis	1525	0.1	0.3	70.6	0.1	16.1	0	0.8	1.2	70.5	59.2
S. saprophyticus	531	0	79.1	7.5	0	1.3	2.1	0.6	0.6	7	15.8

increasing resistance was observed in *E. coli* for this therapeutic class. Thus, this drug class should be used cautiously and, if possible, with lab identification and susceptibility test. Focused and specialized surveillance studies may be an adequate strategy for guiding empirical therapy in community settings and for halting the spread of resistance.

P499

Spectrum of pathogens in community-acquired urinary tract infections and their antimicrobial resistance in a general Hospital

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Objectives: The aim of the study was the etiology and antibiotic resistance of clinical isolates from community acquired urinary tract infections, in a General hospital.
Methods: Between January 1998–2004, a total of 10276 urine specimens were obtained from outpatients. Urine cultures were performed in laboratory according to conventional methods. The identification of urinary isolates was performed using the API20E system (BioMerieux), while their susceptibility was tested by Kirby–Bauer and M.I.C methods (Vitek II, Bio-Merieux), according to NCCLS guidelines.
Results: 2320 out of 10276 urine samples (22.5%) fulfilled the criteria for significant bacteriuria. The most frequent isolates were: *E. coli* 1688 (72.7%), *Klebsiella* spp.182 (7.8%), *Proteus* spp. 170 (7.3%), *E. faecalis* 91 (2.9%), *Ps. aeruginosa* 65 (2.8%), *Candida* spp. 52 (2.2%), CNS 45 (1.9%), *S. aureus* 37 (1.6%). *E. coli* resistance pattern was as follow: ampicillin 41.4%, amoxicillin – clavulanic acid 6.5%, cephalothin 20.0%, cefuroxime 4.8%, nitrofurantoin 5.3%, cotrimoxazole 30.8%, quinolones 7.7%.Six point five per cent of *Klebsiella* spp., 2.3% of *Proteus* spp and 1.1% of *E.coli* were ESBL producers. *E.faecalis* high level gentamycin resistance was 20.1%. There was no resistance to glycopeptides for *Staphylococcus* and *Enterococcus* spp. The resistance of CNS to oxacillin was 35.2%, to gentamycin 16.2%,cotrimoxazole 33.1%, quinolones 20.8% and nitrofurantoin 18.4 %.
Conclusions: *E. coli*, continuous to be the most common clinical isolate in commumity acquired urinary tract infections. About 41.1% of *E. coli* isolates were resistant to ampicillin and 30.8% to cotrimoxazole. Approximately 9.9% of Enterobacteriaceae would be considered as ESBL producers.

P500

Community-acquired enterococcal urinary tract infection

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Objectives: Urinary tract infections in childhood are mainly caused by Gram-negative organisms of the intestinal flora. Enterococcal urinary tract infections are often considered to be hospital- acquired and to affect children with predisposing conditions. The aim of this study was to investigate the frequency, susceptibility patterns, clinical characteristics, imaging findings and outcome of otherwise well children with community-acquired enterococcal urinary tract infections.
Methods: We reviewed the medical and microbiological records of all the 272 cases of proven urinary tract infections in children hospitalized in the Department of Pediatrics of a General Hospital during the 5-year period 1998–2002, with emphasis on patients with enterococcal urinary tract infections.
Results: *Enterococcus faecalis* was isolated in 14 episodes of urinary tract infection in 13 children and was the third more common uropathogen, accounting for 5.14% of urinary tract infections. *E. faecalis* was the single pathogen isolated in 9 cultures, whereas in the remaining 5, *E. coli* was isolated as a second pathogen. All strains were susceptible to ampicillin, vancomycin and nitrofurantoin and resistant to cephalosporins and quinolones and exhibited intrinsic, low-level aminoglycoside resistance. Intravenous ampicillin plus gentamicin was administered in 7 children, ceftriaxone in 3 and amoxicillin-clavulanic acid in the remaining 4 cases. Urine cultures obtained under treatment were sterile in all cases.Imaging studies revealed major urinary tract abnormalities in 9 children Follow-up information was available for 11 children for a period of 21 to 75 months. Four children (31%) suffered recurrences, due to *E. faecalis* in one patient and to Gram-negative organisms in the remaining three. Four children underwent corrective surgery. Despite antibiotic prophylaxis, which was given to all 13 children, 4 of them suffered breakthrough infections and 3 developed renal scarring.
Conclusions: These findings suggest that children with community-acquired enterococcal UTI form a high-risk group, characterised by considerable frequency of urinary tract abnormalities, recurrences, renal scarring, and need for surgical intervention. Thus, special attention is required on appropriate antibiotic regimens, early imaging investigation and prophylactic antimicrobial treatment.

P501

Presence of difficult-to-culture organisms in urogenital specimens of healthy men

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Objectives: *Chlamydia trachomatis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, *Trichomonas vaginalis* are considered as the most common causative agents of nongonococcal urethritis in men and have been reported to possibly cause chronic prostatitis syndromes. The aim of this study was to determine the frequency of isolation of *C. trachomatis*, *M. hominis*, *U. urealyticum*, *G. vaginalis*, *T. vaginalis* from urethral swabs (US) and expressed prostatic secretions (EPS) of healthy men.

Methods: In 2003–2004 45 healthy volunteers without symptoms of urethritis, prostatitis and other urinary tract infections as well as with the absence of white blood cells in EPS and who had not taken antibiotics in previous month were included in the study. The Meares–Stamey bacteriologic technique and commercial PCRs (Amplisens, Russia) with primers specific for *C. trachomatis*, *M. hominis*, *U. urealyticum*, *G. vaginalis* and *T. vaginalis* on US and EPS were performed for each person.
Results: All healthy volunteers were negative by Meares–Stamey technique in terms of the absence of culturable uropathogens in EPS. According to PCR data, *U. urealyticum* have been detected in 6 (13.3%) individuals, *C. trachomatis*–in 4 (8.89%), *M. hominis*–in 2 (4.4%), and *G. vaginalis*–in 1 (2.2%) person. *T. vaginalis* have not been detected in the examined healthy volunteers.
Conclusion: *C. trachomatis*, *M. hominis*, *U. urealyticum*, *G. vaginalis* are found in US of asymptomatic men. The most common detectable microorganism was *U. urealyticum*. *T. vaginalis* have not been revealed in the examined asymptomatic volunteers. Frequent detection of difficult-to-culture organisms in healthy men requires further careful investigations of their causative role in urinary tract infections.

P502

Role of *Escherichia coli* adhesins encoded by the eae A, pap, and drae genes in urinary tract infections related bacteraemia

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Objectives: The role of *Escherichia coli* genes in Urinary Tract Infections (UTI) and blood stream infections remains to be well elucidated. In this study we used PCR and RT-PCR for the detection and expression of the eaeA, pap and drae genes, encoding respectively the intimin, P fimbriae and Dr-II adhesin, in *E.coli* from patients UTI and or Bacteremia.
Methods: *E. coli* were isolated from patients with UTI (n = 10), UTI related blood infection (n = 14), non UTI related blood infection (n = 17) and control isolates from sputum and pus (n = 5). Three reference strains harbouring the three genes were used as positive controls. Strain identification of all isolates was done by Random amplified polymorphic DNA (RAPD)analysis.
Results: Our data have shown that the percentage of pap, eaeA, drae genes detected by PCR and RT-PCR, respectively, was as follows in the different study groups: patients with UTI 60%&0%, 100%&80%, 40%&20%; UTI associated blood stream infection 100%&100%, 100%&76.5%, 100%&100%; non UTI associated blood stream infection 100%&78.5%, 100%&35.7%, 100%&100%, and isolates from pus and sputum 0%&0%, 100%, 0%&0%. Genotyping data revealed 36 different RAPD patterns among the 41 isolates, indicating the involvement of multistrains in these infections.
Conclusion: The high prevalence of pap and drae genes in isolates recovered from patients with UTI related and UTI non-related bacteraemia, highlights the potential involvement of these genes in this serious condition.

P503

Serum sensitivity of beta-lactamase-producing *Escherichia coli* strains isolated from urine of nonhospitalised patients

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Objectives: Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* has emerged in many hospitals worldwide, increasing dissemination and long-term carriage of this organism within community.Until recently, most infections caused by ESBL-producing *E.coli* had mostly been described as nosocomially acquired, or nursing home related. Some recent data suggest that infections due to ESBL-producing organisms might be an emergent problem in outpatients in different countries, and that ESBL-producing *E. coli* is an emergent cause of urinary tract infections in nonhospitalized patients. Better characterization of these strains is needed. The aim of this study was to determine the prevalence of ESBL-producing *E. coli* among the strains isolated from urine of outpatients in Zagreb region, and to assess the sensitivity of those strains to bactericidal action of normal human serum.
Methods: During the five-month study period (January to May 2004) a total of 2,451 *E. coli* strains were isolated from urine of nonhospitalized patients with significant bacteriuria. The isolates were screened for ESBL production by a double disk diffusion technique and 39 ESBL-producing strains were collected, as well as 45 randomly chosen non-ESBL-producing *E. coli* strains. Antibiotic susceptibility was determined using a standard disk diffusion NCCLS procedure. Bacterial susceptibility to serum killing was measured by assessing regrowth after incubation in serum according to Schiller and Hatch method.
Results: The prevalence of ESBL-producing *E. coli* was 1.59%. Statistically significant difference in bacterial susceptibility to serum killing was observed between two groups of strains ($p = 0.03$). Out of 45 non-ESBL-producing strains tested, 25 (55.5%) were serum-resistant, while only 12 out of 39 (30.7%) ESBL-producing strains were resistant to serum killing.
Conclusion: The decreased prevalence of serum resistance among ESBL-producing isolates may be related to chronic colonization or recurrent infection of urinary tract.

P504

Uncomplicated urinary tract infections in women: cost-effectiveness of implementing national guidelines in Finland

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Objectives: National, evidence-based guidelines for urinary track infections (UTI) was introduced in Finland in 2000. The aim of this study is to evaluate the cost-effectiveness of implementing the guidelines in the treatment of uncomplicated UTI (occasional lower urinary track infections with no predisposing factors to infection) in women. Cost-effectiveness comparison will also be done with a modelled situation, where adherence to the implemented guidelines would have been even more comprehensive.
Methods: A 5-year randomized trial was conducted in 30 health centres around the country. All women between the ages of 15 and 55 diagnosed with uncomplicated UTI consulting the health centres, for the first time for this episode of illness, during a 1-week period in pooled years 1998–1999 (n = 293) and 2001–2002 (n = 204) were included in this study. Total costs of an infection period were estimated from a societal perspective. The main outcome measures are the health benefits, the costs and the incremental cost-effectiveness ratio. Both direct and indirect costs arising from the infection were prospectively monitored for a period of two weeks. The measure of effectiveness is being both symptom free and having had no health care re-visits or re-medication for UTI during the follow-up period two weeks after the initial consultation.Results:The cost of an uncomplicated UTI infection period stayed relatively similar before and after implementing the guidelines while the percentage of effectively treated patients increased from 65% to 78%, respectively. At this point the modelled scenario seems to have

moderate decreasing effects on the total costs while the effectiveness of the treatment stays around the same level with the actual situation after the implementation of the guidelines.
Conclusion: Implementing national, evidence-based guidelines in the treatment of uncomplicated UTI had positive effects on the treatment effectiveness with minor influence on total costs. The focus of this study was on the short-term effects. Long-term effects, including development of complications and resistant strains, may have an effect the results.

P505

Management on the phone of acute uncomplicated cystitis in women

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Objectives: Acute uncomplicated cystitis (AUC) is a benign illness with no long term medical sequelae, although significantly impacts on quality of life of women. Moreover, behavioural and genetic risk factors can predispose to relapses. European guidelines consider deep clinical history sufficient for AUC diagnosis; further diagnostic examinations are not suggested in health young women. A 3 days antibiotic therapy with cotrimoxazole or fluoquinolones are recommended, according to local bacterial resistance rate. There is evidence that young women with no complicating factors and specific urinary symptoms can be treated efficaciously and safely over the phone without office visit. In the Italian context there is incomplete compliance to AUC guidelines. Aim of this research was to define a diagnostic, therapeutic algorithm to manage AUC on the phone in order to optimize time consumption in GP ambulatory practice, providing appropriateness and patient satisfaction.
Methods: All literature on telephonic management of AUC published between year 1995 and 2004 was critically reviewed: Ovid MEDLINE database was used, with keywords 'telephone management and acute cystitis'.
Results: Six studies regarding telephonic management of AUC in selected women (from 18 to 65 years) in the GP setting were retrieved. The studies showed: standardization of proper prescribing attitudes; clinical outcomes equivalent to the ambulatory practice; reduction of sanitary costs (i.e. ambulatory visit and laboratory tests); higher satisfaction of patients. On this basis an algorithm for telephone management of AUC by GPs in Italy was designed including 4 steps: elegibility of patients (with no complicating factors, for instance fever >38°C, pregnancy, history of relapses or diabetes); informed oral consent; therapy indications; counselling (information regarding possible complications during therapy and prevention of relapses).
Conclusion: Due to literature evidence and cost-efficacy consideration, AUC empirical management on the phone seems a reasonable option within the Italian ambulatory setting. This proposal needs to be confirmed by well designed studies comparing AUC management on the phone with ambulatory practice.

Borrelia

P506

The prevalence of Lyme disease spirochete *Borrelia burgdorferi* sensu lato in ticks (Ixodes ricinus) collected in Lithuania

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The bacterium *Borrelia burgdorferi* belonging to the family Spirochaetaceae is the etiological agent of human infectious disease Lyme borreliosis. The pathogen can be transmitted to humans and animals by various tick species of the genus Ixodes, and in Europe by Ixodes ricinus. *Lyme borreliosis* is the most common tick-borne infectious disease in humans and domestic animals. In Europe, *Lyme borreliosis* is associated with three genospecies: *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*. These species can cause distinct clinical manifestations of Lyme disease. *B. burgdorferi* s.s can cause arthritis, *B. garinii* serious neurological manifestations and *B. afzelii* a distinctive skin condition known as acrodermatitis chronica atrophicans. Each of the three Borrelia species causes characteristic erythema migrants. They cannot, however, be differentiated morphologically.
Objectives: The purpose of this study was to determine the prevalence and genospecies of *Borrelia burgdorferi* s.l. in I. ricinus ticks collected from different Lithuanian regions by molecular genetic methods.
Methods: A total of 1559 ticks (151 nymphs, 777 females and 631 males), collected from 18 regions in Lithuania, were examined for the presence of *Borrelia burgdorferi* s.l. All ticks were analysed individually by the polymerase chain reaction (PCR) with fla gene specific primers. From total amount (1559) the 243 ticks were analysed by multiplex PCRs using genospecies-specific primers for *B. burgdorferi* s.s., *B. garinii* and *B. afzelii*.
Results: We found that 13.4% (209/1559) ticks were infected for *B. burgdorferi* s.l. Females and males were more infected (respectively 113 of 777, 14.5% and 81 of 631, 12.8%) than nymphs (15 of 151, 9.9%). The prevalence in the 18 regions varied from 1–32%. Borrelia genospecies identified were as follows: 66% (25/38) *B. afzelii*, 31% (12/38) *B. garinii* and 3% (1/38) *B. burgdorferi* s.s. Mixed infections were not detected.
Conclusions: The prevalence of *B. burgdorferi* sensu lato was detected in ticks in all regions of Lithuania. The molecular tools allow identifying three Borrelia genospecies which are important for pathogenesis, diagnostic and preventative implications.This study was supported by ENLINO (Environmental Studies in Lithuania and Norway) and the Lithuanian State Science and Studies Foundation.

P507

Detection of neuroborreliosis in patients from Aarhus county, Denmark

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Objectives: To determine the incidence of Neuroborreliosis (NB) in Aarhus County and to examine the relationship between positive intrathecal synthesis of *Borrelia burgdorferi* antibodies (BBAB) and elevated white blood cell count (WBC) in cerebrospinal fluid (CSF). Furthermore to examine the extent of retesting initially BBAB negative CSF in patients suspected of having NB.
Methods: Detection of intrathecal BBAB by parallel CSF and serum analysis using ELISA capture measuring optical density

(OD) has been performed in our laboratory since 1994. The index between the CSF and serum levels was calculated for IgG and IgM respectively and indexes > 0.3 and/or individual IgG or IgM levels in CSF OD > 1.0 were recorded as a positive test. Data obtained from the computer systems used in Clinical Microbiology (MADS) and Clinical Biochemistry (LABKA) was analysed for all patients having had a test for NB.

Results: Since1997 test activity has been found to be stable with an average of 370 patients tested each year. The positive rate was also found to be stable at about 5% when excluding retesting of known positive cases. About 18 new cases were detected per year. This corresponds to an incidence of 3/100,000/year. From 2001 to 2003 53 new cases were detected, 87% of these had biochemical tests performed on CSF of which 96% showed elevated WBC in CSF (median: 105 mill/L, range: 10–2790 mill/L). In 53 randomly selected BBAB negative patients from the same period, 83% had biochemical tests of CSF, of these 25% showed elevated WBC (median 14 mill/L, range 6–139 mill/L). Of at total of 2842 patients with negative tests 55 (1.9%) were retested within 3 months. One of these was positive.

Conclusion: The incidence of NB in Aarhus County corresponds to the expected national level. The absence of elevated WBC in CSF is a strong predictor of not having NB. Patients with elevated WBC in CSF and negative intrathecal BBAB synthesis should be retested within 2–4 weeks if NB is still suspected. The current rate of retesting BBAB negatives therefore seems insufficient.

P508

Evaluation of a Bayesian decision support system for diagnosis of Lyme disease

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Objective: Evaluation of a Bayesian network constructed to calculate the probability of Lyme disease, adjusted to individual patient characteristics. The system is designed to give a probability of Lyme disease, based on facts from the scientific literature, local clinical prevalence and laboratory data.

Methods: A dataset of 100 Danish patients randomly extracted from a larger survey of the clinical use of Lyme disease serology was presented to two specialists in clinical microbiology on a standardized form together with free text comments on some of the patients. Each case of possible Lyme disease was scored on a 5-step ordinal scale and compared to the output from the Bayesian decision support system. Furthermore a user evaluation of the decision support system was conducted by two general practitioners.

Results: The two experts had a high rate of agreement (92% complete agreement or a difference of one score). 80% of the patients were scored as having no or a low probability of Lyme disease. The average score of the decision support system was in agreement with the experts. But some patients were assessed differently by the bayesian system, as the clinical expert may consider additional more subtle clinical information (in this setup the free text comment). In the user evaluation the general practitioners were positive about the decision support system, and suggested that it be used for educational purposes, as they felt it impractical to use it in the daily routine.

Conclusion: The decision support system did perform quite well on the average, but the 'human' experts add an individual assessment, based on more subtle patient characteristics. However the system is only designed to calculate the average probability of Lyme disease in subgroups of patients with certain characteristics and thus support an evidence based diagnostic decision. The final diagnosis has to be made by the clinician only after considering the complete history and examination of the patient. There are very few studies published on clinical evaluation of expert systems. The data from the present study will be used for further development of the expert system and prepare for a prospective clinical evaluation. If an expert system is to be used in everyday busy clinical life, integration into the electronic health care record is necessary. As a stand-alone system it may be used for educational purposes.

P509

Characteristics of children with clinical suspicion of Lyme neuroborreliosis

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Objectives: The clinical diagnosis Lyme neuroborreliosis and the differential diagnosis of other neurological syndromes in children is a challenge. The objective is to describe symptoms suggestive of Lyme neuroborreliosis and testing for *Borrelia burgdorferi* s.l. intrathecal specific antibody production (BbISAP).

Methods: Retrospective evaluation of the patient record: Group 1. All 29 patients tested for BbISAP (2001–2003) at the pediatric department. Patients were scored by a specialist in Neuropediatrics. Group 2. 44 patients selected with discharge diagnoses of Lyme disease, Bells paresis or headache and who were tested for BbISAP (1996 to 2001).

Main Results: Group 1. 29 patients (81%) did not have neuroborreliosis, 3(8%) had a definite diagnosis with positive BbSiap and the remaining 4(11%) were scored as possible. Group 2. Looking at the initial symptoms in the 17 patients with positive BbISAP: facial paralysis 5, non specific symptoms (headache 5, fever or tiredness 5), meningeal signs 1 and erythema migrans with pain in one arm1 patient. 3 more developed facial paralysis on admission. Concerning sequelae at follow up, 7 patients had none, 3 had light signs of facial paralysis after 3, 6 and 12 months respectively, 1 patient had irritability, speech problems and wrist pain at 18 months. 6 patients were not seen after discharge. In both groups patients with positive BbISAP had a mean leukocyte count of 198 × 106/l (range 13–832)

Conclusion: All patients with positive BbISAP also had elevated leukocyte count in the spinal fluid in both patient groups. Non-specific symptoms as headache, fever and tiredness seem to be common at the start of clinical neuroborreliosis. 4 of 17 (25%) patients had some remaining symptoms many months after discharge.

P510

Contribution to post-Lyme syndrome: the frequency of symptoms in long-term outcome of patients with neuroborreliosis

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Objective: The 'post-lyme' syndrome is a complex of symptoms that can be found in patients after having adequately been treated for *Lyme borreliosis*. The most frequent symptoms include: fatigue, headaches, usually mild cognitive deficit, joint and fibromyalgias and yet, nevertheless, the complaints can persist for months to years, while typically no direct laboratory hallmarks of infection can be found. Repeated antibiotic treatment often does not result in clinical improvement. Because the patients are repeatedly examined and treated by different medical specialists the post-lyme syndrome creates a relative consumptive medical problem.The long-term outcome of a prospective organised study of patients with neuroborreliosis was used to assess the incidence of possible post-lyme syndrome in this type of Lyme disease involvement.

Methods: 88 patients with early forms of neuroborreliosis (diagnosis based on the typical neurological involvement and positivity of either antibody index CSF/serum or PCR in CSF) were evaluated for late outcome (1–6 years; average 35 months). All patients were treated intravenously by antibiotics in hospital.
Results: Subjective complaints of patients disappeared till 6 months after treatment in 42 patients (49%), till 1 year in the next 10 (8%) and till 3 years in 3 (2.5%). 33 patients (37.5%) had problems longer than 3 years. The most frequent symptoms in this group were: headache (65 patients, 74%), psychic problems (tensions, depression, psychic concentration disturbances in 61 patients, 69%), articular pains (33.38%), muscle pains (20.23%), cognitive deficit (14.17%). Notwithstanding the frequency and duration of subjective troubles, the objective neurological involvement disappeared substantially more quickly. Immediately after treatment neurological status was considered normal in 49 patients (56%) whereas, after 6 months, neurological normalisation was found in 72 of those tested (82%) and in another 11 people only very mild residues existed.
Conclusion: The genesis of 'post-lyme' syndrome could be expected in one third of patients with correctly treated neuroborreliosis. Because all patients connected their complaints with their history of Lyme disease, better knowledge on the part of medical staff is necessary and new diagnostic tools (DNA based) are desirable. It is necessary to realise that repeated examination and anti-infective treatment is expensive and less effective.

P511

Genospecies of *Borrelia burgdorferi* in patients with erythema *migrans*

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Objectives: *Borrelia burgdorferi* sensu lato(B.b.), a causative agent of Lyme disease (L.d.) is a heterogeneous group of spirochetes. Clinical manifestations of L.d. are genospecies dependent, and the most common form of early localized L.d. is erythema migrans (E.m). This study was performed to determine genospecies of B.b.in patients with E.m.
Methods: Thirty two patients from West Pomerania region in Poland with final diagnosis of E.m. were admitted to study after antibiotic treatment. Blood samples of patients were tested to detect genospecies of B.b. using genospecies-specific primers for DNA of *Borrelia garinii*, *Borrelia afzeli* and B.b. sensu stricto.
Results: The spirochetes DNA were found in 25 blood specimens (25/32, 78.1%), seven were negative. Among 25 positive samples, ten contained *B. garinii* DNA (20/25, 40%), five-*B. afzelii* (5/25, 20%), four-B.b sensu stricto (4/25, 16%)and in six samples both *B.garinii* and *B. afzelii* DNA was present (6/25, 24%).
Conclusions: 1. DNA of B.b. can be detected in patients with E.m. after antibiotic treatment. 2. The dominant genospecies of B.b. among patients with E.m. from West Pomerania region is *B. garinii*. 3. In patients with E.m. multi genospecies infection can occur.

P512

Serological diagnosis of culture confirmed early Lyme disease (erythema migrans) by two different EIAs and three Western Blot tests in an Italian population

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Objectives: To comparatively evaluate the performances of two different EIA methods and three Western blot tests for the diagnosis of *Lyme borreliosis*.
Methods: 60 sera were serially obtained from 30 Italian patients suffering from acute culture confirmed Lyme disease. All the patients received antimicrobial therapy at the enrollment: the follow up period was 1 month. 65 cross-reacting serum specimens were also evaluated. The following EIAs were used: Anti-Borrelia plus VlsE (Euroimmun) and Quick ELISA C6 (synthetic peptide based from Immunetics). As confirmatory methods, three different Western blot (WB) tests were used: Euroline-WB (Euroimmun), Qualicode *B.burgdorferi* WB (Immunetics) and a multispecies 'home made' test.
Results: The highest sensitivity was found for Anti-Borrelia plus VlsE IgG (56.6%) whereas the sensitivity of Quick C6 was lower (33.3%). In addition, since 17 sera from the very early disease were positive only for the IgM response when tested by Anti-Borrelia plus VlsE IgM, this test performed as a whole system with a sensitivity of 85.0%. The specificity of Anti-Borrelia plus VlsE was 98.5% for the IgG and 78.5% for the IgM: these values suggested the need of a confirmatory testing by WB. The three WB methods showed comparable performances for the detection of the IgM. On the other hand, the WBs performed differently for the IgG detection: the most sensitive was the 'home made' method (71.7%), ranking at second place is the Euroline-WB (68.3%). The Qualicode test was the least sensitive (26.6%). The specificity of the 'home made' and of Qualicode tests was 100%, whereas the Euroline WB IgG identified 12 cross-reacting specimens as border line, giving a specificity value of 80.0%.
Conclusion: The Anti-Borrelia plus VlsE ELISA showed to be a better test than the Quick C6 but it still need to be followed by a confirmatory test in any case of positivity. The need of WB still remains in Europe, where the etiology of Lyme disease is complicated by more than one pathogenic genospecies.

P513

Low-avide IgG antibodies against the newly discovered Borrelia main antigen VlsE correlate to early stages of borreliosis

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Objectives: In various infectious diseases, the detection of low-avide IgG is used to differentiate early from late stages of the disease. To date, such information for infections with borrelia is limited. In the present study, the avidity of serum IgG antibodies against the newly identified borrelia main antigen VlsE was tested in recent and long-lasting borrelia infections.
Methods: The EUROIMMUN 'Borrelia EUROLINE-WB', a combination of a western blot, containing a whole extract of *Borrelia afzelii* plus a membrane chip with a printed line of recombinant VlsE was used to incubate 28 clinically characterized sera from the CDC Lyme panel according to the standard protocol (30 min serum, 3 × 5 min tris buffered saline (TBS), 30 min Anti-human IgG/alkaline phosphatase, 3 × 5 min TBS, 10 min substrate NBT/BCIP). In a parallel incubation, in the first of the three washing steps after the serum incubation, 8 M urea was added to the TBS. Intensity of the VlsE band was automatically evaluated using the computer programme EUROBlotScan. A relative avidity index (RAI) was calculated as follows: RAI(%) = intensity VlsE band (urea)/intensity VlsE band (without urea) × 100.
Results: In erythema migrans patients (early borreliosis, n = 19), 79% of the sera displayed an RAI of 0–40%, 11% exhibited an RAI in the range of 40–70%, and 10% an RAI higher than 70%. In borrelia arthritis patients (late stage of borreliosis, n = 9), no serum displayed an RAI of 0–40%, 44% showed an RAI in the range of 40–70% and 56% had an RAI higher than

70%. Two sera from patients with erythema migrans contained high-avide antibodies, possibly caused by a reinfection.
Conclusions: Low-avide antibodies of class IgG against VlsE appear in early borreliosis. In later stages, the antibodies mature and the RAI increases. The EUROLINE-WB system enables the diagnosis of fresh borrelia infections by identification of low-avide antibodies. In order to verify the significance of avidity-testing in borreliosis, larger, more representative serum panels have to be analysed.
Acknowledgement: We thank Dr. Martin Schriefer from CDC Atlanta for kindly providing the sera.

P514

Evaluation of an internally controlled real-time PCR targeting the OspA gene for detection of *Borrelia burgdorferi* (sensu lato) DNA in cerebrospinal fluid

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Objectives: PCR on skin biopsy specimens has a high sensitivity when used for the diagnosis of erythema migrans and Lyme arthritis. In neuroborreliosis, however, the sensitivity of standard PCR tests on CSF samples is only 10–30%. The aim of the study was to evaluate the sensitivity of the real-time PCR for CSF samples.
Methods: An internally controlled real-time PCR targeting the OspA gene was developed using *Borrelia burgdorferi* (sensu lato) specific primers and Taqman probe. DNA from *B. burgdorferi* and an internal control (Phocine herpes virus) were amplified and detected simultaneously using probes labelled with 6-carboxy-fluorescein (FAM) and indodicarbocyanine (Cy5) respectively. Analytical sensitivity was evaluated on DNA extracted from 33 *B. burgdorferi* sensu lato strains, including *B. burgdorferi* sensu stricto (n = 6), *B. garinii* (n = 12), *B. afzelii* (n = 9), *B. valaisiana* (n = 4), *B. japonica* (n = 1) and strain A14S. Specificity was evaluated with DNA extracted from relapsing fever (RF) Borrelia sp. (n = 4), belonging to all 3 phylogenetic clusters, and on 31 viral, bacterial and fungal samples. Quantitative sensitivity was determined using limiting dilution series of known DNA concentrations. Subsequently, cerebrospinal fluid (CSF) samples were spiked using limiting dilutions of freshly cultured *B. garinii*. DNA was extracted from CSF samples by QIAamp DNA mini blood kit columns. In addition, three CSF samples from culture-positive patients as well as 13 samples from other patients with neuroborreliosis in which culture was not done were tested in the assay.
Results: The real-time PCR detected DNA of all *B. burgdorferi* (sensu lato) species and was negative for DNA from RF Borrelia sp. and all other specimens. The sensitivity of the assay was 1–5 spirochetes. In spiked CSF samples the sensitivity was not diminished. Two out of three culture-positive CSF samples were positive in the PCR, whereas the third sample was inhibited. Two out of 13 samples from other patients with neuroborreliosis were positive in the PCR.
Conclusion: The real-time PCR assay developed in this study provides sensitive and specific detection of all *B. burgdorferi* sensu lato species tested. The QIAamp mini blood kit columns are suitable to extract Borrelia DNA from CSF, and combination with real-time PCR provides a sensitive assay. However, even when using this assay the yield from CSF samples from patients with neuroborreliosis is low.

P515

Impact of long-term antibiotic therapy on symptoms evocative of chronic lyme disease

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Objectives: chronic lyme disease (CLD) could be partly due to the persistence of Borrelia. The aim of our study is to determine the effect of long term antibiotic therapy on heterogeneous symptoms evocative of CLD.
Methods: 100 patients (pts) (65% female, mean age 45 y) with a diagnosis of CLD were included in an open study. A clinical score was designed based on the following items: erythema migrans (56% of cases), positive serology for Borrelia (51%), tick bite (69%), combination of categories of signs or symptoms: systemic (88%), neurologic (94%), articular (91%), cutaneous (76%), psychiatric (77%), cardiorespiratory (73%) or muscular (67%). Diagnosis was classified as very probable (67%), probable (25%) or uncertain (8%), according to the clinical score. An antibiotic therapy was given for 3 to 6 months (penicillin G, ceftriaxone, amoxicillin, doxycycline or clarithromycin). The number (No) of subjective symptoms (SS) and objective signs (OS) was measured at day 0 (D0), month 3 (M3) and M6.
Results: The No of pts with 4 or more categories of signs or symptoms was 82% at D0, 34% at M3 and 31% at M6. The mean No of SS was 12 at D0, 6 at M3 and 5 at M6. The mean No of OS was 2.7 at D0, 1.4 at M3 and 1 at M6. The differences were significant for very probable or probable cases ($p < 0.001$).
Conclusion: This study shows an important improvement of the clinical conditions of pts with CLD treated with a prolonged course of antibiotic. A controlled randomized trial with a strict case definition and a follow up longer than 3 months is needed.

Antibiotic usage

P516

Antibiotic use and resistance in Gram-negative hospital pathogens

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Objectives: To determine the relationship between use of different antibiotics and resistance patterns in hospital gram negative pathogens.
Methods: In early fall, each hospital in Ontario was mailed a survey requesting information about antibiotics included in their formulary, their in-hospital use of specific antimicrobials and their resistance rates in a number of hospital pathogens. A representative from the pharmacy and microbiology laboratory servicing each hospital was identified and served as the contact for obtaining ethics approvals and collecting requested data.
Results: To date, 5 hospitals (3 acute care tertiary centres, 1 community hospital and 1 chronic care facility) have completed the survey. The median DDDs/1000 patient days varied for each drug between the reporting hospitals; ciprofloxacin 51.2 (range 11.2–107.6), ampicillin 24 (range 0–44.9), trimethoprim/sulfamethoxazole 7.8/39 (range 1.7/8.3–36.1/180.4), ceftriaxone 14.8

	Ampicillin Median (range)	Ceftriaxone Median (range)	Ciproflox Median (range)	SXT Median (range)	Imipenem Median (range)
E.coli	58 (52-68)	97 (92-98)	80 (77-84)	73 67-74)	-
Klebsiella spp.	-	97 (87-100)	94 (88-100)	90 (85-94)	-
P.aeruginosa	-	10 (0-21)	94 (88-100)	-	87 (72-93)

(range 0.9–77.5). Four of the 5 hospitals reported antibiotic use data for three years. In 2003, 2/4 reported an increase in ceftriaxone use over that of 2001 (71.9 vs 77.5 and 0.4 vs 0.9 DDDs/1000 patient days), 2 reported a modest increase in ciprofloxacin use (49.6 vs 51.2 and 11.7 vs 11.9 DDDs/1000 patient days). Only one facility reported an increase in more than one type of antibiotic. Although the medians and ranges of resistance rates varied (shown in table below), they did not appear to correlate with the DDD use data reported for the respective antibiotics.
Conclusions: Although antibiotic use and resistance varied between the reporting hospitals, the sample was not adequate to determine correlations between the two for the gram negative hospital pathogens reported.

P517

Outpatient utilisation of antibiotics in Zagreb (Croatia) 2001–2003

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Objective: To analyze outpatient utilization of antibiotics in Zagreb during a 3-year period (2001–2003) using the Anatomical-Therapeutic-Chemical (ATCD) drug classification system and defined daily doses (DDD).
Method: Data on outpatient drug utilization were obtained from Zagreb Municipal Pharmacy, its units accounting for 16% of all city pharmacies, and extrapolated to total number of city pharmacies. All drugs were classified according to ATC drug classification system. Utilization data on each individual drug were expressed as the number and size of packages, and as the cost expressed in HRK based on wholesale price. These data were used to calculate the number of DDD and DDD per 1000 inhabitants per day (DDD/TID).
Results: From 2001 to 2003, the utilization of systemic antibacterials (J01) decreased by 18.18%(from 55 to 45.01 DDD/TID); in all three years, the highest consumption was recorded for penicillins (J01C), which accounted for a half of total antibiotic utilization (25.4, 24.43 and 20.87 DDD/TID in 2001,2002 and 2003, respectively), showing a decline by 17.83%. Cephalosporins (J01D) ranked second in all three years, showing a decline by 33.46% from 2001 to 2003. Macrolides (J01F) ranked third; from 2001 to 2003 their consumption decreased by 17.68%, from 6.9 to 5.68 DDD/TID; quinolones (J01M) ranked fourth and showed a 7.22% increase (from 3.6 to 3.88 DDD/TID). In all three study years, amoxicillin+clavulanic acid was the most commonly prescribed antibiotic (14.7,17.25 and 14.64 DDD/TID in 2001, 2002 and 2003)followed by amoxicillin (7.3, 6.84 and 5.93 DDD/TID in 2001, 2002 and 2003, respectively). Cefurohime ranked third in 2002 and 2003 with 5.28 and 4.56 DDD/TID, respectively, whereas in 2001 ceftibuten ranked third to decline to seventh place according to utilization in 2002 and 2003. In all three years, doxycycline ranked fourth, and azithromycin fifth according to utilization in Zagreb.
Conclusion: Utilization of antibiotics in Zagreb is uneconomical, mostly due to the high use of amoxicillin+clavulanic acid, which has no rational ground in professional guidelines. The analysis showed expensive antibiotics to be largely prescribed in Zagreb representing 50% of total national drug utilization and pointing to the need of efficient measures to make the utilization of antibiotics in Croatia more rational.

P518

Antimicrobial prescribing pattern in primary care: a comparison between general practitioners and family physicians in Bahrain

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Objectives: The aim of this study was to determine antimicrobial prescribing pattern in primary care and to find out whether prescribing pattern is influenced by physicians' training background.
Methods: A retrospective prescription-based study was carried out in 17 out of 20 primary health care centres in Bahrain. Prescriptions (n = 4367) were collected randomly during a weekday in July 2003.
Results: Systemic antimicrobials ranked the fourth most commonly prescribed therapeutic classes of drugs. The top-ten most commonly prescribed antimicrobials, in descending order, were amoxycillin (14.3%), cephalexin (5.3%), erythromycin (1.1%), cloxacillin (0.8%), metronidazole (0.8%), ciprofloxacin (0.5%), cefadroxil (0.4%) cotrimoxazole (0.3%), tetracyclines (0.3%), norfloxacin (0.1%) and procaine penicillin (0.02%). Amoxycillin, cephalexin, erythromycin and cotrimoxazole were prescribed by general practitioners (GPs) more often than by family physicians (FPs) ($p < 0.05$). With respect to prescribing of other antimicrobials and anthelmintic mebendazole the differences between GPs and FPs were nonsignificant. Topical antimicrobial preparations for ear and nose infections were prescribed by GPs in a rate significantly higher than by FPs ($p < 0.05$); of these topical antimicrobials, chloramphenicol and locacorten vioform (flumethasone + clioquinol) ear drops and sulphacetamide eye drops were more often prescribed by GPs ($p < 0.05$). There were no significant differences in prescribing patterns between GPs and FPs as regards various topical antimicrobials used for oropharyngeal, skin, and vulvo-vaginal infections.
Conclusion: Antimicrobial prescribing differences in primary care were observed between GPs and FPs. The generalizability of these findings is uncertain. Judicious use of antimicrobials should be encouraged to reduce the dramatic increase in the prevalence of resistant microorganisms worldwide.

P519

Antibiotic usage in a university hospital in a country with a low prevalence of antimicrobial drug resistance

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Objectives: To describe the overall usage of antibiotics over two decades in Aarhus University Hospital (AUH). To compare the use of antibiotics with the patterns of antimicrobial drug resistance in micro-organisms causing blood-stream infections in AUH.
Methods: Data on micro-organisms causing blood-stream infections from 1981 to 2004 were compiled from the microbiological computer system MADS at AUH. Data on antibiotics were obtained from the hospital pharmacy. Three different blood culture systems were used during the period, BacT/ALERT® since 1994. Sub-cultivation and identification was done according

to standard methods. Unit of measurement for antibiotic use is DDD (Defined Daily Dose).
Results: At AUH the yearly use of antibiotics increased 30% from 1981 to 2004. Expressed as DDD per bedday, however, the use of antibiotics more than tripled during the period and amounted to 0.55 DDD/bedday in 2004. For the periods 1981–1985 and 2000–2004, the relative distribution of the usage in DDD for the various antibiotics was penicillins (76% vs. 66%), cephalosporins (3% vs. 14%), aminoglycosides (7% vs. 4%), quinolones (0% vs. 9%), macrolides (6% vs. 7%), and glycopeptides (1% vs. <1%), respectively. Use of cephalosporins and quinolones increased substantially in the ICU. MRSA was found in <0.1% of blood-stream infections with *S. aureus* from 1981–2000, but in about 3% for 2001–2004. Ampicillin resistance in *E. coli* increased from 27% to 44 % during the period, whereas gentamicin resistance was found in <2% of *E. coli* from 2000–2004. Candida species in blood cultures increased more than 10 times over the years and ranked first among micro-organisms isolated in the ICU in 2003.
Conclusion: AUH is still a hospital with a modest prevalence of drug resistance and use of antibiotics. Changes in relative distribution of antibiotics (marked increase in use of quinolones and cephalosporins) in the latter years seem to coincide especially in the ICU not only with a selection of MRSA but also in the distribution of micro-organisms causing blood-stream infections. Monitoring drug resistance and use of antimicrobial agents in the hospital is a prerequisite for choice of adequate empiric treatment of blood-stream infections and also for an evaluation of the antibiotic policy.

P520

Effect of aminoglycoside/beta-lactam combination therapy versus beta lactam monotherapy on the emergence of antimicrobial resistance: a meta-analysis of randomised controlled trials

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Background: The addition of an aminoglycoside to a beta-lactam has been suggested to have a beneficial effect in delaying the development of antimicrobial resistance.
Objectives: To study the effect of aminoglycoside/beta-lactam combination therapy versus beta-lactam monotherapy on the emergence of resistance.
Design: Meta-analysis of randomized controlled trials (RCTs).
Data sources: Data for this meta-analysis were identified from PubMed, Current Contents, Cochrane central register of controlled trials, and references from relevant articles.
Study selection: RCTs comparing aminoglycoside/beta-lactam combination therapy with beta-lactam monotherapy and reporting data regarding the emergence of resistance (primary outcome) and/or development of superinfection, treatment failure, treatment failure attributable to emergence of resistance, treatment failure attributable to superinfection, all cause mortality during treatment, and mortality due to infection.
Data extraction: Data for one primary and six secondary outcomes were extracted by two investigators.
Data synthesis: A total of 9 RCTs were included in the analysis. Beta-lactam monotherapy was not associated with more emergence of resistance compared to the aminoglycoside/beta-lactam combination (OR = 0.91; 95% CI 0.55–1.49). Actually, beta-lactam monotherapy was associated with fewer superinfections (OR = 0.62; 95% CI 0.41–0.93) and less treatment failure (OR = 0.65; 95% CI 0.49–0.86). Treatment failure attributable to emergence of resistance (OR = 1.56; 95% CI 0.59–4.09), treatment failure attributable to superinfection (OR = 0.72; 95% CI 0.31–1.64), all cause mortality during treatment (OR = 0.70; 95% CI 0.40–1.25), and mortality due to infection (OR = 0.74; 95% CI 0.45–1.21) did not differ significantly between the two regimens.
Conclusions: Compared to beta-lactam monotherapy, the aminoglycoside/beta-lactam combination was not associated with a beneficial effect on the development of resistance of initially sensitive isolates.

P521

Cefuroxime as antibiotic prophylaxis in cardiac surgery

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Introduction: Infectious complications have a major impact on the surgical patient and are associated with substantial morbidity and mortality. For this reason, antibiotic prophylaxis is widely used in cardiac surgery in order to reduce the postoperative infection rate.
Objectives: To elucidate the prophylactic efficacy of a second generation cephalosporin given as single dose for patients who are submitted to Coronary Artery Bypass Grafting after 10 years of constant use.Methods: Compared were two groups of patients. The first group consisted of 429 patients who underwent CABG in 1994 and the second group of 803 patients who were submitted to CABG in 2003. All patients received cefuroxime intravenously as single dose (3 gr) at the induction of anaesthesia. Patients with pre-existing infection or receipt of antibiotics during the last 2 days before surgery, with hypersensitivity to cefuroxime or pre-existing IABP were excluded from this study.
Results: The overall postoperative infection rate was 4.9% in 1994 (21/429) and 5.6% in 2003 (46/803). Respiratory tract infections were 2.3%(n:10) in 1994 and 0.6%(n:5) in 2003 while incisional or deep surgical site infections were 0.7%(n:3) in 1994 and 3.2%(n:26) in 2003. In 1994 catheter-related infections were 0.93%(n:4) vs. 0.87%(n:7) in 2003, urinary tract infections 0.47%(n:2) vs. 0.25%(n:2), infective endocarditis 0.23%(n:1) vs. 0.12%(n:1) and sepsis 0.23%(n:1) vs. 0.62%(n:5). In both periods of time, the predominant pathogens were staphylococcus species (aureus and epidermidis), Gram positive cocci, Gram negative bacilli and fungi.
Conclusions: The increase in surgical site infections is due to a more meticulous surveillance of postoperative infections in the last years as only one case required a sternum revision and debridement and the remaining patients had only a minor infection of the sternal or donor site incision. A single-shot of cefuroxime prophylaxis (3 gr IV) given just after the induction of anaesthesia remains effective throughout the years, despite its continuous use confirming that single dose regimen with a second generation cephalosporin is the method of choice.

P522

Evaluation of antibiotic consumption and resistance patterns in a tertiary care surgical ICU and a medical ICU

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Objectives: A continuous and critical analysis of antibiotic consumption and a knowledge of resistance patterns are essential for the control of health care expenses and microbial resistance, and can additionally be of help in targeting the necessary interventions. The aims of this work were to reveal the characteristics and trends in parenteral, systemic antibiotic use and to

gain an insight into the most important resistance patterns in a 12-bed medical ICU and a 6-bed surgical ICU between 1999 and 2003.
Methods: The annual quantities of antimicrobials delivered to the units during the study period were determined retrospectively from the central pharmacy's database. Antibiotic usage was converted into grams and finally expressed as defined daily doses (DDD) per 100 bed-days. Microbiological data were screened for duplicates and only relevant cultures were taken into account.
Results: Our results can be summarized in the following table:

DDD/100bed-days

	Systemic anti-bacterial		Penicillins		Cephalo-sporins		Carba-penems		MLS		Ami-nogly-cosides		Quinolones		Glyco-peptides	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1999	71.8	137.6	29.5	39.2	6.6	42.7	7.4	6.2	0.3	1.8	8.3	4.7	7.4	18.8	10.2	6.4
2000	69.3	133.7	19.9	44.0	8.9	30.8	9.4	7.4	1.2	5.0	4.5	4.2	6.5	16.9	15.6	12.1
2001	99.2	164.8	19.3	32.4	10.5	47.8	17.2	18.3	4.2	3.7	5.6	6.7	9.7	15.8	30.3	28.0
2002	99.5	153.5	22.6	30.4	7.9	32.9	14.3	25.3	1.6	2.5	5.3	10.4	14.5	13.4	32.6	31.1
2003	78.9	11.9	26.0	28.7	8.9	40.9	12.5	15.6	0.6	2.7	7.7	1.5	13.1	6.8	9.6	9.4

1. Medical ICU
2. Surgical ICU

	S.aureus/ MRSA		CNS/MRCNS		KES/3rd generation cephalosporin R KES		*P. aeruginosa*/ imipenem R *P. aeruginosa*		*E. coli*/ ciprofloxacin R *E.coli*	
	1	2	1	2	1	2	1	2	1	2
1999	57/0	18/1	44/24	30/26	16/1	26/7	12/4	40/11	43/1	16/0
2000	61/1	19/0	54/30	17/11	24/1	26/1	21/4	25/1	65/5	26/3
2001	52/1	14/0	63/33	31/22	18/2	35/3	46/9	22/2	66/2	19/3
2002	62/0	39/0	89/47	47/34	19/1	40/5	43/2	34/9	63/14	24/1
2003	61/5	47/4	68/37	40/27	10/1	63/11	32/7	24/1	61/14	37/0

1. Medical ICU
2. Surgical ICU
VRE and MDR Acinetobacter was not found

Conclusions: As concerns the antibiotic consumption, positive trends could be observed both in quantity and in quality in both settings. Dangerous resistances have newly emerged (e.g. MRSA) or increased in number (e.g. MRCNS). However, these findings did not attain an alarming level. Local susceptibility patterns should be taken into account in empiric antibiotic therapy.

P523

Self-medication with antibiotics in Poland

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Objectives: The study was undertaken as a part of the Self-medication with antibiotics in Europe (SAR) founded by DG SANCO and coordinated by the University Groningen, Netherlands. The aim of the study was to characterise utilisation of antibiotics in Poland in order to evaluate the prevalence, reasons of self-medication, sources and structure of antibiotics used for self-medication.
Methods: The survey was conducted using a self-administered postal questionnaire obtained from SAR coordinating centre. The outcome measures were: reported use of antibiotics in the last 12 months, prescribed or used for self-medication. Self-medication assessment included intended use and storage of antibiotics at home. Study population: adult population selected from region with average age and sex distribution in a country. The randomly selected sample consisted of 1500 individuals from a middle size city (population between 150000 and 250000) and 1500 from a rural area (population between 5000 and 10000), both in northern part of Poland.
Results: The overall response rate was 31%, without significant difference between city and rural populations. Overall, 29% of respondents indicated to use antibiotics in the last 12 months. The most common reasons were: acute laryngitis/tracheitis, acute bronchitis/bronchiolitis, teeth/gum symptom/complaint, and cough, skin infection and urethritis. The most frequently used antibiotics were: amoxicillin, amoxicillin/clavulanic acid, doxycycline, clindamycin, sulfamethoxazole/trimethoprim. Inspite of all antibiotics for systemic use in Poland being currently available only on prescription, 12.5% of respondents indicated its use as self-medication without prescription. The main reasons for using antibiotics as self-medication were: sore throat, runny nose/cold, cough and fever. The main sources of antibiotics used for self-medication were: leftovers from previous illness available at home and antibiotics intended from relatives or friends. Overall 18% of the respondents indicated to keep antibiotics at home during the study period.
Conclusions: Although all antibiotics for systemic use are available in Poland only with medical prescription, the self-medication with antibiotics occurs in substantial proportion of population. There is a need for community directed interventions to limit the use of antibiotics without adequate medical attention.

P524

Antibiotic usage peculiarities in Lithuanian hospitals revealed by national prevalence study

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Objectives: Study antibiotic usage in Lithuanian hospitals and identify week points of antibiotic consumption monitoring system. Monitoring antibiotic consumption is one of the key activities in combating antimicrobial resistance. Lithuania is one of the few countries in Europe that is just starting to obtain antibiotic consumption data, primarily from hospitals.
Methods: Point prevalence study in representative number of Lithuanian hospitals was performed. Data on current antibiotic treatment (antibiotic name, route of admission, reason) as well as on infections were collected for all hospitalized patients during bed rounds. All antibiotics were recorded: those offered by hospital as well as those prescribed by doctors but bought by patients themselves (last mentioned drugs are not included in official hospital's consumption data). The study included 10102 patients in 39 hospitals.
Results: One third of patients were given antibiotics (3140 patients, 31.1%) and in 77.9% of cases it was monotherapy. In general, 3943 antibiotics were prescribed and most of them (74.8%, 2948) were prescribed for the treatment of infections while 24.8% (979) as prophylaxis (unknown - 0.4%, 16). Most often used were penicillin (23.4%), aminopenicillins (19.2%), aminoglycosides (21.2) and cephalosporins (15.5%). When structure of antibiotic was compared with antibiotic consumption data officially obtained from hospitals some differences were detected. During our study it was determined that 6.2% of all used antibiotics were obtained by patients themselves (these are not coming in official consumption data). Great variations between hospitals and units in this respect were observed. There were 5 hospitals where more than 20% (up to 50%) of antibiotics were bought by patients. More often patient's own antibiotics were used in surgical departments: orthopaedic/trauma (12.9%), general surgery (7.3%). It was determined that antibiotics for prophylaxis (12.3%) are tend to be more often bought by patients than for treatment (4.0%). It also was found out that patients more often are asked to buy certain antibiotics: III–IV gen. cephalosporins (27.8%), quinolons (18.4%), I–II gen. cephalosporins (13.7%).
Conclusion: Antibiotic consumption monitoring based only on data from hospital pharmacies might be inaccurate, especially for prophylactic usage, total consumption in surgical departments and some certain drugs (III–IV gen. cephalosporins, quinolons).

P525

An additional measure for quantifying antibiotic use in hospitals

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Objectives: The number of Defined Daily Doses (DDD) per 100 patient days is often used as an indicator for the selection pressure exerted by antibiotics in the hospital setting. This unit of measurement does, however, not fully describe the selection pressure and is sensitive to changes in hospital resource indicators. Additional information is required to facilitate interpretation of this indicator. The number of DDD per 100 admissions could be a valuable additional tool. The aim of this study is to investigate the importance of units of measurement in quantifying antibiotic use data with regards to antibiotic resistance risks.
Methods: Trends in antibiotic use in acute care Dutch hospitals between 1997–2001 were studied. Antibiotic use was expressed in DDD per 100 patient days and in DDD per 100 admissions.
Results: From 1997 to 2001 total systemic antibiotic use significantly increased from 47.2 to 54.7 DDD per 100 patient days whereas expressed in DDD per 100 admissions it remained constant. For some individual antibiotics increases in DDD per 100 patient days were not accompanied by increases in DDD per 100 admissions and vice versa. The average length of stay and the number of admissions decreased between 1997 and 2001.
Conclusions: Correction for variation in resource indicators and additional expression of the data in DDD per 100 admissions is imperative for a meaningful understanding of observed trends in antibiotic use expressed in DDD per 100 patient days. Further research is needed to determine the correlation between different measures of antibiotic use and the level of antibiotic resistance.

P526

Antibiotic prescribing on general surgical wards – room for considerable improvement

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Objectives: The inappropriate use of antibiotics leads to the emergence of resistant organisms, causes unwanted adverse effects and results in unnecessary costs for the health services. Attempts to improve prescribing habits of doctors have been largely unsuccessful. This study was undertaken to determine what could be done to improve antibiotic prescribing habits in our hospital.
Methods: An audit of current antibiotic prescriptions on three general surgical wards was carried out over a four week period in Beaumont Hospital, a tertiary referral 650-bedded teaching hospital in Dublin. Prescriptions were deemed inappropriate if the choice did not conform to hospital antibiotic guidelines for empirical therapy; if duration of antibiotic treatment exceeded guidelines; if dose or dosage interval was incorrect, or if an alternative agent was indicated by available sensitivity results. In each instance of inappropriate treatment, a recommendation to change the prescription was made in the medical chart and the surgical team was contacted with this advice. Whether or not this advice was followed, was then documented.
Results: Treatment was inappropriate in 58% (100/171) of prescriptions studied - the advised change was implemented in half of these (49/100). Prolonged administration of peri-operative prophylaxis (53/58) and the co-administration of metronidazole with coamoxiclav (32/60) were the most common inappropriate prescriptions.
Conclusions: This initiative was time-consuming (48 hours over 4 weeks) and yielded only moderate results in terms of compliance with our advice. However two major areas of inappropriate antibiotic use were identified and interventions have been implemented to try to effect change, including enhanced education of prescribers, liaison with surgeons and greater input by ward pharmacists. Re-audit is currently underway.

P527

Antimicrobials usage in orphanages: results of the unique prospective multicentre study CORPUS

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Objectives: Pattern of the use of antimicrobials has been shown to be an independent risk factor for emergence and spread of resistant microorganisms. It has been previously shown that orphanages are considered to be reservoirs of resistant strains, which spread to day-care centres and into clinical settings. A special study was designed to evaluate patterns of use of antimicrobials in children from different orphanages located in geographically distinct Russian cities.
Methods: Analysis of the use of antimicrobials in previous 12 months was performed based upon reviews of medical histories of 743 children < 7 years from 11 orphanages in 4 cities of European Russia (Moscow, Saint-Petersburg, Smolensk, Karachev).
Results: In general, prescription of antimicrobials varied from 0.31 courses per child/per year in infant orphanage No. 7 in Saint-Petersburg to 3.63 courses per child/per year in Karachev. In all orphanages, b-lactams were the most frequently prescribed (55.3 to 100% of all antimicrobials). Among them, predominant ones were (in order of frequency): ampicillin/oxacillin (164 courses), amoxicillin/clavulanate (148), cefazoline (128), cefotaxime (119) and ampicillin (103). In general, parenteral compounds were predominantly used in orphanages with the proportion from 61.9 to 100%. Macrolides/lincosamides were the second most frequently prescribed in 6 out of 11 orphanages, with azithromycin being prescribed more often (98 courses), exceeding sum of all macrolides: both co-trimoxazole and aminoglycosides were the second in 2 out of 11 institutions.
Conclusions: There were substantial variations in frequency of the antimicrobial prescriptions with no reflection of geographic location, size of city or specific children population. It was a tendency in all orphanages to prescribe parenteral antimicrobials in spite of the presence of suitable oral alternatives. Enforcement measures have been implemented in order to prohibit unlicensed use of some antimicrobials in studied populations.

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Two consecutive point prevalence studies on indications for antibiotic use in selected Latvian hospitals

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Objectives: The objective of these surveys was to test simple surveillance system for the assessment of the prevalence of nosocomial infection and use of antibiotics.

Methods: Seven and nine Latvian hospitals were included in years 2003 and 2004 respectively. One trained personnel in each hospital has visited all clinical departments over the month (May) period. All administered antimicrobials were recorded according to the purpose of the administration: treatment of community and hospital acquired infection, prophylaxis, or the purpose was unclear to investigator. Data were double entered and analysed by EpiData 3.02 and Epi Info 2000 software packages.
Results: The number of patients treated with antibiotics was 855 (28.9% out of 3150 in 7 hospitals) and 988 (26.2% out of 3776 in 9 hospitals) in years 2003 and 2004 respectively. The prevalence of patients treated for nosocomial infection was 3.6% in both 2003 and 2004. In 21% (2003) and 12% (2004) of patients antibiotics were prescribed without clear clinical indications. Five most commonly used antibiotics were cefazolin (26% of all antibiotics in 2003 and 22 % in 2004), gentamicin (11% and 10%), ampicillin/amoxicillin (14% and 12%), metronidazole (9% and 8%), and ciprofloxacin (9% and 7%). Ten different antibiotics were used for surgical prophylaxis. Cefazolin accounted for 58% and 63% from them in 2003 and 2004 respectively. For treatment of infection most commonly used antibiotics were cefazolin (19% and 14%), ampicillin/amoxicillin (15% and 14%), and gentamicin (12% and 11%).
Conclusions: Surveillance protocol was equally applicable for all hospitals. The results of the study indicate the main problems in antibiotic prescription: overuse of antibiotics, inconsistent surgical prophylaxis, and different pattern of empirical choice in various hospitals. It also gives an insight for the problem of nosocomial infections in Latvian hospitals.

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Another white spot on the Europe map is gone: first results of the Russian monitoring of antimicrobial consumption

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Objectives: The joint project of the Institute of Antimicrobial Chemotherapy and The Remedium Group of Companies (TRGC) was established to monitor the patterns of antimicrobial (AM) consumption in Russia with a long term aim to improve prescription habits and reduce antimicrobial resistance.
Methods: Data on the use of AM in Russia were collected from the national projects Retail Pharmaceutical Market Audit and Hospital Audit that are conducted by TRGC since 2000. The pharmacy and hospital audits are realized in 51 and 27 regions of Russia, respectively. Imprecision of the data of both audits does not exceed 10%. The final reports contain products names, Anatomical Therapeutic Chemical classification codes, drug forms, dosages and packages quantity. The use of AM was expressed as the number of defined daily doses (DDD) per 1000 inhabitants per day – DDD/1000 inhabitants/day (DID).
Results: The mean total consumption of AM for systemic use (J01) in 2001–2002 in Russia was 11.9 DID (2001 – 12.0, 2002 – 11.8). The use of tetracyclines (J01A), amphenicols (J01B), beta-lactams, penicillins (J01C), other beta-lactams (J01D), sulfonamides and trimethoprim (J01E), macrolides, lincosamides and streptogramins (J01F), aminoglycosides (J01G), quinolones (J01M), other antibacterials (J01X) was, respectively, in 2001 – 1.88, 0.38, 3.71, 0.30, 1.92, 1.03, 0.99, 1.00, 0.81 DID and in 2002 – 2.12, 0.47, 2.90, 0.41, 1.97, 1.04, 0.81, 1.14, 0.97 DID. Among J01C group aminopenicillins were prescribed above all (57.4% in 2001, 63.4% in 2002) and their use in 2001 was 2.13 DID and in 2002 - 1.84 DID. From 2001 to 2002, the use of ampicillin decreased from 1.67 to 1.10 DID. However, its oral form was mainly prescribed (2001 – 1.3 DID, 76.5%, 2002 – 0.81 DID, 72.7%). Whereas, the consumption of amoxicillin increased from 0.46 DID in 2001 to 0.74 DID in 2002. Cephalosporins use increased from 0.29 DID in 2001 to 0.40 DID in 2002. Between J01F AM macrolides were the most often prescribed group with 0.84 DID in 2001 (81.6%) and 2002 (80.8%).
Conclusions: Data on antibiotic consumption on a country level could be applied for improvement of the usage patterns.

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Evolution in antibiotics consumption in a Belgian general hospital between 1996 and 2003

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Objectives: The excessive and inadequate consumption of antibiotics (ATBs) has serious consequences for public health, such as the increase of bacterial resistance. Therefore, our hospital evaluates trends in antimicrobial use since 1996.
Methods: A 666-bed general hospital in Belgium (186 235 bed-days in 2003, including 65% acute beds). Antibiotic committee was created in 1998 and has many missions like to develop formalized treatment guidelines for commonly encountered infectious diseases and control the consumption of extended-spectrum (E-S) ATBs. The annual consumption of ATBs (Class J01 in the ATC classification from WHO 2004) in Defined Daily Doses (DDDs) and total patient days (PD) for 1996 through 2003 were determined from electronic capture of billing records. The DDD/100 PD was calculated for each antimicrobial. The microbiology laboratory provides the committee with data about the frequency of various resistant pathogens (Sirscan expert system).
Results: Total antimicrobial use (J01) between 1996 and 2003 was 30.80, 31.44, 31.79, 30.87, 30.20, 36.63, 42.95 and 45.5 DDD/100PD, respectively. The most dispensed antimicrobials are the combinations of penicillins, including beta-lactamase inhibitors (J01CR) with an average DDD/100PD of 15.90. The cephalosporins (J01DA) and the fluoroquinolones (J01MA) have an average DDD/100PD of 5.45 and 4.28, respectively. Incidences of nosocomial Methicillin resistant *Staphylococcus aureus* (MRSA) acquisition, methicillin resistance and glycopeptides consumption were stable until 2001, then increase (+23.43% for the resistance; +128.52% for the consumption) between 2001 and 2003.
Conclusion: Total consumption of ATBs has not changed significantly between 1996 and 2000, but in 2001 there is an increase of almost all classes of ATBs. In 2003, a 72-hour stop order system of prescribing was implemented to control the use of E-S ATBs. This system, which is hoped to have an impact on the epidemiology of nosocomial bacteria, is presently under evaluation.

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Study of quinolone consumption in Greek hospitals: results of a Hospital Network

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Background: Resistance to quinolones is growing both in the community and the hospitals of Greece. For this reason, quinolone use is restricted in both settings.
Methods: We studied quinolone consumption in Greek hospitals, through a network that we established and includes 5 hospitals located in different parts of Greece: 'G. Gennimatas'

General Hospital (GGH) of Athens (tertiary care, 700 beds), 'A. Fleming' General Hospital (AFH) of Athens (300 beds), 'A. Papandreou'? General Hospital of Rhodes Island (RH, Southeastern Greece, 335 beds), 'Vostaneio' General Hospital of Lesvos Island (LH, Northeastern Greece, 225 beds) and Zakynthos Island General Hospital (ZH, Western Greece, 125 beds). Antibiotic consumption for the year 2003 was calculated in Defined Daily Doses (DDDs) per 1000 patient days.

Results: Overall, RH had the greatest consumption (1403.3 DDDs /1000 patient days), followed by ZH (1300.1), LH (1210.6), GGH (926.5) and AFH (851.3). In general, cephalosporins and penicillins+inhibitors were the most popular antibiotics. Quinolone consumption was very high in LH (275.5) ranked 2nd among all antibiotic groups. Furthermore, quinolones were ranked 3rd in other 2 hospitals (240.1 DDDs in RH and 90.2 in AFH,). Finally, their consumption was 106.9 DDDs in ZH (ranked 5th) and 61.7 DDDs in GGH (ranked 4th). Considering the ratio new (respiratory) quinolones/ all quinolones the numbers were: 63.4% for RH, 44.4% for ZH, 22.7% for LH, 20.7% for GGH and 15.5% for AFH.

Conclusions: According to our data, quinolones are widely used in Greek hospitals. Consumption of new (respiratory) quinolones is greater in hospitals located far away from Athens. It is obvious that there is need for the design and implementation of a new antibiotic policy, since the existing one is not effective, especially in the hospitals of the periphery. Meanwhile we are planning an audit of the use of these agents in order to clarify the reasons for this prescribing behavior.

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Trends in antibiotic consumption and antimicrobial resistance of major bacterial pathogens over a 5-year period in a general hospital in Athens, Greece

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Methods: We recorded antibiotic consumption in our hospital, a 300-bed General hospital, from 1999 to 2003 and correlated it with the resistance rates of major Gram(-) and Gram (+) pathogens isolated during the same period.

Results: Antibiotic consumption (DDDs/1000 patient days) was 612.2, 687.5, 707.8, 722.9, 851.3, for the years 1999, 2000, 2001, 2002 and 2003 respectively (overall 39% increase). Consumption of all major classes of antibiotics except penicillins increased, especially that of macrolides (>100% increase), quinolones (>200%) and glycopeptides (>600%). Furthermore, penicillins + β-lactamase inhibitors (from 28.4% to 30%), macrolides (from 6% to 9%), quinolones (from 4.8% to 11%) and glycopeptides (from 0.2% to 1.2%) had a proportional increase among antibiotic groups used. During the same period: *E. coli* resistance rate increased for ampicillin (28.7% to 55.2%), co-amoxiclav (22.7% to 27.6%), cefotaxime (0.7% to 5.5%), ceftazidime (1% to 4.5%) and gentamicin (1.8% to 3.2%), decreased for cotrimoxazole (33.2% to 22.4%) and ciprofloxacin (2.2% to 1.8%) and remained 0 for imipenem. For *P. aeruginosa*, resistance rates increased for imipenem (13.9% to 15.4%), ceftazidime (12.8% to 17.6%), gentamicin (30.2% to 35.3%), ciprofloxacin (16.8% to 28.6%) and decreased for amikacin (27.9% to 25.7%), aztreonam (29.6% to 18.3%) and piperacillin/ tazobactam (19.3% to 13.2%). For *K. pneumoniae* resistance rates increased for co-amoxiclav (20% to 25.2%), cefotaxime (7% to 10.1%), ceftazidime (5% to 9%), ciprofloxacin (2% to 4%), imipenem (0 to 5.6%) and remained stable for cefoxitin (~8%) and gentamicin (6%). For *S. aureus* resistance increased for methicillin (24.6% to 35.2%) and ciprofloxacin (13% to 21.6%), decreased for rifampicin (11.6 to 7.2%) and cotrimoxazole (18.8% to 8%) and remained stable for gentamicin (~18%). For enterococci, resistance increased for ampicillin (14.4% to 26.7%), ciprofloxacin (50.5% to 83.6%) and rifampicin (18.3% to 40.5%). Furthermore, Vancomycin-Resistant Enterococci (VRE) were isolated for the first time during 2001 and in 2003 4/116 strains of enterococci were VRE.

Conclusions: During the study period, consumption increased significantly in our hospital for almost all major classes of antibiotics and this was accompanied by an overall increase in resistance for the major pathogens both Gram (+) and Gram (–) and the emergence of VRE. The existing antibiotic policy has to be reinforced or possibly changed.

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Trends in antibiotic consumption and bacterial isolation rate over a 5-year period in a general hospital in Athens, Greece

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Objectives: The aim of this retrospective study was to record the antibiotic consumption in our hospital, a 300-bed General Hospital over a five year period (1999–2003) and to try to correlate it with the bacterial isolation rate of the same period.

Results: During the study period, total antibiotic consumption expressed in DDDs/1000 patient days was 612.2, 687.5, 707.8, 722.9, 851.3, for the years 1999, 2000, 2001, 2002 and 2003 respectively (overall 39% increase). Consumption of all major classes of antibiotics except penicillins increased, especially that of macrolides (>100% increase), quinolones (>200%) and glycopeptides (>600%). Further analysis showed that penicillins + β-lactamase inhibitors (from 28.4% to 30%), macrolides (from 6% to 9%), quinolones (from 4.8% to 11%) and glycopeptides (from 0.2% to 1.2%) had a proportional increase among antibiotic groups used, with penicillins + inhibitors and cephalosporines being the leading groups in all years. During the same period the ratio of Gram negative/ Gram positive bacteria isolated in our hospital was 3.0, 2.5, 3.3, 1.8, 1.6 for the years 1999, 2000, 2001, 2002 and 2003 respectively. A. Gram (–): *E. coli* isolation rate among Gram (–) remained stable during the study period (around 51%),while for *P. aeruginosa* the rate declined (from 19.6% to 14.4%) and the same was true for *K. pneumoniae* (from 11.4% to 10.5%) and *A.bowmannii* as well (from 3.6% to 3.0%). At the same time isolation rate of *E. cloacae* and *P. mirabilis* increased (from 3% to 5.5% and from 5.1% to 8.3% respectively). B. Gram (+): *S. aureus* isolation rate among Gram(+) declined from 24% to 21.4% and the same was true for enterococci as well (from 38.7% to 19.8%). On the other hand, coagulase (–) staphylococci (CNS) showed a great increase (from 37.3% to 58.8%).

Conclusions: During a 5 year period antibiotic consumption increased significantly in our hospital and this was true for almost all major classes of antibiotics. At the same time changes in bacterial isolation rate were observed, both among Gram(+) and Gram(–), with *E. coli*, however, still remaining first among all bacteria isolated, despite the striking increase in Gram(+) isolation.

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The role of inadequate prescribing for the development of drug resistance in respiratory infections in Russia

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Incorrect antimicrobial prescribing increases the risk of the development of drug resistance in pathogen. There have been few studies to detect levels of resistance in respiratory pathogens in Russia but a recent study in Samara Region of resistance among *M. tuberculosis* strains showed very high levels of resistance including multi-drug resistance.

Objectives: To determine the patterns of antimicrobial prescribing for respiratory prescribing including tuberculosis (TB) among Russian physicians in Samara Region and it's possible role in development of resistance.

Methods: 1) point-prevalence cross-sectional survey involving 28 primary care, general and TB institutions; in total 425 physicians took part in the questionnaire-based survey which aimed to look at empirical management of patients with respiratory diseases including the 'common cold'. 2) Subsequently analysis of clinical notes of 491 consecutive patients with respiratory diseases were reviewed to assess actual prescribing habits.

Results: Only a small group of doctors (1.7%) said that they would prescribe antibiotics for the simple 'cold'. Review of clinical cases showed that antibiotics (ABs) were prescribed for 20% of patients with a simple upper respiratory tract infection. At least one AB was prescribed for the majority of patients (90.8%) with acute bronchitis; 80.0% of patients with exacerbation of chronic obstructive pulmonary diseases received one AB and 18.0% received two. When there was no clear evidence base for an AB in treatment of acute tonsillitis, Russian doctors prescribed at least one AB for nearly all (98.9%) of these patients and 18.0% received two ABs. Ciprofloxacin was widely but inappropriately used. Eight out of twelve patients with suspected but unconfirmed tuberculosis received rifampicin monotherapy alone.

Conclusions: Current AB over prescribing in Russia may contribute to the development of resistance in respiratory pathogens. There remains a need to restrict the prescribing of antibiotics by doctors in general and to provide further training. Further studies are needed to assess the level of patients' expectations in receiving an AB as well as the role of over-the-counter prescribing and the true levels of resistance in TB and other respiratory pathogens.

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Outpatient intravenous antibiotic therapy

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Introduction: Outpatient intravenous antibiotic therapy (OIAT), has been proved to be both, safe and effective, on patients with several infectious conditions who are discharged for treatment and follow up at home. Yet there is an open debate on whether we should maintain the antibiotic indicated in hospital or use a different one more suitable for an outpatient setting.

Objective and design: To review a long series of (OIAT) indicated in our (OHD). A full recording of the clinical data available from patients treated between 1996 and 2004 was done: diagnosis, indicated therapy, duration of treatment and outcome.

Results: 990 (OIAT) were indicated. A total of 824 patients were treated with a mean age of 60. The mean duration of treatment was 14.1 days.The infection diagnosis were: respiratory 47% post surgery 11.5%, skin and soft tissues 7.7%, bacteriemias 9.9%, osteoarticular 5.5%. The most used antibiotics were: ceftriaxone 25.8%, aminoglycosides14.9%, piperacilin-tazobactam 9.7%, ceftazidime 9.3% teicoplanine 4.3%, ganciclovir 4.3%. Intravenous infusion pump devices were used in 389 of the treatments. 124 patients needed to be readmitted to hospital (34 secondary to therapy failure). Number of hospital inpatient stays avoided: 11.963.

Conclusions: (OIAT) is both, safe and effective, with a low rate of complications and admittances to hospital in a wide variety of infections. We show in this review that it is not necessary to change the antibiotic indicated during the inpatient treatment if we use intravenous infusion pump devices that allow us to use different antibiotics and regimens.

Antibiotic policy intervention

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Audit of antibiotic prescribing in primary paediatric care for acute tonsillopharyngitis and its progress

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Backgrounds: Audits on the diagnosis and treatment of respiratory tract infections (RTIs) have been performed in primary paediatric care in Slovakia for the past 5 years. Respiratory tract infections are most often the reason for antibiotic indication in ambulatory practice. One representative of RTIs is acute tonsillopharyngitis as clinically narrowly definite problem.

Objectives: The aim of the present study was to evaluate the progress or changes of antibiotic prescribing for acute tonsillopharyngitis in Bratislava and in Zvolen where paediatricians have participated in the study frequently and possibly to improve antibiotic prescribing for acute tonsillopharyngitis via audit, feed back of results and educational activities.

Methods: Four weeks prospective studies in years 2002 and 2003 in two Slovak cities; Same protocols and methodology was applied for multicentric study; the entrance of paediatricians to diagnosis and antibiotic prescribing for acute tonsillopharyngitis were monitored on the basis of resistance surveillance in positive regions.

Results: Out of 1308 patients in February 2002 (whole study), to 1127 (86.2%) were indicated antibiotic treatment and in November 2003 out of 4480 patients (whole study), to 3303 (72.7%). Positively after intervention the prescribing of macrolides decreased in both cities. In Bratislava from 28% to 17% and in Zvolen from 18% to 13%. Indication of penicillins to acute tonsillitis had good tendency in Bratislava (from 45% to 51%) but in Zvolen prescription of penicillins declined (from 63% to 57%). Although aminopenicillins and co-aminopenicillins are

contra-indicated for acute tonsillopharyngitis, they are still more often prescribing in both cities. Prescribing of cephalosporines declined in Bratislava (from 18% to 9%) but in Zvolen increased (from 6% to 10%).
Conclusions: Educational programmes with the aim to stop the growth of antibiotic resistance, in this case macrolides resistance of main pathogen *Streptococcus pyogenes*, shows that it is possible to improve prescription habits of paediatricians by frequently using the prescribing audit.It is important to support the implementation of antibiotic prescribing and effective intervention methods for improving antibiotic prescribing in the regions.

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A controlled intervention study to improve antibiotic use in a Russian paediatric hospital

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Objectives: Antibiotic use is increasing in Eastern Europe promoting antibiotic resistance. There have been few reports on antibiotic use from Russian hospitals. We aimed to implement guidelines for rational antibiotic use in a Russian 600-bed paediatric hospital.
Methods: We performed a prospective controlled intervention study to improve antibiotic use. One dep. of gastroenterological infections and one dep. of respiratory tract served as intervention deps. One dep. of gastroenterological infection and one dep. of respiratory tract infections served as control departments. During October through December 2002 demographic data, diagnoses, antibiotic allergy, bacteriological findings, antibiotic use, duration of stay, costs, and outcome were recorded in all patients. Thereafter we conducted repeated whole day meetings where rational antibiotic policy was promoted. Written consensus guidelines for rational antibiotic use based on the local resistance pattern were developed and implemented. The same data as in the baseline period were recorded during October though December 2003.
Results: At the intervention department of gastroenterological infections the percentage of patients treated with antibiotics decreased from 96% to 50%. At the intervention department of respiratory tract infections the percentage of patients receiving antibiotics decreased from 91% to 69%. There was a slight reduction in antibiotic use at one control dep. There was no increase in mortality or duration of hospital stay.
Conclusion: There was a significant decrease in antibiotic use and costs at the intervention departments and a slight reduction at one control department. The decrease of antibiotic use did not compromise quality of patient care.

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Multidisciplinary interventions and system orientated strategies do have an impact on improving antimicrobial prescribing

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Observations by the clinical microbiology team in our institution suggested that patients were not receiving all doses of vancomycin prescribed. A prospective month-long audit in September 2003 revealed that 160/1007 prescribed vancomycin doses (16%) were withheld inappropriately. Much confusion existed among healthcare professionals in ordering and interpreting vancomycin therapeutic drug monitoring (TDM) levels. In order to avoid generating a culture of blame between the various healthcare professional groups involved in the TDM process, measures to improve vancomycin administration focussed on system-change. These included changes in computer ordering, replacing various vancomycin TDM levels with a single 'pre-dose' level and an agreement from the phlebotomy department to take TDM levels rather than relying on medical staff. In addition, multidisciplinary hospital-wide and ward based educational sessions were undertaken. Following these changes, a second monthly audit in February 2004 revealed that 150/1161 prescribed vancomycin doses (13%) were held inappropriately. The main location of confusion appeared to be at ward level, where both medical and nursing staff had difficulties in ordering and interpreting vancomycin TDM levels. In order to address this, a senior pharmacist undertook to coordinate ward-based pharmacists in assisting staff interpret levels, stickers and TDM interpretative charts were designed for the drug charts of each patient receiving vancomycin and feedback by ward pharmacists to our department was encouraged. Further multi-disciplinary audit feedback sessions, including feedback to hospital management and a plan for ongoing education were undertaken. A third audit in September 2004, revealed a further decrease: 78/782 (10%) prescribed doses were inappropriately withheld. Overall our various interventions resulted in a 37.5% reduction in inappropriately held vancomycin doses over a one-year period. Whilst we feel that we have significantly improved vancomycin prescribing, 10% doses are still being held inappropriately. This study highlights the difficulties identifying barriers to change and changing healthcare worker behaviour despite multiple multidisciplinary system orientated interventions.

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Two-year experience of cephalosporins rotation in the cardiosurgery intensive care unit

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Objectives: Cephalosporins are the drugs of choice in cardio surgery patients as agents with broad antibacterial spectrum and high level of safety. Traditionally, antibiotic rotation means to use the various classes of drugs. Different activity of cephalosporins against some gram-negative bacteria, causing nosocomial infections, gives the opportunity to rotate the drugs within this class. We evaluated the results of two years experience (2002–2003) of antibiotic rotation, based on monitoring of acquired resistance profile of gram-negative bacteria isolated from prolonged ventilated (more than 72 h) adult cardiosurgery patients in intensive care unit (ICU).
Methods: Before rotation ceftriaxon was used as a drug for empirical treatment. The proposed antibiotic rotation scheme was the 6-month cycles of cephalosporins: cefoperazon, then ceftazidim, then cefepim, based on the results of preceding microbiological monitoring. Additional antibiotics of other groups were used when indicated. Total of 151 consecutive nosocomial isolates of gram-negative enterobacteria and non-fermenting bacteria were studied. According to NCCLS recommendations, disc-diffusion test was used to determine bacterial sensitivity. Level of infectious complications and additional use of some reserve antibiotics were evaluated during each cycle.
Results: 2269 patients were admitted. The use of *P. aeruginosa*-active cephalosporins of the III and IV generations resulted in elimination of this pathogen from ICU. We registered the decrease of the number of positive haemocultures from 38/221 (17.2%) before rotation to 16/164 (10.7%) by the 3th rotation cycle. In parallel, the total rate of postoperative infectious

Drug	KES (%)			*P.aeruginosa (%)*			*A.baumannii (%)*		
	1 cycle (n = 26)	2 cycle (n = 37)	3 cycle (n = 20)	1 cycle (n = 15)	2 cycle (n = 18)	3 cycle (n = 3)	1 cycle (n = 9)	2 cycle (n = 14)	3 cycle (n = 9)
Ceftriaxone	19.2	10.8	5	0	0	0	0	0	0
Ceflazidime	77	54.1	30	73.3	77.8	100	22.2	42.8	0
Cefoperazone	11.6	10.8	5	13.3	44.5	33.3	0	0	0
Cefepime	80.8	78.4	55	80	83.3	100	66.7	85.8	55.5
Imipenem	100	100	100	33.4	55.5	66.7	100	100	100
Meropenem	100	100	100	33.4	55.5	66.7	100	100	100
Cefoperazone/ Sulbactam	–	67.6	70	–	94.5	100	–	100	89

complications also decreased (47/657 (7.2%) vs. 22/489 (4.5%), p = 0.015). The need of carbapenems and vancomycin did not increase. Antibiotic susceptibility of KES (Klebsiella, Enterobacter, Serratia), *P. aeruginosa*, *A. baumannii* during our study in ICU is listed in the table (% of sensitive strains).

Conclusion: Cycling of cephalosporins under microbiological monitoring in ICU is an effective method for the management of nosocomial infections in cardiac surgery.

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Impact of infectious diseases division interventions on hospital-acquired infections and antimicrobial resistance in the neurosurgical department of a Greek university hospital

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Objective: The purpose of the present study was to estimate the impact of Infectious Diseases Division interventions on the incidence of Hospital Acquired Infections (HAIs) and antimicrobial resistance in the neurosurgical department of a Greek University Hospital.

Methods: Data concerning the year 2002, 2003 were derived from a database of HAIs in our hospital. Antimicrobial resistance data were obtained from the microbiology department and antibiotic consumption from the pharmacy, expressed as the number of DDDs per 100 bed days. The interventions in year 2003 included quality rounds, close monitoring of antibiotic consumption and strict guidelines for the prophylactic antibiotic. The goal was to reduce the consumption of 3d generation cephalosporins, carbapenems and vancomycin. Educational meetings of the personnel regarding the hand hygiene took place.

Results: In year 2002, 1035 patients were hospitalized in the neurosurgery department and 756 of them had at least one operation. During that year 69 HAIs (6.6%) were detected (11 LRIs, 22 SSIs, 7 BSIs, 8 UTIs, 5 shunt infections and 6 others). In year 2003, 1110 patients were hospitalized, 810 of them had at least one operation. 44 HAIs (3.9%) were detected (7 LRIs, 9 SSIs, 9 BSIs, 5 UTIs, 2 shunt infections and 10 others). There was significant reduction especially in SSIs ($p < 0.001$). The antibiotic consumption was decreased during 2003 from 53.68 DDDs/100 bed days to 42.6 with the exception of piperacillin/tazobactam (from 1.95 to 2.42) and aminoglycosides (from 5.8 to 6.6). Penicillins in total were reduced from 15.81 to 16.87, 3d generation cephalosporins from 2.68 to 2, fluoroquinolones from 2.1 to 1.4, glycopeptides from 1.86 to 1.4 and carbapenems from 1.9 to 1.6. The median duration of the prophylactic antibiotic use in surgery was 4 days in 2002 and 1 in 2003. Antimicrobial resistance in Acinetobacter spp. was reduced from 78% to 39% for piperacillin/taz and from 22% to 10% for imipenem. Antimicrobial resistance in Pseudomonas aueroginosa was reduced from 31% to 6% for piperacillin/taz, from 20% to 8% for imipenem and from 25% to 10% for gentamycin.

Conclusion: The interventions of the Infectious Diseases Division resulted in decrease of the number of HAIs, especially in SSIs. Despite the small amount of species the study indicates that the reduction of the antibiotic consumption might have an impact on antimicrobial resistance.

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Impact of scheduled antibiotic change in the intensive care unit of a tertiary referral hospital in Malaysia

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Introduction: Resistance to antimicrobial agents is driven by the selective pressure of its usage in confined areas such as the Intensive Care Unit (ICU). Immunocompromised patients receiving broad spectrum antibiotics and being cared for in a confined space by busy doctors from various disciplines act as a conducive environment for organisms to develop resistance, multiply and spread. Pathogens of concern at hospital Sultanah Aminah, a tertiary referral hospital for the southern region of Malaysia, include multiply resistant gram-negative organisms, the most common being *Klebsiella pneumoniae* (22%), Acinetobacter spp. (20%) and *Pseudomonas aeruginosa* (18%). The incidence of extended spectrum β lactamases among *Klebsiella pneumoniae* was 41.1% while that of *Escherichia coli* was 29%. The frequency of *Pseudomonas aeruginosa* hyper-producing AmpC – cephalosporinase was 57%.

Objective: In view of the growing population of multiresistant organisms in the ICU, a prospective study was carried out to determine the impact of scheduled formulary change in the antibiotic used for empiric treatment of suspected gram-negative bacterial infections.

Methods: During the pre-interventional period, ceftazidime was used for empiric therapy, which was replaced with cefepime during the one year study period, from January to December 2003.

Results: The incidence of extended spectrum β lactamases among *Klebsiella pneumoniae* was reduced from 41.1% to 36.45% ($p > 0.05$) while that of *Escherichia coli* from 29% to 19.2% ($p > 0.05$). *Pseudomons aeruginosa* which hyper-produced AmpC –cephalosporinase was also reduced from 57% to 37.25% ($p > 0.05$).

Conclusions: These data suggest that a scheduled change in the antibiotic class, by restricting the use of third generation cephalosporin in favor of a fourth generation cephalosporin, resulted in a favorable reduction in the nosocomial bacteria producing extended spectrum â lactamases and the Richmond and Sykes class 1 chromosomally mediated AmpC – β-lactamases.

P542

The impact of prescription intervention on antibiotic resistance patterns of *K. pneumonia* and *P. aeruginosa* in a tertiary care hospital

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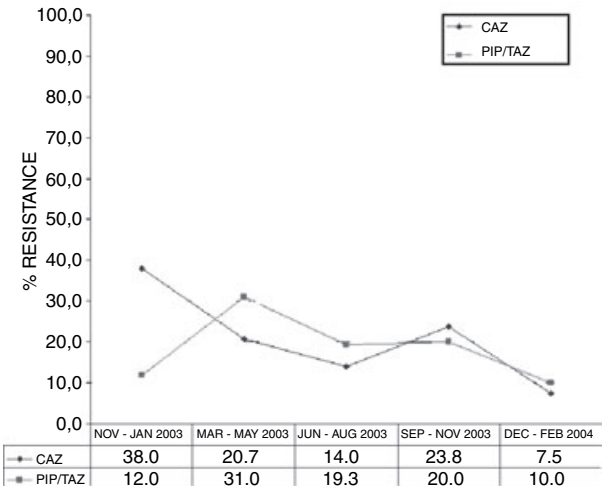
Objective: To evaluate the impact of replacing third generation cephalosporins (CEP) by piperacillin/tazobactam on antibiotic resistance patterns of *K. pneumoniae* (K.p) and *P. aeruginosa*.

Methods: This was a prospective, open-label, observational, antibiotic usage interventional study. Bacterial resistance data and the antibiotic consumption rates were collected from the internal medicine, the surgical wards and the ICU. There were

two periods. At the first 3 month period, consumption data were collected for piperacillin/tazobactam and CEP without any intervention. At the second 12 month period an antibiotic restriction policy was applied. Bacterial susceptibilities of *K. pneumoniae* as well as the rate of ESBL+ *K.p.* and imipenem resistant *P. aeruginosa* were determined. ESBL detection was performed using the NCCLS 2003 guidelines.
Results: The consumption of CEP and specifically that of ceftazidime (CAZ) was decreased from 79.9 DDD/100 bed-days to 19.8 DDD/100 bed-days while that of piperacillin/tazobactam was increased from 112.8 DDD/100 bed-days to 370.8 DDD/100 bed days respectively. The resistance levels of CAZ to *K. pneumoniae* were decreased from 38.0% to 7.5% and that of piperacillin/tazobactam decreased from 12.0% to 10.0% for the respective periods (Fig 1). The rate of ESBL+ *K.p.*

K.pneumonia RESISTANCE



	NOV - JAN 2003	MAR - MAY 2003	JUN - AUG 2003	SEP - NOV 2003	DEC - FEB 2004
CAZ	38.0	20.7	14.0	23.8	7.5
PIP/TAZ	12.0	31.0	19.3	20.0	10.0

dropped from 5.7% during the observation period to 0% during the last 3 months of the study. Regarding the *P. aeruginosa* ImpR there was a decrease in the incidence rate from 32.8% to 12.2%.
Conclusion: Antibiotic formulary intervention by restriction of CEP and their replacement by piperacillin/tazobactam might be useful for reducing high antibiotic resistance rates of *K. pneumoniae* and the incidence of *P. aeruginosa* ImpR .

P543

Survival outcome by initial antimicrobial appropriateness in severe infections of medical patients: a pilot study

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Objective: To estimate impact of initial antimicrobial regimen (Rx) appropriateness (Appr) on survival of patients with severe infection (SI).
Patients-Methods: Prospective entry of all demographic, clinical and microbiology data entry of medical patients with severe infection (3 or more SIRS criteria and/or evidence of organ dysfunction). Most Rx based on currently accepted guidelines. Only infections documented by bacteriology were evaluated. Outcome endpoint: death during hospital stay. Period: March to September 2004. Analysis in PC, by SPSS (Windows-XP) programme. Sensitivity as by Kirby-Bauer.Results: Documented SI pts were 46 (M: 19-F: 27) m.age 72.4 years. Chronic disease present in 84%, and documented infections were 31 bacteraemias (many of urinary origin) and 15 urinary tract without bacteraemia. More frequent pathogens were *E. coli* (17), *S. aureus* (6) *S. faecalis* (5) and 3 each of *Pseudomonas*, *Acinetobacter* and *Candida sp*. Mortality during stay was 8/46 [17.4%]. Appr Rx was noted 34 pts (83%), 4 deaths in each arm (Appr and non Appr. Rx) of bacteraemias ($p = 0.02$ by Fisher exact test) while all urinary SI survived. A stronger correlation to mortality was noted for co-morbidity, previous hospitalization and Rx history, Gram+ cocci or Candida isolation.
Conclusions: The factor of initial Rx appropriateness is, reasonably, important for the survival of patients with severe infections, but only one of several parameters to be taken into account for a multi-disciplinary approach for optimal management, particularly upon pre-existing co-morbidity. An increased number of pts will allow for more solid evidence.

P544

The impact of a sore throat score and a rapid antigen test on clinical management in streptococcal pharyngitis

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Objective: To establish the impact of a sore throat score and a rapid antigen test for group A streptococci on clinical management of patients with acute pharyngitis focusing on reducing the antibiotic prescription.
Methods: This study included 98 patients hospitalized in Infectious Disease Hospital, Lasi, Romania. From each patient we collected clinical data for the score and two pharyngeal swabs: one for rapid antigen test (One Step Strep A Test Cassette–AccuBioTech- a two side sandwich immunoassay) and the second one for culture. Catalase negative, gram positive cocci in chains were serogrouped using a latex-agglutination kit (Slidex Strepto A – BioMerieux). All patients had no antibiotic therapy prior examination and no other infectious diseases. We compared physicians intent for prescribing antibiotics based on clinical data with physicians practice after doing the score and rapid antigen test. We have to state that the hospital standard procedure for diagnosis of acute pharyngitis doesn't include the score and the rapid antigen test. The patients were hospitalized 5–7 days.
Results: Of 98 patients 32 (32.65%) had group A streptococcal pharyngitis, and from 17 (17.34%) patients we isolated non group A beta hemolytic streptococci. Combination of score and rapid test didn't miss any positive result (95%CI: 82.35%–99.87%) and had a good specificity: 93.75% (95%CI: 69.77%–99.84%). Based upon intent the physicians would have prescribed antibiotics for 85 patients including 36 (73.46%) with negative culture and all 17 patients with non group A beta hemolytic streptococci. After doing the score and receiving the results of the rapid antigen test the physicians prescribed antibiotics only for 33 patients (reducing the overall prescription for antibiotics with 67.17%).
Conclusions: The score higher than 3 and the rapid antigen test improved the use of antibiotics as follows: 1. All patients with group A streptococcal pharyngitis received the proper course of antibiotics. 2. Only one patient received unnecessary therapy. 3. The combination of score higher than 3 and rapid antigen test reduced the prescription of unnecessary antibiotics with 98.11% on a first visit of a patient with sore throat. 4. The number of hospitalization days would have been reduced from 5–7 to 2 days in 66.33% of cases.

P545

An intervention programme to optimise the levofloxacin use

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Introduction: Levofloxacin (LF) is a wide spectrum antibiotic with an exceptional oral biodisponibility. It is used mainly in respiratory infections and in other infections especially in penicillin allergic patients. A quick switch from intravenous (IV) to oral (OR) formulation is possible with this antibiotic. In a former study we detected an important use of the IV formulation with economic consequences because IV-LF is particularly more expensive than OR-LF (a difference of 23.5 €/doses). In order to optimize this therapy we thought of start of a programme of early switch from IV to OR formulation.
Objectives: to evaluate the results of a new intervention programme in order to manage an early switch to OR formulation in patients treated with IV-LF.
Methods: An observation in the prescription order with the text 'consider oral formulation by its qualified biodisponibility' was added in all the Costa del Sol in-patients with IV LF prescription. We have analysed the LF consumption in two periods: first before the intervention programme (BI), from july 2002 to june 2003; second after the intervention (AI), from july 2003 to june 2004. Total study and by department analysis were performed.
Results: A total of 3619 doses were used in the BI period: 2514 (30%) were OR and 1105 (70%) IV formulation. In AI time we used 3541 doses (79%) OR and 983 (21%) IV. Prescription from General Internal Medicine (GIM) and Neumology formed 90% of total prescriptions. The additional 10% were in other Medical Units, Surgery and Traumatology. In GIM the lowering of IV doses were from 32% to 22% and in Neumology from 25% to 17%. In the other services, although with lower consumption a reduction in IV doses was observed too (Cardiology from 39% to 25%, Gastroenterology from 39% to 26%, Nephrology from 50% to 26% and Traumatology from 50% to 35%). Altogether a reduction in IV used from 31% to 22% was observed, therefore we have estimated a saving of 398 IV doses and this means a gain of 9610 €.
Conclusions: The initiation of a elementary, easy and quickly intervention programme in antibiotic prescription can contribute to a more careful antimicrobial employ and to a best used of economical resources in the public health system.

P546

MIKSTRA Programme – a five-year follow-up of indication based antibiotic prescribing in primary health care

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Objectives: To follow indication based antibiotic use in primary care to see if prescribing practises change after implementing new treatment guidelines.
Methods: A prospective, controlled before-after study. The data was collected during one week in November each year from 1998 to 2002 in 30 health centres with a total population of 820 000. Twenty control health centres were recruited in 2002. National guidelines for six major infections (otitis media, sinusitis, throat infection, acute bronchitis, urinary tract infections and bacterial skin infections) were published and distributed to all doctors between October 1999 and March 2000. An intervention was carried out in study health centres by training one of the doctors from each health centre to be a trainer at work site and by producing educational and feedback material for their use. Training at work site was carried out by the trained doctor using either problem based learning-method or academic detailing with feedback.
Results: The six most common infections under intervention, together with common cold, comprised 80–85% of all infections each year. The proportion of each infection was very stable over the years. The proportion of patients, who were prescribed antibiotics did not change significantly or differ from that of control health centres in any diagnosis. However, there were changes towards the recommended in antibiotic choices in several infections. Use of first-line antibiotics as the antimicrobial chosen at the first consultation increased in all infections except throat infection. The increase was statistically significant in sinusitis and urinary tract infections. Also the percentage of antibiotic treatments of recommended duration increased significantly in all infections under intervention except throat infections. The treatment practises in throat infections were, however, well in concordance with the guidelines already at the base-line. Results of the study health centres did not differ statistically significantly from those of the controls in 2002. There were no drop-outs among health centres during the five year follow-up but over one third of the doctors changed during the follow-up years.
Conclusions: Training intervention changed antibiotic choices and duration of treatment towards recommended but did not decrease prescribing in general. Changing professional practice is not easy. It takes time, needs simple and targeted messages and repetition.

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Use of symptomatic medication in acute otitis media

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Objectives: To study what kind of symptomatic medication is used in acute otitis media in primary care and whether the use is in accordance with national guidelines.
Methods: The data was collected in a five-year MIKSTRA Programme in 30 primary health care centres throughout Finland during one week in every November from 1998 to 2002. Physicians and nurses collected data about the diagnosis, prescription-only medicines and over-the-counter medicines prescribed or recommended for all patients with an infection during the study weeks (www.mikstra.fi).
Results: A total of 3229 patients were diagnosed as having first consultation for an episode of otitis media (14% of all patients with first consultation for an infectious disease). Of these only the 3059 patients, who had the diagnosis of otitis media alone, were included in this study. One third of the patients at the age group older than 15 years and a quarter of those under 15 years of age got some other medicine than a systemic antimicrobial agent for otitis media. Symptomatic medication was prescribed or recommended for 25% of the patients, who also had been prescribed systemic antimicrobials (n = 2 880) and for 45% of the patients, who had not got a systemic antimicrobial (n = 179). Non-steroidal anti-inflammatory agents (NSAIDs) and painkillers were the most commonly prescribed symptomatic medicines (7% collectively). Antihistamines and topical eardrops with analgesic-anti-infective combination for earache were prescribed for 4% of the patients respectively. Compared to adults over 25 years of age, children under 2 years of age were recommended less systemic flu medicines (9% vs. 4%), topical ear medicines other than those for earache (14% vs. 1%) as well as cough medicines (9% vs. 2%).

Conclusion: According to the national treatment guidelines for acute otitis media, medication, which relieves earache, should be used as a supportive medication especially if prescription of antibiotics is postponed. According to our results the use of NSAIDs and painkillers as well as local analgesic eardrops should be increased in acute otitis media. By improving the symptomatic relieve of pain in acute otitis media the use of watchful waiting-policy in the treatment of this infection could become more acceptable and prescription of systemic antimicrobial agents could be decreased.

P548

Investigation of divergent piperacillin and piperacillin/tazobactam activities to support appropriate formulary decisions

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Objectives: The use of generic antibiotics over branded versions can bring significant costs savings. However, the use of a cheaper generic compound in preference to a branded version of the same compound in combination with an inhibitor, should be carefully considered in the context of current susceptibility data, ensuring appropriate antibiotic use. The use of generic piperacillin (PI) instead of piperacillin combined with tazobactam (PT) is a case in point. This investigation was done to establish the extent to which the antimicrobial activity between PI and PT diverge with respect to their in vitro activities against key gram-negative bacilli.
Methods: The Surveillance Network (TSN) collects all routine susceptibility data from >300 U.S. sites on a daily basis. The majority of data is derived from Vitek and Microscan diagnostic devices, approved by the FDA. Data from Jan 00–Feb 04 was analysed to compare PI and PT activities against isolates of *C. freundii* (CF)[n = 8.649], *E. coli* (EC) [n = 218.331], *K. pneumoniae* (KP) [n = 58.499], *P. mirabilis* (PM) [n = 31.489], *P. aeruginosa* (PA) [n = 109.191], *S. marcescens* (SM) [n = 14.070], and Acinetobacter spp. (AB) [n = 9.925]. Only organisms tested at the same time to the selected antibiotics using approved NCCLS methodologies were included in this analysis.
Results: Overall resistance (R) rates for PI were consistently higher than those observed for PT, respectively for: CF (22.2/6.8%), EC (26.2/1.1%), KP (14.8/4.2%), PM (9.1/0.5%), PA (16.0/10.1%), SM (8.2/4.3%) and AB (46.8/21.0%). During the same time period: for enterics, 93.5–99.7% of PI-R isolates and 99.4–100.0% of PI-I strains were susceptible to PT; for non-enterics, 83.1–90.0% of PI-R isolates and 99.8% of PI-I strains (AB only) were PT-susceptible.
Conclusions: The superior in vitro activity of PT over PI, even among PI-R strains, clearly demonstrates the crucial role played by tazobactam to inhibit expressed beta- lactamases. These two agents should not be considered 'interchangeable' in the face of formulary decisions. Similar analyses must be undertaken when evaluating the appropriate use of any antibiotic whose 'off-patent' status revitalizes its potential formulary status.

Tuberculosis: diagnosis, resistance and immunology

P549

Rapid cultivation of *Mycobacterium tuberculosis* and other mycobacterial species by using culture supplements

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Objectives: Rapid growth of mycobacteria and susceptibility testing in liquid media has greatly reduced the time by which the overall processing of culture-positive samples is completed. This procedure has also reduced efforts invested in culture negative samples. The detection of minimal growth at an early stage combined with the use of nucleic acid-based tests for mycobacterial species identification and their drug susceptibility markers provide a unique opportunity for rapid diagnostics. However, the time required for growth and less than optimal media are still limiting factors for the performance of culture systems. Our aim is to improve the sensitivity and reduce the time required for the detection of mycobacteria in clinical specimens.
Methods: Clinical specimens investigated for the presence of mycobacteria were cultivated in the BACTEC MGIT 960 culture system. The specimens were cultivated in the regular BBLTM Middlebrook 7H9 medium with and without the addition of culture supplements. The *Mycobacterium tuberculosis* strains isolated were typed by using RFLP typing.
Results: Among the eleven hundred clinical samples investigated for the presence of mycobacteria, the culture supplements improved the recovery of *M. tuberculosis* from patients and increased the sensitivity of *M. tuberculosis* cultivation by 20%. Furthermore, the mycobacterial culture supplements reduced the time required for the detection of growth of *M. tuberculosis* by 5.4 days from an average of 16.2 days without supplement addition to an average of 10.8 days with supplement. IS6110-based RFLP typing of *M. tuberculosis* demonstrated that the isolates that grew quicker with supplements represented various RFLP types, and not only a sub-cluster of strains. The sensitivity and rapidity of culture were also enhanced for the detection of other mycobacterial species, but to a various extent in the different entities isolated. *M. avium* isolates that grew faster with culture supplements were found in neck abscesses in children and sputum from immunocompromised patients.
Conclusion: Significantly shorter cultivation time and 20% higher sensitivity in *M. tuberculosis* cultivation was achieved by adding culture supplements to liquid Middlebrook 7H9 medium. Also, a number of non-tuberculous mycobacterial species grew faster with culture supplements addition than without. This results hold promise for significant improvements in culture-based mycobacterial diagnostics.

P550

Evaluation of the Amplified *Mycobacterium Tuberculosis* Direct test for direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens

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Objectives: Evaluation of the Amplified *Mycobacterium Tuberculosis* Direct (AMTD) test (Gen Probe Inc, bioMérieux) by comparing the sensitivity, specificity and positive (PPV) and negative (NPV) predictive values of the test with the acid fast smear and the mycobacterial culture.

Methods: We studied 147 respiratory specimens: sputum (98), bronchoalveolar lavage (22) and bronchial aspirate (27), submitted to the microbiology laboratory from patients suspected of pulmonary tuberculosis (TB). The AMTD results were compared with acid fast smear, MB/Bact system liquid culture (bioMérieux) and Lowenstein-Jensen solid medium (L J).All samples were subjected to the Than-Thiam-Hok (bioMérieux) staining for acid fast bacilli (AFB) detection, decontaminated according to the NALC-NaOH method (N-acetyl-L-cysteine and sodium hydroxide) using the commercial Mycoprep kit (Becton Dickinson) and inoculated in parallel in the MB/Bact system and in the L J medium. The mycobacteria isolated were identified by DNA probes (Accuprobe, Gen Probe, bioMérieux).
Results: Based on mycobacterial culture and on the clinical diagnosis, 70 samples were from patients with TB and 77 from no TB patients. 57 specimens were culture positive (identified as *Mycobacterium tuberculosis* complex -Mt), and 47 were smear positive. 74 specimens were positive by AMTD. After resolution of discrepancies, the sensitivity, specificity and PPV and NPV for the AMTD test were 98.6%, 93.5%, 93.2% and 98.6% respectively.
Conclusions: The results suggest that the AMTD test in combination with AFB and culture is a fast and accurate test for suspected TB patients.

P551

Comparison of a LightCycler real-time PCR method and the BDProbeTec ET system for direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens

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Objectives: Detection of micobacteria by culture takes between one week and two months.To reduce the delay in tuberculosis diagnosis, we compared a home-made LightCycler real-time PCR method to the BDProbeTec ET system (Becton Dickinson, Sparks, Md.), for direct detection of *Mycobacterium tuberculosis* in 60 clinical samples and to the conventional mycobacterial culture.
Methods: The real-time assay amplifies a region from the IS6110 sequence and the BDProbe Tec amplifies a region of the mycobacterial 16S rDNA. Conventional mycobacterial culture was performed with BACTEC MGIT 960 and Lowestein-Jensen medium. Sixty respiratory specimens were used (36 sputa, 14 pleural fluid and 10 BAL) from patient with negative smear microscopy and a high probability of tuberculosis were assessed by the three methods.
Results: Of 60 specimens, 10 grew MTBC (and in one culture grew one *M. avium*-intracellulare). BDProbeTec ET detected 9 of the 10 MTBC culture-positive specimens and gave a positive result in one of the 49 negative cultures. LC real-time PCR detected the same 9 MTBC culture-positive specimens and gave a positive result in another – but different from that of the BDProbeTec ET – of the 49 negative cultures. Thus, the sensitivities of the real-time PCR method and SDA were similar for respiratory specimens.
Conclusion: The data demonstrate that both LC real-time PCR and BDProbeTec assays are highly sensitive and specific techniques for the rapid detection of *M. tuberculosis* comples in respiratory smear negative specimens.

P552

Comparative evaluation of different methods for the identification of mycobacteria other than tuberculosis

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Rapid, easy and prompt identification of mycobacteria to the species level is of utmost importance, since treatment varies according to the species.
Objective: To evaluate several techniques that allow the identification of mycobacteria other than tuberculosis (MOTT).
Methods: A total of 70 clinical strains of MOTT isolated in Löwenstein-Jensen medium were included in the study. Identification has been performed to species or to complexes level using 1) Conventional methods, 2) Accuprobe (Gen-Probe), an hybridization protection assay and chemiluminescent detection of rRNA presented in living organisms, and 3) Genotype (HAIN Lifescience), that consists of PCR amplification, reverse hybridization of PCR products to the strips and detection and interpretation of the results according to the interpretation chart.
Results: The 70 MOTT strains were identified as follows:

Groups of MOTT	Conventional methods	AccuProbe	Genotype
Scotochromogenes			
M. gordonae	23 presumptive	23	23
M. scrofulaceum	-	-	1
Non chromogenes			
M. avium–avium	-	6	7
M. intracellulare (avium complex)	-	3	2
Rapid growers			
M. fortuitum	19 presumptive		19 type 1
M. peregrinum	-	-	6
M. chelonae	2 presumptive	-	2
M. fortuitum–chelonae complex	12	-	-only band number 10

Conclusion: The differentiation of MOTT species by conventional methods is difficult, time consuming and many times unreliable. The commercial tests of AccuProbe and Genotype have replaced the conventional biochemical tests for identification of mycobacteria. The AccuProbe test has a limited number of species that can be identified. The Genotype strip technology detects simultaneously much more species and also gives the opportunity of identification of *M. tuberculosis* complex together with MOTT strains. Although the new methods are more rapid and accurate, all strains were not identified to complex or species level. Seventeen per cent of the isolates would need to be identified by sequencing.

P553

An insight into the evolution of *Mycobacterium tuberculosis* complex using a novel molecular technique – multiple interspersed repetitive units

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Objectives: Many molecular techniques have been utilised in an attempt to elucidate the phylogeny of *Mycobacterium tuberculosis (M. tb)*. Despite these advances the evolution of this

organism is still poorly understood. This study aimed to assess a new molecular amplification technique, Mycobacterial Interspersed Repetitive Units (MIRU), to discover if the phylogeny of *M. tb* complex could be defined using this novel tool.
Methods: A defined *M. tuberculosis* population of 316 isolates indexed by neutral genetic variation was analysed using MIRU. In addition, 50 *M. bovis* isolates were examined to determine if they could be separated from *M. tb* by MIRU typing alone.
Results: Previously neutral genetic variation within the *M. tb* complex genome demonstrated that *M. tb* could be divided into four lineages with *M. bovis* clearly separated on another branch of the tree. Analysis with MIRU established that each lineage could in turn be defined by a specific MIRU code made up of only 2 or 3 loci. Each MIRU code could define between 85–98% of isolates within their respective lineage. However, some markers were not as conserved as others. A MIRU code for *M. bovis* was determined which could define 83% of isolates. This code was unique to *M. bovis* and not observed in any *M. tb* isolates.
Conclusions: MIRU can define evolutionary lineages within *M. tb* and *M. bovis* populations without the need for sequencing. *M. bovis* isolates can easily by differentiated from *M. tb* isolates using this technique. MIRU is a valuable tool for phylogenetic studies and could define an evolutionary uncharacterised population of *M. tuberculosis* complex.

P554

Comparison of RFLP-IS6110 and MIRU-VNTR typing of *Mycobacterium tuberculosis* strains

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Objectives: The discovery of insertion elements and other repetitive DNA elements in the genome of *Mycobacterium tuberculosis* has led to many methods for differentiating clinical isolates, becoming an important tool in the epidemiology of tuberculosis. The use of RFLP-IS6110 (restriction fragment length polymorphism) method allows a high degree of *M. tuberculosis* isolates discrimination and is considered the gold standard. The introduction of MIRU-VNTR (variable number tandem repeats of mycobacterial interspersed repetitive units) has become an alternative to the classical method. Moreover, isolates having low copy numbers of IS6110 can not be clustered precisely, and since MIRU-VNTR analysis detects polymorphisms in 12 different loci, it would be a useful method for subdivide this isolates. The objective of this study is to evaluate the discrimination power of MIRU-VNTR technique analysing a set of *M. tuberculosis* strains isolated in Lisbon hospitals.
Methods: 53 *M. tuberculosis* isolates were typed using RFLP-IS6110 and MIRU-VNTR techniques.
Results: Individually, the total of the 52 strains analysed by RFLP-IS6110 produced 45 strains in previously defined clusters (4 strains in cluster R2; 3 strains in cluster R4; 2 strains in cluster R7; 1 strain in cluster Lisboa2; 4 strains in cluster Lisboa3; 1 strain in cluster Lisboa9; 3 strains in cluster T1; 2 strains in cluster T4; 3 strains in cluster F; 4 strains in cluster B; 1 strain in cluster I1; 1 strain in cluster I2; 1 strain in cluster X1; 1 strain in cluster Y; 1 strain in cluster Q; 1 strain in cluster K; 1 strain in cluster M; 1 strain in cluster L10; 1 strain in cluster J; 3 strains in cluster N2; 2 strains in cluster N3 and 4 strains in cluster O1); 3 of these clusters had low (<5) copy numbers of IS6110 (clusters N2, O1 and N3). MIRU-VNTR analysis of the same isolates with more than 6 copies of IS6110 generated a total of 19 different VNTR allele profiles which is in agreement with the classical technique. However, the analysis of the clusters with low IS6110 copy numbers produced 7 different profiles suggesting a major discrimination power.
Conclusion: The results obtained show that the congruence between genetic relationships inferred from MIRU-VNTR and RFLP-IS6110 typing was highly significant. Compared with RFLP-IS6110, MIRU-VNTR typing has the advantages of being fast and appropriate for all *M. tuberculosis* isolates, including strains with a few IS6110 copies.

P555

Evaluation of novel tandem repeat loci as potential *Mycobacterium tuberculosis* molecular typing VNTR regions

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Objectives: The international community involved with molecular typing of *M. tuberculosis* isolates has recently focussed on Variable Number Tandem Repeat (VNTR) loci. VNTR-PCR involves amplification of the repeat region with primers in the flanking sequences. The number of repeat units is determined from the size of the product on an agarose gel. To date the majority of investigations into *M. tuberculosis* VNTRs have focussed on 40–100 bp repeat-unit VNTRs. In this study we looked at a range (3–78 bp repeats) including smaller repeat-unit VNTRs to investigate allelic variation in our collection of isolates and determine their usefulness in a molecular typing VNTR panel.
Methods: A collection of 23 isolates was used to investigate repeat-number variation at novel VNTR loci ranging from 3 bp to 78 bp in unit length. PCR of tandem repeat sequences were performed using primers in the 5′ and 3′ flanking regions. Pyrosequencing technology, non-denaturing High Performance Liquid Chromatography (non-dHPLC) and Beckman CEQ8000 fragment analysis were used to determine VNTR number for these loci. Beckman capillary sequencing was used to confirm VNTR copy number from sequence for anomalous fragment sizes and to further characterise the individual loci. VNTR copy number was determined from these fragment sizes using a simple calculation. These data were entered into Bionumerics software for phylogenetic analysis of isolates.
Results: Several novel VNTR loci tested were found to vary in copy number between strains in our isolate collection. The number of alleles varied for several of the novel VNTR loci. Certain loci were found to be useful to differentiate the ancient genotypic group one isolates from the more recent groups two and three. For example, all non-genogroup one isolates (n = 15) had one copy of VNTR12, while genogroup one isolates (n = 8) all had greater than one copy of this repeat. Further results characterising each novel locus will be presented.
Conclusion: Results from this study indicate that other tandem repeats, outside the 40–100 bp unit range, should be considered for investigations into highly discriminative markers for *M. tuberculosis* molecular typing. These investigations could help augment the final VNTR panel for an international strain-typing of *M. tuberculosis*. Our results may indicate that certain repeat loci within the genome could enable modelling of *M. tuberculosis* evolution and better inform the role of these repeat sequences in the chromosome.

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Sensitivity of PCR for IS2404 insertion sequence of *Mycobacterium ulcerans* in diagnosis of Buruli ulcer from punch biopsies

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Objectives: Mycobacterium ulcerans disease (Buruli ulcer) manifests as a nodule, papule, plaque or oedematous lesion

prior to ulceration and the usual form of treatment is surgery. Clinical diagnosis can be confirmed by histopathology, detection of acid fast bacilli (AFB) and culture from surgically excised lesions but if non-surgical treatment is planned an alternative diagnostic technique is needed. In the present study 4 or 6 mm punch biopsies were used to compare the sensitivity of detection of AFB, culture for *M. ulcerans*, polymerase chain reaction (PCR) for *M. ulcerans* and histopathology in making the diagnosis of *M. ulcerans* disease in a field setting.

Methods: 4 or 6 mm punch biopsies were taken before surgical excision of 69 lesions suspected clinically to be due to *M. ulcerans* disease. They were analysed by Ziehl- Neelsen stain (ZN) for AFB, culture on Löwenstein Jensen slopes, histology and PCR. PCR for the insertion element IS2404 was modified to include uracil-N-glycosylase and dUTP instead of dTTP to reduce the risk of cross contamination.

Results: Out of 69 clinically diagnosed cases of *M. ulcerans* disease, 50 were confirmed on the basis of culture or histopathology and all were PCR positive. Nine had negative culture and AFB but histopathology was compatible with *M. ulcerans* disease and all had positive PCR. Out of the remaining 10 cases in which PCR was negative, AFB and culture were also negative and histopathology was incompatible with *M. ulcerans* disease in all but one case showed inflammation compatible with but not diagnostic of *M. ulcerans* disease and the clinical presentation was not typical. Therefore the modified PCR was 100% sensitive and there was only one possible false negative result. The sensitivities of microscopy, culture and histology were 42%, 50% and 83% respectively. A 4 mm punch biopsy was preferred to a 6 mm biopsy since it was less likely to bleed and to need stitching.

Conclusion: This field study showed that PCR for IS2404 was the most sensitive diagnostic test for *M ulcerans* disease, with high specificity and it gave a rapid result. Given adequate technical expertise and controls the PCR was viable in this teaching hospital setting in Ghana and, in routine practice, we would recommend ZN staining of biopsies to detect AFB, followed by PCR in AFB negative cases only, in order to minimise costs. Histology and culture remain important as quality control tests, particularly in studies of treatment efficacy.

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Reverse hybridisation as tool for the identification of mycobacteria other than tuberculosis from clinical respiratory samples

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Objectives: To evaluate molecular methods for the identification of non-tuberculous mycobacteria (mycobacteria other than tuberculosis, MOTT) isolated from clinical respiratory samples.

Methods: Nineteen MOTT strains were selected isolated from respiratory clinical samples and cryopreserved at –80°C. Previously decontaminated samples were cultivated in MGIT broth and incubated in BACTEC MGIT 960 system (Becton Dickinson). The strains were identified by hybridization with specific labelled probes (AccuProbe, GenProbe) or identified by Reference National Center in Madrid (CNM). The strains included in the study were *M. abscessus* (1), *M. alvei* (1), *M. avium* (5), *M. avium* complex (1), *M. celatum* (1), *M. fortuitum* (2), *M. gordonae* (1) *M. kansasii* (2), *M. mucogenicum* (1), *M. peregrinum* (1), *M. senegalense* (1), *M. simiae* (1), and *M. terrae* (1). The strains were partially thawed and recovered in MGIT broth until to obtain positive signal growth. DNA was extracted from 0.2 ml and amplification by PCR from rRNA 16S–23S spacer region was done in PE-9600 thermocycler (Perkin Elmer). Amplified product were visualized by electrophoresis agarose gel checking a 400–550 bp band and positive samples were identified by reverse hybridization with labelled probes fixed to nitrocellulose following manufacturer's indications (InnoLipa Mycobacteria v2, Innogenetics).

Results: Three strains (15.7%) cannot be recovered (*M. alvei*, *M. celatum* and *M. fortuitum*). Other 3 strains were identified as Genus Mycobacteria only (*M. senegalense*, *M. mucogenicum* and *M. terrae*) due to not include specific probes for those species. The rest of strains were identified as *M. abscessus* (1), *M. avium* (5), *M. avium* complex (1), *M. fortuitum–M. peregrinum* complex (2), *M. gordonae* (1), *M. kansasii* (2) and *M. scrofulaceum* (1).

Discussion: All amplified strains were correctly identified except one (93.7%). Only *M. simiae* was identified incorrectly as *M. scrofulaceum* by reverse hybridization. Innolipa is a convenient and accuracy method for the identification of MOTT and is more versatility that AccuProbe due to have a more complete probes collection.

P558

Rapid detection of multi-drug resistant *Mycobacterium tuberculosis* by pyrosequencing

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Objectives: After the introduction of effective antimycobacterial drugs, tuberculosis (TB) was thought to be under control. Today, *Mycobacterium tuberculosis* is still one of the most harmful human pathogens worldwide, causing 8 million new cases and between 2 and 3 million deaths each year. The upsurge of TB has been accompanied by the emergence of drug-resistant strains of *M. tuberculosis*. Primary multi-drug resistance (MDR), resistance to at least isoniazid (INH) and rifampin (RMP), is an increasing problem in both developed and developing countries. DNA-sequencing studies have demonstrated that >95% of the RMP resistant *M. tuberculosis* strains have a mutation within the 81-bp hot-spot region of the gene encoding for the b subunit of DNA dependent RNA polymerase (rpoB). INH resistance is most frequently associated with a specific mutation in katG (codon 315) gene, that encodes the catalase-peroxidase enzyme in *M. tuberculosis*. Since early diagnosis of the disease and the rapid identification of resistance to primary anti-TB agents are essential for the efficient treatment and control of MDR-TB, rapid methods for early detection of resistant *M. tuberculosis* isolates are required. The aim of this study was to develop a rapid and reliable method for detection of MDR-TB, applicable to both routine and research laboratory work.

Methods: Pyrosequencing is sequencing by synthesis, a simple and rapid technique for accurate analysis of DNA sequences. A pyrosequencing assay for the detection of rpoB and katG315 mutations causing rifampin and isoniazid resistance was developed. The method was evaluated using 42 clinical *M. tuberculosis* isolates. Pyrosequencing results were compared to the conventional sequencing results and to the phenotypic susceptibility test results.

Results: The concordance of the pyrosequencing and conventional methods was 100% for rifampin resistance and 81% for isoniazid resistance. Nine different rpoB mutations were detected, which all have been previously characterized. The Ser531Leu was the most common mutation found in 16/27 RMP resistant isolates. None of the susceptible isolates were found to contain mutations. The pyrosequencing results were obtained in two days, compared to at least 5 days with the conventional sequencing (in our laboratory settings).

Conclusion: The pyrosequencing method was found to be a cost-effective, rapid and reliable method for detection of MDR-TB.

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M. tuberculosis drug resistance and clinical outcome in Greek and immigrant patients

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Objective: We studied Isoniazide (INH), Rifampicin (RIF) and (MDR) resistance (INH+RIF) of *M. tuberculosis* strains derived from Greek and immigrant patients of 'Sotiria' hospital, in the years 1993–1995 and 2001–2003. We also studied the clinical outcome in the same patients.
Methods: In 1993–95, 1391 *M. tuberculosis* strains were studied, 1209 from Greeks and 182 from immigrants, while in 2001–03, 1270 strains, 960 from Greeks and 306 from immigrants. Cultures and susceptibility testing were performed, classically and automatically, by Bactec 460 and MGIT 960 systems.
Results: In 1993–95, 81/1209 (6.7%) *M. tuberculosis* isolates from Greeks and 26/182 (14.3%) from immigrants were resistant to either INH or RIF. 'Sotiria' TB unit followed up 64 (79%) Greeks and 24 (92.3%) immigrants. Successful therapy was for 50 (78%) Greeks and 13 (54%) immigrants, while contact was lost with 14 (22%) Greeks and 11 (46%) immigrants. In 2001–03, 144/964 (14.9%) isolates from Greeks and 103/306 (71%) from immigrants were resistant to either INH or RIF. 'Sotiria' TB unit followed up 103/144 (71%) Greeks and 63/306 (85%) immigrants. Successful therapy was for 72 (70%) Greeks and 51 (82%) immigrants, while contact was lost with 31 (30%) Greeks and 12 (19%) immigrants. In 1993–95, 14/1209 (1.16%) Greeks and 4/182 (2.20%) immigrants were MDR. TB unit followed up 11 (78.6%) Greeks and 4 (100%) immigrants, with a successful outcome for 7 (63%) Greek and 2 (50%) immigrant patients and lost contact with 3 (27%) Greeks and 2 (50%) immigrants. One Greek patient died. In 2001–03, 36 (3.73%) Greeks and 18 (5.88%) immigrants were MDR. TB unit followed up 25 (69.4%) Greeks and 17 (94.4%) immigrants and lost contact with 5 (20%) Greeks and 3 (17.6%) immigrants. Successful outcome was for 13 (52%) Greeks and 8 (47%) immigrants, while 5 (20%) Greeks and 5 (29.4%) immigrants are still under therapy. 2 (8%) Greeks and 1 (6%) immigrants died.
Conclusion: There is a remarkable increase of *M. tuberculosis* resistance to INH, RIF and MDR, both in Greeks and immigrants, but the per cent of successful clinical outcome remains almost the same. Greek authorities should organize a modern and functional TB monitoring system.

P560

Mycobacterium tuberculosis primary resistance during 1995–2004 in a general universitary hospital, Alicante, Spain

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Introduction: The treatment of tuberculosis as global emergency of public health, fail by drug resistance, inadequate regimen, or noncompliance with prescribed treatment. The resistance to antituberculous agents is primary when it is present before treatment has been started and secondary when it happens during treatment course.
Objectives: To know the prevalence of resistant *Mycobacterium tuberculosis* in primary infections during the period of time 1995–2004.
Methods: The results of susceptibility tests in positive cultures for *M. tuberculosis* were analysed. Cultures in solid medium Lowestein-Jensen and liquid medium Middlebrook 7H12 were made, and, drug susceptibility testing was done by using a radiometric BACTEC 460 system (Becton Dickinson Diagnostic Systems). The antituberculous drugs tested have been as following: streptomycin, isoniazid, rifampicin, ethambutol and pyrazinamide.
Results: In a total of 490 patients with diagnosis of tuberculosis and positive culture, 89.8% (440) of cases shown sensibility to all antituberculous agents, and 10.2% (50) of them were resistant at least to one drug. The 76% (38) of cases in the resistant group were just to one drug, and 24% (12) were resistant to more than one agent. There were 1.4% of multidrug-resistant tuberculosis (MDR-TB) (rifampicin and isoniazid resistance) and, of them, 3 cases were resistant also to a third or fourth drug, pyrazinamide, streptomycin and/or ethambutol. In the period 1995–1997 we have found two cases of MDR TB, another case in the period 1998–2000, and four cases in 2001–2004.
Conclusions: The resistance rate in our area was of 10.2%. The antituberculous agent with higher number of resistances was isoniazid (8.8%), followed by rifampicin (2%). Ethambutol and streptomycin (0.6%) were found in a lowest percentage. The global multidrug-resistant tuberculosis was 1.4%, influenced mainly by increasing number of cases in last years.

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Prevalence of mutations associated with drug resistance in Beijing and non-Beijing *Mycobacterium tuberculosis* strains from the Southern Ukraine

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Tuberculosis (TB) incidence in Ukraine has virtually doubled since 1992, reaching 77.5/100 000 in 2003. Recent studies have demonstrated that Beijing strains of *Mycobacterium tuberculosis* dominate in certain regions of Russia and some countries of the former Soviet Union (FSU). The spread of Beijing strains and their strong association with high levels of drug resistance are believed to be major factors contributing to the TB epidemic in the FSU. Limited data regarding drug resistance rates are available only for selected regions of Ukraine and the prevalence of different genotypes and their contribution to the TB epidemic in Ukraine is unknown.
Objective: To determine the prevalence of Beijing strains of *M. tuberculosis* in two regions in the Southern Ukraine and their association with drug resistance and to determine prevalence of specific mutations associated with rifampicin and isoniazid resistance.
Materials and methods: Total of 110 *Mycobacterium tuberculosis* strains isolated from patients with pulmonary TB from Odessa and Nikolaev oblasts were analysed. Beijing strains were identified using spoligotyping. The prevalence of mutations in rpoB, katG and inhA associated with rifampicin and isoniazid resistance was evaluated using a dot-blot hybridization macroarray based on reverse hybridization of biotin-labelled PCR products to oligonucleotide probes immobilized on membranes.
Results: Spoligotyping of 110 *M. tuberculosis* strains yielded 12 clusters and 28 individual patterns with the largest cluster consisting of 34 isolates. Forty (36.4%) strains were identified as the Beijing strains. Six Beijing isolates had incomplete Beijing spoligotyping profiles with spacers 37, 37–38, and 38–40 missing. Mutations conferring multidrug resistance were most prevalent in Beijing strains (47.5% versus 22.9%). In rifampicin resistant isolates mutations were seen in rpoB codons 512–519, 523–529, and 528–534 in 18.9%, 32.4%, and 51.4% respectively. Mutations in codons 528–534 of rpoB were strongly associated with Beijing strains. Previous evidence about association of mutations in katG codon 315 with the Beijing genotype was not supported in our study.

Conclusions: Molecular genetic analysis shows relatively high genetic diversity of strains circulating in the Southern Ukraine compared with those seen in Russia. High rates of drug resistance are likely to be associated with the spread of Beijing strains and with exogenous infection.

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The pattern of drug resistance in hospitalised patients with drug resistant tuberculosis in an Iranian hospital

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Introduction: Tuberculosis is a common infectious disease these days; it has the highest mortality rate among all infectious causes only after AIDS. The emergence of *Tuberculosis bacillus* species resistant to multiple drugs has become a serious global threat to the human race. Resistance is either primary, or it may evolve in the course of treatment due to patient's non-compliance or an inadequately and inappropriately selected drug regimen. This study has been done to evaluate drug resistance and to determine the type of resistance in drug resistant tuberculosis patients.
Methods: The files of patients hospitalized in Massih Daneshvari clinical mycobacteriology ward during the past 2.5 years due to suspected drug resistance tuberculosis were evaluated. Those who had a sputum antibiogram indicating resistance to at least one anti-TB drug were included in the study. Data, including demographic data, radiological findings, sputum smear, sputum culture and antibiogram was recorded in a specified questionnaire. Analysis was done for central indices using the SPSS software.
Results: 43 cases met the inclusion criteria. 27 of them (63) were male and 16 (37) were female. The youngest patient was 16 and the oldest was 80 years old. The mean value was 36.9 years (SD = 16.76). 30 cases (70%) were Afghans and 13 (30%) were Iranian. Antibiograms of 38 patients (88%) showed resistance to at least INH and RIF; these cases were considered to be MDR. In 24 cases (56%) the tubercles bacillus was resistant to all four drugs (INH, RIF, STM, EMB). 36 patients (85%) had resistance to STM at least, and 26 patients (60%) were at least resistant to EMB. Bacillus drug sensitivity for PZA was not specified.
Conclusion: Most drug resistant cases of TB were seen among Afghan emigrants. 95% of cases had a history of treatment at least once, and the resistance was secondary. Despite discontinuation of streptomycin usage as an anti-TB drug in Iran in recent years, the most common type of resistance was related to this drug, occurring in 85% of cases. Confirming different studies in other countries, the lowest resistance to first line anti-TB drugs was for EMB, detected in 56% of cases.

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Rapid drug sensitivity testing for *M. tuberculosis* using slide culture

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Objectives: MDR-TB has worsened the tuberculosis crisis in the developing world. Lack of resources limits access to rapid drug sensitivity tests like BACTEC. We conducted in a tertiary care public sector hospital in South India, a study to compare drug sensitivity testing of *M. tuberculosis* on LJ medium with the Rapid Slide Culture (RSC) method using human blood medium (HBM).
Methods: Isolates of *M. tuberculosis* grown on LJ medium were subjected to sensitivity testing on LJ medium and by RSC (Absolute Concentration Method). For RSC, citrated HBM (antibiotics and antifungals added to prevent contamination) was used. Outdated human blood was obtained from the hospital blood bank. Antituberculous drugs – INH, Rifampicin, Ethambutol and Streptomycin were added to HBM.Standardised inocula of *M. tuberculosis* were smeared on to the lower 1/3rd of sterile glass slides, immersed in HBM and were incubated at 37°C for 8 days. Presence of microcolonies were demonstrated by ZN staining. Growth was graded according to the size of microcolonies and the drug sensitivity patterns were documented.
Results: 41 isolates were tested for drug sensitivity on LJ medium.25/41 (60.9%) were sensitive to all 4 drugs, 6/41 (14.6%) resistant to a single drug and 10/41 (24.3%) resistant to multiple drugs [6 resistant to all 4 and 4 to INH and Rifampicin]. Drug sensitivity was reported at 28 days. 37/41 isolates (90.2%) were grown by RSC method. Results were read on 8th day. 26/37 (70.2%) were sensitive to all 4 drugs, 3 resistant to single drug (8.1%) and 8 resistant to multiple drugs (21.6%) [2 resistant to all 4 drugs, 3 to INH, Rifampicin and Ethambutol, 3 to INH and Rifampicin]. Agreement between the 2 methods were calculated by kappa statistics. Significant agreement was shown for INH (.871) , Rifampicin (.924) and Ethambutol (.874). Streptomycin (.226) failed to show agreement.
Conclusion: 1. Rapid Slide Culture shows agreement with the standard method for sensitivity testing of *M. tuberculosis* especially for the clinically important drugs INH and Rifampicin. 2. The results of drug sensitivity are obtained considerably early than the standard. 3. This simple inexpensive technique has potential for adaptation to developing economies lacking sophisticated rapid drug sensitivity tests like BACTEC.

P564

LCD-array for molecular analysis of isoniazid and rifampin resistance in *Mycobacterium tuberculosis*

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Objective: To evaluate a Low Cost Density Array (LCD-Array; Chipron, Berlin. Germany) for molecular analysis of isoniazid and rifampin resistance in *Mycobacterium tuberculosis*.
Methods: A total of 44 *M. tuberculosis* isolates with a previously characterised resistant mechanism were evaluated. One LCD-Array contains eight identical microarrays, separated in prestructured reaction chambers. Each chamber is designed to detect mutations within the 90 bp rpoB region, codon 315 in the katG gene and mabA-inhA regulatory region. The capture probes the sequence region of amino acid position 315 for the katG gene span. One probe represents the wild type (wt) (315-Ser) and two probes harbour the sequence for the mutations 315-Thr and 315-Asp. The capture probes of the mabA-inhA regulatory region have been designed for three point mutations in the 5′ non-coding region (-8T-C, -15C-T and –17G-T). One capture probe spanning all three positions has been designed for the wt. The capture probes for the rpoB gene cover a region coding for amino acids 504–534. Six capture probes, representing the wt sequence, are included. Below there are 12 capture probes for the most frequent mutations. A multiplex-PCR for rpoB, katG and mabA-inhA was performed with 1 μl of DNA in 25 μl reaction volume. Hybridizations were performed according to the manufacturer's instructions.
Results: Of the 22 strains with a katG 315 mutation (20 Ser315Thr, 1 Ser315Arg and 1 Ser315Asn) all were correctly identified by the LCD-Array except for the strain with Ser315Asn which is not represented in the LCD-Array. Nevertheless, this strain did not hybridise with the wt probe.

Nineteen strains with alterations in the mabA-inhA regulatory region (2 with-8T-C, 16 with -15C-T and 1 with –17G-T) were included. The LCD-Array correctly identified 16 mutated strains. Three strains (2 with -15C-T and 1 -8T-C) did hybridise with both the wt and mutated capture probes. Thirteen out of 15 strains with mutations in the rpoB region were correctly classified. One strain with an Asp516Phe did not hybridise with the wt type probes for aminoacids 514–519 and the capture probe for this rare mutation was not included in the panel.
Conclusion: LCD-Array is an easy, rapid and convenient method to characterise codon 315 mutations in the katG gene and rpoB region. Capture probes for the mabA-inhA regulatory region should be optimised in order to suppress cross hybridisation of wt with the -15C-T capture probe.

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Mutations in the rpoB, katG and inhA genes leading to rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* isolates from Turkey

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Objectives: This study was carried out to specify mutation within subunit b of the rpoB gene in rifampin (RIF) resistant and within katG and inhA gene in isoniazid (INH) resistant *M. tuberculosis* isolated from TB patients in Turkey.
Methods: 227 RIF, 255 INH resistance strains were included in this study. Strains were identified by standard biochemical tests and molecular method, of PCR-RFLP. Rifampin resistance was determined by proportion method using LJ medium. In order to detect the presence of mutation, amplification was performed using primer rpo95-rpo397 for rpoB, TB86–TB87 for katG, TB92–TB93 for inhA genes as described previously. The same primers were used for DNA sequence analysis of both strands, using the automated Applied Biosystems, ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, Calif).
Results: In 227 RIF resistant *M. tuberculosis* isolates, twenty different types of point mutation at eight different codons, two deletions and one insertion were detected. The codon most frequently involved in mutation were codon 531 (53.3%) and codon 526 (18.5%). The most frequent allelic mutation was the mutated codon TTG in position 531 (Ser® Leu) (49.3%). The other mutations were codon 516 (10.1%), 522 (3.5%), 533 (1.8%). We couldn't find any mutation 11 (4.9%) isolates. A three nucleotide deletion between codon 514 and 516 at two rifampin resistant isolates, and a three nucleotide insertion between codon 514 and 515 was found in one rifampin resistant isolates. In 255 INH resistant *M. tuberculosis* isolates, seven different types of point mutation at three different codons, one deletion were identified. The codon most frequently involved in mutation was codon 315 (66.7%). The most frequent allelic mutation was the mutated codon ACC in position 315 (Ser®Thr) (60.4%). The other mutations were codon 314 (0.4%), 299 (0.8%). We couldn't find any mutation 82 (32.2%) isolates. Four codons deletion between codon 301 and 306 at one INH resistant isolate. We studied inhA gene mutation on 113 of 255 INH resistant *M. tuberculosis*. We found only one type point mutation codon TGA in position 209 (Arg® Stop)
Conclusion: The most frequently encountered mutation is at codon TTG 531 for rpoB gene and at codon ACC 315 for katG gene and at codon TGA 209 for inhA gene.(The project was supported by Government Planning Organization, Turkey)

P566

Second-line antituberculous drugs: usefulness of the E-test for susceptibility testing of multidrug-resistant *Mycobacterium tuberculosis*

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Objective: Tuberculosis caused by multidrug-resistant strains represents a therapeutic challenge for the selection of appropriate antimicrobial agents. Therefore is important to obtain results of susceptibility to second-line antituberculous drugs earlier and accurately. The aim of this study is to demonstrate the usefulness of the E-test to obtain susceptibility to second-line antituberculous drugs against *M. tuberculosis* multidrug-resistant (MDR-TB) strains.
Methods: From 1992 to March 2004 we isolated 20 MDR-TB. Second-line susceptibility testing was performed retrospectively by the proportions method and for amikacin (AK), claritromycin (CH), ofloxacin (OF) and rifabutin (RF) by BACTEC 460TB, as reference methods (NCCLS M24-T2), and by E-test according to the manufacturer's recommendations. We tested isoniazid (IZ), rifampin (R), streptomycin (SM), ethambutol (EB), AK, CH, and OF by two methods. Levofloxacin (LE), moxifloxacin (MO), and linezolid (LZ) was only tested by the E-test and RF only by the reference method. We used *M. tuberculosis* H37Rv as a quality control.
Results: Percentages of MTB strains resistance comparing reference methods versus E-test were as follows: IZ (100% vs 100%), R (100% vs 100%), SM (27% vs 27%), EB (27% vs 33%), AK (13% vs 20%), CH (67% vs 73%), OF (33% vs 40%), RF (87%), LE (27%), MO (7%), and LZ (7%). These results were read after 21 days by reference method following NCCLS recommendations. Nevertheless, the E-test was read before 10 days.
Conclusions: The E-test is an easy and accurate method for the rapid detection of resistance to second-line anti-TB drugs. Linezolid and moxifloxacin showed the best activity against all MDR-TB. Therefore, E-test is a rapid and valid alternative for susceptibility testing in a clinical laboratory routine.

P567

Temperature mediated heteroduplex analysis of rpoB mutations in *Mycobacterium tuberculosis* DNA: direct detection of rifampicin resistance in clinical specimens

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Objectives: The frequency of resistance to rifampicin in some countries can be as high as 23%. Rifampicin and isoniazid are first-line drugs, and up to 90% of rifampicin resistant isolates are also isoniazid resistant, defined as Multi-Drug Resistant TB. (MDR-TB). 96% of rifampicin resistant isolates carry mutations in a 81-bp region of the rpoB gene. Therefore, it is of clinical importance to be able to rapidly detect rifampicin resistance in patients that are at risk of MDR-TB. Mutations in the rpoB gene of *M. tuberculosis* have been analysed by Temperature Mediated Heteroduplex Analysis (TMHA) previously, however, we make the first report of direct detection of rpoB mutations from clinical specimens.
Methods: 100 rifampicin resistant isolates and 100 rifampicin sensitive isolates from the West Midlands, UK cultured in 2000–2004 had DNA extracted using the QIAamp DNA Mini-Kit. Primers were designed to amplify a 400 bp fragment of the

M. tuberculosis rpoB gene that includes the 81-bp 'hotspot' region. Forward and reverse DNA sequencing defined mutations in the rpoB gene. From July 2004, all sputum samples that were rifampicin resistant frozen at –70°C and DNA was as above. An equal number of rifampicin sensitive sputum samples were also examined. To establish technique reproducibility each individual mutation was analysed in triplicate. After PCR and heteroduplexing, ìL of each PCR reaction was loaded onto a DNASep® Cartridge in a WAVE® DNA Fragment Analysis System (Transgenomic Inc., Omaha, NE). The gradient for separation was a 5.8-min gradient (400 bp) at a flow rate of 0.9 ml/min at 68°C.

Results: The 100 rifampicin resistant isolates possessed a range of defined rpoB mutations. The most common mutation was S531L, with H526Y being the second most prevalent. Mutations conferring rifampicin resistance in all of the 100 isolates were readily detected by the WAVE® System. No false positive results were obtained when the 100 isolates with no resistance conferring mutations were analysed by dHPLC on the WAVE® System. Mutation heteroduplexes were reproducible when analysed in triplicate on the WAVE® System. All mutations defined in cultured isolates from the Midlands were detected directly in clinical sputum specimens.

Conclusion: Temperature mediated heteroduplex analysis of rpoB mutations conferring resistance to rifampicin in *M. tuberculosis* isolates and clinical specimens is an accurate, sensitive, cost-effective and reproducible technique.

P568

Comparison of an ELISPOT assay based on the RD1 selected peptides and intact proteins with QF-TB gold assay in the surveillance of health care workers

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Objective: To evaluate the usefulness of different immunological tests for the diagnosis and surveillance of tuberculosis (TB) infection among health care workers (HCW).

Methods: We enrolled 100 HCW coming from 3 hospitals located in Roma, Italy. Among them, 57 came from wards at high risk of exposure to patients with active TB (pneumology and infectious disease wards). All HCW gave a written informed consent. Samples were analysed by 1) a novel ELISPOT assay based on RD1 selected non-overlapping peptides, 2) an ELISPOT assay based on intact RD1 protein and, 3) a whole blood commercial kit (QF-TB Gold; Cellestis, Australia) which uses overlapping peptides spanning the whole length of RD1 proteins. The results were then compared with tuberculin skin test (TST) score and size; TST >5 mm was considered positive.

Results: Overall, positive response to RD1 selected peptides was 11/100, 2/11 were BCG-vaccinated and 7/11 had a TST >10 mm. A positive response to RD1 proteins was found in 28/100, 10/28 were BCG-vaccinated and 12/28 had a TST >10 mm. A positive response to QF-TB was found in 23/100, 7/23 were BCG-vaccinated and 12/23 had a TST >10 mm. Among those QF-TB positive (assay that is going to be considered the gold standard of TB infection), we identified 10 peptide positive and 13 peptide negative. Among those peptide positive 7/10 (70%) had a TST >10 mm, whereas only 5/13 peptide negative (38%) had a TST>10.

Conclusions: The results indicate that the response evaluated by the QF-TB Gold (23/100), is similar to the response obtained by ELISPOT assay with RD1 protein stimulation (28/100) whereas the response to the selected peptides is found only in a smaller group of the HCW (11/100) and it correlates with the TST-size. Our study suggests that the RD1 peptides response may have an higher specificity in identifying these HCW with an higher risk of developing active TB during life time, compared with other immunological in vitro tests.

P569

Lymphocyte subpopulations in patients with active pulmonary tuberculosis

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Objectives: Tuberculosis remains the leading causes of mortality among human infectious diseases in the world. B cells, natural killer cells, $CD4^+$ T cells, other T cell subsets such as, gamma-delta, $CD8^+$ and CD1 restricted T cells have roles in the immune response to *M. tuberculosis*. How T cells help them perform this task remains poorly understood. A study was undertaken to evaluate the profile of the $CD4^+$, $CD8^+$ T cells and $CD19^+$, CD (16 + 56)+ population in patients with active pulmonary tuberculosis.

Methods: Forty-nine active (mean age 35.76 ± 15.8) and 13 inactive (mean age 43.23 ± 20.5) pulmonary tuberculosis cases and 20 healthy subjects (mean age 38.20 ± 17.6), having no disease and at the similar age range were evaluated. $CD4^+$, $CD8^+$ T cells and $CD19^+$, CD (16+56)+ cells percentages were analysed by using Coulter EPICS XL-MCL (Beckman Coulter U.S.A.) flow cytometry. Active tuberculosis patients were divided into three groups according to the treatment and X-ray findings. So, the results were analysed in the context of antituberculous treatment stages and X-ray findings in patients with active pulmonary tuberculosis.

Results: $CD4^+$ cell percentages were significantly lower in active pulmonary tuberculosis patients than in healthy subjects ($p < 0.001$). CD (16 + 56)+ cell percentages were significantly increased in patients with active pulmonary tuberculosis compared with healthy controls ($p < 0.01$). $CD8^+$ T cells were significantly decreased ($p < 0.05$), and CD (16 + 56)+ cell percentages were significantly increased (0.01) in inactive pulmonary tuberculosis patients compared with healthy controls. These parameters were not different statistically when compared the active and inactive pulmonary tuberculosis patients (Table). $CD4^+$, $CD8^+$, $CD19^+$, CD (16 + 56)+ cells percentages did not show difference at the different time of the antituberculous regimen and X-ray findings of the three different groups of active tuberculosis patients.

	Active PT	Inactive PT	Healthy subjects
$CD3^+/CD4^+$	35.48 ± 9.18	39.28 ± 8.17	43.36 ± 6.01
$CD3^+/CD8^+$	25.72 ± 9.37	22.13 ± 5.75	27.16 ± 7.01
$CD3^-/CD19^+$	11.76 ± 5.97	14.13 ± 4.10	11.86 ± 3.26
$CD3^-/CD(16+56)^+$	16.56 ± 8.46	17.36 ± 7.48	11.03 ± 6.71

Conclusion: Active pulmonary tuberculosis patients have a reduced number of circulating $CD4^+$ cells and an increased number of CD (16 + 56)+ cell compared with healthy individuals. These cells could be played an important role in the immune response to *M. tuberculosis* infection. Table: CD4+, CD8+ T cells and CD19+, CD (16 + 56)+ cell percentages (means of % ±SD) among active pulmonary tuberculosis (PT), inactive pulmonary tuberculosis and healthy subjects

P570

The serum/pleural IL-6 ratio in the differential diagnosis of the tuberculous pleural effusion

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Introduction: Interleukin 6 (IL-6) is locally produced at the sites of inflammation of the pleural space. Pleural IL-6 leaks to circulation to cause its systematic effects. The amount of IL-6 produced in the pleural space as well as the amount that leaks to circulation depends on the particular disease.
Objectives: To study the serum/pleural IL-6 ratio as a specific marker in the diagnosis of tuberculous pleural effusion.
Method: Patients: IL-6 value was assessed in 77 serum/pleural fluid pair samples from patients with exudative pleural effusions with Quantakine IL-6 Immunoassay (4.5 hour solid phase ELISA).
Results: The serum/pleural IL-6 ratio was always ≤0.01 in tuberculous, ≥0.1 in malignant (with exception mesotheliomas) and ≥0.02 in parapneumonics pleural effusions. Pleural IL-6 was >100 U/ml in all par pneumonic and <100 U/ml in all malignant effusions.
Conclusion: Serum/pleural IL-6 ratio in association with pleural fluid's value is a specific and sensitive marker for the quick diagnosis of the tuberculous pleural effusion.

P571

CD14 and IL-10 gene polymorphisms in relation to tuberculosis

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Objectives: Over 2 billion people are estimated to be infected with virulent *Mycobacterium tuberculosis* but fewer than 10% develop clinical tuberculosis within their lifetime. Resistance to *M. tuberculosis* involves a complex interaction between the bacteria and the host immune system. Genetic differences in immune responses may affect susceptibility to mycobacterial infection, but no specific genes have been implicated in humans. The aim of this study was to evaluate whether polymorphisms on CD14 or IL-10 genes might be involved in determining human susceptibility to mycobacteria.
Methods: Eighty nine tuberculosis adult patients were recruited from Regional Center for Pulmonary Diseases in Lodz, Poland. Active tuberculosis was detected by sputum smear staining and culturing of mycobacteria. Control group consisted of eighty six healthy adult volunteers, who have never had tuberculosis. Genomic DNA was isolated from EDTA-anticoagulated whole blood according to the QIAamp DNA Mini Kit from Qiagen (Hilden, Germany). Genotype frequencies for CD14 (-159C/T) and IL-10 (-1082G/A) polymorphisms were analysed using allele specific PCR methods. The levels of serum sCD14 were tested by ELISA (Quantikine TM sCD14, R&D, MN, USA).
Results: The frequency of the CD14 genotypes (C/C, C/T and T/T) in patients and controls was similar. About half of the individuals were C/T heterozygous in both groups. The 34% TB patients and 27% healthy controls were C/C homozygous. The association between serum sCD14 levels and CD14 promoter polymorphisms was noticed for neither controls nor patients. We could see no significant differences between sCD14 levels in sera form C/C, C/T and T/T individuals, however we observed significantly higher levels of serum sCD14 in tuberculosis patients (3178 ng/ml) compared with controls (1582 ng/ml). The distribution of the IL-10 genotypes (A/A, A/G and G/G) was similar in both groups being under the study. The 53% TB patients and 43% controls were A/G heterozygous. A/A and G/G genotypes were observed in about 30% and 20% tuberculosis patients and controls respectively.
Conclusions: Our results suggest that genetic variants of CD14 and IL-10 genes may not be involved in determining the susceptibility to tuberculosis. The increase of serum sCD14 levels in patients with tuberculosis has no genetic background, but probably is a consequence of *M. tuberculosis* infection. Supported by MNiI, Grant No. 3PO5B09124.

P572

Monitoring the efficacy of anti-tuberculous treatment by the expression of phagocyte Fcg receptors

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Background: Receptors for IgG (FcgRs) on phagocytic cells are important in host defence against infection.
Objectives: We have studied the expression of FcgRs by peripheral blood monocytes (M), M cultured for 72 hours (M/Mø), and granulocytes (G) in patients with active Tuberculosis (TB), during anti-TB therapy (anti-TB-Rx) and, after completion of anti-TB-Rx.
Methods: The expression of the three type of FcgRs, FcgRI, FcgRII and FcgRIII, on M, M/Mø and G were analysed by flow cytometry in 87 HIV-negative patients with TB (59 men and 28 women), at diagnosis, and monthly thereafter until completion of anti-TB-Rx. FcgRs expression was assessed on resting M, M/Mø and G, and on these cells after stimulation by culture with IFNg.
Results: The expression of FcgRI and FcgRIII by M, M/Mø and G was enhanced in patients with active TB by: 42 ± 4% and 22 ± 2% for M, respectively ($p < 0.001$), 56 ± 6% and 41 ± 4% for M/Mø, respectively ($p < 0.001$) and, 119 ± 9% and 37 ± 3% for G, respectively ($p < 0.001$). The expression of FcgRIIA by M, M/Mø and G was decreased by –31 ± 1% ($p = 0.02$), –46 ± 3% ($p < 0.001$), and –23 ± 1% ($p = 0.002$), respectively. These alterations of FcgRs expression normalized from the 8th week until the end of effective anti-TB-Rx. The expression of FcgRI, FcgRIIA and FcgRIII by M, M/Mø or G from patients with active TB was significantly increased by culture in the presence of IFNg ($p < 0.001$), returning to normal after 8 wks of anti-TB-Rx. Setting a cut-off value = 25% of the mean fluorescence intensity over controls for FcgRs surface expression and, assuming a prevalence range of active TB between 25 and 80% among patients undergoing confirmatory tests, results in a range of sensitivity, specificity, positive and, negative predictive values of: 57%–96%, 48%–97%, 34%–74%, and 59%–98%, respectively for M-FcgRIIA, 48%–73%, 51%–96%, 38%–74% and 68%–97%, respectively for M-FcgRIII, 31%–58%, 64%–93%, 37%–79% and 69%–95%, respectively for G-FcgRI and, 52%–71%, 88%–98%, 49%–77% and 81%–93%, respectively for G-FcgRIIB.
Conclusions: Mø and G from patients with active TB exhibit an altered expression of FcgRs that disappear after effective anti-TB-Rx. Thus, Mø and G FcgRs expression may help in predicting the response to anti-TB therapy.

P573

Study of the Th1/Th2 response and IgG/IgE isotypes in patients with tuberculosis in the Warao indigenous population, Venezuela

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Objectives: Tuberculosis is an important health problem in Venezuela. The Warao people of the Delta Amacuro State, Venezuela, have the highest prevalence of adult TB (115 cases per 100,000 habitants) in this country. We studied some immunological factor that could explain that special susceptibility, like the Th1/Th2 response (IL-4, IL-12 and gamma IFN) and the isotypes IgE and IgG, in mononuclear cell culture and serum respectively.
Methods: We evaluated 86 adults, 40 Warao patients (34 tuberculin skin test (TST) positive and 6 negative), 22 Creole patients (11 TST positive), 12 Warao controls (11 TST positive) and 12 Creole controls (10 TST positive). The antibodies and cytokines were measured by ELISA.
Results: We found that Warao patients showed a high response of IgG4 and IgE against H37Rv antigens in contrast to Creole patients. On the other hand, Creole patients had the highest percentage of positive individuals in IgG1 and IgG3 against PPD antigen. This was correlate with higher levels of IL-4 in Warao patients in 24-hour cell cultures in comparison to Creole patients. IL-12 was found increased in Creole patients in 24 and 48-hour cultures. We could not find any difference in gamma IFN between both populations.
Conclusion: Warao patients showed a immune profile characterized by Th2 response. These differences between Creole and Warao patients were found in humoral and cellular response.

P574

Comparative study of initial and acquired drug resistance in pulmonary tuberculosis in a national research institute of tuberculosis and lung disease, Iran

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Resistant to anti-tuberculosis agents particularly multiple drug resistant (MDR) *Mycobacterium tuberculosis* is an important obstacle in the treatment and control of tuberculosis in the world. Between Sep. 1996 and March 2000 for 273 smear and culture positive pulmonary tuberculosis patients (both old = 86/273 and new = 187/273) pretreatment susceptibility tests of isolated bacilli to INH, RIF, EMB and STM were performed by standard proportional method and the results were classified in three groups: i) Newly diagnosed without any history of treatment; ii) Patients with history of treatment with one course; iii) Patients with history of treatment for two or more courses supposed to be MDR cases. The results were collected for each drug individually and different combinations of two, three and four medications. Resistance to single drug, two drugs, three drugs and four drugs was significantly increased in group III in comparison to group II and I, also significantly increase in group II when compared to group I. We observed a high rate of primary resistance to INH and STM in group I and II and a high rate of MDR (INH and RIF resistance) in groups II and III. The duration of bacilli exposure to anti-tuberculosis agents in the past is a major factor in developing resistance. In contrast to WHO's guideline, due to high rate of primary resistance especially to STM in our area, we don't recommend addition of STM for treatment of patients whose initial four-drug regimens have been failed (group II).

P575

Evaluation of the BacT/ALERT 3D system for susceptibility testing of *Mycobacterium kansasii* to first-line drugs: comparison with the radiometric BACTEC 460TB system

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Objective: *M. kansasii* is one of the most frequent and pathogenic nontuberculous mycobacteria isolated from human beings. There is evidence that the in vitro susceptibility of *M. kansasii*, based on the same interpretative criteria used with *M. tuberculosis*, correlates adequately with clinical response. The aim of this study was to evaluate the fully automated BacT/ALERT 3D system for susceptibility testing of *M. kansasii* to four major antimycobacterial drugs and to compare the results to those obtained by the radiometric BACTEC 460TB system.
Methods: A total of 32 clinical isolates of *M. kansasii* (one per patient) were tested for antimicrobial susceptibility by BacT/ALERT 3D, and by BACTEC 460 as reference method. *M. kansasii* ATCC 12478 and *S. aureus* ATCC 29213 were also processed as quality control. Four antimycobacterial drugs were studied at different critical concentrations: isoniazid (0.4, 1, and 5 mg/L), rifampicin (1 mg/L), streptomycin (1 mg/L with BacT/ALERT 3D and 2 mg/L with BACTEC 460) and ethambutol (5 mg/L).
Results: All isolates were identified as *M. kansasii* genotype I. The data obtained with BACTEC 460 were as follows: 32 resistant to isoniazid at a concentration of 0.4 mg/L, 15 resistant to isoniazid at a concentration of 1 mg/L, 3 resistant to isoniazid at a concentration of 5 mg/L, 2 resistant to rifampicin and 2 resistant to ethambutol. The overall agreement between the two systems was 76.6%. Forty-five discrepancies were detected: 2 major (false-resistant) errors (ME) for streptomycin, 25 very major (false-susceptible) errors (VME) for isoniazid 0.4 mg/L, 12 VME and 1 ME for isoniazid 1 mg/L, 1 VME for isoniazid 5 mg/L, 1 VME and 1 ME for rifampicin and 2 VME for ethambutol. When isolates with discordant results were tested again, the overall agreement between the two systems was 78.1% and forty-two discrepancies were observed: 25 VME for isoniazid 0.4 mg/L, 12 VME for isoniazid 1 mg/L, 1 VME for isoniazid 5 mg/L, 1 VME and 1 ME for rifampicin, and 2 VME for ethambutol. The contamination rate for BacT/ALERT 3D was 0.5%, and results were obtained within an average rate of 6.4 days (range: 4 to 10 days).
Conclusions: A poor correlation between BacT/ALERT 3D and BACTEC 460 for susceptibility testing of *M. kansasii* was observed, specially, with the lower concentrations of isoniazid studied. Therefore, the current protocol for susceptibility testing of *M. tuberculosis* by the BacT/ALERT 3D seems not to be useful for *M. kansasii*.

P576

NKT cells in the peripheral blood of patients with active and inactive pulmonary tuberculosis

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Objectives: Cell mediated immunity is critical in *Mycobacterium tuberculosis* infection. NKT cells are important in viral and intracellular infection. In this study, it was aimed to investigate whether a difference of NKT cells ratio between active and inactive tuberculosis patient.
Methods: Forty-nine active and 13 inactive pulmonary tuberculosis patients and 20 healthy individuals, having no disease and at the similar age range (35.76 ± 15.8, 43.23 ± 20.5 and

38.20 ± 17.61 yrs, respectively) were included in the study. Twenty-five of all active patients at pre-treatment period, 18 of them were in 2nd month and six patients of them were at 6th month of the treatment period. Two ml venous blood samples were drawn from all patients and healthy subjects for studying NKT cells. Total T cells and NKT cells [$CD3^+$, and $CD3^+$/CD(16+56)+] were established from EDTA blood. The blood cells were analysed by Coulter EPICS XL-MCL (Beckman Coulter U.S.A.) flow cytometry equipment.

Results: Percentages of total T cells were not different between active and inactive pulmonary tuberculosis cases, but they were significantly lower than in controls (64.9 ± 9.6, 65.2 ± 5.4, 73.2 ± 7.1, respectively, p = 0.001 for active, p = 0.003 for inactive patients compared with controls). NKT cells percentages were not different statistically between active and inactive patients. In addition, NKT percentages in active and inactive patients did not differ statistically then healthy subjects (5.25 ± 3.93, 5.11 ± 2.48, 5.91 ± 6.09 respectively).

Conclusion: Many studies showed NKT cells can play an important role for protection from *M. tuberculosis* infections but in this study, we could not find any difference for NKT cells among active, inactive tuberculosis and healthy controls.

P577

Investigation of T cell receptors in the peripheral blood of patients with active pulmonary tuberculosis

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Objectives: Human immune responses to *M. tuberculosis* are characterized by activation of multiple T cell subsets including $CD4^+$, $CD8^+$, and gamma-delta T cells, and the role of alpha-beta TCR+ T cells in this response is poorly understood. In this study, it was aimed to compare gamma/delta (gd) and alpha/beta T cell receptors (ab TCR) at active and inactive period of patients diagnosed as tuberculosis.

Methods: Forty-nine active and 13 inactive pulmonary tuberculosis patients and 20 healthy individuals, having no disease and at the similar age range (35.76 ± 15.8, 43.23 ± 20.5 and 38.20 ± 17.61 yrs, respectively) were evaluated in the study. Gamma-delta TCR and alpha-beta TCR percentages were analysed by flow cytometry (EPICS XL-MCL; Beckman Coulter U.S.A.). Active tuberculosis patients were divided into three groups according to the treatment and X-ray findings. So, the results were analysed in the context of antituberculous treatment stages and X-ray findings in patients with active pulmonary tuberculosis.

Results: Percentages of $CD3^+$ cells and $CD3^+$/alpha-betaTCR+ were significantly lower in both active and inactive pulmonary tuberculosis than in healthy subjects. Percentages of $CD3^+$/gdTCR+ and $CD3^+$/abTCR+ were not different statistically between active and inactive patients of pulmonary tuberculosis. In addition, $CD3^+$/gdTCR+percentages in active and inactive patients did not differ statistically than in healthy subjects (Table).$CD3^+$, $CD3^+$/abTCR+, $CD3^+$/gdTCR+ percentages did not show difference at the different time of the antituberculous regimen and X-ray findings of the three different groups of active tuberculosis patients.

	Active PT (n = 49)	Inactive PT (n = 13)	Healthy subjects (n = 20)
Total Lymphocytes	24.86 ± 11.02	37.79 ± 7.23	30.13 ± 7.65
$CD3^+$/ab TCR^+	58.49 ± 9.84[a]	59.02 ± 5.01[d]	67.73 ± 7.26[a,d]
$CD3^+$/gd TCR^+	5.12 ± 2.71	5.18 ± 2.74	5.25 ± 1.45
T Cells	64.89 ± 9.59[b]	65.19 ± 5.45[c]	73.24 ± 7.14[b,c]

[a]p = 0.001; [b]p = 0.001; [c]p = 0.003; [d]p = 0.001

Conclusion: Recent studies have established that besides $CD4^+$ abTCR+ cells, both $CD8^+$ abTCR+ cells and gdTCR T cells have a role in the cellular immune response to mycobacteria. On the contrary, it was found no difference between active and inactive pulmonary tuberculosis and healthy subjects in terms of gdTCR T cells but abTCR T cells were lower at patients then in healthy subjects in this study. It was concluded that abTCR+ T cells might have a protective role for tuberculosis infection.Table: Alpha-betaTCR, gamma-deltaTCR and $CD3^+$ cell percentages (means of % ±SD) among active pulmonary tuberculosis (PT), inactive pulmonary tuberculosis and healthy subjects.

P578

MB/BacT system and E-test versus standard methods for drug susceptibility testing of *Mycobacterium tuberculosis*

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Objectives: To evaluate the reliability of MB/BacT system (Organon Teknika, USA) and E-test (AB BIODISK, Sweden) for drug susceptibility testing (DST) of *Mycobacterium tuberculosis* (MTB).

Methods: Susceptibility testing for Isoniazide (INH), Streptomycin (SM), Rifampin (RMP) and Ethambutol (ETB) using the MB/BacT system was performed on 63 MTB strains isolated from clinical specimens since 2000 in our laboratory. According to the protocol used, final drug concentrations were the following: INH 0.1 μg/ml, SM 1 μg/ml, RMP 1 μg/ml, ETB 5 μg/ml. 20 strains of them, isolated last year, were also tested by E-test. The inoculum turbidity was adjusted to McFarland 3.0 standard and Middle brook 7H11 agar plates were inoculated and preincubated (37°C, 7–10% CO_2) for 24 h after which time, the E-test strips were placed. MICs were easily read within 5 to 10 days of incubation. All the isolates were sent for confirmation to the National Mycobacterium Reference Laboratory where the proportion method on L-J media was used.

Results: 48 out of 63 isolates were susceptible to all drugs tested according to the reference method. 43 of these strains were also detected as susceptible to the four drugs by MB/BacT system. There were only 2 multi-resistant isolated strains. Full agreement of DST between MB/BacT system and L-J media was attained in 241/252 individual tests (95.6%) from 56/63 isolates (88.9%). The sensitivity was 90%, 100%, 87.5% and 87.5% for the detection of resistance to INH, SM, RMP and ETB respectively, while specificity was 96.2%, 94.4%, 98.2% and 96.4%. There was full agreement (100%) between E-test and the reference method for all the 20 strains tested, while the discrepancy with the MB/BacT system was 94%.

Conclusions: MB/BacT system is fully automated and it seems to perform quite well in comparison to the media proportion method. E-test was found to be rapid, accurate and reliable but not so easy to perform. Both methods need further evaluation.

P579

Molecular characterisation of isoniazid and/or rifampicin-resistant *Mycobacterium tuberculosis* clinical strains isolated in Spain

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Isoniazid (INH) and rifampicin (RIF) represent the backbone of short-course chemotherapy for *Mycobacterium tuberculosis* infection. Multidrug-resistant strains of *M. tuberculosis* (MDR-TB)

defined as resistant to INH and RIF have been increasing over the years. Resistance to RIF has been shown to be caused by an alteration of the B-subunit of RNA polymerase, encoded by the rpoB gene. More than 95% of RIF-resistant strains are associated with specific mutations, insertions and deletions in 81-bp region of this gene termed the rifampicin-resistance determining region (RRDR) and these mutations have been reported by several authors in several countries. In contrast, resistance to INH is more complicated as mutations in a different number of genes can lead to drug resistance. In most INH resistant strains, mutations have been found in the katG gene and the promoter region of the mabA-inhA regulon. The molecular mechanism of resistance is unknown in the 10–20% INH-resistant strains. In this study, the objective was to characterize mutations in the rpoB and katG genes just as in the mabA-inhA regulon of RIF and INH Spanish resistant strains. We analysed a total of 278 RIF-resistant and 413 INH-resistant isolates (233 MDR). The resistance to RIF was determined by automated DNA sequencing whilst the resistance to isoniazid was determinated by multiplex-PCR and DNA sequencing. The analysis revealed that 266 (96%) of the RIF-resistant had any mutation in the RRDR. The codons most frequently involved in mutations were the codon 531 (52%), the codon 526 (26%) and the codon 516 (11%). Other codons involved in resistance to rifampicin were 511, 513, 522, 530 and 533. All of them showed point mutations. We detected deletions of the codon 514, 515 and 519 and insertions between the codon 512 and 513. The change of codon 531 (TCG to TTG) was found with highest frequency (47%), the second in frequency was the mutation of codon 526 (CAC to TAC)(10%)followed by the mutation of codon 516 (GAC to GTC) (9%). The prevalence of katG315 AGC > ACC allele and inhAC-15T mutation is 48% and 23% respectively whist others mutations in the 315 codon are found in 5% of the isoniazid resistant strains. The 2% of the INH-resistant strains had mutations in both genes. In 22% of the INH-resistant strains the mechanism of resistance was unknown. The frequency of the different changes was similar to other studies both the rifampicin resistance and the isoniazid resistance.

Brucella

P580

Human brucellosis in Crete, Greece

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Background: Brucellosis is a zoonosis with a worldwide distribution and may present with a variety of clinical manifestations and complications. The aim of this study was to investigate the epidemiological and clinical features, the complications and the laboratory findings of brucellosis in adult patients during a 4-year period.
Methods: The medical records of 37 patients with brucellosis, hospitalized between 1998 and 2001 were retrospectively reviewed. The diagnosis was based on clinical symptoms, cultures and serological tests. Hepatitis was defined as elevated liver transaminase enzymes 2 times over the upper normal value.
Results: The mean age of the patients was 41 (median 33, range 15–79). Twenty-nine (78%) were males. Occupational exposure was mentioned by 54% while 64% had consumed unpasteurized diary products. The most frequent symptom was fever, observed in 34 (94%) with median duration 17 days (range 4–60). Other symptoms were sweating (66%), backache (45%), and malaise (18%). Focal disease was present in 16 patients (43%). The most common sites were spondilitis (24%) and sacroilitis (19%), epididymoorchitis (5%), erythema nodosum (5%) and meningitis (3%). Hepatitis was found in 6 patients (16%). The mean CRP value was 2.3 + 1.3 mg/dL. Leucopenia and thrombocytopenia were observed in 2 patients. Blood cultures were positive for brucella spp. in 74%. The organism was isolated from the cerebrospinal fluid in one patient (3%). All patients received combination treatment. Doxycycline and rifampin was the most common regimen (19 patients; 51%). Relapse occurred in 3 patients with osteoarticular disease who responded to second line therapy.
Conclusion: Brucellosis is endemic in Crete and presents mainly with fever, osteoarticular involvement and hepatitis. The outcome was favorable in most of the patients. Relapse was rare.

P581

Brucellar spondylitis: review of 24 cases and compared to inpatients without spondylitis

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Objective: Brucellosis has a worldwide distribution but in the Mediterranean and Middle East countries it is still a major public health problem. The frequency of osteoarticular involvement of brucellosis is high and the most prevalent manifestation is spondylitis. This study was carried out to describe the clinical and laboratory evaluations, treatment combinations of 24 patients with *brucellar spondylitis* and compared with those of patients with nonspondylitic brucellosis.
Methods: A total of 125 cases of brucellosis were diagnosed and followed up prospectively between January 2002 and August 2003.
Results: Of these patients with brucellosis, 24 (19.2) were diagnosed with spondylitis. In spondylitis patients, one had servical involvement, two had thoracic, 20 had lumbosacral and one had multiple involvement seen in MRI. Nine patients (%37.5) had paravertebral abscess. Back pain (%100), fever and sweating (%91.7), and arthralgia (%72.5) were the most common symptoms for patients with spondylitis. The duration of antimicrobial combination therapy for cases with spondylitis was 60–365 days (median 156 day). Three of these patients underwent surgical abscess drainage. Neurologic sequelae was observed one of patients with spondylitis. No relapse was observed in spondylitis patients. Patients with spondylitis were more likely to have longer durations of symptoms ($p < 0.05$). Cultures of blood specimens from 11 (%45.8) of patients with spondylitis were positive Brucella spp. Erythrocyte sedimentation rates (ESR) and age were higher than those of patients with brucellosis who did not have spondylitis ($p < 0.05$). Serum transaminase levels were higher in nonspondylitis group than spondylitis group ($p < 0.05$).
Conclusion: Our study has shown that *brucellar spondylitis* affects predominantly the lumbosacral spine. Older age and duration of symptoms before therapy are risk factors for

brucellar spondylitis. Antibiotic treatment should be administered for a long period in patients with *brucellar spondylitis* than in systemic brucellosis without spondylitis.

P582

Human brucellosis on a Mediterranean island: Crete, Greece

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Objective: Brucellosis is endemic in Mediterranean area. We investigated the epidemiological characteristics of human brucellosis in the island of Crete.
Methods: All cases of human brucellosis diagnosed from January 1999 to October 2004 were included in this study. Age, sex, animal contact, annual and monthly distribution, phase and type of infection as well as the diagnostic methods were studied.
Results: During this period, 67 (51 males) patients with brucellosis were detected. Mean age was 41.1 and the range was 8–80 years. Among them 50 were occupationally infected (shepherds or goatherds, slaughters, laboratory technicians), 3 were visitors in the island, and 7 were children. Six of the children lived in villages and their parents were farm-workers. Regarding annual and monthly distribution, the higher incidence was detected in 2000, and about half of the cases (31) occurred during the spring months. Most patients (46) were undergoing the acute phase of the disease, 11 the subacute, 3 the chronic, and 7 were in relapse. About half of the patients (33/67) presented a focal disease: bone-joins, 19; epididymo-orchitis, 10; neurobrucellosis, 2; and chronic enteric disease, 2. Hepatosplenomegaly was found in 2 patients, nodular erythema in 1, and thrombocytopenia also in 1 patient. In 29 cases the diagnosis was based on serological tests in association with clinical signs and symptoms. A successful culture for brucella confirmed the diagnosis in 38 patients. Brucella was isolated from blood in 28 patients, from bone marrow in 2, from bone-joins in 2, from CSF in1, from both blood and bone marrow in 3 patients, and from both blood and bone-joins in 2. Unfortunately, in 13 cases no samples for culture were obtained. Subsequently the sensitivity of the culture was at 70%. All cultures were performed by BacT/Alert blood culture system. Time to determination ranged from 43.2 h to 84 h with a mean value of 61.6 h. All isolates were identified as *B. melitensis*.
Conclusion: Brucellosis remains a health problem in our region mainly as an occupational disease caused by *B. melitensis* exhibiting increasing during spring. Blood culture is a useful tool in the diagnosis of human brucellosis.

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Human brucellosis in Northern Greece

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Objectives–Aims: To present the clinical and epidemiological aspects of the disease at a specialized hospital, where most cases of human brucellosis in Northern Greece are referred.
Material–Methods: 67 adult cases (43 men and 24 women, mean age 45.1 {range 14–78} yrs-old) were analysed retrospectively, admitted between 2000 and 2003. All of them were hospitalized at first, and had a follow-up period from 1 to 43 (range 15.9) months afterwards. Diagnosis was based on clinical symptoms and confirmed by serology and/or positive blood cultures.
Results: Fifty two (77.6%) patients had acute, 7 (10.4%) subacute and 8 (11.9%) chronic brucellosis. Forty one (61.2%) had an occupational association with animals, 37 (55.2%) mentioned consumption of diary products, since 30 (44.8%) had not evident affinity with animals or their products. All but two (97%) lived or worked in rural areas. Fifty three (79.1) presented with typical and 14 (20.9%) with atypical symptoms. Thirteen (19.4%) suffered from serious complications, {5 (7.5%) from neurobrucellosis, 2 (3%) from endocarditis, 2 (3%) from spondylitis, 2 (3%) from orcheoepididymitis, 2 (3%) from compression}. Blood cultures were positive at 35 (52.2%) cases (*Brucella melitensis*). A combination of 3 antibiotics (an aminoglucoside plus doxycycline plus rifampin/cotrimoxazol/quinolone) was administered to all patients from the beginning, followed by a combination of 2 or 3 antibiotics (after the withdrawal of the aminoglucoside). Mean duration of treatment was 11.5 (range 4–20) weeks in uncomplicated cases. Relapses were observed in 12 (17.9%) cases. Final, higher than 1/160 titre (range 1/320–1/800) of standard tube Brucella agglutination test (SAT), was observed in 15 (22.4%) treated cases, after the follow-up period. No patient had a fatal outcome.
Conclusions: Human brucellosis still remains a considerable problem in Northern Greece. Atypical symptoms and serious complications are not rare in endemic areas. Initial administration of a combination of more than 2 antibiotics in all cases and duration of treatment longer than 8 weeks may reduce the morbidity and mortality rate. High SAT final levels don't rule out the successful management of the disease.

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Cytokine levels in patients with brucellosis and their relations with the treatment

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Objectives: Cytokines appear to have an important role in the pathogenesis and outcome of brucellosis, and the Th1/Th2 balance may be involved in the susceptibility or resistance to the disease. In vitro experiments demonstrated *B. abortus* induces human and murine monocytes to secrete proinflammatory cytokines such as TNF-alpha and IL-1 beta. The aim of this study was to determine the serum levels of proinflammatory and some Th1/Th2 cytokines in brucellosis and those alterations with treatment and outcome.
Methods: This study was conducted in 28 acute and 7 subacute brucellosis patients who had positive clinical findings, brucella standard tube agglutination tests and/or positive blood culture and 20 healthy volunteers who had any complaints. Eighteen (51.4%) Brucella species were isolated in the blood cultures. All patients were treated with combination of doxycycline or tetracycline with one of the rifampin or streptomycin for 6 weeks. Cytokine levels of pre and post-treatment period serum samples were measured by ELISA method according to the protocol of the manufacturer.
Results: It was found that mean serum levels of IL-6, IFN-gamma and TNF-alpha (mean ± SEM, 15.7 ± 2.7 pg/ml, 12.9 ± 2.9 pg/ml, and 53.3 ± 10.1 pg/ml, respectively) were significantly higher in acute brucellosis compared to the control group (mean ± SEM, 1.1 ± 0.8 pg/ml, 6.9 ± 1.3 pg/ml, and 8.4 ± 1.2 pg/ml, respectively) ($p < 0.05$). Whereas, no significant differences were found between patient and control groups in terms of IL-1beta, TGF-beta, IL-2, IL-4 and IL-8 levels. There was a positive correlation between IFN-gamma, TNF-alpha and IL-6 levels with CRP levels. IL-6, IFN-gamma and TNF-alpha levels measured after treatment were statistically significantly lower than pretreatment values ($p < 0.001$). No differences were found in the levels of these cytokines between acute and subacute

patients with brucellosis. Cytokine levels at patients with positive blood cultures did not show statistically significant differences than the ones with negative.
Conclusion: It was concluded that IL-6, IFN-gamma and TNF-alpha levels were higher in patients with brucellosis that is acute or subacute. It was determined that the levels of these of cytokines were decreased significantly with effective and adequate treatment. However, it was observed that these increases did not correlate with the extent or activity of the disease.

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Liver injury at brucellosis acuta

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Objectives: To present the frequency of liver injury among the patients with brucellosis acuta one department.
Material and methods: 517 patients (33.7% female, 66.3% male) were admitted to one hospital under diagnose of brucellosis acuta. Among them 153 (29.6%) were afflicted by liver disease in anamnestic sile. Patients were treated with concomitant antibiotic therapy associated with hepatoprotective therapy. We used standard laboratory and biochemistry analysis, serological investigations, ultrasonography and liver scanning.
Results: Liver injury comprised 153 (29.6%) of total cases with brucellosis acuta. Slight hepotemegaly with hypodensity was observed at 41/153 (27.3%) patients. Rise activity of aminotransfer enzyme (about two times large) was found at 105/153 (68.7%) which has normalized for 3 weeks. Three times and higher serum transaminase was found at 43/153 (28.3%), value than retain 4–5 weeks. In 147/153 (93.3%) patients obvious serum transaminase activity and liver ultrasonography improvement were noted to the end of antibiotic therapy.
Conclusion: Liver injury is the part of immunopathology mechanisms of brucellosis. Liver injury is not so rare in our material (29.6%) and it is lightly and transitory. Liver injury is not tightly related with severity of clinical signs. Also it is not related with high levels of serologic values.

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Utility of Brucellacapt in the follow-up of patients with acute brucellosis

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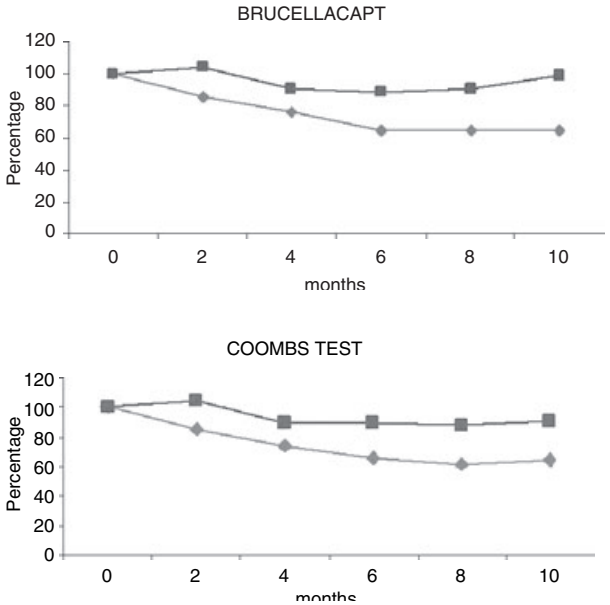
Objectives: The aim of these study is to know the utility of a new serological test (immunecapture-agglutination test (Brucellacapt@)) in the follow up of patients with acute Brucellosis. We compare the evolution curves of the titres of the brucellosis patients with clinical antecedents of previous brucellosis and without these antecedents.
Methods: It has been studied that 258 sera from 51 patients with acute brucellosis. From these 22 patients presented antecedents of previous brucellosis at least 12 months before of the new brucellosis process and 29 patients did not. The diagnosis of brucellosis was based on CDC criterions. We consider acute brucellosis when the patients did not have any brucellosis symptom after 3 months of the beginning of specific treatment. Sera were taken at the moment of first clinical consultation (baseline sera (BS)) and in the 2nd, 4th, 6th, 8th and 10th month after have begun treatment (evolutive sera). All sera were tested by agglutination test tube (SAW), Coombs test anti-Brucella, and Brucellacapt@.
Results: The evolution curves of SAW, Coombs test and Brucellacapt were very similar. From the 1st month till the 6th month after the specific treatment was began, the titres were decreasing in the patients with antecedents of previous brucellosis till 60% of the titres obtained by the baseline sera in all techniques studied. In the patients with antecedents of previous brucellosis the titres of the sera from the 2nd till the 10th month were very similar to the titres of the baseline sera. The titres from the patients with and without antecedents of previous brucellosis presented statistical differences from the 2nd till the 10th month of evolution (SAW BS $p = 0.35$; 2nd month $p = 0.038$; 6th month $p = 0.03$, 8th month $p = 0.025$) (Coombs test BS $p = 0.42$; 2nd month $p < 0.001$; 4th month $p = 0.042$; 10th month $p = 0.028$) (Brucellacapt@ BS $p = 0.15$; 2nd month $p = 0.004$; 4th month $p = 0.042$; 6th month $p = 0.004$).



Conclusions: The patients with acute brucellosis and with antecedents of previous brucellosis present titres statistically higher than patients without antecedents between 2nd month and the 10th month of evolution.

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Native and prosthetic valve endocarditis caused by Brucella Spp.: evaluation of eight cases

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Objectives: Brucellosis and its complications are still a problem in developing countries. Eight cases of infective endocarditis caused by Brucella spp. were reported.
Material and methods: All cases of infective endocarditis were recorded in previously prepared forms prospectively between January 2000 and January 2004 by one of the authors. Duke Criteria were used for the diagnosis of infective endocarditis. BACTEC 9240 Becton Dickinson blood culture system were used for the incubation of blood bottles. Identification of microorganisms were done with classical methods and anti sera were used for *Brucella melitensis* (Murex Biotech Lmtd. UK, Lot No: CM02).
Results: A total of 88 cases with infective endocarditis were recorded and 8 of these (9%) patients were identified as having endocarditis caused by Brucella spp. Six patients were male and two were female. Median age was 44 (range) and median time from the beginning of complaints to the admittance to the

hospital was 17 days (range 5–60 days). Underlying heart diseases were prosthetic valve in 5 patients and native valve sequel secondary to acute rheumatic fever in 3. Splenomegali and hepatomegali were found in 6 patients. Osler node and janeway lesion were found in only one patient. Romatoid factor were found to be positive in 3 patients. All of the patients had positive results of Wright agglutination with titres greater than 1/1280. *Brucella melitensis* were isolated in blood cultures of 6 patients. Vegetation, abscess, corda rupture and new dehiscence of prosthetic valve have been seen either TEE or TTE in 8, 5, 1 and 1 of 8 patients respectively. Although TTE were found to be normal, TEE revealed vegetation in 4 (3 prosthetic, one native valve) of 8 patients. Diagnosis of infective endocarditis was definite in all of the cases. All of the patients were treated with trimetoprim–sulfametaxazole, rifampicin and doxycyclin combination for 12 months. Surgical intervention was done for 7 patients within a median of 18 days (range 7–45). All of the patients were alive after 12 months of follow up.
Conclusions: Brucella spp. should be considered in patients with infective endocarditis, especially in countries where the disease still endemic. TEE should be done in case of normal TTE findings. Early surgical intervention can reduce mortality.

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Laboratory diagnostics of Brucella infection – risk of laboratory infection

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Objectives: Brucellosis is a rare disease in Norway. Brucellosis is caused by Brucella, a small, non-motile, Gram-negative aerobic coccobacillus. The genus Brucella comprises the six species *B. melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. neotomae* and *B. canis*. Even though Brucella is divided in different species based on host specificity and biochemical characteristics, they may be regarded as one species based on DNA sequence information. Here we present a case of laboratory-acquired brucellosis.
Methods: Brucella is oxydase- and catalase-positive, and how fast they become urease-positive is used as a phenotypic criterion of differentiation. Brucella grows slowly in Bactec aerobic blood culture medium, but more than 95% of the positive cultures are positive in less than seven days. In subculture Brucella grows on blood agar in less than two days. When conventional methods are insufficient for identification of a bacterium, sequencing of the 16S rDNA will give the correct genus and often also the species.
Results: Case 1 is a 54-year-old man who was admitted to the Department of Neurology after one day of acute lumbago ultimo July 2003. Seven days later the patient in addition to severe back pain got fever and was transferred to the Department of Infectious Diseases. After three days small Gram-negative rods grew out in aerobic blood cultures. The microbe was identified as Brucella sp based on standard bacteriological techniques and sequencing of 16S rDNA. The patient had not been abroad the last two years, and had no known exposure to Brucella. Case 2 is a 30-year-old woman who had worked with the blood culture isolate from case 1. From January 10th 2004 she had undulating fever, initially regarded as an influenza-like illness. She was admitted to the Department of Infectious Diseases ultimo January 2004 under the diagnosis: 'Fever of unknown origin, Brucella?' Aerobic blood culture taken at the time of admission was positive after three days of incubation, and also in this case Brucella sp was identified. The patient had no other risk of brucellosis than her work in the laboratory five months earlier.
Conclusions: During bacteriological identification of the blood culture isolate from case 1, parts of the identification work (catalase reaction) was done on open bench. After the identification of this isolate as Brucella, all bacteriological diagnostic work that can produce infectious aerosol, are done in biological safety cabinets.

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Trends in the epidemiology of brucellosis in Northern Greece 1994–2003

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Objectives: In 1994, the vaccination control programme with Rev-1 vaccine against *Brucella melitensis* infection in sheep and goats, was discontinued in Greece. Soon, the incidence of brucellosis in animals and humans increased. Thus, in 1998 an emergency mass-vaccination programme for animals with the same vaccine was resumed. The aim of this study is to present and analyse the cases of brucellosis attended to the adult Department of Thessaloniki Infectious Diseases Hospital during 1994–2003. Our hospital attends patients from all Northern Greece.
Methods: We reviewed retrospectively the brucellosis unit archives of our Department and found the patients attended as outpatients during this period, including the hospitalized ones after their dismissal. Sex, age, year of first diagnosis, mode of infection and outcome of disease were included in the study.
Results: 947 patients were identified, 684 males, 263 females, from 14 to 83 years old, mean age ($x \pm SD$) 43 ± 16 years. Between 1994–1998, 258 cases were identified, 27% of the sum of study patients. Between 1998–2000 there was an increase in the number of cases, 411 cases, 43% of the sum. The increase was significant ($p < 0.001$). Between 2001–2003 a decrease in the number of cases was observed, 277 cases, 30% of the sum, $p < 0.001$. The possible source of infection was identified in 660 cases. 499 patients (75%) had direct contact with animals (398 were farmers, 28 veterinarians and 73 had other occupational exposure) and 161 (25%) patients had reported consumption of unpasteurized dairy products. Concerning the complications, 112 patients (12%) had a focal or complicated illness. Osteoarticular complications were the most frequent focal forms (50 patients had spondylitis, 14 sacroiliitis and 13 other forms of arthritis). 21 males (3% of all males) had orchiepididymitis. 10 patients (1%) had neurobrucellosis and 4 (0.4%) patients had endocarditis.
Conclusion: A significant increase of human brucellosis took place in Greece from 1998 to 2000 due to the discontinuation of animal vaccination. Once a new vaccination campaign had started again, the incidence in humans decreased. Furthermore, the main way of brucellosis transmission is occupational contact and the most common type of complication is osteoarticular

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Pancytopenia in patients with *Brucella melitensis* infection

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We studied retrospectively the incidence of pancytopenia in patients with *Brucella melitensis* infection in an endemic area of Northern Greece. Nine out of 88 (10%) patients that were diagnosed and treated at our Hospital over a period of 7 years developed pancytopenia during the course of their disease. Pancytopenia was mild in 7 patients and moderate/severe in 2 patients (Hb 7.4 g/dl, white cell count 1680/μl, neutrophils 900/μl and platelets 48000/μl in the first patient and Hb 8 g/dl,

white cell count 1800/μl neutrophils 1050/μl and platelets 51000/μl in the second patient. The majority of patients (51%) developed mono- or bi-lineage cytopenias (41 and 10% respectively). Anaemia was the commonest cytopenia (28 patients, i.e., 32% of all patients), while leukopenia or thrombocytopenia developed in 4% of patients. Concomitant anaemia and thrombocytopenia were the commonest cytopenias (6%) in those patients with bi-lineage cytopenias. The cytopenias were mild in all cases except 3 patients in whom Hb was 7.3 g/dl, white cell count 1180/μl (neutrophils 580/μl) and platelets 21000/μl. In the vast majority of patients the cytopenias were not of clinical significance neither did they require specific treatment. Blood counts promptly returned to normal upon commencement of specific antibiotic treatment and response of the disease.
Conclusion: Pancytopenia is a relatively common complication in patients with Brucellosis and it is usually mild and without clinical significance. However, it can cause diagnostic problems, especially outside endemic areas.

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Sensorineural hearing loss in three cases with neurobrucellosis

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Objectives: Brucellosis is an endemic disease in our country. It can be in acute, subacute or chronic form. Central nervous system involvement (neurobrucellosis) in brucellosis is less than 5% of all cases. The most common complication is meningitis.
Methods and Results: In our study we evaluated three patients whose main complaint were sensorineural hearing loss (SNHL) among our neurobrucellosis (NB) patients. All of our patients aged 39, 54 and 60 years old were males. Diagnosis of NB depended on positive blood and cerebrospinal fluid (CSF) culture in the first patient and by positive CSF culture in the 3rd patient and by positive brucella agglutination titre in blood and CSF and by positive Brucella IgG titre in CSF in the second patient. In all of the cases the period between the beginning of the complaints and the diagnosis exceeded 6 months. Involvement of cranial nerves are rare complications reported as sporadic cases in NB. In 2000's among diagnostic criteria of NB; 'positive CSF culture for Brucella spp' or 'positive Brucella IgG agglutination titre in CSF' gained importance due to isolation of responsible agent.
Conclusions: SNHL is a rare complication of NB which has not attracted enough attention among known and reported manifestations May we suggest neurologists and otologists to be aware of this evidence as much as we do? Because, necessity of a multidisciplinary approach to diagnosis and therapy of NB is inevitable.

Toxoplasmosis

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Genetic engineering of *Toxoplasma gondii* P30 (SAG1) antigen for improved antibody detection in immunoassays

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Objectives: To improve the immunoreactivity of the recombinant *T. gondii* P30 (SAG1) immunodominant antigen in serologic tests for the detection of human anti-Toxoplasma (Toxo) IgG and IgM antibodies.
Methods: The immunodominant *T. gondii* P30 (SAG1) antigen was expressed and purified from *E. coli* as a soluble fusion protein to the *E. coli* Maltose Binding Protein (MBP-P30). A series of MBP-P30 deletion mutants were constructed and evaluated in a Toxo IgG and IgM immunoassay. Based on these data, we completed total gene synthesis of the P30 gene with a mixture of mutant oligonucleotides designed to replace the non-essential cysteine residues with alanine, removal of the signal peptide, and removal of C-terminal residues to improve antigen immunoreactivity.
Results: The MBP-P30(52–300aa)Ala5 recombinant antigen contains deletion of the signal peptide, deletion of 36 amino acid residues at the C-terminus of P30 including one cysteine residue, and substitution of the five remaining C-terminal cysteine residues in P30 with alanine. The immunoreactivity of this antigen was improved 2–3 fold over MBP-P30. The performance of the MBP-P30(52–300aa)Ala5 antigen was evaluated in a recombinant antigen based Toxo IgG (in combination with the *T. gondii* P35 antigen(GRA8)) and Toxo IgM immunoassay (alone) by testing a pregnant women patient population and comparing the results to a commercial Toxo IgG and IgM immunoassay which employs tachyzoite-derived antigen for antibody detection. The relative sensitivity and specificity of the recombinant Toxo IgG assay was 100% and the relative sensitivity and specificity of the recombinant Toxo IgM assay was 97.2% and 95.1%, respectively.
Conclusion: The MBP-P30(52–300aa)Ala5 can replace the tachyzoite antigen in an immunoassay for the detection of human anti-Toxo IgG and IgM antibodies.

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Avidity of toxoplasma specific immunoglobulin G: results of a new Vidas test on 603 sera

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Objectives: A new version of the serological test Vidas Toxo IgG IV has been recently developed with antigens obtained from tachyzoïtes cultured on cells and not on mice. Avidity of Toxoplasma-specific IgG antibodies has been shown to be an important tool for discrimination between recent and distant infection. The aim of this study was to assess the value of the determination of avidity on the new Vidas test on 553 sera obtained from pregnant women whose dates of seroconversion were known, and on 50 sera obtained from immunocompromised patients.
Methods: A total of 603 sera (563 patients) were tested by the Vidas Toxo IgG IV Avidity test. The datation of the seroconversion was determinated between the date of two samples, the first negative one and the second positive, or before the first sample (positive IgM and negative or weakly positive IgG followed by a twofold or greater increase). The sera of pregnant women and

immunocompromised patients with past infection (more than 15 months) showed weakly positive IgG antibodies and negative IgM. The Vidas Toxo IgG IV Avidity test was performed by the Vidas instrument that automatically produces results and their interpretation. The avidity test was performed on sera with positive Vidas Toxo IgG IV result (>6 IU/ml).
Results: In cases of infection occurring less than 4 months before sampling, the avidity index was <0.3 (low and borderline) on 266 among 267 sera. The serum with high avidity was sampled one month after the infection and it concerned a patient for who the avidity index performed on another serum sampled two months after the infection was low. In the group of infection occurring more than 15 months before sampling, the avidity index was high for 44/46 sera. The avidity results of sequential sera of 13 patients (3 sera per pregnant women) showed that the increase of avidity index was slow and the rate of increase was variable for the different patients. The avidity threshold of 0.3 was reached between 4 and 15 months after infection for 551 sera among 553. The avidity indexes were high for the 47 patients with different types of immunocompromission and past toxoplasmic infection.
Conclusion: The new version of avidity test was helpful primarily to rule out that a patient's infection occurred within the prior 4 months. An appropriate decision on how patients with low- or high-avidity test result should be managed must be made using also results of other serologic methods.

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AF146527 as target gene for detection of *Toxoplasma gondii* by LightCycler PCR

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Objective: Early detection of toxoplasmosis by PCR in the transplanted immunocompromised host is important to improve the outcome of this patient group. We compare sensitivity and accuracy for detection of *Toxoplasma gondii* when targeting two different repetitive genes, the 35-fold repetitive B1gene and the 200–300-fold repetitive AF146527 gene, using the LightCycler instrument. Further, an AF146527 TaqMan assay was tested with two different internal amplification control systems to investigate their impact on PCR performance.
Methods: DNA was quantified and quality controlled with the NanoDrop® ND-1000 Spectrophotometer. Sensitivity and accuracy of similar B1 and AF146527 LightCycler SYBR green assays were compared using ten-fold serial dilutions of *T. gondii* DNA. Performance of the AF146527 TaqMan assay was tested using two different internal amplification control systems. One of the internal amplification control systems tested was amplified by the primers used for detection of the parasite and the other by a different set of primers.
Results: LightCycler PCR is more sensitive when targeting the more repeated AF146527 gene compared to a similar assay targeting the less repeated B1 gene. Performance of the AF146527 TaqMan assay with internal amplification control is similar to the corresponding AF146527 SYBR green assay without internal amplification control. Performance is not affected by an internal amplification control amplified by the same primers as used for detection of *T. gondii* compared to an internal amplification control amplified by a different set of primers.
Conclusions: For increased sensitivity and accuracy when using LightCycler PCR, our results suggest that the AF146527 gene is a preferred PCR target for detection of *T. gondii*. This 200–300-fold gene allows the use of an internal amplification control without loss of performance. However, the conserved nature of this newly described gene still remains to be further confirmed analysis of additional strains of *T. gondii*.

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Seroepidemiology of toxoplasmosis in female population in Greece

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Objectives: The study of *Toxoplasma gondii* immunological status in female population of reproductive age in Greece as toxoplasmosis is a common and important cause of congenital disease.
Methods: 3016 women who attended the Outpatient Dpt of our Hospital from January 2003 to November 2004 were tested for Toxoplasma IgM and IgG antibodies. Of 3016 women 1208 were immigrants (40.05%) and 1808 were Greek. The detection of Toxo IgM and IgG antibodies was performed by MEIA method (AxSYM, Abbott) in 1779 samples and ELFA method (VIDAS, BioMerieux) in 1327 samples. To the last 1327 samples (577 immigrants, 750 Greek), avidity test was performed for the Toxo IgM/ IgG positive sera. All Toxo IgM positive women were tested again after three weeks.
Results: In the total of 3016 women the percentage of Toxo IgG positive women was 25.40% and Toxo IgM positive was 2.22%. The percentage of Toxo IgG positive immigrants was 45.11% and Toxo IgM positive was 2.89%. The percentage of Toxo IgG positive Greek women was 12.22% and Toxo IgM positive 1.76%. Out of 1327 samples tested by ELFA method 35 (2.63%) were Toxo IgM positive: a) 3 samples gave Toxo IgG negative result (8.57% of the Toxo IgM positive) and avidity test has not been performed, second test after three weeks showed seroconversion (Toxo IgG positive). b) 6 samples (avidity <0.300) showed recent infection (17.15%), second test after three weeks showed an increase in Toxo IgG, decrease in Toxo IgM and avidity test >0.300. c) 26 samples (avidity >0.300) (74.28%) were probably excluded from recent infection. In 24/26 samples were no significant changes after the second test. In 2/26 samples test by molecular method was recommended because the IgM/IgG changes could not be sufficiently evaluated.
Conclusions: The seroprevalence of *Toxoplasma gondii* IgG is 25.40% in female population in Greece (Toxo IgM positive 2.22%). Prevalence is considerably higher in immigrants (45.11%) than in Greek women (12.22%). As 74.6% of tested women were seronegative, antenatal control should be recommended in order to avoid intrauterine infection with consequent congenital disorders.

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Handling toxoplasmosis in pregnancy: the Verona experience

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Background: Assessing seroprevalence of the infection from *Toxoplasma gondii* is of pivotal importance to estimate the risk for mothers of becoming infected with toxoplasmosis during pregnancy. Informing non-immune women about which behaviours should be avoided in order not to get infected is therefore important. Even more important is to devise early diagnosis techniques in order to treat the mother and possibly the foetus and to monitor the newborn in the case of an infection: avoiding risk behaviours is sometimes not sufficient to avoid the infection.
Objective: The aim of this study was to estimate: i) the prevalence of antibodies against *Toxoplasma gondii* in pregnant women in Verona; ii) the incidence of primary infection during pregnancy; iii) the prevalence of congenital toxoplasmosis. By

means of procedure we wanted to test: i) the validity of the pre-birth monthly screening in women susceptible to infection as a function of seroprevalence; ii) the feasibility of an early diagnosis; iii) the validity of the diagnostic-therapeutic iter for women with an acute infection.
Methods: Seroprevalence was prospectively analysed in 1801 pregnant women visited at the general hospital during 2001: assays were performed to detect immunoglobulins IgG, IgM, IgA and IgG avidity antibodies. In case of positive IgM a second serum sample was requested and processed in parallel with the first one. Determination of IgG avidity antibodies allowed us to define three stages of infection: acute, possible and past. Congenital infection was determined prenatally by PCR in amniotic fluid and/or postnatally by serology and clinic of the newborn in 27 acute infection cases screened between 1999 and October 2004.
Results: Seroprevalence of toxoplasmic infection in pregnant women in Verona was 17.5%. The incidence of primary infection in pregnancy was 4.7/1000 susceptible pregnant women. All women with serological evidence of toxoplasmosis during pregnancy were given spiramicin to stop placental infection. Transmission was 7.4%. No children showed clinical signs of congenital toxoplasmosis.
Discussion: Our seroprevalence figures (17.5%) are very low and justify the screening of all pregnant women. Monthly screening of susceptible pregnant women has allowed us to early recognise an occurred infection in the mother. This way we could put in action prevention and therapy strategies against the foetus infection, which occurred only in the 7.4% of the studied cases.

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Immunoreactivity of predicted antigenic determinants of *Toxoplasma gondii* ROP 4 protein

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Objectives: The purpose of this study was to determinate and evaluate diagnostic relevance of antigenic epitopes encoded by open reading frame for *Toxoplasma gondii* ROP 4 protein (Serine/Threonine protein kinases, catalyticdomain).
Methods: Four potential antigenic epitopes of *Toxoplasma gondii* ROP 4 protein have been predicted by bioinformatics analysis. Recombinant genes encoded selected amino acids sequences have been constructed from synthetic oligonucleotides by using PCR reaction. Proteins were expressed in *E. coli* as hybrid protein with Glutathione S-transferase. To study antigenic property of new proteins 25 well defined positive (N = 15) and negative (N = 10) serum samples were tested. All serum samples were previously tested by three commercially available assays for the detection of IgG anti *Toxoplasma gondii*. In absence of known IgM positive samples and high discordance between commercially available assays for the IgM detection the 3rd International Standard for ANTI-TOXOPLASMA SERUM, HUMAN (TOXM, NIBSC, UK) was used. Also 200 serum specimens from normal blood donors were tested.
Results: Predicted antigenic determinants were located at position 30–159 aa, 447–467 aa, 491–505 aa and 548–558 aa. Gene encoded amino acid sequences of ROP 4 protein at position 30–145 aa (RH2) and artificial mosaic gene encoded amino acid sequences at position 447–467 aa, 491–505 aa, 548–558 (RH1) aa were synthesized. The pure samples of two recombinant proteins were obtained. All proteins were weak immunoreactive with IgG antibody of anti *Toxoplasma gondii* positive serum samples. When TOXM was tested RH1 and RH2 were able to detect 1. 6 preliminary units of IgM anti-toxoplasma antibodies. None of specimens from normal blood donors were tested as IgM positive with new recombinant proteins. Antigenic epitopes located at 30–159 aa was found as most immunoreactive for the specific IgM detection.
Conclusion: The results indicated that 4 antigenic epitopes have been predicted of the ROP 4 protein of *Toxoplasma gondii*. All proteins used in this study demonstrated a significant diagnostic potential as candidates for the development of diagnostic assays for the detection of anti-*Toxoplasma gondii* IgM activity in serum specimens.

Clinical mycology

P598

An outbreak of *Pichia anomala fungaemia*

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Objectives: To report an outbreak of *Pichia anomala fungaemia* (formerly *Hansenula anomala* the perfect state of *Candida pelicullosa*).
Methods: Report of an outbreak of fungaemia caused by a rare yeast. Clinical investigation was supported by a case-control study and by molecular studies.
Results: In the period comprising October 2002 to November 2003, 16 cases of fungaemia due to *P. anomala* occurred in a pediatric intensive care unit (ICU) at Porto Alegre, Brazil. We performed a case-control study to determine the potential risk factors for *P. anomala* fungaemia, defining cases (n = 16) as those patients in whom *P. anomala* was isolated from at least one blood culture, in association with signs and symptoms of sepsis. Controls (n = 48) were randomized among all patients hospitalized for at least 2 days in pediatric ICU during the outbreak period. During these studies, multiple environmental cultures were also obtained, as well as hand cultures of all health care workers. Weekly surveillance swabs (mouth, rectum, umbilicus, and groin) were done in all children admitted to ICU. Compared to controls, cases had higher duration of stay in intensive care (mean 16.9 versus 9.3 days, $p < 0.01$), and more days of mechanical ventilation (13.4 versus 5.8, $p < 0.01$); they also used more H2 blockers (93.8% versus 56.3%, $p < 0.01$), central venous catheters (93.8% versus 56.3%, $p < 0.01$), and antibiotics (mean 4.2 for 19.2 days, versus 3.1 for 13.4 days, $p < 0.05$), and they were more frequently submitted to neurosurgery (25.0% versus 6.3%, $p < 0.05$). Mortality rate was higher in cases then in controls (43.8% versus 6.3%, $p < 0.01$). At multivariate analysis, however, only the presence of central venous catheter was associated with *P. anomala* fungaemia ($p = 0.009$, OR 17.1, CI 2.0–145.7). Despite the high rate of yeast colonization among pediatric patients during surveillance study (>80%), *P. anomala* was not found in these patients, in health care workers or from environmental. Molecular studies showed that only one strain of

P. anomala was responsible for the outbreak, and the outbreak was controlled after institution of oral nystatin prophylaxis and improvement of care of central venous catheters.
Conclusion: In accordance to previous studies, we reinforce that *P. anomala* can be exogenously acquired.

P599

Nosocomial candidemia in patients using antifungals (breakthrough): comparison to non-breakthrough episodes

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Objectives: To describe all consecutive cases of breakthrough candidemia (BT) that occurred in a large Brazilian hospital, and to compare risk factors, etiology, and the outcome of the infection among patients with breakthrough (BT) and non-BT candidemia.
Methods: Retrospective cohort study (1995–2003). BT candidemia was defined as the occurrence of candidemia in a patient receiving at least 3 days of systemic antifungal therapy.
Results: During the period of study, 20 patients had BT candidemia. Major underlying diseases were solid tumors (25.0%) and hematological malignancies (15.0%), and 60.0% were in use of amphotericin B. Species other than *C. albicans* caused 75% of candidemias in BT patients, mainly *C. parapsilosis*. Comparing to non-BT patients with candidemia (n = 171), BT patients had more frequently mucositis, longer stay in the intensive care unit, and longer periods of hyperalimentation, mechanical ventilation, urinary catheters and large spectrum antibiotics. Candida isolation from sites other than blood occurred more frequently in BT patients ($p = 0.028$), as well as the Candida isolation from central venous catheters ($p = 0.057$). Mortality rate and Candida species distribution were similar among groups.
Conclusions: Based on these observations, it seems that the source of BT candidemia can be not only endogenous, but also exogenous.

P600

Correlation between zinc level of serum and recurrent vulvovaginal candidiasis

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Objective: Alteration in cell-mediated immunity have been seen in some patients suffering from recurrent vulvovaginal candidiasis. Zinc, an essential trace element has been shown to influence several cell-mediated immunologic mechanisms. Most people in Iran suffer from zinc deficiency. We want to determine whether zinc deficiency in serum is a risk factor for recurrent vulvovaginal candidiasis.
Method: This is a cross-sectional controlled study on 100 women who had experienced at least three documented episodes of vulvovaginal candidiasis within the previous 12 months. 25 women of 100 cases who did not have any other risk factors for recurrent vulvovaginal cadidiasis were selected as case group and 50 matched women without recurrent vulvovaginal candidiasis as a control group. Blood samples were drawn for measurement of plasma zinc level. Zinc level of serum determined by Flame Atomic Absorption Spectrophotometry.
Results: We found no significant difference in the mean zinc concentration of plasma between patient and the control group (P value = 0/09).
Conclusion: It is well known that zinc not only has a major impact on different immune function, but also participates in growth and morphogenesis of Candida Albicans. In spite of, in Iran zinc deficiency is common, our results could not confirm the previous hypothesis that zinc deficiency of serum is a risk factor for recurrent vulvovaginal candidiasis. Supported by Azad Islamic University.

P601

Emerging issues of HIV-associated cryptococcosis after versus before the availability of highly active antiretroviral therapy: an analysis of 72 disease episodes

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Objective: Aim of our investigation is to compare the frequency and clinical and mycological features of AIDS-related primary and relapsing cryptococcosis, before and after the introduction of highly active antiretroviral therapy (HAART).
Patients and Methods: The 58 disease episodes occurred at our reference centre of Bologna (Italy) were compared with the 14 cases occurred since the year 1997.
Results: An abnormal clinical presentation, disease localization(s), and evolution (which may involve the so-called 'immune reconstitution syndrome') have been noticed since the HAART era ($p < 0.001$ to p0.04). An early negative culture search and direct microscopy, observed with higher frequency during the HAART era ($p < 0.03$), led to a more extensive resort to polysaccharide antigen testing as the apparently most sensitive diagnostic assay, although a late positivization (after > 1 week of disease) became more common in patients undergoing HAART. Although disease recognition may be delayed, the reduced severity of concurrent immunodeficiency and the availability of an enhanced spectrum of potent antifungal drugs, led to a significant drop of mortality and relapse rates during the HAART time ($p < 0.0001$).
Discussion: An elevated clinical suspicion for HIV-associated cryptococcosis should be maintained in the HAART era too, to obtain an early diagnosis in immunocompromised patients, or in subjects who are benefiting of a rapid HAART-driven immune recovery. In fact, cryptococcosis may occur with a proportionally increased frequency in absence of an evident neurological involvement, and positive microscopy and culture. The large-scale introduction of HAART is probably responsible for a significant clinical and laboratory pathomorphism of AIDS-associated cryptococcosis, parelleling the remarkable reduction of absolute morbidity of this typical opportunistic disease, compared with the pre-HAART era. The different clinical and laboratory presentation of this fungal disease just in the HAART era recommends to maintain an elevated attention when facing patients at risk for impaired immune defences, or under initial HAART.

P602

A retrospective study of fungal infections in patients with haematologic malignancies

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Invasive Fungal Infections (IFI) represent a significant problem for patients with haematologic malignancies undergoing intensive chemotherapy. The incidence and severity of IFI vary and result from the interaction of the following risk factors: previous

fungal infection, state of immunosuppression and organ dysfunction.
Objectives: In this retrospective study we estimated the incidence of fungal infections and the toxicity of antifungal treatment.
Patients and methods: 77 patients of median age 66 years admitted in our department between November 2001 and June 2003. The median time of hospitalization was 30 days. They were stratified in nine groups: Acute myelogenous leukaemia:40, acute lymphoblastic leukaemia:8, myelodyspoastic syndrome:7, multiple myeloma:4, myeloproliferative disease:3, non Hodgkin lymphoma:11, chronic myelogenous leukaemia:1, aplastic anemia:1, Hodgkin's disease:1. A fungal infection was determined as definite, if biologic fluids cultures or a lesion tissue biopsy were positive for fungi, as probable if imagine technique findings were indicative for fungal infection and possible if patients became afebrile after empirical antifungal treatment. The period of neutropenia was approximately 15 days. The antifungal medication included liposomal amphotericin in 38/77, amphotericin B lipid complex (ABLC) in 29/77, voriconazole in 1/77 and caspofugin in 2/77.
Results: 18/77(24%) had a definite, 17/77(22%) a probable and 42/77(54%) a possible fungal infection. Blood cultures were positive for Candida species in 8/77(10%). Positive urine cultures were eliminated in 3/77(4%), sputum cultures in 5/77(6.5%), whereas one patient had positive CSF culture for cryptococcus. Aspergillus spp. was eliminated from the pleural cavity of a patient with empyema during neutropenia period. A 6.5% of patients had both bacterial and fungal infection. Computerized scanning findings were suspicious of fungal infection in 14/77(18.2%). Side effects: ABLC caused fever, renal dysfunction and decreased potassium serum level in 73%, whereas liposomal amphotericin in 63%. The incidence of side effects was not statistically different between the two amphotericin arms ($p = 0.36$). The mortality was 68% and 42% on ABLC and liposomal amphotericin group respectively ($p = 0.039$).
Conclusions: Toxicity was more severe on ABLC arm and Candida species were the most frequent eliminated fungi. The duration of neutropenia and immunodeficiency seemed to be important risk factors for fungal infections.

P603

Biofilm production and the genotypes of *Candida parapsilosis* recovered from bloodstream and non-bloodstream sources

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Objectives: Biofilm production has been implicated as a potential virulence factor in *C. parapsilosis* causing catheter-related bloodstream infections. In addition, the epidemiology of *C. parapsilosis* candidemia is still undefined and may involve sources such as the hands of health care workers (HCWs). We investigated the distribution of *C. parapsilosis* genotypes in isolates from blood, all other sites from patients, and the hands of medical personnel, and its relation to biofilm production.
Methods: A total of 121 *C. parapsilosis* strains were analysed, including 53 bloodstream isolates from catheter-related fungaemia, 39 clinical isolates from other body sites, and 29 isolates from the hands of HCWs. Organisms were grown in Sabouraud's dextrose broth containing a final concentration of 8% glucose to induce biofilm formation, which was determined using both visual and spectrophotometric methods. Electrophoretic karyotyping with pulsed-field gel electrophoresis was used to genotype the *C. parapsilosis*.
Results: Eighty-three per cent (44/53) of the blood isolates were biofilm positive, while only 46% (18/39) of the isolates from all other sites were biofilm positive ($P < 0.01$). Isolates from medical personnel showed high biofilm production (72%, 21/29), like the blood isolates. Electrophoretic karyotyping differentiated all the isolates into three (I, II, and III) genotypes. All the isolates from the bloodstream or the hands of HCWs belonged to genotype I, whereas the clinical isolates from other body sites belonged to genotypes I (64%), II (20%), and III (15%). Biofilm positivity was observed in 78% (83/107) of genotype I isolates versus 0% (0/14) of genotype II and III isolates. There was no significant difference in biofilm production among the genotype I isolates from blood (81%), other clinical specimens (72%), or the hands of HCWs (73%).
Conclusion: This study found a difference in biofilm production among three genotypes of *C. parapsilosis* isolates. Most of the *C. parapsilosis* isolates from the bloodstream or the hands of HCWs belonged to genotype I, which has the ability to produce a biofilm and is associated with catheter-related bloodstream infections.

P604

Cryptococcus neoformans infection in Hong Kong – a three-year retrospective review

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Objectives: *Cryptococcus neoformans* is encapsulated yeast capable of producing life threatening meningitis and disseminated infection. Its importance is widely recognized in patients with immunosuppressive conditions such as AIDS. In geographical areas where the seroprevalence of HIV is low, Cryptococcus infection is often not clinically suspected. This may result in a delay in initiating appropriate antifungal treatment. Therefore, we aimed to analyze the clinical characteristics of *Cryptococcus neoformans* infections occurring in the past three years.
Methods: A retrospective analysis was conducted on all patients' records with microbiological confirmation of *Cryptococcus neoformans* infection in the Prince of Wales Hospital of Hong Kong from Nov 2001 to Oct 2004.
Results: A total of 14 non-duplicate patients were diagnosed to have *Cryptococcus neoformans* infection during the time period. Meningitis was found in 7 patients (50%). The remaining patients consisted of 4 blood stream infection, 2 pneumonia, and 1 retinits. The male to female ratio was 1:1. The mean age was 54.8 years old (range: 25–78 years; S.D. 16.5 years). Initial presentation included headache (5/14), pneumonia (2/14), sepsis syndrome (3/14), confusion (1/14), blurred vision (1/14), mania (1/14), and gastrointestinal bleed (1/14). Among the 5 patients presenting with headache, three had hydrocephalus requiring neurosurgical intervention. Six patients had underlying diseases including malignancies (3/14), systemic lupus erythematosus (1/14), HIV infection (1/14) and ventricular septal defect with concurrent infective endocarditis (1/14). In patients with meningitis, cerebrospinal fluid showed lymphocytosis in 6 out of 7 patients, and Indian Ink preparation revealed encapsulated yeast cells in 5 of them. Cryptococcal antigen testing was positive in all cases of meningitis (Range: 1:8–1:2048). Antigen testing was also positive on sera from 6 patients with meningitis, and 1 patient with pneumonia. Antifungal treatment was started in 10 out of 14 patients, the remaining 4 patients had the disease diagnosed post-mortem. Crude mortality rate was 50%.
Conclusion: *Cryptococcus neoformans* infection is an important disease with high mortality. A high index of suspicion with simple appropriate investigation such as Indian Ink test on cerebrospinal fluid can often reveal the diagnosis, which will allow timely initiation of appropriate antifungal therapy.

P605

The Finnish experience with caspofungin: retrospective study of patients treated during 2001–2004

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The aim of the study: To evaluate retrospectively the use of caspofungin in treatment of confirmed and suspected invasive fungal infections in Finland.
Materials & Methods: All adult patients (=/>16 years) treated with caspofungin in five Finnish hospitals between years 2001–2004 were included in the study and a patient records were reviewed.
Results: 78 patients were enrolled in the study. These five centres used over 92(of nation wide use of caspofungin during the study period. The median age of patients was 55 years. Underlying disease was haematological malignancy in 37 (47%), diabetes in 11 (14%), solid organ transplantation in6 (8%), HIV in 1 (1%) and other disease in 23 (30%) patients. 26 (33%) patients were neutropenic. The risk factor for fungal infection was use of broad-spectrum antibiotics in 66 (85%), central venous catheter in 51 (65%), and prior surgery in 19 (24%) patients. 51 (65%) patients had confirmed invasive fungal infection and 27 (35%) patients received empirical treatment. 20 (26%) patients had invasive aspergillosis and 31 (40%) invasive candidiasis. 13 (17%) patients received caspofungin as primary therapy. Of the remaining patients 45 were refractory to and 14 intolerant of previous medication. The mean duration of caspofungin therapy was 32 days. Caspofungin was discontinued in 4 (5%) patients (3 patients had elevated liver enzymes and 1 neuropathy). Five patients received concomitantly cyclosporine without any major adverse events. The response rate was 45% in Aspergillosis and 77% in Candidiasis. At the end of the follow up 40 (51%) patients were alive. 9 of 38 deaths were caused by fungal infection.
Conclusion: Caspofungin has been used successfully as salvage therapy in Finland. The low incidence of adverse events and interactions make it an important alternative in treatment of confirmed and suspected invasive fungal infection. Efficacy evaluation is difficult in a retrospective study with heterogenous underlying diseases and requires further controlled prospective studies.

P606

Fungal burn wound infection

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Objective: Infection remains the major problem of the burn patients. Most common infectious complication involving the burn wound. Fungal burn infections occurs most often in patients with extensive burn wound injury, that recently emerged as a distinct clinicopathological entity, responsible for both mortality and morbidity.
Materials and methods: During a 2 year September 2002 to September 2004, of patients with thermal injury admitted to hospital burn, Swab specimens were obtained from areas of the burn wound suspected to be harbouring infection. Diagnosis of fungal burn wound confirmed by microscopic examination and culture of the specimens on SDA medium with antibiotic chloramphenicol. Fungi Sp. was cultured at any time during their hospital cause were selected for study.
Results: 25% (250 case) of these patients had colonization with fungi species one or more site during acutely hospitalized study, although 10% of these patients had positive microscopy examination. Patients with fungal burn wound infections had age a mean of 28 years and a mean burn size of 63% of the total body surface area. A mean burn day was 10 day. Majority to have third degree burn (75%) and majority of this patients were male (70%). 2% (5 case) of the patients with fungal burn wound infection died disseminated fungal disease. All of the patients had culture positive been receiving broad spectrum antibiotics with an average of 15 days. 40% (100 case) *Candidia* Sp, 30% (75 case) *Aspergillus* Sp. and 20% (50 case) *Zygomycet* Sp. were organisms fungi most frequently responsible for clinical mycotic burn wound infections. Candida albicans was predominant species isolated, remained *Aspergillus fumigatus*, *Candida tropicalis*, *Phycomyces* Sp., *Fusarium* Sp., *Acromonium* Sp., *Malassezia* Sp., *Penicillum* Sp., *Geotrichum* Sp. were aetiological agent fungal burn wound infections.
Conclusion: In burn patients lose of the normal flora of the body impairment of immunity system, contribute to the increased susceptibility to fungal burn wound infection. Among the fungi candida albicans which is an opportunistic microorganism and one the normal flora to create more difficult burn patients. In conclusion although fungal infections remained a significant source of morbidity, it was associated with a low rate of mortality, large areas of open wound and exposure borad-spectrum antibiotic were associated with the development of fungal burn wound infections.

P607

Factors associated with death in candiduria

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Background: Candida species are responsible for causing serious systemic infections among hospitalized patients. Although mortality rates in candiduria range from 18–30%, no study has thus far focused on identifying factors associated with this high mortality.
Objectives: To identify factors associated with mortality among hospitalized patients with candiduria at our centre.
Methods: We prospectively studied the microbiological and clinical characteristics of 77 consecutive cases of candiduria [≥10,000 colony forming units (cfu)/mL] hospitalized between Jan and Aug 2003 [eight months]. Odds ratios [OR] for suspected risk factors were calculated using the Mantel-Haenzel test. Analysis was performed using the SPSS version 10.0 software.
Results: Among 77 patients, 20 [26%] died during their hospital stay. Analysis showed the following factors to be significantly associated with mortality: use of urinary diversionary devices [OR 8.8; 95% confidence interval (CI) 1.1–70.5; p = 0.04]; use of two or more classes of antibiotics [OR 4.1; 95% CI 1.2–13.9; p = 0.02]; ICU admission [OR 3.3; 95% CI 1.1–9.3; p = 0.03]; and renal failure [OR 2.9; 95% CI 1.1–8.2; p = 0.05]. Besides, the following factors approached statistical significance: fungal colony counts ≥100,000 cfu/mL [OR 4.5; 95% CI 0.9–21.4; p = 0.06]; acidosis (serum bicarbonate ≤17 meq/L) [OR 3.6; 95% CI 0.9–13.7; p = 0.06]; positive urine nitrite test [OR 3.2; 95% CI 0.8–12.3; p = 0.09]; serum albumin ≤3 g/L [OR 3.2; 95% CI 0.8–12.9; p = 0.10]; absence of any treatment interventions for candiduria [OR 2.9; 95% CI 0.9–9.0; p = 0.07]; age ≥ 65 years [OR 2.8; 95% CI 0.9–8.5; p = 0.07]; and treatment with antibiotics for >7 days prior to isolation [OR 2.5; 95% CI 0.9–7.1; p = 0.09]. Infection with non-albicans Candida and co-infection with bacterial urinary pathogens was not associated with increased mortality.
Conclusions: Factors associated with increased mortality in candiduria are those that might be expected to occur in critically ill patients. However, the associations with absence of treatment interventions and higher colony counts in urine culture indicate

that fungal virulence characteristics may be important determinants of mortality. These factors might achieve statistical significance in larger case series of candiduria.

P608

Recurrent vaginal candidiasis: management of the asymptomatic partner

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Objectives: Treatment of acute yeast infections results in relief of symptoms and eradication of yeasts in the majority of cases. Recurrence is not uncommon and is always a problem for gynecologists all over the world. Treatment of symptomatic partner is imperative while simultaneously treatment of symptom-free men is controversial. The purpose of our study was to assess the importance and usefulness of treatment of the asymptomatic partner of women with diagnosed recurrent vulvovaginal candidiasis.
Methods: One hundred and forty-eight married women of reproductive age with a history of at least four or more episodes of recurrent vulvovaginal candidiasis in the preceding year presenting to our clinic with a new episode were enrolled in the study together with their partners. All women were symptomatic while all men were asymptomatic. Vaginal cultures were positive for *Candida* spp. in all cases. Identification of strains and antibiotic susceptibility testing were carried out using the VITEK System, ATB Expression, Biomerieux, France. All women were treated with itraconazole. Couples were randomly allocated into two groups according to application of treatment to male partner. Group A comprised those with treatment and group B those with no treatment which served as controls. One month after treatment cultures were obtained to assess the cure rate and a follow-up was performed at 6 months.
Results: *Candida albicans* was the most frequently isolated yeast (90.5%). One month after treatment the cure rate was 93.2% in group A and 89.2% in group B. The persistent asymptomatic positivity rate was 5.4% and 8.1%, respectively, while the clinical recurrence rate was 1.4% and 2.7%, respectively. Six months after treatment the recurrence rate was 35.1% and 32.4%, respectively.
Conclusion: Our data suggest that treatment of the symptom-free partner might not be necessary and should be limited to difficult situations.

P609

Micafungin (FK463) versus fluconazole in a randomised, double-blind, multinational, oesophageal candidiasis study

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Objective: To determine the efficacy and safety of intravenous micafungin (FK463) 150 mg versus intravenous fluconazole 200 mg in the treatment of patients with esophageal candidiasis.
Methods: A multicentre, multinational, two arm, double-blind, randomized (1:1), parallel group, non-inferiority (10% delta), comparative study of patients 16 years of age and older with esophageal candidiasis confirmed by endoscopy and documented by clinical signs and symptoms. Patients received daily infusions of micafungin and fluconazole for a minimum of 14 days or 7 days after clearing, whichever is longer. The primary efficacy endpoint was the comparative incidence of success or endoscopic cure rate at end of treatment. Endoscopic cure rate was defined as clearing of esophageal lesions (mucosal grade of zero). Successful clinical response was defined as a total symptom score = 0 (cleared) or reduction in total symptom score from baseline by 2 or more grades and no increase in grade for any individual symptom (improved). Safety was assessed for all patients who received at least one dose of study medication based on the incidence of adverse events and changes from baseline in clinical laboratory profile.
Results: A total of 518 patients took at least one dose of study medication. Treatment groups were well balanced with respect to age, gender, race, baseline conditions, underlying disease, and severity of disease at baseline. Micafungin was found to be as effective as fluconazole in clearing esophageal lesions as documented by endoscopy. Clinical responses were rapid and similar in the two study groups. Relapse rates measured at post treatment weeks 2 and 4 were also comparable to fluconazole. There were no statistically significant differences between either arm with respect to overall incidence of treatment emergent adverse events (p = 0.156). The most common adverse events (≥5% of patients) regardless of relationship included: fever (13.1% micafungin vs. 8.5% fluconazole), diarrhoea (10.4% vs. 11.2%), phlebitis (15.4% vs. 4.7%), nausea (7.7% vs. 8.9%), headache (8.5% vs. 8.1%) and pneumonia (9.6% vs. 5.0%). No clinically meaningful changes in mean or median values for any laboratory parameters were noted.

	Fluconazole (n = 258)	Micafungin (n = 260)
Endoscopic Cure Rate at ET	227 (88.0%)	228 (87.7%)
Clinical Response at ET	237 (91.9%)	239 (91.9%)
Relapse Rates during Post Treatment		
Week 2	25/225 (11.1%)	35/227 (15.4%)
Week 4	27/200 (13.5%)	24/192 (12.5%)

Conclusion: Micafungin is as effective and is a safe alternative in treating esophageal candidiasis patients when compared to Fluconazole.

P610

Candidaemia: retrospective analysis of a seven-year experience

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Objectives: To investigate the isolation and distribution rate of Candida spp. in blood cultures and to evaluate the antifungal susceptibility during a 7-year period (1997–2004) at a tertiary care hospital.
Methods: Positive blood cultures (BacT/Alert, Organon Teknika) were examined microscopically directly for yeasts or pseudohyphae and subcultured on Sabouraud agar (Difco). Candida isolates were screened by germ tube test and identified using API20CAUX (Biomerieux). Antifungal M.I.C was carried out by E-test (Biodisk) on RPMI-2% glucose agar, for the following antifungals: Amphotericin B (A), 5-fluorocytocin (5F), itraconazole (I), ketoconazole (K) and fluconazole (F). The last two years voriconazole (V) was also included in this study.
Results: During the study period there were 142 (2%) yeast isolates from a total of 6937 patients exhibiting positive blood culture. All patients with fungaemia were immunocompromised. The causative species were: *C. albicans*–*C.a* 80 strains

(57%), *C. glabrata–C.g* 16 (11%), *C. tropicalis–C.t* 14 (10%), *C. parapsilosis–C.p* 13 (9%), *C. lipolytica–C.l* 6(4%), *C. guillermondii–C. guil* 4 (3%), *C. krusei–C.k* 3 (2%), *C. stellatoida–C.s* 1 (1.5%), *C. humicola–C.h* 3(2%). Both *C. glabrata* and *C. krusei* were isolated for the first time during 2001. Two patients had non Candida fungaemia with *Trichosporon* spp. *Candida* spp. excibited different susceptibility profile to antifungals agents. Sensitivity rates (%) of *C.a* to A was 98, to 5-F 92, K 81, I 73, F 87, V 98. Respective results of *C.g* were A 81, 5-F 81, K 69, I 31, F 56, V 100 of *G.t* A 86, 5-F 78, K 86, I 64, F 78, V 100, of *C.p* A 92, 5-F 92, K 92, I 85, F 85, V 100, of *C.l* A 100, 5-F 67, K 100, I 50, F 67, V 100, of *C. guil* A 100, 5-F 100, K 100, I 100, F 75,V 100, of *C.h* A 100, 5-F 67, K 100, I 34, F 100, V 100, of *C.k* A 100, 5-F 34, K 34, I 67, F 34, V 100 and of *C.s* A 100, 5-F 100, K 100, I 100, F 100, V 100.
Conclusions: Candidemia is predominantly caused by *C. albicans* (57%). The frequency of candidemia due to Candida non albicans is quite high (43%). *Candida glabrata* although the data is limited however was the second most frequent isolate followed by *C. tropicalis* and *C. parapsilosis*. The highest degree of resistance was observed to ketoconazole, itraconazole and fluconazole whereas voriconazole was very active against Candida.

P611

Prognostic indices for death in ICU patients with invasive aspergillosis

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Objective: In ICU patients invasive aspergillosis (IA) is associated with a grim prognosis. The objective of this study was to identify risk factors for mortality in ICU patients with IA.
Methods: Retrospective cohort study (1997–2003). Definite IA was defined as: (1) positive microscopic examination with septate hyphae and positive Aspergillus culture on tissue biopsy, or (2) positive culture of a normally sterile site obtained by aseptic invasive techniques. Probable IA is defined as (1) clinical symptoms of infection, (2) any positive culture from a nonsterile site, (3) chest X-ray or CT of lungs suggestive for IA and (4) either (a) positive results of microscopic examination and culture of BAL fluid or (b) predisposing host risk factors for IA: (i) haematological/oncological malignancy treated with cytotoxic agents, (ii) neutropenia, (iii) glucocorticoid treatment, and (iv) congenital or acquired immunodeficiency. All criteria must be fulfilled for the diagnosis of probable IA (1 + 2 + 3 + either 4a or 4b). Mortality was defined as in-hospital mortality.
Results: During the study period 83 ICU patients experienced IA. The overall mortality was 77% (64/83). In univariate analyses no differences between survivors and non-survivors were observed in age (53 ± 16 years vs. 59 ± 14 years; P = 0.125), APACHE II scores (28 ± 5 vs. 28 ± 10; P = 0.999), delay in start of antifungal therapy (2.5 ± 1.9 days vs. 2.1 ± 2.9 days; P = 0.125), absence of antifungal therapy (0% vs. 19%; P = 0.059), hematologic or oncologic disease (21% vs. 27%; P = 0.769), neutropenia (16% vs. 13%; P = 0.708), chronic use of corticosteroids (37% vs. 53%; P = 0.297), need for renal replacement therapy (37% vs. 41%; P = 0.999), need for mechanical ventilation (95% vs. 97%; P = 0.547), and need for vasoactive drugs (84% vs. 91%; P = 0.421). Survivors had a longer length of ICU stay (44 ± 29 days vs. 16 ± 13 days; P < 0.001) and were mechanically ventilated for a more extended period (37 ± 24 days vs. 15 ± 13 days; P < 0.001). A logistic regression model could not identify independent risk factors for mortality.
Conclusion: In ICUs, IA affects patients with a high index of disease severity as assessed on basis of the APACHE II score. It is also associated with a high rate of severe organ derangements. Due to the high rate of severe comorbidities among survivors and nonsurvivors, and the high overall mortality this study did not have sufficient discriminative power to identify independent risk factors for death.

P612

An open-label comparison of oral voriconazole and itraconazole for long-term treatment of paracoccidioidomycosis

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Objectives: Paracoccidioidomycosis, previously named South American Blastomycosis, is a subacute to chronic systemic mycosis caused by *Paracoccidioides brasiliensis*. The aim of this study was to investigate the efficacy, safety, and tolerability of voriconazole in the long-term treatment of acute or chronic paracoccidioidomycosis, with itraconazole as control treatment.
Methods: This was a randomized, multicentre, open-label, comparative study conducted in Brazil in 2000–2002. Subjects were randomized (2:1) to receive oral therapy with voriconazole or itraconazole for up to 12 months. Satisfactory global response (incorporating clinical, mycologic, radiologic, and serologic assessments) at end of treatment (EOT) was compared for the two treatment groups.
Results: Fifty-three subjects received at least one dose of study drug: 35 received voriconazole and 18 received itraconazole. All but 4 subjects with confirmed paracoccidioidomycosis (3 on voriconazole, 1 on itraconazole) received at least 6 months of continuous study treatment. The response rates in these treatment-evaluable patients were 100% for both treatment groups, and there were no relapses after 8 weeks of follow-up in either group. The majority of cases had lung involvement at baseline; one case with both lung and CNS involvement responded well to voriconazole. The most common treatment-related events included abnormal vision, chromatopsia, rash, and headache in the voriconazole group, and bradycardia, diarrhoea, and headache in the itraconazole group. Two voriconazole subjects were withdrawn prematurely, as required by the protocol, due to study drug related elevated alkaline phosphatase and hepatic enzymes (ALT, AST, and GGT). The frequency of liver function test abnormalities was slightly higher in subjects receiving voriconazole compared to itraconazole, but the median changes in these parameters from baseline values were similar between treatment groups. One voriconazole subject expired after 52 days because of a rupture of an aortic aneurysm: an autopsy was performed and was negative for paracoccidioidomycosis.
Conclusions: This is the first study to demonstrate that voriconazole is well tolerated and effective for the long-term treatment of paracoccidioidomycosis.

Clinical use of vaccines

P613

Immunogenicity and safety of the pneumococcal conjugate vaccine in elderly patients: comparison to the 23-valent polysaccharide vaccine

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Background: Patients ≥70 years are a target population for pneumococcal vaccination but immunogenicity of the 23-valent polysaccharide vaccine (23vPS) in this age is limited. Pneumococcal conjugate vaccines induce enhanced, T-cell mediated immunity and have been shown to be safe and effective in infants.
Objective: To compare immunogenicity and safety of pneumococcal conjugate vaccine (PnC) to 23vPS in patients ≥70 years.
Methods: 443 ambulatory elderly patients aged ≥70 years without prior pneumococcal immunization were randomized in an open label study to receive 23vPS, 7vPnC (Prevnar® 2 μg saccharide/dose, except 4 μg for 6B), or higher doses, achieved by reconstituting 9vPnC with 7vPnC and giving 0.5 or 1.0 ml respectively to produce formulations with 4 or 8 μg saccharide / dose, (except 8 or 16 μg for 6B, 2 or 4 μg for types 1 and 5, present in 9vPnC). Blood samples were obtained prior to vaccination and one month later. Antibody responses were measured by ELISA and OPA.
Results: Only data for the lowest PnC dose (7vPnC) and 23vPS are presented in this abstract. Samples from 439 subjects were analysed. Mean age was 79.9 years (range 69–90); 56.4% were male 43.6% females. Pre-immunisation antibody levels were similar in all groups. One month after vaccination ELISA GMCs of 7vPnC and OPA GMTs were 2–3 times higher than 23vPS, with exception of 19F. GMCs of all three conjugate doses were superior to 23vPS. Pain at the injection site (7vPnC 38% vs. 23vPS 23%), redness (7vPnC 32.4% vs. 23vPS 21%) and swelling (7vPnC 19.6% vs. 23vPS 16%) were the most frequent local reactions. Fever over 38°C was observed in n = 3 (23vPS) and n = 1 (7vPnC) vaccinees. No vaccine related serious adverse events were observed.

	ELISA GMC (μg/ml)						
	4	6B	9V	14	18C	19F	23F
7vPnc (2 μg)	3.1	8.2	10.1	17.5	13.4	5.6	12.5
23vPS	1.4	4.4	3.6	8.4	6.7	4.4	3.8
	OPA GMT						
7vPnc (2 μg)	–	1380.7	2999.4	–	1389.5	188.7	1329
23vPS	–	795.4	966	–	444.6	195.5	301.9

Conclusion: The antibody response after PnC in elderly patients ≥70 years of age was superior to 23vPS. Local reactogenicity for PnC was slightly more common than after 23vPS and highest in the top level dose of PnC. These results support further investigations.

P614

Genetic characterisation of pneumococcal surface protein A

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Objectives: *Streptococcus pneumoniae* is an important pathogen causing invasive diseases such as sepsis, meningitis and pneumonia. The dramatic increase of antibiotic resistance of pneumococci has stressed the need for the development of an effective vaccine. In recent years, several studies have shown that pneumococcal surface protein A (PspA) is a possible candidate for vaccine development. The aim of this work is to map the genetic variability of PspA among representative clinical isolates from Germany.
Methods: PspA genes of 40 clinical pneumococcal isolates were sequenced using a primer set amplifying the central region of the gene and analysed using the HUSAR Bioinformatics Platform (Biocomputing Service Group, Heidelberg, Germany). Strains were serotyped using the Neufeld Quellung Reaction.
Results: The serotype distribution of the strains was as follows: 4 (n = 3), 6B (5), 9V (2), 14 (8), 18C (6), 19F (5), 23F (6) and 7F (5). The pspA genes of these strains could be assigned to two families both containing 20 genes. Family I could be divided into two clades, with 17 genes in clade 1 and 3 genes in clade 2. Family II could be subdivided into three subgroups (clades 3–5) containing 16, 3 and 1 sequence respectively. Clade 1 and clade 3 represent 82% of the genes. Serotypes were distributed evenly over the clades and families.
Conclusions: The distribution of the pspA gene families in Germany differs widely from that found in other countries such as, the US and Sweden. An effective vaccine for Germany could be based on conserved N-terminal regions found in both clade 1 and clade 3 sequences.

P615

Prevalence of pneumococcal serotypes in invasive disease in children

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Objectives: The aim of this study was to the investigate the prevalence of pneumococcal serotypes in invasive disease in children.
Methods: Navarra is a province in northern Spain with a population of 570,000 inhabitants. From September 2000 we have been referring all pneumococcal isolates to the Centro Nacional de Microbiología in Majadahonda for serotyping. Although the health authorities in the province have not included the heptavalent pneumococcal vaccine in the official vaccination schedule, this vaccine has been widely used by paediatricians since late 2001. We have split the study period into 4 seasons (from September to October).
Results: During the study period, 88 pneumococcal strains were isolated, of which 86 were typable and two were non-typable. 19 were recovered during the first season, 22 in the second, 22 in the third and 25 during the last season. During the first season the most frequent serotypes were: 1 (4 isolates), 6A (3), and 19 (2 isolates, one 19A and one 19F); For the second season: 6 (7 isolates, 4 6A and 3 6B), 14 (4) and 18C (2) and 19 (2 isolates, one 19A and one 19F); for the third season: 19 (13 isolates, 5 19A and 8 19F), 1 (2) and 7 (2), and for the fourth season: 19 (9 isolates, 4 19A and 5 19F), 14 (5), 18C (2) and 24 (2). 11 of 19 isolates from the first season were not included in the heptavalent vaccine (57.9%), 6 (27.3%) from the second, 6 (27.3%) from the third and 8 (30%) from the fourth season.
Conclusion: Despite the use of pneumococcal vaccine, the number of cases of invasive infection has not decreased in the Navarra region. Moreover, some serotypes included in the vaccine (14, 19A and 19F) have become more prevalent. Changes in circulating serotypes, incidence of cases between vaccinated and non-vaccinated children and changes in the population (rapid growth due to immigration) could explain the findings presented here.

P616

Safety, reactogenicity and immunogenicity/ priming following 2 vs.3 doses of a meningococcal C conjugate vaccine given concomitantly with DTaP-IPV-HBV/Hib vaccine to infants

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Objectives: Meningococci are important pathogens in young children. Fewer injections may increase compliance with existing infant vaccination schedules.
Methods: Healthy infants (241) in ten paediatric practices in Germany were randomized to receive either two or three doses of a MCC vaccine (Menjugate®, Chiron Vaccines, Marburg, Germany) at age 2, 3, 4 or 2 and 4 months, co-administered with a DTaP-IPV-HBV/Hib combination vaccine (Infanrix®hexa, GSK, München) at age 2, 3, 4 months. At 12 months a plain meningococcal A/C polysaccharide challenge was given. Blood samples were taken prior to the administration of the first vaccine dose, four weeks after the second or third dose, just prior to the challenge vaccination and either 7 or 28 days thereafter.
Results: There was no serious adverse event related to vaccination. Local reactions were similar to those with hexavalent vaccine, systemic reactions occurred with a similar frequency in both groups. One month after 2 or 3 MCC doses, GMTs (bactericidal assay with human complement; hBCA) were 337 (95%CI: 260–437) and 445 (95%CI: 346–572), respectively. 98% and 100% of vaccinees, respectively, showed hBCA >1:8 one month after primary immunization. Two injections of MCC given at 2 & 4 months induced immunological priming In children primed with 2 MCC doses, there was an 19 fold hBCA increase at 28 days following the Men A/C polysaccharide challenge as compared to 1.09 fold increase in a group of unprimed historical control children. Antibody concentrations to hepatitis B were similar in both groups.
Conclusions: In this group of 241 children, the MCC vaccine Menjugate® was safe, well tolerated and highly immunogenic following administration of either 2 or 3 doses in infants and primed immunologic memory responses at 12 months.

P617

Antibiotic resistance, serotypes, and clonal composition of *Streptococcus pneumoniae* in clinical trials of acute otitis media pre- and post-launch of the 7 valent pneumococcal conjugate vaccine

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Objectives: *Streptococcus pneumoniae* is among the most common bacterial pathogens associated with acute otitis media (AOM). Although high levels of efficacy for the 7 valent pneumococcal conjugate vaccine (PCV–7) have been reported for invasive pneumococcal disease, the efficacy with regards to AOM is considerably less clear. Earlier studies involving three prospective, multinational AOM clinical trials, reported on penicillin resistance and serotypes of pre and post PCV-7 *S. pneumoniae* isolates. The present study expands the analysis of these same samples to include genotyping of all 187 isolates for which penicillin resistance and serotyping data were available.
Methods: The genotyping involves our expanded MLST approach, in which all of the customary 7 MLST loci are sequenced not only for the usual 500 bp internal fragment, but for the entire locus, with the addition of several housekeeping loci adjacent to MLST genes. The resulting data set includes approximately 9000 base pairs of sequence data for each isolate, including as a subset, the usual MLST sequence data. These data provide us with high resolution clonal designation of the pre and post PCV-7 samples, while also allowing us to correlate our clonal composition data with the pneumococcal MLST database.
Results: In general, the same clones were present in the pre and post PCV-7 samples; however, there tended to be a proportional increase within clones of non-vaccine serotype in the post PCV-7 set (e.g. clones of serotype 11, 29, and 22 increased in proportional representation in post vaccine years). The changes associated with clones of vaccine or vaccine related serotype were more variable, with some clones not changing in proportional abundance (e.g. clones of 19F, 9V and the mixed serotype clone known as Spain 23F-1), others decreasing (e.g. clones of 23F, 14, and 6A), and others increasing (e.g. one 19F clone). One clone of particular interest was represented by 19A isolates pre PCV-7, and in post PCV-7 years was, with the exception of a single serotype 19A isolate, represented entirely by serotype 15B (a serotype not present in the pre PCV-7 set of isolates).
Conclusions: Although it is difficult to determine if the introduction of PCV-7 directly caused the changes in the AOM clones documented in this study, our results are consistent with a mixture of clonal expansion of non-vaccine serotypes and occasional serotype switching mediated by the selective pressure of the vaccine.

P618

Effect of the 7-valent conjugate pneumococcal vaccine on the serotype distribution of drug-susceptible and drug-resistant *Streptococcus pneumoniae* colonising healthy children attending day-care centres in Lisbon

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Objectives: Evaluate the impact of the 7-valent pneumococcal conjugate vaccine on the serotype distribution among drug-susceptible (DS) *Streptococcus pneumoniae* (Pn) and drug-resistant (DR) Pn isolates colonizing children.
Methods: Nasopharyngeal samples were collected from children under 6 years old attending 5 day-care centres in Lisbon, Portugal. A total of 243 children were sampled before vaccination (May 2001-baseline period); and 127 samples from the vaccinees were obtained afterwards (March 2003). All Pn strains were characterized by antibiotype and serotype.
Results: Frequency of Pn carriage was 74% in the baseline period and 64% after vaccination. Carriage of DSPn isolates with vaccine serotypes (VT) decreased from 24% in the baseline to 5% after vaccination, while carriage of DSPn with non-vaccine serotypes (NVT) increased from 76% in the baseline to 95% after vaccination. In the baseline period, we found six VT, among 23 DSPn isolates, being serotype 19F (39%) the most frequent one, followed by serotypes 23F, 9V, 14, 18C and 6B in decreasing order of prevalence. We also found 15 NVT among 74 DSPn isolates, being serotype 11A (18%) the most frequent one; other serotypes were: 16F, 15B, 23A, 6A, 19A, 7F, 10A, 35F, 33F, 3, 15C, 17, 18A, 34 and 3 non-typeable (NT). After vaccination, only two VT were found: serotypes 19F (2 isolates) and 6B (1 isolate). In addition, we found 14 NVT among 57 isolates: serotypes 6A and 11A were the most frequent ones - 18%, followed by 3, 10A, 15B, 16F, 15C, 21, 17, 9N, 19A, 23A, 34, 35F and 1 NT isolate. Carriage of DRPn with VT decreased from 81% in the baseline to 5% after

vaccination, and carriage of DRPn with NVT increased from 19% to 95% after vaccination. In the baseline period, we have identified five VT among 64 DRPn, being serotype 6B (31%) the most frequent one, and serotypes 23F, 14, 19F and 9V more rarely found. Among 15 DRPn isolates we found four NVT being serotype 15A (33%) the most prevalent, followed by serotypes 6A, 15C, 33F and 5 NT isolates. After vaccination, only one VT isolate was identified (9V). In addition, we found 20 NVT isolates, being serotype 19A (25%) the most frequent. The other serotypes found were: 23A, 15A, 33F and 9 NT isolates.
Conclusions: Vaccination did not affect carriage of Pn, however decreased carriage of DRPn and DSPn isolates with vaccine serotypes and increased carriage of Pn populations of non-vaccine serotypes. Serotype diversity in DSPn is higher than in DRPn isolates and this trend is not affected by vaccination.

P619

Immune response in measles-mumps-rubella of young adults aged 16–25 years vaccinated with one or two doses of MMR vaccine

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Objectives: Serologic evidence of immunity and immunization status were evaluated in healthy adolescents and young adults 4–10y after completing their immunization for MMR.
Methods: Blood samples were collected from 286 healthy adolescents and young adults aged 16–25y (47.7%) female; (52.3%) male on April 2002 in the province of Chania. IgM and IgG antibodies were detected using microparticle enzyme immunoassay, Pambio for measles and mumps and MEIA for rubella.
Results: 220 (80.7%) individuals had received the combined vaccine for measles-mumps-rubella , whereas 40 (19.2%) individuals had received monovalent vaccines: 33 for measles, 22 for rubella, 32 for mumps. In addition, 16 (5.6%) individuals were unimmunized. Furthermore, the combined vaccine and the monovalent vaccine were compared with regard to induced serum antibody titres. Concerning measles, the mean antibody level (positive > 11) was 11,394 after administration of the monovalent vaccine, compared to 10,947 after administration of the combined vaccine (not significant difference). Concerning rubella, the mean antibody level (positive > 0.5) was 69.8 after administration of the monovalent vaccine, compared to 71,535 after administration of the combined vaccine (not significant difference). Finally, concerning mumps, the mean antibody level was 0.6879 after administration of the monovalent vaccine, compared to 0.777 after administration of the combined vaccine ($p < 0.001$). The mean value of antibody titre after administrating a single dose ($n = 122$) compared to two doses ($n = 107$) of the MMR-vaccine was as follows: concerning measles 11.09 and 10.74 (not significant difference) respectively, concerning rubella 65.59 and 76,068 (not significant difference), whereas concerning mumps 0.712 and 0.851 ($p < 0.001$) respectively.
Conclusions: The mean values of antibody activities against measles and rubella did not differ significantly after vaccination with monovalent or combined vaccine. However after vaccination with combined vaccine the mean antibody values were significantly higher against mumps. The administration of one dose of the vaccine for measles and rubella produces similar immune response compared to two doses of vaccination, whereas concerning mumps two doses of MMR vaccine can provide sufficient seroconvertion. Adolescents with naturally acquired immunity had significantly higher titres than adolescents with vaccine-induced titres.

P620

DNA vaccination fails to induce protective immunity against herpes simplex virus type 1 in chronic morphine treatment mice

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Objective: Chronic morphine treatment has been shown to alter a number of immune parameter including suppression of cellular immunity. In this study, we compared protection against lethal HSV-1 challenge following live virus immunization(avirulent strain)with that following vaccination with a plasmid encoding HSV-1 glycoprotein B (gB-DNA) in chronic morphine treated mice.
Methods: HSV-1 was propagated on vero cell line. Mice received (50 mg/kg) morphine s.c daily; on day 11 chronic morphine treated group and control group (PBS treated) immunized with one i.d. injection of HSV-1(KOS). For DNA vaccination chronic morphine treated group and control group (PBS treated) received 100 microgram of DNA vaccine and two booster dose injections i.m. were one week apart. Neutralizing anti body assayed two weeks after the second vaccination. Cell mediated immunity (CMI) was evaluated by measuring footpad immune response(DTH) to inactive HSV-1(KOS). All mice challenged with 4MLD50 of virulent HSV-1 two weeks after final immunization. Survival rate was followed for 14 days.
Results: Chronic morphine treated mice, which immunized with DNA vaccine impaired to induce enough neutralizing anti body. CMI as measured by DTH response was also impaired in the two types of vaccines in chronic morphine treated mice. The protection rates of the vaccinated mice against the lethal i.p challenge were shown as 1) KOS-morphine treated (57.14%) control group(85.71%), 2) DNA vaccine-morphine treated (16.66%) control group(66.66%). There were no survival of the PBS vaccinated mice that treated with morphine or PBS.
Conclusion: The cellular requirements for induced immunity against HSV-1 has been shown differ between the two types of vaccines. The results showed chronic morphine treated mice that immunized with DNA vaccine failed to induce protective immunity, DTH and anti body response. However live virus vaccination impairs DTH in mice, but it can induce protective immunity against lethal HSV-1 challenge.

P621

Hepatitis B vaccine coverage among health care workers in Malaysia

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Due to the nature of work, health care workers (HCW) are at great risk of acquiring blood borne infections such as hepatitis B virus, hepatitis C virus and human immunodeficiency virus from patients. Universal Precautions Policy guidelines were introduced more than a decade ago to minimise exposure to diseases spread by blood and certain body fluids. This study looked into one of the aspects of hepatitis B prevention among HCW in Malaysian context.
Objective: To assess hepatitis B vaccine coverage among HCW working in hospitals in Malaysia.
Methods: This is a cross sectional study involving pre-tested questionnaires. The duration of the study is seven months, from February 2001 to August 2001. Hospital staff working in wards or diagnostic laboratories in Hospital Kuala Lumpur (HKL) and Hospital Universiti Kebangsaan Malaysia (HUKM) as well as Universiti Kebangsaan Malaysia (UKM) undergraduate

students undergoing clinical attachments in HUKM were randomly chosen. All subjects volunteered to participate in this study. The hospital staff comprised nurses and medical laboratory technologists while the students included student nurses, medical students, biomedical students and dental students.

Results: A total of 625 subjects were enrolled and only 58.4% had taken a complete hepatitis B vaccination. Majority of the last dose taken by subjects was less than five years ago. However, 82.2% have taken at least one dose of the hepatitis B vaccine and are supposed to complete the schedule in due course. About 17.8% HCW never received hepatitis B vaccination with the most number from HKL (26.6% from the total staff in HKL). Approximately 22% admitted that anti-HBs test was done a month post vaccination giving a desirable response rate of 84.8%.

Conclusion: Not all HCW are protected against hepatitis B. Preventing hepatitis B in HCW should be one of the priorities of the hospital management as it is definitely cheaper than managing chronic hepatitis B cases.

P622

Factors affecting medical students' participation in measles-rubella immunisation campaign in Iran

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Objectives: Immunization against measles and rubella can prevent a remarkable part of morbidity and mortality. The effect of these vaccines on public health depend largely on their coverage. In Iran, the measles vaccine coverage has been above 95% in children under 5, while rubella immunization is not part of the routine immunization programme. In December 2003, a mass campaign was carried out with the aim of increasing coverage for both vaccines. This study aimed at estimating the participation of medical students (as one of the target groups for the immunization campaign) and the factors affecting this participation.

Methods: This was a cross-sectional study on 250 medical students in Tehran University of Medical Sciences, Tehran, Iran. The students completed a 25-item self-administered questionnaire about participation in MR mass immunization campaign, demographic data, their knowledge (3 questions)and attitude (5 questions). A pilot study was done to assess the internal validity of the questionnaire.

Results: Mean age of the students was 20.7 (SD: 2.4) years and 42% were female. 3% were married. Eighty-two per cent of the students had participated in the mass campaign. The most important source of their information regarding the campaign were the radio and television (69.3%). Male sex (OR = 4.9; 95% CI: 2.0–12.6) and living in private home (OR = 4.6; 95% CI: 1.7–12.4) were the most important determinants of non-participation. Age and years spent at medical school did not affect participation. There was no significant association between knowledge and participation in the mass campaign. The participant's attitude towards vaccination was significantly more positive than the non-participants. More participants, rather than non-participants, believed that vaccination increased immunity against the virus and improved public health.

Conclusion: Students who did not participate in the immunization campaign had more negative attitude towards the vaccine and the programme. Since medical students are instrumental in such public health activities, proper methods of informing and training them (other than the mass media) are necessary to increase their participation.

P623

The investigation of seroprevalance of vaccine preventable diseases among medical students

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Objective: Medical students, like other healthcare workers, often come into contact with patients and their infected materials. They are therefore at risk for various infectious diseases, some of which are preventable through vaccination. There is insufficient study indicating seroprevalance of these infections among medical students in our country and there is no vaccination programme. We aimed to detect the seroprevalance of vaccine-preventable diseases in the fifth grade medical students and emphasize the importance of immunization of adults.

Methods: In this study HbsAg, antiHBs, antiHBcTotal, which are serological indicators of HBV infection, and measles, mumps, rubella, varicella, tetanus IgG and HAV total antibody titres were investigated by ELISA method in students who were in their fifth year of medical school.

Results: HbsAg, antiHBs, antiHBcTotal, measles, rubella, mumps, varicella, tetanus IgG and HAV Total were detected as positive respectively, 2.8%, 67.2%, 41.2%, 56.5%, 96%, 92.1%, 56.5%, 80% and 74%. There was no correlation between illness and vaccination history according to their student survey and seropositivity.

Conclusion: This study indicated that a large proportion of the students were at risk for measles, varicella, hepatitis A and B. Susceptible students should be vaccinated. Medical students should be tested serologically for these infections prior to beginning their medical studies or at least prior to clinical studies, and an immunization programme should be established for all medical students lacking immunization to these infections

Molecular methods for respiratory bacterial pathogens

P624

porA sequencing for subtype characterisation of *Neisseria meningitidis* group B

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Objectives: Meningococcal C conjugate vaccine is not part of the national vaccination scheme, but volunteer vaccination of children and adolescent occurs in large scale since the winter of 2003. This is the basis for the registered decrease of invasive disease due to meningococcal group C, in Portugal. During the epidemiological year of 2003–2004, *Neisseria meningitidis* group B represented 71.7% of all strains that cause laboratory confirmed meningococcal disease (MD), according to the Portuguese laboratory-based surveillance system for MD (VigLab DM). The aim of this study is the fully characterization of the subtype

of *Neisseria meningitidis* group B, by sequencing of porA. Genosubtyping will be useful for epidemiological surveillance as well as to predict the efficacy of a future vaccine based on meningococcal group B outer membrane vesicle.

Methods: We studied 78 strains of *Neisseria meningitidis* group B, isolated from clinical samples during the epidemiological year of 2003–2004. We amplified VR1 and VR2 of porA, and the PCR products were sequenced.Nucleotide sequences were analysed using the computer programme Bioedit and submitted to the *Neisseria meningitidis* PorA Variable Regions Database (http://neisseria.org) for subtype classification in family and variants.

Results: We found 39 different subtypes, the most frequent being from family 14 and its variants (26 strains). VR2 could not be amplified from 3 strains and VR1 was not amplified from one strain. Two new nucleotide sequences from VR2 were submitted to the PorA variable region database.

Table 1. Most frequent genosubtypes and its correspondent phenotypes

Molecular Characterization		Immunologic Characterization	
No. of Strains	Genosubtype	No. of Strains	Phenotype
		3	B:NT:P1.12
4	P1.12-L13-1	1	B:1:P1.12
		1	B:2b:P1.14
		2	B:1:P1.14
		4	B:NT:P1.14
		2	B:NT:NST
10	P1.22.14	1	Not done
		5	B:NT:P1.14
		2	B:NT:NST
8	P1.22-1.14	1	Not done
		3	B:1:NST
		1	B:14:NST
5	P1. 22,14-6	1	B:NT:NST
1	P1.7-2,14	1	B:NT:NST
		1	B:NT:P1.14
2	P1. 18-1,14	1	B:NT:NST
5	P1.19,15	5	B:NT:P1.15
		2	B:NT:P1.7
4	P1.7.16	2	B:NT:NST
2	P1. 17,16-3	2	B:NT:NST

Conclusions: Considering the diversity of genosubtypes of the studied strains and the geographic distribution of them, we did not identify any focus or outbreak during the period of this study. Sequencing of porA seems to be a good approach for better characterization of *N. meningitidis* serogroup B, taking in account the high number of nunsubtypable strains.

P625

New clone of *Neisseria meningitidis* Y causing invasive meningococcal disease in the Czech Republic

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The serogroups of *Neisseria meningitidis* isolated from invasive meningococcal disease (IMD) in the Czech Republic have been investigated since 1970. For this period, the disease has been caused mainly by serogroups B and C. Serogroup Y was not isolated from IMD in the period 1970–1992 and since its first isolation from IMD in 1993 it has caused invasive disease very rarely (1.1% of IMD). Serogroup Y is found among isolates from healthy carriers and/or from respiratory disease at a higher percentage than among isolates from IMD (4.1% and 4.2% respectively). Since 1995 IMD caused by *N. meningitidis* Y has shown an increasing annual incidence and the case fatality rate (CFR) is the highest for IMD caused by *N. meningitidis* Y (27.8%) compared to the total CFR (11.0%) and/or CFR caused by serogroup B (8.1%) and serogroup C (12.7%). For this reason, *N. meningitidis* Y was investigated using classical typing and molecular microbiological methods to assess the clonality of this agent. Serogrouping was performed by slide agglutination with hyperimmune antisera and sero/subtyping by Whole Cell ELISA with monoclonal antibodies. Electrophoretic types (ET) were investigated by multilocus electrophoresis (MLEE) and sequence types (ST) by multilocus sequence typing (MLST). Among *N. meningitidis* Y isolates from IMD the prevalent phenotype was Y:2c:P1.2,P1.5, which occurred in 1995, i.e. the year after the annual incidence of IMD caused by *N. meningitidis* Y started to increase in the Czech Republic. Clonal analysis by MLEE and MLST showed that *N. meningitidis* Y:2c:P1.2,P1.5 belong to the ST–23/Cluster A3 clonal hyperinvasive complex. Recent increased incidence of IMD caused by *N. meningitidis* Y in the Czech Republic is in correlation with antigenic and genetic shift of this strain. Our findings are in accord with a similar situation found in Maryland (USA) recently. A new clone of *N. meningitidis* Y caused an increase of invasive meningococcal disease in the Czech Republic recently. The availability of vaccine against *N. meningitidis* Y was recommended. This work was partially supported by research grants NI/7109–3 and NJ/7458–3 of the Internal Grant Agency of Ministry of Health of the Czech Republic and made use of the Multi Locus Sequence Typing website (http://pubmlst.org/neisseria) sited at the University of Oxford and funded by the Wellcome Trust and European Union. We thank Dr. K. Jolley (University of Oxford, UK) for kind editing of the text.

P626

Evaluation of touch-down real-time PCR based on SYBR green I fluorescent dye for the detection of *Neisseria meningitidis* in clinical samples

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Objectives: LightCycler (Roche®) technology with the use of SYBR Green I® dye provides a convenient, high sensitive and rapid way to detect any PCR amplified product. The present study outlines the development and evaluation of a touch-down real-time PCR for the detection of *N. meningitidis* in clinical samples with the use of SYBR Green I® dye and melting curve analysis.

Methods: A total of 293 samples were obtained from 255 patients with confirmed suspected or propable IMD over a 2 year period (2002 and 2003), were tested with both conventional PCR (Robocycler®, Stratagene) and Real-time PCR (LightCycler. Roche®). For the identification of *N. meningitidis* the crgA primers were used, and a 230 bp PCR fragment was identified by both gel electrophoresis and melting curve analysis.

Results: The sensitivity of both PCR assays for culture-confirmed cases was 93% and the specificity was 97.8%. Agreement between the two PCR assays was 96%. The inter- and intra-assay variations revealed a high variation of Tm values among the different runs. Differences of 1.40C (mean Tm = 87.95°C) were observed. The inter-assay SD was equal to 0.36. In contrast, the intra-assay SD was lower than 0.1 in experiments in which 2.5 pg of DNA were amplified in 3 different capillaries in the same run.

Conclusions: Our results showed a good correlation between the conventional PCR and the Real-time PCR. The use of SYBR Green I dye, showed an excellent alternative for the rapid and reliable diagnosis of menigococcal disease in biological fluids.

P627

Simultaneous detection of rifampicin resistant and penicillin intermediate phenotypes in *Neisseria meningitidis* by real-time multiplex PCR

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Objectives: Detection of antibiotic resistance in *Neisseria meningitidis* is generally performed by phenotypic assays, which require the isolate to be cultured and tested against different drugs. This needs time and bacterial culture may be negative when patients have already started antibiotic treatment prior to specimen collection. Rifampicin and penicillin are the two reference antibiotics for prophylaxis and therapy, respectively, of meningococcal invasive disease. In this study, we developed a Real-Time multiplex PCR for simultaneously detecting rifampicin resistant (RifR) and/or penicillin intermediate (PenI) *N. meningitidis* strains.
Methods: The assay, developed on the LightCycler Real-Time PCR platform (Roche Diagnostics), uses two primers and two hybridization probe sets in the same reaction mixture. To distinguish between fluorescence emitted by each hybridization probe set, two probes, detecting penA and rpoB genes respectively, were labeled with a different fluorophore (LC-Red640 or LC-Red705, respectively) and read in two different channels. The results were compared with those obtained by the E-test and by sequencing the amplified genes.
Results: A total of 77 *N. meningitidis* strains, isolated from patients with meningococcal invasive disease, and collected by the Reference Laboratotory (RL) , were tested. Among them, 30 were PenI and 7 RifR; 1 strain was both PenI and RifR. RifR strains showed a Tm of 49°C compared to a Tm of 55°C of RifS strains by reading the emission of fluorescence in the 705 channel. PenI strains showed a Tm of 55°C and PenS strains a Tm of 45.5°C by reading the emission of fluorescence in the 640 channel. The results were easy to be interpreted and always in agreement with those obtained by E-test and by sequencing.
Conclusion: This molecular assay is able to distinguish RifR and PenI meningococcal phenotypes in the same reaction tube. The sensitivity and specificity of this assay make it suitable for direct use on clinical samples to obtain a rapid result of the antibiotic susceptibility.

P628

Quantitative detection of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* in lower respiratory tract samples by real-time PCR

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Objectives: Quantitative lower respiratory tract culture is an important diagnostic tool for bacterial respiratory tract infections, but is relatively insensitive and takes at least 24 h. Recently the interest for a rapid and more sensitive diagnostic method has lead to evaluation of the properties of PCR, but a consistent problem has been distinguishing colonization from infection. To our knowledge, the potential for overcoming this problem by using quantitative PCR (Q-PCR) has not been investigated. We have therefore evaluated the usefulness of Q-PCR for increasing significant pathogen detection in lower respiratory samples.
Methods: We investigated 203 (106 sputum, 51 bronchial brush, 46 BAL) consecutive respiratory tract samples collected at Karolinska University Hospital, Solna. Following DNA extraction samples were subjected to Q-PCR, using primers targeting the genes ply (*S. pneumoniae*), frdB (*H. influenzae*) and copB (*M. catarrhalis*). DNA extracted from positive controls with known CFU/mL was included in all PCR-runs, allowing later estimation of CFU/mL for the test samples. In parallel, a blinded assessment of quantitative cultures from all samples was performed. Significant pathogen finding by culture and Q-PCR was defined as ≥100.000 CFU/mL, for Q-PCR an estimate from the amount of DNA present.
Results: Significant pathogens were found in 45/203 (22.2%) samples with culture and in 68/203 (33.5%) with Q-PCR. The absolute increase in pathogen finding with Q-PCR was 23/203 (11.3%). The largest increase in significant pathogen finding was seen for *H. influenzae*, where a net increase of 13/203 (6.9%) was observed with Q-PCR (19 additional, 6 missed). Significant culture and insignificant Q-PCR was only found in sputum samples; in 2/203 (1.0%) samples with *S. pneumoniae*, in 6/203 (3.0%) samples with *H. influenzae*, and in 1/203 (0.5%) samples with *M. catarrhalis*.

Significant Q-PCR findings (n = 203)	Significant culture findings (n = 203)			
	S. pneumoniae	*H. influenzae*	*M. catarrhalis*	None of these
S. pneumoniae	12 (5.9%)	–	–	11 (5.4%)
H. influenzae	–	16 (7.9%)	–	19 (9.4%)
M. catarrhalis	–	–	8 (3.9%)	2 (1.0%)
None of these	2 (1.0%)	6 (3.0%)	1 (0.5%)	141 (69.5%)

Conclusions: Q-PCR substantially improved significant pathogen detection in the examined samples. The relative increase in significant pathogen finding with Q-PCR compared to culture was 51.1%. Underestimation of CFU/mL with Q-PCR could be due to inhibition of the PCR reaction or alternatively to mutations in target genes leading to lower affinity of the primer. Q-PCR is a rapid method that can be implemented in most laboratories. In an optimized setting, a Q-PCR result can be achieved within 5 h after collecting the sample from a patient.

P629

Assessment of the MagNa pure compact system for extraction of *Neisseria meningitidis* and *Streptococcus pneumoniae* DNA from clinical CSF and blood specimens

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The use of molecular diagnostic tests in medical microbiology has highlighted the need for a simple, rapid and efficient method of nucleic acids extraction. The evaluation of the new MagNa pure compact system (Roche Diagnostic Ltd.) was performed to detect bacterial DNA in CSF and blood samples. The recovery of DNA of the two major pathogens causing bacterial meningitis was analysed with an automatic extraction and real-time PCR amplification of the ctra gene (capsular transport gene) for *Neisseria meningitidis* and ply gene (pneumolysin gene) for *Streptococcus pneumoniae*. CSF and whole blood samples were spiked with a strain of *Neisseria meningitidis* 2711 and a strain of *Streptococcus pneumoniae* ATCC 6305 in order to obtain dilutions of 10 000 to 1 bacteria per millilitre. Two hundred microliters were directly extracted with the nucleic acid isolation kit I and the blood protocol of the MagNa pure compact system after a short pre-treatment of enzymatic and buffer lysis. Fifty seven specimens of CSF samples were also tested with the same protocol. For the two pathogens, the sensitivity of DNA extraction was 100 bact/ml for CSF samples and 1000 bact/ml for blood sample. This corresponds to 1–2 and 10 bacteria in our assay of CSF and blood, respectively. Among the 57 CSF tested, 12 were PCR positive for *Streptococcus pneumoniae* and 3 for *Neisseria meningitidis*. Cases positive by culture (n = 10 for *S. pneumoniae*, n = 1 for *N. menigitidis*) were all identified by our assay. Furthermore, we detected on additional 3 cases of infection. No inhibition of amplification

was recorded. The MagNa pure compact system allowed an efficient and reliable DNA extraction of both gram–positive and gram-negative bacteria in about twenty minutes, provided a pre-lysis step is included. Implementation of such strategy is currently evaluated in our routine procedure.

P630

Analysis of mutations within quinolone resistance determining regions of *S. pneumoniae* clinical isolates collected from TARGET surveillance during 2003

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Objectives: To compare fluoroquinolone (FQ) susceptibilities and determine the prevalence of QRDR mutations in recently circulating *S. pneumoniae* (SP) strains.
Methods: SP were collected from centres within Europe, USA, Mexico and South Africa. LEV, gatifloxacin (GATI) and moxifloxacin (MXF) MICs were determined by microbroth dilution. Approx. 20% of SP with LEV MIC of 1 mg/L and all SP with LEV MIC of 2 and above were chosen for analysis. QRDR regions for parC, parE, gyrA and gyrB were amplified by PCR and DNA sequences determined.
Results: No isolate of intermediate susceptibility to LEV (MIC = 4 mg/L) was found. Out of 3233 SP submitted 39 were LEV resistant (res). Of these, 33 were res to GATI and 10 res to MXF. QRDR amino acid changes are indicated in the Table. [Key: 1 ParC-S79F, 2 ParC-S79Y, 3 ParC-S79R, 4 ParC-D83N, 5 ParC-D83Y, 6 ParE-D435N, 7 GyrA-S81F, 8 GyrA-S81Y, 9 GyrA-E85A, 10 GyrA-E85G, 11 GyrA-E85K, ND not determined.] Other QRDR changes were also found but these were not related to FQ resistance (data not shown). Few isolates with LEV MIC of 1 mg/L possessed '®silent' mutations, but 42% of LEV MIC 2 mg/L did. These changes were mainly in the S79 residue of ParC (1 SP carried a S81F GyrA change). These '®silent' SP are only 1 step away from full LEV resistance. The mode GATI or MXF MIC for these SP was 0.5 or 0.25, respectively. Most LEV-res SP (MIC ≥ 8 mg/L) had 2 QRDR changes and those with additional changes had higher LEV MICs.
Conclusions: A large proportion of borderline LEV-susceptible isolates possessed silent parC mutations. These have the potential to select full FQ resistance in the future. The use of more potent FQs, such as MXF, that show less cross-resistance to LEV than GATI may reduce the likelihood of this occurring.

	LEV MIC mg/L (N)					
QRDR change	1 (508)	2 (57)	8 (18)	16 (16)	32 (4)	128 (1)
None	502	33	–	–	–	–
1	1	12	–	–	–	–
2	1	4	–	–	–	–
3	–	–	–	–	–	–
4	–	1	–	–	–	–
5	–	1	–	–	–	–
6	–	5	–	–	–	–
7	–	1	1	–	–	–
1 & 7	–	–	6	11	–	–
1 & 9	–	–	2	–	–	–
1 & 10	–	–	4	1	–	–
1 & 11	–	–	–	1	1	–
2 & 6	–	–	1	–	–	–
2 & 7	–	–	2	–	–	–
2 & 8	–	–	–	1	1	–
2 & 11	–	–	–	–	1	–
3 & 8	–	–	–	1	–	–
4 & 7	–	–	1	–	–	–
6 & 7	–	–	1	1	–	–
1,7 & 5	–	–	–	–	1	–
2,5,7 & 9	–	–	–	–	–	1
ND	4	–	–	–	–	–

P631

Non-haemolytic isolates of serotype 1 pneumococci

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Introduction: The pneumococcus can be divided into over 90 different serotypes on the basis of capsular polysaccharide which is one of the primary virulence factors of the organism. Pneumococci can be carried asymptomatically in the human nasopharynx and only rarely go on to cause invasive pneumococcal disease (IPD). Pneumococci of serotypes 4, 9V, 14, 19F, 23F, 18C and 6B are more prevalent in IPD and are included in the recent heptavalent conjugate vaccine. Serotype 1 is not included in this vaccine. However serotype 1 pneumococci have been linked to disease outbreaks, cause a high burden of disease in developing nations and has been associated more with disease than carriage. The cholesterol dependent cytolysin pneumolysin (Ply) is an important multi-functional virulence factor of the pneumococcus. The important activity of the toxin in serotype 1 disease remains unclear as some invasive serotype 1 pneumococci express a non-haemolytic/very weakly haemolytic version.
Objectives: To investigate variability in gene sequence protein sequence, expression levels and activity of Ply from serotype 1 clinical isolates of *S. pneumoniae*. The gene sequences and functional activities of Ply from 30 serotype 1 pneumococci were investigated
Methods: MLST analysis to determine relatedness of isolates. Amplification of ply gene by PCR and DNA sequencing of amplicons. Haemolytic Assays of cell lysates to determine in vitro haemolytic activity. SDS PAGE, Western blot and Elisa to determine in vitro expression levels.
Results: The 30 isolates were made up from four ST types; the majority of isolates belonged to two related STs (ST306 and ST227) and were considered part of the same clonal group. Over half of the isolates were non-haemolytic despite producing high titres of Ply. The majority of non-haemolytic isolates belonged to ST306. We also identified an 8aa insertion from a non-haemolytic ST227 isolate. Preliminary sequence analysis shows that some serotype 1 Ply alleles differ from the 21 alleles observed previously from clinical isolates in our laboratory and are unique when compared to known Ply sequences.
Conclusions: Some serotype 1 pneumococci express novel Ply alleles. Some serotype 1 pneumococci isolated from IPD produce a non-haemolytic or very weakly haemolytic form of Ply. Non-haemolytic forms of Ply are associated with ST306

P632

Phenotypic and genotypic characterisation of 'atypical' *Streptococcus pneumoniae* isolates by MLST

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Objectives: The aim of this study was to analyze the phenotypic and genotypic characteristics of *Streptococcus pneumoniae* isolates presumptively identified as 'atypical' pneumococci and the comparison with the novel species *Streptococcus pseudopneumoniae*. We describe the genetic relationships and critically discuss the value of conventional identification tests.

Methods: We analysed 12 atypical pneumococcal isolates from patients with invasive diseases from Germany, Spain and France. The following identification procedures were performed: optochin sensitivity (in 5% CO_2 and ambient air), bile solubility, capsular serotyping using the Neufeld Quellung Reaction, DNA probe test (AccuProbe) and biotyping (API Strep32). The analysis of the genotypic characteristics included autolysin (lytA), pneumolysin (ply) and pneumococcal adhesin A (psaA) detection by PCR, 16S rRNA sequencing and multilocus sequence typing using seven housekeeping genes (aroE, gdh, gki, recP, spi, xpt, ddl).
Results: Nine isolates were positive by the DNA test. The remaining three isolates showed typical pneumococcal characteristics such as bile solubility, optochin sensitivity or positive Quellung reaction. Interestingly, seven isolates showed optochin susceptibility identical to *S. pseudopneumoniae*: they were resistant after incubation in 5% CO_2 and sensitive when they were incubated in ambient atmosphere. Nearly all strains were uncapsuled (rough); only three isolates were serotype 14. Pneumococcal genotypic characteristics could be determined as follows: ply (all isolates n = 12), lytA (n = 6) and psaA (n = 2). MLST demonstrated all strains to belong to new sequence types.
Conclusions: Differentiation of *S. pneumoniae* from certain other 'viridans group' and *S. pseudopneumoniae* remains difficult. The detection of pneumococcal virulence genes for ply or lytA seem to be problematic because novel studies reported of their presence in S. mitis or oralis. Especially the description and classification of 'atypical' pneumococci often leads to presumptive exact results which have to be regarded critically because of the great DNA-similarity within the group of streptococci. MLST is a useful tool for differentiation of 'atypical' pneumococci.

P633

Real-time PCR based on the amplification of a gene fragment of pneumococcal surface antigen A in the identification of *Streptococcus pneumoniae*

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Objectives: It has recently been found that some alfa-hemolytic streptococci other than pneumococci may harbour genes encoding pneumococcal virulence factors, mainly pneumolysin (ply). This could present a problem, as many PCR methods for *S. pneumoniae* are based on detection of pneumolysin gene. Pneumococcal surface antigen A (PsaA) has previously been demonstrated in all of the 90 currently known serotypes of *S. pneumoniae* (1). Our aim was to develop a real-time PCR method for rapid detection of a fragment of PsaA encoding gene, to analyse pneumococcal and nonpneumococcal strains using the method and to compare results to those obtained using our previously published ply real-time PCR method (2).
Methods: The forward primer used was adapted from Morrison et al. (1). A new reverse primer was designed using VectorNTI Suite v6 (Informax). A hybridization probe pair was designed and synthesized at TibMolbiol and at the same time additional nucleotides were added to the 5′ end of the primers to get a higher melting temperature of primers. Real-time PCR was done using the LightCycler (Roche Diagnostics). Specificity of the PsaA PCR method was studied by analysing 28 nonpneumococcal bacterial strains, including DNA extracted from freshly grown *S. mutans* (1 strain), *S. mitis* (2), *S. agalactiae* (2), *S. bovis* (1), *S. pyogenes* (2) and *S. sanguis* (2), human DNA and cytomegalovirus DNA. Additionally, 97 nasopharyngeal alfa-hemolytic streptococcal strains were studied, among which were 65 strains identified as pneumococci (optochin sensitive, bile soluble) and 9 strains previously found difficult to identify.
Results: The PsaA real-time PCR method was found to specifically detect pneumococcus. None of the other DNA:s tested were amplified and detected using the method. All 65 pneumococcal strains were positive by PsaA real-time PCR. All 9 alfa-hemolytic streptococcal strains that had given aberrant results in different identification tests previously (optochin test, bile solubility, ply PCR) were negative by PsaA PCR.
Conclusions: PsaA real-time PCR is a rapid method for detecting and identifying pneumococci. Since increasing numbers of studies report that other alfa-hemolytic streptococci may harbour the ply gene, PsaA real-time PCR could be used for confirmation of pneumococci in samples that give a positive or aberrant result in pneumolysin based PCR. Ref: (1) Morrison et al. 2000, J Clin Microbiol 38:434–7. (2) Saukkoriipi et al. 2002, Mol Cell Probes 16:385–90.

P634

Chlamydophila psittaci in fulmars on the Faroe islands – a disease risk to humans?

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Background: Birds are natural hosts for *Chlamydophila psittaci*, but also mammals and humans can be infected. In the 1930ties *C. psittaci* caused severe epidemics with 20% fatality rate on the Faroe Islands. The infections were associated to catching of the seabird fulmar *Fulmarus glacialis*. The knowledge of the *C. psittaci* prevalence among fulmars and its implication for risk of human disease is scarce.
Objective: To investigate the prevalence of *C. psittaci* in fulmars on the Faroe Islands and relate it to reported zoonotic infections.
Methods: Juvenile fulmars were caught on the sea surface in the northern archipelago of the Faroe Islands. Fecal swab samples were collected and after transportation to Sweden. DNA was prepared and examined by real time PCR (DeGraves et al, J Clin Microbiol 2003, 41:1726–9). DNA sequencing of ompA was done by using terminator labeled cycle sequencing in a modified version of a previous description (Herrmann et al Appl Environ Microbiol 2000, 66:3654–6). Information about human *C. psittaci* infections were obtained from The Chief Medical Office.
Results: Of 431 juvenile fulmars were 43 (10%) detected to be infected by *C. psittaci*. The prevalence differed between 8% and 21% in different catchment areas, suggesting variation in *C. psittaci* attack rate on different nesting rocks. Analysis of the ompA gene from 29 *C. psittaci* cases resulted in identical sequences that also was identical to the type strain 6BC, that was isolated from a parakeet. Considering previously reported strain variation of *C. psittaci* among birds, it was surprising that ompA was conserved in fulmars and identical to the prototype strain isolated 63 years ago. Catchment and preparation of juvenile fulmars for domestic food implies heavy exposure to eventual occurrene of *C. psittaci*. Nevertheless, on average only one case per year (range 0–8) of psittacosis has been reported from the Faroe Islands since 1954, and of these no case was fatal.
Conclusion: The risk for humans to acquire symptomatic *C. psittaci* infection from fulmars, and probably from all wild birds, is very low.

P635

Suitability of a single throat swab specimen for the detection of *M. pneumoniae*, *C. pneumoniae* and *Legionella* spp. in respiratory specimens by nucleic acid amplification techniques

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Introduction: The advantage of nucleic acid amplification techniques (NAAT) is their extreme sensitivity and specificity when compared to traditional techniques. Multiplex formats might solve the practical shortcoming of detecting only the infectious agent that is searched for. Real-time assays enable a one-tube assay, reducing the assay time and limiting potential contamination between samples. A comparison between different NAAT is needed.
Objectives: To compare the sensitivity and specificity of conventional nucleic acid sequence-based amplification (cNASBA), real-time (RT) mono- and multiplex NASBA, and mono-PCR for the detection of *M. pneumoniae* (M. pn), *C. pneumoniae* (C. pn) and *Legionella* spp. (Leg) on a single throat swab from hospitalized and outpatients with community-acquired pneumonia (CAP).
Methods: 215 dry throat swab specimens from patients with CAP, collected all over Europe, were investigated by in-house mono PCR, conventional NASBA, RT mono-NASBA, and RT multiplex NASBA, respectively. Definition of the expanded gold standard used to calculate the sensitivities of the tests applied: true positive if positive by at least two NAAT.
Results: 5/215 swabs were found to be positive for C. pn. by all NAAT. Two additional specimens were found to be C. pn positive by conventional NASBA. Leg was detected in 2, 1, 0, 0 swabs by PCR, cNASBA, RT mono- and multiplex NASBA respectively. Total numbers of positives for C. pn. and Leg were too low to calculate sensitivities and specificities. M. pn was detected in 12, 16, 10, 8 swabs by PCR, cNASBA, RT mono- and multiplex NASBA respectively The sensitivities of the different techniques, compared to an expanded gold standard, were 69%, 100%, 63% and 50% for detection of M. pn by PCR, cNASBA, RT mono- and multiplex NASBA respectively. If positive by any method, the sensitivities were 71%, 94%, 59% and 50% for detection of M. pn by PCR, cNASBA, RT mono- and multiplex NASBA respectively.
Conclusions: Previously obtained results on sensitivity were confirmed: the slightly lower sensitivity of the multiplex assay for the detection of M. pn and Leg when compared to the mono real-time and conventional assay. Lower sensitivities of the NASBA assays for the presence of M. pn and Leg species were observed, which could be due to RNA degradation.

P636

Evaluation of the NucliSens mini MAG RNA extraction and real-time NASBA applications for the detection of *M. pneumoniae* and *C. pneumoniae* in throat swabs

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Introduction: A new nucleic acid isolation method, the NucliSens mini MAG (bioMérieux, The Netherlands), was developed based on the Boom nucleic acid extraction method in combination with magnetic silica particles. Real-time NASBA applications have been developed for the detection of *M. pneumoniae* and *C. pneumoniae*.
Objectives: To evaluate the performance of the mini MAG combined with real-time NASBA applications for the detection of *M. pneumoniae* and *C. pneumoniae* in a single throat swab from hospitalized patients with community-acquired pneumonia (CAP).
Methods: Nucleic acids were extracted from aliquots of 215 throat swab specimens from patients with CAP, collected all over Europe, by manual Boom extraction and, retrospectively, by using the NucliSens mini MAG. Nucleic acid extracts from the former were investigated by in-house real-time mono NASBA, from the latter by real-time NASBA applications containing an internal control RNA (bioMérieux). Real-time detection was done on the NucliSens EasyQ Analyzer (bioMérieux).
Results: All five *C. pneumoniae* positive samples were positive by all techniques used. No invalid results were reported. Fifteen specimens were found to be *M. pneumoniae* positive: 4/15 swabs were positive by all techniques used, 5/15 were positive by PCR and the mini MAG in combination with the real-time NASBA application, 4/15 were only positive by manual Boom extraction and in house real-time NASBA and 1/15 was only positive by PCR. When a truly positive was defined by positivity in both amplification or extraction methods, sensitivities of PCR, manual Boom extraction and minimag were 100%, 40% and 90% and specificities were 100%, 98.0% and 100%, respectively. Fourteen samples (6.5%) gave an invalid *M. pneumoniae* result due to the absence of the signal of the internal control when using the *M. pneumoniae* real-time application.
Conclusions: The NucliSens mini MAG and the real-time NASBA applications were successfully combined to detect *C. pneumoniae* and *M. pneumoniae* RNA in throat swabs. This combination is simple, easy and fast.

P637

Investigation of *Chlamydia pneumoniae* and *Mycoplasma* spp. in clinical specimens from patients with arthritis

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Objectives: Chlamydia-induced arthritis is the most frequent form of reactive arthritis in Western countries. Recent advances in the standardization of polymerase chain reaction techniques has accounted for considerable progress in identification of Chlamydia. Although *C. pneumoniae* is often detected by molecular assays, it is rarely investigated with a panel of multiple genes in different human fluids. We mainly focused in the amplification and detection of DNA and or mRNAs fragments from Chlamydia and Mycoplasma genome.
Methods: PCR and RT-PCR were used to assess bacterial DNA and mRNA transcripts on 40 specimens from 12 patients with undefined arthritis. Specimens including sinovial fluid, PBMC and serun samples, were analysed by either nested polymerase chain reaction (n-PCR) or touchdown PCR for Mycoplasma and *C. pneumoniae* with primer sets targeting the 16S-rRNA and urease gene of *Mycoplasma* spp. and *U. urealyticum* and the major outer membrane protein (MOMP), the 16S rRNA and the Heat shock Protein 60–70 (Hsp-60–70) of *C. pneumoniae*. A molecular study to evaluate genetic diversity among isolates of both pathogens and to compare bacterial sequences was done.
Results: Five out 12 (41.6%) patients (10/33 specimens) were found n-PCR positive for *C. pneumoniae* Momp (21.2%) or 16s rRNA (24.4%); two patients (16.6%) did also show HsP-60 gene. One *C. pneumoniae* negative specimen was found positive for Mycoplasma hominis. Four specimen (including those HsP+) were also positive by rt-PCR. Chart analysis revealed that

C. pneumoniae positive specimens were from patients with psoriatic arthropaty, atipic arthritis, rheumathic polymialgy and reactive arthritis. The Mycoplasma hominis positive specimen was from a patient with psoriatic artrhopaty. Previous serological analyses provided no evidence of recent exposure to both pathogens.
Conclusion: Molecular biology not only has improved the ability to detect Chlamydia in the joint for diagnostic purposes but also has extended the current understanding of whether the organism triggers or perpetuates disease. In two patients, the detection of MOMP and 16s rRNA *C. pneumoniae* gene in either blood or sinovial fluid was together with the metabolic active HsP-60 transcripts. This finding together with the genetic background of the patients, may indicate an alternate state used by Chlamydia to escape the immune system of the host and cause worsening of disease.Grants of MIUR 04, CA.RI.FE. and CA.RI.CE. Foundations

P638

Comparison of five PCR methods and culture in detection of *Chlamydia pneumoniae* in mouse lungs

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Objectives: Chronic *C. pneumoniae* infection has been associated with atherosclerosis and pulmonary diseases. The aim of this study was to evaluate five PCR methods and culture in detection of *C. pneumoniae* in the lungs of infected mice at different disease stages.
Methods: NIH/S mice were infected intranasally with *C. pneumoniae* K6 or K7 isolates, and reinfected 56 days later. Samples from the lungs were taken after both infections. Samples were cultured in Vero cells, and DNA was extracted using QIAamp Tissue Kit. Five PCR methods were used: 1) single-step hot start PCR, 2) nested PCR, 3) hot start PCR-TRF (time-resolved fluorescense), 4) and 5) Light Cycler real-time quantitative hot start PCR with two different primer-probe –sets. In methods 1 and 2, PCR products were visualized in agarose gel, and in methods 3 to 5, liquid hybridisation was used. Standard DNA was obtained from purified elementary bodies of isolate K7.
Results: With the purified C.pneumoniae DNA, the sensitivity of all the five PCR methods was <2 genome equivalents. In the mouse lung specimens taken on days 3–14 after primary infection, the sensitivities of all methods were high. However, at day 24 p.i., the sensitivities of PCR methods 1 and 2 and culture were markedly lowered compared to the methods 3–5. After reinfection, the decreased sensitivities of PCR methods 1 and 2 and culture compared to methods 3 to 5 became obvious already at day 9 p.i., and after that, no positive findings were obtained with methods 1 and 2 and culture. Among 20 samples obtained at days 21 and 31 after reinfection, 11 were positive with one, and 8 with at least two of the methods 3 to 5. As a whole, the sensitivity of methods 3 to 5 was >90% in all culture-positive, and 60 to 71% in culture-negative samples. The sensitivity of methods 1 and 2 decreased below 90% when the IFU counts in culture decreased below 1000–10000 IFU/sample, and was only 2 to 7% in culture-negative samples. The correlation between genome quantification and IFU counts was weaker after the reinfection than after the primary infection.
Conclusions: As a conclusion, the sensitivities of PCR methods and culture correlated very well during the acute phase of *C. pneumoniae* infection, but, along with decreased numbers of viable chlamydia, the correlation decreased. In addition, the selection of PCR method affects positive findings. This may also demonstrate difficulties in detection of *C. pneumoniae* especially in chronic infections.

P639

Real-time multiplex PCR for the detection of *Legionella* species in respiratory samples

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Objectives: *Legionella* species cause life-threatening pneumonia and a fast diagnostic method is crucial for the correct treatment and the survival of the patients. Since 1998, a conventional PCR with agarose gel detection (LA-PCR) has been in use at our laboratory for the detection of *Legionella* species in respiratory samples. We sought to develop a real-time multiplex PCR with an internal control using the SYBR Green format (LC-PCR) and melting point analysis, with the advantage of even quicker, and possibly, more specific results.
Methods: Sixty-two specimens from patients previously found positive by the LA-PCR (positive reaction for *L. pneumophila*), of which 37 were confirmed by culture or other methods, 26 specimens with a positive reaction for *Legionella* species in the LA-PCR, and 117 LA-PCR negative specimens were the study material. Seven primers targeting the 5 S rDNA and the mip genes were applied with the FastStart SYBR Green using the LightCycler (Roche), together with an internal synthetic DNA control (IPC), amplified by two of the primers. The IPC was titrated to ensure minimal competition for the primers.
Results: The sensitivity of the LC-PCR was 50 genome copies. Melting points for the 5 S rDNA product was 82.2 ± 0.6°C for the positive control and 82.0 ± 0.3°C for the positive patient samples. All specimens positive for *L. pneumophila* by the LA-PCR were positive with the LC-PCR. Interestingly, only the amplification product representing the 5S rDNA target was visualised in the fluorescence plot, whereas an ensuing agarose gel electrophoresis revealed both the 5 S rDNA and the mip products. This is probably due to preferential binding of the SYBR Green to the 5 S rDNA product rather than the mip product (Giglio et al. 2003). Of the 26 samples positive for *Legionella* species by the LA-PCR, only 11 (of which 8 were from patients with confirmed *Legionella* infection by other methods) could be confirmed by the LC-PCR.
Conclusion: A multiplex real-time PCR for the detection of *Legionella* species was established. With the LC-PCR, response time can be shortened by several hours, with a sensitivity comparable to the LA-PCR and with a higher specificity.

P640

International pilot proficiency programme for the molecular detection of *Legionella pneumophila* on respiratory samples

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Objectives: To assess the proficiency of laboratories performing of nucleic acid amplification technologies (NATs) for the detection of *L. pneumophila* in European and other countries.
Methods: Bronchoalveolar lavage specimens (BAL) were pooled from patients with other diagnosis than legionellosis. Each specimen was individually tested negative by PCR and cultured for *Legionella* before pooling. The presence of inhibitors in the PCR was assessed. The test panel consisted of 10 heat-inactivated BAL or phosphate buffered saline (PBS) samples, composed of 6 samples spiked with 18, 1.8×103 or 1.8×10^5 cfu/ml of *L. pneumophila* sg 1 reference strain ATCC 33152 and four negative samples. The panels were distributed by courier at room temperature. To compare performance, a scoring system was applied: 2 points for a correct result, 1 point

for an equivocal result and 0 point for an incorrect result. A maximum score of 20 points was attainable.
Results: A total of 38 data sets were returned from 34 participant laboratories (85%). Fourteen countries were represented. The mean performance score was 15.6 and 13.0 for in-house and commercial assay methods, respectively. Thirty-four (89.5%) data sets reported qualitative results only and 4 (10.5%) data sets reported both qualitative and quantitative results. Correct results for the entire panel were reported in 3 (7.9%) data sets, one incorrect or equivocal results was reported in 8 (21%) data sets and 27 (71.1%) data sets had two or more incorrect or equivocal results. BAL samples were more frequently identified correctly compared to PBS samples. False negative results were reported in 68 (29.8%) of all tests performed on positive samples. False positive results were reported in 6 (4%) negative samples.
Conclusion: The mean performance score (15.6) was very satisfactory, considering the inclusion of two challenging low inoculum samples (18 cfu/ml). Higher performance scores (18–19) were obtained if those samples are excluded. Twenty-seven participant centres (71.1%) achieved a score of 16 or higher. Single PCR showed a lack of sensitivity compared to real-time PCR based assays.

P641

An in-field hygiene-epidemiological demonstration of the true relations between a case of legionellosis and single spot exposure to *L. pneumophila* from shower on board a Norwegian seismic vessel in the Gulf of Mexico

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Introduction: Legionellosis is a life-threathening illness which to a high degree is shown to be caused by *Legionella* pneumophila. Most well known are the outbreaks from air condition systems, which regularly are seen from hotels and hospitals around the world. Although outbreaks connected to showers and bobble-pooles are discussed, specific demonstration of true genetical relations between infection and specific exposure have only infrequently been presented. In this paper we present a case of legionellosis, occurring on board a ship in the Gulf of Mexico, diagnosed at a hospital in Norway, epidemiologically surveyed on board and identified as being connected to one certain shower on board the ship.
Material and Methods: The hygiene-epidemiological surveys were done by water samplings and swabs from showers in all cabins on board (n = 39). In addition, swabs were taken from each of the shower hoses and taps giving a total number of samples of 136. The samplings were analysed by screening using *L. pneumophila*-specific RealTime PCR (Roche), cultivation of the positive RT-PCRs, serotyping (BioRad)of the live isolates and further genotyping (PFGE).
Results and discussion: The RT-PCR screening revealed positive results of *L. pneumophila* genetic material from 18 of the 39 cabins (46%). Cultivation rendered live *L. pneumophila* from 22 spots and of these, 21 were *L. pneumophila*, serotype 1. The epidemiological relations were determined by PFGE analyses showing four different genotypes, of which one was dominant in the environment. Among the other three genotypes, two were found in more than one spot, while one of the genotypes only was found in one spot. The latter was identical to the infection genotype, and was not found in the shower of the cabin belonging to the patient but from the shower in a guest cabin not in ordinary use, but used in connection with sunbathing on deck. In conclusion, use of screening investigations by of specific *L. pneumophila*-PCR seem to be a good and rapid tool to indicate specific exposure. By further analyses including cultivation, serotyping and genotyping, the true source are possible to identify also in environments which are strongly contaminated with *Legionella*.

P642

Detection of *Legionella* spp by a multiplex real time PCR based on the rnpB gene

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Objective: To develop a multiplex real time PCR for detection of the most important species in the *Legionella* genus.
Methods: The rnpB gene, coding for the RNase P RNA, was determined for 40 species specific reference strains of *Legionella* and all 15 serogroups of *L. pneumophila*. A set of three primers and a TaqMan probe for specific analysis of *L. pneumophila* was designed. In addition seven primers and a group specific probe were selected for detection of *L. anisa, L. bozemanii, L. dumoffii, L. feeleii, L. gormanii, L. longbeachae* and *L. micdadei*. Species identification of non-pneumophila species was performed by terminator labeled cycle sequencing of the amplification product.
Results: The rnpB gene had species specific sequences for 38 species, while identical sequences were found for *L. micdadei* and *L. maceachernii*. For *L. pneumophila* 8 sequence variants were found. In the real time PCR the detection limit for *L. pneumophila* was 10 genome copies per reaction. For other *Legionella* spp the sensitivity was typically between 10 and 60 copies when using a mix of primers. In addition to the above mentioned species, nine other *Legionella* spp of less medical importance were detected at varying copy numbers. A sensitivity comparison between our method and the ProbeTec assay for detection of *L. pneumophila* (BectonDickinson) showed that our method could detect a 200 fold higher dilution of a DNA preparation from a reference strain. To analyse the detection capacity of the real time PCR five clinical isolates/reference strains of the above mentioned *Legionella* species and 20 strains of *L. pneumophila* were tested. All strains were detected, except one strain of *L. feelii*. This strain had no mismatching sequence in the primer/probe regions that could explain this failure. The specificity was assessed by using a panel of DNA from 12 representative bacteria species and the PCR resulted in no amplification. Our method was further assessed by examination of a quality assurance panel from the European Working Group of *Legionella* Infections and all 10 specimens of *L. pneumophila* were detected.Evaluation of the method for detection of *Legionella* spp in clinical respiratory tract samples and environmental water samples is ongoing.
Conclusion: We have developed a sensitive and specific multiplex real time PCR for detection of the most important *Legionella* species in clinical diagnostics.

Molecular methods for enteric pathogens

P643

Comparison of VNTR analysis with PFGE and REP-PCR for genotyping of pathogenic *Yersinia enterocolitica*

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Objective: Analysis of variable-number tandem repeat (VNTR) sequences has proved to be a useful tool for molecular epidemiology studies in bacteria. Identification of a VNTR locus in the genome of *Yersinia enterocolitica* (located at the 3′ side of orf528) has recently been described. We have evaluated the usefulness of this polimorphic region analysis in comparison to other well-established methods such as pulsed-field gel electrophoresis (PFGE) or repetitive extragenic palindrome PCR (REP-PCR) to genotype clinical isolates of *Y. enterocolitica*.
Methods: We studied a total of 51 clinical isolates of *Y. enterocolitica* serobiotype O:3/4 with no evident epidemiological relationship, collected from different patients over seven years, and clustered by VNTR analysis in 8 different alleles according to the number of CCAGCA repeats they contained: 14 repeats (n = 6), 13 (n = 14), 11 (n = 5), 9 (n = 3), 8 (n = 9), 7 (n = 8), 5 (n = 1) and 4 (n = 5). These were all analysed by REP-PCR, and 16 isolates, representative of the different alleles, were further characterized by PFGE (NotI restriction).
Results: REP-PCR did not identify separate clusters among the *Y. enterocolitica* isolates, as all showed identical patterns. NotI generated 4 different PFGE-patterns with minor differences between them. The combination of PFGE and VNTR analysis divided both of the two predominant PFGE types into five subtypes, increasing discrimination.
Conclusions: REP-PCR does not discriminate among *Y. enterocolitica* isolates of the same biotype. PFGE identifies different genotypes but less so than the analysis of the polimorphic VNTR locus. VNTR analysis provides a method that notably increases the discriminatory power of other typing methods.

P644

Evaluation of molecular markers (PFGE, PCR-RFLP-flaA, MLST and PCR-RFLP-LG) in Spanish *C. jejuni* isolates

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Campylobacter jejuni has been established as the microorganism to be the major cause of acute diarrhoeal disease in humans. Bacteria showed high polymorphism and molecular diversity.
Objectives: The aim of this study was to evaluate the discriminatory power of different molecular methods in strains closely related (three outbreaks) and between strains non related.
Methods: Forty two *C. jejuni* strains were studied (thirty three non relate isolates and nine isolates from three outbreaks). All strains were molecularly characterized by pulsed-field gel electrophoresis (PFGE) digested separately with SmaI and KpnI, flaA restriction fragment length polymorphism (PCR-RFLP-flaA) using Dde I as the restriction enzyme, multilocus sequence typing (MLST) based on the DNA sequences of an aproximate 450 bp size fragments of aspA, glnA, gltA, glyA, pgm, tkt and uncA housekeeping genes and LPS genes (LG) restriction fragment length polymorphism (PCR-RFLP-LG) digested separately with DdeI and CfoI. Serotyping was performed on strains with a passive hemagglutination technique and non-absorbed antisera, using a set of 33 different antisera produced by ourselves.
Results: PFGE provided 25 and 30 different types for SmaI and KpnI respectively (DI: 0,968 and 0,990). Using PCR-RFLP-flaA 19 types were obtained (DI: 0,941). MLST identified 21 different sequence types (DI: 0,949). By means of PCR-RFLP-LG genotyping method isolates were grouped in 15 and 14 types with the enzymes DdeI and CfoI respectively (DI: 0,905 and 0,877). Ten serotypes were found (DI: 0,911), one isolate reacted with two antisera, cross-reactions (1 + 44) and seven isolates were untypeable. All related strains within each outbreak showed identical types in all tested methods.
Conclusions: All genotyping methods showed high Discrimination Indexes being PFGE the methods with the most discriminatory power. The unambiguous nature of MLST, based on sequence data, represents a significant advantage over PFGE and other gel-based methods and it is very useful for longitudinal studies of population structure. The results obtained suggest that the combine use of PCR-RFLP-flaA and PFGE (with KpnI digestion) methods could be a good option for identifying identity among isolates.This work has been supported by the I + D + I project MCYT n°: AGL2002 - 04480- C03 - 02.

P645

Comparison of different isolation and identification methods for thermophilic *Campylobacter* species

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Objectives: In this study different culture methods were compared in order to optimize the recovery of thermophilic *Campylobacter* species those were accepted to be associated with diarrhoea in human. After cultivation all isolates were identified to the species level by conventional and molecular methods for comparison.
Methods: Rectal swab samples taken from 514 children with diarrhoea were examined. All samples were diluted in PBS and inoculated on to two CAT agar plates and one non-selective agar plate by using the membrane filter method. One of the CAT agar plates was incubated at 42°C, the other one was incubated at 37°C with the non-selective agar plate. At the identification stage, performance of biochemical reactions and restriction enzyme analysis (REA) were compared. Bacterial DNA was extracted by CTAB (Cetyltrimethylammonium bromide) method and PCR was performed by using the previously described primer pair THERM 1 and THERM 4. Following PCR amplification products were cut by AluI and Tsp509I.
Results: Thirty six (7%) *Campylobacter* strains were isolated. The sensitivities of the CAT agar at 42°C, CAT agar at 37°C, membrane filter were calculated as 86.1, 58.3 and 52.8% respectively. CAT agar at 42°C was found significantly more effective than the others in recovering thermophilic *Campylobacter* spp. But five (13.9%) isolates were recovered only by the membrane filter method. One of these five isolates was *C. upsaliensis*. Thirty two (88.9%) isolates were defined by biochemical reactions while all of the isolates were succesfully identified to the species level by REA. REA was found reliable and faster than the conventional biochemical reactions. The isolation rates of the species were calculated as *C. jejuni* 83.3%, *C. coli* 13.9% and *C. upsaliensis* 2.8%.
Conclusions: According to our findings we recommend the usage of CAT agar at 42°C and the filter method in combination.

Conventinal methods for identification of thermophilic *Campylobacter s* to the species level are cumbersome but REA is an easy method to set up in a routine laboratory and can shorten the time required for diagnosis.

P646

Identification of plasmids by PCR-based replicon typing

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Objectives: The dissemination of antimicrobial resistance is largely attributed to the acquisition of resistance genes by horizontal transfer mediated by plasmids. Plasmids are usually classified in incompatibility (Inc) groups. Incompatibility is a manifestation of relatedness, relying on common replication controls (replicons, rep) or equipartitioning elements. The identification of plasmid by rep typing could represent a very useful method to trace the diffusion of plasmids conferring antimicrobial resistance to unrelated bacterial strains, and to follow the evolution and spread of emerging plasmids. However, plasmid characterization by incompatibility typing is limited by the laborious and time-consuming methodology required, based on conjugation, transformation or on multiple hybridization experiments performed with low-specific inc/rep probes. These methods cannot be easily applied on a large number of strains.The necessity of tracing plasmids conferring drug resistance prompted us to develop a PCR-based replicon typing method.

Methods: In this method, 18 couples of primers were devised to perform 5 multiplex- and 3 simplex-PCRs, recognizing the repFIA, repFIB, repFIC, repHI1, repHI2, repI1, repL/M, repN, repP, repW, repT, repA/C, repK, repB/O, repX, repY repF, and repFII replicons.

Results: Eighteen different replicons were cloned and sequenced and used as positive controls in the PCR-based replicon typing. The specificity of the method was investigated on a collection of 62 reference plasmids, belonging to known Inc groups. The method was also applied to a collection of 25 multidrug resistant *Salmonella enterica* strains isolated from animals in Italy, previously characterized respect to the resistance genes. In 19 on 25 strains, plasmids of the A/C, I1, N, X and HI2 were detected, demonstrating the circulation of prevalent plasmids associated to specific resistance genes.

Conclusion: The PCR-based inc/rep typing could represent the easiest way to perform a first screening of a large number of strains, or can be used to trace the diffusion of multi-drug resistant plasmid. The application of this method could have a great impact on epidemiological investigation of the horizontal transfer of resistance genes in different bacterial strains.

P647

Simple sequence repeat analysis for *Vibrio cholerae* strains typing

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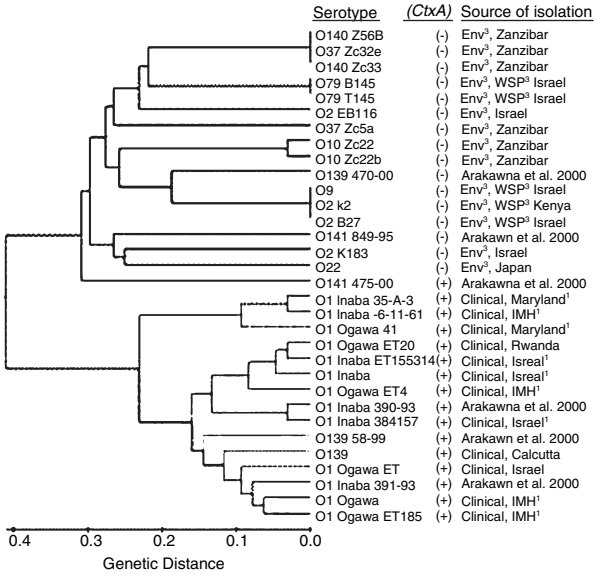
Background: One of the most dynamic components of bacterial genomes are the Simple Sequence Repeats (SSR). SSRs, also termed VNTR, are simple DNA motifs tandemly repeated at a specific locus. A subgroup of SSRs are the mononucleotide repeats (MNR). Being highly mutable, SSR provide a ready source of genomic polymorphism used in bacterial typing. However, to date, practical typing of *Vibrio cholerae* is mainly serological and requires about 200 antisera. *V. cholerae* is the etiological agent of cholera. The pathogenic and non-pathogenic isolates are divided into different serological groups, of which only O1 and O139 serogroups are associated with cholera pandemics.

Objectives: Development of an SSR-based method that combines the variation seen in highly mutable SSR loci, with that of shorter, relatively more stable MNR loci , for accurate and rapid typing of *V. cholerae* isolates. This approach will enable accurate phylogenetic and evolutionary studies, as it offers the ability to distinguish both far- and closely-related strains.

Methods: The complete genomic sequence of *V. cholerae* was screened for perfect SSR (≥3). Two strategies were applied for the identification of SSR polymorphism: capillary fragment size analysis for the SSR loci, and sequencing of the selected MNR loci.

Results: In silico screen of the *V. cholerae* genome revealed thousands of SSR tracts with an average frequency of one SSR every 152 bp. The longest SSR and MNR loci were chosen for further analysis. A panel of 32 *V. cholerae* strains, representing both clinical and environmental isolates, was tested for polymorphism in SSR loci. All nine SSR loci were polymorphic, displaying two to 13 alleles per locus. Also, sequence analysis of nine MNR-containing loci provided information of both variations in the MNR tract itself, and single nucleotide polymorphism (SNP) in their flanking sequences. These multiple sequence variations presented high levels of polymorphism, with four to eleven distinct sequence-types per locus. Phylogenetic analysis of the combined SSR data showed a clear discrimination between the clinical strains belonging to O1 and O139 serogroups, and the environmental isolates. Furthermore, discrimination between most of the strains was achieved.



Conclusion: Results suggest that SSR-based typing methods, which combine both SSR length polymorphism and MNR multilocus sequence typing (MNR-MLST) could be efficiently applied for *V. cholerae* typing.

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Identification of potential faecal contamination markers of coliforms and thermotolerant coliforms isolated from chicken feed using tRNA intergenic length polymorphism analysis (tDNA-PCR)

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As part of a National Salmonella Programme, raw material and processed feed from feed mills producing poultry feed in Denmark are regularly tested for coliforms (Gram-negative and lactose-positive bacteria) as a measure of possible faecal contamination of the feed. However, no further identification of the bacteria contained in this group of coliforms is performed. In this study the species distribution for coliforms and thermotolerant coliforms (TTC) used as indicators for faecal contamination were investigated. Coliforms were isolated together with TTC's (coliforms grown at 44°C) that increase the specificity of faecal contaminants. Application of classical phenotyping methods alone was not sufficient to identify species within the coliforms and TTC's in the raw material and feed. Therefore, intergenic length polymorphism analysis (tDNA-PCR) was employed as a reliable and rapid method to allow final speciation. A total of 290 isolates were identified representing 110 coliforms and 180 TTC's, with only 23 (21%) and 67 (37%) of the isolates were identified as *E. coli*, respectively. Other coliforms represented *Enterobacter* spp. (44%), *Klebsiella* spp. (26%) and *Citrobacter* spp. (5%), whereas TTC's constituted *Enterobacter* spp. (29%), *Klebsiella* spp. (23%) and *Citrobacter* spp. (4%). Among the coliforms and TTC's, 8 isolates could not be identified using tDNA-PCR. 16S rRNA sequence analysis will establish whether these constitute a new taxon under the family of *Enterobacteriaceae*. The present investigation showed that tDNA-PCR represents a quick and reliable method for identification of coliforms and TTC's obtained from feed and feed raw materials. In addition, the heterogeneity of taxa identified as TTC's seems to indicate that TTC's should not be used as an indicator of faecal contamination of feed and feed ingredients.

P649

Improved PCR detection and subtyping of CTX-M-beta-lactamase-encoding genes

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Background: Rapid detection and differentiation of CTX-M beta-lactamases is of need in many laboratories as this family of ESBLs is rapidly disseminating. PCR has been used widely to detect blaCTX-M genes but detection of all the known variants usually required multiple reactions with specific primers for different CTX-M subtypes. Consensus primers have also been described but the recent emergence of new CTX-M variants (e.g. CTX-M-25, -26) made them no longer versatile. In this study we describe a novel real-time PCR technique for detection of all CTX-Ms and a single-enzyme post-PCR restriction analysis for differentiation between the 5 CTX-M genetic clusters.
Methods: A pair of primers matching conserved sequences at positions 205 to 227 and 724 to 706 with respect to the CTX-M translational starting point was designed to amplify a 519-bp fragment of all the known blaCTX-M genes using a real-time approach with SYBR Green product detection. A computer analysis was used to identify BseDI as a restriction endonuclease capable of distinguishing the subtypes of blaCTX-M genes. PCR products were digested with BseDI and digests analysed by agarose gel electrophoresis. Bacterial strains producing known beta-lactamases (CTX-M-2, -3, -4, -5, -9, -15; TEM-1; SHV-1; KluA) were used for quality control and assessment of specificity of PCR and discriminative power of the restriction analysis.
Results: A single DNA fragment of the expected size was amplified in all CTX-M-positive control strains. Melting curve peaks were detected at 85, 86.5 and 88°C for CTX-M-2, -3 and CTX-M-9 genetic subtypes, respectively. No increase in fluorescence was detected for CTX-M-negative isolates. Further restriction analysis produced the predicted restriction patterns and, therefore, allowed to distinguish different CTX-M subtypes.
Conclusions: The proposed real-time PCR is a versatile and specific tool for rapid detection of CTX-M beta-lactamases. When needed, the post-PCR restriction analysis may be used to confirm differentiation of CTX-M genetic subtypes.

P650

Detection of extended-spectrum and plasmid-mediated AmpC beta-lactamase in cefoxitin resistant *Klebsiella pneumoniae* clinical isolates

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Objectives: Clinical isolates of *Klebsiella pneumoniae* are often multi-resistant and the major hosts for extended-spectrum beta-lactamases (ESBLs) and plasmid-mediated AmpC beta-lactamases (PABLs). Many clinical laboratories have problems for ESBL detection in the presence of an AmpC beta-lactamase. Nonetheless, identification of ESBLs is important, since the activity of the extended-spectrum cephalosporins (ESCs) in vivo may not be accurately predicted by susceptibility tests using the traditional National Committee for Clinical Laboratory Standards (NCCLS) breakpoints. This study was conducted to evaluate two phenotypic confirmatory tests for the detection of ESBLs and to identify families of PABL genes in clinical isolates of cefoxitin-nonsusceptible *K. pneumoniae*.
Methods: A total of 55 clinical isolates of *K. pneumoniae* at the Bundang CHA Hospital that were non susceptible to cefoxitin were collected between June 2004 and September 2004. Susceptibility testing was performed by Vitek GNS card. To detect ESBL and AmpC production, double disk synergy test (DDS), NCCLS ESBL phenotypic confirmatory test, and mHodge test with some modification were performed. ampC mutiplex PCR developed by Perez-Perez and Hanson was performed (JCM, 2002).
Results: According to the susceptibility for ESCs and result of ESBL by automated Vitek system, isolates were categorized to three groups. By phenotypic ESBL confirmatory test, 37 of the 40 ESC-non susceptible (NS) and Vitek ESBL(−) and all of the 10 ESC-NS and Vitek ESBL(+) isolates, but none of the 5 ESC-susceptible (S) and ESBL(−) isolates, produced ESBL. DDS was more excellent than NCCLS confirmatory test. Thirty-six of the 40 ESC-NS and Vitek ESBL(−), all of the 5 ESC-S and Vitek ESBL(−), and 2 of the 10 ESC-NS and ESBL(+) isolates were shown to contained ampC-type genes by PCR. Of the 43 PABL producing isolates, 39 (90.7%), 2 (4.7%), 2 (4.7%), and 1 (2.3%), respectively, demonstrated DHA, CIT, EBC, and MOX bands. One isolate demonstrated CIT and DHA bands. The results of mHodge test with some modification were well correlated with that of PCR
Conclusions: Among the *K. pneumoniae* isolates with decreased cefoxitin susceptibility, PABL-producing strains were prevalent. In the case of Vitek ESBL(+) and cefoxitin non susceptible, only a few strains contained ampC genes. The most common type of family-specific AmpC beta-lactamase genes within *K. pneumoniae* at our institution in Korea was DHA group.

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Pulsed field gel electrophoresis of *Salmonella enterica* subspecies *enterica*: the presence of plasmid DNA has implications for the interpretation of PFGE data

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Objectives: Pulsed field gel electrophoresis (PFGE) is currently regarded as the 'Gold Standard' for the typing and strain identification of isolates of *Salmonella enterica* subspecies *enterica* and other organisms. Plasmid profiling, on the other hand, has been used previously in epidemiological investigations but is not now in common use. This study examines the contribution of plasmid DNA to the PFGE patterns obtained and assesses the significance of plasmids for the interpretation of PFGE data for Salmonella.
Methods: All isolates of *Sal. enterica* from human infection in Scotland are submitted to the reference laboratory. Isolates are serotyped and, where applicable, phage typed. Since the beginning of 2003, all isolates have been examined for the presence of plasmid DNA and subjected to PFGE following chromosomal digestion with Xba1. Analysis utilised BioNumerics software (Applied Maths, Belgium). Plasmid sizes were calculated and stored in a database. PFGE patterns were analysed (excluding fragments below 30 kbp in size), PFGE types were assigned to each isolate and stored in a database together with plasmid profiles.
Results: Plasmid and PFGE data has been collated on over 1800 isolates. Plasmid DNA was detected in 1525 (about 85%) of these isolates, ranging in size from 1.9 to 180 kbp. It was generally observed that bands arising from smaller plasmids (<30 kbp) were not visible on the PFGE gels. Larger plasmids could be observed as bands on the PFGE pattern. The virulence plasmids of serotypes Typhimurium, Enteritidis and Dublin appeared as bands of the expected size. However, some other plasmids were found to contribute bands of lower molecular weight than expected, or no observable band, probably dependent on the number of restriction sites present on the plasmid. During an outbreak of serotype Saintpaul, PFGE differences of up to 4 bands were observed as the result of the presence/absence of different plasmids.
Conclusion: PFGE is widely used as an epidemiological tool and is rightly regarded as the 'Gold Standard' for outbreak investigation of Salmonella. Correlation of plasmid profile with PFGE typing results has indicated that plasmids can make a significant contribution to the banding patterns obtained with PFGE. Plasmid profiling should be considered as a relatively easy and inexpensive addition to PFGE in order to simplify the interpretation of PFGE results in the identification of outbreaks and strain relatedness.

P652

Molecular characterisation of Salmonella by multilocus sequence typing, pulsed-field gel electrophoresis and amplified fragment length polymorphism

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Objectives: The variability of *Salmonella enterica* strains were studied using three genotypic typing techniques and the discriminatory ability, reproducibility and ease of analysis were compared.
Methods: Three molecular typing methods were used to characterize 110 *Salmonella enterica* strains that had been isolated from human or veterinary sources in Denmark between 1995 and 2001. All strains were genotyped by multilocus sequence typing (MLST) using the genes aroC, dnaN, hemD, hisD, purE, sucA and thrA, pulsed-field gel electrophoresis (PFGE) with XbaI and amplified fragment length polymorphism (AFLP) with BglII and BspDI.
Results: The number of alleles for the seven housekeeping genes ranged from 15 to 22. Sequence types (ST) were assigned based on the allelic profile and in total 43 STs were identified. Most of the strains with the same serotype had the same ST or only one isolate accounted for an additional ST. Eleven of the strains could not be typed with the conventional PFGE method because of DNA degradation and were given the PFGE type, NT. The last 99 isolates generated fingerprints with between 11 and 22 bands and 73 unique PFGE types were identified. The profiles were used to generate an UPGMA tree using the dice similarity coefficient, and in most cases isolates with the same serotype clustered together. The 110 AFLP fingerprints generated between 80 and 100 bands and in total 78 AFLP types were assigned. When making an UPGMA tree using the dice similarity coefficient, isolates with the same serotype clustered in the same lineage of the tree supported by bootstrap values above 70%.
Conclusion: When making genetic trees, all three methods resulted in similar clustering which in most cases corresponded with serotype. Of the three techniques, MLST was the easiest to interpret and compare between laboratories. The MLST genes used here lacked diversity and the ability to discriminate between isolates were higher with both AFLP and PFGE. The discriminatory ability of AFLP and PFGE were similar but PFGE fingerprints were both easier to reproduce, interpret and less time consuming to analyze when compared to AFLP.

P653

Characterisation of *Salmonella enterica serovar Typhimurium* DT104 strains isolated in the Slovak Republic

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Objectives: Multidrug-resistant S. *enterica serovar Typhimurium* (*S. Typhimurium*) definitive phage type (DT) 104 is now acknowledged as an internationally distributed zoonotic pathogen. In the SR this phage type has been isolated for the first time in 1997 and in 2003 predominated among the *S. Typhimurium* strains. The aim of this study were both molecular and biological analysis of 70 DT104 strains isolated from sporadic human cases.
Methods: The all isolates were tested by the disc diffusion method for susceptibility to ten antimicrobial drugs, according to standard procedure of NCCLS (1997). The plasmid DNA was isolated by the alkaline lysis technique of Birnboim and Doly (1979). PCR with specific primers for integron detection was performed as described by Lovesque et al. (1995). PCR technique was also used for detection of virulence genes. The hydrophobicity was determined on the basis of bacterial adherence to hydrocarbon-xylene (BATH) and salt aggregation test with ammonium sulphate. The assay of motility was performed on the semisolid agar medium and the biofilm formation was examined in the tube test (Bonafonte, 2000).
Results: The majority of DT104 strains were isolated from patients of age 0–18. However, fourty-nine strains were multidrug-resistant, the dominant resistance type was ACSSuT (58.6%). The analysis of plasmid DNA revealed that 69 (98.6%) strains possessed 90 kb serovar specific virulence plasmid. Fourty-four DT104 strains contained integrons of 1000 and

1200 bp and 40 of they exhibited the ACSSuT phenotype. Two strains with the same multiresistance contained only integron of 1200 bp. All studied strains were positive for presence of virulence genes stn and iroB. Adhesion was found to be high in 48 (68.6%) strains. Motility of 55 (78.0%) strains was high. The biofilm after cultivation in a starvation medium was formed by 63 (90.0%) strains.

Conclusion: The results involving the multidrug-resistance, the content of the class 1 integrons as well as the high virulent potential of studied strains suggest the rapid spread of DT104-ACSSuT phenotype strains in recent time in the SR. They possess a significant threat for possible the horizontal transfer of resistance determinants to other pathogens of importance to human health.

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P654

Amplified intergenic locus polymorphism as a basis for bacterial typing of *Listeria* spp. and *Escherichia coli*

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Objectives: Identification and typing of microorganisms have become major objectives over the past decade in microbiology. Recent advances in molecular biology have resulted in the development of numerous DNA-based methods for discrimination among bacterial strains. The high number of polymorphic sites present among closely related bacterial genomes is the basis for the presented method. The method identifies multi-locus genomic polymorphisms in intergenic regions termed AILP – Amplified Intergenic Locus Polymorphism. The experiments described here were carried out to evaluate the potential of this new strain typing methodology for representative gram positive and gram-negative bacterial species, *Listeria* and *E. coli*.

Methods: For each locus, a pair of unique PCR primers was designed to amplify an intergenic sequence from one ORF to the adjacent ORF. Presence, absence and size variation of the amplification products analysed by gel electrophoresis were identified, and used as genetic markers for rapidly differentiating among strains.

Results: Polymorphism was evaluated using eighteen AILP sites among 28 strains of *Listeria* monocytogenes and six strains of *Listeria* spp., and 30 AILP markers among 27 strains of *Escherichia coli*. Up to four alleles per locus were identified among *Listeria* strains and up to six among *E. coli* strains. In both species, more than half of the AILP sites revealed intra-species polymorphism. The AILP data were applied to phylogenetic analysis among *Listeria* and *E. coli* strains. A clear distinction between *L. monocytogenes* and *Listeria* spp. was demonstrated. In addition, the method separated *L. monocytogenes* into the three known Lineages and discriminated the most common virulent serotypic group 4b. In *E. coli* AILP analysis separated the known groups as well as the virulent O157:H7 isolates. These findings for both *Listeria* and *E. coli* are in agreement with other phylogenetic studies using molecular markers.In conclusion, the AILP method provides a rapid and simple initial identification of isolates as a basis for epidemiological investigation, clearly discriminating between different strains or revealing similarities that can be further tested using high discriminatory power typing methods. Thus, the AILP should be a useful addition to the available methodologies for rapid initial microbial strain typing.

P655

Detection of quinolone resistance mutations in gyrA, gyrB, parC and parE by denaturing high performance liquid chromatography using standard HPLC equipment

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Objectives: Denaturing high performance liquid chromatography (DHPLC) has become widely used in recent years as a method of detecting single nucleotide polymorphisms (SNPs). As such, DHPLC can be used for the detection of mutations that confer quinolone resistance, e.g. mutations in DNA gyrase (gyrA, gyrB) or DNA topoisomerase IV (parC, parE). Dedicated machines for DHPLC are available but expensive and many laboratories have existing HPLC equipment. In this study the use of a standard HPLC machine (Agilent A1100) and a generally available column (Varian HelixTM DNA column) was investigated for detection of mutations in the gyrA, gyrB, parC and parE genes of *Salmonella enterica*.

Methods: A panel of Salmonella strains with known different mutations in gyrA (n = 9), gyrB (n = 2), parC (n = 2) and parE (n = 1) were assembled. Wild type (WT) *Salmonella enterica serovar Typhimurium* NCTC 74 was used as control strain. The DNA of respective strains was amplified by PCR using Optimase® proofreading DNA polymerase and prepared for DHPLC as previously described. Duplex DNA samples (5 μl) were analysed using an Agilent A100 HPLC system with a HelixTM DNA column (Varian) and Varian buffers A and B according to the manufactures recommendations. DHPLC analysis was performed at temperatures of both 61°C and 63°C to detect mutations in gyrA and gyrB and at both 62°C and 64°C to detect mutations in parC and parE.

Results: Using the A1100 HPLC system mutations in gyrA, gyrB, parC and parE were readily detected by comparison with control chromatograms although in some cases this required

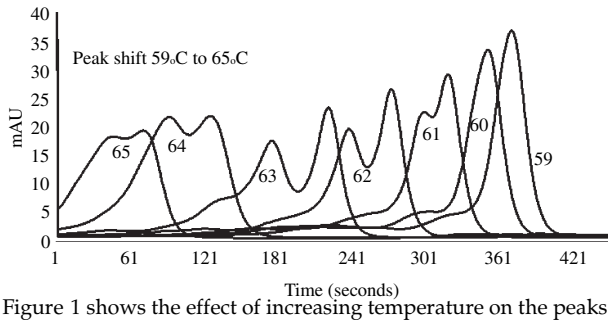

Figure 1 shows the effect of increasing temperature on the peaks observed for an Asp87-Asn mutation in gyrA.

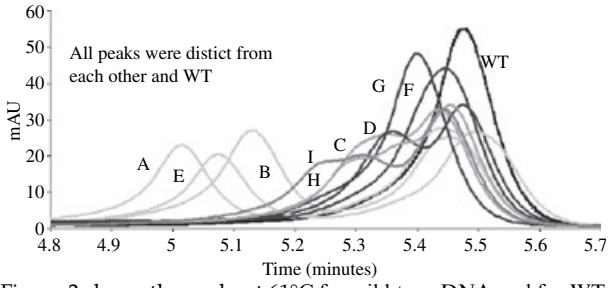

Figure 2 shows the peaks at 61°C for wild type DNA and for WT and mutant DNA from strains with mutations in the following regions of gyrA - Ser83-Phe + Asp144-Asp (A), Asp114-Asp (B), Asp 87-Asn (C), Ser 83-Phe (D), Ser 83-Tyr (E), Asp 87-Gly (F), Asp87-Gly + Asp144-Asp (G), Asp 87-Tyr (H), Asp82-Asn (I).

analysis at two temperatures. In most cases the strains with different mutations gave unique profiles from each other.
Conclusion: The results showed that it is possible to use standard HPLC equipment and readily available columns for detection of mutations in gyrA, gyrB, parC and parE. However, interpretation of results can be subjective when only small differences between WT and mutant or between mutants and mutant are seen and appropriate controls should always be used.

P656

Replicon typing of emerging plasmids conferring resistance to ceftriaxone and carbapenems

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Objectives: A resistance gene that has emerged on a plasmid may be transferred to other strains and species, enabling it to penetrate into niches not accessible to its original host strain. This implies that plasmid-mediated antimicrobial resistance is a global problem that does not respect any boundaries, among bacterial species and genera. The replicon typing of plasmids is a potent tool to classify plasmids into families on the basis of their replication controls. However, this approach has been limited by the laborious and time-consuming methodology required. We have developed a replicon typing method to screen and classify plasmids by PCR. In this study the method was applied to well characterized plasmids, belonging to the collections of resistant Gram-negative bacteria isolated in USA, Greece and Italy. The aim of this study was to verify the sensitivity of the method when applied to plasmids circulating among human pathogens, conferring resistance to antimicrobials of relevance for human therapy.
Methods: The PCR-based inc/rep typing method recognizes 18 different replicons (rep) and has been applied to 31 previously characterized plasmids from our collections of multi-drug resistant Enterobacteriacae and Pseudomonas, showing resistance to extended-spectrum cephalosporines or carbapenems by the presence of the blacmy-2, blacmy-4, blacmy-13, blavim-1, blavim-2, blavim-4 or blaimp-12 genes.
Results: The repA/C and repI1 were identified on the highly diffused blacmy-2-plasmids isolated from ceftriaxone resistant *Salmonella enterica* and *Escherichia coli* in USA. Three repA/C plasmids, carrying the blacmy-4 and blavim-4 genes have been also identified in clinical isolates of Klebsiella pneumoniae and Enterobacter cloacae from Italy, suggesting a wider diffusion of these kind of plasmids. Nine blavim-1-carrying plasmids, identified in Enterobacteriacae recently isolated in five hospitals of Athens, were all positive to the repN replicon. This information represents a new tool to monitoring the spread of these plasmids. However, the method failed in identifying three plasmids from Pseudomonas spp. isolated in Italy, suggesting that additional replicon sequences should be analysed to increase the sensitivity of the method.
Conclusion: The possibility to trace plasmids by PCR may extend the knowledge of the mechanisms of horizontal gene transfer of antimicrobial resistance, facilitating the development of control strategies against this phenomena.

P657

A role for rmoA in IncF plasmid mediated biofilm formation in *E. coli* K12

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Objectives: Bacterial biofilms cause serious health problems because these sessile cells can withstand immune response and show an increased resistance to antimicrobial agents. Non pathogenic *E. coli* have a limited ability to form biofilms, but carriage and transmission of conjugative plasmids enhance biofilm formation. We are interested in understanding the role plasmids play in promoting biofilm formation and the molecular mechanisms behind these effects.
Methods: To create mutations in biofilm associated genes random transposon mutagenesis of IncF plasmid R1–16 was performed using miniTn5 transposition. To exclude mutations in transfer genes, a selection screen requiring conjugative transfer of mutant plasmids was employed. Interrupted genes were identified using arbitrary PCR and DNA sequencing. Strains harbouring mutant plasmid derivatives were tested for biofilm forming ability in microtiter format. This in vitro model of biofilm formation that relies on removal of planktonic cells and quantification of surface attached bacterial mass by crystal violet staining. Subsequent specific inactivation of the rmoA gene in R1–16 was performed using the lambda Red system of bacteriophage lambda. To obtain information about the expression of the gene of interest Northern Blot analysis and Reverse Transcriptase PCR were performed.
Results: As a result of the transposon mutagenesis rmoA was found to be important for biofilm formation. *E. coli* strains carrying R1–16 with a deletion of rmoA showed a 5-fold decrease in biofilm formation compared to wildtype R1–16. This effect was observed in full and in minimal media. Kinetic experiments of biofilm formation revealed that the rmoA phenotype is already developed in an early stage of biofilm formation. Northern Blot analysis and Reverse Transcriptase PCR indicate that rmoA is transcribed as part of an operon.
Conclusions: Genes homologous to rmoA are present in Yersinia enterolitica, Salmonella typhimurium, and on many plasmids including plasmids of pathogenic *E. coli*, Shigella flexneri and Salmonella typhi. The amino acid sequence of RmoA shows strong homology to the Hha protein of *Escherichia coli* and the YmoA of Yersinia enterolitica, which are modulators of bacterial gene expression in response to environmental stimuli. Thus, RmoA may act to regulate plasmid or host genes that are important to bacterial biofilm formation.

P658

Subtyping of *Escherichia coli* verocytotoxin 1: first report of VT1d subtype in humans

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Objectives: Verocytotoxins are highly potent cytotoxins and are important virulence factors of verocytotoxin producing *Escherichia coli*. Two major types of toxins are known: verocytotoxin 1 (VT1) and verocytotoxin 2 (VT2). Several variants of VT2 have been identified, whereas the VT1 type appears to be more homogenous with only one variant VT1c, besides the VT1. However, in 2003 a new variant of VT1 of bovine origin was identified and named VT1d.358 vtx1 positive strains, isolated from 1983–2003 from Danish patients with diarrhoea, were vtx1 subtyped. The vtx1 subtypes were corre-lated to clinical manifestations such as diarrhoea, bloody diarrhoea, fever, vomiting, HC and HUS.
Methods: A PCR-RFLP assay was used to distinguish the vtx1 and vtx1c subtypes. PCR products obtained with Gannon F and R primers were independently digested with cfoI and rsaI. The vtx1d subtype was revealed by sequencing.
Results: The vtx1c subtype was found in 54 strains, predominantly those without the eae gene (see table). The vtx1c positive VTEC strains belonged to 17 different O-serogroups, the predominant serotypes were O146: H21 (25.9%) and O128ab:H2 (18.5%). Only one O157:H- was found. Moreover,

vtx1c was most frequently associated with the vtx2d subtype. Vtx1c strains (without vtx2) were less associated with fever (P = 0.0275) and had a higher incidence amongst women (P = 0.0020). We found that the vtx1c subtype in combination with vtx2 had a protective effect on bloody diarrhoea (P = 0.0084). We found 8 strains, isolated from 1998–2003, containing the vtx1d subtype. The vtx1d strains belonged to five different O-serogroups (O64, O41, O79, O84 and O154). The vtx1d subtype was significantly associated to vomiting (P = 0.0249).

Table. Distribution of VT1 subtypes according to the virulence profile

	VT1	VT1c	VT1d
vtx1	38	20	7
vtx1 + *vtx2*	23	32	0
vtx1 + *eae*	183	1	1
vtx1 + *vtx2* + *eae*	52	1	0

Conclusion: This is the first reported finding of the vtx1d variant in humans. Transmission of the vtx1d, probably from the bovine reservoir, is not a new phenomenon; since we found this variant from strains isolated already in 1998. This study emphasizes the need to develop easy screening methods that take vtx1 variants into account and the need for more knowledge of the clinical significance of vtx1 variants.

P659

Rapid detection and characterisation of extended-spectrum beta-lactamases by Pyrosequencing™

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Objectives: Since the first detection of extended-spectrum beta-lactamases (ESBLs) two decades ago this group of beta-lactam hydrolysing enzymes has become a major problem in the usage of cephalosporins. ESBLs are mainly found in *Escherichia coli* and *Klebsiella pneumoniae* and are able to hydrolyse and inactivate most beta-lactams including aztreonam and third generation cephalosporins. So far, there are about 340 different ESBLs described whereas the most common and clinical relevant ones are the TEM and SHV beta-lactamases. Different techniques are in use for the detection of ESBLs like Etest, Vitek, PCR-RFLP, and sequencing to mention the most relevant ones. However, all these methods are either time consuming or cost intensive which makes them unattractive for high throughput screening and characterisation. Thus, the goal of the present study was to develop a fast and cost-efficient method for the detection and characterisation of ESBLs. Pyrosequencing is a novel non-electrophoretic sequencing method for the detection of single nucleotide polymorphisms (SNPs) by rapid sequencing of short sequences of DNA. All TEMs and SHVs show very high homology with differences in only a few distinct nucleotides. With Pyrosequencing 96 different SNPs can be detected within 10 min. The costs for one sample are between 0.2–1$. The built-in quality control allows the exact and easy confirmation of the quality of the obtained data.

Methods: Plasmid preparation followed by phenol/chloroforme-extraction; PCR with biotinylated primers; gelelectrophoresis; purification of PCR products (Streptavidin); pyrosequencing reaction

Results: Different polymorphic sites (SHV: amino acids Leu-31, Arg-201, Gly-234 and Glu-235; TEM: amino acids Gln-37, Glu-102, Arg-162, Met-180, Gly-236) were chosen for identification of SHV-1, -2a, -3, -4, -5 and TEM-1, -2, -6, -7, -10, -20, -21 and -52. Representative results are shown for the characaterisation of SHV-4 (table 1).

table 1	Site1	Site2.1	Site2.2.1	Site2.2.2	Site2.2.3
SNP :	T/A	G/T	G/A	G/A	G/A
nuc pos:	92	602	700	703	705
aa pos:	31	201	234	235	235
result :	T	T	A	A	A

Conclusion: Pyrosequencing has been demonstrated to be a suitable and cost-saving technique for the rapid an accurate identification of clinically relevant SHV and TEM beta-lactamases. Further work will include the implementation of multiplexed assays for PCR and pyrosequencing to increase cost and time efficiency. Pyrosequencing has proven to be a very cost efficient and reliable method for the detection and characterisation of TEMs and SHVs.

P660

Identification of major pathogenic markers in shigatoxigenic *Escherichia coli* by the use of multiplex PCR in beef meat

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Shiga toxin-producing *E. coli* (STEC) are a very important cause of gastrointestinal diseases in humans, often with severe complicating problems that include bloody diarrhoea, haemorrhagic colitis (HC)and haemolytic uraemic syndrome (HUS). Cattle appear to be the main reservoir of STEC which are transmitted to humans through food (especially raw beef) contaminated with faecal material. Moreover, transmission of STEC by person-to–person contact, drinking water or unpasterurised milk has also been described.

Objectives: The aim of the present study was to develop and evaluate two multiplex PCRs (mPCR) systems for rapid and specific identification of major virulence genes (stx1, stx2, eaeA, hlyA) of STEC detected in raw beef meat samples.

Methods: The 25 g raw beef samples were inoculated with different numbers of *E. coli* of reference strain EDL 933. Thus, they were added into 225 ml of TSB broth and incubated at 37°C for 18 h with shaking (150 rpm). The first mPCR test (mPCR-1) was established to identify Shiga toxin 1 and 2 genes and, as an internal control of amplification, the specific for *E. coli* 16S rRNA gene. The second mPCR assay (mPCR-2) allowed the identification of two STEC-associated virulence marker genes encoding intimin (eaeA) and enterohaemolysin (ehlyA). Each PCR amplification was performed in PCR mixture consisting of DNA template, 1 X Taq buffer, 2 U Taq DNA polymerase (MBI, Lithuania), dNTPs, 5 mM of Mg+, specific primers and water to the final volume of 50 ul. All amplifications were run in a thermocycler (PTC-100, MJ Research, USA), under the following conditions: initial DNA denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 53°C for 1 min and 72°C for 1 min. The final extension step was performed at 53°C. The amplified PCR products were visualized in 1.5% agarose gel.

Results: The first mPCR resulted in different amplification products (348 bp for stx1 gene, 584 bp for stx2 gene and 798 bp for 16S rRNA gene), which could be readily distinguished in agarose gels. In the second test (mPCR-2), which uses the primers specific for intimin and enterohemolysin and the *E. coli* 16S rRNA, the amplicons of 837 bp, 534 bp and 401 bp, respectively, were generated.

Conclusion: The obtained results demonstrate the high specificity of the mPCR systems developed and their possibility for rapid and specific identification of the major virulence factor genes in STEC originated from raw beef meat samples.

P661

Commensal and uropathogenic *Escherichia coli* strains: do they have differences in the presence of pathogenicity islands?

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(Barcelona, E)

Objectives: To detect the different PAI markers in commensal and uropathogenic *E. coli* strains and to stablish the relationship between PAIs and the ECOR phylogenetic group.
Methods: 50 different *E. coli* commensal strains from feces of healthy women and 100 uropathogenic *E. coli* (UPEC) strains (50 from pyelonefritis and 50 from urinary sepsis), were studied. Molecular typing by ERIC-PCR was used to assess the clonal relationships among the commensal strains. The PAIs (I, II, III and IV) described in UPEC 536, PAI I and II in UPEC J96 and PAI I and II in UPEC CFT073 were detected by PCR using specific primers. The resolution of the phylogenetic group was done by PCR. The results were analysed statistically using the SPSS 10X software.
Results: *E. coli* commensal strains showed a significant presence of PAIs (42%), but lower than UPEC strains (91%). The eight different PAI markers detected showed the same distribution in commensal and UPEC strains, being lower in the commensal group. PAI IV536 was the most frequently found in 38% of commensal vs 88% of UPEC strains, followed by PAI ICFT073 (26% vs 73%), PAI IICFT073 (14% vs 45%), PAI IIJ96 (8% vs 34%), PAI I536 (8% vs 33%) and PAI II536 (4% vs 20%). PAI III536 was only detected in 2% of the UPEC strains and PAI IJ96 was never detected. The distribution of the phylogenetic group was antagonic, being group B2 the less frequent in commensal strains (18%) and B1 the majority (34%), whereas in UPEC strains the group B2 was the most common (67%) and B1 the minority one (18%). From a total of 49 PAIs detected in commensal *E. coli* strains, the 74.4% were harboured in strains from group B2, 14.3% group D, 8.2% group A, and 6.1% group B1. From a total of 295 PAIs observed in UPEC strains, the 84.4% were exhibited in strains from group B2, 9.2% group D, 4.4% group A, and 2.0% group B1. In both groups, commensal and UPEC strains, B2 showed association to the presence of PAIs ($P < 0.001$). The average number of PAIs accumulated per strain was 0.98 in commensal and 2.95 in UPEC strains; nevertheless the comparison of PAIs per cell in the different phylogenetic groups, showed that B2 group had 3.9 in commensal vs 3.7 in UPEC strains, while in the remaining phylogenetic groups was lower than 1.6 in both commensal and UPEC strains.
Conclusion: Although group B2 *E. coli* strains were uncommon in commensal flora, they are highly virulent and represent a potential reservoire for strains that cause urinary tract infection.

P662

Different virulence factors in pathogenic enterohaemorrhagic *Escherichia coli* O157 and non-O157 isolated from humans compared to isolates from cattle

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Objectives: Verocytotoxin producing *E. coli* can cause bloody diarrhoea and haemolytic-uremic syndrome among humans and especially children. Additional traits besides the verocytotoxin production seem however to increase the virulence. Intimin is an adhesin that enables some isolates of EHEC to tightly attach to epithelial cells of the intestine. EHEC-hemolysin is plasmid encoded, and the hemolysin lyses erytrocytes and provides hemoglobin as a source of iron. The objectives of the present study were to study additional, possible virulence factors in verocytotoxin producing *E. coli*.
Methods: In the present study, we have used PCR with primers detecting four additionally adhesin genes other than the intimin gene and two other genes than E-hly supporting the bacterial access of iron. Isolates of different clonality according to PFGE of both human and cattle origin have been studied.
Results: We found that *E. coli* possibly compensate the absence of the eaeA gene coding for intimin by another adhesin gene coding for type 1 fimbriae and that the absence of EHEC-hemolysin may be compensated by the gene coding for a siderophore aerobactin or the usually chromosomally encoded gene coding for alfa-hemolysin. Interestingly, verocytotoxin producing *E. coli* isolated from cattle, the main source of human infection, do not have the genes coding for aerobactin or hemolysin to the same extent as human isolates.
Conclusion: We propose that this difference concerning presence of the genes coding for intimin, aerobactin and alfa-hemolysin might be the reason for the relatively low incidence of symptomatic EHEC infections among humans in relation to the high number of verocytotoxin producing *E. coli* among cattle. Verocytotoxin as well as intimin, aerobactin and a-hemolysin seem to be important virulence factors for EHEC.

P663

Phylogenetic origin of the uropathogenic *Escherichia coli* resistant and susceptible to fluoroquinolone and trimethoprim-sulfamethoxazole

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Objectives: To assess the role that the relationships between resistance and virulence may play in the uropathogenic *E. coli*, we compared *E. coli* strains resistant to fluoroquinolone (FQ) and trimethoprim-sulfamethoxazole (TMP-SMZ) with the susceptible ones.
Methods: A total of 150 *E. coli* uropathogenic strains (50 from cystitis, 50 from pyelonephritis and 50 from urinary bacteraemia) were analysed for virulence factors genes: papA, papGI, papGII, papGIII, fimH, afa/draBC, sfa/focDE, hlyA, cnf1, iutA, fyuA, kpsMTII, ibeA, traT, malX; ECOR phylogenetic groups: A, B1, B2 and D by PCR. O antigens associated to UTI (O1, O2, O4, O6, O7, O18 and O83) were determined using a agglutination microtechnique. Antimicrobial susceptibility to FQ and TMP-SMZ by standard disc-diffusion techniques.
Results: From the 150 *E. coli*, 12% were resistant to FQ and 29% to TMP-SMZ. FQ and TMP-SMZ susceptible and resistant *E. coli* were equally distributed among the 3 clinical syndromes. Phylogenetic group B2 was associated to FQ susceptible (76% vs 28% in resistant, $P < 0.001$) and TMP-SMZ susceptible isolates (80% vs 45% in resistant, $P < 0.001$), in contrast group B1 was associated to FQ resistance (11% vs 6%, $P < 0.001$) and TMP-SMZ resistance (14% vs 4%, $P < 0.001$), and group D to TMP-SMZ resistant (25% vs 7%, $P < 0.001$). *E. coli* susceptible strains were associated to high virulence score (mean of 7.3 virulent traits for FQ susceptible strains vs 3.94 for resistant strains, $P < 0.001$; and 7.90 virulent traits for TMP-SMZ susceptible strains vs 6.45 for resistant strains, $P < 0.05$). malX, papA, papGII, fimH, afa/draBC, sfa/focDE, kpsMII, hlyA, cnf1, fyuA and UTI-O antigens were statically associated to FQ susceptible *E. coli* ($P < 0.05$) and malX, sfa/focDE, kpsMII, fyuA and UTI-O antigens to TMP-SMZ susceptible strains ($P < 0.05$).
Conclusions: *E. coli* isolates susceptible to FQ or TMP-SMZ belonged predominantly to ECOR phylogenetic group B2,

harbouring a great number of virulent traits and consequently showed a high virulence score. On the contrary, strains resistant to these antibiotics belonged predominantly to B1, possesses a little number of virulent traits and consequently showed a lower virulence score. Our results suggest that resistant strain probably are not the consequence of the acquisition of the resistance elements by a susceptible strain, but come from others phylogenetic origins.

P664

Virulence factors and antibiotic resistance in uropathogenic *Escherichia coli*

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Objectives: To use multiplex polymerase chain reaction (PCR) to screen a large number of *Escherichia coli* clinical isolates for virulence factor genes and to find, if present, a correlation between pathogenicity and antibiotic resistance in these strains. As some in vitro studies have recently suggested that decreased pathogenicity of *E. coli* is associated with the acquisition of quinolone resistance, our study aimed to investigate, in particular, the virulence factors in fluoroquinolone-resistant, compared with fluoroquinolone-susceptible, uropathogenic *E. coli* strains.
Methods: In two hundred and twenty-four *E. coli* isolates from the urine of patients with urinary tract infections the sensitivity to fluoroquinolones (ciprofloxacin, levofloxacin and norfloxacin), beta-lactams (ampicillin, amoxicillin-clavulanic acid, cefaclor and ceftazidime) and co-trimoxazole was investigated by a microdilution technique according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS). ESBL production was investigated by the double-disk synergy test. On the basis of antibiotic susceptibility pattern, one hundred and twenty of the tested strains were chosen and screened for type 1 fimbriae, P fimbriae, hemolysin, aerobactin and cytotoxic necrotizing factor 1 genes by multiplex PCR. Hemolysin production was tested on sheep-blood agar plates. The presence of type 1 fimbriae was determined on the basis of mannose-sensitive agglutination of Saccharomyces cerevisiae.
Results: Type 1 fimbriae were present in more than 90% of the strains; the gene for aerobactin was present in approximately two thirds of the isolates; genes for P fimbriae, hemolysin or cytotoxic necrotizing factor 1 were present in approximately one fourth of the strains. But these last three genes were present in a very small percentage or absent in the fluoroquinolone-resistant strains.
Conclusion: Our results suggest that resistance to fluoroquinolones is associated with a great decrease in the presence or the expression of some virulence factors in uropathogenic *E. coli*, while resistance to beta-lactams is not. Where phenotypic information was available, it was consistent with the genotypes identified by PCR.

HIV

P665

HIV-proviral DNA and plasma-RNA sequencing for detection of drug resistance mutations in antiretroviral naive patients

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Objectives: Genotypic antiretroviral resistance testing is commonly used in drug naive HIV-patients (pts). Plasma-RNA is taken as representative of the actively replicating viral population. However, in the absence of drug pressure, resistant variants may revert to or be outcompeted by more fit wild type viruses. We tested if DNA obtained from peripheral blood mononuclear cells (PBMC) provides better or complementary informations than plasma (PL) about drug resistance mutations (DRM) transmitted or superinfections in naive pts.
Methods: PL and PBMCs from 34 pts diagnosed in 2004 as HIV-infected were analysed. The protease (PR) and aa 1–240 of the reverse transcriptase (RT) were sequenced by in-house method, compared for the presence of DRM as defined by IAS, and analysed for drug susceptibility profile by the Stanford HIVdb algorithm.
Results: In 29 pts both genotype and subtype were concordant (Cpt); 4 Cpts had non-B subtype (CRF01_AG subtype in 1, CRF02_AG in 1, F in 1, C in 1), B in the others; several DRMs were detected, conferring resistance to NRTI (2 pts), to NRTI + NNRTI (1 pt), PI (2 pts). 5 pts showed substantial differences between PL-virus and PBMCs-virus (Dpt). While only 2 Dpts showed RT primary DRMs and resistance to NRTI (1 pt) and NNRTI (1 pt) in PL, in PBMCs-DNA of these Dpts also PR-primary DRMs were found, conferring PI-resistance too; other 3 Dpts revealed in PBMCs both RT and PR primary DRMs, and related broad range of NRTI and PI-resistance, with drug sensitive viruses in PL. 3 out of these 5 Dpts had a CRF01_AG subtype in PBMC, and B subtype in PL. A Dpt had a concordant B wild type in a blood sample stored 7 months before.
Conclusions: We found resistance to antiretroviral drugs in 10 out of 34 naive-pts (29.4%), but only in 7 pts, 5 Cpts and 2 Dpts, (20.5%) this resistance was at least partially evident with conventional Plasma analysis. A substantially different virus, often with another subtype alignment, was found in PBMCs of 5 Dpts, revealing a recent superinfection by a resistant virus, or a reversion of an originally infecting resistant virus, with the storage of the less fit variant, still detectable as transient prevalent population in the PBMC-archive. A longitudinal analysis will allow to clarify these findings, but we suggest that a periodical analysis of PBMCs is useful in naive pts to detect drug resistant variants that may be stored and may compromise future therapy options.

P666

HIV-1 DNA load in naive and successful antiretroviral treated patients

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Objectives: In antiretroviral treated patients the size of the HIV DNA reservoir in PBMCs is associated with disease progression. This parameter can be relevant for treatment maintenance, simplification or discontinuation in patients with viro-immuno-

logical success after several years on highly active antiretroviral therapy (HAART). We evaluated the association between HIV-DNA load and viro-immunologic and clinical parameters in antiretroviral naive (NPts) and in treated (TrPts) patients, selected for successful long term HAART.

Methods: This cross-sectional study was performed on 31 NPts and 49 TrPts selected on the basis of plasma RNA load (50 cp/ml within 6 months of starting their first regimen, without either replication (blips) or treatment interruption, and a (12 months treatment period. To quantify the HIV-DNA integrated copy number in PBMC, we used a real time PCR in-house protocol performed on ABI-PRISM 7000 (App. Bios.), with a detection limit of 20 cp/106 CD4.

Results: Considering NPts stratified according to the median HIV-DNA level of 27299 cp/106 CD4 (range 189–525675), subjects with lower DNA level had more frequently higher CD4 ($p = 0.042$), and higher CD4 percentage ($p = 0.13$). No statistically significant association with plasma RNA levels was found. After a median of 30 months of HAART (12–93 months), 6 out of 49 (12.2%) patients had undetectable DNA level. Considering TrPts stratified according to the median DNA level (1908 cp/106 CD4), no statistically significant differences in terms of age, gender, risk factors for HIV, pre-HAART HIV-RNA level, PI- or NNRTI-based HAART, duration of HAART were found. Conversely, subjects with lower HIV-DNA level had more frequently higher pre-HAART CD4 cell count and percentage, higher CD4 cell count and percentage at last follow up ($p < 0.001$ for all assays). Considering TrPts by CD4 count strata (<200, 201–350, >350), the distribution of HIV-DNA load was statistically correlated in these groups ($p < 0.001$). After adjusting for epidemiological and other confounding variables, only pre-HAART CD4 cell count (OR 0.1, 95% CI 0.01–0.92, $p < 0.001$) was found to be independently associated to low DNA values.

Conclusions: In NPts HIV-DNA load was inversely correlated with immunological status, but not with RNA load. Patients with persistently undetectable RNA load during HAART have a wide range of proviral DNA (<20–13142 cp/106 PBMC). Low DNA level was independently associated with high pre-HAART CD4 cell count.

P667

Prevalence of HIV-1 drug resistance-associated mutations in multi-treated infected patients

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Objectives: Drug resistance mutations in the human immunodeficiency virus type 1(HIV-1) Reverse Transcriptase (RT) and Protease genes lead to lower sensitivity to antiretroviral agents and are an important cause of drug failure. We evaluate the presence of mutations in antiretroviral multi-treated patients.

Methods: The plasma of 527 HIV-1 infected patients previously treated with different therapeutic regimens were analysed. Most patients were males (72%), mean age 41 years (range 73–21). Plasma of patients were collected for genotyping analysis to identify resistance mutations in RT and Protease regions using ABI ViroSeq HIV-1 Genotyping System (Applied Biosystem). Changes in susceptibility were assessed by a computer programme that interprets drug resistance: Standford database (http:/hivdb.standford.edu/hiv/).

Results: On the HIV-1 Reverse Transcriptase region 1730 mutations were detected, 1076 (62.2%) of them were primary mutations correlated with high level of drug resistance. 470 mutations were implied in the resistance to the non nucleoside analogues of the reverse transcriptase inhibitors (NNRTIs). On the Protease gene 1669 mutations were found, 1239 (74.2%) of them were secondary mutations. Mutations al codons 184, 215 and 103 were most frequent in the RT region. L63P was the most prevalent Protease mutation. Multi-drug resistant mutation Q151M was observed in 10 patients. Wild type sequence was detected in 29 cases. Genotyping was not available because not enough cDNA were obtained in 59 (11.2%) samples. Presence of mutations related with high-level of resistance to Zidovudine were detected in 39% of the patients, 39% to Lamivudine, 51% to NNRTIs and 69% to Protease Inhibitors (Nelfinavir presented the high-level of resistance 25%).

Conclusion: The detection of key mutations associated with HIV-1 antiretroviral resistance is very useful because it enhances clinical management and permits to chose the optimal therapeutic regimens in treatment-experienced patients.

P668

Thymic size and CD4+ cell recovery in HIV-infected patients during long-term highly active antiretroviral therapy

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Objectives: The human thymus is suggested to be contributing to de novo T cell synthesis even late in life. Such synthesis can be assessed by analyzing thymic output markers such as thymic size. In HIV-infected patients reconstitution in the peripheral CD4+ cell pool during the first phase of highly active antiretroviral therapy (HAART) seems to be thymic-associated. To investigate whether this is a prolonged effect 25 HIV-infected patients in steady state HAART and with known thymic size were followed prospectively with CD4 counts and HIV-RNA measurements every third month in a follow-up period of 12 months.

Methods: Thymic size was determined by computer tomography (CT) at inclusion in the study and T-cell subpopulations were determined at follow-up by flow cytometry.

Results: The correlation between thymic size and CD4 counts was significant at inclusion in the study ($r = 0.498$; $P = 0.011$) and remained almost unaltered at 3, 6 and 9 months of follow-up ($r = 0.449$, $P = 0.036$ after 3 months, $r = 0.517$, $P = 0.012$ after 6 months, $r = 0.478$, $P = 0.021$ after 9 months). However, with regard to CD4 increase no difference was found between patients with minimal and abundant thymic tissue, respectively, at any time of follow-up.

Conclusions: The measurement of thymic size at baseline may be of importance regarding the initial increase in CD4+ cells in HIV-infected patients receiving HAART, but does not seem important to a continued increase in CD4+ cells during long-term HAART in a follow-up period of 12 months. Whether thymic size is important to a continued increase in CD4+ recent thymic emigrants estimated by measuring other thymic output markers such as naïve CD4+ cells and TREC-containing CD4+ cells is still not known. The included patients in this study are still followed and the abovementioned measurements at follow-up will be performed.

P669

Immune reconstitution syndrome in HIV patients

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Introduction: immune reconstitution syndrome (IRS) is an inflammatory process which happen from few days to months after beginning of antirretroviral treatment (ART). IRS give rise to a variable clinical picture from only fever and malaise to fatal outcome.

Objectives: To describe the clinical feature of patients suffering from HIV infection and IRS.
Method: We have compiled all the patients suffering from HIV infection and IRS attended in our Hospital from August-2003 to August-2004. Diagnosis Criteria of French (AIDS 2004; 18: 1615–1627) were used. We have describe the clinical and immunological status, the probable cause of IRS and the outcome.
Results: In the study-time 108 patients began initial or rescue ART. 7 were diagnosed of IRS. 6 were infected when used intravenous drug (all were co-infected by hepatitis C virus) and the other by sexual transmission. Mean age was 37 years and the time from HIV diagnosis was 10 years. The number of previous ART and the drug patterns at this time were variable. Mainly CD4 were less than 50 and viral load (VL) were high. Clinical data appeared between weeks 2 and 8 from the ART beginning, mainly with good tolerated fever. VL decrease and CD4 increase were observed. In 3 cases the associated infections was disseminated tuberculosis, 1 lymph node tuberculosis, 1 disseminated MAI, 1 pneumonia by *P. carinii* and 1 liver necrosis by hepatitis B virus. All of them required treatment as in-patients. Steroid therapy was indicated in 4 cases with satisfactory outcome and 3 cases were no treated and treated only with non-steroidal anti-inflammatories.
Conclusions: 6.4% of patients who start initial or rescue ART suffered from IRS, mainly related with mycobacterial infection. Symptoms appear between weeks 2 and 8 from de ART beginning with good response to therapy with steroids or non-steroidal anti-inflammatories.

P670

Hyperlactateamia during highly active antiretroviral therapy. Frequency and clinical-therapeutic consequences in a prospective case-control study

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Introduction: Notwithstanding the increased frequency of HAART-associated hyperlactataemia, causes, pathogenesis, management, and consequences on HIV disease course remain uncertain.
Methods: A case-control study was conducted on 1027 HIV-infected patients (p) referring to our centre, to prospectically assess the frequency and features of hyperlactataemia. Among p taking HAART with adherence levels (90%, p with serum lactic acid levels (18 mg/mL were compared with p with normal lactatemia, on the basis of a wide series of variables.
Results: Of 755 evaluable p, 272 (36%) had (1 event of hyperlactatemia (mean value, 24.7 mg/mL). Two or more subsequent alterations were found in only 56 p (7.4%), with progressively worsening values in 73.2% of cases, while a grade 4 (<39.6 mg/dL) hyperlactataemia was found in 5 p only (0.7%). Comparing the 272 p with elevated lactatemia with the 483 controls, no significant differences as to age, gender, risk for HIV, duration and stage of HIV disease, CD4+ count, HIV viraemia, as well as prior or present nucleoside analogue (NA) treatment and its duration. P with hyperlactataemia experienced a significantly longer anti-HIV therapy ($p < 0.004$), and a more elevated frequency of lipodystrophy ($p < 0.005$), hypertriglyceridaemia ($p < 0.02$), and altered serum skeletal muscle enzymes ($p < 0.02$ for CPK, and $p < 0.001$ for aldolase). The prevalence of clinical signs and symptoms (nausea, vomiting, abdominal pain, fatigue, myalgia) was limited to 2–3%, and comparable in the two study groups, and therefore difficult to be related to hyperlactataemia. Elevated serum lactic acid levels never was responsible for HAART interruption, while regimen modification was precautionarily modified in <5% of p.
Discussion: While hyperlactatemia may involve ~20 of HAART-treated p, according to the literature the development of lactic acidosis is an exceedingly more rare event (1.7–25.2 cases per 1000 treated p-year), and usually interested p receiving NA (d4T, followed by ZDV, ddI, 3TC, ddC, and ABV). In our experience, asymptomatic hyperlactatemia emerged as a frequent HAART complication, although acidosis was never observed. An association with a more prolonged HAART duration and other adverse events like lipodystrophy, dyslipidaemia, and skeletal muscle toxicity, was noticed. When facing a metabolic acidosis, ventilatory assistance may become needed, as well as prompt fluid, bicarbonate, and vitamin and coenzyme administration. NA should be replaced with same-class drugs borne by a lower risk of acidosis, such as tenofovir, 3TC and ABV.

P671

Do oestrogen receptors have a role in the pathogenesis of HIV-associated lipodystrophy?

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Objective: Epidemiological data of increased risk of HIV-associated lipodystrophy in women and reports of sex hormone abnormalities in patients receiving highly active antiretroviral therapy (HAART) led us to investigate whether sex steroid hormone receptors were involved in this syndrome.
Methods: 14 male HIV-positive patients were evaluated, including 6 naive patients who were starting a HAART protocol (group 1) and 8 who were switching from a HAART regimen including PIs to a PI-free treatment (group 2). Five patients of group 2 had lipodystrophy. Patients underwent a thorough endocrine and metabolic examination and were submitted to a needle biopsy of abdominal subcutaneous fat before starting HAART therapy (group 1) or before switching from PIs (group 2) and 6 months after the first evaluation. Expression of genes encoding sex steroid hormone receptors, aromatase, and factors produced by adipose tissue and involved in adipogenesis was evaluated by real-time RT-PCR.
Results: Anthropometric evaluation showed that lipodystrophic patients had lower BMI, total and subcutaneous thigh adipose tissue, but higher WHR and visceral adipose tissue than non-lipodystrophic patients. However, 6 months HAART administration in group 1 naive subjects and PI switch in group 2 did not substantially modify fat mass and fat distribution. Endocrine evaluation demonstrated that lipodystrophy patients had similar hormone profile than non-lipodystrophic subjects and that HAART did not significantly modify hormone levels. Analysis of transcript levels in adipose tissue showed that oestrogen receptor beta expression was significantly reduced in the subcutaneous adipose tissue of HIV-infected lipodystrophic patients, down-regulated by HAART regimens including protease inhibitors (group 1), and restored following switch from protease inhibitors (group 2). Regarding adipose tissue marker genes, adiponectin mRNA levels in lipodystrophic patients were significantly lower than in non-lipodystrophic subjects, but not modified by HAART.
Conclusions: This study introduces oestrogen receptor beta as a new player in the scenario of HIV-associated lipodystrophy, opening perspectives for the investigation of selective oestrogen receptor modifiers for the management of this syndrome.

P672

Effect of HAART on HIV-related hospital admissions and morbidity

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Background: AIDS related hospital admissions and morbidity have decreased after the introduction of HAART.
Objective: To assess the changes in hospital admissions and morbidity with the improvement of HARRT.
Methods: We studied the hospital admissions and morbidity over three different periods pre-HAART (January 1992 to December 1994), early-HAART (January 1995 to December 1998) and post-HAART (January 1999 to October 2004), in HIV infected patients followed at our centre. Results are expressed for periods pre-HAART, early HAART, and post-HAART respectively. Mean (SD) CD4/mL were: 16 ± 5, 37 ± 11 and 118 ± 33 ($p < 0.0001$). Undetectable plasma viral load (%) were: 0, 16 and 43 ($p < 0.0001$). Patients with a prior diagnosis of AIDS were: 91%, 68% and 36% ($p < 0.001$). The rate (%) of women/heterosexuals was: 6/2, 11/5 and 21/11 ($p < 0.01$) and, that of IVDA (%) was: 96, 88 and 75 ($p = 0.03$). Hospital admissions due to AIDS-defining illnesses decreased ($p < 0.001$) with a significant increase in the rate of respiratory tract infections ($p < 0.005$), digestive tract ($p < 0.01$) and liver diseases ($p < 0.001$). The proportion of AIDS-defining illnesses decreased after HAART ($p < 0.01$), whereas the rate of liver diseases increased ($p < 0.001$).
Conclusions: The HAART era has been associated with a progressive decrease in hospital admissions due to AIDS-defining conditions, and a steady enhancement of the spectrum of admissions by non-AIDS-defining conditions has increased.

P673

Persistence of chronic premature priming for apoptosisin HIV-infected individuals during HAART and short structured interruption

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Objectives: Despite the benefits produced by HAART, persistent apoptosis in HIV-infected patients receiving efficient antiretroviral therapy occurs. Aim of this study was to assess priming for apoptosis and susceptibility to undergo spontaneous apoptosis in PBMC from HIV-1 infected patients. Moreover, the influence of continuous HAART and short structured therapy interruption (STI) have been investigated.
Methods: In-vivo priming for apoptosis was detected in freshly isolated PBMC by analysis of 'tissue' transglutaminase protein synthesis and the phosphatidylserine exposure on plasma membrane. The susceptibility of PBMC to undergo spontaneous apoptosis was analysed ex-vivo, after 48 h of in-vitro culture in absence of growth factors, by quantification cells showing DNA fragmentation. In addition, the anti-apoptotic Bcl-2 down-modulation and caspases 8 and -9 activation were monitored, to identify the main apoptotic pathway prematurely initiated. The relationship between priming and spontaneous apoptosis markers and antiretroviral treatment or stopping was investigated in two groups of HIV-infected patients: a group of 15 HIV-infected patients, naïve for HAART (with CD4 cell counts (500 cells/mmc and plasma HIV-1 RNA levels (10,000 copies/ml), at baseline and during 6 months of HAART; and a second group of 20 HIV-infected patients during a short period of STI and reintroduction of therapy.
Results: Our findings highlight that a non-physiological persistent priming for apoptosis is induced in PBMC of HIV-1 infected individuals. Introduction of efficient antiretroviral therapy in naïve patients does not modify the quote of PBMC primed for apoptosis, that remains almost unchanged and statistically not influenced from the modification of viral load. Neither successful HAART associated with short STI can modify this situation. By contrast, the susceptibility of primed cells to undergo spontaneous apoptosis closely correlates with viral load. The accumulation of caspase-8 suggests that the non-physiological chronic priming for apoptosis may be sustained by death receptors signalling, likely mediated by viral components.
Conclusions: Further studies on priming for apoptosis in HIV-infected PBMC may be relevant for the definition of alternative immune intervention targets aimed to prevent the premature priming for apoptosis of immune cells, rather than the inhibition of activation of programmed cell death, should be planned and analysed.

P674

Polymerase chain reaction detection of *Leishmania* from blood in HIV-infected patients. What does it mean?

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Objectives: Interpretation of Polymerase Chain Reaction (PCR) of Leishmania in blood samples from asynthomatic HIV infected patients
Patients and methods: PCR for *Leishmania* detection in blood and urine, latex agglutination test and serology was done in 250 HIV infected outpatients. LnPCR was performed following author's instructions and consists in two amplification reactions: the first includes primers R221 and R332 specific for kinetoplastida and the second consists on the reamplification of the first PCR product with a new set of *Leishmania*-specific primers, R223 and R333. KAtex detects antigen present in a urine sample by agglutination.
Results: We studied 250 HIV infected patients. None of them had history of recent or past visceral leishmaniasis and all subjects were asympthomatic. Mean age was 35 years and 184 (73.6%) were men. Blood PCR was positive in 46 patients (18.4%); 8 subjects (3.2%), all with blood PCR positive, had urine PCR positive and 6 (2.4%), also with blood PCR positive, had positive the determination of LATEX in urine. None of the 42 patients had positive serology for *Leishmania*. All patients were controlled for, at least, 2 years and none of them had suspected or confirmed visceral leishmaniasis. During the study, a second strange was obtained only in 17 subjects and only one of them maintained positive blood PCR without clinical manifestations of visceral leishmaniasis.
Conclusions: These data suggest that isolated positive PCR for *Leishmania* can not be associated with active visceral leishmaniasis. According to our data positive PCR cannot predict developing visceral leishmaniasis, at least, during study period. More studies are necessary for knowing the real meaning of positive PCR for *Leishmania*.

P675

Cryptococcaemia: aetiological agent and clinical outcome

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Objectives: To report the underlying diseases, the type of etiologic agent involved, severity and outcome of the disease of patients with cryptococcal fungaemia.

Methods: Between April 1995 and November 2002, we retrospectively studied all patients with at least one positive blood cultures for *C. neoformans* at Mycology Laboratory, Santa Casa Complexo Hospitalar, Brazil. The clinical records of these patients were reviewed, and the clinical outcome was based on the report of hospital discharge or death of the patient while hospitalized. Disease severity was estimated using APACHE II score.
Results: During the period of study, 28 patients with cryptococcemia were included in this study. These patients were mainly white (89.3%) and male (78.6%), and their mean age was 36 years old. With the exception of one immunocompetent patient, the remaining patients had the following underlying diseases: AIDS (89.3%), lung transplantation (3.6%) and liver cirrhosis (3.6%). The CD4 cells mean count was 49 cells/mm^3. The varieties of *C. neoformans* isolated from these patients were identified as variety grubii in 26 patients (92.8%) and variety gattii in the remaining two patients (7.1%). With one exception, *C. neoformans* var. grubii infected 25 patients with AIDS. *C. neoformans* variety gattii was recovered from an immunocompetent patient and from a lung transplanted patient. Besides blood cultures, *C. neoformans* was also isolated from the following body sites: cerebrospinal fluid (89.3%), respiratory tract (35.7%), urine (35.7%), skin lesions (3.6%) and prostate (3.6%). Systemic antifungal therapy was initiated within 24 hours after positive blood culture in 95% of the patients. There were a strong association between APACHE II >14 and mortality ($p < 0.01$).
Conclusions: The majority patients with cryptococcaemia in our study (89.3%) had AIDS, supporting previous epidemiological studies involving variety grubii as the etiologic agent. The high mortality rate among patients with cryptococcaemia in our study (41%) showed strong association with the APACHE II score ($p < 0.01$).

P676

Outcome of HIV-associated tuberculosis in the early- and late-HAART era

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Objectives: To assess outcome of pulmonary tuberculosis (TB) diagnosed in HIV-infected patients in the early and late time period of the widespread introduction in clinical practice of highly active antiretroviral therapy (HAART) in Italy (during 1996).
Methods: We reviewed clinical charts of 121 HIV-infected patients with culture-confirmed pulmonary TB hospitalized at National Institute for Infectious Diseases ''*L. Spallanzani*'', Rome from January 1996 to December 2002. 42 patients diagnosed in 1996–1998 (early-HAART patients) were compared to 79 patients diagnosed in 1999–2002 (late-HAART patients). Differences in categorical variables were analysed with the use of the 2 test or Fisher's exact test, as appropriate. The Kaplan–Meier method was used to estimate survival. In univariate analysis, survival differences between the two groups of patients were assessed using the log-rank test.
Results: At onset, patients diagnosed in 1999–2002 were more likely to be foreign born (39% vs 21%, $p < 0.05$), and less likely to be injection drug user (54% vs 74%, $p < 0.05$). Late-HAART patients had a higher CD4+ median count (155 vs 113/mm^3). Moreover, 1999–2002 patients had more frequently started an HAART regimen at least 3 months before TB diagnosis (43% vs 21%, $p < 0.05$), and were less frequently on prior Nucleoside Reverse Transcriptase Inhibitors (NRTI) therapy before starting an HAART regimen (6% vs 48%, $p < 0.001$). By the end of the study period (December 31, 2003) 12 patients had died (15%) in 1999–02 period and 17 patients (40%) in 1996–98 period ($p < 0.005$). The survival proportion at 6, 12 and 24 months was 95%, 93% and 89% in patients diagnosed in 1999–2002, while it was 83%, 76% and 66% respectively in 1996–1998 patients ($p = 0.016$, log-rank). Compared to patients who started HAART when antiretroviral naive risk of death was significantly increased in patients who started HAART after NRTI therapy or did not take HAART at all ($p < 0.001$ for both comparison).
Conclusions: We found that patients diagnosed in the late-HAART era had a significantly longer survival. Our data suggest that the increasing use of HAART has continued to modify the survival of patients with HIV-associated TB, probably because of the increased proportion of patients starting HAART when antiretroviral naive in the late-HAART era.

P677

Screening for tuberculosis infection using a new blood test in a population of HIV-positive individuals

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The tuberculin skin test (TST) used to detect *Mycobacterium tuberculosis* infection has many drawbacks, and a new diagnostic test for latent tuberculosis (QuantiFERON-TB GOLD) has recently been introduced. This test measures the production of IFN-g in whole blood upon stimulation with the antigens ESAT-6 and CFP-10, which are not present in BCG vaccine strains or the vast majority of nontuberculous mycobacteria. This test overcomes some of the drawbacks of the TST including lowered specificity in BCG vaccinated individuals. The test has been reported to perform with specificity of 98.1% and a sensitivity of 89.0% when tested in a population of 216 BCG-vaccinated Japanese adults and 118 patients with culture-confirmed *M. tuberculosis* infection. Another study reported a test performance with sensitivity at the level of TST and an excellent agreement between the two tests of 94%, (kappa value 0.866) in a population of BCG unvaccinated young Danish individuals. As the QuantiFERON-TB GOLD test is based on cellular immune responses, the test is believed to have impaired performance when used on individuals with immune suppression, as seen in HIV infected. However, this group is in particular risk of developing tuberculosis and a test to detect latent as well as active TB would be of particular interest in this group. We therefore designed a study involving HIV positive individuals attending an outpatient unit at a department of infectious disease in Copenhagen. Blood samples from 250 HIV positive individuals has been collected and tested with the 3. generation of the QuantiFERON TB technology; the QuantiFERON-TB Gold in tube test (in tube QFT). This test uses the addition of a third TB specific antigen to ESAT-6 and CFP-10 and blood is drawn directly into antigen coated collection tubes, which can be incubated immediately. Because of the study group under investigation we also added mitogen (PHA) as a positive control in order to reveal individuals with a degree of impaired cellular immune response that would make the test inadequate.

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Altered phagocyte Fcg receptors expression in HIV-infected patients with active tuberculosis

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Background: Phagocyte receptors for IgG (FcgRs) are important in host defence against infection.

Objectives: We have studied the expression of FcgRs by peripheral blood monocytes (M), monocytes cultured for 72 hours (M/Mø), and granulocytes (G) in HIV infected patients with active Tuberculosis (TB), during anti-tuberculous therapy (anti-TB-Rx) and, after completion of anti-TB-Rx.
Methods: The expression of FcgRI, FcgRII and FcgRIII, on M, M/Mø and G (resting and cultured with IFNg) were analysed by flow cytometry in 51 HIV infected patients with active TB (45 men and 6 women), at diagnosis of TB, and monthly thereafter until completion of anti-TB-Rx.
Results: The expression of FcgRI and FcgRIII by M, M/Mø and G was enhanced in HIV infected patients with active TB by: 34 ± 4% and 19 ± 3% for M, respectively ($p < 0.001$), 43 ± 6% and 29 ± 3% for M/Mø, respectively ($p < 0.001$) and, 54 ± 6% and 21 ± 3% for G, respectively ($p < 0.001$). The expression of FcgRIIB by M, M/Mø and G was decreased by −24 ± 3% ($p = 0.02$), −33 ± 4% and, −19 ± 2% ($p < 0.005$), respectively. These alterations of FcgRs expression normalized from week 12th until the end of effective anti-TB-Rx. The expression FcgRI, FcgRIIA and FcgRIII by M, M/Mø and G from HIV patients with TB was significantly enhanced by culture with IFNg ($p < 0.005$). Setting a cut-off value = 25% of the mean fluorescence intensity over controls for FcgRs surface expression and, assuming a prevalence range of active TB between 25 and 80% among HIV patients undergoing confirmatory tests, results in a range of sensitivity, specificity, positive and, negative predictive values of: 61–86%, 58–96%, 64–89%, and 57–90%, respectively for M-FcgRIIA, 56–84%, 63–91%, 58–79% and 68–97%, respectively for M-FcgRIII, 61–88%, 54–87%, 44–87% and 69–91%, respectively for G-FcgRI and, 42–75%, 88–98%, 49–77% and 86–98%, respectively for G-FcgRIIB.
Conclusions: Our results suggest that, the alterations of Mø and G Fcg receptors expression in HIV infected patients with active TB can be used to predict the response to anti-tuberculous therapy.

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High prevalence of admission for AIDS-associated opportunistic infections in a Brazilian teaching hospital

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Objectives: The aim of this study was to describe the profile of patients admitted in an Infectious Diseases ward of a large Brazilian tertiary hospital during the period comprising December 2002 to December 2003.
Methods: Retrospective observational study.
Results: During the period of study, 125 patients were admitted to the Infectious Diseases ward of Santa Casa Complexo Hospitalar, Brazil. Most of these patients were male (68.0%) and mean age was 39.4 years old. Most of the patients (57.6%) were in their first hospital admission. During hospitalization, the diagnosis of AIDS was performed in 76.8% of patients. Mean CD4 count was 134 cells/mm^3 and mean HIV-1 viral load was 671,267 copies/mL. Previous HIV serologic status was known by 50.0% of those HIV patients admitted for the first time. Tuberculosis was the main diagnosis in patients admitted to the Infectious Diseases ward (20.0%), and 92.0% of these patients had HIV co-infection. Tuberculosis was also the main cause of death (36.0% of deaths); all the patients who died had HIV co-infection. However, cryptococcal meningitis was the more lethal disease (lethality rate of 57.1%). Other co-infections presented in patients with HIV infection were hepatitis C virus (28.0%), hepatitis B virus (6.4%), HTLV (2.4%), and Chagas disease (0.8%).
Conclusions: Despite the policy of providing free antiretroviral in Brazil, AIDS is still a frequent cause of hospital admissions, mainly in those patients who were unaware of their HIV serological status and in those who had the diagnosis in late stages of the disease. Tuberculosis continues to be a important diseases for these patients.

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Pneumococcal disease in HIV-infected patients: vaccination an unresolved issue

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Background: Pneumococcal disease is preventable in HIV-infected patients but the efficacy of pneumococcal vaccine in this population remains controversial. The aim of this study was to describe the characteristics of pneumococcal disease and analyze the vaccine response in a population of HIV patients.
Methods: In a retrospective study of the clinical records and microbiological database we analysed the incidence of pneumococcal infection in patients visited at the HIV Unit of our hospital over a 9-year period (1996–2003). Vaccine use and immunological and virological status were assessed in all patients. The study was carried out in a universitary tertiary hospital.
Results: A total of 74 (4.9%) pneumococcal episodes were recorded in 65 of 1502 HIV-infected patients. Mean age was 40 + 9 years (18–79), 53 males (71.6%), 55% IDU, 39.2% were C3 and 16.3% were B2-B3 stages, and mean of HIV infection was 135 months. Baseline CD4 count was 276 cell/mm^3 and viral load was 251,240 cop/ml. *Streptococcus pneumoniae* was isolated from respiratory specimens in 40 patients (54.1%) and 29 (39.2%) patients had pneumococcal bacteraemia. Mean CD4 during the infectious process was 289 cell/mm^3 and viral load was 369,000 cop/m^3. Only 35 (47.3%) patients received 23 valent pneumococcal vaccine. Mean basal CD4 count for vaccinated and no vaccinated patients were 362 and 206 cell/mm^3, respectively ($p < 0.04$). Mean CD4 counts and HIV RNA viral loads at vaccination and 3 months after vaccination were 362 and 368 cell/mm^3 and 41,791 and 72,996, respectively ($p < 0.7$ and 0.1). In 17/35 (57.1%) patients the episode was diagnosed after a mean of 799 (55–2582) days of vaccination. The CD4 at vaccination was 343 cell/mm^3. Patients without pneumococcal episode after vaccination showed a mean CD4 of 313 ($p < 0.5$).
Conclusions: Prevalence of *Streptococcus pneumoniae* infections in our HIV population was 4.9%. Less than half of patients had been vaccinated, mainly due to low CD4 count. Vaccination did not significantly modify CD4 count or HIV RNA viral load. However, pneumococcal disease developed in 57% of those previously vaccinated.

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Neoplasia associated with HIV infection in the HAART era

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Background: Surveillance data indicate that, the incidence and type of HIV related neoplasias has changed after the introduction of HAART.
Objective: To determine the incidence and types of cancers in the pre-HAART and post-HAART eras, and the differences between women and men.

Methods: Retrospective record review of HIV infected patients with cancer from January 1991 to December 2003 at a teaching hospital.
Results: A total of 192 HIV patients with cancer were identified, 158 men (age: 38 ± 11 years, CD4: 123 ± 39/μL) and 34 women (age: 44 ± 16 years, CD4: 68 ± 27/μL), of them 90 were detected in the pre-HAART (47%) and 102 in the post-HAART era (53%, p = n/s). AIDS defining cancers (Total: 139, 72.4%; of them 108, 77.7% in men vs. 31, 22.3% in women) were more frequent pre-HAART (Total: 76, 54.7%; of them 60, 43% in men vs. 16, 11.5% in women) than post-HAART (Total: 63, 45.3%; of them 48, 34.5% in men vs. 15, 10.8% in women). Non AIDS defining cancers (Total: 53, 27.6%; of them 34, 64% in men vs. 19, 36% in women) were less frequent pre-HAART (Total: 14, 26.4%; of them 9, 17% in men vs. 5, 5.3% in women) than post-HAART (Total: 39, 73.6%; of them 25, 47.2% in men vs. 14, 26.4% in women). Total cancer related mortality was higher pre-HAART (61% vs 52%, $p < 0.05$) and in women (60% vs 55%, p = n/s) than post-HAART (Total: 53, 27.6%; of them 36, 18.8% in men vs. 17, 8.9% in women). AIDS defining cancer related mortality was higher pre-HAART (65.8% vs 36.5%, $p < 0.01$) in both sexes ($p < 0.01$), while non AIDS defining cancer related mortality was higher post-HAART (76.9% vs 35.7%, $p < 0.01$) in both sexes ($p < 0.01$).
Conclusions: The incidence of non AIDS defining cancer increased, although the total incidence of HIV related neoplasia has not changed after HAART. Total cancer related mortality is higher pre-HAART and in women. Both sexes have a higher mortality by AIDS defining cancer pre-HAART and, by non AIDS defining cancer post-HAART.

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Genotyping HIV-1 non-B subtypes in Ireland: comparison of env and pol methods

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Characterisation of HIV-1 subtypes provides important epidemiological information and also has a potential impact on diagnosis, and therapy. The dominant HIV-1 strain in Ireland is subtype B, however other strains have also been identified. Most subtyping in our institution is performed using pol nucleotide sequence analysis.
Aims: To establish methods for amplification and subtyping of HIV-1 env (V3 region) gene and to compare pol and env genotypes in non subtype B HIV-1 samples.
Methods: HIV-1 viral pol sequences of stored samples were obtained and an approximate subtype assigned using the online HIV-1 Genotyping Tool (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi). 25 samples (viral loads 440–500,000 copies/ml) containing non subtype B viral pol regions were chosen for further analysis. Viral RNA from the env region of each sample was amplified and nucleotide sequences generated. The env and pol sequences were inputted into the above online genotyping tool, sequences were aligned using ClustalW and a neighbor-joining phylogenetic tree was created. (Joe Feldstein, http://evolution.genetics.washington.edu/phylip.html).
Results: 15 subtype categories were defined by pol genotyping. When compared to env genotyping, four samples (16%) had the same, fourteen (56%) similar and seven (28%) samples possessed divergent env and pol genotypes.
Conclusion: We have developed a feasible method for obtaining env V3 region sequence and subtype data. Only 16% of samples tested possessed truly the 'same' subtype. Overall, data presented in this study illustrates that subtyping based on simply the pol or env region may overlook interesting nuances of subtype variation and recombination and therefore efficient and effective methods for rapid subtyping of both regions, must continue to be developed. Larger studies may provide a clearer picture of the similarity between pol and env region genotypes. Such data could prove to be a helpful tool for more thorough and comprehensive analyses of patient subtype distributions.

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CD27+ (memory) B cell reduction as an independent marker of progression in HIV-1-infected patients

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Objectives: Dysregulation of B cells and their decreased ability to respond to HIV-1 antigen are likely to contribute to the increase in bacterial infections observed in advanced HIV disease in adults. However, the mechanisms of B cell dysfunction during HIV-1 infection are poorly understood. The aim of this study was to determine the phenotype of peripheral B cells in drug-naive HIV-1 positive patients. In addition, the relationship between B cell subsets, quantity of CD4+ T cells, regulatory T cells and plasma viral load was examined.
Methods: A group of 32 drug-naive patients with newly diagnosed HIV-1 infection was investigated. Phenotyping of B and T cells was performed in the peripheral blood by three-color flow cytometry (FACSCalibur, Becton-Dickinson, USA). B cells were characterized using monoclonal antibodies (mAb) anti-CD19 (FITC), anti-CD27 (PE), anti-CD38 (PerCP). CD27+ B cells were recognized as memory B cells. T cells were analysed using mAb anti-CD3 (FITC), anti-CD4 (PE), anti-CD25 (PerCP) (all Becton-Dickinson, Germany). Plasma HIV RNA viral load was detected by PCR (Roche Diagnostic Systems, Switzerland).
Results: We observed a positive correlation between CD4+ T-cell count and the percentage of CD27+/CD38(B cells ($r = 0.49$, $p < 0.01$). Also, the percentage of CD27+/CD38- B cells negatively correlated with plasma viral load ($r = (0.501$, $p < 0.01$). Additionally, we found no correlation between the levels of B cell subsets and the percentage of CD25+ regulatory T cells.
Conclusions: These findings suggest that HIV-1 infection induces a remarkable phenotypic alteration of B cells. Additionally, CD27+ B cell reduction is associated with the decrease of CD4+ T cells, as well as the increase of plasma viral load. This indicates that defects of humoral immunity during HIV-1 infection may play a role in disease progression. Therefore, further study to investigate of these phenotypic alterations after the reduction of HIV-1 loads by effective antiretroviral therapy is required.
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Immune activation markers in HIV-1 infected patients from India

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Objectives: The dynamic interaction between the human immune system and HIV-1 leads to the altered expression of T-cell surface markers, reflecting the various aspects of HIV immunopathogenesis. We studied the changes in the T-cell surface expression of peripheral blood lymphocyte subpopulations in HIV-1 subtype C infected Indian patients.
Methods: Blood samples were collected from 78 ART naïve HIV-1 seropositive and 25 seronegative individuals. Samples

were processed by standard FACS Lyse/Wash method and stained for three-color flowcytometry with panels of CD3, CD4, CD8, CD45RA, CD45RO, CD62L, CD38 and HLA-DR antibodies. Appropriate leucogate and isotype controls were included and samples were acquired on a BD FACSCalibur instrument using Cell Quest software. The mean, median and SD values were determined and expression levels of T-cell subsets were compared using the nonparametric Mann–Whitney test.
Results: CD4 and CD8 absolute counts and percentages varied significantly between the HIV-1 infected individuals and healthy controls ($p < 0.000$). The CD4:CD8 ratio was inverted in HIV-1 infected individuals as compared to healthy controls ($p < 0.001$). The distribution of naïve CD4+ T cell subset (CD45RA+CD45RO-CD4+) and memory CD4+T cell subset (CD45RO+CD45RA-CD4+) was significantly decreased in HIV infected as compared to HIV seronegative individuals ($p < 0.000$). However, both naïve and memory CD8+ T cell expression was significantly higher in HIV-1 infected individuals. The homing receptor expression on naïve CD8+ T-cell subset (CD62L+CD45RA+) was significantly reduced as compared to CD4+ T-cell subset ($p < 0.004$). Co-expression of activation markers HLA-DR and CD38 was significantly elevated on CD4+ and CD8+ T cells in HIV infected individuals as compared to healthy individuals ($p < 0.000$). Expression of CD38 on CD8+ T cells was found to be augmented in HIV-1 infected patients ($p < 0.000$).
Conclusion: The results suggest extensive immune alterations in lymphocyte subpopulations in HIV-1 subtype C infected Indian population similarly to the data reported for other subtypes.

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Rape and defilement as a cause of AIDS and HIV infection

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Objectives: High rate of defilement and rape has greatly increased the spread of AIDS among the young girls in Uganda.
Methods: There is a very high rate of AIDS and HIV infection in Uganda as a result of school girls being raped. Methods used include: teachers have become a great menace to their students. Infected teachers have resorted to the young girls without any protection. However, the Ministry of Education has resorted to moving around the schools sensitizing both the students as well as the teachers on the effects of such actions. This it has done by showing films on related topics and how one can easily fall in such traps, teaching the students different dramas to be acted out by them so that a practical picture of the disease is given. The Government has also made laws whereby the people who commit such crimes like defiling children are charged in the courts of law. Parents have also been sensitised on the dangers of making their children get close to the male teachers because such closeness can lead to rape and finally to the spread of AIDS. The media has also played a great role in fighting the spread of AIDS and HIV infection. The media has come up with material targeting the young ones in school. This has played a great role in the fight against such. With such they have taught the young ones defensive measures once they are found in such situations like avoiding being with the opposite sex in isolated places alone. The young ones have been advised to confide in their parents in case of anything. This greatly brings about transparency and avoids doing things secretly.
Results: Children have picked up the messages being passed on to them and have greatly reduced the high levels of HIV infection. They have started talking to their parents and contributing to articles in the media.
Conclusion: With the help of the Ministry of Education, Non Government Organisations, Media, Reading materials, Drama in schools, Ministry of Health and the parents/guardians, AIDS and HIV infections have greatly reduced in Uganda.

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Who wishes to be tested for HIV?

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Objectives: Slovenia is a country with a low incidence of HIV infections. Since 1988 Maribor Teaching Hospital offers the possibility of free examination, counseling and testing for HIV infection in the so-called AIDS OPC for all who do not wish to visit their family doctor. Here the testing is done either by name, confidentially or anonymously. In our prospective study we included all patients who visited the OPC between 1 January 1998 and 31 December 2003.
Methods: Using an inquiry form we followed sex, age, profession, marital status, risk factors, the reason for testing, eventual contacts with HIV-infected persons, the general condition. Blood screening was carried out by two different ELISA methods, and in case of suspected infection additional confirmative tests were done – Western blot, Immuno blot and PCR.
Results: The men:women ratio of our patients was equal, most of them were from the age group of 21–30 years, students or persons with a high level of education. Only 4% wished to be tested anonymously, 8% had HIV-positive acquaintances. In more than half of the patients the stated reason for the visit of the OPC was changing of sexual partners and prostitution, one third had no risk factor and were tested as a preventive measure, 9% were IV drug addicts. Only 5% of patients were either homo- or bisexual. 75% of patients had no permanent sexual partner, 16% were either married or in a permanent relationship. 6% of patients stated feelings of ill-health, 11% had enlarged lymph nodes. From among 396 tested patients, 4 cases of HIV infection were revealed.
Conclusions: Among our patients we discovered only 4 HIV-positive, all belonging in high-risk groups. The majority of HIV-positive cases are still discovered due to clinical diseases. The majority of tested patients are educated persons coming for examination and testing on account of general concern and when changing sexual partners. Despite the possibility of anonymous testing, most decide on testing by full name, nevertheless they do wish to avoid their family doctor.

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The utility of cytomegalovirus (CMV) quantitative polymerase chain reaction in diagnosis of CMV disease in patients with AIDS

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Objective: HIV infected individuals on no or failing antiretroviral therapy are at risk of CMV disease. Reliable diagnostic tests are important to identify patients at risk, to diagnose manifest disease and to monitor therapy. Studies have shown various capacities of diagnostic tests and have often been limited by lack of autopsy, important because CMV disease is often first diagnosed after death. The objective of this study was to explore the diagnostic utility of CMV quantitative PCR in AIDS patients who died and had a subsequent autopsy.
Methods: Patients included in this retrospective study were HIV and CMV seropositive and died during the period 1991–2002 during follow-up at Ullevaal University Hospital, Oslo. A full autopsy including neuropathological examination was required for inclusion. Paraffin-embedded sections were rou-

tinely stained with haematoxylin–eosin and immunohistochemistry for CMV was performed in a few cases of doubt to confirm CMV disease. In the case of CMV retinitis, ante-mortem diagnosis was based on typical ophthalmoscopic findings. Otherwise, characteristic histopathological features of cytomegalocytes with inclusions were required for diagnosis of CMV disease. The last one or two stored plasma samples (according to availability) either before diagnosis of CMV alive or before death were analysed by COBAS AMPLICOR CMV Monitor (Roche).
Results: A total of 125 patients were included. 55 patients were diagnosed with CMV disease, of which 39 were first diagnosed at autopsy. 70 patients did not have CMV disease. A total of 85 plasma samples were tested before diagnosis in patients with cytomegalovirus disease and 117 samples were analysed from patients without CMV disease. Median time between the last sample and diagnosis for patients with and without CMV disease were 69 and 74 days, respectively. Geometric mean virus titre in the last sample in patients with viraemia with and without CMV disease were 5894 (median 3140) and 1850 (median 1860) copies/ml, respectively. Results of sensitivity, specificity, positive and negative predictive values of the test are presented in table 1.

Table 1. Sensitivity, specificity, Positive and negative predictive value for CMV disease by changing viraemia cut-off, time of sampling and number of positive tests.

Quantitative PCR result	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
Any viraemia	0.51 (0,38-0,64)	0.87 (0,77-0,93)	0.76 (0,60-0,87)	0.69 (0,59-0,78)
Viraemia> 2 000 copies/ml	0,29 (0,19-0,42)	0,96 (0,88-0,99)	0,84 (0,62-0,95)	0,63 (0,54-0,78)
Vireamia> 10 000 copies/ml	0,16 (0,09-0,28)	1,00 (0,95-1,00)	1,00(0,70-1,00)	0,60 (0,51-0,68)
Sample < 30 days$^{\alpha\beta}$	0,50 (0,29-0,71)	0,87(0,53-0,98)	0,90 (0,60-0,98)	0,44 (0.23-0,67)
One of two tests positive$^{\alpha}$	0,58 (0,38-0,73)	0,84 (0,72-0,91)	0,65 (0,46-0,80)	0,78 (0,66-0,87)
Two of two tests positive$^{\alpha}$	0,23 (0,12-0,41)	1,00 (0,94-1,00)	1,00(0,65-1,00)	0,71 (0,60-0,80)

CI = Confidence interval
α Any viraemia.
β Patients with samples taken within 30 days of either CMV diagnosis Calive or post-mortem or before death.

Conclusion: CMV disease continues to occur in HIV infected individuals even at a time of widespread use of HAART. In this retrospective autopsy based study quantitative PCR had high specificity and positive predictive value in patients with high viral load or recurring viraemia but was limited by poor sensitivity and negative predictive value.

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ICU admission in patients infected with the human immunodeficiency virus over ten years

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Objective: To describe the profile of patients with infection HIV who enter in the intensive cares unit (ICU) throughout 10 years and to evaluate the potential impact of highly active antirretroviral therapy (HAART) of in the admission patterns and survival
Methods: Data were recorded all HIV-infected patients admitted to our ICU since January 1994 to December 2003, to a general ICU located in Marbella (south of Spain). We analyze age, sex, epidemiologic dates of HIV infection, HAART, diagnosis in ICU, mortality and use mechanical ventilation.
Results: To total of 41 HIV-related admissions were included. Average age 40 ± 11, average stay 9 ± 9. Half was not previously known diagnose VIH. In the known cases, the average time of diagnose of infection HIV was of 28 months and were naive to the ART 65%. Only 15% were in HAART. The medium one of CD4 was of 198 and the VL of 234.000. The reason for entrance in ICU in 50% was respiratory insufficiency, (65% PCP), followed of sepsis in 25%. More than 75% required mechanical ventilation. Mortality was of 50%. When analyzing mortality no significant differences were found in the different years neither in the reason for entrance nor in the virológica and immunological situation nor in if they were with HAART.
Conclusions: The HIV patients who enter in ICU do not know infection HIV in half of the cases, usually they are not in ART and the most frequent reason for the entrance is respiratory insufficiency with use of mechanical ventilation. No significance difference in the last 10 years mortality was found in spite of the HAART introduction.

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Management of lower respiratory tract infections in HIV-infected adults

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Objectives: To asses the causative organisms and efficacy of antibiotic treatment of bacterial lower respiratory tract infections (LRTI) in HIV infected patients.
Methods: Retrospective study of 113 patients diagnosed with LRTI from January 2002 through December 2003, the susceptibility to antibiotics of microorganisms isolated from sputum samples and the management of the illness.
Results: We included 56 males (78% smokers) and 46 females (70% smokers), with median age 35 years old (limits: 18–72 years old). After clinical findings, chest radiographs and bacteriological results, we diagnosed 44 pneumonia (P), 31 acute exacerbation of chronic bronchitis (AECB), 28 episodes of super infection of bronchectasis (B) and 5 pulmonary abscesses (PA). Bacteremia was more common in P. The CD+ lymphocytes count was low (median 110/mmc). The length of hospital stay was longer in patients with AP (median 18 days) compared with the other LRTI (median 10 days). We isolated 152 strains, the most common pathogen being *Haemophilus Influenzae* (63 admissions), followed by *Streptococcus pneumonia* (30 admissions), *Staphilococcus.aureu* (12 admissions), *Klebsiella pneumoniae* (14 admissions), *E. coli* (8 admissions), 7 admissions of each *Ps.aeruginosa* and *Acinetobacter* spp, *Enterobacter* (5 admissions), *Proteus mirabilis* (3 admissions) and 1 admission for each *Citrobacter* and *Comamonas testosteroni*. Amoxicillin/clavulanate represented empirical treatment in most of cases (67 patients). Ciprofloxacin and third generation cephalosporin were the most administrated therapy after the susceptibility tests. Bacteriological cure was obtained in 110 patients, 3 patients expired (1 *Klebsiella* spp. bacteraemia, 1 *Ps. aeruginosa* PA and 1 *Streptococcus pneumoniae* bacteraemia).
Conclusions: LRTI are frequent causes of admission in HIV infected patients. TMP-SMX prophylaxis is not useful for LRTI in most of cases. A rapid diagnosis and the use of an susceptible antibiotic can be the key of success, even in immunocompromised patients.

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Prevalence of HIV infection and other sexually transmitted diseases in gay saunas in Taiwan

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Objectives: Gay bathhouses have been described as public venues for transmitting human immunodeficiency virus (HIV) and sexually transmitted diseases (STDs). This study aimed to

investigate (1) the prevalence of HIV infection, syphilis, hepatitis A, hepatitis B, amoebiasis, gonorrhoea, and chlamydial infection, and (2) risky sexual behaviors for STDs acquisitions among men who have sex with men (MSM) attending gay saunas in Taiwan.
Methods: An ongoing cross-sectional study has been conducted in six gay saunas since September 2004. Men visiting bathhouses who agreed to participate in this study were invited to complete a one-page, anonymous questionnaire and to receive STDs screening tests. Serology studies were tested for HIV, syphilis, hepatitis A, hepatitis B, amoebiasis and urine specimens were tested for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by polymerase chain reaction (PCR).
Results: One hundred and sixty MSM were enrolled, including 138 (86.3%) questionnaires, 158 (98.8%) blood tests, and 69 (66.7%) urine tests. The mean age (±SD) of 138 participates was 30.2 (±8.7) years (range: 18–74). Seropositivity for HIV antibody (Ab) was 7.9% (12/152). Reactivity rate for *Treponellma pallidum* hemagglutinin (TPHA) test with a 160-fold or higher dilution was 21.3% (32/150), in which 14 (45.2%) of 31 men also tested for rapid plasma reagin showing reactive with a 4-fold or higher dilution. Seroprevalence for hepatitis A and B Ab, hepatitis B antigen, and amoebiasis [IHA titre (1:128)] were 57.6% (57/144), 59.6% (87/146), 20.5% (30/146), and 4.8% (7/146), respectively. Prevalence rates of Chlamydia and gonorrhoea infection were 7.2% (5/69) and 4.3% (3/69). In univariate analysis, seroreactivity for TPHA (P = 0.001, odds ratio, 6.5; 95% confidence interval, 1.99–22.34) and using party drugs (P = 0.001, odds ratio, 7.1; 95% confidence interval, 1.89–26.36) were associated with the risk for HIV infection. Men with TPHA reactivity reported having more unprotected oral sex partners than men who were seronegative for syphilis (2.36 vs. 1.27, t = 0.23, P = 0.03).
Conclusions: The preliminary results showed that high prevalence rate of syphilis among MSM and the association between syphilis, using party drugs and HIV infection. Unprotected oral sex was associated with TPHA reactivity.

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The introduction of highly active antiretroviral therapy paradoxically increased a late diagnosis of HIV infection, often coinciding with full-blown AIDS

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Introduction: Notwithstanding the availability of HAART since 1996,AIDS notifications continue to occur, with increasing frequency among patients (p) who were not aware of their condition, or neglected specific monitoring and treatment.
Patients and Methods: All cases of AIDS newly diagnosed since 2001 at our centre were compared with all AIDS episodes observed in the decade preceding HAART introduction (1986–1995),on the ground of a broad spectrum of epidemiological and clinical variables.
Results: Compared with the pre-HAART era, the expected drop of frequency of overall AIDS cases were seen: from a mean of ~58 p from 1986 to 1995, to ~17 p per year since 2001 ($p < 0.001$). An increased mean age at diagnosis ($p < 0.003$), a more frequent involvement of women ($p < 0.02$), an increased sexual exposure vs i.v. drug abuse ($p < 0.001$), and a higher proportion of foreign p ($p < 0.04$),were found. After HAART introduction, the most evident drop of incidence of AIDS-associated opportunism involved all diseases linked to a very deep immunodeficiency (as expressed by a CD4 count (50–100 cells/μL), while a proportional increase of lymphomas, tuberculosis, and bacterial pneumonia occurred. Both examined periods were characterised by the persistence of *Candida* esophagitis and pneumocystosis as the most frequent AIDS-defining diseases, followed by neurotoxoplasmosis, wasting syndrome, and AIDS dementia. Both absolute number and proportion of p with multiple concurrent AIDS-related disorders had a paradoxical increase just during the HAART era, as well as AIDS diagnoses notified at death ($p < 0.001$), while AIDS-associated immunodeficiency did not change significantly. Surprisingly, a prior use of antiretrovirals was more common among AIDS p until 1995, vs p diagnosed since 2001 ($p < 0.001$), since during recent years an AIDS diagnosis tends to be made concurrently with the first positivity of HIV serology.
Discussion: When facing p with suspected opportunistic illnessess, the Clinician should maintain an elevated clinical suspicion for an advanced, but occult and untreated HIV disease. An increased attention to this emerging problem may allow a more rapid recognition of suspected p, and an appropriate therapy of p who could not benefit from HAART, since they were unaware of their disease, or 'removed' their problem (when p lost to follow-up or p who previously refused follow-up and cure, are of concern).

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Basic clinical-applied pharmacoeconomic features of antiretroviral therapy. A pilot single-centre study performed on around 1000 HIV-infected individuals receiving combined antiretroviral treatment

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Objective: Aim of our study is to assess the frequency and composition of prescriptions taken by 990 HIV-infected patients (p) followed in a reference centre of Northern Italy during the last 15 months.
Methods: All drugs are directly distributed at monthly interval. Antiretrovirals administered for the longest period to each single p were considered, for our 15-month assessment. Physicians in charge of HIV-infected p evaluated all anti-HIV treatments on the ground of internationally-recognized efficacy-safety parameters, but not pharmacoeconomic issues. Updated pharmacy expenses were linked to the 24 most commonly used therapeutic plans, covering 80.1% of prescriptions.
Results: AZT-3TC-efavirenz was the most prescribed combination (7.3%), followed by AZT-3TC-nevirapine (7.1%), 3TC-d4T (6.2%), and AZT-3TC-lopinavir (5.2%). Mean daily costs of treatment started from a minimum of dual 3TC-d4T association (10.7 [a]), up to a maximum of 25.8 a/d (+241.1%) for AZT-3TC-lopinavir. After excluding dual nucleoside analogues (accounting for 9.8% of prescriptions), regimens based on a NNRTI usually had a lower cost vs the majority of those including 1–2 PI. The increased expense of each combination is compared with the cheapest selection, and costs of all triple associations were compared.
Discussion: NNRTI-based regimens accounted for 29.3% of treatment administered to our p cohort (with nevirapine at 15.1% and efavirenz at 14.2%),while PI were used in 37.3% of cases: lopinavir–ritonavir 13.6%, nelfinavir 9.9%, indinavir 9.2%. Triple nucleoside/nucleotide analogues accounted for 4.1% of regimens. Dual nucleoside analogues had the lowest expense (10.7–11.6 a/d), while triple nucleoside/nucleotide analogues costed 18.5–20.4 a/d. Among NNRTI, nevirapine-based combinations costed 18.3–18.7 a/d, while efavirenz is 10% more costly (19.2–20.1 a/d). A broad range of associations and related prices was found with PI-based HAART, but apart indinavir and saquinavir combinations (15.7–21.7 a/d), all other regimens had greater costs, ranging from 23.4–24.3 a/d for nelfinavir associations, up to 24.9–25.8 a/d for lopinavir. Considering nelfinavir- and lopinavir-containing therapies, the difference with NNRTI vary from 11.6% between efavirenz and nelfinavir, up to 41% when nevirapine and lopinavir are compared.

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Effectiveness and durability of tenofovir and didanosine as backbones in anti-retroviral naive patients

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Objective: To determine the durability and effectiveness of the anti-HIV combination of didanosine (ddI) 250mg once daily given with tenofovir (TDF) 300 mg once daily plus a protease inhibitor or NNRTI in HIV+ anti-retroviral naive patients over a 3-year period
Method: Charts of all patients receiving ddI/TDF as a once daily regimen were reviewed. Parameters to be measured included (1) the total number started on this regimen; (2) the per cent reaching viral suppression as measured by PCR-RNA; (3) the per cent remaining on the regimen for 2 years or more; (4) side-effect profile
Results: 66 patients were identified. 7 were lost in follow-up: 2 were incarcerated and their regimen switched; 2 moved out of state and 3 are lost to follow up. Of the 59 remaining, 47 have been on the regimen over 36 months and 17 for 30 months. All remain undetectable with viral loads less than 50 as measured by PCR. CD4 counts increased by 53 with a range from (110 to 215.
Discussion: This differs from data recently reported by BMS that was done by Podzamczer et al and JM Gtell. In our patient population adherence was apparently good or better and clients tolerated this regimen over a long period regardless if the anchor drug was atazanavir, efaverinz, or niverapine. There was no evidence of lactic acidosis or other complications. It is our feeling that this is a strong backbone to give with a protease or NNRTI in naive patients.

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Study on the effect of prolonged treatment with zidovudine, lamivudine and abacavir on a T-lymphoblastoid cell line

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Objective: Cellular factors, such as altered activation and/or decreased accumulation of drugs has been associated to a decrease of the antiretroviral activity of several drugs. The aim of this study was to determine whether the prolonged in vitro treatment of cell culture with zidovudine (ZDV), lamivudine (3TC) and abacavir (ABC) may select cellular factors able to reduce the intracellular concentrations of drugs.
Methods: CEM T-cell line was treated with increasing concentrations of ZDV, 3TC and ABC. After 6 months of culture, we obtained a cell line, here called CEMZLA. The antiviral activity of drugs was evaluated by measuring p24 viral antigens. Enzymatic assays, western blot assays and real-time PCR analysis were used through the study to identify the mechanism/s underlying the phenomenon of cellular resistance.
Results: CEMZLA were markedly resistant to the antiviral activity of ZDV (25-fold), moderately resistant to the antiviral activity of 3TC (5-fold) and fully sensitive to ABC. Parallel experiments showed that ABC, alone or in combination, is not able to select cells with a resistant phenotype. Assays, performed to evaluate thymidine kinase (TK) and deoxycytidine kinase (dCK) activities indicated that CEMZLA exhibits an approx. 60-fold decrease in TK activity compared with the parental line. On the contrary dCK activity from CEMZLA was not decreased compared with the parental CEM cells. The lower TK activity in CEMZLA correlates with a lower amount of TK protein and to a decrease in the intracellular concentration of ZDV-TP, being ZDV-TP levels 20-fold lower in CEMZLA than in CEM. Interestingly, TK mRNA levels were unchanged in CEMZLA when compared to parental line, suggesting that the reduced activity of TK was not due to an alteration in the transcription process. To explore whether an ABC transporter protein played a role in the resistance to 3TC in CEMZLA, we evaluated the expression of MDR1, MRP1, MRP4, MRP5 mRNA levels in CEM and CEMZLA. No significant differences were observed in the expression of these mRNA in CEM and CEMZLA.
Conclusions: The prolonged treatment with ZDV, 3TC and ABC leads to a selection of cells highly resistant to ZDV (due a defect of TK activity), moderately resistant to 3TC (the mechanism is still under study) and fully sensitive to ABC. Prolonged treatment with ABC does not lead to the expression of cellular factors involved in the decrease of the antiviral activity of the drug.

HCV: risk factors, diagnosis, clinical presentation and treatment

P695

Hepatitis C exposure and the health care worker in New Zealand. A review of 10 years of blood and body fluid exposure reports

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Objective: Evaluate the risk of Hepatitis C infection in the Health Care Worker (HCW)Who had sustained a Blood or Body Fluid (BBF) exposure, where the source BBF was Hepatitis C positive or the source was of unknown.
Methods: Blood and Body fluid Exposure Reports submitted during the period 1993–2003 were reviewed. Source: The person whose blood or body fluid was involved in the injury HCW: The injured person. Testing of the source person's blood for evidence of Hepatitis C antibodies was introduced in 1993. The BBE exposure reports containing evidence that the source was Hepatitis C positive or unknown were analysed for the following factors: Exposure type: A: Needle stick or other sharp injury B: Mucous membrane exposure C: Contamination of fresh wounds D: Bite, scratches Employment Group Serological testing of HCW and Source:
Results: 3302 BBF reports from staff were received during the 10–year period.510 were selected for review. 349 (68.4%) were from blood or body fluid exposure where no source was identified. 161(31.6%) had an identifiable Hepatitis C positive source. Exposure type A was reported in 395(77%) was, however only 84(17%) had a documented Hepatitis C source. Exposure type B was reported in 65(13%)and Exposure type C and D 30 and 20 respectively. 51(10%) of the HCW s had left employment prior to follow up. 17 of these had documented Hepatitis C exposure. All had received information of the exposure risk at time of injury and the need Hepatitis C serology

6 months post injury. No communication regarding Hepatitis C infection has been received from that group No Seroconversion to Hepatitis C was recorded at 6 months follow up.
Conclusion: All Health Care Workers who sustain an BBF exposure are concerned that they might get infected with an blood borne virus one of which are Hepatitis C. The risk of acquiring Hepatitis C is probably a rare event. 10 Years of monitoring BBF exposures in a District Health Board with 6000 employees failed to document any seroconversion event.

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Striking changes in the prevalence of hepatitis C virus genotypes in Greece in the last five years

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Objectives: To estimate the relative frequency of Hepatitis C Virus (HCV) genotypes in Greece over the last 5-year period in comparison to their respective prevalence in the 1990's and to investigate for possible associations with epidemiological, demographic and other variables.
Methods: 1090 consecutive patients with chronic HCV infection and detectable HCV RNA, referred to the Hepatitis Research Laboratory of Athens University over the last five years, were included in this study. Sera were first subjected to HCV RNA amplification by PCR (Amplicor-Cobas, Roche) and the PCR product was then genotyped by a line probe assay (Innolipa, Versant, Bayer) according to the manufacturer's instructions. The results were analysed by age, sex, duration of infection, intravenous drug abuse (IVDU), other modes of transmision and other variables. Results were also compared to previously reported data on the prevalence of HCV genotypes in Greece in the 1990's.
Results: The overall prevalence of HCV genotype 1 was 43%, of genotype 2 9%, genotype 3 29% and genotype 4 19%. The prevalence rates in the 1990's of genotypes 1, 2, 3 and 4 were 54%, 9%, 22% and 11% respectively. Among HCV-infected individuals born after 1955, genotype 1 was detected in 36% and genotype 3 in 44% of them, compared to 65% and 7% respectively, in those born before 1955. There was no correlation between HCV genotype and the sex of the patients. Genotype 3 accounted approximately for three quarters of HCV infections among intravenous drug users. These secular changes of HCV genotypes in Greece appeared to be related to alterations in prevailing modes of HCV transmission as well as to increased immigration and extensive population mix.
Conclusions: The distribution of HCV genotypes is in continuous temporal change in Greece. The prevalence of HCV genotypes 3 and 4 is progressively increasing, genotype 1 decreasing and contrary to most European countries and USA, HCV genotype 3 is becoming the predominant type accounting for almost half of new HCV infections and for their majority among intravenous drug users. These results are of clinical, epidemiological and particularly of therapeutic relevance.

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Hepatitis C virus infection among dialysis patients in Tunisia: prevalence and molecular evidence for nosocomial transmission

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Objectives: In order to give evidence for nosocomial transmission of hepatitis C virus (HCV) in Tunisian haemodialysis patients, 431 patients from 11 haemodialysis units were enrolled in a prospective study between November 2001 and November 2003.
Methods: HCV serological and virological statute was initially determined, respectively using third generation ELISA test and RT-PCR qualitative assay. Anti-HCV negative haemodialysis patients were followed monthly with measurements of alanine aminotransferases (ALT). Every rise in ALT value led to the screening for HCV-RNA in order to detect potential de novo infection. In case of confirmation of recent contamination, the genotype of the HCV isolate was determined by sequencing NS5B region. The issue of nosocomial transmission was addressed by sequencing the HVR-1 region of the E2 gene.
Results: The overall prevalence of HCV antibodies in investigated haemodialysis patients was 19%; HCV RNA was detected in 72% of the anti-HCV-positive patients. Two cases of de novo HCV infection were identified during the virological follow up (incidence = 0.84%) with a significant increase in ALT and a seroconversion during the second sampling round. The first novel HCV-positive case (anti-HCV-negative patient, HCV RNA positive)belonged to HCV genotype 1b, unlike isolates from already infected dialysis patients in the same unit. In the second case, the new case like other HCV positive patients were infected with the same genotype 1b; the sequencing of the HVR-1 region of the E2 gene provided strong evidence that the isolate from newly infected patient and an other isolate from already infected dialysis patients were closely related (bootstrap value 100%).
Conclusion: Molecular tools and phylogenetic analysis allowed to exclude nosocomial transmission in the first case and to confirm it in the second one. Epidemiological investigation exhibited that these two patients were dialysed on the same day, on the same shift but in different areas, suggesting nosocomial transmission intermediating medical staff and/or nursing and emphasizing on strict adhesion to universal hygiene standards and routines in dialysis units.

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Sources of infection in patients with chronic hepatitis C virus

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Since the discovery of hepatitis C virus (HCV), much has been learned about its epidemiology and risk factors for infection. The aim of this study was to evaluate risk factors for hepatitis C virus infection, and to obtain a predictive value between some factors and fibrosis and hepatocellular carcinoma.
Subjects and methods: The study included 755 persons with chronic hepatitis C virus infection. Screening for HCV antibodies was carried out on serum samples using a third-generation enzyme-linked immunoassay and confirmed by third-generation line immunoblot assay. Samples were also tested for hepatitis B virus surface antigen using ELISA and all positive samples were confirmed by Access-HBsAg. Anti-HIV antibodies were sought by ELISA and positives were confirmed by Western blot. For statistical analysis, Chi-square test was used. P-values of <0.05 were considered statistically significant.
Results: The global population was divided into drug users and non drug users. In the drug users group, 431 were intravenous drug users (IVDU) and 60 had high alcohol intake. Viral coinfection with HBV and HIV was higher for IVDU than for the other group (65.9% vs 15% for HBV; 63.6% vs 10% for HIV). When alcohol intake was elevated, GOT value was significantly higher than GPT value (65% vs 35%). However, IVDU presented GPT value higher than GOT value.In the non drug users group,

the risk factors were: iatrogenic sources (41.6%), blood transfusion (33.3%), high-risk sexual behavior (5.54%), hemodialysis (3.78%), had lived in endemic countries (3.03%), tattooing (2.65%), needle stick accidents (1.13%), transplants (1.13%) and unknown (8.7%). This group was older than drug users group. In the iatrogenic sources, digestive surgery and endoscopic exploration had elevated values of viral transmission. HCV infection by blood transfusion had an elevated HBV coinfection. When the risk factor as unknown, they had HIV viral coinfection rate higher than the rest of non drug users group (8.7% vs 4.5%).
Conclusion: In the drug users group, the multivariate analysis showed a strong association between alcohol intake, hepatocellular carcinoma, GOT value, fibrosis and inflammation, and non viral coinfection. On the other hand, HIV and HBV positive, GPT value and non hepatocellular carcinoma items were strongly associated. However, in the non drug users, the multivariate analysis showed a strong association between GOT value, fibrosis and inflammation, and viral coinfection.

P699

HCV infection and pregnancy. Prospective series of 29,260 consecutive cases

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In 29,260 consecutive pregnant women we evaluated the prevalence of HCV chronic infection, its course during pregnancy and the rate of HCV vertical transmission.
Patients and methods: Anti-HCV was tested by EIA III and confirmed by RIBA III. ALT, anti-HIV and HCV-RNA were tested at enrollment, at delivery and six months after. The same determinations were also performed in all new-borns of anti HCV positive mothers, at birth (on cord blood only during the initial 36 months of the study) and at 6-month intervals up to 24 months.
Results: Anti-HCV positivity was found in 767 cases (2.6%), 65% of whom were HCV-RNA positive. The proportion of those hypertransaminasemic decreased from 48% before pregnancy to 4% in the last trimester and increased again after delivery (49% at 6 months) without concomitant changes in the proportion of viremic ones. Twenty-two new-borns were HCV-RNA positive but 20 negativized and nineteen others became positive within 6 months; thus the proportion of anti-HCV and HCV-RNA positive new-borns was 4.5% at 1 years (19/418), all with the same genotype of the mother. Type of delivery and feeding and maternal HIV status did not influence the rate of HCV transmission.
Comments: This series confirm, on a large scale, previous data concerning the prevalence of HCV infection in pregnant women and strongly support a favourable (possibly immuno-mediated) effect of pregnancy on liver cell necrosis in anti HCV positive women. Data from new-borns also indicate that cord blood test for HCV RNA is not advisable due to its negligible positive predictive value.

P700

Evaluation of a new kinetic amplification based assay for quantification of hepatitis C virus (HCV) RNA

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Objectives: The authors are developing an automated kinetic amplification assay targeting the 5′-UTR for quantification of HCV RNA. Analytical performance characteristics of this assay were evaluated, including: sensitivity, linearity, the ability to detect genotypes 1–6, and specificity.
Methods: Viral RNA was extracted from panels and donor samples using an automated method with magnetic silica beads. Reverse transcription, amplification, and detection were subsequently performed. Analytical performance of the assay, currently in development, was evaluated to determine sensitivity, dynamic range, specificity, and equivalence of genotype quantification. Linearity and limit of detection were evaluated with HCV 1a viral panels. Additionally, HCV 1a transcript quantified by phosphate analysis and A260 was used to evaluate linearity over an extended range. Transcripts representing HCV subtypes were similarly quantified and used to evaluate genotype detection. Specificity was evaluated using serum from HCV antibody negative donors.
Results: The assay was linear over the range tested (25–5×10^8 copies/reaction) with subtype 1a transcripts as target. HCV 1a virus panels were detected >95% of the time at 20 IU/mL with a 0.5 mL sample volume. Preliminary experiments with transcripts representing genotypes 1–6 demonstrated equivalent quantification. Assay specificity was >99% using negative donor serum samples.
Conclusions: The new automated HCV viral load assay under development has been demonstrated to detect HCV RNA at very low concentration. The assay displays a wide dynamic range with excellent specificity.

P701

Evaluation of the total hepatitis C virus core antigen in children born to HCV positive mothers

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Objectives: The aim of this study was to evaluate an immunoenzymatic assay for the detection and quantification of HCV core antigen (HCV core Ag) and examine the time course and pattern of HCV antibody response and viral load in infants born to HCV positive mothers. We also studied the correlation between viral load and HCV core Ag levels.
Methods: We studied 67 blood samples collected from 22 children born to a HCV positive mothers group, between birth and more than 24 months, in the period August 2000–July 2004. Samples were stored frozen at –80°C. Specimens were tested for the presence of anti-HCV antibodies using a third generation screening assay, Abbott AxSYM® HCV 3.0. Cobas Amplicor® Test (Roche Diagnostics) was used to detect and quantify HCV RNA in patients serum samples. Detection and quantification HCV core Ag was measured by track-C® assay (ORTHO). All assays were carried out following manufacturer instructions.
Results: All children except five were anti-HCV negative after 24 months of birth. Fifty-five samples showed agreement with PCR (83.6%), twenty-three were PCR and HCV core antigen positive and thirty-three were negative in both markers. The correlation coefficients (r) observed between HCV core Ag (pg/ml) and HCV RNA (UI/ml) was 0.72. Eleven sera showed disagreement between HCV core Ag (significantly higher than in disagreement profiles (622 ± 0 UI/ml) ($p < 0.001$). However, among agreement profiles there is no significantly difference of viral load between Anti-VHC negative patients (389190 ± 272974 UI/ml) and positive patients(411241 ± 293342 UI/ml). On the other hand, mean VHC core Ag concentration in agreement profiles 92 ± 105 pg/ml)is also significantly higher than in disagreement profiles (17 ± 24 pg/ml) ($p < 0.001$).

Conclusion: The HCV Ag core quantification is useful to identify viraemia in anti-VHC negative and positive and antigen levels correlated with quantitative HCV RNA levels. However, track C® could fail to detect viraemia in patients with very low HCV core Ag concentrations.

P702

A new anti-HCV EIA based on recombinant antigens derived from different sequence variants of hepatitis C virus

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Objective: The purpose of this study is the development of a new anti-HCV EIA by using recombinant antigens derived from different sequence variants of different HCV virus genotypes.
Methods: The 9 sequence variants of recombinant antigens comprising major epitopes from core, NS3, NS4 and NS5 HCV proteins have been selected for the anti-HCV assay development. This new EIA was evaluated using serum specimens (n = 511) obtained from patients infected with different HCV genotypes from different parts of the world. The HCV status of these specimens was confirmed using RIBA HCV 3.0 (ORTHO Diagnostic Systems Inc., USA), and Cobas Amplicor HCV Test, v.2.0 (Roche Diagnostics, USA). 376 samples were confirmed as anti-HCV positive, 57 samples were tested anti-HCV 'Indeterminate', and 78 sera were tested anti-HCV negative. Among anti-HCV RIBA negative specimens 3 samples were HCV RNA positive. Additionally, anti-HCV Mixed Titer performance Panel PHV 205, anti-HCV Low Titer Performance Panel PHV 103 (BBI Inc., USA) and 15 anti-HCV seroconversion panels (BioClinical Partners, Inc., USA) were tested. The anti-HCV negative panel was composed of 200 specimens (BBI Inc., USA).
Results: New EIA detected anti-HCV activity in 100% of specimens tested anti-HCV positive or/and anti-HCV indeterminate by RIBA HCV 3.0 and HCV RNA positive. 92% of RIBA 'indeterminate" samples were immunoreactive with more than one antigen used for the development of the new anti-HCV EIA. Moreover, three anti-HCV negative but HCV RNA positive samples were immunoreactive with NS3 antigens used in this new EIA. Additionally, the EIA was able to detect seroconversion point earlier in 3 anti-HCV seroconversion panels than commercially available anti-HCV EIA. Two anti-NS4 HCV negative but anti-core, anti-NS3 and anti-NS5 positive specimens from PHV 103 and PHV 205 were found anti-NS4 positive by using the NS4 antigen from the new EIA. None of the used anti-HCV negative specimens were tested as positive with new anti-HCV EIA.
Conclusion: A new highly sensitive and specific anti-HCV detection assay was developed using various sequence variants of HCV antigens. This assay may be used for screening sera from patients infected with different HCV genotypes with almost equal efficiencies.

P703

Seroprevalence of HIV, HBV, HCV and CMV infection among Polish orthopaedic surgeons

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Background: Orthopedic surgeons are at risk for occupationally acquired infections with blood borne pathogens.
Objective: to estimate the prevalence of infection with hepatitis B and C viruses (HBV, HCV), cytomegalovirus and human immunodeficiency virus (HIV) among orthopaedic surgeons and correlate the results with occupational and nonoccupational risk factors.
Design: A voluntary, anonymous serosurvey at an annual meeting of Polish Asso-ciation of Orthopedic Surgeons held in Szczecin, Poland in September 2004. Serum samples were tested for HIV antibody, for CMV IgG antibody, for HCV antibody and for markers of HBV infection: hepatitis B surface antigen and total antibody to hepatitis B antigen.
Results: Of 1000 eligible orthopaedic surgeons at the annual meeting, 101 (10%) participated. 76 (75.2%) reported a percutaneous blood contact in the previous month. Among participants without reported nonoccupational risk factors for HIV infection none was positive for HIV (0%; 95%CI: 0–3.7%). One participant (1%), 26 years in profession, had anti-HCV. There was evidence of infection with hepatitis B in 10 of 74 (13.5%) participants who had reported having no nonoccupational risk factors and in 5 participants with such risk factors. None of them developed a chronic infection. From 15 doctors infected with HBV, 5 knew their serological status (3 immunized themselves against hepatitis B despite such knowledge), 10 did not know the fact and had been immunized with hepatitis B vaccine, 4 revaccinated. The immunization rate in our study was 79%. The prevalence of infection with HBV was not associated with number of procedures and of percutaneous contacts with blood in the past month ($p > 0.94$ and $p > 0.14$ respectively) and the use of protective barriers ($p > 0.01$), but was associated with the age ($p < 0.02$) and the participation in the training sessions on occupational infections ($p < 0.05$). The seroprevalence for CMV was 63/98 (62%); it decreased with age ($p < 0.0003$).
Conclusions: 1. Although these findings may not be generalizable to all orthopaedic surgeons, we found that despite infection control precautions and availability of hepatitis B vaccine, orthopaedic surgeons remain at risk for acquiring bloodborne viral infection. CMV posed the highest risk, followed by HBV and HCV. 2. As the vast majority of HBV infected doctors did not know their serological status and underwent immunization with hepatitis B vaccine, testing for HBc antigen before every vaccination remains crucial.

P704

Insertion of a new V3-like domain in nonstructural 5A protein of hepatitis C virus genotype 1b

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Objectives: Hepatitis C virus (HCV) nonstructural 5A (NS5A) protein has been disputably implicated in the resistance of HCV to interferon therapy in both Japanese and European clinical studies, particularly for HCV genotype 1b. Many interactions have been reported between NS5A and cellular proteins, but its function in the life cycle of HCV is unknown despite an interaction with NS5B. Some authors have reported insertions–deletions of small sequences, from 1–12 nucleotides long, in different regions of the viral genome, 5' UTR, HVR1, ISDR. In this study, we report on a novel insertion in the HCV genome in the V3 region of NS5A protein.
Methods: We amplified the second half of the NS5A gene including ISDR and V3 domains in 131 patients with HCV genotype 1b (n = 113) and mixed genotype 1, 1a-1b or 1b-4a (n = 18).
Results: Among these patients, we found five (3.81%), infected by HCV-1b, with an insertion in the V3 region of the NS5A gene without disruption of the open reading frame. Two sizes of inserts were found: four patients had an insert of 31 amino acids and one patient had an insert of 27 amino acids. The 31 amino acid insert was very similar to the V3 domain (2353–2379) but it

was not the same sequence in the five cases (homology from 54.8–71%).
Conclusion: The consequences of this duplication on protein function are not yet clear. The NS5A protein needs further study to understand its implication in replication, transcription activation, or resistance to treatment of HCV.

P705

Serological profile and virological evaluation of hepatitis B and hepatitis C virus infection among HIV-infected patients in Greece

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Objectives: To evaluate the prevalence of serological markers of HBV and HCV infections among a large cohort of HIV-infected patients and to examine the serum levels of HBV-DNA and HCV-RNA in a subgroup of HIV/HBV and HIV/HCV coinfected patients respectively.
Methods: We retrospectively analysed 737 HIV-infected patients followed-up at the Department of Infectious Diseases, 'Andreas Sygros' University Hospital of Athens, Greece, since 1995, for the presence of serological markers of HBV and HCV infection. Virological evaluation (HBV-DNA, HCV-RNA) and HCV genotyping, before the initiation of ART, was available in a subgroup of HIV/HBV and HIV/HCV coinfected patients.
Results: Overall 88.6% (653/737) of the study population were men and 11.4% (84/727) women whereas the vast majority of them (453/737, 61.5%) were homosexual (HOM) men. Thirty-nine of 737 patients (5.3%) reported intravenous drug use (IVDU) and 17 of them were both HOM/IVDU. Among the 737 HIV-infected patients 89 (12.1%) were HBsAg(+) and the majority of them (54/89, 60.7%) were HBeAg(+) whereas 33.7% (30/89) of HBsAg(+)/HIV(+) patients were HBeAg(−)/anti-HBeAg(+). Anti-HBc seropositivity was detected in 48.1% (355/737) of the study population. The serum levels of HBV-DNA in this subpopulation with available virological data were 5.75 ± 1.66 (−log10 copies/ml) and HBeAg(+) HIV/HBV coinfected patients had significantly higher baseline serum HBV-DNA levels than HBeAg(−)/AntiHBe(+) coinfected ones (7.40 ± 0.64 vs 4.59 ± 1.01, −log10 copies/ml, respectively, $p < 0.001$). Fifty-one of 625 HIV-infected patients, of which anti-HCV test was available, were anti-HCV positive (8.2%). Quantitative calculation of HCV-RNA and HCV genotyping were available in 28 HIV(+)/anti-HCV(+) patients. The vast majority of them (24/28, 85.7%) had HCV-RNA levels more than 700.000 IU/L and the most common HCV-genotype was genotype-1 (12/28, 42.9%) followed by genotype-3 (10/28, 35.7%), genotype-4 (4/28, 14.3%) and genotype-2 (2/28, 7.1%). Five patients (0.68% of the total study population) exhibited triple infection (HIV/HCV/HBV).
Conclusion: HIV/HBV coinfection is observed in a significant proportion of HIV-infected patients in Greece and the majority of them are HBeAg (+), exhibiting extremely high HBV-DNA levels, in contrast to general Greek population. Genotype-1 and high HCV-RNA levels are the most common findings in HIV/HCV coinfected patients, characterizing a difficult to treat population.

P706

Acute transverse myelitis and hepatitis C virus

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Objectives: We report a case of acute transverse myelitis developing 4 years after documented HCV seropositivity, associated with intrathecal anti-HCV protein IgG synthesis.
Case report: A 60-year-old woman, seropositive for HCV since 1996, was admitted to the hospital in July 2000 with persistent fever and acute transverse myelitis. Blood parameters were normal but serum antibodies against HCV and HCV-RNA (genotyping 1) were tested positive. Cerebrospinal fluid (CSF) analysis showed mild lymphocytic pleocytosis (40/mmc) with increase of total proteins (116 mg/dl); and isoelectrofocusing (IEF) evidence of oligoclonal bands not found in serum. Neurotropic virus RNA and DNA were not found. The patient was treated with ampicillin, gentamicin and acyclovir for 3 weeks. Leg weakness slightly improved and was discharged. In November 2001, she was admitted to the hospital with worsening of leg weakness. She was treated with intravenous methylprednisolone (1 g for 3 days) responding with slight improvement in clinical parameters. In July 2002, due to renewed worsening of clinical parameters, a lumbar puncture was performed. CSF analysis showed normal protein levels and no pleocytosis but IEF confirmed several oligoclonal bands. PCR testing for HCV-RNA was negative but antibodies against HCV c22–3 protein were found by immunoblot assay. Further treatment with intravenous methylprednisolone was undertaken. The patient is currently unable to walk and shows hyperreflexia with Babinski sign and moderate urinary urgency. In order to test for an intrathecal immune response to HCV, we performed IEF of the first and last serum and CSF specimens of the patient and a multiple sclerosis (MS) patient as control, before and after immunoabsorption with recombinant structural and non structural HCV proteins immobilized on nitrocellulose strips (Chiron, CA, USA). Identical patterns of oligoclonal IgG were found in the first and last CSF sample of our patient. After immunoabsorption with recombinant HCV proteins, some IgG bands disappeared or became markedly faint in the last CSF sample, whereas no change was observed in IgG oligoclonal pattern of CSF from the MS patient
Conclusion: To our knowledge, this is the first report of acute myelitis developing some years after demonstration of HCV seropositivity and associated with intrathecal immune response against HCV proteins.

P707

Prevalence of chemokine receptor polymorphisms in HIV/HCV co-infected subjects

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Objective: The protective role of chemokine receptor polymorphisms has been well established in HIV infection. Still under debate is the association between the CCR5-delta32 allele and the acquisition, natural evolution or treatment response in chronic HCV infection. To date, the prevalence of chemokine receptor polymorphisms in the context of HIV/HCV-coinfection has not been extensively studied. Our objective was to determine the prevalence of 5 chemokine receptor polymorphisms in HIV/HCV-coinfected patients, in comparison with HIV-infected patients and uninfected, healthy volunteers.
Methods: The respective allele frequencies of CCR5-delta32, CCR2–64I, SDF1–3'A, and genotype distributions of CCR5–59029 and CX3CR1 were determined for 43 HIV/HCV-coinfected patients, 245 HIV-infected patients, and 158 uninfected volunteers using PCR-restriction fragment length polymorphism(RFLP)-based methods.
Results: Compared to controls and HIV-infected patients, HIV/HCV-coinfected patients did not show any difference in terms of prevalence of CCR5-delta32, CCR2–64I and SDF1–3'A alleles (respective p-values in comparison with controls (0.27; 0.24 and 1 and respective p-values in comparison with HIV-infected (0.62;

0.63 and 1). The distribution of CCR5–59029 and CX3CR1 genotypes was comparable in each group (respective p-values in comparison with controls (0.88 and 0.35 and respective p-values in comparison with HIV infected (0.28 and 0.121). The Hardy–Weinberg Equilibrium was respected for all polymorphisms in the HIV/HCV-coinfected group.
Conclusion: The chemokine polymorphisms investigated in this study are not positive selection factors for the HIV/HCV-coinfection status.

P708

Is it justifiable to withhold treatment for HCV-chronic hepatitis from drug addicts?

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Objectives: Current therapies for HCV-chronic hepatitis have a low success rate and are not well tolerated in drug addicts. In fact literature data show that 20% of these patients drop out vs. 7% of patients from general population. Our purpose was to improve adherence to therapy and to closely evaluate side-effects in our patients, particularly depression.
Methods: Patients attending addiction treatment clinics have been evaluated for HCV serology and then for their family and job stability. Furthermore, an assessment of depression was made through Beck test. Then the patients could be enrolled and treated with pegylated interferon 2b (PEG-IFN 2b) and ribavirin for 24 or 48 weeks, according to the genotype. Periodic haematological tests were performed to investigate blood abnormalities and virological response; Beck test was repeated every 3 months.
Results: Seven addiction treatment units of our area took part to the study and 41 patients were enrolled (39 males and 2 females). The population mean age was 32 (20–52) years old. The mean HCV infection duration was 7.5 years and the mean duration of addiction was 10 years. HCV genotypes distribution was as follows: genotype 1 = 8 pts, genotype 2 = 1 pt, genotype 3 = 30 pts, genotype 4 = 2 pts. Ten patients had undergone previous treatment with IFN. Up to now 20 patients (49%) have reached an end- of- treatment response (ETR), 14 pts (34%) are on treatment and 7 pts (17%) have stopped the therapy, of whom 5 pts (12%) because of adverse events and only 2 pts (5%) have dropped out. Seventeen out of 41 patients (41.5%) presented adverse events (someone had more than one): 7 pts weight loss, 3 pts nausea, 2 pts rashes, 4 pts fatigue, and 5 pts reported other symptoms (mialgyas, dizziness, panic attacks and insomnia). In 4 patients (9.8%) there was evidence of blood abnormalities (3 pts showed anemia and 1 pt reduction of platelets number).
Conclusions: In our experience, the low rate of drop-outs in our population (5%) vs 20% reported in literature is both due to a careful selection of patients and to a close clinical and psychological follow-up.

P709

Erythema multiforme related to pegylated interferon alpha 2a plus ribavirin treatment for chronic hepatitis C

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Objectives: Pegylated interferon and ribavirin combination therapy is effective and safe for chronic hepatitis C infection. Adverse affects are similar to interferon alpha and a variety of rashes including erythema multiforme have been noted. We report a case of erythema multiforme related to pegylated interferon alpha 2a plus ribavirin treatment for chronic hepatitis C virus infection.
Case: In May 2004 a 56-year-old woman was admitted to hospital because of anti-HCV positivity. The ALT and AST levels were found in normal range. HCV RNA level was found as 616619 copy/mL. Histopathological examination of biopsy material was showed chronic hepatitis by a Knodell score 9. Pegylated interferon alpha 2a 180 microgram/week and ribavirin 1200 mg/day combination therapy was started. Flu like syndrome was seen after the first dose of interferon. The patient was readmitted because of fever and rashes on thigh with pruritis 3–4 hours after the second dose of interferon. In physical examination multiple maculopapular lesions in various sizes were seen on thigh and leg bilaterally. WBC, platelet, ESR, IgE, ALT, AST, TFT, ANA, Anti-DNA, ASMA, AMA, cryoglobulin, thyroglobulin, anti-thyroglobulin. anti-TPO were searched and found in normal range. Skin biopsy was performed and erythema multiforme was confirmed by histopathological examination. The treatment was started with oral antihistaminic (fexofenadine hydrochloride 180 mg/day) and topical corticosteroid (Fluticasone propionate 0.005%). Skin lesions were regressed in 10 days.
Conclusion: Interferon alpha have been widely used to treat chronic hepatitis C virus infection and pegylated interferons are novel agents in this area. Side effects are common; they are usually minor but are problematic for a significant proportion of patients. Major adverse events can occur, but life-threatening adverse events have been rare in large surveys. Erythema multiforme can be seen after or during various infections and with administration of various drugs. In this case report, erythema multiforme probably belong to pegylated interferon alpha 2a was reported.

Bacteriology: role of selective media and identification systems

P710

Evaluation of selective culture media for *Lactobacillus*, *Bifidobacterium* and *Bacteroides* to study the intestinal flora

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Objectives: False positivity rates of selective culture media to study *Lactobacillus* (Lac) and *Bifidobacterium* (Bif) in the intestinal flora, range from 9–85% and 29–50% respectively. The aim of our study is to evaluate sensitivity and specificity of established and recently described selective culture media used for enumeration of Lac, Bif and *Bacteroides* (Bac) from faecal samples.
Methods: For Lac 8 selective media (LAMVAB, Sorbitol agar, 2 Rogosa variants and 4 MRS variants) were studied with 24 Lac strains and 14 faecal (non-Lac) strains. For Bif 3 selective media (NPNL, MTPY and RB) were studied with 22 Bif strains and 44 faecal (non-Bif) strains. For Bac 2 selective media (BBE and

Schaedler (VN) were evaluated with 31 faecal (non-Bac) strains. All plates were incubated anaerobically at 37°C for 72 h.
Results: The MRS medium, most widely used for enumeration of Lac from faecal samples, is not selective (specificity = 0%) but sustains the growth of all Lac strains tested (sensitivity = 100%). MRS with aerobic incubation improves specificity of the medium (67%) but reduces sensitivity (90%). Similar data were obtained for Rogosa medium: high sensitivity (100%) but low specificity (7%), which was increased to 50% by the addition of metronidazole. LAMVAB medium, recently described by Hartemink et al. (J. Microbiol. Meth., 1999) has the highest specificity (100%) with acceptable sensitivity (83%). The *L. acidophilus* group is not detected using this medium. NPNL, the reference medium for selective enumeration of Bif has a sensitivity of 100% but a specificity of only 32%. RB medium showed similar results. MPTY medium, recently described by Rada et al. (J. Microbiol. Meth., 2000) demonstrated a sensitivity of 95% and a specificity of 100%. Out of the selective media for Bac, BBE showed the highest specificity (100%) compared to only 48% for Schaedler (VN. BBE detects only the *B. fragilis* group.
Conclusions: Several media used for the selective enumeration of Lac, Bif and Bac allow the growth of faecal contaminants, leading to an insufficient specificity. LAMVAB is the optimal medium for Lac. MTPY is the optimal medium for Bif and BBE is the medium of choice for selective enumeration of Bac.

P711

Comparative evaluation of a new biochemical test kit for the presumptive identification of *Candida albicans*

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Objectives: Conventional methods for the presumptive identification of *Candida albicans* such as the germ tube test (GT) requires expertise, a microscope and is time consuming to perform. In this study a direct comparison was made between the GT and the new Oxoid OBIS albicans test. OBIS albicans is a rapid card-based chromogenic test that detects the presence of two enzymes specific to *C. albicans* and *C. dubliniensis*: β-galactosaminidase and L-proline aminopeptidase. OBIS albicans uses non-carcinogenic substrates and does not require the use of UV light sources.
Methods: A total of 219 fully characterised clinical strains were blind tested, comprising: *C. albicans*, including control strain ATCC 10231, *C. dubliniensis*, other *Candida* spp., *Saccharomyces cerevisiae*, *Blastoschizomyces capitus*, *Geotrichum candidum* and *Rhodotorula rubra*. All organisms were grown on Sabouraud Dextrose Agar for 48 hours at 30°C. Material equivalent to three to five 1-mm colonies was removed from the agar plates using a paddle pastette and spread across the surface of the test area. One 50 μl drop of OBIS Rehydrating Solution was added to each test area. The cards were placed in a plastic sleeve and incubated at 37°C for 60 min. Following incubation, 50 μl of OBIS NaOH Developer was added to each test area and the reaction noted. Formation of a distinct yellow colour at the point of inoculum indicated the presence of β-galactosaminidase and was therefore a positive result. 50 μl of OBIS DMAC Developer was then added to each test area and the reaction noted. A distinct magenta colour indicated presence of L-proline aminopeptidase and was therefore a positive result. A positive reaction for both enzymes provided presumptive identification of *C. albicans*. A negative reaction for either one or both enzymes indicated that the organism was not *C. albicans*. All organisms were also tested by the germ tube test.
Results: Sensitivity and specificity of OBIS albicans was 100%. *Candida albicans* and *C. dubliniensis* are phenotypically similar and both species produce the target enzymes and form germ tubes.
Conclusion: OBIS albicans performed equivalently to the germ tube test in differentiating *C. albicans/dubliniensis* from other *Candida* spp.

P712

Evaluation of chromogenic *Candida* agar

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Objectives: Several chromogenic *Candida* media have been developed to aid differentiation and identification of yeast cultures in clinical specimens, in particular, *C. albicans*, *C. tropicalis* and *C. krusei*. Differentiation is based upon reactions with chromogenic substrates and colonial morphology on the medium. In this study, direct comparison was made of a new medium, Oxoid Chromogenic Candida Agar (OCCA), CHROMagar Candida (CHROM) and Candida ID (CID).
Method: 214 fully characterised clinical cultures were tested blind, comprising strains of: *C. albicans*, *C. krusei*, *C. kefyr*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. lusitaniae*, *C. dubliniensis*, *C. guilliermondii* and other yeast species. Mixed cultures comprising *C. albicans* mixed with one other *Candida* spp. were also tested. Using the diminishing streak technique, each pure and mixed culture was plated onto OCCA, CHROM and CID. The plates were incubated for a minimum of 24 h and a maximum of 48 h at 30°C. Plates were examined for growth and morphology at both time intervals.
Results: After 24 h OCCA revealed a sensitivity and specificity of 100% and 97.8% respectively for *C. albicans* (n = 64), compared to 98.4% and 98.5% for CHROM and 100% and 96.3% for CID. All media yielded false positive results with *C. dubliniensis*. Sensitivities for *C. tropicalis* (n = 30) after 48 h incubation for OCCA and CHROM were 100%. Specificities were 100% and 94.7% respectively. *Candida glabrata*, *C. dubliniensis* and *Saccharomyces cerevisiae* yielded false positive *C. tropicalis* results on CHROM. Grouped sensitivity and specificity for *C. tropicalis*, *C. guilliermondii*, *C. kefyr* and *C. lusitaniae* on CID was 100% and 98% respectively. CID yielded 3 false positives in this group including *S. cerevisiae*, *C. famata* and *Rhodotorula rubra*. The sensitivity and specificity for *C. krusei* (n = 30) after 48 h on OCCA was 96.7% and 100% respectively, with 1 false negative, compared to 100% for both on CHROM. The grouped sensitivity and specificity for other yeast species on CID after 48 h was 90.7% and 98.3% respectively, yielding 8 false negatives and 2 false positives.
Conclusion: The new Oxoid Chromogenic Candida Agar (OCCA) is recommended for the differentiation and presumptive identification of clinically important *Candida* spp. After 24 h incubation, OCCA detected and differentiated *C. albicans* more reliably than CHROM. After 48 h incubation, both OCCA and CHROM were more specific than CID.

P713

Usefulness of selective media for isolation of *Aeromonas* spp. from stool samples

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Objectives: Convincing evidence suggests that some aeromonads do cause gastroenteritis. Seriousness vary from acute watery diarrhoea up to chronic problems. Diarrhoea caused by *Aeromonas* spp. are located principally in warmer regions, where

aeromonads are accounted among the major enteric pathogens. Nevertheless it still remains unclear, how far aeromonads could be involved in diarrhoeal disease in our region. Isolation from stool samples is difficult and successfulness of isolation depends on the cultivation method used. Use of an appropriate selective media for isolation of aeromonads is necessary.

Methods: All *Aeromonas* isolates were obtained from stool samples during a routine survey in 2003–2004. Two cultivation methods were compared. Beside Deoxycholate Citrate agar, the common medium for isolation of enteric pathogens, CIN agar (Cefsulodin Irgasan Novobiocin) was included in the routine processing of stool samples in 2003. In 2004 *Aeromonas* agar (Bile Salt Irgasan Brilliant Green), on which also aeromonads susceptible to ampicilin are able to growth, was added.

Results: Two years of microbiological examination of stool samples from patients suffering from acute gastroenteritis proved, that *Aeromonas* isolates are not so rare. 49 aeromonads were isolated from 11 371 stool samples in 2003, whereas in 2004 already 116 aeromonads were obtained from 11 695 stool samples. From all bacteriologically positive stool samples (any diarrhoeal agent) aeromonads were detected in 2.2% (2003) resp. 4.5%(2004) cases. *Aeromonas* agar was more efficient and considered to be better selective medium than CIN agar. Unlike CIN agar, detection of positive oxidase reaction was possible directly from *Aeromonas* agar. In comparison with aeromonads other enteric pathogens as *Salmonella*, *Campylobacter* and *Yersinia* were more frequent. On the other hand only in 29% cases aeromonads were isolated together with another enteric pathogens mentioned above.

Conclusion: Very young children or old people were often predisposed to an acute gastroenteritis due to aeromonads. We noticed, that aeromonads were repeatedly isolated from patients, who had been treated for dyspepsia before (10% of *Aeromonas* isolates). Change of the use of selective media for aeromonads significantly increased the number of isolates and thus it contributed to the clarification of cases of unknown diarrhoea. Our project is being supported by IGA of Ministry of Health of the Czech Republic, Id.code: NR/8011–2.

P714

Identification of Gram-negative bacilli in BD Phoenix™ using the new low inoculum mode

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Objectives: Identification of gram-negative bacilli using automated microbiology systems has been a common and accepted laboratory practice for many years. The systems that provide rapid results typically require the user to make an inoculum concentration that is equivalent to a 0.5 McFarland standard. Occasionally, due to an insufficient number of colonies on the primary isolation media, this inoculum requirement is not readily achieved . In order to address these cases, a low inoculum mode has been developed for The BD Phoenix™ Automated Microbiology System (BD Diagnostics). The low inoculum mode utilizes the same panels as the regular system, but contains new databases and only requires an inoculum density equivalent to a 0.25 McFarland standard. This study investigates identification accuracy of the Phoenix System, set to low inoculum mode, for frequently encountered and clinically significant gram-negative bacilli.

Methods: A total of 866 gram-negative bacilli, comprised of 15 genera, were tested in the BD Phoenix System set on low inoculum mode (PSLI). The majority of strains had been biochemically referenced using classical methods, while a small portion were run in parallel using the BD Phoenix System on regular mode (PSRM) as the reference. The PSRM was set up according to the users manual and the PSLI was set up using the new BD PhoenixSpec nephelometer at an inoculum density ranging from 0.2 to 0.3 McFarland units.

Results: Of the total 866 isolates tested, the PSLI correctly identified 839 (96.9%) to the species level and 851 (98.3%) to the genus level. There were 15 cases (1.7%) which listed 2 or 3 choices under instrument ID requiring a supplemental test to produce a final ID. The PSLI yielded 18 (2.1%) incorrect results to the species level and 6 (0.7%) to the genus level, while 9 (1.0%) strains resulted in no identification. Average time to results came out as 4.58 hours for the 839 strains that produced a correct ID to species level.

Conclusion: The newly developed low inoculum mode of the BD Phoenix System provides acceptable performance for the identification of routine and clinically significant gram-negative bacilli while using half of the normal inoculum density.

P715

Performance of BD Phoenix™ in low inoculum mode for the identification of frequently isolated Gram-positive cocci

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Objectives: Gram-positive cocci from the genera *Staphylococcus*, *Enterococcus*, and *Streptococcus*, make up a significant portion of the frequently isolated bacteria to be identified in the clinical microbiology laboratory. Because the colonies that these organisms form on a primary isolation plate are often few and small, it is not unusual to subculture for next day testing. This is done to ensure enough growth is present to make a suspension equivalent to a 0.5 McFarland standard – generally the minimum required for ID systems. Utilizing a newly developed low inoculum mode, and databases, the BD Phoenix™ Automated Microbiology System (BD Diagnostics) provides the user with an option of running isolates at an inoculum density equivalent to a 0.25 McFarland standard. The panels that are used with the low inoculum mode are the same as those used with the regular system. This study examines identification accuracy of frequently isolated gram-positive cocci using the BD Phoenix System configured to low inoculum mode.

Methods: Using the new BD PhoenixSpec nephelometer, 730 gram-positive cocci were set up in Phoenix GP panels at an inoculum density of 0.2–0.3 McFarland units and tested in low inoculum mode (PSLI). The isolates were comprised of 14 species which were referenced by a combination of conventional testing and the BD Phoenix System set at regular mode (PSRM).

Results: Accuracy of identification with PSLI was determined to be 97.1% (709/730) to the species level and 99.6% (727/730) to the genus level for the isolates tested. Incorrect identification rates were found to be 2.6% (19/730) to species level and 0.1% (1/730) to genus level. For 1.0% (7/730) of the isolates the PSLI did not give a final ID but required a supplemental test to separate 2 or 3 organisms listed under instrument ID, and for 0.3% (2/730) of the isolates no identification was obtained.

Conclusion: Frequently isolated gram-positive cocci, tested at low inoculum density, were accurately identified in the BD Phoenix System run in the newly developed low inoculum mode.

P716

Evaluation of the new Gram-negative identification card for the VITEK 2

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Objective: The objective of this study was to evaluate the new Gram-negative identification card (GN) for the VITEK® 2 with API® 20E and API® 20NE as the reference method. The three components of the study were reproducibility and Quality Control (QC) testing, challenge testing, and clinical and stock isolate testing. The performance criteria for the challenge and clinical isolates with the GN card were set at >96% overall correct identification, >11% low discrimination, >2% incorrect identification, and >4% unidentified organisms within a >95% confidence interval. The evaluation was performed at three laboratory sites.
Methods: Ninety-four (94) challenge and 562 clinical and stock isolates were included in this study. The same challenge organisms were tested once at all three sites. Reproducibility testing included 7 different strains that were tested for 10 days, and QC was performed for 20 days with 7 ATCC® species. All microorganisms were inoculated to the GN card and either an API® 20E and API® 20NE strip, depending on the suspected identification of the microorganism. Appropriate QC was performed on the GN card and the reference methods according to the manufacturer's package insert. Identification was assessed to be correct when the microorganism was identified by the GN card as the single first choice identification to the genus and species level, or to one of two closely related species of the same genus, or when the need for supplemental tests was specified owing to low discrimination.
Results: The challenge microorganism testing with the GN card showed 98% overall correct identification, 2.5% low discrimination ID, 2.0% incorrect ID and 0% unidentified organisms with the GN card. The clinical strains demonstrated 96.8% overall correct ID, 6.4% low discrimination ID, 3.0% incorrect ID and 0.2% unidentified microorganisms. Of the 3% (17/562) clinical isolates that were defined as giving incorrect ID only 3 strains were incorrect at the genus level. All QC testing yielded expected results within a 95% confidence interval except for three microorganism and biochemical combinations.
Conclusions: The results of the reproducibility, challenge and clinical isolates tested indicate that the GN card meets all performance criteria within a >95% confidence interval.

P717

Analysis of the comparative work-flow and accuracy of the VITEK®2 Compact and the ATB Expression® system

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Objectives: The aim of this study was to analyse the impact of introducing into our routine microbiology laboratory the VITEK®2 Compact (V2C), bioMérieux France, a new automated identification (ID) and susceptibility testing system (AST). Primary focus was to measure potential gains in laboratory productivity and operating costs. Secondary focus was to evaluate the quality of ID/AST results analysed by the system test validation softwares.
Methods: The evaluation took place in our 532-bed hospital from Sept to Oct 2004. 400 routine isolates, except fastidious bacteria: 57% Gram-negative, 40% Gram-positive and 3% yeasts were tested using our in-house method, ATB Expression (ATBEx) in parallel with the V2C. This represented 91% of laboratory activity, from 370 various clinical samples. Data were collected on 4 parameters:
Productivity: Independent analysis and chronometric time measurement of the general work process: from specimen reception, ID/AST set-up to result validation.
Cost: The total ID/AST cost including technician time (cost per hour) was calculated.
ID Accuracy: Tests were performed in parallel and discordant results were tested by molecular technique.
AST Confidence: Comparison was made of ATBEx Expert software and V2C Advanced Expert System™ (AES) for result agreement. The Medical microbiologist expertise provided final results on any discordant results.
Results: The global process time gain with the V2C was 12 hrs due to a) the more rapid ID/AST result reporting time (mean detection of 6 hrs for V2C and 18 hrs for ATBEx), b) the faster manipulation time (1.5 min vs 5.5 min), c) the faster AES validation. The overall operating cost was reduced by 15% with V2C.The overall ID agreement between the systems was 94%. Global agreement with AST expertise was more than 95% with both systems.
Conclusions: We underline the benefits of the V2C in the setting of a medium sized microbiology laboratory. V2C compared well to our robust ATBEx overnight method and provided time and cost gains. The V2C AES validation tool allowed technicians to perform first level ID/AST validation confidently before biologist final approval. Further investigations are planned to study clinical impact of rapid ID/AST reporting with this innovative instrument in a unit of our hospital.

P718

Comparison of the identification performance of the VITEK 2 system to the PHOENIX™ system under laboratory conditions

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Objectives: It is essential to obtain fast and reliable identification of clinical isolates to adjust patient drug treatment. To reach this goal, different automated systems are now available on the market. Two of them were evaluated in our routine laboratory: the VITEK 2 system, equipped with the new colorimetric menu, and the PHOENIX™ system.
Material and methods: In total, 501 clinical isolates were tested; 247 were Gram-positive and 254 Gram-negative. Half consisted of fresh clinical isolates and the rest, stock collection strains. All were subcultured on Columbia Blood Agar and tested in parallel. In case of result discrepancies, the strains were retested on both systems. If the discrepancy remained, supplemental tests were performed including molecular identification methods to define the reference identification. In the analysis, the repeats were also considered for the performance calculation.
Results: The performance including repeat results is shown in the table. Overall, 81 (16%) strains were repeated due to discrepant results at the first test: 13 (16%) due to incorrect answer on both systems, 9 (11%) and 59 (73%) due to VITEK 2 and PHOENIX systems, respectively. A higher level of discrepancies with the PHOENIX system was observed for the identification of Gram-positive and stock collection isolates at the first test.

Method		Total	Correct ID		Mis Identification		No Identification	
		#	#	%	#	%	#	%
GP	VITEK 2	247	236	95.6	4	1.6	4	1.6
	PHOENIX	247	232	93.9	13	5.3	2	0.8
GN	VITEK 2	254	243	95.7	10	3.9	0	0.0
	PHOENIX	254	237	93.3	15	5.9	1	0.4

Conclusion: Overall, the VITEK2 system using the new colorimetric menu gives better correct identification than the PHOENIX™ system after the first test. With regard to Gram-positive and stock collection isolates, its performance was clearly superior.

P719

The performance of the new streptococci panel for BD Phoenix™

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Introduction: Identification of streptococci (beta-hemolytic and viridans streptococci) can cause problems with conventional biochemical test or identification kits. We investigated the performance of a new streptococci identification panel for the automated system BD Phoenix.
Methods: Ninety-two clinical isolates of streptococci from sterile body sites were investigated. Thirty-nine were beta-hemolytic streptococci (Lancefield group A, B, C, G), 17 pneumococci, and 36 viridans streptococci. Reference methods were latex agglutination for beta-hemolytic streptococci, optochine susceptibility and bile-solubility for pneumococci and 16S sRNA and sequencing for all other streptococci, including some pneumococci. The Phoenix panels were inoculated from a fresh culture and processed as advised by the manufacturer.
Results: Seventy-seven (84%) strains were identified correctly, including all beta-hemolytic streptococci, 12 (13%) strains were identified incorrectly, and 3 (3%) strains could not be identified at all by the system. Only one third (4/12) of the *S. mitis* strains, which is an important pathogen in hematological patients, were identified correctly.
Conclusion: Beta-hemolytic streptococci and pneumococci were reliably identified by the automated system. Identification of viridans streptococci esp. *S. mitis* is less reliable. Therefore further attention is needed to better identify viridans streptococci.

P720

Evaluation of LIAISON® treponema screen, a novel recombinant antigen-based chemiluminescence immunoassay for the diagnosis of syphilis

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Objectives: The laboratory diagnosis of syphilis is a crucial point in the epidemiological and diagnostic evaluation of the disease. In this study we compared the performances of a new chemiluminescent assay, LIAISON® Treponema Screen, with those obtained by ELISA, TPHA and WB methods.
Methods: Study group. 2500 serum samples were obtained from two blood banks in France (Tours and Lille). 131 serum samples were from patients attending the STD outpatients clinic of the St Orsola Hospital in Bologna, Italy, and suffering from different stages of syphilis.Commercial serologic tests. The following tests were used: Syphilis screening EIA, (Radim, Pomezia, Italy), and TPHA (AlfaWasserman, Milan, Italy). Testing was performed following the manufacturer's instructions and all the specimens showing an equivocal result were tested twice. WB. A native 'home made' WB was used. An IgG test was considered positive when at least three out of the four following *T. pallidum* antigens were present: TpN15, TpN17, TmpA and TpN47. LIAISON® Treponema Screen. LIAISON® Treponema Screen (DiaSorin, Saluggia, Italy) is a qualitative fully automated method for determination of specific antibodies to *T. pallidum* in human serum or plasma. This new method is a one step sandwich chemiluminescence immunoassay (CLIA).
Results: Blood donors serum samples. All the blood donors samples were screened by CLIA: 2 sera showed a border-line result. These 2 samples were further analysed by all the others methods and they were scored as negative. The specificity of the CLIA was 99.92% (95% CI: 99.71–99.99%). Syphilis serum samples.128 out of 131 samples were identified as positive by the CLIA test; 2 samples showed a border-line result and 1 sample was negative by the chemiluminescent assay. ELISA test showed the following performance: 125 sera were positive, and 6 samples were scored as border-line. Finally, all the 131 samples were WB positive, whereas TPHA identified 124 positive specimens. The concordance between CLIA and ELISA was 99.24% (95% CI: 95.82–99.98%).

Stage of syphilis	ELISA	CLIA	TPHA	WB
Primary (n = 7)	4 3 border-line	6 1 border-line	3	7
Secondary (n=31)	31	31	31	31
Latent (n = 77)	74 3 border-line	75 1 border-line	74	77
Tertiary (cardiovascular) (n = 5)	5	5	5	5
Tertiary (neurological) (n = 6)	6	6	6	6
Congenial syphilis (n = 5)	5	5	5	5
Total number (n = 131)	125 6 border line	128 2 border-line	124	131

Conclusions: The LIAISON® Treponema Screen assay showed to be a very sensitive and specific method for the laboratory diagnosis of syphilis and it can be considered a good alternative to the conventional tests.

P721

Evaluation of the BINAX-Now urinary antigen test for establishment of pneumococcal aetiology of adult community-acquired pneumonia with bacteraemia

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Objectives: Community-acquired pneumonia (CAP) is a serious problem and *Streptococcus pneumoniae* is the leading cause. Recently recommended strategies include optimization of etiological diagnosis because of emerging resistance. For this purpose the development of tests that can rapidly and reliably identify the causative agent has been encouraged. The aim of the present study was to evaluate the use BINAX-Now urinary antigen test (BNUAT) in clinical practice of a general hospital.
Material and methods: From November of 2001 through October of 2004, 1161 patients with suspected CAP were analysed with BNUAT; this test was solicitated according to clinician criteria of results were compared with isolation of *Streptococcus pneumoniae* from blood culture (77% of the patients).
Results: BNUAT was positive in 174 cases (15%), negative in 982 (84.6%) and not conclusive (weak reactivity) in 5 (0.4%). We do not evaluate the specificity of the technique because we have not a valid gold standard, specially in patients ongoing antibiotic therapy. The sensibility of the method: of the 894

patients with blood culture was of 70% (*Streptococcus pneumoniae* was isolated in 26 patients, having positive antigen 18 of them). In the same period of time *Streptococcus pneumoniae* was isolated from blood cultures of other 54 patients with pneumonia that had not been requested BNUAT.
Conclusions: Although recently it has been recommended strategies for CAP diagnosis include rapid urinary antigen tests we believe that the sensitivity is not high in bateremic patients; also it is not clear in which patients this assay is needed. In our experience BNUAT was only solicitated in 26 out of 80 patients (32.5%) with pneumococcal bacteraemia. In addition cost-effectiveness analysis is needed.

P722

Evaluation of the combined use of Statens Serum Institut enteric agar and the LOUIS test screening protocol in a Scottish microbiology laboratory

G. Wilson *(Stirling, UK)*

Objectives: Statens Serum Institut (SSI) enteric agar has previously been reported as a suitable single plate agar for the isolation of enteric pathogens except *Campylobacter* spp and anaerobic bacteria. This study sought to compare the combined use of SSI enteric agar and the LOUIS test screening protocol with that of a more traditional 3-plate system, for the detection of *Salmonella* and *Shigella*, employed in the microbiology laboratory of a Scottish District General Hospital.
Methods: Over a 6-month period 780 outpatient stool samples were compared by both methods.The traditional method consisted of direct plating of faecal material onto MacConkey and DCLS agars and after overnight selenite enrichment on to XLD agar (all Oxoid, UK). Suspect colonies were identified using overnight API 20E identification strips (bioMerieux, France). For the SSI method 10 microlitres of a faecal suspension was inoculated onto SSI agar (Statens Serum Institut, Denmark) and after selenite enrichment 10 microlitres to a further SSI plate. Suspect colonies were screened by the 3 h LOUIS test protocol. The API rapid ID32E gallery (bioMerieux, France) was used for full identification. Polyvalent and group specific sera (Murex, England) were used for serological identification.
Results: The traditional method resulted in a total of 205 identifications. Duplicate identifications from MacConkey and DCLS were counted as one for purposes of analysis. The SSI method resulted in 149 identifications. The difference was statistically significant ($p < 0.001$). Both methods detected 31 *Salmonella* strains after enrichment but differed significantly ($p = 0.027$) on direct detection (SSI = 20, traditional = 12). One isolate of *Shigella sonnei* was detected by both methods and in addition the SSI agar allowed the isolation of a *Vibrio parahaemolyticus* and a *Yersinnia enterocolitica* without the need for additional agars. The application of the LOUIS test protocol on SSI isolates enabled 20 *Salmonella* and 1 *Shigella sonnei* to be presumptively reported 3 h after direct isolation and a further 11 *Salmonella* after enrichment. Only 2 false positive *Shigella* isolates required full identification after LOUIS test screening.
Conclusions: The use of SSI enteric media can significantly reduce the amount of false positive workload. The combined use with the LOUIS test protocol can effectively screen out false positives and allow presumptive reporting of most enteric pathogens 3 h after isolation.

P723

Matrix assisted laser desorption/ionisation time-of-flight mass spectrometry provides fast and reliable identification of *Staphylococcus haemolyticus*

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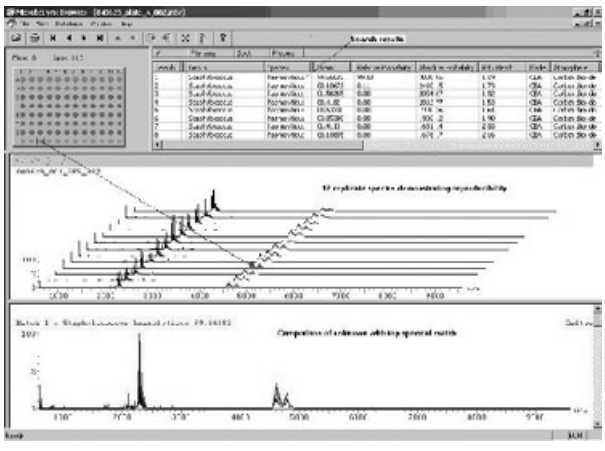
Objective: *Staphylococcus haemolyticus* is an emerging pathogen more resistant to antibiotics than most MRSA. It causes symptoms indistinguishable from *Staphylococcus aureus* infections and is responsible for some outbreaks in orthopaedic and neonatal wards mimicking MRSA infection. Analysis of these isolates can mistakenly be perceived and identified as low-grade pathogenic coagulase negative staphylococci. However, a fast and reliable identification is needed in order to institute appropriate antibiotic therapy. To achieve this we have successfully used the ''Peterborough Collection'' of clinical isolates to establish a MALDI-TOF MS database for *Staphylococcus haemolyticus*.
Method: Intact *Staphylococcus haemolyticus* cells are transferred from a culture plate to a MALDI target plate and overlaid with the MALDI matrix 5-Chloro-2-mercaptobenzothiazole. The co-crystallised sample is then irradiated with a N2 laser and the resulting plume of positive ions separated using time-of-flight mass spectrometry. This produces a characteristic mass spectral fingerprint pattern, which forms the basis of identification against a database containing representative spectra of the species. The MALDI-TOF MS technique also allows for high sample throughput and rapid identification of isolates, since the collection and analysis of spectral data against a substantial database requires ~1.5 hours for a 96 well MALDI target plate.
Results: In this study 71 (93.4%) of the 76 clinical strains of *Staphylococcus haemolyticus* were identified correctly against a database of more than 3600 spectral entries representing over 500 different bacterial species, Figure 1. Furthermore conclusive identification was confirmed for the majority of these strains since all 8 top matches were correct to species. Comparison of three misidentified test spectra with their corresponding spectral matches demonstrated unique high mass ions exclusive to the matching *S. haemolyticus* spectra. Therefore filtering the matches for these ions it is possible to correctly identify 74 (97.4%) of the 76 clinical strains to species level.



Conclusion: MALDI-TOF MS has successfully identified 97.4% clinical isolates of *S. haemolyticus*, demonstrating the techniques provides fast reliable identification of this emerging pathogen.

P724

External quality assessment for microbiology – a review of participant performance with coryneform bacteria

C. Walton *(London, UK)*

Objectives: To assess the results from clinical diagnostic laboratories taking part in the United Kingdom National Quality Assessment Service general bacteriology scheme with EQA specimens containing coryneform and other Gram positive bacteria increasingly associated with infections in the immunocompromised, and to compare performance with repeat specimens.
Methods: Quality assessment of general bacteriology (n = 781; 301 UK, 480 overseas) for the isolation and identification of bacterial pathogens, was performed on isolates including *Erysipelothrix*, *Rhodococcus*, *Bacillus*, *Arcanobacterium*, *Rothia* and *Nocardia*.
Results: The overall level of performance since 1990 with specimens containing coryneform bacteria was 71% of participants' reporting a correct result; which compares with 94% for all identification specimens in the general bacteriology scheme over the same period. A correct result was assigned where participants' had reported identification to at least genus level. Over a series of specimens, participants' identification to species level was met with varying success. *Erysipelothrix* were fully identified by participants with an average success rate of 63% (range 72–88%); *Rhodococcus*, 69% (range 59–79%); *Arcanobacterium*, 62% (range 58–66%); and *Nocardia*, 17% (range 11–24%). For organisms distributed only once, the percentage of participants fully identifying a *Bacillus cereus* was 52% and a *Rothia mucilaginosa*, 47%.
Conclusions: These data provide an interesting insight into overall performance with the coryneform bacteria in diagnostic microbiology laboratories. The level of performance seen with isolates such as *Erysipelothrix* was generally better than with others, such as *Nocardia* spp. or *Rothia mucilaginosa*. Performance over time with repeat specimens was variable however, a significant improvement was seen with *Arcanobacterium*. More unusual organisms including coryneform bacteria are increasingly implicated in infections of the immunocompromised patient. The Gram stain and other simple phenotypic tests are critical in differentiating these organisms, before a commercial identification system is considered, and laboratories need to be aware of some of the pitfalls in identification. EQA has an important role as an educational tool available to participating laboratories, highlighting some of the common problems faced in routine diagnostic testing and where appropriate, providing some practical advice.

P725

Evaluation of the ATB STREP 5 and ATB ENTEROC 5 tests for Gram-positive pathogens antimicrobial susceptibility determination

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Objectives: The aim of this study was to evaluate ATB STREP 5 and ATB ENTEROC 5 tests (bioMerieux) for antimicrobial susceptibility determination of streptococci and enterococci.
Methods: A total of 200 clinically significant Gram-positive bacterial pathogens were included in the study. The isolates belonged to two *Streptococcus* species (*S. pneumoniae* n = 79 and *S. pyogenes* n = 21) and two species of *Enterococcus* (*E. faecalis* n = 7, *E. faecium* n = 93) collected from patients in different medical centres in Poland. The results of the analyses were compared with those obtained by reference microbiological methods in accordance with the NCCLS guidelines. The enterococcal isolates were tested for the presence of the HLAR phenotype using the agar screening method. Vancomycin-resistant enterococci were identified by agar screening procedure and positive results of the test were confirmed by PCR detection of vanA and vanB genes. Penicillin-resistant *S. pneumoniae* strains were identified by two methods: the oxacillin disc and broth dilution methods. The E-tests were used for determination of penicillin and cefotaxime MICs to *S. pneumoniae*. Characteristic parameters of the automatic methods of drug susceptibility determination were defined. *E. faecalis* ATCC 29212, *E. faecalis* ATCC 51299, *S. pneumoniae* ATCC 49619 strains were used as reference strains.
Results: A high correlation was found between the results obtained by the ATB STREP 5 and ATB ENTEROC 5 and by the dilution reference methods. The total concordance of resistance categories was 99% for streptococci and 98.5% for enterococci. The concordance of MICs values for *S. pneumoniae* determined by ATB STREP 5 and E-tests was 98.7% for penicillin and 97.4% for cefotaxime. For enterococci 98% accuracy, 100% sensitivity and 97.8% specificity was achieved for HLAR, 100% accuracy, specificity and sensitivity for VanA and 97% accuracy, 98.8% sensitivity and 96.3% specificity for VanB.
Conclusion: The ATB STERP 5 and ATB ENTEROC 5 tests represent an accurate and acceptable means for antimicrobial susceptibility testing of clinically relevant Gram-positive cocci.

P726

Evaluation of a new chromogenic agar medium for isolation and identification of group B streptococci from clinical samples

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Objectives: Isolation procedures for *Streptococcus agalactiae* (Haemolytic streptococcus Group B) are routinely performed in many clinical laboratories. *S. agalactiae* is particularly sought in genital samples from ante-natal patients due to the risk of transmission to the neonate during delivery. The aim of this study was to evaluate the effectiveness of a new chromogenic medium for isolation of *S. agalactiae* from genital samples.
Methods: Culture plates of a new chromogenic medium, Strepto B ID, were kindly provided by bioMérieux. A total of 134 swabs routinely referred to our department for ante-natal screening were tested. All swabs were either high vaginal or endocervical swabs from pregnant women. Each swab was emulsified in 1 ml of sterile physiological saline and a 100 μl sample of the resulting suspension was then inoculated onto both Columbia blood agar and Strepto B ID. Cultures on Blood agar were incubated for 20 h anaerobically at 37°C and cultures on Strepto B ID were incubated for 20 h in air at 37°C. 112 of the 134 swabs were also enriched overnight in selective Todd Hewitt broth and cultured onto the same media under the same conditions. Haemolytic colonies on Blood agar and red colonies on Strepto B ID medium were identified using latex agglutination and standard biochemical tests.
Results: A total of 20 strains of Group B streptococci were recovered in this study using any medium (19 on direct culture and one strain via enrichment). All of these strains were isolated as red colonies on Strepto B ID. Of the 20 strains, 19 strains were isolated using Blood agar. A total of 24 additional haemolytic colonies required further investigation on Blood agar but were shown to be species other than Group B streptococci. In contrast only one false positive strain, *Streptococcus equisimilis*, generated red colonies on Strepto B ID.

Conclusions: Strepto B ID was more sensitive and more specific than Blood agar for isolation of Group B streptococci. All strains of Group B streptococci formed distinct red colonies that were very easy to differentiate from other flora.

P727

Accelerated blood culture diagnostic using the bioMérieux Vitek 2™

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Objectives: Identification (ID) and antimicrobial susceptibility testing (AST) from positive blood cultures normally require overnight culture. We evaluated a method to obtain ID and AST within 1 day.
Methods: After detection of bacterial growth, 10 ml of each blood culture bottle (Becton Dickinson Bactec™) was centrifuged thrice (10 min 500 *g* to remove erythrocytes, 2 × 5 min 2500 *g* to remove the supernatant). A suspension with 0.9 McFarland was then inoculated into the bioMérieux Vitek 2. Results of direct ID and AST were compared to those obtained from agar cultures according to manufacturers guidelines.
Results: 346 bottles containing 198 gram-positive cocci and 148 gram-negative rods were investigated during a period from December 2003 to July 2004. Identification and AST were excellent for gram-negative rods and enterococci, whereas direct identification of staphylococci required confirmation by other rapid tests (e.g. slide agglutination for clumping factor). AST results showed major discrepancies for 0.2% (gram-negative rods) to 1.2% (gram-positive cocci) of all tested antibiotics.
Conclusion: This method is suitable for same day ID and AST of typical blood cultures isolates. While gram-negative bacilli and enterococci were determined with high accuracy, differentiation between *S. aureus* and coagulase-negative staphylococci requires complementation by additional techniques.

16S rRNA

P728

Evaluation of QIAamp DNA min kit for removing of inhibitors in detection of *Cryptosporidium parvum* oocysts in water samples by a nested PCR assay

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In recent years, there has been a dramatic increase in the occurrence of waterborne disease outbreaks caused by the *Cryptosporidium parvum*, and presence of this protozoan parasite in drinking water supplies is a significant health problem faced by the water industry. Therefore regulatory agencies are urging that source and finished water be screened for this organism. A new strategy for detection of *Cryptosporidium parvum* oocysts in water samples is PCR–based techniques. In this study a nested–PCR assay was designed for the specific amplification of a 199 bp DNA fragment of the gene encoding the hsp70 of *Cryptosporidium parvum* oocysts. In order to prevent the inhibition of PCR amplification by substances contained in water samples, three DNA purification methods including QIAamp DNA min kit, InstaGene Matrix and MagExtractor–Genome were compared in concentrates of tap water samples spiked with the oocysts. After determining the most efficient purification technique, the efficiency of QIAamp and immunomagnetic separation for nested–PCR assay of various environmental water samples was compared. The results show that QIAamp provide a useful and rapid mean for removing PCR inhibitors to facilitate screening clean water samples (turbidity < 2 NTU) to detection *Cryptosporidium parvum* oocysts, by nested-PCR. It seems that QIAamp purification- nested PCR assay is a sensitive, rapid and cost effective method for detection of *Cryptosporidium parvum* oocysts in clean water samples.

P729

Amplification of conserved 16S rDNA sequences in the diagnosis of subdural empyema complicating sinusitis

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Objectives: To demonstrate the usefulness of broad-range PCR amplification and sequencing of 16S rDNA in the diagnosis of culture-negative subdural empyema (SDE), complicating sinusitis.
Methods: DNA was extracted from sinus discharge and SDE samples. Broad-range amplification of bacterial DNA was carried out using primers aimed at the 16S rRNA gene. PCR amplicons were directly sequenced and resulting sequences were analysed using BLAST analysis.
Results: Case1: a 12-year-old male child was admitted to the pediatric ward because of severe headache accompanied by fever (400°C) and vomiting. Bilateral ethmoid and maxillary sinusitis was demonstrated by CAT scan without brain involvement. After 6 days of treatment with intravenous ceftriaxone and metronidazole, he developed hemiplegia, aphasia and disorientation. CAT scan demonstrated SDE. The SDE and sinuses were drained. Subsequent course was uneventful. Case 2: a 15-year-old male, presented with headache, fever and nasal discharge. He had been treated with oral amoxicillin-clavulanic acid for several days before admission. CAT scan demonstrated pansinusitis. He received intravenous ceftriaxone and metronidazole. Three days later the patient developed focal convulsions. CAT scan demonstrated a large SDE. The empyema and sinuses were drained. No pathogens were demonstrated by Gram stain and aerobic and anaerobic cultures obtained from both patients. Broad-range 16S rDNA PCR resulted in positive amplicons in both patients' SDE samples. Direct sequencing of the positive amplicons identified nucleotide sequences specific to *S. intermedius* in case 1, and to *S. Pyogenes* in case 2.
Conclusion: Intracranial infections complicating sinusitis are not common, but may be devastating. Microbiological diagnosis is complicated by a wide range of causative pathogens and jeopardized by the fact that many patients are already treated with antibiotics at presentation. Broad range PCR sequencing provides a rapid and accurate methodological solution in cases of partially-treated bacterial infections of unknown etiology, giving clues to the etiologic agents and probably better guide antimicrobial therapy.

P730

Application of 16S rRNA PCR and DGGE to demonstrate differences in local bacterial colonisation within a single chronic venous leg ulcer

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Objectives: Chronic wounds represent an enormous, but often neglected health care problem. Pressure sores, venous leg ulcers and diabetic foot wounds require long-term professional care and are associated with a significant socio-economic impact. It is estimated that around 50–60 thousand patients in Denmark suffer from wounds needing professional treatment, with annual costs to the Danish healthcare sector of £100–200 million. Wound healing represents an extremely complex physiological process, which is often detrimentally influenced by the presence of colonizing bacteria (even in clinically non-infected wounds). Moreover, in polymicrobial wounds they may act synergistically to the further detriment of wound healing. The purpose of this study was to investigate local differences in bacterial colonisation within a chronic wound.
Methods: In this investigation, multiple biopsy and swab samples were obtained as part of skin graft operations performed on chronic venous leg ulcers in order to study microbial diversity. The wound was sampled prior to excision of the whole wound bed. Sampling was undertaken at multiple locations across the wound and at the wound edge. Swab samples and biopsies were subjected to standard culture analysis and 16S rRNA PCR, denaturing gradient gel electrophoresis (DGGE) and sequencing.
Results: Within the wound samples DGGE identified the major wound microflora components and established the extent of local differences in bacterial diversity. Confirmatory sequence analysis demonstrated that *Staphylococcus aureus* was maintained across the wound area, but differences in the occurrence of catalase-negative *Staphylococcus* species an *Escherichia coli* were detected.
Conclusion: This ongoing investigation has verified DGGE as a powerful, novel tool for use in the elucidation of the clinical microbiology of a chronic disease state.

P731

Ribosomal DNA sequencing: experiences from use in the Danish national reference laboratory for identification of bacteria

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Objectives: Diagnostic tools for identification have increased dramatically in the last decade. Sequencing of genes coding for rRNA have revolutionized the insight in phylogeny and taxonomy of bacteria as it has put new demands for the service given by national reference laboratories for identification of bacteria. Experiences relating to phenotypical as well as 16S rDNA sequencing of the first 175 strains examined are presented.
Methods: Submitted, clinically important, bacterial strains, from the years 2002 and 2003, difficult to species designate were, in addition to phenotypic characterization, examined by partial 16S rDNA sequencing. Phenotypic characterization of strains were based on internationally accepted guidelines. For molecular analysis, DNA was extracted from strains and analysed by real-time PCR of part of the 16SrRNA gene. Amplicons produced were DNA sequenced and a BLAST search in the NCBI GenBank was done. Obtained results were compared.
Results: Of the 175 strains examined, approximately 2/3 were Gram-positive and 1/3 Gram-negative, respectively. One fifth of strains were anaerobes, while 4/5 were either facultative anaerobic or aerobic strains. Most strains showed methodological agreement at species or genus level. Methodological disagreements were relatively rare. In 1/6 of strains valuable information was obtained from sequencing results, while for some strains identification was based primarily on the phenotypic results. Only few strains could not be clearly identified by either method. A very large number of strains representing taxons ranging from facultative anaerobic to aerobic and anaerobic species and genera, as well Gram-positive as Gram-negative, were successfully examined. Of the submitted strains many have only rarely been encountered as human pathogens.
Conclusion: Thus, genotypic identification may result in recognition of hitherto seldomly or unrecognized bacteria as human pathogens, which will lead to a deeper understanding of the nature of human infections. It is evident to focus on slowly growing, fastidious or suspected troublesome organisms when using sequencing for national reference purposes. Short sequences (450–650 base pair) seem sufficient for most identifications. There is no doubt that in the future molecular bacterial identification will be a powerful tool for national reference laboratories enhancing both the speed and validity of performed examinations.

P732

Using polymerase chain reaction for evaluation of positive results of two automated blood culture systems which remain negative by smear and subcultures

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Objectives: Automated blood culture systems have improved the detection rate of blood-borne pathogens. False positivity of these systems is between 1–10%. In this study we aimed to evaluate whether smear and subculture-negative positivities observed in two automated blood culture systems (BacTAlert (Biomérieux) and Bactec (Becton Dickinson)) were due to real microbial growth or false positive signalling.
Methods: Microbial DNA was extracted from 90 aerobic BacTAlert and 65 aerobic Bactec blood culture bottles which gave positive signals but smear and sub-cultures were sterile. Each bottle belonged to a different patient. Eubacterial 16SrDNA and fungal ITS region directed PCR were performed to determine the presence of microorganisms in these samples. Positive results were subjected to automated sequence analysis and nucleotide sequences were matched from the GeneBank.
Results: None of the bottles gave positive results by fungal PCR. Only 10 BacTAlert bottles gave positive results by eubacterial PCR. These bottles gave positive signals between 2nd and 5th days of incubation (mean 3.5 days). Sequence analysis results were as follows: *Pasteurella multocida* (1), *S.epidermidis* (2), *S.hominis* (1), *Micrococcus* spp (1), *S.pneumoniae* (1), *Corynebacterium* spp (2), *Brachibacterium* spp (1), *Arthrobacter/Rothia* spp. (1).
Conclusions: *P. multocida* was determined from a patient who had undergone surgery and *S. pneumoniae* was from a patient who was admitted to emergency department. In other patients there were underlying diseases such as hematological malignancies or solid tumors. Although most of the determined bacteria were the inhabitants of the skin flora, characteristics of

the host make them medically important for diagnosis. As these bacteria are easy to grow on laboratory media, their inability to grow on subculture may be due to their low number, or the antibiotic usage of the patient. Incubating these bottles for longer periods may increase the number of microorganisms and subcultures may become positive. In patients with underlying diseases, molecular methods can be used for quick identification of the microorganisms.

P733

Detection of bacterial pathogens by polymerase chain reaction in case of endophthalmitis, by using universal eubacterial primers

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Infectious endophthalmitis, despite its being relatively rare, is potentially one of the most devastating ocular infections. Therefore, the most effective treatment is based on rapid diagnosis of the disease and the identification of a causative agent. The most common bacteria that cause acute endophthalmitis are *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Streptococcus* spp. Delayed postoperative endophthalmitis is caused by microorganism such as *Propioniobacterium acne*, *Actinomyces israelli*, or *Corynebacterium* spp. The tests for presence of these bacteria in classic culture of aqueous humor (AH) and vitreous fluid (VF) are often negative, between 24–80% of which, lead to a clinical dilemma over the cause of the inflammation. A rational approach to the use of antibiotics and steroids necessitates determining, whether the inflammation is infectious or sterile. The evolution of new molecular techniques adapted to the field of medical microbiology is a promising tools, for the rapid and sensitive identification of bacterial pathogens.

Purpose: The goal of this study is to determine the usefulness of the PCR method in the diagnosis of endophthalmitis.

Materials and methods: 30 clinical specimens 19 AH and 11 VF were obtained from 20 eyes with the clinical diagnosis of endophthalmitis. These included: 14 cases after cataract surgery, 1 case post trabeculectomy, 2 cases after penetrating traumas, and 3 cases after endogenous endophthalmitis. The same samples were analysed using 2 different methods: 1. conventional microbiological techniques (microscopy and diagnostic culture) and 2. PCR directed at 16S rDNA using universal primers.

Results: In the aqueous humor the causative pathogen was identified in 1 case (5.2%) by using diagnostic culture compared with 7 cases (36%) by using PCR methods. In the vitreous samples the pathogen was identified in 1 case (9%) by using conventional method compared with 5 cases (45.4%) by using PCR. Microscopic preparation was difficult to evaluate in all samples.

Conclusions: PCR performed on aqueous humor and vitreous fluid is a reliable tool for diagnosis of causative organism particularly in smear and culture negative specimens. By using universal primers we are able to detect the presence of pathogen in case of endophthalmitis. This plays the main role in a successful therapy.

P734

Detection of *Tropheryma whipplei* in Portuguese patients by molecular methods

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Tropheryma whipplei is the causative agent of Whipple's disease, a rare multisystemic bacterial infection with variable clinical manifestations. The diagnosis of this disease is usually based on histopathological study of the infected tissues by periodic acid-schiff staining (PAS). More recently, molecular identification methods were developed based on PCR amplification of a intergenic region between the 16s and 23s ribossomal DNA.

Objective: Detection of *T.whipplei* in clinical samples of patients with suspicion of Whipple's disease, from different Portuguese hospitals.

Patients: Different clinical samples of 6 hospitalized patients were tested. From patients 1 and 2, serum and cerebrospinal fluid were collected, from patient 3 serum and urine, from patient 4 only serum and from patients 5 and 6 a duodenal biopsy.

Methods: DNA of all samples was obtained by using the Qiamp DNA mini Kit (Qiagen, Izasa, Portugal) and amplified by Nested-PCR, with primers Tws 1F/2R in first amplification and Tws 3F/4R in the second amplification. The same DNA was also amplified using the Real-Time PCR (by the tecnique of Faststart DNA Master Sybr Green–Roche Farmacêutica Química, Portugal) using the primers Tws 3F/4R, followed by sequence of positive samples. The sequences obtained were then compared to the *T. whipplei* reference sequence in NCBI Blast.

Results: In 3 of the 6 patients, DNA of *T.whipplei* was detected. Two of the 3 positive results were only positive by Real-Time PCR. Complete sequence homogeneity was found between the 3 positive results and the reference sequence for *T. whipplei*.

Conclusion: For the first time in Portugal the diagnosis of Whipple's disease was confirmed by the presence of *T. whipplei* in clinical samples by molecular methods. Until now, the detection of it's presence was made by histopathological study (PAS-positive) of the infected tissues or by exclusion with other disease when the histological analysis was inconclusive. The molecular identification methods made possible the confirmation of positive results of PAS and the detection of *T. whipplei* in patients were histopathology analysis was not possible or inconclusive. The Real-Time PCR is a very important tool for detection of intracellular and fastidious microrganism which are very difficult to detect by classical diagnosis methods of microbiology.

P735

Evaluation of universal PCR assay and endonuclease digestion for rapid direct identification from positive blood cultures

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From Sep/2003 to Apr/2004, positive BacT/Alert blood culture bottles were collected for evaluating the accuracy of a direct universal PCR assay and endonuclesae digestion method to achieve rapid bacterial identification at Tri-Service General Hospital. A total of 168 clinical relevant samples including 104 gram-negative and 64 gram-positive bacteria were evaluated. Results of the rapid PCR method were compared with conventional identification systems used in our clinical microbiology laboratory. Overall, 135 (80.4%) were correctly identified to species level. Thirty-three (19.6%) strains could not be identified due to reasons of new or identical patterns. The rapid universal PCR method proved more accurate for identification of Gram-negative bacteria (91%; 95/104) than that of Gram positive ones (63%; 40/64). Most discordances corresponded to identical patterns (17.2%; 3.5% for Gram-negative and 13.7% for Gram-positive bacteria) while new patterns (database not included in reference study) were found in 2.4% (1.8% and 0.6% for Gram-negative and Gram-positive bacteria, respectively).The universal PCR method is a directly reliable method for identification of

Gram-negative but not for Gram-positive organisms. Compared with conventional methods that require 1 or 2 days, this method can make same-day reporting possible so that it may permit better patient management.

P736

Two possibly novel *Alistipes* species isolated from clinical specimens of human origin

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Objectives: In this study, two groups of unusual bacteria, which phenotypically resemble members of *Alistipes*, but phylogenetically display ≥ 3% 16S rRNA sequence divergence with their mostly closely related species, *Alistipes finegoldii* were fully characterized.
Methods: The 7 isolates (4 for group I and 3 for group II) were biochemically characterized by using a combination of conventional tests as described in the Wadsworth and VPI anaerobic manuals plus the API ZYM, rapid ID 32, and Rapid ANA II systems. The 16S rRNA genes of the isolates were sequenced by an automatic 373A DNA sequencer. Sequence data was analysed by using the programmes FASTA and DNATools.
Results: Phylogenetically and phenotypically, the unidentified bacteria displayed a relatively close association with each other. However, a 16S rRNA gene sequence divergence of approximately 4% between the two unknown bacteria, as well as distinguishable biochemical characteristics such as different activities of *β*-glucosidase, a-arabinosidase and p-nitrophenylphosphatase, demonstrate that these organisms are genotypically and phenotypically distinct and each group represents a previously unknown sub-line within the *Alistipes* phylogenetic cluster. The closest described species to both of them is *A. finegoldii* (approximate 97% similarity), but group I can be differentiated from *A. finegoldii* by positive *β*-glucosidase reaction, and group II can be distinguished from *A. finegoldii* by being a-arabinosidase positive.
Conclusion: Additional studies are in progress, but based on the phenotypic and phylogenetic findings to date, it appears that these two groups may represent two novel species in the genus *Alistipes*.

P737

Evaluation of molecular diagnostics of bacteria in culture-negative human infections

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Objectives: In this study universal bacterial PCR and subsequent DNA sequencing was evaluated for demonstration and identification of bacteria in culture-negative human infections. As no 'golden standard' is available for comparison when culture is negative, the performance of the molecular analyses was estimated by 1) Ability to detect bacterial DNA in culture-negative and culture-positive clinical samples from normally sterile sites 2) Agreement between bacterial identities obtained by the molecular methods and phenotypic identification 3) Confirmation of results by other laboratory tests in culture-negative samples.
Methods: Clinical sample material from normally sterile sites was examined by microscopy and culture. DNA extracted from the specimens was analysed by real-time PCR of part of the 16S rRNA gene. Bacterial identities were established by DNA sequencing of amplicons. 1) Results of PCR were evaluated in culture-negative and –positive samples. 2) DNA sequence based bacterial identities were compared to those reported from culture. 3) In cases of molecular bacterial identification in culture-negative samples, efforts were done to seek confirmation of the obtained identities by other laboratory tests.
Results: 1) Bacterial DNA could not be detected in unselected culture-negative samples, but PCR was positive in two thirds of microscopy-negative samples with monobacterial growth. 2) Generally, culture and DNA sequencing resulted in the same bacterial identities. Species identification was obtained by DNA sequencing for bacteria that are only identified to the genus level by routine culture, 3) In many culture-negative cases, bacterial identities obtained by DNA sequencing could by confirmed by culture of the same organism from other sites or at other times, by species-specific PCR, or by pressence of antigens or antibodies.
Conclusions: Few data are available on the reliability of DNA sequencing for diagnosis of culture-negative bacterial infections. The results show that: 1) The technique may be useful in culture negative samples when there is particular suspicion of bacterial infection, also when microscopy is negative, but its routine application on unselected samples is futile. 2) Bacterial identities obtained by culture-independent DNA sequencing are highly reliable, and may be more accurate than culture. 3)DNA sequence based identification of bacteria in culture-negative samples can often be confirmed by other laboratory tests.

P738

Correlation between biochemical tests and 16S rRNA gene sequencing to identify bacteria

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Objectives: The objective of this work was to analyse the use of PCR amplification and DNA sequencing of the 16S rRNA gene to identify clinical isolates which are difficult to identify by conventional biochemical tests.
Methods: Twenty-three bacteria isolated from blood were analysed. PCR amplification of the 16S rRNA gene was carried out using 8F and 806R primers which amplified a region of 798 pb. The reaction was performed with 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. Amplified DNA products were resolved by electrophoresis in agarose gels (2%, w/v) containing 0.5 mg/L of ethidium bromide. PCR products were recovered directly from the agarose gel and purified. The DNA obtained was sequenced and identified by comparison in the GeneBank.
Results: After DNA sequencing 11 (48%) out of the 23 bacteria showed DNA homology ranging from 98% to 100% and 12 (52%) demonstrated homology ranging from 91% (only one strain) to 97% with 16S rRNA sequences found in the GeneBank. Biochemical methods allowed identification of 12 (52%) of these 23 isolates at a species level. Among these 12 isolates, 4 showed the same species with 16S rRNA (concordance of 33.3%). Five out of the remaining 11 isolates were identified at a genus level with biochemical tests and 6 isolates were not. Among the 5 isolates identified at a genus level 3 were identified with 16S rRNA (concordance of 60%). The most difficult species to identify by biochemical testing were *Nocardia* sp. and *Capnocytophaga* sp. as well as anaerobes such as *Fusobacterium* sp.
Conclusions: This study shows that 16S rRNA gene sequencing can be a useful and rapid technique to identify fastidious and some anaerobic bacteria which are difficult to identify by biochemical methods.

P739

Catabacter hongkongensis gen. nov. sp. nov., a novel anaerobic Gram-positive bacillus associated with bacteraemia

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Objectives: To characterize a novel anaerobic Gram-positive bacillus, *Catabacter hongkongnesis gen. nov. sp. nov.*, isolated from patients with bacteraemia.
Methods: We isolated two strains of an unidentified anaerobic Gram-positive bacillus from the blood cultures of two patients. The isolates were characterized phenotypically by conventional phenotypic tests, commercial identification systems and scanning electron microscopy; and genotypically by amplification and sequencing of their 16S ribosomal RNA genes and study of their G + C contents.
Results: Two strains of an anaerobic, non-sporulating, gram-positive bacillus was isolated from the blood cultures of two patients, a 48-year-old man with intestinal obstruction and secondary sepsis (strain HKU16T) and a 39-year-old man with acute appendicitis (strain HKU17). Both isolates are unidentified by conventional phenotypic tests and commercial identification systems. They produce catalase and are motile with a tuft of flagellae inserted on one side. Bacterial cells are coccobacilli tapered at both ends on electron microscopy. 16S ribosomal RNA gene sequence analysis showed more than 16% nucleotide difference between the sequences of both isolates and those of the most closely related bacteria. The G + C content of strain HKU16T is 40.2 ± 2.2%.
Conclusion: Based on their phylogenetic position, unique G + C content and phenotypic characteristics, we propose a new genus and species, *Catabacter hongkongensis gen. nov. sp. nov.*, to describe the bacterium. The gastrointestinal tract may have been the source in both patients. The isolation of a catalase-postiive, motile, non-sporulating anaerobicGram-positive bacillus in clinical laboratories should raise the possibility of *C. hongkongensis.*

Clinical mycology: case reports

P740

Severe disseminated fusariosis in a leukaemic child successfully treated with posaconazole and white blood cell transfusions

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Objective: *Fusarium* infections are an increasing threat to patients with severe neutropenia. Mortality rates exceed 70% in patients with disseminated disease. posaconazole (POS) is an investigational extended-spectrum azole antifungal agent that is active against *Aspergillus* spp and various other moulds, including *Fusarium* spp. We report the clinical outcome of a child with leukaemia who underwent white blood cell (WBC) transfusions and POS therapy for disseminated fusariosis after conventional antifungal therapies failed.
Case Report: A 14-year-old girl with high-risk lymphoblastic leukaemia began induction chemotherapy and prophylactic antimicrobial therapy with oral amphotericin B (AMB) and cotrimoxazole. An episode of *Escherichia coli* bacteraemia was successfully managed; however, on Day 39 of chemotherapy while still neutropenic, the patient became febrile. Multiple ecchymotic skin nodules developed, and a large area at the catheter insertion site became inflamed. Liposomal AMB and caspofungin therapy was initiated empirically, but the patient's condition continued to deteriorate. Existing skin nodules worsened, new lesions appeared, and 2 nodules became necrotic. Cultures of specimens from skin biopsy, catheter insertion site, sputum, stool, and blood all grew *Fusarium moniliforme*. Chest radiography showed 2 condensations in the right lung consistent with fungal pneumonia. Therapy was changed to POS oral suspension (200 mg qid) and the patient received WBC transfusions 24 and 48 hours later. The central venous catheter was replaced; cultures of the first catheter also grew *F. moniliforme*. Subsequently, the patient's condition rapidly improved. Cultures of all blood samples drawn after the start of POS therapy were negative, and no new skin lesions appeared. Fever and neutropenia resolved, and existing skin lesions gradually improved. One pulmonary lesion disappeared, and the second evolved into a cavitation with an air-crescent sign. POS was administered for 10 months, with concomitant consolidation chemotherapy. POS was well tolerated with no drug-related adverse events. The patient is alive, is in remission from leukaemia, and has no residual fungal lesions except for a thin-walled cavity in the right lung.
Conclusion: POS, in conjunction with WBC transfusions, resulted in rapid clinical improvement in a child with leukaemia who had an extensive *Fusarium* infection. As suggested by other reports of successful outcomes, POS appears promising for the treatment of invasive fusariosis.

P741

Successful combination therapy with posaconazole and amphotericin B for multifocal disseminated zygomycosis in a patient with relapsed acute lymphoblastic leukaemia

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Objectives: Few therapeutic options exist for zygomycosis, a rare but life-threatening fungal infection that affects immunocompromised patients. Posaconazole (POS), an investigational, extended-spectrum triazole antifungal, has in vitro activity against various *Zygomycetes*. We report the efficacy of POS and liposomal amphotericin B (LAMB) in a patient with multifocal disseminated zygomycosis.
Case Report: A 22–year-old man underwent induction chemotherapy for relapsed acute lymphoblastic leukaemia. One week after initiation of chemotherapy, the patient was febrile, had throat, neck, and chest pain, and was noted to have hyperthyroidism. Vomiting and weight loss developed, and chest radiography showed evidence of a right upper lobe infiltrate. Caspofungin 50 mg qd was initiated. Chest/abdomen computed tomography revealed multiple bilateral pulmonary lesions, an area of consolidation, multiple thyroid nodules, and a large left renal infarct. Invasive pulmonary aspergillosis was suspected, and therapy with LAMB (7.5 mg/kg/d) and voriconazole (4–6 mg/kg bid) was added. Culture of a biopsy specimen from

the lung grew *Mucor* species. Voriconazole and caspofungin were discontinued due to their limited activity against *Zygomycetes*. Surgical resection of affected tissues was not performed due to the extent of dissemination. The patient was enrolled in an open-label clinical trial and received POS oral suspension 800 mg/d in divided doses. Therapy with LAMB was continued, but the dose was increased to 10 mg/kg/d. Signs and symptoms improved over the next 3 months and the LAMB dose was eventually reduced to 5–7.5 mg/kg/d, depending on renal function. Eight months after diagnosis of zygomycosis, the patient underwent successful autologous stem cell transplantation (SCT) without recurrence or worsening signs or symptoms of zygomycosis. LAMB therapy was discontinued after 11 months because of renal insufficiency. The patient was considered to have had a partial response to POS and has not required hospitalization since SCT. To date, the patient has received POS for 18 months and continues to tolerate it well.
Conclusion: This is the first reported case of successful POS/LAMB combination treatment for multifocal disseminated zygomycosis. Importantly, POS was well tolerated during long-term treatment and allowed the patient to undergo SCT without aggravating zygomycosis. Treatment outcome suggests that POS may be an effective alternative treatment for zygomycosis.

P742

Effects of posaconazole salvage therapy in five patients with zygomycosis

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Objectives: Zygomycosis is a severe, life-threatening disease that can occur in immunocompromised or debilitated patients, including those with diabetes and acquired immunodeficiency syndrome (AIDS). We describe our experience in treating zygomycosis with posaconazole (POS), a potent, extended-spectrum, investigational azole antifungal that has shown promising in vitro activity against various *Zygomycetes*.
Methods: Patients with proven or probable invasive fungal infections were enrolled in a multicentre, open-label trial in which participants received monotherapy with POS oral suspension 800 mg daily in divided doses after failing other therapy or becoming intolerant to their therapy.
Results: Three men and 2 women (age range: 46–62 years) were treated with POS for proven zygomycosis at our institution. Underlying conditions included diabetes (n = 2); diabetes, AIDS, and liver cirrhosis (n = 1); idiopathic pulmonary fibrosis treated with lung transplantation (n = 1); and lung cancer with neutropenia (n = 1). Sites of primary infection were the sinuses (n = 3, 1 with brain involvement) and lungs (n = 2). Treatment with amphotericin B (deoxycholate and lipid formulations) (n = 5) and voriconazole (n = 1) (median, 24 days; range, 10–150 days), alone or in combination with surgical debridement (n = 3), failed to produce sustained clinical improvement. Additionally, 3 patients became intolerant to amphotericin B. POS therapy was administered to the 5 patients for a median of 169 days (range: 77–214 days). Extensive surgery for rhinosinusal mucormycosis was carried out during POS treatment in 1 patient and before and during POS treatment in 2 patients. Successful outcomes were seen in all 5 patients: 4 achieved a complete response and 1 had a partial response. The 2 patients with lung infection died; however, both deaths were considered related to underlying conditions, not to the mycosis or to POS. POS was generally well tolerated. Patients' overall safety profiles reflected their underlying disease, with no occurrence of treatment-related adverse events.
Conclusion: POS demonstrated promising activity in patients with refractory zygomycosis or intolerance to therapy and produced successful outcomes in all 5 patients treated. It is important to note that POS was well tolerated during long-term treatment (up to 214 days), even in patients who were intolerant of other therapies.

P743

Efficacy of posaconazole for chronic refractory *Alternaria* infection

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Objectives: *Alternaria*, a dematiaceous fungus and common plant saprophyte, may cause invasive disease in immunocompromised human hosts, but invasive alternariosis in healthy persons is rare. We describe a case of an immunocompetent woman with a 25–year history of invasive *Alternaria* infection in whom conventional antifungal and surgical therapy failed. The disease subsequently responded to treatment with posaconazole (POS), a new extended-spectrum triazole antifungal.
Case Report: In 1978, a 22–year-old immunocompetent woman developed a painful ulcer of the hard palate after root canal. Biopsy revealed maxillary osteomyelitis with fungal hyphae and culture grew *Alternaria* spp. Despite treatment with amphotericin B deoxycholate (AMB), the palatal ulcer recurred 1 year later; culture grew *Alternaria*. Retreatment with AMB produced an initial response, but the disease relapsed with bony destruction of the maxilla and nasal cartilage. During the next 18–20 years, multiple courses of ketoconazole, terbinafine, fluconazole, AMB lipid complex, liposomal AMB, and itraconazole (ITR) were given, but the disease eventually relapsed. In vitro susceptibility testing showed voriconazole (VOR) MIC of 0.5 μg/mL and VOR was initiated. Clinical resolution was achieved and biopsies were negative after 16 months. After 21 months of VOR treatment, she developed recurrent nasal skin nodules, rhinorrhea, and pansinusitis. Sinus biopsy and maxillary bone cultures revealed *Alternaria* and an increased VOR MIC (8 μg/mL). Combination treatment with caspofungin and liposomal AMB led to clinical remission after 8 months of treatment, but sinus and bone biopsies continued to show fungal hyphae despite negative cultures. Signs and symptoms returned and the disease relapsed again. Because in vitro susceptibility testing showed a POS MIC of 0.5 μg/mL, POS oral suspension (400 mg Q12 hr) was begun. After 1 month all symptoms and signs of infection resolved. Multiple skin and sinus biopsies were negative on histopathologic examination and culture. POS therapy was stopped after 18 months (August 2004). Currently, the patient has shown no evidence of recurrent infection.
Conclusion: POS resolved all symptoms and signs of disease in a patient with chronic alternariosis that was refractory to all available antifungal therapies, including VOR. The successful outcome suggests POS may be an alternative option for refractory *Alternaria* infection, including those that do not respond to other azoles.

P744

Efficacy of posaconazole salvage therapy in paediatric subjects with invasive fungal infections

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Objectives: We report the efficacy of posaconazole (POS), an extended-spectrum, investigational triazole antifungal, against invasive fungal infections (IFIs) in pediatric subjects.

Methods: Subjects ≥13 years of age with IFIs were enrolled in a multicentre, open-label clinical trial in which participants were administered POS oral suspension 800 mg daily in divided doses after becoming intolerant of, or developing disease refractory to, other antifungal therapy. Complete or partial response was considered a successful outcome; stable disease or treatment failure was considered a nonsuccessful outcome.
Results: Of the 330 subjects who were treated in the study, 11 were <18 years of age (range: 8–17 years). Six were boys and 5 were girls. Underlying diseases included haematological malignancies (n = 9), bone marrow transplantation (n = 4), and inherited immunodeficiency (n = 1). Previous antifungal therapy included amphotericin B (n = 11, deoxycholate and lipid formulations), itraconazole (n = 4), and other antifungal medications (n = 4). Successful outcomes were observed in 5 subjects with IFIs caused by *Aspergillus* spp (n = 3), *Candida* spp (n = 1), and *Coccidioides immitis* (n = 1). The remaining 6 subjects, who had nonsuccessful outcomes, had IFIs caused by *Aspergillus* spp (1), *Candida* spp (n = 2), *Zygomycetes* (1), and *Fusarium* spp (n = 2). Mean POS plasma concentrations in pediatric subjects were similar to those in adults (776 ng/mL and 817 ng/mL, respectively). Occurrence of treatment-emergent adverse events (AEs) was similar in pediatric and adult subjects; the most commonly reported AEs were gastrointestinal events, fever, and headache.
Conclusion: Treatment with POS resulted in successful outcomes in 45% (5 of 11) of pediatric subjects. This rate of success was similar to that seen in the overall population (50%). Pediatric subjects who received POS had similar steady-state plasma concentrations, comparable success rates, and similarly favorable AE profiles to those of adults. Although the number of subjects in this analysis is small, these data suggest that POS may have clinical utility in the treatment of refractory IFIs in pediatric subjects.

P745

Difficult-to-treat central nervous system HIV-unrelated cryptococcoma cured with voriconazole

R. Manfredi, S. Sabbatani, F. Chiodo *(Bologna, I)*

Introduction: When considering cryptococcosis in a setting other than HIV disease,very few episodes are documented,and CNS cryptococcoma is an absolutely rare event.
Case report: The authors describe an exceptional cerebral cryptococcoma occurred in a chronic nephropathic HIV-negative patient (p) suffering from homocystinuria, favorably cured with combined neurosurgery and voriconazole, after a limited response to high-dose fluconazole. In our case, after surgical intervention confirming a diagnosis of cryptococcoma, a first treatment line carried out with iv fluconazole did not lead to a sustained improvement, as the new antimycotic derivative voriconazole did at 400 mg/day, later in the disease course. After patient's death due to a underlying progressive renal-vascular disease,necropsy examination excluded any sign of infiltration due to *Cryptococcus neoformans*; voriconazole administration can be therefore considered eradicating.
Discussion: When clinicians face an expansive cerebral mass, before starting therapy it is mandatory to establish an early presumptive diagnosis, in order to target antimicrobials and recommend eventual surgical drainage. Among a broad spectrum of infectious etiologies, it is suggested to take into consideration also of a fungal abscess, whose evolution would certainly be disadvantaged by symptomatic steroids (like happened in our p before that a specific diagnosis was confirmed), and/or antibacterial agents. In our case, both surgical and medical therapy of a brain cryptococcoma in a non-HIV-infected p have been performed: the surgical management helped to remove the majority of the brain abscess and to formulate the diagnosis, as well as to release an initial hydrocephalus. Also in HIV-infected p, cryptococcoma is a relatively rare feature, and it hardly appears isolated,and not associated with a meningeal cryptococcosis. As far as we know, there are no prior reports of primary cryptococcomas in chronic nephropatic, HIV-negative p, as well as in p with homocystinuria. Although being a rare occurrence, cerebral cryptococcoma may represent an emerging issue in the next future, because of its relationship with a broadening range of risk factors, including malignancies, neutropenia, end-organ failure, bone marrow and solid organ transplantation, and multiple primary-secondary immunodeficiency. With the mounting increase in the frequency of p with end-organ kidney disease,it is possible that also cryptococcosis may emerge as a more frequent complication.

P746

Voriconazole proves effective in the management of a brain cryptococcoma apparently resistant to fluconazole

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Introduction: The yeast *Cryptococcus neoformans* shows an apparently homogeneous in vitro response to fluconazole, but particular sites of infection and prolonged disease may be difficult to treat. A rare case of cerebral cryptococcoma occurred in a HIV-negative patient (p) suffering from chronic kidney failure and pancytopenia is reported, since fungal clearance was obtained only after voriconazole treatment, despite previous surgery and long-term high-dose fluconazole use.
Case report: A brain cryptococcoma was diagnosed in a 54–year-old p after brain surgery, histopathological examination, and culture. Despite i.v. fluconazole treatment at 600–1000 mg/day, the clinical and neuroradiological course was progressively worsening, so that i.v. voriconazole (400 mg/day), was introduced. The concurrent renal failure contraindicated amphotericin B administration. After obtaining a certain diagnosis of this rare fungal complication in a non-HIV-infected p, the first-line high-dose fluconazole administration did not obtain a significant ameliorement, while the subsequent 50–day voriconazole therapy led to negative neuroradiological, scintigraphic and microbiological examinations associated with significant clinical improvement. After patient's death occurred 7 months later due to the progression of kidney failure and overwhelming cardiovascular events, the necropsy examination of the brain and other organs excluded *C. neoformans* lesions, thus confirming definitively the eradicating efficacy of voriconazole therapy.
Discussion: Although brain cryptococcoma remains a rare occurrence, especially in the absence of HIV infection, this shifty pathology may represent an emerging infection in the next future. Its pathogenesis is supported by a broad variety of risk factors, including malignancies, neutropenia, severe end-organ failure, bone marrow and solid organ transplantation,and other primary or secondary (i.e. iatrogenic) immunodeficiency. When facing a cerebral mass, an early diagnosis is needed to start an appropriate medical and/or surgical therapy. In the broad spectrum of etiologies of brain abscesses the role of fungi cannot be underestimated to avoid empiric steroid administration and proceed with an adequate antimycotic treatment. The novel antifungal voriconazole combines an enhanced spectrum with an increased intrinsic potency with a favorable cerebral concentration which allows the treatment of difficult-to-treat brain fungal infections.

P747

Cutaneous and disseminated histoplasmosis of autochthonous origin in Italy

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Introduction: *Histoplasma capsulatum* is a dimorphic fungal pathogen involved in self-limited respiratory infection of the immunocompetent host, while it may be responsible for severe airway or disseminated disease when immunodeficiency is of concern. Outside the endemic areas of America, Africa, and South-Eastern Asia, it is a very rare, typically 'imported' pathogen. However, anecdotal reports of unequivocal autochthonous histoplasmosis suggest the possible existence of favorable environmental conditions in our continent, and in Italy too (Po river valley).
Case report: An Italian i.v. drug addict with advanced HIV disease (as expressed by a CD4+ lymphocyte of 70 cells/μK) and a negative history for travels outside our country), came to our attention owing to an irregular hyperpyrexia, weight loss, chest pain, pancytopenia, and hepato-splenomegaly persisting since 2 weeks. Plain radiological and CT studies of the chest detected diffuse nodular infiltrates and a prominent mediastinal lymphadenopathy. One week later, multiple papulous-ulcerate skin lesions of the face allowed the histopathological recognition of the typical H. capsulatum blastoconidia, either intra- or extracellular in site, with respect to phagocyte cells. A 5–week course of i.v. liposomal amphotericin B at 5 mg/Kg and oral itraconazole achieved a complete clinical and mycological resolution of this rare disseminated and cutaneous opportunistic mycosis.
Conclusions: Our patient represents the seventh case of autochthonous AIDS-related histoplasmosis observed in Italy. Epidemilological and environmental studies of habitats supporting the growth of *H. capsulatum* in Italy and Europe could be of particular interest, given the increased risk of opportunism encountered in the immunocompromised host. Notwithstanding its rare occurrence, this fungal infection causing a broad spectrum of clinical presentations, cannot be disregarded in the differential diagnosis of opportunistic disorders related to advanced HIV disease.

P748

Reversible dilated cardiomyopathy associated with amphotericin B treatment

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Objectives: We report two patients who developed reversible dilated cardiomyopathy secondary to amphotericin B (AmB) treatment
Case series: Patient 1: A 64–year-old man with a history of mild hypertension was treated with 6 cycles of rituximab – chemotherapy for non-Hodgkin's lymphoma (CHOP). The cumulative dose of doxorubicine was 300 mg/m^2. A baseline transthoracic echocardiogram (TTE) was normal with an ejection fraction (EF) of 59%. After the last cycle he was treated with liposomal AmB (5 mg/kg, total 350 mg qd) for febrile neutropenia. Seven days later he developed congestive heart failure and a new TTE revealed dilated cardiomyopathy (severe global impairment of the left ventricular (LV) systolic function (EF = 13%) with LV end-diastolic diameter 61 mm and end-systolic diameter 54 mm). Antifungal treatment was discontinued and the patient was treated with inotropic agents and diuretics. Within 24 hours vital signs were improved and EF returned to 30%. One year later he had a normal EF (56%).
Patient 2: A 23–year-old woman was admitted because of pulmonary empyema. She had a history of mild systemic lupus erythematosus (SLE) and she reported use of intravenous drugs. A baseline TTE was normal. She was started on liposomal AmB 4 mg/kg (250 mg qd) for candiduria and 8 days later she developed dyspnea and pulmonary oedema. A TTE revealed a dilated LV with end diastolic diameter 59 cm, end systolic diameter 47 cm and an EF of 35%. Diuretics were administered and AmB was discontinued. Her vital signs were improved and EF was increased to 48% five days later. One month later the EF was 50%.For both patients, other causes of heart failure such as septicaemia, viral myocarditis, valvular disease, or acute coronary syndrome were excluded on the basis of negative blood cultures, viral serology, and biochemical, and echocardiographic findings. Doxorubicin cardiotoxicity, is unlikely due to the low cumulative dose of the drug, the rapid evolution and the reversibility of this condition.
Medline search: there are only 2 reports describing dilated cardiomyopathy as side effect of AmB. In both reports the patients were treated with AmB deoxycholate for coccidioidomycosis. All four reported patients (including ours) had comorbid cardiac conditions such as coccidioidomycosis, chemotherapy with anthracyclines, SLE and use of illicit drugs.
Conclusion: AmB might be cardiotoxic in patients with other concurrent factors causing cardiac dysfunction.

P749

Caspofungin treatment in two infants with persistent fungaemia due to *Candida lipolytica*

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Caspofungin has been used successfully in adults with invasive candidiasis. There is limited experience with caspofungin in pediatric patients. We report two infants with persistent candidemia unresponsive to traditional antifungal therapy who were successfully treated with amphotericin B and caspofungin.
Case 1. Ten day-old male patient underwent intestinal resection with anastomosis due to jejunal stenosis. On 10th postoperative day, the patient became febrile associated with elevated white blood cell counts and CRP levels. Broad spectrum antibiotics and fluconazole were initiated. Because of positive blood cultures for *Candida albicans* and *Candida lipolytica*, central venous catheter of patient was removed and fluconazole therapy was replaced with conventional amphotericin B. However, blood cultures continued to be positive for *C. lipolytica*. After the possibility of a focal infection, such as endocarditis, was excluded caspofungin was initiated with a dose of 1.6 mg/kg/day. Following addition of caspofungin all blood cultures have remained negative.
Case 2. A 4–month-old female with gastroesophageal reflux, unresponsive to medical therapy was, admitted to the pediatric surgery service. She was treated for nosocomial pneumonia due to methicillin-resistant *Staphylococcus aureus*. On the follow up, her temperature was elevated. Broad spectrum antibiotics and fluconazole were initiated. Because of positive blood cultures for *Candida albicans* and *Candida lipolytica*, central venous catheter of patient was removed and fluconazole therapy was changed to conventional amphotericin B. Focal infection could not be demonstrated, however, blood cultures continued to be positive for *Candida lipolytica*, then treatment changed to liposomal amphotericin B. Due to persistence of positive blood cultures, caspofungin was also initiated with a dose of 1.6 mg/kg/day. The patient have since remained afebrile, and blood cultures have remained negative. Caspofungin may be considered in infants with persistent candidemia that are not responsive to

standard therapy. Carefully controlled *in vivo* studies and well-designed randomized clinical trials investigating dosage and duration of the treatment with caspofungin, and investigation of its pharmacokinetics, interactions and potential toxicities are necessary before caspofungin is routinely recommended in children.

P750

Paracoccidioidomycosis in the breast: case report in a fertile female patient

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Objectives: To report the case of a fertile female patient with disseminated paracoccidioidomycosis involving the breast.
Methods and Results: A 32–year-old smoker female patient was admitted to treat a breast abscess. Four years before, she was admitted in other hospital to evaluate hoarseness – in that occasion, she had the diagnosis of a vegentant lesion in the larynx caused by *Paracoccidioides brasiliensis*. She had received treatment with ketoconazole, but she abandoned treatment. Six months before admission she went to the emergency room to have an abscess in her left breast drained. Two months later, she returned to the hospital because of the same problem. At admission, she complained of weight lost (4 kg), productive cough, and purulent sputum. She denied fever. An abscess was drained in the left breast, and culture revealed *Paracoccidioides brasiliensis*. Painful left axillary lymphadenopathy was observed (~1.5 cm), and there was no oral lesions neither hepatosplenomegaly. Chest radiography showed a bilateral interstitial infiltrate, with small nodules and cavities. *P. brasiliensis* grew in sputum culture. Osteolytic lesions were seen in the 5th, 6th, and 7th left ribs. HIV serology tests were negative, and fasting glucose was normal. This patient has 4 children, and normal menstrual cycles. Her oestrogen levels were normal. Paracoccidioidomycosis was effectively treated with itraconazole.
Conclusions: Women seem to be protected from paracoccidioidomycosis, and most of cases occur in patients in the post-menopause period. It has been suggested that the marked gender difference seen in adults could be explained by the inhibitory action of oestrogens on the conidia or mycelium-to-yeast transition. Genital paracoccidioidomycosis is a very rare event, mainly in fertile female patients.

P751

Successful treatment of *Trichosporon mucoides* infection with lipid complex amphotericin B and 5–fluorocytosine

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Disseminated infection due to *Trichosporon* species is one of the emerging mycoses in immunocompromised children. In this report, a child with disseminated *Trichosporon mucoides* infection who was successfully treated with lipid complex amphotericin B and 5–fluorocytosine are presented.
Case Report: A 15–year-old boy with acute lymphoblastic leukaemia was admitted to the pediatric intensive care unit because of fever, respiratory failure, and recurrent seizures. He had been operated for cardiac thrombosis due to double-lumen catheter two days before admission. His neurological findings were attributed to hypoxia developed during the operation. On physical examination, his body temperature was 39.2°C, he was unconscious, and he had left hemiplegia. Cracking rales were also detected on the right hemithorax. Laboratory investigations revealed that his hemoglobin was 10.5 g/dL, white blood cell count was 3200/mm^3, absolute neutrophil count was 1100/mm^3, and CRP was 9.7 mg/dL. Chest X-ray examination revealed diffuse infiltration at the lower zone of the right lung. The diagnoses of sepsis and pneumonia were established and vancomycin and meropenem were initiated. On the follow up, he developed pericardial effusion which was treated with pericardial tube drainage. Because of persistent fever, fluconazole was initiated on the 5th day of hospitalization. His blood cultures were positive for Trichosporon mucoides. Because this strain was resistant to fluconazole and itraconazole but susceptible to 5–fluorocytosine and amphotericin B, fluconazole treatment was discontinued and lipid complex amphotericin B (5 mg/kg/day) and 5–fluorocytosine (100 mg/kg/day) were initiated. Patient's fever was resolved on the 5th day of new antifungal regime. Pneumonia was also gradually resolved and antifungal therapy was discontinued at the end of 8th week of therapy. The patient was discharged with almost no neurological sequel.
Conclusion: *Trichosporon mucoides* must be considered as an etiological agent in patients with immunocompromised and/or recently operated. Although optimal therapy of serious *Trichosporon* infections remains poorly defined, amphotericin B in combination with 5–fluorocytosine may be suitable.

P752

Successful treatment of ulcerating keratitis due to *Acremonium recifei* with voriconazole

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Objectives: Fungal keratitis is a common, potentially sight-threatening ocular infection. The majority of cases occur after corneal injury, usually by fungus-contaminated plant material. Occasionally, contact lens wear is complicated by fungal keratitis. A broad spectrum of fungal species has been identified as etiologic agents. Until now, only a single ocular infection due to *Acremonium recifei* has been described in a 48–year-old man who developed corneal ulcer after a piece of coconut flew into his eye while cracking it. He was treated successfully with keratoplasty, local and intravenous miconazole as well as oral ketoconazole.
Methods: A previously healthy 38–year-old woman was admitted for severe, ulcerating keratitis. She had worn hard contact lenses since the age of 12 without complications. Despite topical, subconjunctival, intracameral and systemic broad-spectrum antibiotics, amphotericin B and itraconazole, as well as acanthamoebal therapy, the infection deteriorated. After seven weeks there were satelliting infiltrations of the corneal stroma and a severe anterior chamber reaction with hypopyon. A perforating keratoplasty was performed.
Results: Microscopy of the corneal specimen detected fungal elements. Culture of cornea and aqueous fluid both yielded pure growth of *A. recifei*. Using E-Test (AB Biodisk, Solna, Sweden) the isolate exhibited resistance as indicated by minimal inhibitory concentration (MIC, expressed in μg/ml) to: itraconazole: 16, and amphotericin B: 2. Microdilution testing according to NCCLS revealed the following MIC: fluconazole: >64, itraconazole >8, voriconazole: 8, amphotericin B: 1, and flucytosine: >64. Because of the lacking clinical response and the discrepant susceptibility results for amphotericin B, therapy with voriconazole was started. The vigorous local, intracameral and systemic therapy with voriconazole was tolerated well and resulted in a sustained resolution of the infection. Subsequently, due to corneal haziness and vascularization corneal graft transplantation had to be repeated.

Conclusion: We describe the first case of an ulcerating keratitis due to *A. recifei* associated with contact lens wear which was treated successfully with voriconazole. Voriconazole is a new, highly potent triazole with broad spectrum activity against fungi, including moulds. The present case highlights the potential of voriconazole in the treatment of severe ocular infections even due to rarely encountered fungal species.

P753

Expression of alpha-defensin–1 in chronic hyperplastic candidosis

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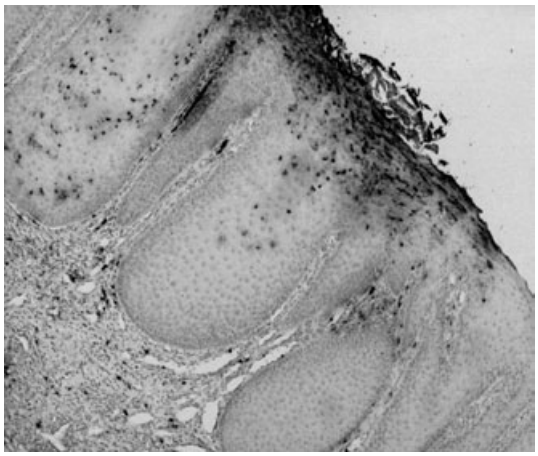
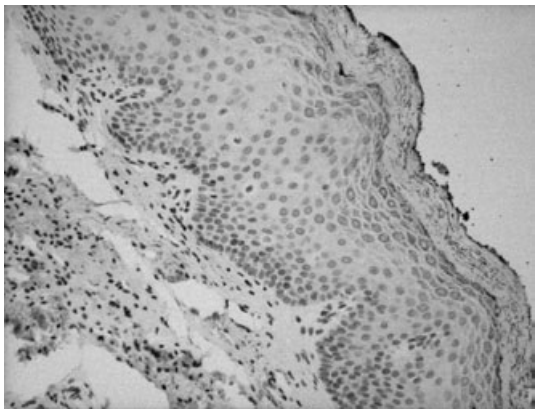
Background/Objectives: *Candida* yeasts are common commensals of the normal oral microbial flora. They may also penetrate into underlying tissues and cause opportunistic infections. Chronic hyperplastic candidosis (CHC) represents a chronic opportunistic candida infection of the oral cavity. The aim of the present study was to clarify the presence, tissue localization and participation of α-defensin–1 in the host response against chronic candidal stimulus.

Methods: Biopsies from ten patients with CHC lesions were examined by immunohistochemistry using α-defensin–1 specific polyclonal antibody. Ten samples from patients with leukoplakia of idiopathic origin (LP) were used as candida negative controls. Sections were also stained with periodic acid-Schiff (PAS).

Results: In CHC lesions, α-defensin–1 was detected intravascularly in neutrophils, interstitially in lamina propria and, in particular, intraepithelially in neutrophils, in part forming intraepithelial microabscesses. In the CHC samples the staining intensity of the individual neutrophils varied in the epithelium and cellular staining was associated with peri- and extracellular staining, in particular in the superficial epithelial cell layers. In contrast, only very few and homogeneously staining α-defensin–1 immunoreactive neutrophils could be detected in the LP samples, mostly intravascularly in lamina propria. No extracellular a-defensin–1 deposits could be discerned in the LP epithelia.

Conclusion: Alpha-defensin–1 containing neutrophils are recruited from the intravascular pool, from where they transmigrate through lamina propria to *Candida* hyphen containing





epithelium. Neutrophils form microabscesses and respond to *Candida* by activation and release of a-defensin–1 to peri- and extracellular matrix. This together with the epithelial cell migration from the basal layer to epithelial surface leads to a-defensin–1 rich protective shield in the most superficial epithelial cell layers.

Enterococci: epidemiology and resistance

P754

The antimicrobial susceptibility of bacteraemia isolates of *Enterococcus faecalis* and *E. faecium* in a London hospital, 1990–2002

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Objectives: Enterococci are common in faecal flora and can cause serious infection in humans. We wished relate the antibiotic susceptibility of enterococci from bacteraemic patients to changes in human and animal use of antibiotics.

Methods: The antibiotic susceptibility of all enterococci isolated from the blood of bacteraemic patients in St Thomas' Hospital, London, between 1990 and 2002 was determined either by broth microdilution or by agar dilution. We noted local changes in clinical services, and thus of antibiotic use (related to the union of two hospitals), as well as published information on the use of growth-promoting antibiotics in food animals.

Results: We isolated 392 *E. faecalis* and 147 *E. faecium* during the 12 years. The annual isolation rate of both species increased markedly from 9 *E. faecalis* and 3 *E. faecium* in 1990 to a peak of 60 and 42 respectively in 2000. All isolates of *E. faecalis* were ampicillin susceptible but 89% of *E. faecium* were resistant. Resistance rates for other antibiotics for *E. faecalis* and *E. faecium* were erythromycin 83% and 99% respectively, vancomycin 5% and 26%, teicoplanin 3% and 22%, chloramphenicol 33% and 12%, ciprofloxacin 60% and 89%, gentamicin (high-level) 44% and 39% and tetracycline 75% and 38%. In addition, 0.5% of *E. faecalis* were linezolid resistant and 2% of *E. faecium* were Q/D resistant. When the years 1990–1996 (use of growth-promoting antibiotics in food animals) and 1997–2002 (relocations of

clinical services) were compared, resistance rates for erythromycin, glycopeptides, ciprofloxacin, and aminoglycosides increased for both species, and for ampicillin, tetracycline, and Q/D for *E. faecium*. However, resistance to Q/D and linezolid was negligible.
Conclusions: The two species of enterococci became more common and more resistant largely in relation to relocation of clinical services commonly associated with high-level antibiotic use. Use of avoparcin and virginiamycin in animals before 1996, had minimal or no potential effect on resistance, since resistance appeared after their animal use had been discontinued, but when local use of their counterparts in human medicine had markedly increased or, in the case of Q/D, begun. Despite the occasional use of Q/D and later linezolid, resistance to these two antibacterials remained rare. Fortunately, newer antibiotics, such as daptomycin, are active against all isolates, including those resistant to virtually all other antibiotics.

P755

Wide dissemination of enterococci highly resistant to aminoglicosides among poultry and healthy humans from Portugal

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Objectives: To characterize enterococcal isolates highly resistant (HLR) to aminoglycosides (AG) from human and poultry samples from Portugal and to establish clonal relatedness among them.
Methods: Fecal samples from 99 healthy human volunteers (HV) and 99 skin or meat samples from poultry (P) were collected in North and Center of Portugal (1999–2001). MICs for gentamicin (Gm), streptomycin (Sm) and kanamycin (Km) were determined by the agar dilution method (NCCLS). Species identification and presence of AG resistance genes [aac(6′)-Ie-aph(2′′)-Ia, aph(2′′)-Ib, aph(2′′)-Ic, aph(2′′)-Id and aph(3′)-IIIa] were determined by PCR. Clonal relatedness among HLRGm enterococci was established by PFGE.
Results: 765 isolates [*E. faecalis* (n = 307), *E. faecium* (n = 301), *E. gallinarum* (n = 68) and *Enterococcus* spp (n = 89)] were identified. Resistance to Sm, Km, or Gm was observed in 72%, 53%, 39%, and 80%, 68%, 13% of P and HV samples, respectively. All HLRGm enterococci contained aac(6′)-Ie-aph(2′′)-Ia: 53 isolates (46 P and 7 HV) had only this gene and 31 isolates (20 P and 11 HV) had aac6-aph2 plus aph(3′)-IIIa. HLRKm but not to HLRGm was found in 180 isolates (70 P and 110 HV), aph(3′)-IIIa being present in 90% of P and 85% of HV isolates. Two HLRGm enterococcal PFGE types were found in both P and HV samples: one *E. faecalis* (4 P and 1 HV) and one *E. faecium* (2 P and 1 HV).
Conclusion: HLRAG enterococci is widely spread among P products and HV from Portugal, aac(6′)-Ie-aph(2′′)-Ia and aph(3′)-IIIa being responsible of HLRGm and HLRKm. Our results confirm that poultry used for human consumption might be an important source for the diffusion of HLRAG enterococci strains and/or their respective resistance genetic elements.

P756

Molecular characterisation of vancomycin-resistant *Enterococcus faecium* from Portuguese hospitals

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Objectives: To analyse the clonal relationship and the presence of antibiotic resistance and virulence traits among vancomycin resistant *E. faecium* (VREF) clinical isolates from hospitals of 3 different Portuguese cities.
Methods: 103 VREF isolates from different patients (1996–2003) 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 characterization was done by an overlapping PCR strategy in 53 selected VREF isolates. Clonal relatedness was established by PFGE. Presence of virulence traits (Esp, Gel, Hyl, Cyl, Agg) was investigated by multiplex PCR.
Results: VREF (102 vanA and 1 vanB2) were mostly resistant to ampicillin, erythromycin, ciprofloxacin and HLR-kanamycin (98, 98, 95, and 67%, respectively). Genes coding resistance to macrolides or aminoglicosides were erm(B), aac6-aph2, and aph3́-IIIa. Fifty-one PFGE types were identified, some of them successfully spread among two (5 clones) or three (one clone) hospitals in distinct years, and wards. Sixteen variants of Tn1546 were found; some of them were observed in different hospitals (PP-2, PP-5), in different dates (PP-2, PP-4, PP-5, PP-13) and different clones (PP-2, PP-4, PP-5, PP-9, PP-13). PP-4, PP-5 and PP-24 contain an ISEf1 which we previously found to be predominant among clinical Portuguese VRE *faecalis* strains. Different combinations of virulence factors were detected, esp being detected in 32% (n = 33/103) of VREF isolates, all resistant to ampicillin.
Conclusions: VREF were frequently found in the institutions studied. The presence of specific and persistent both VREF clones and Tn1546 types might contribute to the wide spread of VRE in the Portuguese hospital environment. The dissemination and establishment of successful VREF clones in the hospital setting amplifying particular Tn1546 types available in local metagenomes, might influence different evolutionary events.

P757

Distribution of enterococci infections and antibiotic resistance patterns in a Greek hospital

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Objectives: Enterococci constitute a major problem due to the increasing incidence of enteroccocal infections and the nosocomial spread of strains resistant to multiple antimicrobials including the threat of VRE strains. The purpose of this study is to assess the prevalence and resistance patterns of enterococci isolates among the patients of our hospital.
Methods: During a 3-year period 350 enterococci strains were isolated from various clinical specimens obtained from 144 (41.2%) outpatients, 59 (16.8%) ICU patients and 147(42%) patients hospitalized in other units. The identification of the isolates and the determination of the antimicrobial resistance to penicillin (P), ampicillin (Am), ciprofloxacin (Cip), high dosage gentamycin, vancomycin (Va), teicoplanin (Tei), and linezolid (Lin) was performed by Auto Scan of D. Behring while the disk diffusion method was used as a confirmatory test.
Results: Among the 350 isolated enterococci, 203(58%) strains derived from urine specimens, 31 (8.8%) from lower respiratory tract, 30 (8.6%) from wounds, 20 (5.7%) from blood cultures and 66 (18%) from other sources. 298 (85.2%) strains were identified as *E. faecalis*, 47 (13.4%) as *E. faecium* and 5 (1.4%) as *Eterococcus* spp. The resistance rates of *E. faecalis* isolated from outpatients, ICU patients and patients of other units were as follows: to Am (0/26/2%), P (5/34/12%), Cip (20/70/30%), Va (0/9/2%), and Tei (0/8/0%) while the antimicrobial resistance for the same groups of patients for *E. faecium* were to Am (0/86/2%), P (75/86/10%), Cip (75/86/78%), Va (0/14/0%) and Tei (0/14/0%) respectively. A total of 18 (5.1%) enterococci isolates

predominately derived from ICU and comprised of 9 strains of *E. faecalis* (3%) and 9 strains of *E. faecium* (19%), were found to be high level Gentamicin resistant. No strains resistant to linezolid were observed.
Conclusions: *Enterococcus* spp. is the causative pathogen for many urinary, lower respiratory tract and blood infections. *E. faecium* shows higher antimicrobial resistance compared to *E. faecalis*. It was observed that isolates from outpatients show a moderate resistance to quinolones, while ICU isolates were highly resistant to penicillin, quinolones and high level Gentamycin. For the present all VRE strains are susceptible to oxazolidinone. The high incidence of resistant enterococci strains to tested antibiotics, especially in ICU, highlighted the need for continual surveillance and rational use of antimicrobial compounds against enterococci.

P758

Vancomycin-resistant enterococci in Greece: a multicentre prevalence study on intestinal colonisation

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Objective: To estimate the prevalence rates of fecal carriage with vancomycin resistant enterococci (VRE) in Greek hospitals and investigate the risk factors.
Methods: Thirteen hospitals (5 university and 8 regional) scattered in all Greek territory participated in the study and 1246 patients were tested. The survey took place on November 1, 2002. The same methodology was exactly performed in every hospital. Fecal specimens were cultured in enterococci broth with 6 μg/ml vancomycin and subcultured on bile esculin azide agar with 6 μg/ml vancomycin. Enterococci were identified by conventional methods and vancomycin resistance was detected by disk diffusion method and E-test. All isolates were sent to a reference centre for further investigation. Van gene characterization was performed by a multiplex polymerase chain reaction (PCR), which also confirmed the identification. Clone distribution was tested by pulsed-field gel electrophoresis (PFGE).
Results: VRE were detected in all except 1 regional hospital and 266 strains were isolated from 255 (20.5%) of 1246 patients. Overall, 79 (29.7%) isolates were *E. faecium*, 12 (4.5%) *E. faecalis*, 153 (57.5%) *E. gallinarum*, 16 (6%) *E. casseliflavus*, 4 (1.5%) *E. avium*, and 2 (0.8%) *E. hirae*. Among 266 isolates, 82 (30.8%) presented vanA, 17 (6.4%) vanB, 152 (57.1%) vanC1, and 15 (5.6%) vanC2/C3 resistance type. When the inducible types (vanA, van B) were separately considered 93 (7.5%) patients were carriers. Van-A type was detected in all university and in 4 of the 8 regional hospitals, while van-B in 1 university and 3 regional hospitals. The percentages of vanA and vanB were significantly higher in university compared to regional hospitals. At the ward level, university hospitals exhibited significantly higher rates for renal and surgical wards, whereas in ICU and medical wards no difference was observed. PFGE analysis revealed the presence of multiple clones. Age, presence of cancer, chronic renal failure, length (days) of hospitalization, use of monobactams and carbapenems and previous ERCP were identified as risk factors independently associated with increased risk of VRE (VanA or VanB) colonization.
Conclusion: This study revealed high VRE colonization rates among Greek hospitalized patients. Van-A appeared as the predominant inducible type. Cancer, chronic renal failure, length (days) of hospitalization, use of monobactams and carbapenems, and previous ERCP were identified as risk factors.

P759

Ampicillin resistant *Enterococcus faecium*: a rapidly increasing problem in hospitals in a Danish region

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Objectives: In 2000, clusters of epidemic spread of ampicillin resistant enterococci (ARE) were registered in two wards and their affiliated intensive care units (ICU). In order to determine the size and dynamics of nosocomial infection with ARE, the study investigates the overall isolation of ARE during an 9-year period in a Danish region. Molecular examination of the ARE strains was used to disclose clonal spread within hospitals.
Methods: The Department of Clinical Microbiology, Aalborg Hospital provides diagnostic bacteriology to the County of North Jutland (approximately 500,000 inhabitants) and serves around 300 general practitioners (GP) and 7 hospitals. The department also undertakes surveillance of nosocomial infections in the county. Enterococci were identified by conventional methods (1), and examined with pulsed-field gel electrophoresis (PFGE). Molecular bands were judged visually according to previous described methods (2). Clinical data was retrieved from the case records. Retrospective data regarding ARE strains were retrieved from the department's laboratory information system (ADBakt, Autonik, Sweden).
Results: The number of ARE strains was: 1996 (2), 1997 (11), 1998 (7), 1999 (5), 2000 (18), 2001 (26), 2002 (44), 2003 (73), January to October 2004 (127). ARE was isolated from various sources including blood cultures, surgical wounds and abdominal abscesses. Fourteen isolates (4.5%) came from GP's and 299 isolates (95,5%) from hospitals. Forty-eight ARE strains isolated locally during 1997–2004 and 8 *E. faecium* and 6 *E. faecalis* strains from a neighbouring county were compared by PFGE. All strains from the neighbouring county were distinct from local strains. Many unique strains were identified both in the community and in hospitals. Clonal spread of ARE strains was disclosed in one department of abdominal surgery and one department of nephrology as well as their affiliated ICUs.
Conclusions: ARE has emerged and become a nosocomial problem in North Jutland County, Denmark. ARE strains seems to be prevalent in the community and following introduction into the hospital setting they may give rise to clonal spread and nosocomial infections.
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P760

A Nordic study of antibiotic susceptibility of enterococci at different ward levels

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Objectives: To study the antibiotic susceptibility of enterococci at different ward levels.
Methods: Initial isolates of enterococci from patients at primary care centres (PCC) (N = 220), general hospital wards (GHW) (N = 215) and intensive care units (ICU) (N = 176) at 16 hospitals and counties, in Denmark, Finland, Norway and Sweden, were collected during 1999–2000. Minimal Inhibitory

Concentrations (MICs) were determined using Etest (AB Biodisk). Species related MIC breakpoints of the Swedish Reference Group for Antibiotics were used for all antibiotics except for moxifloxacin and levofloxacin. For these two antibiotics the European Committee on Antimicrobial Susceptibility testing nonspecies related clinical breakpoints were used.
Results: There were significant differences in the frequency of *E. faecium* among the enterococci at the PCC (7.3%), GHW (13%) and the ICUs (28%) ($p < 0.05$). A total of 0.8% of the *E. faecalis* isolates and 2.7% of the *E. faecium* isolates were low level (MIC 8–16 mg/l) vancomycin resistant (VRE) but teicoplanin susceptible. High level gentamicin resistance (HLGR) was seen among a total of 13% and 17% of the *E. faecalis* and the *E. faecium* isolates, respectively. There were significant higher frequency of HLGR among the *E. faecalis* isolates at both the GHW (20/%) and the ICUs (17%) compared to the PCCs (5.9%) ($p < 0.05$). The *E. faecalis* isolates showed a significant difference in susceptibility to imipenem, moxifloxacin, and vancomycin at the ICUs compared to the PCCs ($p < 0.05$). A significant difference in susceptibility between the GHW and PCC was only seen for moxifloxacin. The *E. faecium* isolates showed no difference in susceptibility between the ward levels.

	Susceptibility PCC/GHW/ICU (%)	
Antibiotic	*E. faecalis*	*E. faecium*
Ampicillin	100/100/98	6.0/19/29
Imipenem	90/88/83	–
Levofloxacin	11/6.2/7.1	6.0/2.7/84
Moxifloxacin	89/78/74	12/11/19
Teicoplanin	100/100/100	100/100/100
Vancomycin	100/100/98	100/97/98

Conclusion: There were significant differences in the frequency of *E. faecium* at the different ward levels, *E. faecalis* showed a high susceptibility against all the tested antibiotics except for imipenem, the quinolones and gentamicin (HLGR). These antibiotics showed a significant difference in susceptibility between the ward levels. Vancomycin and teicoplanin were the only antibiotics that showed high susceptibility against most *E. faecium* at all ward levels.

P761

ESPAR: variation of antibiotic resistance in *Enterococcus* spp. isolates

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Objectives: The idea of ESPAR project was to study emergence, spread and persistence of antibiotic resistance in normal microbiota during 2-year period. The main objective was to follow the variation of antibiotic resistance, caused by a short antibiotic treatment in *Enterococcus* isolates, and to study the evolution of the resistant *Enterococcus* strains.
Methods: *Enterococcus* spp. isolates were collected from 4 healthy subjects, treated with clindamycin for 7 days. Faecal samples were serially collected in nine different time points; Before administration of antibiotic, right after it and then after 21 days, 3, 6, 9, 12, 18 and 24 months. Enterococcae were also isolated from faecal samples, serially collected from 4 control subjects during 6 months. 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. Multiplex-PCR was used for studying macrolide resistant genes, erm (B), erm (TR) and mef (A). Species variation was studied with pyrosequencing method.
Results: When comparing all four subjects, there were no clear correlation between clindamycin treatment and antibiotic resistance. However, results from two subjects showed that clindamycin probably had influence, because there were no resistant strains in 0-sample, but after 7 days resistance rose substantially. The amount of resistant strains in 7 days samples was at least threefold comparing to 0-sample, and the amount of resistant *Enterococcus* strains was largest between 7 and 21 days. In control group there were no clear correlation between resistance and time.
Conclusion: Results showed that due to the clindamycin treatment, the amount of resistant *Enterococcus* strains rose substantially and resistance returns very slowly, if not at all, back to the normal level. However, normal variation in antibiotic resistance in *Enterococcus* isolates makes it more difficult to analyse the real influence of the clindamycin treatment.

P762

Diversity among high-level gentamicin resistant *Enterococcus faecium* in the south east of Sweden

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Objectives: The aim of this study was to investigate genetic relatedness of *Enterococcus faecium* with high-level gentamicin resistance (HLGR) among clinical isolates from patients with clinical indications admitted to three hospitals in the south east of Sweden during a period of nine and a half months.
Methods: High-level gentamicin resistant *E. faecium* isolates obtained from routine clinical specimens submitted to the Clinical Microbiology Laboratory between November 2003 and August 2004 were included in this study. No zone around a disc with 30 μg gentamicin (Oxoid Ltd) was defined as HLGR according to SRGA, the Swedish Reference Group of Antibiotics (www.srga.org). Genetic relatedness was studied among 20 none-replicate *E. faecium* with HLGR and control strains with no HLGR (n = 20). The control strains were matched when possible by year and unit with the HLGR-isolates. Detection of related clones was performed using PFGE of SmaI DNA restriction fragments. *S. aureus* NCTC 8325 was included as control/ladder. The isolates were subjected to PCR for detection of the aac(6′)Ie-aph(2″)Ia gene. *E. faecalis* ATCC 51299 was included as positive control and *E. faecalis* ATCC 29212 as negative control.
Results: Thirteen of 20 *E. faecium* with HLGR belonged to the same clone according to the PFGE patterns. There was similarity between one of the HLGR-isolates and two of the control strains. 19 of 20 isolates with HLGR and the positive control carried the aac(6′)Ie-aph(2″)Ia gene. The gene was not present in the negative control and non-HLGR control strains of *E. faecium*.
Conclusion: Genetic relatedness was seen among 65% of the *E. faecium* isolates with HLGR indicating a clonal spread of these isolates among patients admitted to hospitals in the south east of Sweden. Almost all HLGR isolates harboured the aac(6′)Ie-aph(2″)Ia gene.

P763

Temporal changes in enterococcal species in the human intestine, and persistence of *Enterococcus faecium* SF68 following its administration to healthy volunteers

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Objectives: To investigate the temporal changes in enterococcal species in the human intestine, and the persistence of the probiotic strain, *Enterococcus faecium* SF68, a human volunteer study was conducted.

Methods: A total of 13 healthy volunteers were enrolled in the study. Seven subjects received 2 capsules of *Entercoccus faecium* SF68 (108 CFU/caps.) thrice daily for 5 days, while 6 subjects formed the control group. Faecal samples from all subjects were collected before *E. faecium* SF68 administration (day 0) and on days 4, 10, 20, weeks 5, 7, 9, 11, and months 6, 8, 12. Pre-probiotic administration, stools were screened for enterococcal species and their clonality for all volunteers and further on, only for the control group. Faecal samples were plated on Enterococcal agar and after 48 h of incubation, 10 esculin hydrolysing colonies were followed further. PYR was used for genus identification, while species identification was studied by RAPD-PCR using primer D11344, and in case of inconclusive results, by SDS-PAGE of whole-cell proteins. Clonality was investigated by RAPD-PCR using primers D11344 and D14307, and in case of discordant results between the 2 primer sets, by PFGE using SmaI.
Results: From 127 enterococci isolated on day 0, a total of 5 different species were identified: *E. faecium* (47%), *E. faecalis* (39%), *E. durans* (11%), *E. hirae* (2%) and *E. gallinarum* (<1%). From day 4 to month 12, 744 enterococci were isolated from the control group and identified as: *E. faecium* (59%), *E. faecalis* (26%), *E. durans* (7%), *E. hirae* (3%); other species (*E. casseliflavus*, *E. avium*, *E. mundtii*) were <2%. Distribution of clonal types, especially in *E. faecium* and *E. faecalis*, varied among the control group. During the study period clonal types in *E. faecalis* varied from 1 to 6 and in *E. faecium* between 8 to 14 types, which was on an average twice the number of types/isolation day compared to *E. faecalis*. Post-administration, *E. faecium* SF68 was detected in all 7 subjects on day 4. At week 9, only a single volunteer showed the presence of *E. faecium* SF68 and at week 11 none of the volunteers were found to carry the probiotic strain.
Conclusions: The composition of the commensal enterococcal faecal flora was found to fluctuate over the study period of 1 year with *E. faecium* and *E. faecalis* being the predominate species. This is the first study showing the persistence of colonisation by a probiotic strain in the intestine for such a prolonged period.

P764

Vancomycin-resistant enterococci, colonising the intestinal tract of hospitalised patients in a university hospital in Greece

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Objectives: To explore the prevalence of Vancomycin-resistant Enterococci (VRE) colonizing the intestinal tract of hospitalized patients and to define risk factors.
Patients and Methods: A point prevalence survey of VRE fecal carriage was carried out among patients admitted to a 600-bed teaching hospital for at least 2 days. Resistance to vancomycin was detected by the E-test method. Epidemiological data were recorded for all patients included in the study and were used for the risk factor analysis.
Results: A total of 128 patients hospitalized for at least 2 days were enrolled in this investigation. Thirty-nine patients (30.5%) were colonized with vancomycin-resistant enterococci. Twenty-three of the 39 strains were identified as *Enterococcus faecium*, 13 were identified as *Enterococcus gallinarum* and 3 strains as *Enterococcus casseliflavus*. The risk factors that were significantly associated with VRE colonization included length of hospital stay (13.2 days vs. 8.6 days), age (60.7 years vs. 47.7 years) and the presence of underlying malignancies (28.2% vs. 11.2%). Association was found between VRE colonization and use of antimicrobials with anaerobic activity such as metronidazole, piperacillin/tazobactam and imipenem. The use of vancomycin was associated with VRE colonization in ICU.
Conclusions: VRE colonization must be monitored and risk factors should be determined because they are useful for screening hospitalized patients for VRE colonization in order to establish prevention and control measures.

P765

Persistent single VRE strain is responsible for two outbreaks in an ICU

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Background: Colonisation and infection with vancomycin-resistant Enterococci (VRE) are problems in hospitals worldwide. In October and November 2003 VRE were detected in clinical specimens of five patients on a surgical ICU of our University Hospital with a VRE prevalence of less than 0.7%. In order to prevent further transmission standard isolation procedures were initiated and no additional cases were found. Combined with routine MRSA screening a screening for VRE colonisation was started in March 2004.
Materials and Methods: VRE screening was performed on this ICU with 4 subunits and 28 beds for a 6-month period. 114 patients who stayed for more than 5 days were included and screened weekly. A rectal swab was taken and examined by direct culture on bile esculin azide agar plates containing 6 mg/l of vancomycin. Suspicious colonies were identified to species level using routine criteria and API Rapid ID 32 Strep system. The MIC of vancomycin and teicoplanin was determined by E-test method. In case of VRE detection standard isolation practices were started. Molecular typing was performed by pulsed field gel electrophoresis (PFGE) to determine relatedness of isolates.
Results: End of May 2004 one case of VRE wound infection occurred, rectal screening was negative. During June and July nine patients were found to be VRE positive, eight cases were revealed by rectal screening, one by clinical specimen only. Five of these nine patients developed VRE infection. All isolates were *E. faecium* with vancomycin and teicoplanin resistance. PFGE confirmed a single VRE strain was responsible for both VRE outbreaks. Since August 2004 no additional VRE cases were detected on this ICU.
Conclusions: Probable a single VRE strain can persist undetected for at least 6 month in an ICU setting. Our routine VRE screening seems to be efficient for the detection of colonised patients but did not prevent a second outbreak. Colonised patients are at risk for VRE infection and represent a source for further transmission. VRE outbreaks can be controlled by intervention strategies.

P766

Clonal outbreak of vancomycin and ampicillin resistant *Enterococcus faecium* (VRE) in the haematological unit of a Belgian hospital

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Background: Outbreaks of VRE nosocomial infection have been described in high risk units but remain rare in Belgium. We investigated a cluster of vancomycin resistant *Enterococcus faecium* infection in our hematological unit.

Methods: Case definition was a patient admitted to Erasme Hospital hematological unit and colonized/infected with vancomycin and ampicillin resistant *E. faecium* (VRE) during 2003–2004. Enterococci were identified by Rapid ID-Strep and mobility at 35°C. Susceptibility to ampicillin, gentamicin, streptomycin, clindamycin and doxycycline was determined using the disk-diffusion method. Vancomycin resistance was tested on vancomycin screen agar (6 μg/ml) according to NCCLS. Resistance to glycopeptides and ampicillin were confirmed by E-test method and by PCR for detection of vanA, vanB and vanC genes. SmaI macrorestriction analysis resolved by pulsed field gel electrophoresis (PFGE) was performed. Weekly rectal screening for VRE was undertaken in patients hospitalized in the haematological unit by inoculation on Enterococcosel agar supplemented with vancomycin (10 μg/ml) and with/without enrichment broth with 7.5% NaCl.
Results: From January to June 2004, the incidence of infection with VRE in patients admitted to hematological unit was 0.9% (4/466) as compared to 0% (0/956) ($p < 0.005$) in the previous 12 months. Infections included bacteraemia (n = 2), anchiocholitis (n = 1) and soft tissue abscess (n = 1). At the end of June, outbreak investigation and control measures were implemented combined to weekly rectal screening. Contact precautions (wearing of gloves and gown) and environmental disinfection were implemented for the care of VRE infected/colonized patients. After infection control implementation, no further case of VRE infection was occurred but 7/134 (5%) screened patients were colonized with VRE over a 5-month period. All strains were resistant to ampicillin (MIC > 256 μg/ml), vancomycin (MIC > 256 μg/ml) and teicoplanin (MIC > 16 μg/ml), harbored the vanA gene and belonged to the same PFGE type.
Conclusions: This clonal outbreak of VRE in an hematological ward over a 10-month period was associated with significant morbidity. Early detection of carriers and use of contact precaution have not led to full control of the epidemic as asymptomatic cross colonization still occurred. Active surveillance culture of carriers will be continued to enforce isolation of colonized patients.

P767

First nosocomial outbreak of *Enterococcus faecium* expressing heterogenous vancomycin-resistance at a French university hospital

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Objectives: Vancomycin-resistant enterococci (VRE) are widespread worldwide. Despite growing concern about VRE as nosocomial pathogens, especially in the United States, they are rarely isolated in French hospitals. We report the molecular characterization of the first nosocomial outbreak of VRE at a French University Hospital.
Methods: Strains correspond to either clinical samples or rectal swab specimens for analyzing carriage. Identification, susceptibility testing, molecular characterization and molecular comparison of the strains [pulse field gel electrophoresis (PFGE)] were performed according to standard techniques.
Results: VRE were isolated from 18 patients between August and November 2004, of whom 17 were hospitalized at the department of Nephrology and one at the department of Internal Medicine. Twelve patients were only carriers and six patients had true VRE infections: one bacteraemia, two peritonitis and three urinary tract infections. All of these isolates were *E. faecium* and expressed heterogenous glycopeptide resistance (MIC for vancomycin: 12–256 mg/L and for teicoplanin: 6–16 mg/L). These strains were also resistant to ampicillin, levofloxacin, clindamycin, erythromycin and displayed high-level resistance to gentamicin, kanamycin and streptomycin, but remained susceptible to linezolid and tetracycline. PFGE revealed that these 18 *E. faecium* isolates were undistinguishable or clonally related. They were all of vanA genotype with a genetic organization identical to that described for Tn1546 in the VanA prototype strain *E. faecium* BM4147, except for the presence of IS16 inserted into the vanY gene. Implementation of stringent hand disinfection and environmental disinfection policies, as well as measures for patient isolation contained this outbreak of VRE, but currently did not eradicate totally the vanA *E. faecium* strains from the unit.
Conclusions: This work reports the emergence of VRE in a French University Hospital with very heterogenous glycopeptide expression, thus making their detection very difficult.

P768

The first recorded outbreak of VRE in Hungary involving vanB carrying *E. faecium* isolates from clinical samples and from the hospital environment

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Objective: The aim of this study was to investigate and confirm by phenotypic and molecular methods the first reported outbreak of vancomycin resistant enterococci (VRE) in Hungary and to uncover the epidemiological relationship between the outbreak isolates.
Methods: The isolates were screened on a 6 mg/l vancomycin-BHI plate as recommended by NCCLS. Vancomycin and teicoplanin MIC values were determined for the screen-positive isolates by the E-test. Identification was achieved by basic biochemical tests, motility in SIM medium, the Crystal GP system and by PCR amplification of the ddl and vanC genes. vanA and vanB genes were detected by PCR. Clonal relationship between the VRE isolates was determined by PFGE.
Results: The outbreak strains were isolated at a hematology unit in Budapest during 2003 and 2004. The initial investigation confirmed vanB positive *E. faecium* isolates from six blood cultures, one urine sample, eight faecal samples and three environmental samples. All these isolates were found to be epidemiologically related to each-other by macrorestriction (PFGE) analysis but unrelated to vanB negative *E. faecium* isolates obtained from the same hospital. One vanA carrying *E. faecium* was also isolated from the faecal sample of a hospital administrator which was clearly unrelated to the outbreak strains. VRE carriage was not detected for any other member of the hospital staff, however, a comparatively high percentage of the carriage of vanC positive isolates (*E. casseliflavus* and *E. gallinarum*) was observed.
Conclusions: This is the first report of vanB carrying VRE from Hungary and also the first report of an outbreak caused by VRE. These results suggest that a screening and monitoring system has to be implemented to prevent the further dissemination of VRE in the country.

P769

Genetic relationship, occurrence of esp and antibiotic resistance among blood-culture derived *Enterococcus faecium* isolates

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Background: Enterococci have during the last three decades emerged as a significant cause of nosocomial infections. The

durable nature of enterococci combined with dissemination of antibiotic resistant *Enterococcus faecium* and *Enterococcus faecalis* in hospital settings is an increasing problem since treatment of these infections can be challenging. Enterococcal surface protein (Esp), a virulence factor encoded by the esp-gene, has been associated with immune evasion, biofilm formation and adhesion. Isolates of infectious origin have been found to be enriched with the esp-gene.

Objectives: To characterize *E. faecium* infectious isolates from a Swedish blood-culture collection in terms of antibiotic resistance, occurrence of esp, genetic relationship and to determine if cross-infection of *E. faecium* had occurred between patients.

Methods: A total of 82 *E. faecium* strains isolated from different patients with bacteraemia during a 3-year period at the Karolinska University Hospital, Huddinge, Sweden were analysed. The strains were scanned for presence of the esp-gene by PCR. Minimal inhibition concentration against ampicillin, ciprofloxacin, gentamicin, imipenem, linezolide and vancomycin was determined by the agar diffusion method according to NCCLS. For determination of genetic relationship between the isolates and detection of possible cross-infection between patients pulsed-field gel electrophoresis was used.

Results: About half of all isolates were shown to be esp-positive. The esp-positive strains were less genetically diverse and had higher frequencies of antibiotic resistance compared to the esp-negative ones. Resistance towards ampicillin, imipenem and ciprofloxacin was common while vancomycin resistance was rare. All isolates were susceptible to linezolid. *E. faecium* isolates, identical according to PFGE, were detected from different patients.

Conclusions: Presence of esp seems to be common among the *E. faecium* blood-culture isolates. Dissemination of antibiotic resistant, esp-positive *E. faecium* strains between patients seems to occur in the hospital. The esp-positive *E. faecium* strains seem to have a less diverse genetic origin compared to the esp-negative ones.

P770

Molecular epidemiology of glycopeptide-resistant enterococci isolated in a university hospital, Italy (2003–2004)

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Objectives: Recently *Enterococcus* spp has emerged as a major nosocomial pathogen because of its resistance to multiple antibiotics, including glycopeptides. Actually severe enterococcal infections create therapeutic problems difficult to solve. The aim of the present study was to determine antimicrobial susceptibility pattern and genotypic characteristics of glycopeptide-resistant enterococci (GRE) isolated in Padua Hospital, one of the largest in Northeastern Italy, and to explore the genetic relationship among them.

Methods: In two-year period, 21 GRE were isolated principally from patients who were undergone liver transplantation, hospitalized in different wards. Strains were identified by API 20 Strep (BioMérieux). Minimal inhibitory concentrations (MICs) were determined by broth-microdilution method according to NCCLS guidelines with cation-adjusted Mueller-Hinton broth. The presence of van genes (vanA and vanB) was analysed by PCR assays and molecular analysis was performed by pulsed-field gel electrophoresis (PFGE) after restriction with SmaI enzyme. The presence of esp genes was also investigated by PCR assays.

Results: Of all GRE isolates, 92% were *Enterococcus faecium*. All strains were vanA- positive and resistant to all traditional antibiotics: ampicillin, cefalosporins, tetracycline, ciprofloxacin, gentamycin (high resistance level). Assays to demonstrate the surface protein Esp, recently identified as a marker of clones prevalent among hospitalized patients, were negative for all strains. Genotype analysis by PFGE identified three major patterns (A–C) suggesting patient-to-patient clonal dissemination or presence of colonized residents who serve as a reservoir for GRE.

Conclusions: The molecular typing is fundamental for epidemiological studies which represent the first step to establish control measures directed to contain spreading of multidrug-resistant bacteria responsible of severe infections.

P771

Low prevalence of vancomycin resistant enterococci colonisation in intensive care patients in a Brazilian teaching hospital

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Objective: To determine the prevalence of vancomycin resistant enterococci (VRE) colonization in intensive care units patients in a 400-bed tertiary care teaching hospital, and correlate with vancomycin consumption.

Methods: Anal swab cultures were collected from patients in adult and pediatric intensive care units (ICU) between October-2003 and October-2004. Samples were collected from patients with more than 5 days of admission in these units, and repeated every 7 days until their exits, and incubated in a bile esculine agar with vancomycin (6 mcg/ml) to search for *Enterococcus* sp growth. Strains were then reincubated in a blood agar medium, identified by Vitek® and susceptibility to vancomycin and teicoplanin tested by an E-test. Minimal inhibitory concentrations (MIC) over than 8 μg/ml were reported like resistant. Medical charts and use of antimicrobial were recovered. Defined daily dose (DDD) consumption of vancomycin in entire hospital was also recovered using an informatized supply control programme.

Results: In pediatric and adult ICU were collected 249 samples from 112 patients, media of 2.22 samples per patient (ranging from 1 to 12 cultures), of them 141 in the pediatric ICU (47 patients) and 108 in the adult ICU (65 patients). Vancomycin resistant *Enterococcus* sp grew in 9 patients, 4 from pediatric and 5 from adult ICU, being 5 cases of *Enterococcus gallinarum* and 2 cases of *Enterococcus casseliflavus*, both possibly with no transmissible VanC phenotype. Only 2 patients were colonized by *Enterococcus faecalis* vancomycin resistant. First, a woman with acute intermittent porphyria with a length of stay in the ICU longer than 3 months receiving broad-spectrum antimicrobials (cefepime, vancomycin, anfotericin B and later piperacilin – tazobactam). Another case was a child with an acute fulminant hepatitis A with a prolonged stay in another hospital before his admission, dying in the further 6th day waiting for a liver transplantation. Both patients did not have documented infection by these strains, and in both the VRE were susceptible to ampicilin and also resistant to teicoplanin. Consumption of vancomycin was less than 50 DDD/1000 patients-day in this period.

Conclusion: VRE is not a major concern in our ICUs, despite the literature report. Furthermore, there was not any case of VRE *faecium* colonization. Of note, both strains were susceptible to ampicillin. These findings may correlate with a restrictive use of vancomycin.

P772

Glycopeptide-resistant *Enterococcus faecium* in Spain with vanA genotype and apparent teicoplanin susceptibility

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Objectives: Nosocomial glycopeptide-resistance *Enterococcus faecium* (GREF) is unusual in Spain, however GREF have been isolated in our institution since September 2002. The objectives of this study were to determine the clonal relationship, the phenotype of glycopeptide resistance and the involved genotype and its association with conjugative elements in GREF isolated in our hospital.
Methods: Identification and preliminary susceptibility testing were done with the Walk-Away System. MICs of vancomycin (VAN), teicoplanin (TEI), ampicillin (AMP), erythromycin (ERY), tetracycline (TET), chloramphenicol (CLO), HLR-gentamicin (GEN), HLR-streptomycin (STR), linezolid (LNZ), and quinupristin-dalfopristin (Q/D) were determined by microdilution (MD, NCCLS). MICs of VAN and TEI were also determined with E-test strips. Clonal relationship was determined by both REP-PCR and PFGE (SmaI restriction). VAN-resistance was transferred by conjugation to *E. faecium* BM4105SS. Plasmids were obtained by the alkaline lysis method. The glycopeptide-resistance determinant was identified by multiplex-PCR and PCR with vanA-specific primers.
Results: Between September 2002 and April 2004, 31 GREF were isolated from 28 patients from wound (n = 13), blood (n = 3), or other samples (n = 15). MICs (mg/l) of VAN for a majority of isolates (n = 29) were >256 (MD and E-test). MICs of TEI by MD and E-test were 4–8 and 32–256, respectively, for a majority of isolates (n = 21). The percentages of resistance were: HLR-GEN (77), HLR-STR (23), AMP (100), ERY (100), TET (6), CLO (3), LNZ (0), Q/D (52). Three REP-PCR patterns (25, 5, and 1 isolates) and two PFGE patterns (26 and 5 isolates) were obtained. VAN-resistance was transferred in all cases to *E. faecium* BM4105SS (being the transfer frequencies 10^{-4} for 29 isolates and 10^{-6} for the remainder 2). Several conjugative plasmids were involved in the transfer. MICs (mg/L) of VAN and TEI against transconjugants were >128 and 8–64, respectively. The vanA determinant was identified in all parental isolates and transconjugants.
Conclusions: We have identified two clones of GREF transferring vanA at a high frequency. In our isolates, the E-test was more reliable than MD for detecting the VanA phenotype.

P773

Comparative analysis of virulence factors in enterococci isolated from hospitals

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Objectives: This work presents comparative evaluation of the occurrence of potential virulence factors as hemolysin (Hly), gelatinase (Gel), lipase (Lip), DNAse; esp gene and their association with formation of biofilm among enterococcal clinical samples in Slovakia and Poland.
Methods: Total 225 clinical isolates *E. faecalis* and *E. faecium* with different resistance determinants were involved in this study (109 isolates from Slovakia and 116 isolates from Poland). Antimicrobial susceptibility was determined by the disk-diffusion method according to NCCLS standards. The presence of Hly, Gel, Lip and DNase activities were investigated on TSA with 5% horse blood, BHI agar with gelatine (40 g/l), Spirit Blue agar and DNAse agar. For detection of esp gene PCR with specific primer set was used. The ability to produce biofilm was tested in a microtiter assay.
Results: The occurrence of the respective virulence factors was as follows (Slovak isolates vs. Polish isolates) cytolysin 16.5 vs. 11.2%, gelatinase 47.7 vs. 6.9%, DNAse 16.5 vs. 5.2%, lipase 5.5 vs. 25.9%, esp 55 vs. 53.5%. Both, esp-positive and esp-negative isolates expressed competence for adherence in biofilm experiments; however intensity of biofilm formation was different. The Slovak/Polish enterococcal isolates were also screened for resistance to 4 selected antibiotics with results: gentamicin 100/57.8%, streptomycin 10.1/59.5%, vankomycin 0/5.2% and teicoplanin 0/0.8%.
Conclusion: Distributions of respective virulence factors which may play an important role in enterococcal pathogenesis among strains from Slovakia and Poland was estimated to be diverse. Bacterial adherence to polystyrene surface was proved to be independent with any of studied virulence factors, but seems to be associated with antibiotic resistance.

P774

Occurrence of vancomycin-resistant and high level aminoglycoside-resistant *Enterococcus* spp. isolated in an Italian hospital, Bari, southern Italy

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Glycopeptide-resistant enterococci have become a focus of concern in many countries because options for antimicrobial therapy are limited. It is well known that the use of a cell wall active such as penicillin or a glycopeptide with an aminoglycoside results in synergistic bacterial activity against severe infections due to *Enterococcus*. The efficacy of this bactericidal treatmen is limited by the emergence of high level gentamycin, kanamycin and streptomycin resistance. The aim of this study was to evaluate the occurrence of glycopeptide resistance and high-level aminoglycoside resistance in 1169 strains of *Enterococcus* spp isolated from several anatomic sites of patients admitted to Policlinico Hospital, Bari, Italy. *E. faecalis* and *E. faecium* represent the 82.6% and 15.8% of isolates, respectively. The most common sites of isolation were in the order: urinary tract (64.8%), pus (21.4%), blood (6.3%), CVC (2.1%), materials from genital tract (3.2%) and rectal swab (0.5%). We have identified 19 strains (1.6%) of *Enterococcus* spp resistant to both vancomycin and teicoplanin (GRE). *E. faecalis* represent the 0.7%, whereas *E. faecium* represent the 6.5%. MIC90 to vancomycin was for *E. faecalis* 0.5 and 4 mg/L for *E. faecium*. Corresponding values for teicoplanin were 2 mg/L for *E. faecalis* and 0.25 mg/L for *E. faecium*. GRE represent the 1.6% of *Enterococcus* isolated and this value reflect those found in Europe. GRE were isolated mainly from urine, pus, blood and CVC, and essentially from service of Medicine, Surgery and ICU. High-level resistance to gentamycin and kanamycin were tested by diffusion agar screen method. HLR to gentamycin were detected in the 33.2% of *E. faecalis* and in the 31.3% of *E. faecium*. HLR to kanamycin was found in the 68.5% of *E. faecalis* and in the 81.1% of *E. faecium*. HLR to aminoglycoside were found in isolates from urina, pus, blood and CVC. Data on HLR to gentamycin are in according to that found in other countries of Europe, whereas high percentage of HLR was found for kanamycin. These data indicate a potential for further improvement of antibiotic policies in our hospital.

P775

Mucoid *Enterococcus faecalis*, an emerging phenotype?

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Objectives: The prospective evaluation of the isolation of encapsulated *Enterococcus* spp. with mucoid phenotype, due to the increase of this unusual type of strains.
Methods: Over a 4-year period (July 2000/June 2004) 116487 urine specimens from hospital and community patients were cultured.
Results: 27054 (23.2%) specimens had positive results. *Enterococcus* spp. was recovered from 2744 of them (10.1%) samples. After 24 h. of incubation in cultures from 8 patients, strains of slow growth were isolated, with small colonies on blood agar and pinpoint colonies on cled agar. After 48 h. nonhaemolytic confluent colonies with highly mucoid aspect, resembled that of Gram-negative bacilli. Gram stain corresponded to ovoid Gram-positive cocci arranged in pairs, with a distinctive halo around the cells. Urine sediment showed good quality of the samples, with abundant leukocytes and Gram-positive cocci. All 8 isolates were identified as *E. faecalis*, without atypical reactions. By microdilution, strains were susceptible to ampicillin, amoxicillin-clavulanic, nitrofurantoin, norfloxacin and vancomycin and presented variable results to fosfomycin, gentamicin and cotrimazole. Mucoid aspect persisted after repeated subcultures and after frozen storage. All 8 patients were community patients. Ages were 72, 77, 92, 57, 72, 71, 75 and 39 years and had cronic urinary tract infections, although not previously caused by *E. faecalis*. The only patient with relevant risk factors was the first one, with cystectomy for bladder neoplasia. Evolution was favorable, with negative post-treatment controls in 6 cases. In two patients mucoid *E. faecalis* was reisolated repeatedly despite correct therapy.
Conclusions: 1. In our patients mucoid *E. faecalis* urinary infections were community-acquired.
2. The possibility of initial misidentification with gram-negative bacilli, can delay the microbiological diagnostic.
3. Antimicrobial susceptibility results not expected with a mucoid strain, must orientate to the isolation of encapsulated *E. faecalis*.
4. Routine Gram stain of slow growing strains will help in early detection of these kind of strains.

P776

Detection of high-level gentamicin resistance and genes encoding gentamicin resistance in invasive enterococci in Denmark

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Objectives: Enterococcal infections are often treated with a combination of an aminoglycoside and a cell-wall-active agent such as penicillin or a glycopeptide. In Denmark vancomycin resistant enterococci still have a very low prevalence, whereas high-level resistance to gentamicin (MIC > 500 mg/l) seems to be more common. In the other Nordic countries is high-level gentamicin resistance most frequently detected in *E. faecalis*. Four genes, aac(6′)-Ie-aph(2′′)-Ia, aph(2′′)-Ib, aph(2′′)-Ic and aph(2′′)-Id, are known to encode gentamicin resistance in enterococci. This study investigates the prevalence of high-level gentamicin resistance and the distribution of the different gentamicin resistance genes in invasive enterococci.
Methods: A total of 159 invasive (mainly blood) enterococci were collected between January 2004 and September 2004. Only one isolate from each patient was included in this study, except in two cases where both an *E. faecium* and an *E. faecalis* were obtained from the same patient. The strains were collected at six Danish Departments of Clinical Microbiology (Aalborg, Odense, Hvidovre, Herlev, Hillerød, and SSI) and sent to National Center for Antimicrobials and Infection Control at SSI. All 159 strains were identified to species level by PCR. Susceptibility to gentamicin (128–2048 mg/l) was determined by the micro broth dilution method. Gentamicin resistant enterococci were investigated for the presence of the aac(6′)-Ie-aph(2′′)-Ia, aph(2′′)-Ib, aph(2′′)-Ic, and aph(2′′)-Id genes by PCR.
Results: Of the 159 isolates were 97 (61%) *E. faecalis* and 62 (39%) *E. faecium*. In total were 59 (37%) of all the invasive enterococci resistant to gentamicin, divided into 36 (61%) *E. faecium* and 23 (39%) *E. faecalis*. All gentamicin resistant isolates contained aac(6′)-Ie-aph(2′′)-Ia. None of the other resistance genes were detected in any of the isolates.
Conclusion: In total 38% of the collected invasive strains were gentamicin resis-tant and 61% of these resistant strains were *E. faecium*. This indicates that gentamicin resistance is common in Denmark especially in *E. faecium*. The resistance was mediated by the aac(6′)-Ie-aph(2′′)-Ia gene, which was the only gene detected in the resistant strains.

P777

An unusual resistance phenotype in nosocomial strains of *Enterococcus* species in a Greek hospital

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Objectives: *Enterococcus faecalis* is a common nosocomial agent and usually demonstrates susceptibility to ampicillin, penicillin G and amoxicillin/clavulanicate, while *Enterococcus faecium* is commonly resistant to the above mentioned antimicrobial agents. The aim of our study is the presentation of an unusual resistance phenotype that was recently identified in a Greek tertiary hospital among *A. faecalis* and *E. faecium*, exhibiting resistance to penicillin and susceptibility to ampicillin and amoxicillin/clavulanate.
Methods: A total of 35 non-repetitive enterococcal strains were collected from various hospital infection samples. Identification and susceptibility testing were performed by using the Vitek 2 automated system (Biomerieux, Marcy l′ Etoile, France). The MICs for penicillin G, ampicillin and amoxicillin/clavulanic acid were determined using *E*-test. The production of beta-lactamase was evaluated using the cefinase test.
Results: Among 35 isolates, 33 were identified as *E. faecalis* and 2 as *E. faecium*. All isolates were susceptible to ampicillin and amoxicillin/clavulanic acid with MIC ranging from 0.38 to 1.50 mg/L respectively but they were resistant to penicillin (MIC > 32 mg/L). Using the cefinase test they were found to be negative for beta-lactamase production. PFGE analysis revealed various unrelated clonal patterns.
Conclusions: A spread of this unusual resistance phenotype would affect the usefulness of penicillin that generally retains activity against *E. faecalis*. The aetiology of this phenotype is an object of study.

Epidemiology of ESBL and MBL – II

P778

Low diversity of integrons among non-*Escherichia coli* Enterobacteriaceae clinical isolates producing extended-spectrum beta-lactamases from Spain

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Objectives: To analyse the overall prevalence and diversity of integrons among ESBL-producing non-*Escherichia coli* Enterobacteriaceae, NEC-ESBL(+), from Hospital Ramón y Cajal (1987–2000).
Methods: Sixty-six PFGE clonal types of NEC-ESBL(+) corresponding to 34 *Klebsiella pneumoniae* (KP), 7 *Klebsiella oxytoca* (KO), 14 *Enterobacter cloacae* (ECL), 3 *Enterobacter aerogenes* (EA), 1 *Enterobacter gergoviae*, 4 *Salmonella enterica*, and 3 *Citrobacter* spp. were studied. Fifty-two ESBL-producing *E. coli*, EC-ESBL(+), and 43 EC-ESBL(−), recovered in the same institution were used as a control group. Class 1 and 2 integrons were detected by PCR, typed by RFLP using AluI and HaeIII as restriction enzymes, respectively, and identified by sequencing (one per RFLP type). Class 3 integrons were determined by dot-blot hybridization.
Results: ESBL among NEC-ESBL(+) were identified as 17 TEM, 15 SHV, 3 CTX-M-9, and 31 CTX-M-10 and among EC-ESBL (+) as 16 TEM, 9 SHV, 22 CTX-M-9, 1 CTX-M-14, and 4 CTX-M-10. Class 1 integrons were detected in the 30% of the NEC-ESBL(+) isolates studied, being more common among KP (13/34, 38%) than among all other different enterobacterial species (7/32, 22%). The presence of these integrons among NEC-ESBL(+) was lower than among EC-ESBL(+) or EC-ESBL(−) from the same hospital (30%, 67% and 40%, respectively). Eight RFLP types (size ranged from 0.9 to 2.2 kb) were detected. The most common ones were those identified as aadA1 (7/19, found in KP and ECL species), and dfrA16aadA2 (2/19 found in KP and ECL species). Three other types were detected in KP and one type in EA. Four of these six types were also present in *E. coli*. Two types were only found in ECL and *Salmonella* isolates, respectively. Five isolates contained two class 1 integron types of different size. Class 2 or class 3 integrons were not found.
Conclusions: The lower prevalence of integrons among NEC-ESBL(+) than among EC-ESBL(+), could be related with different content in ESBL genes (CTXM-9, more prevalent in EC, is associated to In6 class 1 integron). The presence of most of the class 1 integron types among different enterobacterial species during the same time period suggests dissemination of common elements (plasmids or transposons) in which integrons are located.

P779

Diversity of integrons and sulfonamide resistance genes among ESBL and non-ESBL Enterobacteriaceae recovered from Portuguese hospitals

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Objectives: To analyse the overall distribution of class 1 and class 2 integrons, and sul1, sul2, and sul3 genes among ESBL and non-ESBL Enterobacteriaceae isolates from different Portuguese nosocomial environments.
Methods: We studied 83 Enterobacteriaceae clinical isolates recovered from patients attending at Portuguese hospitals (October 2002 to May 2004): (i) 50 ESBL-producing clinical isolates corresponding to 21 *Klebsiella pneumoniae*, 2 *Klebsiella oxytoca*, 12 *Escherichia coli*, 4 *Enterobacter aerogenes*, 4 *Enterobacter cloacae*, 4 *Proteus mirabilis*, and 3 *Serratia marcescens*; and (ii) 33 non-ESBL-producing isolates (4 *K. pneumoniae*, 4 *E. coli*, 4 *E. aerogenes*, 2 *E. cloacae*, 13 *S. marcescens*, 2 *P. mirabilis*, 2 *C. freundii*, 1 *M. morganii*, and 1 *P. stuartii*). Bacterial identification was performed by the Vitek System. Antibiotic susceptibility was performed by the standard disk diffusion method. Presence of class 1 and 2 integrons, and sul1, sul2 and sul3 genes, were searched by PCR using primers previously described.
Results: Class 1 integrons were most prevalent than class 2 integrons (61% vs 5%). Presence of class 1 integrons was higher among ESBL(+) than among ESBL(−) strains (36/50, 72% vs 19/33, 58%) but class 2 were more common among ESBL(−) isolates (3/33, 9% vs 1/50, 2%). Resistance to sulfonamides was found in 77% (64/83) of the isolates, sul1 being more commonly found than sul2 or sul3 genes (55/83, 66%; 35/83, 42%; 4/83, 5%). The recently described sul3 gene was detected in four ESBL-producing *K. pneumoniae* isolates from two hospitals. A high number of strains contained simultaneously sul1 and sul2 (n = 29), sul1 and sul3 (n = 4) or sul1, sul2 and sul3 (n = 3) genes.
Conclusion: Class 1 integrons and sul1 and sul2 genes are widely distributed among Enterobacteriaceae clinical isolates from Portugal. The frequent presence of class 1 integrons among ESBL(+) isolates carrying sul genes might contribute to the persistence of these genes in the nosocomial setting. Although described recently in different enterobacterial species, this is the first report of sul3 genes in *K. pneumoniae*.

P780

High diversity of genetic elements associated with CTX-M-9-producing Enterobacteriaceae clinical isolates from Spain

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Objectives: To analyze the clonal relatedness and diversity of the genetic environments among all CTX-M-9 producing enterobacterial clinical isolates recovered in our institution since first description in 1996.
Methods: Seventy CTX-M-9-producing isolates (69 *Escherichia coli* (EC) and 1 *Klebsiella pneumoniae*) from 45 different patients were collected from 1996 to 2002. Forty-five isolates (one per patient) were included in the study. ESBL characterization was performed by isoelectric focusing, PCR and sequencing. Antibiotic susceptibility was determined by the disk diffusion and microdilution methods. Conjugation experiments were done by broth and filter matings Whole plasmid profile was determined in both wild type and transconjugant strains by the Birnboim Doly method. Clonal relatedness was established by XbaI-PFGE and EC phylogenetic groups were determined by a multiplex PCR assay. Characterization of blaCTX-M-9 genetic environment was performed by a PCR overlapping assay based on In60 sequence (GenBank AF174129), an In6-like class 1 integron in which blaCTX-M-9 has been previously reported.
Results: All but one CTX-M-9-producing isolate were identified as *E. coli*. The isolates showed a high clonal diversity (41 PFGE types/45 isolates) and belonged to phylogenetic groups D (54%), B1 (20%) A (15%) and B2 (10%). All isolates were resistant to streptomycin, trimethoprim, sulfonamide, and ciprofloxacin.

Plasmid content of wild type strains varied from 1 to 7 (2 to >65 kb). Transfer of blaCTX-M-9 was achieved in 80% of the cases. Size of plasmids carrying blaCTX-M-9 ranged from 7 to 65 kb, 45% of the transconjugants showed more than one plasmid. In all cases, blaCTX-M-9 was located in In6-like class I integron structures that were divided in four types: (I) similar to that of In60 (n = 30), (II) showing differences in the region located downstream blaCTX-M-9, (IS3000) (n = 5); (III) showing different gene cassette content in the first 5CS'-3CS' (aadA1 vs dhfrA16-aadA2) (n = 4); IV) lacking the first 5CS'-3CS' (n = 2).
Conclusions: CTX-M-9 producing isolates were mainly associated to *E. coli* species belonging to a wide variety of clonal types and phylogenetic groups. Variations in the genetic environment of blaCTX-M-9 suggest a high recombination rate of this structure. The location of In60 elements on different conjugative plasmids might contribute to increase the dissemination of blaCTX-M-9 in our environment.

P781

Characterisation of class1 integrons carrying blaVIM genes in Enterobacteriaceae from Greece

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Objectives: The emergence of VIM-producing Enterobacteriaceae in Greece is a worrisome phenomenon which incited the present study aiming to investigate whether and to what extend the horizontal transfer of metallo-β-lactamase (MBL) genes contributes to the acquisition of these genes by pathogenic bacteria. The blaVIM gene is carried on mobile gene cassettes inserted into class 1 integrons having the potential to move from one bacterium to another.
Methods: Two *Escherichia coli* [bronchial secretions-BS (1) and urine-U (2)], one *Klebsiella pneumoniae* (U), one Proteus mirabilis (CSF), two *Enterobacter cloacae* [blood-B(1) and U(2)], two *E. aerogenes* [B(1) and stool carriage-SC(2)] and one *Citrobacter freundii* (SC) were studied. Antibiotic susceptibility testing and EDTA-disk synergy tests, were carried out to screen for MBL-producing phenotype. PCR and sequence analysis were used to identify and analyse the blaVIM-containing integrons.
Results: All isolates had reduced susceptibility to imipenem (0.5–2 μg/ml) and demonstrated a positive EDTA-disk synergy test. Sequence analysis of PCR products detected the presence of the MBL gene blaVIM-1 as part of class 1 integron to all but one isolates. *E. coli* (1) isolate from BS harboured a class 1 integron that contained only a blaVIM-2 gene cassette. Preliminary PCR-based experiments and RFLP analysis revealed the presence of the same integron carrying blaVIM-1, dhfrI and aadA in three of the strains [*E. coli* (2), *P. mirabilis* and *E. aerogenes* (2)] isolated at different tetriary hospitals in Athens at different periods. *E. cloacae* (1) and *C. freundii* isolated from two not epidemiologically related patients of our hospital carried the same integron containing blaVIM-1 and aac(6')-IIc genes. *E. cloacae* (2), *C. freundii* and *K. pneumoniae* isolates from the same patient hospitalised in the ICU of our hospital harboured different to each other class I integrons with blaVIM-1 gene cassette.
Conclusions: MBLs of the VIM-type represent an emerging threat in Greek hospitals. All of the blaVIM-carrying isolates of our study harboured class 1 integrons. We observed: 1) the diversity of blaVIM-containing integrons in different isolates of the same patient and 2) the integration of the same class 1 integrons in genetically unrelated nosocomial isolates. More research is needed for understanding the way these mobile genetic elements are transferred between bacteria and how they incorporate and disseminate antibiotic resistance gene cassettes.

P782

Rectal carriage of extended-spectrum beta-lactamase producing Gram-negative bacilli in patients admitted to intensive care units

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Objectives: To ascertain the rate of rectal carriage of extended-spectrum beta-lactamase (ESBL) – producing Gram-negative bacilli in patients admitted to two different intensive care units (ICUs).
Methods: Rectal swabs were obtained from all patients within 24 h of admission (and every third day thereafter) to either an adult general medical and surgical ICU (MSICU) or an adult neurosurgical ICU (NICU). Swabs were cultured on Maconkey agar at 37°C in air for 48 h. Presumptive Gram-negative bacilli were identified to species level using API 20E. Screening for ESBLs was conducted using MAST DD and positive isolates were screened further using published molecular methods. Susceptibility was determined using the BSAC disk diffusion method. Demographic details were recorded for all patients in a prospective manner during the study.
Results: Ninety-two patients (median age 61 yr, range 18–88; 47 male) were entered into the study (52 MSICU, 40 NICU). Median length of stay was 4 days (range 1–24 days). ESBL-producing Gram-negative bacilli were isolated from 13/52 (25%) of MSICU and 3/40 (7.5%) NICU patients on rectal screening at admission. A further eight patients (3 MSICU, 5 NICU) became positive after admission. Ten (62%) of 16 patients ESBL+ on admission were admitted to the ICU within 48 h of admission to hospital. Molecular characterization identified ESBLs from the TEM-, SHV- and CTX-M classes.
Conclusions: Rectal carriage of ESBL-producing Gram-negative bacilli was common in this study, particularly on the MSICU. Prior hospitalization was not a reliable indicator of carriage with some ESBL+ patients being admitted direct from the community. These findings may influence the selection of antibiotics for empirical treatment of sepsis in the ICU setting.

P783

First description of bla-ctx-m-2 producing isolate in Spain and characterisation of its genetic environment

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Objectives: To characterize the genetic environment of the first CTX-M-2-producing *Escherichia coli* isolate described in Spain.
Materials and methods: *E. coli* VS27, a fecal isolate collected in 2003 as part of a surveillance study from a healthy volunteer without hospital or antibiotic exposure in the previous 3 months to the sample collection, was analysed. ESBL characterization was carried out by IEF, PCR, and further sequencing. Antibiotic susceptibility was performed by disk diffusion and microdilution (NCCLS). Transfer of blaCTX-M-2 was searched by broth and filter mating methods. Whole plasmid profile was determined by the Kado and Liu technique. To characterize the genetic environment of blaCTX-M-2, an overlapping PCR assay based on the sequence of In35 (GenBank AY079169), an In6-like class 1 integron in which blaCTX-M-2 has been previously reported, was designed. All PCR products were sequenced and further compared to sequences in the GeneBank databases by BLASTN.

Results: *E. coli* VS27 showing resistance to streptomycin, sulfonamide, tetracycline, ciprofloxacin and nalidixic acid, produced an ESBL of pI = 8.1. The strain contained 6 plasmids (three <3 kb, 5, 40 and 70 kb) but broth or filter mating did not achieve transfer of cefotaxime resistance. blaCTX-M-2 was located in a In6-like class I integron that show 100% homology with the In35 sequence except for the gene cassette content of the first 5CS-3CS (sat-aadA1) which has not been previously associated with this element.
Conclusions: Presence of blaCTX-M–2 in Spain is of concern since increases the diversity of blaCTX-M genes in our area, already epidemic for those of clusters CTX-M-1 (blaCTXM-10) and CTX-M-9 (blaCTXM-9, blaCTXM-14). The genetic environment of blaCTX-M-2 in isolates from different continents (showing diversity of the first 5′CS-3′CS and 100% homology with the rest of In35 sequence) suggests recent emergence of this structure and a high recombination rate of the 5′CS-3′CS part of this integron.

P784

Great diversity of ESBL-producing Enterobacteriaceae in a Birmingham hospital, United Kingdom

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Objective: To ascertain the diversity of ESBLs present in Birmingham Heartlands Hospital.
Method: Isolates from clinical specimens submitted from October 2003 to February 2004 which were resistant to any of cefotaxime, ceftazidime, cefpodoxime were screened using the double disk diffusion (DDD) method. Faeces from the same period from hospitalised and community patients with diarrhoea were plated on CAT campylobacter medium which has 8 mg/L cefoperazone and subsequent colonies screened using the DDD test. Positive isolates were identified and primers to amplify blaTEM, bla SHV, bla OXA and bla CTX-M used to generate amplicons, blaTEM/SHV were sequenced. For CTX-M positive isolates, dHPLC heteroduplex analysis was used to identify the CTX-M types. A proportion of these isolates were also sequenced to confirm the accuracy of the heteroduplex analysis.
Results: For clinical specimens, 41 *E. coli* were isolated (29 carrying CTX-M-15, 31 TEM-1, 29 OXA and 1 SHV-12); *Klebsiella* 21 isolates (19 producing OXA, 14, SHV-11, 3 SHV-12, 1 SHV-28, 1 SHV-1, 18 TEM-1 and16 CTX-M-15); *Enterobacter* 11 isolates (9 SHV-12, 8 OXA and 7 TEM-1). In the case of faecal isolates: 12 *E. coli* (12 CTX-M-15, 10 TEM-1 and 5 OXA); *Klebsiella* 14 isolates (13 CTX-M-15, 13 SHV-11, 5 OXA and 1 SHV-12); *Enterobacter* 13 isolates (13 SHV-12 and 5 TEM-1).
Conclusion: The striking finding is that the SHV-11 and –12 ESBL genes are extensively distributed amongst both community and hospital isolates of *Klebsiella* and *Enterobacter*. A single hospital isolate of *E. coli* was found to carry SHV-12, perhaps representing spread from *Klebsiella*, conversely *Klebsiella* were also found to carry high levels of CTX-M-15 in both hospital and community isolates. In contrast, *E. coli* isolates almost only carried CTX-M-15. CTX-M-15 is more widely distributed amongst bacterial species than suggested by the most recent survey in the UK and ESBLs of the SHV type are also highly significant causes of resistance to third generation cephalosporins.

P785

Investigation of a possible outbreak of extended spectrum B-lactamase producing *Enterobacter cloacae* using ERIC-PCR

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Objective: Over the course of 2003 there was a marked increase in the number of extended sprctrum B-lactamase (ESBL)-producing isolates of *Enterobacter cloacae* from clinical specimens submitted to the microbiology laboratory in Aberdeen, Scotland. The molecular epidemiology of these isolates was investigated.
Methods: 59 isolates from 47 patients were investigated. They had previously been identified phenotypically as *Enterobacter* species using the API 20E system and their ESBL activity confirmed by double-disc testing and/or combination disc testing according to NCCLS guidelines. Enterobacterial-repetitive-intergenic-consensus (ERIC)-PCR was used to retrospectively type these isolates of ESBL-producing *Enterobacter*.
Results: 31 isolates were from Aberdeen Royal Infirmary (ARI, tertiary referral hospital for the region), 7 were from Woodend Hospital (acute and long-stay geriatrics), 1 from Roxburghe House (hospital for the terminally ill), 1 from Cornhill Hospital (psychiatric), 1 from the Neonatal Unit at Aberdeen Maternity Hospital and 4 from three community hospitals. 14 samples were from general practices in the region. The isolates originated from a wide variety of clinical specimens including urine (35 samples), wound swabs (8), blood (4), sputum (3), faeces (3), other swabs (2) pus (1), tissue (1), line tip (1) and pleural fluid (1). Six different banding patterns were identified, designated types A to F. It was found that 79% of the isolates typed were type A, and that this percentage rose to 84% when only ARI isolates were considered. The type A isolates from ARI had come from 14 different wards. The remaining five types (B to F) that were identified each accounted for only a small number of isolates. Type B was found in ARI (1 isolate) and a GP specimen (1). Type C was found in ARI (1), Woodend (1) and a GP specimen (1). Type D also came from ARI (1) and Woodend (1). Type E was only found in ARI (1) and type F in a GP specimen (1) and the neonatal unit (1).
Conclusion: A clonal outbreak of ESBL-producing *Ent. cloacae* was identified. The clone is widespread, being found in a large number of wards throughout the hospital and in the community.

P786

Detection of a novel CMY-type beta-lactamase in Italian isolates of *Proteus mirabilis*

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Background and objectives: Plasmid-mediated AmpC-type beta-lactamases (CBLs) are emerging resistance determinants of increasing clinical importance in Enterobacteriaceae. They confer resistance to expanded-spectrum cephalosporins and cephamycins and are poorly susceptible to beta-lactamase inhibitors. In Italy, reports of these enzymes have been exceedingly rare. In this work we report on the detection of a novel CBL of the CMY/LAT lineage, named CMY-16, in clinical isolates of *Proteus mirabilis* from northern Italy.
Methods: Bacterial identification and susceptibility testing were carried out following standard procedures. Beta-lactamase production was analysed by isoelectric focusing (IEF). Beta-lactamase

genes were detected by a custom DNA microarray, and their exact nature was determined by PCR and sequencing.
Results: Eight nonreplicate isolates of *P. mirabilis* showing a unusual beta-lactam resistance phenotype (resistance to: ampicillin, amoxicillin-clavulanate, cefotaxime, ceftazidime and cefoxitin; susceptibility to: cefepime, aztreonam, piperacillin/tazobactam and carbapenems) were obtained from patients from 4 different hospitals in Northern Italy (Novara, Varese, Bergamo and Abbiategrasso) during the period Aug. 2003 to Jun. 2004. Most isolates were from the urinary tract, from inpatients in long-term-care facilities, but one isolate was from an outpatient. Double-disk synergy test with extended-spectrum cephalosporins (ESC) yielded negative results with clavulanate but positive results with some ESC and tazobactam. IEF analysis revealed the presence of beta-lactamases of pI 5.4 and >8.4. Microarray analysis revealed the presence of CMY/LAT- and TEM-type genes. Sequencing showed the presence, in all isolates, of a novel CMY/LAT-type gene encoding an enzyme, named CMY-16, different from CMY-2 by two (A171S and W221R) and from CMY-12 by one (N363S) aminoacid substitutions, respectively. Transferability of the CBL gene by conjugation was not detected.
Conclusions: A novel CBL of the CMY/LAT lineage, CMY-16, was found in *P. mirabilis* isolates resistant to cefotaxime, ceftazidime and cephamycins circulating in northern Italy. Present findings underscore the emerging role of these resistance determinants also in our Country.

P787

Cephalosporin-resistance in Enterobacteriaceae: a new plague?

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Objectives: To study the prevalence, resistance mechanisms and antibiotic susceptibility profiles of third generation cephalosporin resistant Enterobacteriaceae. To study the efficacies of different detection techniques (Phoenix methodology and combination discs) for extended-spectrum Beta-lactamases (ESBLs) and AmpC enzymes.
Methods: All Enterobacteriaceae isolates were subjected to identification and susceptibility testing using Phoenix methodology. All isolates found to exhibit resistance to 3rd generation cephalosporins and/or ESBL production were subjected to confirmatory testing by the combination disc method using cefpodoxime + cefpodoxime/clavulanate, and cefpirome + cefpirome/clavulanate.
Results: ESBL producing Enterobacteriaceae were isolated from 71 patients, 73% of whom were in-patients. Fifty-six per cent of isolates were from urine samples, 14% from respiratory tract samples and 8% from blood cultures. The remaining 15 specimens comprised mainly abdominal drain fluids and wound swabs. Sixty per cent of these isolates were *E. coli* and 30% were *K. pneumonia*. There were 3 isolates of *E. cloacae*, 2 isolates of *K. oxytoca* and 1 isolate of *C. freundii*. All isolates were sensitive to meropenem. Whereas 96% retained sensitivity to amikacin, only 56% were sensitive to piperacillin-tazobactam, 40% to cefepime, 48% to gentamicin and 24% to ciprofloxacin. Several of these isolates appear to be producing AmpC enzymes as well as ESBLs. The prevalence of this dual resistance mechanism was surprisingly high in *E. coli*. Results produced by the different methodologies were largely consistent for ESBL detection. AmpC production could not be confirmed using Phoenix methodology, but was detectable using combination disc methodology, which was also able to detect ESBL and AmpC dual resistance.
Conclusion: Cephalosporin resistance is becoming increasingly common in Enterobacteriaceae, with increasingly diverse and complex mechanisms. Laboratories should therefore have a low threshold for confirmatory testing of ESBL and AmpC production. Phoenix and combination disc methodologies are both reliable for ESBL detection. However, the latter are better for AmpC detection, particularly when co-produced with ESBLs. The carbapenems are now the only reliable agents against ESBL producing Enterobacteriaceae. Amikacin provides an effective alternative in the vast majority of cases.

P788

Widespread detection of VEB-1-type extended-spectrum beta-lactamases among nosocomial *Pseudomonas aeruginosa* isolates in Bulgaria: a nationwide multicentre study

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During the last few years *Pseudomonas aeruginosa* has become one of the most common nosocomial pathogens in Bulgaria. Recently, some isolates that are resistant to all available antimicrobial agents including carbapenems have emerged. We studied the prevalence and molecular epidemiology of VEB-1-type beta-lactamases among *Pseudomonas aeruginosa* strains, isolated since 1998 in more than 20 hospitals from distinct regions of Bulgaria. The producers of VEB-1-type enzyme were highly resistant to ceftazidime, aminoglycosides and quinolones, and moderately susceptible to imipenem and meropenem. Identification was confirmed with VITEK automated system. Resistance to ceftazidime was detected by disk diffusion method of the National Committee for Clinical Laboratory Standards and minimal inhibitory concentrations were determined by E-test. All ceftazidime-resistant strains were additionally tested by double disk diffusion method with carbapenem disk and 3/4 generation cephalosporin disk for presence of synergism. A total of 117 ceftazidime-resistant non-repetitive *P. aeruginosa* isolates were studied during this period. The presence of blaVEB-1 was determined with polymerase chain reaction. We detected VEB-1-type beta-lactamases in 36.8% (43/117) of *P. aeruginosa* strains. All strains that express synergism with double disk diffusion method were confirmed to be blaVEB-1 carriers. VEB-1 is widespread mostly in Asia and is rarely detected in European countries. Nevertheless it appears to have a significant presence among *P. aeruginosa* isolates in Bulgarian hospitals and causes serious impediments in antimicrobial treatment and difficulties in limiting its dissemination in our country.

P789

Occurrence in 2000 of epidemic CTX-M-15 producing Enterobacteriaceae before the UK-wide outbreak was recognised

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Objective: To screen isolates of presumptive ESBL-producing Enterobacteriaceae made during 2000 and 2001 from the University Hospital, Birmingham, for the early appearance of CTX-M-15 producing Enterobacteriaceae.
Method: Sensitivity testing was carried out on the VITEK 1 system and presumptive ESBL isolates were stored at −70°C. Isolates were screened for the presence of blaCTX-M using a PCR generated probe and dot blotting. Primers, which had previously been shown to specifically amplify all groups of

blaCTX-M genes, were applied. For probe positive isolates, DHPLC heteroduplex analysis was employed to identify the CTX-M specific gene. A total of 28 isolates of *E. coli* and 5 other Enterobacteriaceae were obtained in 2000, and 58 and 18 respectively in 2001.
Results: Only *E. coli* were found to caryy blaCTX-M and no isolates were found before July 2000. 19/28 *E. coli* in 2000 and 12/40 in 2001 were blaCTX-M positive.
Conclusion: We have discovered that CTX-M-15 appeared very suddenly in Birmingham (within the Midlands) in July 2000, nearly 2 years before the UK-wide outbreak was recognized. Interestingly the gene was only found in *E. coli* and probably represents the spread of a highly successful clone which subsequently has caused a large-scale and persistent outbreak in a hospital in Shrewsbury (also within the Midlands) starting in late 2002. As CTX-M-15 was first described in the South Asian region and Birmingham has a very large South Asian community, the CTX-M-15 gene may have been introduced in patients returning from India and Pakistan.

P790

Distribution of beta-lactamases in clinical isolates of enterobacteriaceae and nonfermenters from a region of central Italy

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Objectives: In the last decade metallo-β-lactamases (MBL) have widely spread in Italian Hospitals. MBL represent a heterogeneous group of enzymes that have the common feature of hydrolyzing extended-spectrum cephalosporins and carbapenems. The aim of this work was that to investigate the distribution of resistance genes to β-lactams in clinical isolates of Enterobacteriaceae, *Pseudomonas aeruginosa* and other gram-negative nonfermenters from Abruzzo, a region of central Italy.
Methods: Nonreplicate isolates of Enterobacteriaceae (n = 25) *P. aeruginosa* (n = 50) and other gram-negative nonfermenters (n = 5) resistant to carbapenems were collected from 4 regional hospitals (L'Aquila, Pescara, Atri, Avezzano) in Abruzzo (Central Italy). Isolates were from the respiratory or the urinary tract. MBL determinants were screened by colony blot hybridization using DNA probes for blaVIM-1, blaGOB-1, and blaIMP-1 genes. Hybridization-positive colonies were analysed by PCR using specific primers, and the PCR products were sequenced to confirm identification of the gene.
Results: MBL of the VIM and IMP types were detected in 30 *P. aeruginosa* isolates from three of four hospitals. A different distribution was observed for different types: VIM-4 producers were circulating in L'Aquila hospital (located in the internal part of the region), while IMP-13 producers were circulating in Atri and Pescara hospitals (both located on the coast). No MBL were detected in clinical isolates of Enterobacteriaceae or of *Acinetobacter* spp. Genetic analysis of the blaIMP and blaVIM determinants revealed features typical of integron-associated genes.
Conclusion: This report indicates that MBL are widespread in nosocomial isolates of *P. aeruginosa* from our region, although significant geographical differences were observed for different enzyme types. MBL genes have apparently not yet diffused in Enterobacteriaceae or *Acinetobacter*, in this area. Continuous surveillance is of paramount importance to control these important emerging resistance determinants.

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ESBL-strains of *E. coli* and *K. pneumoniae* in GENARS hospitals

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Objectives: The increasing resistance of Enterobacteriaceae to mono-bactams and oxyimino-cephalosporines like cefotaxime, ceftazidime, ceftriaxone and cefpodoxime is due to an increasing spread of strains expressing extended spectrum B-lactamases (ESBL). ESBL rates of more than 50% have already been reported for southern European countries. For Germany, Austria and Switzerland a multicentre study of the Paul Ehrlich Society (PEG) in 2001 detected ESBL-rates of 0.8% and 8.2% for *E. coli* and *K. pneumoniae*, respectively. More recent and detailed information about the occurrence of ESBL phenotypes in Germany is provided by the GENARS project which is funded by the German Federal Ministry of Health and Social Security.
Methods: All laboratories involved determine antimicrobial susceptibility by testing the Minimal Inhibitory Concentration (MIC) in the laboratory routine for about 25 different antibiotics. According to NCCLS guidelines, some of these antibiotics tested, could be used as indicators for an ESBL screening (cefotaxime, ceftazidime, cefpodoxime). The ratio of cefpodoxime and cefpodoxime/clavulanic acid was used for phenotypical confirmation.
Results: In the first half of 2004, the percentage of strains with a positive ESBL-screening amounts to 4.3% and to 10.5% for *E. coli* (n = 1944) and *K. pneumoniae* (n = 382), respectively. Phenotypically confirmed as ESBL are 1.7% of all *E. coli* strains and 7.1% of all *K. pneumoniae* strains. In both species strains were detected in which ESBL's are phenotypically confirmed, but where NCCLS parameters for initial screening failed. Strains with a MIC for cefpodoxime of ≥ 8 mg/L which can not be reduced by clavulanic acid are identified as AmpC expressing strains (*E. coli* 1.1%; *K. pneumoniae* 1.8%). Focused on the departments and the kind of wards in GENARS hospitals the epidemiological situation concerning ESBL producing strains can be assessed. In GENARS hospitals the difference of ESBL rates between departments can be considerable (*E. coli*: 0.7–6.1%; *K. pneumoniae*: 2.7–18.1%). For both *E. coli* and *K. pneumoniae*, ESBL rates are highest in ICU's.
Conclusion: To prevent spread in hospitals, cities and countries, a detailed surveillance of ESBL-strains is necessary. To reach this goal an improvement of our epidemiological knowledge about the occurrence and spread of ESBL-strains is needed. In Germany the MIC-determination as demonstrated for GENARS hospitals allows a correct detection of ESBL producing strains.

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SARI: distribution of extended-spectrum beta-lactamases in German ICUs

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Objectives: The aims of this study were to determine the frequency of ESBL-positive (ESBL+) strains and the occurrence of blaCTX-M encoded CTX-M enzymes in isolates obtained from 34 German ICUs participating in the project 'Surveillance on antibiotic use and bacterial resistance in ICUs' (SARI). These findings were investigated for an association with ICU-based antimicrobial consumption data measured as 'Antimicrobial use density' (AD).

Methods: Within the German SARI project, 323 non-duplicate, mainly ciprofloxacin- or cephalosporin-resistant isolates of *E. coli* and *Klebsiella* spp. were obtained from 34 ICUs, together with ICU - based antimicrobial consumption data. Additionally, for one month the participating ICUs collected 169 isolates of these species irrespective of their antimicrobial susceptibility for determination of ESBL-prevalence.
Results: Eighty isolates were obtained from 15 ICUs tested positive for ESBL. However, 52 strains of *K. pneumoniae* originated from two ICUs. Genotyping confirmed the continuous spread of three ESBL+ clones. In 17 strains from seven ICUs blaCTX-M genes related to the TOHO-1, CTX-M-1 and CTX-M-15 families were detected. Antimicrobial consumption was analysed in 20 ICUs, which had contributed at least 10 isolates of *E. coli* or *Klebsiella* spp. for testing. Comparison of the characteristics of 10 ICUs each with and without detectable ESBL+ strains revealed significant differences ($P < 0.05$) in the average combined antimicrobial usage of cefotaxime and ceftriaxone (AD 91.7 vs. 60.1). Contrarily, usage of other broad spectrum antibiotics like fluoroquinolones and carbapenems did not reveal significant differences.
Conclusions: The frequency of ESBL in 34 German ICUs was estimated at 5% with a considerable variation in prevalence between different ICUs and different blaCTX-M types spreading throughout Germany. This study provides first data on ESBL in German ICUs and the possible influence of infection control and antibiotic use.

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The first incidence of PER-1 ESBL-producing *Pseudomonas aeruginosa* in Poland and identification of a novel OXA beta-lactamase variant

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Objectives: To analyse an outbreak caused by ESBL-producing *P. aeruginosa* in one of Warsaw hospitals.
Methods: Thirty-eight ESBL-producing *P. aeruginosa* isolates were collected between September 2003 and May 2004 from patients in different wards of a Warsaw hospital. The ESBL production was confirmed by the double-disc test. MICs of 14 antibiotics were determined by the NCCLS agar dilution method. Molecular typing was performed by the PFGE analysis of the XbaI-digested bacterial DNA. Beta-lactamases of the isolates were visualised by isoelectric focusing (IEF) of whole-cell extracts. Genes coding for the enzymes were identified by PCR and sequencing of the resulting amplicons.
Results: The susceptibility testing revealed that the *P. aeruginosa* isolates were multiresistant, demonstrating resistance to beta-lactams, aminoglycosides and ciprofloxacin. Their patterns of resistance to beta-lactams were typical for ESBL producers except for the fact that all but 5 isolates were susceptible to piperacillin. The PFGE analysis classified the isolates into two different types; 35 isolates represented PFGE type A and the remaining 3 isolates belonged to type B. The PFGE type A isolates were characterised by beta-lactamase IEF patterns consisting of enzymes with pIs of 5.3, 7.7 and 8.2 (33 isolates), or 5.3 and 8.2 (2 isolates). The PFGE type B isolates produced beta-lactamases with pIs of 5.3, 6.5, 7.7 and 8.2. Of the enzymes observed in the all isolates, the one with the pI of 8.2 corresponded to the species-specific AmpC cephalosporinase, whereas that with the pI of 5.3 was identified as the PER-1 ESBL. The pI 7.7 and 6.5 IEF bands were assigned to OXA-2 and OXA-74 beta-lactamases, respectively. OXA-74, produced exclusively by the PFGE type B isolates, is a novel variant of OXA-10-related oxacillinases. Although no other bands were observed in the IEF analysis, PCR amplicons specific for the blaOXA-50 gene, which naturally occurs in *P. aeruginosa*, were generated for the all isolates.
Conclusions: PER-1-producing *P. aeruginosa* was identified for the first time in Poland. It caused a highly clonal outbreak in one of Warsaw hospitals. It is possible that a blaPER-1 gene-containing determinant was transmitted between two separate clones of the organism observed during the outbreak.

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Detection of extended spectrum beta-lactamases in *Escherichia coli* strains isolated from clinical specimens and their antimicrobial susceptibility

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Objectives: Extended-spectrum beta-lactamases (ESBLs) confer resistance to newer cephalosporins and penicillin with expanded-spectrum activity. ESBLs are mostly plasmid-mediated enzymes. ESBLs were first described in 1983. ESBLs can be detected by double disk synergy (DDS), modified DDS (MDDS) and E test methods. It is important for clinicians for appropriate antimicrobial treatment to find ESBL.
Methods: In this study the presence of ESBL was investigated by DDS and MDDS methods in 100 *E. coli* strains isolated from clinical specimens such as urine (75), blood (8), wound (7) and other sites (10). *E. coli* strains were also tested for susceptibility to aztreonam, cefotaxime, ceftazidime, cefoperazone, cefoxitin and imipenem. Demographic and clinical data of patients were also collected and evaluated.
Results: The presence of ESBL was detected in 12% of isolates by MDDS method. 94.6% of the ESBL negative and 4 of 12 ESBL positive strains were isolated from urine specimens. 8 of 12 ESBL positive strains were isolated from blood (2), surgical site (4) and respiratory tract (2) specimens. The 96.4% of out patient *E. coli* strains was ESBL negative, but 10 of 12 ESBL positive strains were isolated from hospitalized patients. Intravascular catheters, surgical procedures and mechanical ventilation ratios were statistically higher in ESBL positive group. Intermediate susceptible isolates were classified as resistant. In-vitro susceptibilities of 88 non ESBL producing *E. coli* strains were found as 73.9% for aztreonam, 96.6% for cefotaxime, 98.9% for ceftazidime, 96.6% for cefoperazone, 98.9% for cefoxitin and 100% for imipenem. In-vitro susceptibilities of 12 ESBL producing *E. coli* strains to these antibiotics found as 0/12 for aztreonam, 0/12 for cefotaxime, 1/12 for ceftazidime, 1/12 for cefoperazone, 6/12 for cefoxitin and 12/12 for imipenem.
Conclusion: ESBL producing pathogen bacteria was a clinical problem especially in hospitalized patients. ESBL producing strains must be suggested not only for *Klebsiella* spp. but also for hospital acquired *E. coli* strains.

P795

Risk factors and treatment outcome of bloodstream infections caused by *Proteus mirabilis* isolates producing extended-spectrum beta-lactamases

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Objectives: Bloodstream infection (BSI) caused by *Proteus mirabilis* is a relatively uncommon clinical entity. Clinical

significance and diagnostic aspects have received little attention. This study was initiated to evaluate risk factors and treatment outcome of BSI episodes caused by *P. mirabilis* strains.
Methods: From January 1997 to June 2004, 25 BSI caused by *P. mirabilis* strains occurred at the Ospedale di Circolo of Varese (Northern Italy). Isolates were evaluated by phenotypic and molecular methods in order to assess their ability of producing extended-spectrum beta-lactamases (ESBLs). Clinical records of BSI-patients were examined retrospectively. Demographic data, underlying diseases (according to McCabe and Jackson classification scheme and Charlson weighted index), risk factors, antimicrobial therapy, and treatment outcome were investigated by comparing cases due to ESBL-positive to those due to ESBL-negative *P. mirabilis* strains. Student's unpaired *t*-test and the Mann–Whitney *U*-test were used to compare continuous and not normally distributed continuous variables, respectively. V-square test was used to evaluate the treatment outcome.
Results: Eleven isolates were found to express ESBLs, either the TEM-52 or TEM-92 enzyme. The remaining 14 isolates were ESBL-negative and were uniformly susceptible to extended-spectrum cephalosporins and monobactams. Comparison of the two groups showed that previous hospitalization in nursing homes ($P = 0.04$) and use of bladder catheter ($P = 0.01$) were significant risk factors for infections due to ESBL-positive strains. In addition, cases due to ESBL-producing strains showed a significantly higher mortality attributable to BSI ($P = 0.04$). BSI cases due to ESBL-negative isolates uniformly responded to therapy, whereas 5/11 cases due to ESBL-positive isolates failed to respond ($P < 0.01$). Use of carbapenems was uniformly associated with complete response independently of ESBL production.
Conclusion: Therapeutic failure and mortality may occur in BSI episodes caused by *P. mirabilis*. Recognition of ESBL-positive strains appears critical for the clinical management of patients with systemic *P. mirabilis* infections.

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Community and hospital-acquired extended-spectrum beta-lactamase (CTX-m-15)-producing enterobacteriaceae in Lebanon

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Introduction and objective: Extended-spectrum beta-lactamases (ESBLs) are enzymes that encode resistance to broad-spectrum beta-lactam antibiotics. This prospective multicentric point-prevalence study was carried out to detect the presence of ESBL-producing strains within the faecal flora of community- and hospital-based patients and health workers in Lebanon.
Methods: 1500 fecal samples were collected from patients and staff of the intensive care units of six tertiary care general hospitals located in different areas of Lebanon between January and March 2003 and 382 from healthy subjects between April and June 2003. These samples were screened for potential ESBL producers by culture on trypticase soy-5% sheep blood agar containing vancomycin (6 μg/ml), amphotericin (2 μg/ml), ceftazidime (2 μg/ml) and clindamycin (1 μg/ml). Patients were checked for ESBL producing strains upon admission, 72 h later and at weekly intervals over a 3-months period. Other participants were checked once. ESBL producing strains were detected by antimicrobial susceptibility testing and were then identified by the API 20E system. The ESBLs produced were characterized by PCR analysis of bla genes, DNA sequencing and isoelectric focusing experiments. The genome of ESBL producing strains was analysed using pulsed-field gel electrophoresis.
Results: A total of 118 strains including 102 *Escherichia coli*, 9 *Klebsiella pneumoniae*, 6 *Enterobacter cloacae* and 1 *Citrobacter freundii* collected from 61 patients, 2 health workers and 9 healthy subjects were found to produce ESBLs. The characterization of one representative strain by subject ($n = 72$) revealed that the majority (83%) express CTX-M-15 ESBL (pI 8.6). The remaining isolates produced SHV-5a ESBL (pI 8.2). DNA macrorestriction presented 38 different genotypes revealing the absence of clonal link among *E. coli* strains. In addition to the fact that this study is the first in Lebanon to investigate the carriage of ESBL producing strains, it represents the first report of a SHV-5a producing *C. freundii*.
Conclusion: CTX-M-15 type ESBLs are capable of spreading in both hospital and community settings in Lebanon. The high degree of diversity of strains suggested that horizontal transfer of plasmids was likely the main mechanism of CTX-M-15 ESBL spread. The dissemination of plasmids was probably preceded by inter and intra spread in the hospital and the community of several strains

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Accurate, rapid genotyping of CTX-M beta-lactamase by denaturing high-performance liquid chromatography

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Objective: To develop a sensitive, rapid and high-throughput method using DHPLC (Wave® Nucleic-Acid Fragment Analysis System, Transgenomic) for genotyping CTX-M producing ESBLs.
Method: Primers were designed to amplify group specific fragments (250–400 bp) of the ORF of all three groups of blaCTX-M that have been identified in the UK and China. Heteroduplex PCR products were formed using a specific reference strain and the unknown field isolate. The PCR products were then screened using temperature-mediated heteroduplex analysis by DHPLC on the Wave® Nucleic-Acid Fragment Analysis System. 101 isolates were tested blind, having first had the blaCTX-M identified by DNA sequencing.
Results: Unique profiles were obtained for all 5 blaCTX-M types. Characteristic profiles were obtained for each blaCTX-M type for all 101 isolates with a genotyping accuracy of 100%. The distribution of CTX-M types for UK was: CTX-M-15 (61), -26 (8) and China – 14 (28), -3 (9), -1 (3).
Conclusion: This is the first report of the use of DHPLC technology for beta-lactamase genotyping, it offers a highly specific, sensitive, economical and high-throughput approach to screen known, and identify new, CTX-M genotypes in large numbers of clinical isolates. It has great potential for the surveillance and epidemiological monitoring of CTX-M type ESBLs.

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Outbreak of multiresistant *Klebsiella pneumoniae* producing CMY-2 beta-lactamase in a neonatal intensive care unit in Spain

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Objectives: The aims of this study were to investigate the presence of plasmid-mediated AmpC-type beta-lactamases

(PACBL) and of class 1 integrons in multiresistant *K. pneumoniae* isolates from a neonatal intensive care unit and to determine their clonal relationship.
Methods: Identification and susceptibility testing were performed with the Microscan Walkaway System (Dade Behring). PACBL production was inferred from resistance (R) to penicillins, penicillin-beta-lactamase inhibitor combinations, cefoxitin, cefotaxime and ceftazidime; no synergism between cefotaxime or ceftazidime and clavulanic acid; and susceptibility to both cefepime and imipenem. Resistance to cefoxitin was transferred by conjugation to rifampin-R *E. coli* J53. Plasmids were extracted by the lysis-alkaline method. A PACBL was detected by multiplex-PCR and identified by PCR with specific primers and sequencing. Class 1 integrons were investigated by PCR and sequencing. Clonal relationship of isolates was determined by pulsed-field gel electrophoresis (PFGE, XbaI restriction).
Results: Between January and October 2003, 35 neonates were infected or colonized by multiresistant *K. pneumoniae* isolated from urine (n = 16), blood (n = 5) or respiratory samples (n = 14). All isolates were resistant to tobramycin and gentamicin and susceptible to amikacin, with MICs of ciprofloxacin of 0.5–1 mg/L. Eight isolates were selected for further evaluation: these all presented the same PFGE-pattern and transferred resistance to cefoxitin, tobramycin and gentamicin to *E. coli* by conjugation (transfer frequency: ca. 10^{-3}). A > 80 kb plasmid was observed in all transconjugants. All parental isolates and transconjugants produced a PACBL identified as CMY-2 and carried a class 1 integron with aadB, ORF1 and cmlA gene cassettes (but not blaCMY-2).
Conclusions: We have confirmed the presence of an outbreak in a neonatal intensive care unit caused by a multiresistant *K. pneumoniae* producing CMY-2 and containing an integron with the aadB gene cassette.

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Ascertainment of ESBL-producing *E. coli* infections in 2002 and 2003 – survey of English microbiology laboratories

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Objectives: Infections caused by *Escherichia coli* with extended-spectrum CTX-M beta-lactamases are a rapidly growing problem in the UK. A survey of English microbiology laboratories was carried out to audit the methodology used to detect ESBL-producing *E. coli*, to identify the extent of community-acquired urinary tract or bloodstream infections with ESBL producers, and to establish the extent of any public health investigation.
Methods: Questionnaires were sent to all clinical microbiology laboratories across England in January 2004. Analysis of the responses was undertaken.
Results: A total of 142 laboratories returned completed questionnaires. A standard method for detecting ESBLs was used by 72% of the responders though the precise methods varied among sites (16% of responders did not know whether or not they used a standard method, or did not answer the question). ESBL-producing *E. coli* was detected from blood cultures in hospitalised patients by 44% of responders (27% did not know). Fourteen responders (10%) had detected ESBL-producing *E. coli* from blood cultures from community patients or samples (30% did not know). ESBL-producing *E. coli* had been detected in urine samples by 42% of responders from hospitalised patients (32% did not know) whilst 37% reported that they had detected producers in community patients or samples (35% did not know). Thirty-two responders (23%) reported that there had been a local investigation of multi-drug resistant infections caused by ESBL-producing *E. coli* (3% did not know) in the preceding 24 months.
Conclusions: The survey revealed numerous detections and several investigations into potential outbreaks of ESBL-producing *E. coli* across England during 2002 and 2003. These findings support the view, based on routine surveillance and reference submissions, that ESBL-producing *E. coli* are an emerging problem, also that they are occurring in community as well as hospitalised patients. Most laboratories had methods for ESBL detection, but standardised national guidance has recently been published by the Health Protection Agency (http://www.hpa.org.uk). A follow-up questionnaire is due to be sent out early in 2005.

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Frequency of extended spectrum beta lactamase producing *Escherichia coli* strains isolated from central venous catheter related infections quantified by three laboratory methods

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Objectives: The purpose of this study was the ESBL screening of clinical isolates of *Escherichia coli* isolated from patients with central venous catheter related infections, by automatic methods (VITEK system), double-disk synergy test and E-test® ESBL.
Methods: The study was carried out on 51 *E. coli* strains recovered in 2004 from patients admitted for cardiovascular surgery at the Cardiovascular Disease Institute of Bucharest, Romania. The strains identification was performed using the VITEK system. The strains were subsequently tested for antibiotic susceptibility by standard disk diffusion method and the strains suspected of ESBL production were comparatively tested by double disk diffusion confirmatory test using clavulanic acid and the standard E-test® strips, containing of two gradients aligned in opposing directions, one side of the strip cefotaxime (CT) MIC range of 0.5–32 mcg/ml and the other a cefotaxime MIC range of 0.125–8 mcg/ml overlaid with a constant level of 4 mcg/ml clavulanic acid (CTL). MIC ratios of CT/CTL (MIC ratio >8) have been used as a discriminative criteria for the presence or absence of ESBLs.
Results: Out of the total number of strains, 17.64% were given ESBL positive by VITEK system. The results of antimicrobial resistance testing by standard disk diffusion method showed that 35.29% exhibited beta-lactamase pattern, with high resistance levels to ampicillin and low or high resistance to 1st generation cephalosporins and 17.64% of the tested strains were suspected of ESBL production showing decreased sensitivity to cephtazidime and cefotaxime. ESBL screening of strains by double disk diffusion test showed that 5 strains (9.8%) demonstrated clavulanic acid inhibitory effect in the phenotypic confirmatory test. The E-test results confirmed the presence of ESBL only in 4 strains of the suspected of ESBL production (7.84%), the rest being not determinable, probably due to MICs values exceeding the concentration range, or to the overlapping of other beta-lactamases (e.g. AmpCs and inhibitor-resistant TEMs (IRTs) masking the ESBL pattern.
Conclusion: Our results showed that different methods used for the detection of ESBL producing *E. coli* strains gave variable results and showed different sensitivity levels in detecting ESBL strains. These data suggest that clinical microbiology laboratories should not rely on one single method for the screening ESBL producers, complementary tests being necessary for incidence studies.