Antimicrobial resistance mechanisms - III

P1714 Phenotypic and genotypic characterisation of antimicrobial resistance in Egyptian *Escherichia coli* isolates

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Background: A few reports to determine bacterial resistance including resistance patterns in *Escherchia coli* isolated in Egypt were published. No information, however, is available underlying the resistance mechanism. Therefore, we begun our study on β-lactam resistance and found CTX-M-18, -15 and -27 responsible for extended-spectrum β-lactam resistance, which was now extended to resistance towards other antibiotics.
Objective: The study was undertaken to characterise phenotype and genotype of antimicrobial resistance of CTX-M-producing *E. coli* strains.
Methods: Thirty *E. coli* strains were isolated from two hospitals in Cairo in 2001. MIC was determined according to NCCLS guidelines. PFGE was carried out. Strains were screened and mapped by PCR methods for antimicrobial resistant genes, integrons and gene cassettes. Genes were sequenced if necessary.
Results: The resistance Pattern of CTX-M-producing *E. coli* for ampicillin, cefotaxime, ceftazidime, ciprofloxacin (C), tetracycline (T), gentamicin (G), kanamycin (K), streptomycin (S), chloramphenicol (Ch) sulphamethoxazole (Su) and trimethoprime (T) were 100, 100, 83, 43, 100, 90, 77, 100, 97, 100, 97%, respectively. Ninety-seven per cent of *E. coli* are harboured class 1 integron. Variable gene cassettes were obtained. A predominance of gene cassettes that confer resistance to S and T. Sequencing of quinolone-resistance determining regions (QRDR) showed that strains with a reduced susceptibility to C of MIC 1 mg/L have a single mutation in *gyrA* and strains with full resistance to C of MIC >8 mg/L have a double mutations in *gyrA* and single mutation in *parC*. The resistance genes confers G resistance were aac(3*I*)-IIc (93%), aac(6*I*)-Ib (66%) and ant(2″)-1a (7%). The prevalence of aph(3)-Ia in K resistant strains was 83%. The prevalence of Sul1 and *sul2* were 90 and 100%, respectively. The prevalence of *tet*(*A*) is higher than *tet*(*B*) in tetracycline-resistant strains. Enzymatic (*cat1* & *catB*) & nonenzymatic (*cmlA*) mechanisms of Ch resistance were detected. The *floR* was not detected.
Conclusions: The CTX-M producing *E. coli* strains studied do not only have a broad-spectrum β-lactam resistance phenotype due to a different β-lactamases, they also show pronounced resistance to other classes of antibiotics. Genotypic characterisation revealed a high number of different resistance determinants. It is striking that for most classes of antibiotics resistance is due to multiple genes with similar overlapping phenotypic effects.

P1715 *gyrA* mutations and quinolone resistance in *Salmonella enteritidis* and *Salmonella typhimurium*

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Objectives: To know the relation between *gyrA* mutations and resistance to nalidixic acid (NAL) and fluorinated quinolones in *Salmonella enteritidis* and *Salmonella typhimurium*.
Methods: The in vitro activity of ciprofloxacin (CFX) against 29 NAL-resistant *S. enteritidis* and 15 NAL-resistant *S. typhimurium* clinical isolates was determined by the agar dilution method. *gyrA* QRDR was PCR amplified and sequenced in the 44 isolates.
Results: All 29 NAL-resistant *Salmonella enteritidis* isolates (MIC > 128 mg/L) were susceptible to CFX, while the 15 NAL-resistant *S. typhimurium* isolates had reduced susceptibility to CFX (MIC 0.1–0.2 mg/L). Only five *S. enteritidis* isolates had *gyrA* mutations clearly related to quinolone resistance (Asp87Tyr). 13 isolates had no mutations, and nine isolates had mutations whose involvement in quinolone resistance in unclear (seven Val143Gly and two Glu139Asp). 14 of 15 *S. typhimurium* isolates had a Asp87 mutation to Tyr (11 isolates) or Asn (three isolates).
Conclusions: NAL-resistant *S. typhimurium* have usually reduced susceptibility to CFX, and correlate with a Asp87 mutation, which seems to be the first quinolone-resistance mutation to emerge. NAL-resistant *S. enteritidis* are usually CFX-susceptible and in one half of the isolates have no *gyrA* mutations. Further studies are needed to elucidate if NAL-resistance justify CFX use restrictions in *S. enteritidis*.

P1716 First report of a TEM-26 β-lactamase with mutations conferring resistance to piperacillin/tazobactam

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Background: Extended-spectrum β-lactams are commonly included in empirical antibiotic regimens for the treatment of Gram-negative infections. The emergence of extended spectrum β-lactamase (ESBL)-producing bacteria poses a serious threat to the continued use of this family of antibiotics. Since ESBLs have previously been uniformly susceptible to β-lactamase inhibitors (e.g. tazobactam or clavulanic acid), inhibitor/β-lactam combinations (e.g. piperacillin/tazobactam) have been advocated as potential therapeutically effective agents.
Methods: In June 2003, a clinical isolate of *Escherichia coli* was recovered from a 82-year-old woman nursed on the high dependency surgical ward in St James's University Hospital Leeds, UK. The patient had received courses of ceftazidime and piperacillin/tazobactam (Pip/Taz). The organism was isolated from a urine specimen. Speciation was confirmed biochemically with API 20E system. The organism was resistant to ceftazidime, cefotaxime, aztreonam, Pip/Taz and amoxicillin/clavulanic acid and was susceptible to meropenem, imipenem and cefoxitin using a disk diffusion method. ESBL production was sought using the disc synergy and the MAST ID tests. PCR using specific primers was used to screen for the presence of blaSHV and blaTEM. Nucleotide sequence analysis was used to determine the identity of the resistance determinants.
Results: ESBL production was demonstrated with both the disc synergy and the MAST ID tests. PCR showed the organism to harbour a blaTEM. Nucleotide sequence analysis identified the resistant determinant as a TEM-26 β-lactamase with an additional mutation at amino-acid position 69 with the replacement of methionine by valine. This mutation has previously been shown to confer resistance to beta-lactamase inhibitors.
Conclusions: To our knowledge this is the first report of a TEM extended-spectrum β-lactamase also conferring resistance to Pip/Taz. This highlights the continuing global emergence and mutability of this clinically important family of enzymes. It emphasises the importance for vigilance in identifying novel resistance mechanisms and the need for prudent use of antimicrobials to preserve their clinical efficacy.

P1717 New isolation of an integron-located VEB-1 extended-spectrum β-lactamase in a *Proteus mirabilis* clinical strain

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Objectives: The aim of this study was to investigate the molecular basis of resistance observed in a *Proteus mirabilis* strain isolated from an adopted Indian child.
Methods: The susceptibility profile was determined using an automated microdilution MicroScan method and production of ESBL activity was identified by a double-disc synergy test with two

cephalosporins: ceftazidime and cefotaxime. β-lactamases were extracted by ultrasonication. Supernatants containing crude enzyme extracts were analysed by isoelectric focusing on ampholine polyacrylamide gels (pH 3.5–9.5) using a Multiphor II electrophoresis System (Pharmacia Biotech) and β-lactamases were visualised with an overlay of nitrocefin. Previously described primers VEBcasB and VEBcasF were used for detection of the blaVEB-1 gene. Automated DNA sequencing was performed in the amplified product. The 5′ and 3′-CS primers were used in combination with VEB-INV1 and VEB-INV2, respectively, in order to determine the size of the variable region of the integron. A newly design primer PVEB was used to demonstrate the colinearity of the integrase with *blaVEB-1*.
Results: The *P. mirabilis* strain was isolated from an urine sample and showed resistance to all extended-spectrum cephalosporins, aminoglycosides, trimethoprim–sulphamethoxazole and phosphomycin. An unusual synergy was observed between cefoxitin and cefotaxime and cefuroxime using double-disc synergy. Isoelectric focusing analysis revealed the presence of a β-lactamase with a pI of 7.6. DNA sequencing analysis revealed the presence of a *blaVEB-1* gene, which showed 98% identity with the *blaVEB-1* gene found in *P. mirabilis* Lil-1. This gene cassette was located within a class 1 integron of approximately 3 kb. On both sides of the *blaVEB-1* gene there were two unsequenced regions of around 600 bp each, flanked by the integrase gene upstream and the 3′conserved segment (3′CS) downstream.
Conclusions: These data indicate that the *blaVEB-1* gene is part of a genetic environment comprised in a class 1 integron. However, the variable regions of the integron are different in size than previously described integrons containing *blaVEB-1* gene in *P. mirabilis*, *E. coli* or *P. aeruginosa*. Further studies are needed to evaluate which gene cassettes are codified by these variable regions.

P1718 Ceftazidim-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from four Turkish hospitals: identification of TEM-, SHV-, and GES-type β-lactamases

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Objectives: To study the prevalence of TEM-, SHV-, and GES- type β-lactamases among *Escherchia coli* and *Klebsiealla pneumoniae* strains those having ceftazidime MICs higher than 2 μg/L.
Material and methods: A total of 61 *E. coli* and 43 *K. pneumoniae* isolated from four different university hospitals were studied for the existence of TEM-, SHV-, and GES- type β-lactamases. Susceptibility tests were done according to the criteria of National Committee for Clinical Laboratory Standards (NCCLS). MICs were obtained by agar dilution method. Existence of extended-spectrum β-lactamases were assessed by double-disc synergy test. Existence of the above mentioned β-lactamase genes were studied by PCR with specific oligonucleotide primers and further confirmed by isoelectric focusing the bacterial extracts.
Results: None of the isolates were carbapenem resistant. Double-disc synergy tests were positive in 40 (66%) and 35 (81%) of *E. coli* and *K. pneumoniae*, respectively. TEM gene was detected in 40 (65%) and 20 (47%) while SHV gene in 12 (20%) and 17 (40%) of *E. coli* and *K. pneumoniae* strains, respectively. GES gene was not detected.
Conclusions: TEM and SHV genes are highly prevalent among ESBL producing *E. coli* and *K. pneumoniae*. GES type ESBLs are not responsible in the ceftazidime resistance in the region.

P1719 Third generation cephalosporin-resistant *Escherichia coli* from a paediatric hospital in Iasi, Romania

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Objectives: Third generation cephalosporin resistant (3GC-R) *E. coli* strains are frequently involved in urinary-tract infections in the paediatric hospital from Iasi, Romania since 1994. This preliminary study was undertaken in order to obtain information on the epidemiology of these strains and the mechanism of 3GC resistance.
Methods: A random sample of eight *E. coli* strains isolated in the period 2000–2001 were examined. Susceptibility status to various antimicrobial agents was assessed using the Mini Api System (Bio Merieux). Detection of extended spectrum β-lactamases (ESBL) was performed with the Etest method using strips containing ceftazidime plus clavulanic acid. The isoelectric points of β-lactamases produced by each strain were determined by isoelectric focusing (IEF) of crude cell extracts. Typing of the strains was carried out by a PCR-based method using the ERIC2 oligonucleotide primer.
Results: All strains showed resistance to amoxicillin, amoxicillin/clavulanic acid, ceftazidime, cefoxitin. In seven cefoxitin-resistant strains the ESBL detection test was negative. Upon IEF these strains were found to produce β-lactamases with highly basic isoelectric points. It was therefore presumed that these strains produced acquired AmpC enzymes. In the remaining isolate ceftazidime MIC was significantly reduced in the presence of clavulanic acid. The strain expressed a β-lactamase that focused at 8.2 (probably an SHV-5 enzyme). ERIC2 PCR typing showed at least four different banding patterns suggesting a widespread of 3GC resistance mechanism to distinct strains.
Conclusions: The frequent isolation of 3GC-R *E. coli* in the paediatric hospital of Iasi is dues to the dissemination of epidemiologically distinct strains. Resistance can be attributed to production of various β-lactamase types including AmpC (cephalosporinases)and extended-spectrum enzymes. Reduction of the use of 3 GC and enforcement of infection control measures must be applied.

P1720 Characterisation of two novels 6′-N-aminoglycoside acetyltransferase genes, aac(6′)-30 and aac(6′)-31, found in *blaIMP-16* and *blaIMP-1 Pseudomonas aeruginosa* strains carrying integron

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Objectives: Two clinically unrelated *Pseudomonas aeruginosa* strains, 101-4704 and 48-696, were isolated from hospitalised patients in Brasilia and Sao Paulo, respectively. Both isolated expressed class B carbapenem-hydrolysing metallo-β-lactamase (MBL) IMP-16 (101-4704) and IMP-1 (48-696). These genes were found embedded in class 1 integrons that also encoded aminoglycoside-modifying enzymes. The aim of this study was further characterize the two novels aminoglycoside resistant genes (ARG).
Methods: Primers targeting the 5′CS and 3′CS regions of class 1 integron were used to amplify the *blaIMP-16* and *blaIMP-1* containing integron. These primers yielded PCR products, which were sequenced on both strands using DuPont Automated systems. After integrons sequence analysis, the ARG were amplified by PCR and cloned into the expression vector pPCRScriptCam SK. The recombinant plasmids were transferred into *Escherichia coli* DH5alfa and their respective aminoglycoside resistance profile evaluated against gentamicin, amikacin, kanamycin, neomycin, netilmicin, sisomicin, isepamicin and tobramycin. Nucleotides sequences and their deduced protein products, alignments and phylogenetic relationships were determined using the Lasergene software package.
Results: Sequence analysis revealed the presence of two novel aminoglycoside genes just downstream of the *blaIMP-16* and *blaIMP-1*, namely aacA(6′)-30 and aacA(6′)-31, respectively. The aacA(6′)-30 was fused with the following gene, aacA(6′)-Ib′, which formed an open reading frame of 984 bp and potentially encodes a protein of 36.7 kDa. The AAC(6′)-30 possessed most similarity (52.7%) to the previously described AAC(6′)-29b. The aacA(6′)-31 was 555-bp long, encoded a putative protein of 20.5 kDa and was most similar (82.1%) to the aacA(6′)-Ib′ found in the *blaIMP-16* carrying integron strain. *E. coli* strains harbouring the fused form aac(6′)-30/aac(6′)-Ib′ and aacA(6′)-31 showed MICs three to five-

fold-higher than the recipient *E. coli* DH5alfa strain, including to gentamicin and amikacin. The MICs remained unaltered to isepamicin.
Conclusions: The fused form AAC(6′)-30/AAC(6′)-Ib′ is likely to be a bifunctional protein rather than the expression of both AAC(6′)-30 and AAC(6′)-Ib′. The AAC(6′)-30/AAC(6′)-Ib′ and AAC(6′)-31 conferred a resistance profile called AAC(6′)-IV phenotype. The association of mobile MBL genes with ARG presents an immense concern since both enzymes cannot be neutralised by clinically available inhibitors.

P1721 Biological cost of fusidic acid resistance in *Staphylococcus aureus*

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Objectives: Antibiotic resistance due to chromosomal mutations causing structural modifications in the cellular target of the drug is often associated with a fitness burden for the resistant bacteria. This loss of biological fitness, however, can be overcome in some cases by the acquisition of compensatory mutations. As shown recently, certain amino acid exchanges in elongation factor G (EF-G) of *Staphylococcus aureus*, such as H457Y, cause resistance to fusidic acid. Interestingly, fusidic acid-resistant clinical *S. aureus* isolates carrying the exchange H457Y frequently harbour additional amino acid substitutions within EF-G (e.g. S416F) which do not contribute to resistance. The aim of the present study was (i) to analyse the biological costs of the mutation H457Y and (ii) to investigate whether the mutation S416F is able to compensate for this fitness burden.
Methods: The influence of amino acid exchanges within EF-G on the biological fitness of *S. aureus* was analysed by measuring growth kinetics and by means of fitness assays and plasmacoagulase activity assays, using isogenic recombinant *S. aureus* strains carrying either the wild-type EF-G gene (*fusA*) or a mutant *fusA* derivative (H457Y, H457Y/S416F or S416F) on a multicopy plasmid. All assays started with a defined cell number of bacteria and were repeated three times to ensure reproducibility.
Results: The assays showed that the fusidic acid resistance-mediating mutation H457Y causes a marked impairment of biological fitness. The amino acid exchange S416F, however, did not influence the fusidic acid susceptibility and impaired the biological fitness of the bacteria only slightly when present individually. The strain expressing the EF-G derivative with the double mutation H457Y/S416F, however, grew significantly faster, showed enhanced fitness in competition with the wild-type and exhibited a higher plasmacoagulase activity than the strain harbouring the single exchange H457Y.
Conclusions: The presented data provide evidence that the amino acid exchange S416F in EF-G functions as a fitness-compensating mutation in fusidic acid-resistant *S. aureus* harbouring the EF-G mutation H457Y, thereby demonstrating that the biological cost of fusidic acid resistance in *S. aureus* can be considerably reduced by secondary mutations within the bacterial genome.

P1722 Characterisation of In77, a class 1 plasmid-located integron carrying the novel metallo-β-latamase gene *blaGIM-1*

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Objective: GIM-1 was recently described as the fourth type of mobile MBL. Primary studies showed that *blaGIM-1* was embedded in a class 1 integron with unusual size and structure. The aim of this study was to characterise the genetic environment of *blaGIM-1*.
Methods: The integron structure was revealed with a walking sequencing strategy, using custome primers. Sequencing of the fragments was performed in both strands using DuPont Automated systems and the results analysed using DNAstar. The plasmid obtained from the isolate harbouring blaGIM-1 was electroporated into *Escherichia coli* DH5alfa and a rifampin-resistant mutant of *Pseudomonas aeruginosa* pA01 (RifR). Selection was performed in nutrient agar plates containing 4 μg/mL of ceftazidime. Restriction profiles of the plasmid were carried out with different restriction enzymes, to obtain the plasmid size.
Results: In77 is a 6-kb integron showing all the key genetic components commonly found in a class 1 integron (the intI1 integrase gene with its own promoter regions, an attI1 recombination site and in the 3′-CS, the fused structure qacEdelta1/*sul1*). This integron harbour in the first position the recently described *blaGIM-1*. Downstream of the MBL gene was found an aacA4, followed by an aadA1. However, this second aminoglycoside resistance gene was interrupted by the insertion sequence, IS1394, previously described in a *Pseudomonas alcaligenes* isolate. This integron also carried an ESBL gene, *blaOXA-2* in the last position. Two frame shifts were found in this integron, one in the integrase gene and the second in the IS1394. Further characterisation of the In77, revealed that this integron is likely to be located in a nontrasferable 22-kb plasmid.
Conclusions: This work describes a novel integron, In77, carrying the lately mobile MBL gene described, *blaGIM-1*. Distinct features, such as the addA1 interrupted by the IS1394 and the frame shifts that make the integrase and the insertion sequence stationary.

P1723 The effect of large inocula on in vitro activities of penicillin-inhibitor combinations and cefoperazone–sulbactam against ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* strains

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Objectives: Currently, there is little consensus about the clinical effectiveness of β-lactam–β-lactamase inhibitor combinations in treatment of infections caused by ESBL-producing organisms. It has been argued that significant decrease in activity of antimicrobial agents in testing with high inocula may be predictive of a possible therapeutic failure in cases of severe infections. The aim of our study was to determine the effect of large inocula on *in vitro* activities of amoxicillin–clavulanic acid (AMC), piperacillin–tazobactam (PTZ) and cefoperazone–sulbactam (CPS) against *Escherichia coli* and *Klebsiella pneumoniae* strains producing various types of ESBLs.
Methods: Twenty laboratory strains producing the knows ESBLs, TEM-3–TEM-7, TEM-9–TEM-12, TEM-26, TEM-47, SHV-2–SHV-6, CTX-M-3, CTX-M-5, CTX-M-9 and CTX-M-15 and 198 ESBL-producing clinical isolates of *E. coli* ($n = 46$) and *K. pneumoniae* ($n = 152$) collected in 21 Russian hospitals were included in this study. ESBL production was detected by a double-disc synergy test. Activities of AMC (2:1), PTZ (4 mg/L – fixed tazobactam concentration) and CPS (1:1) were determined by broth microdilution tests using standard (5×10^5 CFU/mL) and 100-fold higher inocula. Results were interpreted according to the current NCCLS guidelines. The susceptibility to CPS was determined on the basis of cefoperazone MIC breakpoints.
Results: In testing with standard inocula, the rates of resistance to AMC, PTZ, and CPS were 10.6, 36.2 and 5.5%, respectively. The data on the MICs of each drug tested with different inocula are summarised in the table. The inoculum effect, defined as an eightfold of greater MIC increase on testing with the higher inoculum, was commonly observed with PTZ (84.4%) and less-frequently detected with AMC (28.0%) and CPS (25.7%). The extent of the inoculum effects with these drugs was largely independent of the type of ESBL produced. In high-inoculum tests, all but two (0.9%) strains appeared resistant to FEP and PTZ, whereas 5 and 25.5% of strains remained susceptible to AMC and CPS, respectively.

	% of isolates with n-fold MIC increase						MIC 50; 90% mg/L*	
Drug	$n = 1$	$n = 2$	$n = 4$	$n = 8$	$n = 16$	$n \geq 32$	10^5CFU/mL	10^7CFU/mL
AMC	7.8	34.4	29.8	13.3	7.3	7.3	16; 16	32; 128
PTZ	0.9	7.3	7.3	8.3	9.2	67.0	16; 1024	≥4096; ≥4096
CPS	7.8	32.6	33.9	11.0	7.8	6.9	16; 32	32; 128

*Concentration of a beta-lactam component

Conclusions: A strong inoculum effect detected with PTZ is probably predictive of a high risk of failure if this drug is used for treatment of serious infections caused by ESBL-producing organisms. Based on the lowest resistance rate and the least pronounced inoculum effect, CPS may be considered as the most effective β-lactam–β-lactamase inhibitor combination.

P1724 Tn501-like transposons carrying novel *blaVIM-4* metallo-β-lactamase (MBL) gene cassettes in Poland and GIM-1 in Germany: report from the SENTRY antimicrobial surveillance programme

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Objective: Analysis of the genetic context of MBL containing integrons in carbapenem resistant *Pseudomonas aeruginosa* strains from Poland and Germany.
Methods: Carbapenem resistant strains were analysed by ribotyping and pulsed-field gel electrophoresis. The MBL-containing integrons from these strains were amplified using primers designed to class 1 integron specific 5′ and 3′ conserved sequences (CS). Upstream sequences were amplified by PCR by a novel degenerate primer approach using one primer anchored to the 5′ and 3′ CS sequences and degenerate primers designed to randomly hybridise to upstream and downstream sequences. Sequencing was performed on both strands by the dideoxy-chain termination method.
Results: The 11 Polish isolates all contained an identical class 1 integron containing a novel VIM-4 cassette which contained a 5′ direct repeat of 169 bp of the 3′ portion of the *blaVIM-4* gene. The 11 strains represented four different PFGE types. In all of these isolates the class 1 integron was inserted into the *tnpA* gene of a Tn501 type transposon, the *tnpA* gene having 100% identity to the *tnpA* gene of Tn501. The German isolates were all of an identical ribotype and contained a class 1 integron harbouring the GIM-1 MBL. Interestingly this class 1 integron was also inserted into the *tnpA* gene of a Tn501-like transposon but at a different site.
Conclusions: The Polish integrons harbour an unusual *blaVIM-4* gene cassette that has a 3′ duplication, which can be explained by a mechanism involving deletion of a segment of an ancestral tandem repeat of *blaVIM-4* via slipped strand replication, mediated by a combination of polymerase and integrase. Interestingly both the Polish and German MBL containing isolates contain class 1 integrons that are harboured by Tn501-like transposons. In all cases the integrons are inserted into the *tnpA* gene of the transposon. This is the first time that MBL gene cassettes have been associated with Tn501-like transposons and this observation adds another level of mobility to these gene cassettes.

P1725 Quinolone-resistance mechanisms of *Stenotrophomonas maltophilia* strains from Greek hospitals

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Objectives: *Stenotrophomonas maltophilia* is an important emerging pathogen causing a variety of nosocomial infections. Being inherently resistant to many antibiotics, this species poses a therapeutic challenge to the clinician. Fluoroquinolone (FQ) resistance in Gram-negatives has been attributed to amino acid substitutions in the quinolone resistance-determining regions (QRDR) of DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*). The aim of this study therefore was to determine the sequence of the QRDRs of *gyrA*, *gyrB*, and *parC* genes in nonclonal *S. maltophilia* clinical isolates, resistant to quinolones.
Methods: Six nosocomial isolates from five Greek hospitals, collected during the period April 1998 to May 1999 were studied. Susceptibility testing was performed by the agar diffusion (Kirby-Bauer) test and Etest, according to NCCLS guidelines. Epidemiologic relatedness between the isolates was assessed by pulse-field gel electrophoresis (PFGE) of SpeI-restricted genomic DNA. The QRDR regions of *gyrA*, *gyrB*, and *parC* of all strains were amplified by polymerase chain reaction (PCR) using specific primers and sequenced. Active efflux of ciprofloxacin (CIP), cefepime, meropenem, chloramphenicol, carbenicillin, aztreonam and tetracycline was examined by determining the MICs with broth microdilution method in the presence and absence of CCCP.
Results: Ciprofloxacin MICs ranged from 4 to 32 mg/L. A substitution at position 47 (Arg®) of *gyrA* particle was present in all six strains studied. A strain with MIC of 4 mg/L harboured two more aminoacid changes at positions 127 (Met®) and 137 (Asp®). *GyrB* presented an 11-aminoacid gap in all strains, while no aminoacid substitutions were found in ParC, even in the single strain with a CIP MIC of 32 mg/L. All six isolates showed a fourfold decrease in MICs of the above-mentioned antimicrobials in the presence of CCCP, suggesting the presence of an active efflux system. SmeABC and SmeDEF efflux pumps should be overexpressed in all strains tested, except one in which only SmeDEF pump was overexpressed.
Conclusions: No clear correlation appears between MIC of ciprofloxacin, mutations in QRDRs of *gyrA*, *gyrB*, and *parC*, and active efflux. The resistance of *S. maltophilia* to fluoroquinolones seems to be complicated and further work is required to clarify the mechanisms behind the wide phenotypic variations observed.

P1726 Survey of extended-spectrum β-lactamase in clinical isolates of *Pseudomonas* and *Acinetobacter* species

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Objectives: The incidence of resistance to ESBL antibiotics is increasing in Turkey. In this study, in-vitro activities of 17 antimicrobial agents against 601 *Pseudomonas* and 318 acinetobacter species isolated between 01 January 2000 and 31 August 2003 were studied for the production of ESBLs.
Methods: Identification and susceptibility tests were done using VITEC 2 system. Conventional methods and BBL Crystal (BioMerieux, Fr) were studied additionally when necessary. Minimum inhibitory concentration (MIC) of panresistant strains were determined by agar dilution method.
Results: A total of 177 (87%) of isolated *Acinetobacter* spp was from inpatient clinics, 41 (12.8%) were from outpatient specimens. Intensive care unit was accounted for 36.7% of positive cultures in hospitalised patients. Among *Acinetobacter* species 317 were *Acinetobacter* spp.; saccharolytic, nonhaemolytic, one was *Acinetobacter* spp., asaccharolytic, nonhaemolytic. *Acinetobacter* isolates were generally more resistant than *Pseudomonas* isolates. Fifty-seven per cent of all acinetobacter strains were ESBL-positive. Panresistance rate was 8.4%. Of the antimicrobials tested, imipenem and β-lactams with β-lactamase activity were found most active with susceptibility rates 50–60%. Rates of susceptibility against amikacin, ampicillin, cefazoline, cefotetan, ciprofloxacin, ofloxacin, gentamicin, piperacillin, tobramycin were lower than 40%. Eighty-seven per cent (524) of *Pseudomonas* isolates were *P. aeruginosa*, 1.16% (7) were *P. fluorescence*. One-third of all isolates were from intensive care units. Fifty-six per cent of *Pseudomonas* isolates were ESBL-positive. Imipenem (70%) and piperacillin/tazobactam (83%) have the highest activity against *Pseudomonas* spp. Ciprofloxacin

resistance was 51%. Among aminoglycosides, amikacin was most active with 73% susceptibility rate, followed by tobramycin (56%) and gentamycin (43%).
Conclusion: Imipenem and β-lactam inhibitors are still the first line drugs in nonfermentative bacteria infections in our region.

P1727 Biochemical characterisation of VIM-7, a new VIM variant produced by a *Pseudomomas aeruginosa* clinical isolate from Texas

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Objectives: *blaVIM-7* was the first mobile metallo-β-lactamase described in North America. This plasmid-encoded gene was found in the *Pseudomonas aeruginosa* isolate 07-406, submitted to the CANCER Programme in 2001. The enzyme produced is very divergent from the other member of the VIM-family enzymes. VIM-7 has 77% amino acid identity to VIM-1, whereas VIM-1 to VIM-6 has 89 to 99% identity. The aim of this study was determine the kinetic parameters to VIM-7.
Methods: *blaVIM-7* was amplified and aggregated with Nde I and Bam HI restriction sites by PCR and primarily cloned in PCR Script Cam+. The gene was subcloned in pET-9a expression vector and overexpressed in in *Escherichia coli* DH5a. The VIM-7 protein was purified using a periplasmic preparation by fast performance liquid chromatography (FPLC). The integrity of the enzyme was confirmed using mass spectroscopy and N-terminal sequencing. Kinetic properties were determined with several β-lactam substrates measuring hydrolysis rates under initial rate conditions.
Results: VIM-7 varies from VIM-1 by 31% amino acid residues at the level of the mature protein, being the most divergent VIM variant so far identified. Semi-purified VIM-7 actively hydrolyses imipenem (IMI), meropenem (MER), penicillins and cephalosporins, where the penicilins and IMI appears to be the preferential substrates. The Km (micro molar) values obtained with semi-purified enzyme were 17 to ampicillin, 35 to IMI and 126 to MER. VIM-7 showed no hydrolysis against aztreonan and poor rates against the serine-β-lactamase inhibitors (clavulanic acid and tazobactam). In comparison with VIM-1 and VIM-2, VIM-7 showed remarkable differences.
Conclusions: VIM-7 is a new highly divergent VIM variant, which demonstrates significantly kinetic properties from those values reported for VIM-1 and VIM-2. VIM-7s kinetic profile and its possibility of dissemination in America, suggests that this enzyme will compromise therapies in this region.

P1728 Distribution and association of *mef*(*A*/*E*), *erm*(*B*) and *tet*(*M*) genes in erythromycin resistant *S. pneumoniae* and oral streptococci

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Objectives: A clinical sample of 28 macrolide-resistant streptococci collected in our laboratory from throat swab cultures, including 21 *S. pneumoniae*, four *S. sanguinis*, one *S. mitis*, one *S. milleri* and one *S. morbillorum* was characterised in order to evaluate (i) the distribution of *mef*(*A*/*E*), *erm*(*B*) and *erm*(*A*) genes; (ii) their association with *tet*(*M*) and (iii) the localisation of resistance determinants on the streptococcal genome.
Methods: PCR with primers specific for *mef*(*A*/*E*), *erm*(*B*), *erm*(*A*) and *tet*(*M*) were used. The localisation of resistance genes was obtained by PFGE, Southern Blot and hybridisation with specific probes. Direct automated sequencing of PCR fragments was performed.
Results: *erm*(*B*) was identified in 71.4% of MLSB strains (60.7% in *S. pneumoniae*, 7.2% in *S. sanguinis*, 3.5% in *S. morbillorum*), *mef*(*A*) gene was present in 17.8% of M strains (10.7% in *S. pneumoniae*; 3.6% in *S. mitis*; 3.5% in *S. sanguinis*), while *erm*(*A*) was never found. In all MLSB strains *erm*(*B*) was always associated with *tet*(*M*) in all species. In M-strains, *tet*(*M*) was associated in 7.1% in *mef*(*A*)-positive strains (3.5% in *S. milleri* and 3.6% in *S. sanguinis*) and in 3.5% in *S. pneumoniae mef*(*E*)-positive. Southern blotting analysis indicated that *erm*(*B*) and *tet*(*M*) always hybridised in a single band in all cases, suggesting a localisation in the same genetic element; on the contrary *mef*(*A*) and *tet*(*M*) hybridised on different fragments, demonstrating the different localisation on the streptococcal genome; in only one case *mef*(*E*) and *tet*(*M*) appeared to be associated. The sequence analysis of long-PCR of two strains with *erm*(*B*)/*tet*(*M*) and *mef*(*E*)/*tet*(*M*) showed the presence of Tn1545 in the former and the integration of the MEGA element, which carries *mef*(*E*), in Tn916 containing *tet*(*M*), in the latter. Furthermore, the sequence of long-PCR of *mef*(*A*)/*tet*(*M*) positive strains demonstrated the presence of Tn1207.1 and Tn916, which were never associated with each other.
Conclusions: Our study demonstrated that (i) *erm*(*B*) gene is prevalent in all species; (ii) *tet*(*M*) is always associated with *erm*(*B*) and sporadically associated with *mef*(*A*). Finally, only in *mef*(*E*)–*tet*(*M*) strains, was the integration of MEGA into Tn916 demonstrated, showing an example of genetic evolution of resistance.

P1729 Mechanisms of resistance in 17 unrelated *Escherichia coli* strains of different origins with a multiple-antibiotic-resistance phenotype

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Objective: To characterise the multiple antibiotic resistance mechanisms in 17 unrelated *Escherichia coli* strains of different origins.
Methods: The strains were recovered from foods (four) and faecal samples of healthy animals (eight) and humans (five). Susceptibility testing to 25 antimicrobial agents was performed by agar-dilution method (NCCLS). Mutations in the *gyrA*, *gyrB* and *parC* genes, and the analysis of *blaTEM*, *blaOXA* and *blaSHV* genes were determined by PCR and sequencing. The *tet*, *aac*(3), *ant*(2′′), *aphA*, *sul*, *aadA*, *cmlA* and *floR* genes were detected by PCR, whereas *dfr* genes were identified by PCR and RFLP. Chloramphenicol acetyl-transferase (CAT) activity was also studied. Type 1 and 2 integrons were analysed by PCR and sequencing.
Results: All strains were resistant to nalidixic acid, ampicillin, tetracycline, chloramphenicol and rifampicin, and most also to trimethoprim (14 strains), sulphamethoxazole (16 strains) and streptomycin (16 strains). Four and eight strains were resistant to gentamicin and kanamycin, respectively. Ten strains (ciprofloxacin MIC range 0.125–1 mg/L) showed a single amino acid change in GyrA (S83L). The amino acid changes found in the remaining seven strains (ciprofloxacin MIC range 4–64 mg/L) were (GyrA/ParC changes): S83L+D87N/S80I: four strains; S83L + D87H/S80I: one strain; S83L + A84P/S80I + A108V: one strain; and S83L + D87N/S80I + E84G: one strain. The resistance genes detected were the following (no. strains): *blaTEM1* (17); *tetA* (10), *tetB* (seven); *cmlA* (five); *aac*(3)-*IV* (three), *aac*(3)-*II* (one); *aphA1* (two), *aphA2* (six); *aadA1* (12), *aadA2* (five), *aadA5* (two); *sul1* (10), *sul2* (11), *sul3* (six); *dfrA1* (nine), *dfrA12* (five), *dfrA17* (two), and a new *dfr* gene was also found in one strain. None of the 17 strains had *blaOXA*, *blaSHV*, *ant* (2″) or *floR* genes. More than five different resistance genes were found in 15 of the 17 strains and one strain harboured 11 of the 18 genes detected. Ten strains showed more than one gene implicated in the resistance to the same antimicrobial agent. CAT activity was found in six of the 17 strains. Eleven strains harboured one or two type 1 integrons, which included the following gene cassettes (no. strains): *aadA1* (one), *dfrA1*+*aadA1* (six), *dfrA12* + *orf* + *aadA2* (four), *dfrA17* + *aadA5* (two). Type 2 integron was detected in four strains, which included the *dfrA1* + *sat* + *aadA1* gene cassettes.
Conclusions: The multiple-antibiotic-resistance phenotype in *E. coli* strains of different origins is associated with heterogeneous resistance mechanisms, some of which are located in integrons.

P1730 Genetic analysis of fluoroquinolone-resistant *Acinetobacter baumannii* isolates from Korea

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Objectives: *Acinetobacter baumannii* has recognized as a important opportunistic pathogen of nosocomial infections and the resistance to fluoroquinolone among *Acinetobacter* spp. has rapidly emerged in Korea. The mechanism of resistance to fluoroquinolone has been associated mainly with mutations in quinolone-resistance-determinig regions (QRDR) of the *gyrA* gene of DNA gyrase and the *parC* gene of topoisomerase IV. The genetic analysis of fluoroquinolone resistance in *A. baumannii* isolates in Korea remain to be investigated. To investigate the prevalence of mutations in DNA gyrase and topoisomerase IV, we analysed the QRDRs of the *gyrA* and *parC* genes against ciprofloxacin-resistant *A. baumannii* isolates from Korean hospitals.
Methods: A total of 59 clinical isolates of *A. baumannii* were collected from nontertiary hospitals in 2002–2003. *A. baumannii* isolates were identified by the API 20NE kit and *recA*-RFLP analysis with-Tsp5091. MICs of ciprofloxacin and levofloxacin were determined according to the criteria of NCCLS. The QRDRs of *gyrA* and *parC* genes were amplified by PCR using specific primers. To detect mutations in the QRDRs, digestion of the PCR products with *Hin*f I were analysed and sequencing was performed by the dideoxy-chain termination method.
Results: Ciprofloxacin and levofloxacin resistance rates of *A. baumannii* isolates were 89.8 and 78.0%, respectively. The MIC50 and MIC90 of ciprofloxacin and levofloxacin of these isolates were 32 and 128 and 8 and 16 mg/L, respectively. All of 53 ciprofloxacin-resistant *A. baumannii* had only a substitution of Ser83 with Leu in the GyrA protein. Other mutations in the *gyrA* gene, Gly81Val and Ala84;Pro, did not be detected. Among 53 *A. baumannii* isolates with alteration in Ser83 of GyrA, mutations with substitution of Ser80 to either Leu or Trp in the ParC were found in 41 and one isolate, respectively. Nine isolates had a substitution of Glu84 to Lys and two isolates contained no mutation in the ParC.
Conclusions: In this study, double mutation at codons Ser83 in GyrA and Ser80 in ParC were more frequently found among *A. baumannii* isolates with a ciprofloxacin MIC of 16 mg/L. In ParC protein, the novel substitution of amino acid, Ser80 to Trp, was detected.

P1731 CTX-M-type extended-spectrum β-lactamases in commensal *Escherichia coli* from healthy children living in urban areas of Bolivia and Peru

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Objectives: The CTX-M-type β-lactamases are acknowledged among the most important secondary extended-spectrum β-lactamases (ESBLs) spreading in Enterobacteriaceae. CTX-M-producers have mostly been reported among nosocomial isolates, but recently also from outpatients and animals, pointing to the presence of a large reservoir of these resistance genes. In this paper we describe the detection of different CTX-M genes in commensal *Escherichia coli* from healthy children living in Bolivia and Peru.
Methods: *E. coli* were from faecal swabs of healthy children from four urban areas of Latin America, two in Bolivia and two in Peru. Susceptibility testing and detection of ESBL production were carried out as recommended by NCCLS. Characterisation of β-lactamases was carried out by analytical isoelectric focusing (IEF), and by molecular analysis of genes by PCR and sequencing. The genetic support and transferability of the CTX-M determinants were investigated by mating experiments, plasmid profiling, and Southern blot experiments. The phylogenetic group of *E. coli* isolates was determined by multiplex PCR.
Results: During a large screening (3208 subjects) carried out to investigate antimicrobial resistance in commensal *E. coli* of healthy children from Peru and Bolivia, ceftriaxone-resistant *E. coli* isolates were recovered from four subjects (one from each urban area). The four isolates tested positive for ESBL production in a double-disc synergy test and IEF revealed multiple bands of β-lactamase activity. Molecular analysis showed that three isolates (two from Bolivia and one from Peru) produced the CTX-M-2 enzyme, while one (from Yurimaguas and Peru) produced CTX-M-15. The CTX-M-2 determinants were carried on large conjugative multidrug resistance plasmids (of heteogeneous restriction profiles in different isolates) that also encoded a TEM-1 β-lactamase. Transferability could not be detected for the CTX-M-15 determinant. The isolates producing CTX-M-2 belonged in three different phylogenetic groups (A, B2 and D), while the CTX-M-15 producer in phylogenetic group D.
Conclusions: These findings underscore the widespread distribution of CTX-M genes, and the role that the commensal *E. coli* microbiota can play as a potential reservoir of these resistance determinants. To our best knowledge, this is the first report on CTX-M enzymes in Bolivia and Peru, and on CTX-M-15 in Latin America. ANTRES project supported by EU INCO-DEV, ICA4-CT-2001-10014.

P1732 Characterisation of extended-spectrum β-lactamases in *Escherichia coli* strains recovered in a Spanish hospital

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Objective: To characterise the β-lactam resistance mechanisms in a series of 33 *Escherichia coli* clinical isolates with MIC >2 mg/L of ceftazidime or cefotaxime.
Methods: The strains were recovered from unrelated patients in a hospital of Asturias, (Spain) during a 12-month period. Susceptibility testing to 10 β-lactams was performed by the agar-dilution method. Screening for detection of extended-β-lactamases (ESBL) was carried out by the double-disc test. Genes of TEM, SHV, OXA, CTX-M, CMY and FOX β-lactamases were investigated by specific PCRs and sequencing. Mutations in the promoter region of the chromosomal *ampC* gene were analysed by PCR and sequencing. Clonal diversity was studied by pulsed-field gel electrophoresis (PFGE) by digestion with XbaI enzyme.
Results: The MIC ranges (in mg/L) were as follows: ampicillin, 128 to >256; ticarcillin, 32 to >256; amoxicillin–clavulanic acid, 2/1–32/16; cefazolin, 16 to >256; cefoxitin, 1 to 256; ceftazidime, <0.5 to 128; cefotaxime, 0.25 to >128; ceftriaxone, 0.25 to >128; imipenem, <0.125 to 0.25; and aztreonam, 1 to 256. Genes encoding ESBL were detected in 23 of the strains (13 *blaCTX-M-14*, four *blaCTX-M-9*, one *blaCTX-M-29*, two *blaCMY-2*, two *blaTEM-52*, and one *blaSHV-12*). The −42 point mutation in the promoter of *ampC* gene was detected (not associated with ESBL) in other eight *E. coli* strains. Neither *blaFOX* nor *blaOXA* genes were detected in the 33 strains. The *blaTEM-1* gene was identified in 17 strains, in 11 cases associated with ESBL and in four cases with the mutation at the −42 position of the *ampC* gene promoter. No ESBL or *ampC* mutations were detected in the last two extended spectrum cephalosporin-resistant *E. coli* strains. All the 20 isolates with *blaCTX-M* or *blaCMY* genes showed unrelated PFGE patterns.
Conclusions: *blaCTX-M-14*, and *blaCTX-M-9*, are the main ESBL genes detected in our strains and the unusual *blaCTX-M-29* gene has also been found. The −42 point mutation in the *ampC* gene promoter has not been detected in strains with ESBL genes.

P1733 Molecular characterisation of two multiresistent clinical isolates of *Chlamydia trachomatis*

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Two clinical isolates of *C. trachomatis* resistant to azithromycin and ofloxacin were obtained. The objective of the present work

was studying them for possible mutations that are known as conferring resistance to macrolides and fluoroquinolones.
Methods: Patient 1 was a 25-year-old woman with exacerbation of chronic salpingitis. Cervical specimens were tested for *C. trachomatis* by PCR and cell culture, and both PCR and cell culture results were positive (isolate no. 1). Patient 2 was a 31-year-old woman presented with endocervicitis on May 2000 and had a positive cervical culture for *C. trachomatis* (isolate no. 2). She reported that she had had an episode of *C. trachomatis* infection in 1997 and received spiramycin for it. The patient was treated with doxycycline. Eight months later, PCR test of cervical specimen for *C. trachomatis* was negative. Each strain was titered at 10-fold dilutions and dilution producing approximately 104 inclusions per well, which corresponds to 10–20 inclusion per field under microscope magnification of 400, was used in MIC and MBC determination experiments. The inclusions were detected by direct immunofluorescence using fluorescein-labelled antibodies against major outer membrane protein.
Results and conclusions: Isolates no. 1 and 2 demonstrated resistance to azithromycin (MIC and MBC were above 5.12 mg/mL, compared with between 0.02 and 0.16 mg/mL for the reference strain ATCC VR-885) and ofloxacin (above 256 mg/mL, compared with 4 mg/mL for the reference strain). The isolates demonstrated heterotypic kind of resistance, because only small numbers of inclusions was observed in presence of antibiotic. Partial sequences of genes *gyrA*, *gyrB*, *parC*, *parE*, L22, L4 and 23S rRNA were determined. Both the isolates had mutations A2058C and T2611C (the *E. coli* numbering) in the peptidyl transferase region of the 23S rRNA gene. That mutations are associated with resistance to macrolides in the clinical isolates of pathogenic bacteria. Mutations in a nonconservative region of the L22 gene L22–Gly52>Ser, Arg65>Cys, Val77>Ala – were found in the isolate no. 1. Such mutations have not been found in other macrolide-resistant bacteria and we believe that they are not responsible for macrolide resistance. Though bacterial FQ resistance has been usually associated with certain point mutations QRDR of gyrA, no mutations were observed in the quinolone resistance-determining regions (QRDR) of *gyrA*, *gyrB*, *parC* and *parE* genes.

P1734 The presence of tetracycline resistance determinants and the susceptibility for tigecycline and minocycline

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Background: In Enterobacteriaceae *tetA–tetE* tetracycline resistance determinants may be encountered depending on the species. In *Staphylococcus aureus* the *tetK* and *tetM* determinants are commonly encountered. In some cases cross-resistance is observed with other tetracyclines. Tigecycline is a new tetracycline-like antibiotic with broad activity.
Aim: Study the relationship between the presence of tetracycline resistance determinants and MICs for tigecycline and minocycline in Enterobacteriaceae and *S. aureus*.
Methods: Tetracycline resistant isolates were randomly chosen from a collection of European isolates. MICs were determined using NCCLS methodology. The presence of the tetracycline determinants was established by specific PCRs. *Escherichia coli* ($n = 51$) were tested for the presence of *tet*(*A*)–(*E*), *Klebsiella* spp. ($n = 49$) for *tet*(*A*)–(*D*); *Serratia marcescens* ($n = 48$) for *tet*(*A*)–(*C*), and (*E*), and *Enterobacter* spp. ($n = 50$) for *tet*(*B*)–(*D*). *S. aureus* ($n = 101$) were tested for *tetK* and *tetM*.
Results: Seven *E. coli* isolates contained two determinants, whereas this was observed in only two *Klebsiella* and two *Enterobacter* isolates. None of the tested determinants was present in 30 *Enterobacter* and 45 *Serratia marcescens* isolates, suggesting that other resistance mechanisms are responsible for the tetracycline resistance. For any combination of resistance determinants among the Enterobacteriaceae the MICs for minocycline were higher than for tigecycline. No clear correlation between any combination of resistance determinants and the MICs for tigecycline and minocycline was observed. *S. aureus* isolates carrying the tetM determinant showed significantly higher MICs for minocycline than for tigecycline. When *tetK* alone was present the minocycline and tigecycline MIC distribution was comparable ranging from 0.12–2 mg/L for tigecycline and from 0.06–8 mg/L for minocycline. In *tetM*-positive isolate the ranges were 0.25–0.5 mg/L and 4–32 mg/L, resp.
Conclusions: No clear relation between the presence of tetracycline resistance determinants and the MICs for tigecycline were observed for Enterobacteriaceae and *S. aureus*, whereas such a relation was present for minocycline MICs and *tetM* and *tetK*.

P1735 Correlation of the mutation potential of *Acinetobacter baumannii* clinical isolates with alterations in the *mutS* gene

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Objectives: To analyse the amino acid (aa) sequences of the mismatch-repair (MMR) component *mutS* in sensitive and resistant clinical isolates of *Acinetobacter baumannii* (Ab) and to investigate whether variations in *mutS* correspond with the mutation potential of the isolates in response to challenge with ciprofloxacin (CIP).
Methods: A total of 12 representative Ab isolates were selected according to sensitivities. *mutS* was PCR-amplified with degenerate primers and sequences analysed by alignment using hierarchical clustering. First and second step mutants were generated with 2 × MIC of CIP and mutation frequencies were calculated as the ratio of mutant to viable cells. The *gyrA* (and where appropriate *parC*) quinolone-resistance-determining regions (QRDR) of parents and mutants were PCR amplified, with *Hin*fI restriction and sequencing indicating the presence of target site mutations.
Results: Within a 210 base pair section of *mutS* spanning the mismatch-binding domain, the majority of clinical isolates differed from the control strains at 16 aa positions. The aa sequences of all resistant isolates were identical whereas sensitive isolates varied. Two sensitive isolates (E21 and E33) matched neither the controls nor the other clinical isolates at 17 aa positions. Another sensitive isolate (U51) had an aa sequence identical to the resistant isolates and a higher mutation frequency (2.8×10^{-6}) compared with other sensitive isolates ($6–60 \times 10^{-8}$). Successive generations of U51 exhibited a greater increase in MICs of CIP (parent to second-step, 0.5 to 4–32 mg/L) than other sensitive isolates, their MICs remaining low. One U51 second-step mutant (MIC = 32 mg/L) developed a target site mutation of *gyrA* Ser83 to Leu. No *parC* target site mutation was found, despite the high-level CIP resistance. Six identical aa substitutions were found in the *gyrA* QRDR of both the parents and mutants of E21 and E33. No target site mutations were found.
Conclusions: Mutants generated from a sensitive isolate that displayed an identical aa MutS pattern to resistant strains developed higher levels of resistance to CIP compared with other sensitive strains. This correlated with a higher mutation frequency and a *gyrA* mutation at Ser83 in contrast to mutants of the other sensitive isolates in which no corresponding mutations were seen.

P1736 Examination of the carbapenem resistance gene activation mechanisms of imipenem-resistant *Bacteroides fragilis* isolates from the UK

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Objectives: To examine the resistance mechanism, the presence of the *cfiA* resistance gene and especially the activating insertion sequence (IS) elements of imipenem-resistant *Bacteroides* strains in the culture collection of the Anaerobe Reference Laboratory, Cardiff, UK.

Methods: Susceptibility measurements using Etests for amoxicillin/clavulanate, cefoxitine, clindamycin, imipenem and metronidazole, PCR and PCR-mapping to detect the *cfiA* genes and IS elements, nucleotide sequencing and imipenemase production assays were applied.
Results: Seven imipenem-resistant *B. fragilis* strains were detected that all produced imipenemase and were *cfiA*-positive. The resistance genes were shown to be preceded by IS elements that were already described (IS942, IS1186) in two strains or that were recently detected among isolates from the USA or Nottingham, UK in five strains. Obtaining the the full nucleotide sequence of IS element (IS614B and IS614C) representatives of this latter strains showed that they resemble to IS612 and IS614 detected in imipenem-resistant *B. fragilis* strains in Japan but may be hybrid or mosaic forms of these elements from Japan.
Conclusions: Our results confirm that imipenem resistance is due to *cfiA* genes activated by insertion sequence elements among *B. fragilis* strains and highlight, compared with earlier results, the presence of IS614B and IS614C among isolates from the UK and the USA as the possibly most prevalent ones at least in these countries. (This study was supported by a Royal Society – Hungarian Academy of Sciences exchange fellowship and a Hungarian National Research Fundation grant T037475 to Jozsef Soki.)

P1737 Evaluation of the *Salmonella typhimurium* proteome by semi-automated two dimensional HPLC-mass spectrometry; detection of proteins implicated in multiple antibiotic resistance

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Objective: The proteome of *Salmonella enterica* serovar *typhimurium* was characterised by two-dimensional HPLC mass spectrometry to provide a platform for subsequent proteomic investigations of low level multiple antibiotic resistance (MAR).
Methods: Cells ($2.15 \pm 0.23 \times 10^{10}$ CFU; mean $\pm$ SD) were harvested from liquid culture and proteins differentially fractionated, on the basis of solubility, into preparations representative of the cytosol, cell envelope and outer-membrane proteins (omps). These preparations were digested by treatment with trypsin and peptides separated into fractions ($n = 20$) by strong cation exchange chromatography (SCX). Tryptic peptides in each SCX fraction were further separated by reverse phase chromatography and detected by ion-trap mass spectrometry. Peptides were assigned to proteins and consensus rank listings compiled using SEQUEST.
Results: A total of 816 ± 11 individual proteins were identified which included 371 ± 33, 565 ± 15 and 262 ± 5 from the cytosolic, cell envelope and omp preparations, respectively. A significant correlation was observed ($r^2 = 0.62 \pm 0.10$; $P < 0.0001$) between consensus rank position for duplicate cell preparations and an average of $74 \pm 5\%$ of proteins were common to both duplicates. A total of 35 outer membrane proteins were detected, 20 of these from the omp preparation. A range of proteins ($n = 20$) previously associated with MAR in *E. coli* were detected including the key effectors AcrA, AcrB, TolC and OmpF.
Conclusions: Characterisation of the *Salmonella typhimurium* proteome will provide information for the discovery of protein biomarkers for multiple antibiotic resistance, enable the production of specific tests and thereby, evaluation of control strategies.

P1738 Characterisation of the mechanism of resistance to several antimicrobial agents in *Salmonella* clinical isolates causing traveller's diarrhoea

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Objectives: Analyse the antimicrobial resistance in *Salmonella* isolates causing Traveller's diarrhoea (TD), and characterise the mechanisms of resistance to several antimicrobial agents.
Methods: Sixty-two *Salmonella* strains isolated from patients affected with TD were analysed. The antimicrobial susceptibility to 12 antibiotics: ampicillin (AMP), amoxicillin/clavulanic acid, nalidixic acid (NAL), tetracycline (TET), trimethoprim/sulphametoxazole (SXT), chloramphenicol (CHL), gentamicin, amikacin, imipenem, norfloxacin, ciprofloxacin, ceftazidime, were performed using the agar dilution method. The molecular mechanisms of resistance to several antimicrobial agents was detected by PCR and the chloramphenicol acetyl transferase activity by a colorimetric assay.
Results: Twenty different serovar were identified, being *Salmonella enteritidis* the most prevalent [20 isolates (32.3%) of 62], followed by *S. typhimurium* [with six isolates (9.7%)]. The remaining isolates belonged to a wide variety of serovar. The highest levels of resistance were found against TET and AMP (20.9 and 19.4%, respectively), followed by resistance to nalidixic acid (16.1%). The resistance to NAL was related to the presence of mutations in the amino acid codons 83 or 87 of the *gyrA* gene. In the isolates resistant to ampicillin different β-lactamases were found: OXA-1 (one isolate) and TEM-like (seven isolates, in one case concomitantly with a CARB-2). Resistance to TET, was related to *tetA* (five cases) and *tetB* and *tetG* in one case each. Resistance to CHL was related to the presence of the *floR* gene in one case, while CAT activity was present in one strain. Different dihydrofolate-reductases (dfrA14, dfrA12, dfrA17 and dfrA1) were detected in SXT-resistant isolates.
Conclusions: A wide variety of serovars was found among *Salmonella* isolates causing TD, being *S. enteritidis*, followed by *S. typhimurium* the most frequently isolated. A low percentage of strains presented multi-resistance, however the highest levels of resistance were found for TET, AMP and NAL, being worthy of remark the steadily increase of the resistance to nalidixic acid since 2000. Surveillance for antimicrobial resistance is important to detect any trend and therefore give a more accurate empiric treatment.

P1739 Gene for aac(6′)-Ib, conferring resistance to amikacin, found in four different isolates of enterobacteria

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Objectives: The study of gene cassette for aac(6′)-Ib, conferring resistance to amikacin, found in four different clinical isolates of enterobacteria (*K. pneumoniae* 31, *E. cloacae* 46, *E. cloacae* 58 and *C. freundii* 69).
Methods: Conjugation was performed, using 0.1 mL of stationary cultures from *K. pneumoniae*, *C. freundii* and *E. cloacae* clinical isolates as donors and from *E. coli* 2110nal as recipient. Plasmidic DNA from these strains, were used for dot-blot hibridisation versus probes from aac(6′)-Ia and aac(6′)-Ib. The plasmids were digested with *Bam*HI, and cloned into pACYC184. Transformation with plasmidic DNA obtained from clinical isolates or transconjugants was performed on *E. coli* DH5alpha by cold calcium chloride treatment. To further characterise the amikacin resistance plasmid it was mapped by restriction analysis, and subsequently subcloned by partial digestion. or directed subcloning of restriction fragments. To characterise the aac(6′)-Ib gene and flanking regions of our isolates we used plasmids pMFB33, pMFB331, pMFB332 and pMFB22 for sequencing with CyDye labeled M13 direct and reverse universal sequencing primers in an ALFexpress automatic sequencer.
Results: These four strains resistant harbouring large conjugative plasmids ranging from 31–69 kb. Dot-blot hybridisation of plasmidic DNA from transconjugans, showing a positive result for aac(6′)-Ib. The transformans selected for amikacin resistance, contained a *Bam*HI fragment insert of 3.9 kb leading to the plasmid pMFB2.The restriction map of pMFB2 showed a structure related to that Tn21-derived integrons. The sequence obtained of plasmids pMFb33, pMFB331 pMFB332 and pMFB22 showed a fragment of 267 bp insert upstream of aac(6′)-Ib gene cassette.

Conclusions: We have found that all our isolated strains resistant to amikacin carried a plasmid that included an integron structure with the aac(6′)-Ib gene cassette, in this case fused in frame with an ORF with similarietes to the protein L of plasmid CloDf13 from *E. coli*. As all strains carry the same integron structure this implies a horizontal transmission. In this case the pressure due to the use of amikacin as prophylatic in urology surgery could be the trigger of this outbreak in our hospital.

Antimicrobial susceptibility in Gram-positive bacteria

P1740 Resistance rates and resistance mechanisms of *S. pneumoniae* strains isolated from eight European countries: final report of the PNEUMOWORLD study

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Objectives: An international study named PNEUMOWORLD started in 2001 to monitor the antimicrobial susceptibility and resistance mechanisms of clinical isolates of *S. pneumoniae* collected from adults over 16 years of age from 32 centers of eight European countries (Spain, France, Germany, Austria, Portugal, Switzerland, Italy and Belgium).
Methods: Serotyping was performed with the Quellung method. Microdilution method was done for all strains according to NCCLS with the following antibiotics: Penicillin G, amoxicillin, cefotaxime, cefuroxime, cefpodoxime, clarithromycin, clindamycin, gatifloxacin, levofloxacin, cotimoxazole, tetracycline, chloramphenicol and telithromycin. Macrolide-resistant phenotypes was checked with double disc-diffusion test according to standard methods. Macrolide resistance mechanisms (*ermB* and *mefA*) were done with the RT-PCR. Strains showing no products of *ermB* and *mefA* were checked further for 23S rRNA mutations in four alleles and changes on the ribosomal proteins L4 and L22. Fluoroquinolone resistance (FQ-R) mechanisms were detected by sequencing gyrase A (*gyrA*) and B (*gyrB*) genes and topoisomerase C (*parC*) and E (*parE*) genes.
Results: A total of 2285 clinical isolates of *S. pneumoniae* from sterile body cultures were collected between 2001 and 2003. Patient ages ranged between 17 and 100 years. Resistance rates were variable among the eight European countries. Penicillin resistance (intermediate, resistant) was detected in 566 strains and was highest in Spain (37.8 and 25.4%) and lowest in Austria (4.4, and 0%). The predominant serotypes of penicillin-resistant isolates were serotype 23F (16.8%) and 14 (16.4%). macrolide resistance was detected in 618 strains and was highest in France (45.8%). Predominant serotypes of macrolide resistant strains were 14 (20.4%) and 6B (17%). 81.7% of macrolide resistant strains were cMLSB phenotypes, 3.6% were inducible and 14.7% with an efflux mechanism of resistance. Six macrolide resistant isolates were neither *ermB* nor *mefA* and possessed mutations on 23S rRNA and L4. FQ-R (Gatifloxacin >1 mg/L) was found in 15 strains with amino acid alterations mainly on *par*C at positions 79, 83, 91 and 137. and on *gyrA* at positions 81, 85 and 114.
Conclusions: High rates of macrolide and penicillin resistance were detected in Spain, France and Italy and lower rates were found in Switzerland, Austria and Germany.

P1741 European surveillance study on antibiotic sensitivity of *Propionibacterium acnes* strains

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Objectives: *Propionibacterium acnes* strains are recovered from infections linked to operative procedures, foreign bodies and septicaemia. The aim of this study was to investigate the antibiotic sensitivity patterns of *P. acnes* strains isolated from different infections.
Methods: A total of 304 *P. acnes* strains from 13 laboratories in 13 European countries were studied. The strains were isolated from septicemia (105), skin and soft tissue infections (77), intraabdominal infections (22), head and neck infections (19), bone infections (15), meningitis (14), endocarditis (13), eye infections (13), prosthetic devices (10), lung infections (6), and other infections (10). The organisms were identified using biochemical tests, gas-chromatographic analysis and PCR. Pulsed-field gel electrophoresis was used for further characterisation. The size of chromosomal fragments was 40–400 kb. Mutanolysin mixed with lysozyme was used for inducing lysis. The restriction enzyme for DNA digestion was Spe1. The minimum inhibitory concentrations of four antimicrobial agents (clindamycin, erythromycin, linezolid, and tetracycline) against the *P. acnes* strains were determined by the agar-dilution method according to NCCLS and EUCAST. Brucella base-sheep blood agar with 100 000 CFU of inoculum per spot was used. The agar plates were incubated in anaerobic jars for 48 h at 37°C. The MIC was defined as the lowest concentration of antimicrobial agent resulting in a marked change in the appearance of growth when compared with the control plates.
Results: A total of 18 clusters and 78 banding patterns were identified among the isolates of *P. acnes*. The similarity between major PFGE types ranged from 54 to 100%. The minimum inhibitory concentration values were: for clindamycin, MIC50 0.032 mg/L, MIC90 0.25 mg/L, range 0.032–64.0 mg/L; erythromycin, MIC50 0.25 mg/L, MIC90 0.5 mg/L, range 0.032–256 mg/L; linezolid, MIC50 0.5 mg/L, MIC90 1.0 mg/L, range 0.25–2.0 mg/L; tetracycline, MIC50 0.5 mg/L, MIC90 1.0 mg/L, range 0.032–32.0 mg/L. No strains were resistant to linezolid, 3% of the strains to tetracycline, 15% of the strains were resistant to clindamycin and 17% of the strains to erythromycin. The most resistant strains to clindamycin and erythromycin were found in Croatia and Slovenia and the most susceptible strains in the Netherlands. The blood isolates were dominating among the resistant strains.
Conclusions: Antibiotic resistance among *P. acnes* strains is increasing in Europe.

P1742 Increasing erythromycin MICs within Canadian *Streptococcus pneumoniae* isolates with the M phenotype

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Objectives: Erythromycin resistance is increasing among clinical isolates of *Streptococcus pneumoniae* (SPN) in Canada. We examine the longitudinal trend in the proportion of erm and mef espression (M or MLSB phenotype, respectively) within Canada, and the distribution of erythromycin MICs for each phenotype.
Methods: SPN isolates were collected by the Canadian Bacterial Surveillance Network, which is comprised of private and hospital-affiliated laboratories from across Canada. Laboratories were asked to collect a defined number of consecutive clinical isolates followed by all sterile site isolates of SPN. In-vitro susceptibility testing was performed by broth microdilution using NCCLS guidelines. Erythromycin-resistant isolates were classified as either M or MLSB phenotype based on clindamycin resistance, corresponding to *mef* and *erm* genes respectively.
Results: There were a total of 13 177 isolates, including all patient age groups, were obtained from across Canada between 1997 and 2003. Erythromycin resistance has increased over that time from 6.8% in 1997 to 16.2% in 2003. The percentage of isolates with a M vs. MLSB phenotype has remained constant over that time at 54 and 46%, respectively. The distribution of erythromycin MICs

in MLSB phenotype isolates is unchanged comparing 1997 to 2002, with the majority of isolates having high level resistance (MIC > 32μg/mL). The MIC50 and MIC90 to erythromcyin for M phenotype isolates has increased from 1997 to 2002 (MIC50 2 μg/mL vs. 4 μg/mL, MIC90 16–32 μg/mL).
Conclusions: The proportion of M compared with MLSB phenotypes is consistent over a 10-year period within SPN in Canada. There is a significant shift toward higher erythromycin MICs among M phenotype isolates. This likely indicates either a more efficient efflux pump or increased expression of the mef gene. Despite the increase in erythromycin MICs amongst isolates with the M phenotype there has been no change in the MIC90 of telithromcyin for these isolates since testing began in 1999.

P1743 Comparison of daptomycin and vancomycin MIC results by NCCLS, SFM, DIN and SRGA methods

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Objectives: We determined if daptomycin and vancomycin MIC results obtained with DIN (Deutsches Institut für Normung), SFM (Societe Francaise de Microbiologie), and SRGA (Swedish Reference Group for Antibiotics) are similar to NCCLS (National Committee for Clinical Laboratory Standards) for selective Gram-positive organisms.
Methods: Approximately 250 enterococci, staphylococci and streptococci organisms were tested at three different European laboratories. Both clinical and common stock isolates were tested using media supplemented to 50 μg/mL calcium. Test-specific media were as follows: NCCLS, Mueller–Hinton Broth; SRGA, PDM agar; DIN, Isosensitest broth and SFM, Mueller–Hinton agar. Quality control strains *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212) were tested on each day.
Results: Essential Agreement Rates (% EA ± 1-doubling dilution) compared with NCCLS MICs were:

MIC Method	Daptomycin		Vancomycin	
	n	% EA	n	% EA
DIN	266	99.6	260	100
SFM	259	99.2	253	99.6
SRGA	244	93.0	239	99.6

DIN and SFM daptomycin MICs were within ±1 dilution of NCCLS MICs for all species except *S. pyogenes* (DIN EA% = 96.3, SFM EA = 93.1). SRGA daptomycin MICs were within ±1 dilution for 95% of strains except *E. faecalis* (EA% = 82.1) and *E. faecium* (EA% = 75.9).
Conclusions: There was excellent correlation of daptomycin and vancomycin NCCLS MICs compared with DIN and SFM MICs. There was good correlation of NCCLS and SRGA MICs, with the exception of enterococcal MICs, which will require further study.

P1744 Application of the mutant prevention concentration for *Streptococcus pneumoniae* against azithromycin, clarithromycin and erythromycin

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Objectives: The emergence of macrolide-resistant SP is a growing concern since macrolides are commonly used to treat *Streptococcus pneumoniae* (SP) infections. Two important mechanisms of resistance include ER ribosomal methylase (*erm*) genes and macrolide efflux (*mef*) genes. The mutant prevention concentration (MPC) is a novel approach for determining the likelihood of an antibiotic to select first-step resistant mutants in a large population of cells. We determined MPC values for penicillin-sensitive (PSSP) and penicillin-intermediate (PISP) isolates against macrolide/azalide agents.
Method: Minimal inhibitory concentrations (MIC) were determined by microbroth dilution following NCCLS guidelines. MPC testing was done by plating ≥10 (10) cells onto agar plates containing drug and incubated in CO^2 at 35–37°C for 24 and 48 h. The lowest concentration preventing growth was the MPC. polymerase chain reaction (PCR) was performed on selected organisms (MPCs ≥0.5 mg/L) for presence or absence of macrolide-resistant genes.
Results: To date, 59 clinical isolates (49 PSSP and 10 PISP) of SP have been tested. For azithromycin (AZ), clarithromycin (CL), erythromycin (ER), MIC50/90 (μg/mL), MPC50/90 μg/mL) and mode MIC/MPC were as follows respectively against PSSP: 0.125/0.25, 4/≥8, 0.125/≥8; 0.031/≥1, 0.25/≥4, 0.031/0.25; 0.063/≥8, 0.5/≥4, 0.063/0.5. MIC values were elevated against PSIP. PCR analysis of 16 organisms (MPCs ≥ 0.5 mg/L; MICs ≤ 0.125 mg/L) showed six had acquired macrolide-resistance (two *ermB*, four *mefA*), however, their susceptible parents did not have the macrolide-resistant genes. Mutants were selected from AZ, ER & CL plates.
Conclusions: Macrolide-resistant SP sub-populations were selected on MPC plates from large heterogeneous bacterial populations. MPC may, therefore, be an accurate measurement for determining appropriate dosing strategies to prevent resistance. The rank order of potency based on MPC was ER = CL>AZ. This represents the first report of the MPC concept applied to macrolides.

P1745 Activity of daptomycin against clinical and environmental enterococci from Portugal

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Objectives: To study the activity of the new antibiotic daptomycin against clinical and environmental enterococcal isolates with different antibiotic susceptibilities.
Methods: Enterococci were isolated from the following sources: (i) 251 clinical isolates from patients located at three hospitals (HP) in Central and North Portugal (1996–2003); (ii) 32 sewage water (SW) samples from four hospitals in Porto city collected just before (upstream samples, $n = 13$) and just after leaving (downstream samples, $n = 19$) the hospital setting (2001–2002). SW samples were plated on Slanetz–Bartley agar with and without antibiotics. One isolate/morphology and resistance phenotype was selected. Antibiotic susceptibility was determined using the agar dilution method in Mueller–Hinton media (NCCLS).
Results: From the 464 (251 HP and 213 SW) isolates studied, 246 were identified as *E. faecium* (119 from HP and 127 from SW), 179 as *E. faecalis* (132 from HP and 47 from SW) and 39 as *Enterococcus* spp. (SW). Resistance to different antibiotics was observed in isolates from both sources (HP, SW): vancomycin: 54, 17%; teicoplanin: 51, 12%; tetracycline: 82, 39%; erythromycin: 79, 64%; ciprofloxacin: 81, 72%; chloramphenicol: 20, 14%; gentamicin: 45, 33%; streptomycin: 32, 39%; kanamycin: 67, 58%; and nitrofurantoin: 4, 11%. Multi-resistance was observed in isolates from HP and SW. Daptomycin showed good activity against all enterococci studied, including the multi-resistant isolates. The MIC50/MIC90 values for *E. faecium* were both 4 mg/L and for *E. faecalis* were 2 and 4 mg/L. Differences in the antibiotic activity were not observed in isolates of distinct sources.
Conclusions: The good activity demonstrated by daptomycin against all enterococcal isolates studied makes this compound a good choice to treat the infections caused by resistant enterococci from Portugal.

P1746 Antimicrobial susceptibility patterns and macrolide resistance genes of β-haemolytic streptococci in Korea

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Objectives: This study evaluated the antimicrobial susceptibilities and macrolide resistance mechanisms of clinical isolates of β-haemolytic streptococci in Korea.
Methods: The minimal inhibitory concentrations of seven antimicrobials were determined for 540 clinical isolates of â-haemolytic streptococci between January 1990 and December 2000. Resistance mechanisms of erythromycin-resistant isolates were studied by the double-disc test and the PCR.
Results: Of the isolates, the overall rates of nonsusceptibility to tetracycline, chloramphenicol, erythromycin and clindamycin were 80.0, 22.8, 20.2 and 19.1%, respectively. Among the β-haemolytic streptococci tested, *Streptococcus agalactiae* had the highest nonsusceptible rates to tetracycline (95.8%), erythromycin (25.3%) and clindamycin (28.2%). Of 109 erythromycin nonsusceptible isolates, 69 isolates (63.3%) had the cMLSB phenotype [constitutive resistance to macrolide-lincosamide-streptogramin B (MLSB)], 26 isolates (23.9%) had the M phenotype and 14 (12.8%) isolates had the iMLSB phenotype (inducible resistance to MLSB). The proportion of the three MLS resistance phenotypes of *S. pyogenes* was nearly equal. About three-quarters (74.4%) of *S. agalactiae* had the cMLSB phenotype, whereas two-thirds (66.7%) of group G streptococci had the M phenotype. With the exception of two isolates, all MLSB resistant strains carried the *erm*(*B*) genes in cMLSB phenotype, *erm*(*TR*) genes in iMLSB phenotype, and *mef*(*A*) genes in M phenotype, respectively.
Conclusions: Of erythromycin-resistant isolates, the cMLSB phenotype with the *erm*(*B*) gene was dominant in Korea. Mechanisms and phenotype proportions of MLSB resistance were different by serogroup in β-haemolytic streptococci. It is possible that MLSB resistance genes have transferred among β-haemolytic streptococci because the erythromycin resistance genes are the same in β-haemolytic streptococci.

P1747 *In vitro* activity of daptomycin against a range of *Staphylococcus aureus* and *Enterococcus* isolates: first results from Turkey

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Objectives: The recent increase in infections caused by resistant Gram-positive pathogens, particularly nosocomial infections, have prompted a search for new antimicrobials that can counter these organisms. The cyclic lipopeptide daptomycin acts by inhibiting cell wall synthesis and has a unique chemical structure and a novel mode of action that enables it to target organisms that are resistant to other classes of agents. Currently, this drug is being investigated in a phase III clinical trial as treatment for serious Gram-positive infections in hospitalised patients. To date, there has been no investigation of the efficacy of daptomycin in Turkey. The aim of this study was to evaluate the in-vitro activity of this drug against *S. aureus* and *Enterococcus* strains isolated from infections at a Turkish health centre.
Materials and methods: The organisms tested were 108 *Staphylococcus* and 183 *Enterococcus* strains isolated from infected patients at Baskent University Hospital. minimum inhibitory concentrations (MICs) were determined by Etest on Iso-Sensitest agar medium.
Results: MIC results for daptomycin activity against the 108 *S. aureus* and 183 *Enterococcus* isolates are summarised in the Table. The daptomycin MIC50 values for the MRSA and MSSA strains were the same (0.38 mg/L), and the MIC90 values for the MRSA and MSSA were similar (1.5 and 1 mg/L, respectively). The daptomycin MIC values for the vancomycin-resistant and vancomycin-sensitive enterococcal strains were also similar (Table).

Table. The minimum inhibitory concentrations (MICs) of daptomycin against the *Staphylococcus* and *Enterococcus* strains tested.

Species (No. of isolates tested)	MIC (mg/L)		
	Range	MIC_{50}	MIC_{90}
MRSA (58)	0.047–2	0.38	1.5
MSSA (50)	0.094–1.5	0.38	1
E. faecalis (106)	0.047–4	0.38	1.5
E. faecium (53)	0.047–8	1	2
Other *Enterococcus* spp.* (10)	0.047–2	0.19	1
VRE** (14)	0.094–4	1	1.5

*4 *E. casseliflavus*, 4 *E. avium*, 2 *Enterococcus* spp.
**11 *E. faecium*, 2 *E. avium*, 1 *E. faecalis*.

Conclusions: Daptomycin showed potent activity against all the *S. aureus* and *Enterococcus* spp. that we tested, regardless of resistance to methicillin or vancomycin. Our findings for in-vitro activity of daptomycin against *S. aureus* and *Enterococcus* strains in Turkey are the first such results from our country. Clinical trials will provide more data on the efficacy of daptomycin in such cases.

P1748 Time-kill evaluation of the in-vitro activity of ertapenem against *S. pneumoniae*

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Objectives: (i) To evaluate dynamic bactericidal activity of ertapenem and its effects at sub-MIC level in combination with other drugs against *S. pneumoniae* strains displaying different antibiotic resistance phenotypes and (ii) to explore physiological perturbations (morphological changes) induced by this new carbapenems at sub-MIC concentrations.
Methods: Three *S. pneumoniae* strains for each of the following phenotypes have been studied: penicillin-susceptible (Pen-S), penicillin-intermediate (Pen-I), penicillin-resistant (Pen-R), erythromycin-resistant (Ery-R) and multi-resistant (Multi-R: Pen-Ery-SXT-Ch-R). Bactericidal activity of ertapenem (4 × MIC) was evaluated by time-kill curves following NCCLS suggestions (M26-A). Interactions of ertapenem in combination with clarithromycin (Cla), levofloxacin (Lev), rifampin (Rif) and vancomycin (Van) were studied by the time-kill method. In order to explore the morphological changes induced by ertapenem, cells were exposed to 0.5 × MIC and microscopic observations were performed after 2, 6 and 24 h of incubation.
Results: Ertapenem showed remarkable bactericidal activity against all *S. pneumoniae* tested irrespectively of their antibiotypes, causing >99.9% reduction of the initial inocula within 24 h of exposure for all strains. Results of drugs interactions are depicted in the Table.

Microorganism (*n*)	Cla		Lev		Rif		Ven	
	SYN	IND	SYN	IND	SYN	IND	SYN	IND
S. pneumoniae Pen-S(3)	3	0	3	0	3	0	3	0
S. pneumoniae Pen-I(3)	3	0	3	0	3	0	3	0
S. pneumoniae Pen-R(3)	3	0	3	0	3	0	3	0
S. pneumoniae Ery-R(3)	2	1	3	0	3	0	3	0
S. pneumoniae Multi-R(3)	3	0	3	0	3	0	3	0

Synergism was the prevalent outcome, antagonism was never found. After 2,6 and 24 h of exposure to ertapenem sub-MICs morphological alterations were observed and these were mainly represented by abnormal elongation of cells.

Conclusions: Ertapenem possesses potent bactericidal activity against *S. pneumoniae* strains irrespectively of their resistance to other molecules and is able to interfere with bacterial physiology at sub-MIC levels. Because of these characteristics and of its synergistic behaviour when combined with other anti-pneumococcal drugs, ertapenem is indicated in the treatment of infections caused by this pathogen.

P1749 Activity of ertapenem and 19 other antimicrobials against *Streptococcus pneumoniae* and *Haemophilus influenzae* isolated from the respiratory tract showing resistance mechanisms to betalactam antibiotics

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Objectives: The purpose of this study was to examine the susceptibility to ertapenem and 19 other antimicrobials of 191 *S. pneumoniae* strains resitant or with intermediate resistance to penicillin G and 100 *H. influenzae* strains resistant to ampicillin due to β-lactamase productions.
Methods: All microorganisms had been isolated from clinical respiratory samples collected between 2000 and 2001 in the 21 participating microbiology laboratories geographically distributed in 13 Spanish Autonomous Comunnities. The following antimicrobials were tested: penicillin G, amoxicillin, amoxicillin/clavulanate, cefaclor, cefuroxime, cefpodoxime, ceftibuten, cefixime, cefotaxime, ertapenem, imipenem, erythromycin, clarithromycin, azithromycin, clindamycin, tetracycline, chloramphenicol, ciprofloxacin, levofloxacin and moxifloxacin. Susceptibility to antimicrobials was tested using an agar dilution method based on the guideliness of the NCCLS.
Results: *S. pneumoniae* strains with intermediate resistance to pencillin were highly susceptible to carbapenems, with MIC90 of 0.25 and 0.5 mg/L for imipenem and ertapenem, respectively. Among the penicillin-resistant strains the MIC90 values for imipenem and ertapenem were 1 and 2 mg/L, respectively. Eight isolated in our global series (9.3%) would be classified as resistant to imipenem, while for ertapenem only four strains (4.7%) would be considered resistance. Therefore, and although imipenem in general lines is one dilution more active than ertapenem, other pharmacological considerations and additional clinical data would allow for categorising a greater number of organisms as susceptible to ertapenem comparec to imipenem. Carbapenems were extraordinarily active against the whole group of *H. influenzae* strains, particularly ertapenem, which inhibited 90% of the series at a concentrations of 0.12 mg/L.
Conclusions: Ertapenem is a powerful agent against the most common respiratory bacterial pathogens, including those which have gradually incorporated effective resistance mechanims. Concentrations of 4 mg/L inhibited all except one of the total 291 organisms considered. Ertapenem is at least comparable to imipenem, cefotaxime or the newer fluorquinolones, all of which are known to be very effective against these microorganisms. Such performance, and its favourable pharmacokinetic characteristics, make ertapenem an interesting option for the treatment of respiratory tract infections.

P1750 Daptomycin in-vitro activity tested against 2221 Gram-positive strains collected from European hospitals, 2002

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Background: Daptomycin (DAP) is a cyclic lipopeptide recently approved by the US FDA for the treatment of complicated skin and skin structure infections. We evaluated the contemporary in vitro activity of DAP against a collection of Gram-positive (GP) strains collected in European medical centers in 2002.
Methods: A total of 2221 clinical GP strains were collected from 29 centres. The collection included *S. aureus* [763 strains; 31% oxacillin (OXA)-resistant (R)]; coagulase-negative staphylococci (CoNS; 281 strains; 79% OXA-R), enterococci [260; 4% vancomycin (VAN)-R], streptococci (904) and other GP species (13). The strains were tested by NCCLS broth microdilution in Mueller–Hinton broth with 50 mg/L Ca++ against DAP. More than 20 comparators were also tested.
Results: DAP inhibited all tested strains at ≤4 mg/L and only the enterococcal isolates showed DAP MIC of 4 mg/L (14 strains; 5.4%). All staphylococcal and streptococcal isolates were inhibited at ≤1 mg/L of DAP. The activities of DAP, VAN, teicoplanin (TEI), quinupristin/dalfopristin (Q/D) and linezolid (LZD) are shown in the table:

Organism (no. tested)	MIC_{90}(mg/L)/% susceptible				
	DAP	VAN	TEI	Q/D	LZD
S. aureus (763)	0.5/100	1/100	1/100	0.5/100	2/100
CoNS (281)	0.5/–	2/100	4/98	0.5/99	1/100
S. pnemoniae (SPN;746)	0.25/–	0.5/100	0.5/–	0.5/100	1/100
β-haemolytic streptococci (75)	≤ 0.12/–	0.5/100	≤ 0.12/–	0.5/100	1/100
viridans group streptococci (77)	0.25/–	1/100	≤ 0.12/–	1/100	1/100
E. faecalis (EF;192)	2/100	2/97	1/97	>8/1	2/100
E. faecium (EFM;57)	4/–	2/91	2/93	2/79	2/100

Conclusions: VAN-R and Q/D-R did not influence DAP activity against enterococci, and PEN-R did not affect DAP activity on SPN. DAP showed a significant potency and spectrum against all GP including multi-drug resistant strains and may represent a therapeutic option for infections caused by these pathogens.

P1751 Activity of telithromycin against erythromycin susceptible and resistant *Streptococcus pneumoniae* isolates from adults with invasive infections

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Objectives: To know the activity of different TT concentrations against clinical isolates of *S. pneumoniae* susceptible and resistant to erythromycin, that caused invasive infections in adults in our setting.
Materials and methods: A total of 120 clinically significant *S. pneumoniae* isolates recovered from adults admitted in our institution at the current year (2003) were used in the study. MICs were determined with a microdilution method using cation adjusted Mueller–Hinton broth supplemented with 5% lysed horse blood. Time-kill curves were performed using Todd–Hewitt broth plus 0.5% of yeast extract as culture medium. Antibiotic concentrations were as follows: 2, 1, 0.5, 0.25 and 0.125 μg/mL for TT and 1 μg/mL for erythromycin. ppropriate dilutions were performed at 0, 2, 4, 6 and 24 h in order to know the number of viable bacteria (CFU/mL).
Results: A total of 60 strains were erythromycin susceptible (MIC < 0.06 μg/mL), and the other 60 were erythromycin resistant (MIC > 64 μg/mL). TT had low MICs for erythromycin susceptible (MIC 0.008–0.016 μg/mL) and for erythromycin-resistant strains (MIC 0.125–0.25 μg/mL). TT was uniformly bactericidal (irrespective of the strain's erythromycin susceptibility). In case of erythromycin susceptible strains, TT showed 99.9 % killing of all strains at 6 h of study and in case of erythromycin-resistant strains this descent was achieved at 18–24 h of incubation. The TT bactericidal effect was concentration-dependent against erythromycin resistant strains and was achieved with concentrations one dilution higher than MIC.

Conclusions: Time-kill results confirmed the excellent antipneumococcal activity of TT, irrespective of the susceptibility of strains to erythromycin. Even for strains for which macrolide MICs were >64.0 μg/mL, TT was uniformly bactericidal after 24 h at 0.25 μg/mL.

P1752 *In-vitro* activities of linezolid, moxifloxacin, quinupristin/dalfopristin and tigecycline against recent clinical isolates of *Staphylococcus* spp. in Spain

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Objectives: In a recent point prevalence study in Spain, we demonstrated high percentages of resistance of *S. aureus* (SA) and coagulase-negative staphylococci (CoNS) to many antimicrobial agents as follows: oxacillin (SA/CoNS): 30/63; erythromycin: 33/64; gentamicin: 18/35 and ciprofloxacin: 37/50 (13th ECCMID. Glasgow 2003). These high rates of resistance prompted us to evaluate the *in vitro* activity of new antimicrobial agents against these isolates.
Methods: In 2002 we carried out a point prevalence study in 143 Spanish hospitals collecting all *Staphylococcus* isolated in a single day. A total of 439 *S. aureus* (137 oxacillin-resistant) and 370 CoNS (227 oxacillin-resistant) were studied. Identification and antimicrobial susceptibility testing of all microorganisms was performed at the same laboratory. MICs of linezolid (LIN), moxifloxacin (MOX), quinupristin/dalfopristin (Q/D) and tigecycline (TIG) were determined by the microdilution method using Mueller-Hinton broth (NCCLS recommendations). *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were used as control strains.
Results: The MIC values (mg/L) and ranges of activity of the different antimicrobial agents against *Staphylococcus* spp. are summarised in the table.

	LIN		MOX		Q/D		TIG	
	OXA-R	OXA-S	OXA-R	OXA-S	OXA-R	OXA-S	OXA-R	OXA-S
S. aureus								
Range	≤ 0.25–2	≤ 0.25–2	≤0.06–16	≤0.06–4	≤0.06–0.5	≤0.06–0.5	≤0.03–0.5	≤0.03–0.5
MIC_{50}	1	1	1	≤0.06	0.12	≤0.06	0.25	0.25
MIC_{90}	2	2	4	≤0.06	0.25	0.12	0.5	0.5
CoNS								
Range	≤0.25–2	≤0.25–2	≤0.06–64	≤0.06–8	≤0.06–0.5	≤0.06–0.5	≤0.032–2	≤0.03–0.5
MIC_{50}	0.5	0.5	0.5	≤0.06	≤0.06	≤0.06	0.12	0.12
MIC_{90}	1	1	2	0.5	0.12	0.12	0.5	0.25

OXA: Oxacillin; R: resistant; S: susceptible.

Conclusions: All isolates tested were uniformly susceptible to linezolid, quinupristin/dalfopristin and tigecycline. Moxifloxacin was the less active of the antimicrobials tested. In general, the good activity of the new antimicrobial agents tested shows promise for the treatment of infections due to multiresistant staphylococci. (This study was financed by the Spanish Network for the Research in Infectious Pathology).

P1753 Comparison of four commercially available systems for antimicrobial susceptibility testing (AST) of *Streptococcus pneumoniae*

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Objective: The objective of this study was to evaluate four commercially available systems for AST of a challenge set of *Streptococcus pneumoniae* (SPN) of known serotypes and antimicrobial resistance patterns.
Methods: A total of 108 clinical strains of SPN were included in this study. All strains were tested using two manually read dried panel systems, the Sensititre *Haemophilus influenzae*/*S. pneumoniae* (TREK Diagnostics Systems, Cleveland, OH) and Microscan MICroSTREP plus 1 (Dade Behring, Mississauga, ON), and two fully automated systems: Vitek 2 AST-P506 card (bioMérieux, St Laurent, Quebec) and Phoenix SMIC (BD Diagnostics, Sparks, MD). Testing was performed according to the manufacturers guidelines and all required quality control tests were included. SPN ATCC 49619 was included as a common quality control strain in all systems. Reference broth micro-dilution testing (MIC) was performed by the National Centre for *Streptococcus*, Edmonton AB on all strains according to NCCLS recommendations and was considered the gold standard.
Results: There were 36 different serotypes included in this set. By reference MIC, 57% were penicillin-S, 24% were penicillin-I and 19% were penicillin-R; 36% were erythromycin-R; seven strains (6.4 %) were ceftriaxone-R (meningitis breakpoints) and seven strains (6 %) were ofloxacin-R. The antimicrobials tested were not identical for all systems. For penicillin, there was 90% agreement within interpretive categories for all systems with one major error for Sensititre and Microscan, and the remainder were minor errors (4–10%). For erythromycin, there were five major errors (three-Phoenix, one-Microscan, one-Sensititre) and the remaining were minor errors (1–7%). For ceftriaxone there was one very major error (Vitek 2), 1 major error (Microscan) and the remaining were minor errors (7.4–12%). For quinolones, ofloxacin (MIC and Vitek 2) and levofloxacin (Microscan, Sensititre and Phoenix) were tested. Results for these agents showed that there were only minor errors for all systems (5.5–7.4%) compared with reference MIC.
Conclusions: All systems performed favourably when compared with the reference MIC method. The isolates grew equally well in the dried panels and the automated systems. For those agents commonly used to treat SPN infections, these commercial systems are easy to set up, read and provide equivalent results. The automated systems also provide expert interpretation of AST according to NCCLS guidelines.

P1754 Comparison of the Etest and the agar dilution method for glycopeptide susceptibility testing of staphylococci

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Objectives: To validate the Etest for detecting glycopeptide-resistant staphylococci.
Methods: A total of 450 staphylococcal isolates (150 *Staphylococcus aureus*, 300 coagulase-negative staphylococci) isolated from clinical specimens in four Greek hospitals have been examined from glycopeptide susceptibility by Etest using two inoculum densities, 0.5 and 2.0 Mc Farland. Results obtained were compared with those by reference agar dilution method.
Results: All *S. aureus* isolates expressed susceptibility to vancomycin and teicoplanin according NCCLS breakpoints. Using inoculum 0.5 and 2.0 Mc Farland the Etest vancomycin gave 15 and 20 false-positive results (specificity 90% and 86.6%, respectively). In contrast, no discrepancies have been found between the Etest teicoplanin and the agar dilution method. Among CoNS, 30 isolates expressed resistance to teicoplanin but remained susceptible to vancomycin according the reference method. Etest teicoplanin (0.5 and 2.0 Mc Farland) identified correctly all these isolates (sensitivity 100%). On the other hand, 20 and 40 isolates were falsely characterised as teicoplanin-resistant by Etest with 0.5 and 2.0 Mc Farland inoculum (specificity 92.6% and 85%, respectively). Population analysis revealed that all false-positive isolates were not hetero-resistant and didn't express resistance at a low frequency.
Conclusions: This study indicates that he use of the Etest with an inoculum of 0.5 Mc Farland provides a reliable and sensitive method for the detection of teicoplanin-resistant staphylococci, without incurring too many false-positives.

P1755 Antibiotic resistance of enterococci isolated from blood and urine cultures during 2002–2003

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Objectives: To study the resistance of Enterococci from blood and urine cultures during 2002–2003.
Methods: A total of 136 strains of enterococci were isolated in blood and urine cultures. The identification of the isolated bacteria was performed by standard methods and the API-strep systems(bioMerieux). The susceptibility testing was carried out by disc-diffusion method and by Etest.
Results: Enterococci were the third cause of urinary-tract infections and bacteraemia in order of apperance. Totally 139 strains were isolated:118 strains of enterococci were isolated from 1383 urinary-tract infections and 21 strains from 172 bacteraemias. In three patients the same strain was isolated both in blood and in urine cultures. Of 136 strains of enterococci were isolated 114 strains of *E. faecalis*, 16 *E. faecium*, two *E. durans*, two *E. gallinarum*, one *E. casseliflavus*, one *E. hirae*. A total of 23 strains were found resistant to ampicillin (16.9%), 62 to high level gentamycin (45.6%), 82 to high level streptomycin (60.3%), 91 to ciprofloxacin (66.9%) and three to vancomycin and teicoplanin (2.2%).None of them was able to produce β-lactamase. Of 16 strains of *E. faecium* 15 were resistant to ampicillin.The determination of vancomycin and teicoplanin MICs in strains of VRE (two *E. faecium*, one *E. faecalis*) were tested highly resistant to vancomycin and teicoplanin suggesting the presence of VanA phenotype. Two strains of VRE (*E. faecium*) were isolated in urine cultures and one strain of VRE (*E. faecalis*) were isolated in blood culture.
Conclusions: The high resistance of *Enterococcus* spp. to high level aminoglycosides and the identification of VRE strains is critical for infections control purposes in the hospital environment.

P1756 Resistance to β-lactams, macrolides, ketolides and newer fluoroquinolones in nasopharyngeal pneumococci with reduced susceptibility to ciprofloxacin

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Objectives: To study the susceptibility of pharyngeal pneumococci with MICs of ciprofloxacin >4 μg/mL to β lactams, macrolides, ketolides and newer fluoroquinolones.
Methods: We studied the in-vitro activity of penicillin, cefazolin, cefuroxime, cefotaxime, telithromycin, erythromycin, clindamycin, ciprofloxacin, levofloxacin and moxifloxacin, by the agar-dilution method according NCCLS guidelines, against 49 *S. pneumoniae* strains with MICs of ciprofloxacin >4 μg/mL isolated from outpatients throat.
Results: The MIC range of ciprofloxacin was 4–8 μg/mL. MIC50 and MIC90 of levofloxacin were respectively, 1 and 2 μg/mL, and MIC50 and MIC90 of moxifloxacin were 0.2 μg/mL. No strains resistant to these fluoroquinolones were found. 69.5% of strains were penicillin-susceptible (mode MIC = 0.01 μg/mL). 22% of strains were penicillin intermediate (MIC 0.1–1 μg/mL), and 8.5% were penicillin-resistant (MIC > 1 μg/mL). All the penicillin-resistant or intermediate isolates were resistant to cefuroxime. 45.7% were resistant to cefuroxime (MIC 50 = 1 μg/mL; MIC90 $\geq$ 32 μg/mL) and 8.5% cefotaxime-resistant (CIM > 2 μg/mL) (MIC50 = 0.2 μg/mL; MIC90 = 2 μg/mL). 40.7% of strains were erytrhomycin-resistant (MIC50 = 0.2 μg/mL; MIC90 $\geq$ 32 μg/mL). All of them showed constitutive MLSB phenotype. No telithromycin-resistant strains were found.
Conclusions: Pneumococci with MICs of ciprofloxacin >4 μg/mL have been shown to harbor topoisomerases mutations in at least 22% of cases. Nevertheless, these strains remain, in this study, susceptible to newer fluoroquinolones. Both high level and intermediate resistance to penicillins are moderately lower than in previous studies in Spain.

P1757 Susceptibility of *Streptococcus anginosus* group isolates from dental abscesses and abscesses of fascial spaces of the face and neck

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Objectives: Streptococci belonging to anginosus group are the main facultatively anaerobic bacteria involved in oral and maxillofacial infections. The aim of the present study was to investigate the antimicrobial susceptibility of 59 streptococcal strains of anginosus group isolated (as single microbial agents or in association with other bacteria) from pus samples collected from dental abscesses and abscesses of fascial spaces of the face and neck in Romanian patients.
Methods: The isolates were speciated using the Rapid ID 32 STREP system (Bio-Merieux, France). Antimicrobial susceptibility testing against: penicillin G (PG), erythromycin (EM), clindamycin (CM), chloramphenicol (CL) and tetracycline (TC) was performed by Etest (AB Biodisk, Sweden). In addition, the phenotype of EM resistance was identified by the double disc diffusion test.
Results: The isolates were identified as: *S. anginosus* (53 strains), *S. constellatus* (five strains) and *S. intermedius* (one strain). The MICs (mg/L) ranges were: PG 0.002–0.125, EM 0.016–3, CM 0.016–0.047, CL 0.016–3 and TC 0.125–256. All isolates were susceptible to PG, CM and CL. Only three strains of *S. anginosus* were found resistant to EM and they showed the M phenotype. Intermediate susceptibility (8.5% of all strains) and resistance (40.7% of all strains) to TC were found among isolates of all three species.
Conclusions: (i) PG still remains the drug of choice for the treatment of infections caused by these bacteria, but in mixed infections with β-lactamase producers, amoxicillin/clavulanic acid (Augmentin) or ampicillin/sulbactam (Unasyn) are indicated to be used; (ii) the frequency of EM resistance was low (5%) compared with other reports; (iii) on the basis of these in-vitro results, CM might be an alternative to β-lactam antibiotics in patients allergic to penicillin or in mixed infections with penicillin-resistant microorganisms.

P1758 Comparative in-vitro activity of telithromycin against viridans group streptococci

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Objective: The objective of this study was to evaluate the *in vitro* activity of telithromycin (TEL) comparatively to other antibiotics against viridans group streptococci (VGS) isolates collected in 2002 from clinical specimen in France.
Methods: In total, 123 isolates of VGS were tested including 49 isolates of *Streptococcus mitis*, 49 of *S. oralis*, 13 of *S. sanguis*, eight of *S. salivarius*, three of *S. parasanguis* and one isolate of *S. gordonii*. MICs of TEL, penicillin (PEN), amoxicillin (AMX), cefuroxime (CFM), erythromycin (ERY), clarithromycin (CLA), and azithromycin (AZI) were determined by agar dilution according to the 2003 recommendations of the Comité de l'Antibiogramme de la Societé Française de Microbiologie (CA-SFM). Quality control was performed with the three following strains of *S. pneumoniae* (SP): ATCC49619 (wild strain), Drug 4428 (*mefA*) and Drug 3031 (*ermB*). Susceptibility rate to TEL was calculated according to European breakpoints determined by EMEA: 0.5 to >2.
Results: MIC50/90 (mg/L) and the percentage of susceptibility (%) against VGS strains are presented below:
Overall VGS (n = 123): TEL: 0.06/0.12; 99.2, PEN: 0.12/2; 44.7, AMX: 0.06/4; 74, CFM: 0.5/8; 63.4, ERY: 0.25/4; 85.4, CLA: 0.25/2; 85.4, AZI: 0.25/16; 85.4.
S. mitis **(n = 49):** TEL: 0.06/0.12; 98, PEN: 0.12/4; 49, AMX: 0.12/16; 71.4, CFM: 0.25/16; 65.3%, ERY: 0.25/2; 89.8, CLA: 0.25/1; 89.8, AZI: 0.25/16; 89.8.
S. oralis **(n = 49):** TEL: 0.03/0.12; 100, PEN: 0.12/2; 46.9, AMX: 0.12/4; 75.5, CFM: 0.5/8; 57.1, ERY: 0.12/>128; 85.7, CLA: 0.12/16; 85.7, AZI: 0.12/64; 85.7.

Other VGS ($n = 25$): TEL: 0.06/0.06; 100, PEN: 0.12/1; 32, AMX: 0.25/2; 72, CFM: 0.12/2; 72, ERY: 0.5/>128; 76, CLA: 0.25/32; 76, AZI: 1/32; 76. So, 55.3% of the 123 VGS isolates showed decreased susceptibility to PEN, 41.5% were intermediately resistant and 13.8% highly resistant. 26% were intermediately resistant to AMX. In total, 36.6 and14.6% of the overall VGS showed decreased susceptibility to CFM and macrolides respectively.
Conclusions: These results demonstrated that telithromycin has good in vitro activity against viridans group streptococci including strains with reduced susceptibility to PEN, AMX, CFM and macrolides.

P1759 In vitro susceptibility of *Streptococcus pneumoniae* to ertapenem and other antibiotics

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Objectives: Infections caused by *Streptococcus pneumoniae* continue to be frequent worldwide and are associated with significant morbidity and mortality. The emergence of *S. pneumoniae* strains resistant to penicillin and other antibiotics has complicated the treatment of such infections. The aim of the study was to evaluate the in vitro susceptibility of *S. pneumoniae* to ertapenem, a new carbapenem, in comparison to other antibiotics.
Methods: A total of 80 clinical isolates of *S. pneumoniae* were tested by the Etest method on Muller–Hinton agar supplemented with 5% defibrinated sheep blood. 60 isolates were penicillin resistant (33 intermediate, MIC 0.1–1 μg/mL, and 27 resistant MIC > 1 μg/mL) and 20 were penicillin sensitive. The MIC50 and MIC90 were determined for penicillin (PEN), vancomycin (VAN), erythromycin (ERY), ciprofloxacin (CIP), levofloxacin (LEV), ceftriaxone (CEF) and ertapenem (ERT).
Results: The results of the 60 penicillin resistant isolates are given in the following table:

	MIC50	MIC90	Range	% Resistance
PEN	0.5	2	0.125–8	100
VAN	0.5	0.75	0.25–1	0
ERY	0.094	256	0.023–256	50
CIP	0.5	0.75	0.25–8	0.7
LEV	0.5	0.75	0.38–24	3.3
CEF	0.25	0.5	0.0324	5
ERT	0.19	0.5	0.006–0.75	0

The highest MIC value of 256 μg/mL was found for ERY in 25% of the resistant isolates. In the 20 penicillin sensitive isolates, antimicrobial resistance was found only for CIP (5%). The MIC50, MIC90 for VAN was 0.38 and 0.5 μg/mL, and for ERT 0.008 and 0.16 μg/mL, respectively.
Conclusions: ERT was the most active agent against both penicillin resistance and sensitive isolates. Although all isolates were sensitive to VAN and ERT, the MIC of ERT was always lower in all 80 isolates, a finding that may be meaningful from a pharmacodynamic viewpoint. Ertapenem may be an important, potent agent for the treatment of invasive pneumococcal infections.

P1760 Trend of *Streptococcus pyogenes* antimicrobial resistance in Reggio Emilia, Italy

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Objectives: The recognition of the prevalence of antimicrobial resistance in streptococci has implications for the choice of antibiotic therapy in clinical practice. We evaluated the antimicrobial resistance of *S. pyogenes* (SGA) isolates among patients with upper respiratory tract infections in Reggio Emilia (Italy) and the data were included in the ARES project (Antimicrobial Resistance Evidence Survey).
Methods: The study was conducted over a 12-months period and included patients with symptoms of acute pharyngotonsillitis. The 471 *Streptococcus pyogenes* isolates were identified according to standard laboratory techniques. Catalase-negative and β-emolitic colonies isolated from 5% sheep blood agar were identified using latex agglutination test and Api 20 S system. Sensitivity testing was done with disc diffusion according to the National Committee for Clinical Laboratory Standards (NCCLS). Antimicrobial sensitivity to penicillin, erytromycin, tetracycline, clindamycin, rokitamicin and amoxicillin/clavulanate was determined.
Results: All the 471 isolates were uniformly susceptible to penicillin. Overall 69/468 isolates (14.74%) showed resistance to erytromycin and 33/463 (7.13%) were also resistant to clindamycin (constitutive resistance). Erytromycin resistance trend in our area is variable (30% in 1999, 24.63 in 2000, 12.43 in 2001 and 14.74% in 2003). The prevalence of rokitamicine-resistant isolates is 5.23% in Reggio, Emilia (24/459isolates)and 5.60% (216/3715 isolates) in the National area.
Conclusions: Streptococci may be the most virulent organism, causing septic and nonseptic complications. Failure to eradicate streptococci from patients can occasionally lead to rheumatic fever and rarely to glomerulonephritis. With the emergence of increased treatment failures, it has been necessary to consider alternative therapies for patients who cannot tolerate penicillin. Our date suggest the importance of testing the sensitivity of *Streptococcus pyogenes* to macrolides, particularly 16-atoms suchas Rokitamicin.

P1761 In vitro activity and synergism studies of linezolid alone or in combination with several antimicrobial agents against *Nocardia* spp.

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Pulmonary nocardiosis is a severe complication in heart-transplant (HT) patients. No standardised treatment has been established. Recent data seem to indicate a good clinical efficacy of Linezolid (LZD) against *Nocardia* spp.
Objectives: To evaluate the in vitro susceptibility of *Nocardia* spp. to LZD and several other antibiotics and the efficacy of antibiotic combinations including LZD.
Methods: Six strains of *Nocardia* (*N. asteroides*, two; *N. brasiliensis*, two; *N. farcinica*, one; *N.* spp., one) were isolated from fine-needle biopsy of pulmonary lesions from HT patients with clinical signs and symptoms of pulmonary nocardiosis. Growth was obtained on conventional bacterial media, incubated at 37°C in 5% CO_2 for 24–72 h. Antimicrobial susceptibility studies were performed by Kirby–Bauer method and MIC determination by a microdilution method in Mueller–Hinton broth II. Synergism studies were performed by disc technique on Mueller–Hinton II agar using the following antibiotics: LZD, piperacillin–tazobactam (TZP), imipenem (IMP) and trimethoprim–sulphametoxazole (TMP-SMX) alone or in combination with each other and with ciprofloxacin (CIP), levofloxacin (LVX), ofloxacin (OFX) moxifloxacin (MFX), amikacin (AN), netimicin (NET), tobramycin (NN), gentamicin (GM) and streptomicin (ST). Susceptibility tests were read at 24 and 72 h; for synergism studies, plates were observed up to 144 h.
Results: By Kirby–Bauer method, all strains were susceptible to LZD, IMP and AN; five to doxycycline, cefotaxime (CTX), ceftriaxone (CRO), cefepime; four to TMP-SMX; three to cefamandole and MFX, two to TZP and azithromicin. One strain was susceptible to ampicillin (AM), ampicillin-sulbactam (SAM), CIP and rifampin. The MIC 90 (mg/L) were minor or equal to the following: LZD eight; IMP two; AN one; AM 62; AMS 32; TZP 256/32; CTX four; CRO four; CIP 32; MFX 8; TMP-SMX 8/152. Synergism studies: at 72 h, most of the combinations were indifferent. Synergism was observed for: LZD with AN, GM, NN in one strain; IMP with AN, CIP in three, with GM, NN, TZP, LZD in two,with LVX, MFX, SXT in one; TZP with NET in three, with AN, GM, NN in one; with OFX, LVX, MFX in one; TMP-SMX with NN in three,

with AN in two, with ST in one. Antagonism was observed for LZD with AN in five cases, with TMP-SMX and CIP in three.
Conclusions: LZD shows a good in vitro activity. Based on these preliminary data, the combination of IMP and AN appears suitable as initial treatment of invasive nocardiosis, while LZD may represent a good choice for sequential oral therapy.

P1762 Effect of pH, media and inoculum size on telavancin (TD-6424) in vitro activity

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Objectives: Telavancin (TD-6424), a rapidly bactericidal agent with multiple mechanisms of action, is in phase 2 clinical trials for serious Gram-positive infections. Telavancin (TLV) has potent activity against Gram-positive bacteria and exhibits concentration-dependent behaviour. We studied the effect of pH, media and inoculum size on the in vitro activity of TLV in comparison with that of vancomycin (VAN).
Methods: The effect of pH (6–8) and inoculum size (10^5–10^8 CFU/mL) on MIC was determined against *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus pneumoniae* according to the NCCLS method. The effect of inoculum size (10^6–10^8 CFU/mL) and media consisting of cation-adjusted Mueller–Hinton broth (MHB) and brain–heart infusion broth (BHI) on bactericidal activity was determined for TLV and VAN against *S. aureus* strain MRSA 33591 by time-kill kinetics.
Results: Although an increase in inoculum from 10^5–10^6 CFU/mL had no effect on the MIC of TLV, higher inocula (10^7 and 10^8 CFU/mL) resulted in a two to eightfold increase in MIC. pH variation of 1 U from the normal value had little effect on TLV MIC against staphylococci and enterococci but negatively affected MIC against *S. pneumoniae* (four dilution increase). The bactericidal activity of TLV and VAN was unchanged in either MHB or BHI. Increasing the inoculum size increased the time required to produce 3 or 5 log10 of killing.

	Time (hr) to $\geq 3-\log_{10}$ ($\geq 5-\log_{10}$) reduction of inoculum		
	10^5 CFU/mL	10^7 CFU/mL	10^8 CFU/mL
TLV (16 ug/mL)	8 (24)	24 (24)	24 (48)
TLV (32 ug/mL)	4 (4)	8 (24)	24 (24)
VAN (16 ug/mL)	8 (24)	24 (>48)	24 (>48)
VAN (32 ug/mL)	8 (24)	24 (>48)	24 (>48)

TLV, but not VAN, reduced the inocula to undetectable levels within 24 and 48 hours at 10^7 and 10^8 CFU/mL.

Conclusions: High inoculum had little impact on telavancin inhibitory and bactericidal activity against *S. aureus*. Unlike vancomycin, telavancin was able to decrease an inoculum of 10^8 CFU/mL to undetectable levels at a clinically achievable concentration. This suggests possible advantages in treating infections associated with large inocula of organisms.

Mycology

P1763 *Pneumocystis jiroveci*: a new human saprophyte?

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Recently, it has been shown that patients with chronic lung disease have a high rate of colonization by *Pneumocystis jiroveci* (formerly known as *P. carinii*) and also that acute infection, usually asymptomatic, is common during the first years of life. However, so far it has not been established whether the pathogen can colonise the immunocompetent adult host in a subclinical way.
Objectives: To evaluate the possibility of subclinical infection by *P. jiroveci* (Pc) in the general adult population.
Methods: (i) Population: Prospective study (February–July 2003) in which were included the first 50 patients evaluated in the Unit of Work Health of our hospital, not exposed to hospital environment during the previous year and not meeting the exclusion criteria (confirmed diagnosis or suspicion of chronic lung disease, neoplasm or immunosuppression of any aetiology). Every patient was presented with a clinical-epidemiological survey and oropharingeal washes (OW) samples were obtained by gargling with 10 cc of sterile 0.9% physiological serum for analysis. (ii) Method: The presence of fragments of the mtLSU-rRNA region of Pc was analysed in OW by means of nested polymerase chain reaction (PCR) using the primers AZ1002-E/-H and pAZ102-X/-Y.
Results: The mean age of individuals was 33.9 $\pm$ 9.45 years old. 19 of them (31.6%) were male. 28 (56%) were newly employed medical residents, 13 (26%) common services staff and nine (18%) administrative staff. *Pneumocystis* infection was observed in 13 (26%) of the 50 individuals. All positive subjects were asymptomatic at the time of their enrolement in the study and only one of them had taken steroids for a brief period of time in the 6 months prior to the study. No relationship was established between the presence of Pc infection and age, sex, professional standing or a previous history as smokers.
Conclusions: For the first time a high prevalence of subclinical infection by *Pneumocystis* has been established in the general population. This fact supports the theory claiming the saprophytic nature of the pathogen in humans. Though further studies are necessary to determine the feasibility and transmission of the infection in immunocompetent individuals, our results suggest that the general population might play an important role as a reservoir and source of infection from the microorganism to the immunocompromised host. (Research Project 32/02. Ministry of Health- Junta de Andalucía) and (Eurocarinii Project QLK2-CT-2000-01 369).

P1764 Genetic diversity of *Pneumocystis jiroveci* isolates obtained in the area of Seville, Spain

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Pneumocystis jiroveci (Pc) is one of the most important opportunistic infections affecting immunocompromised individuals but our knowledge about its epidemiological characteristics is still very limited. Molecular techniques have made it possible to identify different Pc genotypes, which could be associated with the virulence and clinical manifestations of the infection. In Spain there are not available data about the distribution of these genotypes.
Objectives: The aim of our study was to provide with some information in this respect.
Methods: We analysed 32 bronchoalveolar lavage samples from 11 individuals with HIV infection and Pc Pneumonia and from 21 individuals with chronic lung diseases colonised by Pc. In all

cases the diagnosis was obtained by means of nested-PCR (primers pAZ102-E and –H and pAZ102-X and –Y). The genotypic characterisation was performed by sequencing a fragment of 360-bp of the locus mtLSU-rRNA of Pc, which enables the identification of four genotypes according to the mutations observed in positions 85 and 248.
Results: See Table.

Genotypes (Cases) Pneumonia	Patients with HIV infection + Pc (n=11)	Patients with chronic lung disease + colonized by Pc (n=21)	P
G-1(19)	7(63.6%)	12(57.1%)	0.98
G-2(3)	1(9.1%)	2(9.5%)	0.54
G-3(10)	3(27.3%)	7(33.4%)	0.95
G-4(0)	0(0%)	0(0%)	–

Conclusions: In our region there are a predominance of genotype 1. The similar distribution of genotypes in individuals with and without HIV infection suggests a common source of infection for both populations or either the transmission of the pathogene from one population to another. Supported by Eurocarinii Project QLK2-CT-2000-01 369 & SAF 2003-06061.

P1765 *Pneumocystis jiroveci* colonisation in lung transplantation receptors with cystic fibrosis

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Background: Bronchopulmonary infections are the most serious complication for patients with cystic fibrosis (CF) and very often lung transplantation (LT) becomes the only therapeutic alternative to improve the survival rate of these patients. The prognosis of LT depends on such factors as the rejection of the graft and the occurrence of opportunistic infections. Recently, the presence of asymptomatic *Pneumocystis jiroveci* carriers has been observed among patients suffering from CF, but their role in the natural history of the disease has yet to be established.
Objectives: To determine the occurrence of *P. jiroveci* colonisation in individuals with CF who have become immunocompromised as a result of LT.
Methods: Prospective study in a cohort of patients with CF, including a group of LT receptors. Every patient filled in an epidemiological survey and sputum and/or oropharingeal lavage samples were obtained. The diagnosis of *P. jiroveci* colonisation was reached by means of nested-PCR.
Results: A total of 52 sputum samples and 47 oropharingeal lavage samples from 98 patients with CF were analysed. All transplanted patients received prophylaxis with cotrimoxazole and none of them developed *P. pneumonia* during the follow-up period of 1 year (see Table).

	Cohort	Lung Transplantation	Non Lung Transplantation
N (Male/Female)	98(44/54)	10	88
Average age ± SD(years)	16 ± 6.8	20.9 ± 6.5	15.5 ± 6.7
P. jiroveci(+)	23 pt(23.5%)	3 pt(30%)	20 pt(22.7%)

Conclusions: Prophylaxis with cotrimoxazole does not prevent colonization by *P. jiroveci* in individuals with CF who have received LT. As a result the development of strains resistant to this drug poses a risk of *P. pneumonia* infection and the possibility of their presence should be monitored in these patients.
(Research Project 11/01. Consejería de Salud Ministry of Health-Junta de Andalucía) and (Eurocarinii Project QLK2-CT-2000-01 369).

P1766 Genotypic resistance to sulpha drugs in patients with *Pneumocystis pneumonia* in southern Spain

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The lack of techniques to obtain *Pneumocystis jiroveci* (Pc) cultures has made it necessary to develop molecular techniques to detect its resistance to different drugs. Punctual mutations 55Thr→Ala and 57Pro→Ser in the DHPS gene are associated with the failure of treatment with sulpha drugs and with the worsening of the prognosis of Pc pneumonia. Nevertheless, the importance the discovery of these mutations may have in the clinical field has still to be established. The aim of our study was to ascertain the occurrence of strains with DHPS mutations in our milieu and its clinical implications.
Methods: We included in our study all patients diagnosed for a period of 2 years in our hospital of whom bronchoalveolar lavage samples and/or sputum were available. The diagnosis was performed by means of nested PCR. Primers DHPS-3/-4 were used to identify the resistant mutations by means of restriction enzymes.
Results: DHPS gene could be amplified in nine of the 12 identified cases (75%) (see Table):

Case	Risk factor	Age	Exposure to sulfa drugs	Genotype	CD4	Response to treatment
1	HIV	41	No	Wt	20	Unfavourable
2	HIV	51	No	Wt and 55	25	Favourable
3	HIV	42	Yes	Wt	212	Favourable
4	HIV	28	No	55/57	406	Favourable
5	HIV	52	No	Wt	35	Unfavourable
6	Renal transplantation	44	No	Wt	NA	Favourable
7	HIV	38	No	Wt	1	Favourable
8	HIV	38	Yes	Wt	105	Favourable*
9	HIV	41	Yes	Wt/57	14	Unfavourable*

Wt: wild strain; 55: mutation in codon 55; 57: mutation in codon 57; 55/57: double mutation; NA: not available; *: pentamidine theraphy

Conclusions: We observed in our area an elevated occurrence of pneumonias provoked by strains of *P. jiroveci* with mutations associated with resistance to sulpha drugs. Supported by Eurocarinii Project QLK2-CT-2000-01 369 & Research Project 55/03 - Consejeria de Salud - Junta de Andalucia.

P1767 Quantitative real-time PCR *Taq*Man assay for detection of *Pneumocystis jiroveci*

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Objective: *Pneumocystosis* remains difficult to diagnose by current microbiology methods in particular in non-HIV immunodeficient patients and rapid diagnostic tools are necessary. We describe a rapid and reproducible real time PCR method for detection and quantification of the tubuline *P. jiroveci* (PJ) gene in bronchoalveolar lavage (BAL) samples.
Method: A quantitative real time PCR was developed for detection and quantification of PJ. Specific PJ primers and fluorogenic probe were chosen in the tubuline gene sequence. A plasmid containing the target sequences was used to construct a standard curve for quantification. DNA extraction was performed with Qiagen Kit according to the manufacturer's recommendations. Different control samples including human DNA, virus, bacterial and fungal species were also tested to investigate the assay specificity. All samples and controls were tested in duplicate. Fifty-three BAL samples sent to the laboratory for diagnosis of pneumocystosis

were prospectively investigated by real time PCR and direct microscopic examinations (DME) using Giemsa stain and direct immunofluorescence.
Results: The PCR assay showed a limit of detection of 50 gene copies per millilitre. A linear signal over a range of 4 log10 of magnitude was obtained. The assay was reproducible as indicated by the intra-assay CV values obtained with the plasmid standard (0.09–1.17%). The PCR assay proved to be highly specific, reacting only with the tubuline PJ gene but with none of the other species tested. All PCR-negative samples were DME-negative. Twenty-four (45%) BAL were PJ-positive by PCR assay. Among these, eight were positive by DME (35 %). The range of PJ DNA gene copies per millilitre were 9.190 to 1.467.628 and 8 to 10.470 for the PCR+/DME+ and PCR+/DME- samples, respectively.
Conclusions: We developed a rapid, sensitive and specific real time PCR for the diagnosis and quantification of PJ in BAL samples. Correlation of our results with clinical data should now be investigated in order to establish clinical predictive values of our method.

P1768 Susceptibility profile of medically relevant *Basidiomycota* species other than *Cryptococcus neoformans*

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Objectives: We have analysed the susceptibility of several fungal species with clinical relevance belonging to the division *Basidiomycota* apart from *Cryptococcus neoformans*. As with other opportunistic fungi, the number of infections due to these species has increased steeply during the past two decades, however, little is known about their susceptibility profile.
Methods: A collection of 130 clinical isolates was tested. All strains were recovered during a period of 9 years (1995–2003) from 45 Spanish hospitals. Each clinical isolate represented an unique isolate from a patient. The susceptibility testing followed strictly the EUCAST-AFST recommendations for testing *Candida* spp. (EUCAST discussion document 7.1), but included minor modifications. Briefly, all microplates were wrapped with film sealer to prevent the medium from evaporating, attached to an electrically driven wheel inside the incubator, agitated at 350 rpm and incubated at 30°C for 48 h. *C. parapsilosis* ATCC22019 and *C. krusei* ATCC6258 were used as quality control strains.
Results: Table displays ranges and geometric means (GMs) of MICs of antifungal agents in mg/L, classified per *Basidiomycota* species:

		Ranges/ GMs				
Species	*n*	Amphotericin B	Flucytosine	Fluconazole	Itraconazole	Voriconazole
Cryptococcus laurentii	7	0.03–0.5/0.12	1.0–128/35.3	4.0–64.0/11.9	0.06–8.0/0.37	0.06–8.0/0.39
Cryptococcus albidus	6	0.06–2.0/0.50	0.25–128/12.7	4.0–64.0/17.9	0.12–1.0/0.39	0.12–4.0/0.44
Cryptococcus humicola	3	0.03–0.50/0.20	8.0–64.0/20.1	2.0–64.0/12.7	0.06–1.0/0.31	0.01–0.25/0.01
Cryptococcus curvatus	1	0.25	1.0	8.0	1.0	0.25
Rhodotorula mucilaginosa	28	0.06–8.0/0.29	0.06–128/0.47	8.0–256/78.0	0.06–16.0/2.56	0.5–8.0/3.05
Rhodotorula glutinis	4	0.03–1.0/0.09	0.25–1.0/0.42	64.0–128/90.5	0.12–16.0/1.0	0.25–2.0/0.63
Rhodotorula aurantiaca	1	0.50	128.0	8.0	0.06	0.12
Trichosporon mucoides	28	0.03–16.0/0.33	0.50–256/26.2	1.0–64.0/7.24	0.03–8.0/0.40	0.01–8.0/0.25
Trichosporon asahii	20	0.12–16.0/4.0	2.0–128/12.9	1.0–64.0/6.5	0.03–8.0/0.57	0.03–8.0/0.23
Trichosporon inkin	18	0.03–1.0/0.19	0.25–128/24.4	0.5–32.0/2.42	0.03–2.0/0.21	0.03–2.0/0.13
Trichosporon cutaneum	9	0.03–16.0/0.31	4.0–128/20.1	0.25–64.0/2.51	0.01–8.0/0.13	0.01–8.0/0.05
Trichosporon ovoides	3	0.5–16.0/3.17	8.0–128/40.3	1.0–6.4/8.0	0.12–1.0/0.25	0.03–0.25/0.06
Pseudozyma spp.	2	0.12–0.25/0.19	0.50–128/3.0	16.0–64.0/32.0	0.06–0.25/0.12	0.12–0.25/0.19

Conclusions: (i) Resistance *in vitro* to amphotericin B among *Basidiomycota* species is not common with the exception of *T. asahii* and *T. ovoides*. (ii) *Cryptococcus* spp. and *Pseudozyma* spp. exhibit high MICs of flucytosine and fluconazole (iii) *R. mucilaginosa* is resistant *in vitro* to azole agents (iv) These findings reinforced the need of continued surveillance programs that analyse species distribution and antifungal susceptibility profiles of medically important fungal isolates.

P1769 Investigation of Pal's agar to detect *in vitro* phenoloxidase activity of *Cryptococcus neoformans* strains and to differentiate *C. neoformans* from *Candida albicans*

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Objectives: *Cryptococcus neoformans* can be isolated on most routine mycologic or bacteriologic media. However its growth from the sputum and urine samples of particularly HIV-infected patients may be confused and/or obscured by *Candida albicans* on conventional media. This is due to the fact that both *C. neoformans* and *C. albicans* produce white colonies on the first days of isolation. Brown colonies of *C. neoformans* due to melanin formation on media containing phenolic compounds such as caffeic acid containing Staib's (Guizotia abyssinica) agar (SA) is typical marker for the presumptive identification of *C. neoformans*.We assessed another medium Pal's agar (PA) containing caffeic acid from *Heliantus annus* to detect phenoloxidase activity in *C. neoformans* strains and to differ *C. neoformans* colonies from those of *C. albicans*.
Methods: A total of 57 *C. neoformans* strains were used: 49 were human isolates (47 HIV+) and eight were environmental isolates. *C. neoformans* ATCC90112 and eight *C. albicans* refference strains were used for control. To detect phenoloxidase activity the strains were streaked on PA and SA plates and incubated at 30°C for 2–5 days. Colony colour was examined visually every 24 h for up to 5 days. In order to investigate the differentiation ability of PA between *C. neoformans* and *C. albicans* as a primary culture medium randomly selected 10 *C. neoformans* strains were used together with the reference *C. albicans* strains in dual combinations. SA and SDA were used along with PA in each test. Plates were inoculated by streaking preadjusted inoculum of mixed suspensions and incubated at 30°C for 2–5 days. Colony colour and count was read visually every 24 h for up to 5 days.
Results: All yeast isolates tested grew well on PA. All *C. neoformans* isolates grew typical brown colored colonies and selectively detected on both PA and SA plates after 48–96 h of incubation. Indistinguishable white colonies growth from mixed suspensions on SDA. Colony count has no significant meaning on differentiation suggesting that both PA and SA do not selectively stimulate *C. neoformans* growth but only differ the colony colour
Conclusions: PA is an excellent primary, differential medium to evidentiate *C. neoformans* isolates, easy and inexpensive to prepare from readily available seeds. Its routine use in microbiology laboratories could be very useful for easy and rapid diagnosis of *C. neoformans* infection from a wide range of patient populations including particularly HIV + individuals and HIV- at risk patients

P1770 Cryptococcosis in non-HIV infected patients: a report of 29 cases

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Objectives: To determine the patients characteristics, clinical features, and outcomes of cryptococcosis in non-HIV-infected patients.
Methods: We retrospectively reviewed the medical records of non-HIV-infected patients who had positive culture for *Cryptococcus neoformans* in Ramathibodi Hospital from 1987 to 2003.

Results: During 17 years, 33 non-HIV-infected patients with cryptococcosis were identified. Of these, 29 patients had available medical records for the study. The mean age was 46 years (range, 18–72 years) and 76% were female. Twenty patients (69%) had at least one associated underlying medical condition. The three most common underlying conditions included systemic lupus erythematosus (36%), diabetes mellitus (21%) and malignancies (16%). *C. neoformans* was recovered from cerebrospinal fluid (CSF) (41%), sputum/bronchoalveolar lavage fluid (39%) and blood (18%). Other sites of cryptococcal infection were gastrointestinal tract, bone and joints, lymph nodes, and skin and soft tissue. Eight patients (28%) had disseminated cryptococcosis. who presented as meningitis and abnormal chest radiography. Serum cryptococcal antigen was tested in 20 patients and yielded positive titres, ranging from 1:4 to more than 1:1024, in 12 patients (60%). CSF cryptococcal antigen was tested in 18 patients and yielded positive titres, ranging from 1:4 to more than 1:1024, in 15 patients. Most of patients were treated with amphotericin B and subsequent fluconazole. Two patients were initially misdiagnosed and treated as tuberculosis. Two patients were lost to follow-up. The overall mortality rate was 17%.
Conclusions: Cryptococcosis is not uncommon in non-HIV-infected patients. Cryptococcosis should be recognised as a possible cause of meningitis and pulmonary infection especially in patients with underlying immunocompromised condition(s). Cryptococcal antigen may be helpful but culture is needed for the diagnosis. Early recognition of cryptococcosis and appropriate antifungal therapy in these patients may improve clinical outcomes.

P1771 In vivo interaction of flucytosine in combination with amphotericin B against flucytosine-resistant and flucytosine-susceptible *Cryptococcus neoformans* clinical isolates

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Objectives: To evaluate the in vivo interaction of flucytosine combined with amphotericin B against one flucytosine-resistant (MIC = 64 μg/mL) and one flucytosine-susceptible (MIC = 4 μg/mL) clinical isolate of *Cryptococcus neoformans* using fungal burden as endpoint.
Methods: Mice were infected with an inoculum size of 5×106 CFU per mouse. Treatment was started 24 h postinfection and was continued for 5 days. Mice were treated with the drugs alone or in combination. Amphotericin B was given intraperitoneally (IP) at 0.25 or 0.5 mg/kg/day and flucytosine was given per os (PO) at 100 or 250 mg/kg/d. The four possible combinations were tested. Control groups receiving either water PO or glucose 5% IP were included. Each group contained five mice. On day 6 mice were sacrificed and quantitative cultures of brain, lungs and spleen were done.
Results: For the flucytosine-susceptible isolate a decrease of approximately 1 to 2 log 10 CFU/g was observed in all three organs for flucytosine monotherapy and only in the lungs and spleen for amphotericin B monotherapy. Combination of flucytosine at 250 mg/kg/day and amphotericin B at 0.5 mg/kg/day significantly decreased the fungal burden in all three organs compared with each drug alone, with the highest efficacy in the lungs. Antifungal interactions were considered to be synergistic. Against the flucytosine-resistant isolate, flucytosine alone at both concentrations was ineffective in all three organs and amphotericin B alone was ineffective in brain, poorly active in spleen (decrease of approximately 0.5 log 10 CFU/g) and active in the lungs (decrease of approximately 1.5 log 10 CFU/g). Combination of flucytosine at 250 mg/kg/day and amphotericin B at 0.5 mg/kg/day was considered to be synergistic with a significant decrease of the fungal burden in the brain and in the spleen, but not in the lungs.
Conclusions: Synergistic interaction was observed *in vivo* between amphotericin B and flucytosine, even in the brain, in both flucytosine-susceptible and -resistant isolates.

P1772 In vitro interaction of flucytosine in combination with amphotericin B against flucytosine-resistant *Cryptococcus neoformans* clinical isolates

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Objectives: To determine *in vitro* if the addition of flucytosine to amphotericin B will modify the activity of amphotericin B against flucytosine-resistant clinical isolates of *Cryptococcus neoformans* with three different techniques.
Methods: Ten flucytosine-resistant (MIC $\geq$ 32 μg/mL) clinical isolates of *C. neoformans* were used. Checkerboard studies were performed in accordance with NCCLS M-27 A2 document modified to assess antifungal interactions. Microplates were read spectrophotometrically after 72 h of incubation and interactions were interpreted by FIC indices. Time-kill studies were done in RPMI pH 7 with a starting inoculum of 1.0×10^5 CFU/mL. Flucytosine alone and in combination was tested at a concentration of 64 μg/mL and amphotericin B at 0.125, 0.25 and 0.5 μg/mL. Interactions were evaluated after 72 h of incubation. Susceptibility by Etest® was tested on RPMI-agar (pH 7) and MICs of the antifungals were determined after 72 h of incubation.
Results: MICs of the drugs alone determined by NCCLS methodology ranged from 32 to $\geq$128 μg/mL (geometric mean MIC of 120 μg/mL) for flucytosine and ranged from 0.5 to 1 μg/mL for amphotericin B. By checkerboard studies synergy was observed for 70% and additivity for 30% of the tested isolates. Antagonism was not observed. Antifungal interactions observed by time-kill studies were synergistic for four isolates, additive for one and indifferent for four isolates. It was not possible to detect synergy for any of the strains by Etest® with the chosen technical conditions.
Conclusions: Synergy between amphotericin B and flucytosine can be detected *in vitro* on flucytosine-resistant isolates of *C. neoformans*. Studies on the different mechanisms of resistance to flucytosine are warranted to determine whether it influences the type of interaction of this drug with amphotericin B.

P1773 First isolation of *Sporopachydermia cereana* in humans

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Objectives: Yeasts with identical, but unknown biochemical profile (ID 32 C, Bio Mérieux, France) were isolated from five hospitalised patients in a university hospital (blood culture, $n = 1$, tracheal secretion, $n = 4$) between 2001 and 2002.
Methods: The isolates were identified by sequencing the ITS-regions. Phenotypically, the SDS-PAGE patterns and the susceptibility to antimycotic agents were investigated, genotypically a PFGE- and RAPD-analysis was performed.
Results: Sequence analysis of the ITS 1 and ITS 2 region of the five patient's isolates revealed a homology of 93–100 % (ITS 1) and 88–95% (ITS 2) to the cactophilic species *Sporopachydermia cereana*. The identification was confirmed by the CBS, the Netherlands. In the SDS-PAGE the patient's isolates and two reference strains (CBS 6644, 6645) showed identical protein patterns. The two reference strains differed in their PFGE patterns (three and five bands, respectively, in the range of 0.9–2.5 Mb) from the patient's isolates, which showed nearly identical patterns with six bands. Analysis of the isolates in the RAPD-PCR with primers previously used to type aspergilli revealed identity of the patient's isolates, whereas the reference strains were different. All seven isolates were susceptible to amphotericin B, 5-fluorocytosine, fluconazole, itraconazole, and voriconazole, as determined by a microdilution assay and Etest.
Conclusions: This is the first description of *Sporopachydermia cereana* in humans. The source of the strains remains unknown, since no epidemiological link was found between the patients.

P1774 Outcome of fungal peritonitis in patients on continuous ambulatory peritoneal dialysis: analysis of an 11-year experience in a single centre

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Objective: Fungal peritonitis is a serious complication in patients on continuous ambulatory peritoneal dialysis (CAPD) and is associated with high rates of morbidity and mortality. We retrospectively reviewed 35 episodes of fungal peritonitis in our hospital over an 11-year period (1992–2002) in an attempt to identify risk factors and determine the clinical outcome of these patients.
Methods: Patients on CAPD with fungal peritonitis were identified from our computerised mycology laboratory database. An audit template was designed and used to collect relevant information of these patients from their medical records.
Results: There were 43 episodes of fungal peritonitis in 41 patients during this period. There were 35 episodes of fungal peritonitis in 34 patients whose medical notes could be reviewed. *C. albicans* and *C. parapsilosis* were the two predominant species isolated from peritoneal effluent in this study. 76.4% of the patients had received antibiotics in 2 months prior to the episode of fungal peritonitis. 55.8% of patients had intraperitoneal antibiotics for management of bacterial peritonitis. The attributable mortality from fungal peritonitis was 26.4% despite therapy that included early removal of the peritoneal dialysis catheter and prolonged antifungal therapy. Peritoneal dialysis catheter was removed in 82% of the patients.
Conclusions: Fungal peritonitis is a relatively uncommon complication in patients on CAPD, but it is associated with significant morbidity and mortality. Prior antibiotic use and recent bacterial peritonitis were found to be risk factors in developing fungal peritonitis. Treatment requires the administration of antifungal agents and the early removal of the peritoneal catheter. The prompt diagnosis of fungal peritonitis and the early institution of therapy are essential for better outcome in the management of fungal peritonitis in patients on CAPD.

P1775 *Fusarium fungaemia* in four Danish haematological patients

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Objective: *Fusarium* species, the majority of which are soil saprophytes or plant pathogens, cause severe disseminated infections in immunocompromised patients with granulocytopenia, often despite antimycotic treatment. We describe four cases of *F. fungaemia*, in three of which the microorganism was not initially recognised as *Fusarium*, due to lack of familiarity with the organism.
Methods and results: During a 3-year period, we diagnosed four cases of fungaemia with *Fusarium* species in Danish haematological patients. All four patients had clinical sepsis, three of four had pneumonia. Characteristically, three of the four patients presented with painful localised erythromatous cutaneous papular lesions mimicking pyoderma gangraenosum. Despite intensive antifungal treatment, three of the four patients died. Results of *in vitro* susceptibility test of relevant amtimycotic drugs using a modified NCCLS microdilution method and the E-test method showed some discrepancies especially for amphotericin B. Voriconazole seems to be a promising new agent.
Conclusions: The majority of cases of disseminated *Fusarium* infections has been reported from warm climate zones. From the Nordic countries only a single case has up till now been published. As both macroscopic and microscopic appearance of *Fusarium* species is rather characteristic and as *Fusarium* is resistant to most available antimycotic agents, we find it relevant to increase microbiologists awareness of the occurrence of disseminated fusariosis also in colder climates in severely immunocompromised patients.

P1776 The aetiologic agents of onychomycosis in Latvia

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Objectives: Onychomycosis is a global problem. From 5 to 20% of inhabitants suffer from fungal nail infections. The causative agents are classified in different groups – dermatophytes, moulds and yeasts. The dystrophic nails harbour a large amount of bacteria and secondary invading yeasts and moulds. The role of moulds and yeasts in aetiology of onychomycosis or aggravation of the condition is quite questionable. The aim of this study was to establish the main causative agents of fungal nail infection in Latvia. Before this there was no research about onychomycosis in Latvia – about the main causative agents, optimal diagnostic methods, susceptibility to the clinically used antifungal preparations.
Methods: The present research work was done at the laboratory of The State Centre of Skin and sexually transmitted diseases within last 3 years. In the research were involved 671 patients with clinically changed nails and suspicion to onychomycosis. Microscopy and cultivation methods were used (Mycoline, bioMerieux) with further identification of *Candida* spp. by Fungi fast/twin (Arbor medical).
Results: The presence of pathogenic fungi was confirmed for 511 patients (76.15%). The most often causative agent was *Trichophyton rubrum* (64%) as being the only causative agent of monoinfection and *T. rubrum* in mixed infection with *C.* spp., *Penicillium* spp.and *Aspergillus* spp. (5.65%). Other dermatophytes (*Epidermophyton floccosum*) and different species of *Trichophyton* (*T. mentagrophytes*, *T. tonsurans*) were detected (2.72%). Totally the dermatophytes as the causative agents of onychomycosis were isolated in 72.37%. The second largest group of causative agents was *Candida* spp. (17%). The moulds were isolated in the remaining 10.63%.
Conclusions: Tinea unguis most often is caused by dermatophytes – *T. rubrum* and *T. mentagrophytes*. *C. albicans* and *C. parapsilosis* are the main causative agents from yeasts. From nondermatophytes the most often isolated was *Scopulariopsis brevicaulis*.

P1777 Rapid identification of opportunistic fungi with generic PCR and pyrosequencing

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Background and objectives: The incidence of life-threatening fungal infections is rising in proportion to the increasing number of subjects at risk, including AIDS, transplant, cancer and elderly patients and premature infants. At present, identification of the most frequently encountered fungal pathogens is based on an array of different laboratory methods and often requires up to several days. Simple and rapid diagnosis of fungal infections warranting timely and efficient therapy remains a goal sought after. In this study we have used the internal transcribed spacer (ITS) regions within the ribosomal DNA gene complex as molecular targets in a generic assay for the identification of pathogenic fungi.
Methods: DNA of cultured yeast and filamentous fungi was extracted or released for subsequent amplification of the polymorphic ITS2 spacer using oligonucleotides priming at regions of ribosomal genes 5.8 S and 28 S which are universally conserved throughout the fungal kingdom. Short amplicon fragments were sequenced using pyrosequencing analysis. For species determination, direct local BLAST against our fungal ITS database was performed.
Results: Over 300 isolates and laboratory strains comprising the clinically most significant opportunistic yeast and mould pathogens were analysed. A 40-m long ITS2 sequence signature showed to provide enough discriminatory power for unambiguous identification of all species included in the study. Highly significant E-values were obtained in all BLAST searches against local and public GenBank databases. In contrast to conventional fungal

identification methods, the species identification time was the same for all organisms, nearly 6 h.
Conclusions: Rapid analysis of short ITS sequence signatures of fungi by generic PCR amplification and pyrosequencing shows considerable potential for the diagnosis of invasive fungal infections.

P1778 Cyclic peptides and depsipeptides: markers of fungal infections

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Objectives: Fungi produce cyclic peptides and depsipeptides by multifunctional synthetases. The nonribosomal biosynthesis is not specific with respect to possible amino-, hydroxy-, fatty-acids, etc. used as substrates, and so very often one can meet 'weird' residues forming the cyclopeptide molecules. Specificity of fungal synthetases thus makes it possible to use cyclic peptides as very selective markers of fungal infections useful both in human or veterinary medicinal diagnostics and even in agriculture and safety food profiling. Most of clinically important fungi strains have been described as 'small' cyclopeptide or peptide producers. These secondary metabolites can be found both in strains responsible for cutaneous infections (some *Trichophyton* species produce ferrichrome) and more importantly in systemic fungal infections (e.g. some aspergilli and *Candida* generate gliotoxin, etc.).
Methods and results: We have cultivated a series of fungal pathogens, e.g. of the genera *Aspergillus*, *Fusarium*, *Paecilomyces*, *Beauveria*, *Metarhizium*, *Alternaria*, *Emericella*, *Trichothecium* and *Pseudallescheria*. Particular attention has been dedicated to the isolation of pure spores. Crude extracts were purified by LC and HPLC. Results of this part are represented by series of novel cyclic peptides from genera: *Trichothecium*, *Metarhizium*, *Beauveria* and *Paecilomyces*. We have also described a new series of cyclic peptides from true *in vivo* isolated pathogen *Pseudallescheria*. In addition, we have developed several mass spectrometric protocols for cyclic peptide sequencing. Their application in HPLC/MS configuration can be used for sensitive detection and unambiguous cyclic peptide identification even in complex biological fluids.
Conclusions: We suggest to use cyclic peptides and depsipeptides as an extremely specific marker family for fungal infection diagnosing. Cyclic peptides have been described so far in fungi (also basidiomycetes like *Amanita phalloides*), some bacteria (e.g. *Microcystis* and *Nodularia*), plants and few marine animals only. Thus, any molecule of this category found in mammal clinical sample should indicate the corresponding infection.
Acknowledgement: Czech Science Foundation (203/02/1417 and 203/04/0799).

P1779 Identification and characterisation of clinical isolates of *Trichosporon* species from Kuwait

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Objective: Disseminated trichosporonosis is an emerging disease in immunocompromised patients particularly those with persistent granulocytopenia. This study was carried out to identify local clinical isolates of *Trichosporon* species by biochemical and molecular methods. Molecular typing was also performed for intraspecies diversity.
Methods: Twenty-nine isolates of *Trichosporon* species recovered from clinical specimens from Kuwait were first speciated by VITEK 2 yeast identification system. PCR amplification assays based on sequences within internally transcribed spacer (ITS)-1 and ITS-2 of rDNA were developed for specific detection of three *Trichosporon* spp. viz. *T. asahii*, *T. mucoides* and *T. jirovecii*. The methodology established with reference strains was then applied for species-specific amplification of genomic DNA from the clinical isolates. The results were confirmed and extended by direct DNA sequencing of ITS-1 and ITS-2 of rDNA. The typing of the isolates for intraspecies diversity was performed by randomly amplified polymorphic DNA (RAPD) analysis using two different primers.
Results: The VITEK 2 yeast identification system identified all 29 clinical isolates from Kuwait as *T. asahii*. However, using *T. asahii*-specific primers, genomic DNA of only 25 of 29 isolates was amplified, while no amplification was obtained for the remaining four isolates. The identity of *T. asahii* isolates was further confirmed by direct DNA sequencing of ITS regions of rDNA of three randomly selected isolates. The identity of the remaining four non- *T. asahii* isolates as *T. asteroids* was established by amplification of rDNA, with panfungal primers followed by sequencing of ITS-1 and ITS-2. The RAPD patterns for *T. asahii* isolates exhibited little intraspecies diversity. However, the typing patterns for the four *T. asteroids* isolates were different from the patterns obtained from the *T. asahii* isolates.
Conclusions: The currently available VITEK 2 yeast identification system does not adequately discriminate between different *Trichosporon* species. Molecular methods based on rDNA amplification and sequencing may also be needed for species-specific identification of *Trichosporon* species. Supported by Research Admn. projects grants MY 02/02 and the college of Graduate Studies, Kuwait University.

P1780 Phylogenetic positions of clinical isolates within the filamentous fungal genus *Trichoderma*

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Objectives: The imperfect filamentous fungal genus *Trichoderma* is already on the growing list of opportunistic fungal pathogens. Most of the *Trichoderma* infections are reported from patients undergoing peritoneal dialysis and from immunocompromised transplant recipients. As the rapid and accurate identification of opportunistic fungi is crucial for the choice of adequate therapeutic interventions, we studied the taxonomic positions of 12 clinical *Trichoderma* strains including 10 human pathogenic and two nasal mucus isolates.
Methods: The examined clinical isolates were originally identified based on their morphology. As the identification of *Trichoderma* strains is difficult if only morphological characters are considered, we used molecular techniques to confirm the identity of the examined clinical *Trichoderma* isolates and to study their phylogenetic positions within the genus. Sequence analysis of the internal transcribed spacer region was carried out with primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). Random amplified polymorphic DNA analysis was carried out using the OPA random primer kit of Qiagen. Isoenzyme analysis of glucose-6-phosphate-dehydrogenase, glucose-6-phosphate-isomerase, peptidase B, phosphoglucomutase and 6-phosphogluconate-dehydrogenase enzymes was performed by cellulose acetate electrophoresis.
Results: The applied molecular techniques revealed that certain *Trichoderma* strains involved in the study should be reidentified. ITS-data confirmed the identities of nine isolates, while three strains, originally described as *T. pseudokoningii* (IP 2110.92), *T. citrinoviride* (UAMH 9573) and *T. koningii* (CM 382) proved to be *T. longibrachiatum*. This was supported by data from isoenzyme analysis.
Conclusions: Our results support the hypothesis that most of the clinical *Trichoderma* isolates are restricted to section Longibrachiatum of the genus, and that previous reports about the isolation of other *Trichoderma* species from immunocompromised and peritoneally dialised patients may not have been correct. However, the identitity of the *T. harzianum* isolate involved in the study could be confirmed with molecular techniques, suggesting that strains belonging to other species of the genus may also cause infections in humans, but with a lower frequency than *T. longibrachiatum* strains. This work was financially supported by grant F037663 of the Hungarian Scientific Research Fund.

P1781 Growth of *Aspergillus fumigatus* is inhibited by *Candida* species on sealed Sabouraud agar plates, but not on sealed Columbia agar plates

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Objectives: Randhawa *et al*. (1) reported that some *Candida* species inhibit growth of *Aspergillus fumigatus* when cocultured on peptone-glucose agar (PGA), especially on sealed agar plates. *C. glabrata* and *C. albicans* proved to be the major inhibitors in this study. The authors speculate that colonisation by *Candida* species may delay a definite diagnosis of aspergillosis from sputum or other clinical specimens. To assess the possible impact of these findings for clinical diagnostics of *A. fumigatus*, we questioned the role of used media.
Methods: The experiments of Randhawa *et al*. (1) were repeated with Sabouraud dextrose agar (SDA), *Candida*-II agar (C-II), Columbia agar (CA) and CLED agar on sealed and unsealed agar plates for cocultivation of *A. fumigatus* with *C. albicans* (six strains), *C. glabrata* (one strain), *C. krusei* (three strains) and *C. tropicalis* (three strains).
Results: On SDA, growth of *A. fumigatus* was strongly inhibited by *Candida* species, especially on sealed plates. On unsealed plates, *C. glabrata* and *C. albicans* were strong inhibitors as well, whereas *C. tropicalis* and *C. krusei* were only weak inhibitors. After cocultivation with strong inhibitors for 48 to 72 h, no visible growth of *A. fumigatus* was obtained. These results were in concordance with those reported for PGA (1). There was no difference in growth inhibition between SDA and C-II. In contrast, on CA, all tested *Candida* species were weak inhibitors on sealed plates. On unsealed plates, a relevant inhibition was registered for *C. tropicalis* and *C. albicans*, whereas *C. glabrata* and *C. krusei* were only weak inhibitors. After 48 to 72 h of cocultivation, visible growth of *A. fumigatus* was obtained, regardless of the tested *Candida* strain. Growth inhibition on CLED was comparable with that on CA.
Conclusions: To avoid contamination, sealed agar plates are often used for isolation of *A. fumigatus* in clinical laboratories. When specimens that might be heavily contaminated by *Candida* species are cultured on SDA, delayed growth or growth inhibition of *Aspergillus* might be a concern. Our results suggest that this problem might be overcome by simultaneous inoculation on a glucose-poor medium, e.g. CA plates, and prolonged incubation of these plates for more than 48 h.
Reference:
1. Randhawa HS et al. (2002). *In vitro* inhibition of *Aspergillus fumigatus* by *Candida* species, especially *C. albicans* and *C. glabrata*. Current Science 82, 860–865.

P1782 Phenotyping by Fourier-transform infrared spectroscopy versus restriction- and amplification-based genotyping of *Candida tropicalis* isolates: a comparison

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Invasive candidiasis has increased dramatically over the past decades. The contribution of non-albicans *Candida* spp. including *C. tropicalis* to invasive infections is rising, yet little is known of their molecular epidemiology. Therefore, effective typing systems are required for tracing epidemiological distribution patterns. In addition to restriction endonuclease analysis of genomic DNA (REAG) followed by pulsed-field gel electrophoresis (PFGE), different amplification-based methods were studied: Arbitrarily primed (AP)-PCR under low-stringency conditions with a set of 38 different 10mer arbitrary oligonucleotides (G + C content: 30–90%), interrepeat (IR)-PCR based on micro- and minisatellite sequences and eucaryotic telomeric motifs using eight different primers. These results were compared with those obtained by phenotyping the isolates with Fourier-transform infrared spectroscopy (FTIR). For fingerprinting, 60 *C. tropicalis* reference strains and clinical isolates from different European and Asian centers were included into the study. The rank order of discriminatory ability among the genotyping methods was as follows: REAG using BssH II >> AP-PCR > IR-PCR. In contrast to other restriction enzymes used, BssH II revealed a series of distinct bands in the region from 100–300 bp to approximately 2–3 kb. Regarding AP-PCR fingerprinting, random primers with a G + C content of 50% showed the best discriminatory power if prolonged ramp times (5 min) were applied. In contrast, IR-PCR was shown to be not suitable for genotyping *C. tropicalis* isolates. Using the 'Ward' algorithm and the first derivates of the spectra, FTIR showed a main branching into two groups, which was further divided in several subgroups depending on the heterogeneity level selected. In general, FTIR was less discriminatory than REAG, but easier to perform. The classifications derived from REAG and FTIR were not completely congruent. Strains representing the same major profile but being epidemiologically unrelated appeared to be widespread in several centres included. These findings indicate that REAG followed by PFGE is the most useful molecular method for the investigation of inter-strain variation within the species *C. tropicalis*. Dependent upon primer design, AP-PCR may offer a fairly well alternative molecular fingerprinting method. In addition, the whole-organism fingerprinting by FTIR provides a tool with sufficient resolving power to distinguish isolates of this *Candida* species.

P1783 Development of a lovastatin resistance-based transformation system for *Rhizomucor miehei*

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Objectives: Various members of the class Zygomycetes are known as agents of opportunistic infections in men and animals. The thermophilic organism, *Rhizomucor miehei*, is a practically and theoretically significant member of this fungal group. The aims of the present study were to clone and characterise the 3-hydroxy-3-methylglutharyl coenzyme A reductase (HMG-CoA reductase) gene (hmg) of *R. miehei*, and to elaborate a direct selection method for the transformants based on lovastatin resistance,which does not require the usage of auxotrophic markers.
Methods: Genomic DNA library and genomic DNA used as templates in PCR reactions were derived from the wild-type *R. miehei* ATCC 46344. The genomic library screening, subcloning, extraction of DNA, sequencing and construction of plasmids were performed using the standard techniques. PEG mediated protoplast transformation was used to introduce the expression vectors into *R. miehei*. 8 μg/mL lovastatin was used as selecting agent. The transformants were analysed by PCR and Southern hybridisation.
Results: A genomic library was constructed from the chromosomal DNA of *R. miehei* and it was screened using a probe amplified by PCR with degenerated primers designed for the most conserved regions of the hmg. As a result of this analysis, a genomic insert of about 8000 bp containing the entire hmg gene was cloned into pBluescript vector and it was used for sequencing. As a result, the complete nucleotide sequence was determined and analysed. The putative protein sequence proved to be 1020 aa in length. *R. miehei* was transformed with plasmids containing the hmg gene. Transformants could be selected for lovastatin resitance. Analysis of transformants and optimisation of transformation were also done.
Conclusions: Lovastatin is a competitive inhibitor of HMG-CoA reductase and wild-type *R. miehei* cannot form colonies on plates containing 4–16 μg/mL lovastatin. We have cloned and characterized the hmg gene from *R. miehei*. This gene catalyses the conversion of HMG-CoA to mevalonate: this is the rate-limiting step of the acetate/mevalonate pathway leading to sterol and isoprene biosynthesis. Transformantions with hmg harbouring vectors resulted transformants resistant to lovastatin, because they contained hmg in high copy number. We elaborate a transformation method based on hmg gene, which could open the way for further molecular studies, such as analyses of possible virulence factors.

P1784 Identification of *Rhizomucor* species by means of biochemical and molecular methods

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Objectives: Expanding set of data demonstrate the opportunistic pathogenic role of the *Rhizomucor* species. The genus involves two species: *R. pusillus* and *R. miehei*. Their thermophhilic nature and characteristic morphological features clearly distinct them from other members of the Mucorales. Identification of *Rhizomucor* isolates at species level seems to be more problematic frequently resulting misidentifications or species names remained to be determined. The purpose of the present study was to broaden the basis of knowledge affording a methodically simple, quick and more unambiguous identification of the two *Rhizomucor* species.
Methods: Carbon source utilisation analysis of 17 *Rhizomucor* isolates using 87 different compounds. Isoenzyme analysis of 18 isolates testing five enzyme system. Random amplified polymorphic DNA (RAPD) analysis of 23 isolates with 7–10 bp oligonucleotide primers. Lovastatin inhibition tests of 30 isolates.
Results: Four carbon compounds were identified whose utilisation showed a clear difference between the *R. miehei* and *R. pusillus* isolates. These were sucrose, glycine, phenylalanine and alanine. Staining patterns of three enzyme system also showed two different electromorphs correlating perfectly with the two investigated *Rhizomucor* species. RAPD analysis revealed higher genetic variability. However the genetic variability was found to differ in *R. pusillus* and *R. miehei*: the latter revealed less-intraspecific polymorphism. As the results of highly different amplification patterns of the two *Rhizomucor* species, RAPD bands characteristic of *R. miehei* or *R. pusillus* isolates could be identified. The susceptibility of *R. miehei* and *R. pusillus* to lovastatin under different culturing conditions was investigated also. *R. pusillus* proved to be significantly more sensitive to lovastatin than *R. miehei*. Malt extract agar (pH 3.5) supplemented with 10 μg/mL lovastatin inhibit the growth of *R. pusillus* but allows the vigorous growth of *R. miehei*.
Conclusions: Besides determining easily countable characters for the identification of *Rhizomucor* isolates, the experimental data provide information concerning the genetic variability of these species. The extent of genetic polymorphism is different in the two *Rhizomucor* species: while substantial polymorphism was found among the *R. pusillus* strains, the investigated *R. miehei* strains proved to be almost homogenous. We elaborate a simple and very reliable method for species level differentiation.

P1785 *In vitro* antifungal activity, cytotoxicity and cellular accumulation of new water-soluble amphotericin B (AMB) – polyvinylpyrrolidone (PVP) formulations

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Objectives: Amphotericin B, the drug of choice for the treatment of systemic fungal infections, is characterised by (i) a negligible solubility in water and therefore a poor bioavailability for host cells and (ii) a cellular toxicity. Liposomal or colloidal formulations have been developed to palliate these drawbacks. Conjugation of the aqueous-insoluble drug to water-soluble biodegradable polymeric carriers also constitutes an attractive approach for improving AMB bioavailability. In the present work, we have investigated the in vitro antifungal activities of new water-soluble AMB-PVP complexes, and their accumulation in macrophages, in relation with their cytotoxicity.
Methods: Two new AMB–PVP complexes were synthesised as described earlier (Patent Certificate: 1003871, Greek Industrial Property Organization), namely AC2 (MW = 24 kDa), and AC5 (MW = 90 kDa). MICs and MFCs of *Candida* spp. and *Aspergillus* spp. were determined according to NCCLS guidelines. Killing activity at 2 × MIC against *Candida* was also determined. Cytotoxicity was evaluated by haemolysis of sheep red blood cells and release of LDH from macrophages. Cellular accumulation in J774 macrophages was determined after measuring AMB concentration by HPLC.
Results: MIC of AMB-PVP complexes were up to 20 times lower as compared with AMB, (with AC2 showing the lowest MICs and MFCs) against *Candida* spp. and slighty lower to equal against *Aspergillus* spp. Killing activity after 2 h exposure of *C. albicans* to AMB and AMB-PVP complexes at 2 × MIC, reached −2 log for complexes as compared with −1 log for AMB. Uptake in J774 macrophages after 24 h was three times lower for complexes than for AMB. Haemolytic activity and LDH release were approximately three times lower for complexes than for AMB.
Conclusions: AMB-PVP complexes showed improved antifungal activity when compared with AMB against *Candida* spp. and *Aspergillus* spp. They accumulate to lower levels than AMB in eucaryotic cells, which may explain their lower toxicity. These data demonstrate the potential benefits of these new formulations and suggest future applications.

P1786 Two cases of severe phototoxic reactions related to long-term outpatient voriconazole treatment

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Introduction: Voriconazole is an alternative for amphotericin B in the treatment of invasive aspergillosis. Voriconazole has a high oral bioavailability and is therefore promising for outpatient treatment. It is well tolerated, but one noncomparative study reported (mild) cutaneous side-effects in 8.6% of the patients. Case 1, a man (24 years) with chronic granulomatous disease was diagnosed with probable invasive pulmonary aspergillosis according to the EORTC/MSG definitions. Outpatient treatment with oral voriconazole (200 mg BID) was started and the patient improved. After 8 weeks, he developed profound erythema in sun-exposed areas (face, hands). Voriconazole was stopped and the erythema resolved within 2 weeks. Six weeks later the patient relapsed. Voriconazole was reinstituted. Despite protective measures, he redeveloped profound skin erythema and friability in sun-exposed areas after 4 weeks. After 6 weeks, lesions evolved to desquamation and small ulcers at the hands. After 8 weeks, the patient had large areas with bulla, desquamation and superficial ulcerations on lips, face and hands. Voriconazole was switched to itraconazole oral solution and all lesions healed within 1 week. Case 2 (man, 52 years, diabetes mellitus) underwent aortobifemoral vascular grafting. He was reoperated for thrombotic problems. Multiple cultures of the explanted graft yielded *Aspergillus fumigatus*. The vascular graft infection was complicated with acro-ileiitis and arthritis of the right knee and ankle. Amphotericin B was given for 1 month. Thereafter the patient was discharged with oral voriconazole (200 mg BID). After 2 months, asymptomatic hyperpigmentation in sun-exposed areas was noticed. After 20 weeks small vesicles appeared in sun exposed areas (face, neck, arms and legs). The vesicles evolved to bulla (diameter 1–2 cm) and ruptured after a few days. Crustae were formed and the lesions healed in 2 weeks. New lesions continued to appear while old lesions were healing. Voriconazole was switched to itraconazole oral solution and all lesions resolved within 2 weeks.
Discussion: Skin reactions, mainly rash, pruritus and phototoxic induced erythema, are known but mostly mild side-effects of voriconazole. More severe facial erythema and cheilitis were reported in five patients. We reported severe late-onset phototoxic skin reactions in two patients. Further postmarketing surveillance on the incidence and impact of these late-onset cutaneous side-effects is warranted.

P1787 Outcome and aqueous drug levels of voriconazole in four patients with fungal keratitis treated with voriconazole

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Objectives: To report on outcome and antifungal concentrations in aqueous humor and plasma of four patients with fungal keratitis treated with topical and/or oral voriconazole.
Methods and results: Voriconazole eye drops were prepared by supplementing the voriconazole lyophilisate for the i.v. administration

by 0.9% sodium chloride, yielding a 1% solution. An ophthalmic nurse administered the voriconazole eye drops daily one drop hourly. Aqueous humor of the affected eye and plasma samples were collected concurrently 1 h after the last eye drop and 12 h after oral voriconazole administration in order to obtain trough concentrations of both routes of application in both compartments. Samples were immediately frozen at −20 °C until analysis with HPLC/UV (plasma) and LC/MS (aqueous humor). Three patients with *Candida albicans* keratitis and one patient with *Aspergillus fumigatus keratitis* were treated with systemic (800 mg b.i.d. p.o) and topical (one drop of 1% i.v. solution hourly) voriconazole. After 2 days of systemical and topical administration the voriconazole concentrations in aqueous humor were 2.5–3.6 and 3.2–4.2 μg/mL in plasma. Clinical improvement was seen in all patients. After switching to topical administration of voriconazole, the concentrations in aqueous humor fell to values of 0.08–0.9μg/mL after 2–14 days and the voriconazole concentrations were undetectable in plasma (<0.2 μg/mL). Recurrence of fungal keratitis occured within 14 days in the patient suffering form keratitis due to *A. fumigatus*. After switching back to systemic voriconazole clinical improvement was again observed in this patient. The other three patients remained well with topical voriconazole.
Conclusions: Treatment with systemic voriconazole resulted in aqueous humor concentrations well above MIC for *Aspergillus* and *Candida* species, which was associated with a good response in all patients. Systemically applied voriconalzole is a good alternative with excellent response rates for fungal keratitis whereas topical voriconazole resulted in lower intraocular concentrations, and in one patient, recurrence of clinical symptoms.

P1788 Voriconazole for the treatment of drug-resistant fungal infections: experience from a Compassionate-Use Programme in Spain

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Objective: To describe the safety and efficacy of voriconazole in patients treated within the compassionate-use programme.
Methods: Retrospective study of patients (pts) received voriconazole through a compassionate-use programme in Spain for treatment of an invasive fungal infection if they were refractory to or intolerant of approved antifungal therapy. Depending on tolerability voriconazole was administered either intravenously or orally. I.v. voriconazole was administered as a loading dose of 6 mg/kg/12 h on day 1 followed by 4 mg/kg/12 h thereafter. Oral voriconazole was administered as a loading dose of 200 or 400 mg/12 h on day 1 followed by 100 or 200 mg twice a day per patients weighing <40 or >40 kg, respectively. When feasible the route of administration of voriconazole was changed from i.v. to oral. Outcome was assessed by investigators at the end of therapy or at the last visit as success (complete or partial response), stable infection, or failure, based on protocol-defined criteria.
Results: During the period of April 2003 through November 2003, 59 patients (35 males/24 females) were reviewed. The age of the patients ranged from 15–79 years. The most common underlying disease was leukaemia (22%). Nineteen patients (19%) presented neutropenia. The most common fungus isolated was *Aspergillus* spp. (47%), followed by *Candida* spp. (20%). A total of 17 fungal isolates were recovered from tissues, followed by blood and bronchoalveolar lavage. Prior to voriconazole, 60 patients received amphotericin B, 19 itraconazole, 16 fluconazole and four caspofungin. The reason for using voriconazole was failure of other antifungal drugs (69%), intolerance (11%), and both failure and intolerance (20%). The advert events reported were generally mild to moderate in severity, transient in nature, and reversible. Twenty-nine patients (78%) responded to voriconazole therapy, in four cases (4%) infection progressed.
Conclusion: Voriconazole provided high rated of cure with very good overall tolerance. These data confirmed the efficacy of voriconazole in the treatment of invasive fungal infections specially invasive aspergillosis even in patients who are intolerant of or refractory to conventional antifungal therapy.

P1789 Lack of hepatic effect of anidulafungin

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Introduction: There is a need for safe and effective antifungals. ANID is a novel echinocandin antifungal agent with demonstrated efficacy in mucosal and invasive infections due to *Candida* spp., and is currently under regulatory review. Hepatic toxicity is a common reason for failure of new drugs, and rules have been proposed to detect and evaluate the hepatic safety of new agents via monitoring hepatobiliary parameters for potential signals of toxicity.
Objectives: To evaluate the hepatic safety of ANID.
Methods: Patients (pts) with confirmed oesophageal candidiasis were enrolled in a multinational, randomised, controlled, double blind, double-dummy, phase 3 clinical trial. After obtaining informed consent, pts were randomised to receive i.v. ANID 50 mg or oral fluconazole (FLU) 100 mg, plus corresponding placebo. Pts returned daily for 14–21 days of therapy, and at follow-up (FU), 2 weeks after end-of-therapy (EOT). Hepatobiliary parameters (ALT, AST, ALP, Bili) were obtained at baseline, days 3, 7, 14, EOT (if EOT was not day 14) and FU. Thresholds for clinically significant (CS) increases from baseline were prospectively specified prior to unblinding, as were various significant combinations of changes, such as ALT >3× the upper limit of normal (ULN) plus bili >1.5× ULN.
Results: A total of 601 pts, most with AIDS, were enrolled. Most (98%) took one or more nonantifungal medication concomitantly. The number and percentage of pts with CS increases are shown in the table for ALT. Similar results were obtained for the other hepatic values noted above.

Table: Patients with CS* ALT increases from baseline

ALT	ANID n/N (%)	FLU n/N (%)
Day 3	1/265 (0.4)	0/268
Day 7	2/258 (0.8)	3/260 (1.2)
Day 14	2/237 (0.8)	8/243 (3.3)
EOT	0/43	1/37 (2.7)
FU	2/240 (0.8)	5/240 (2.1)

*CS = 3 fold increase from baseline, and at least 3× ULN

There was also no difference between the arms when various prospectively defined combinations of values were considered.
Conclusions: In this immunocompromised population of pts on multiple medications, no signal of hepatic effects of anidulafungin was apparent.

P1790 *Ex vivo* combined activity of posaconazole and granulocyte-macrophage colony-stimulating factor-treated neutrophils against *Scedosporium prolificans*

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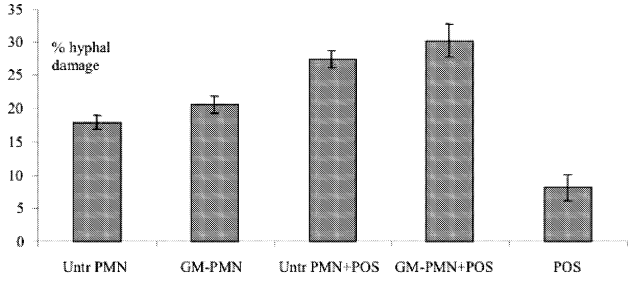
Objectives: *Scedosporium prolificans* causes therapy-refractory invasive infections with high mortality in immunocompromised patients. We tested the combined effect of posaconazole (POS), an investigational antifungal triazole, and granulocyte-macrophage colony-stimulating factor (GM-CSF) on ex vivo activity of neutrophils (PMNs) against *S. prolificans*.
Methods: Balb/c mice were treated with 50–250 ng of murine GM-CSF or saline sc daily for 4 days. PMNs, isolated from GM-CSF- or saline-treated mice, were incubated with POS (1μg/mL) and hyphae at effector cell: target ratio 5:1. Per cent damage of *S. prolificans* hyphae was assessed by XTT metabolic assay. ANOVA with Tukey test were used for multiple comparisons.

Results: PMNs from GM-CSF (100 ng)-treated mice did not induce significantly increased damage of *S. prolificans* hyphae *ex vivo* compared with untreated controls ($n = 8$, $P = 0.15$). POS (eight studies) had synergistic effects when combined *ex vivo* with either untreated ($P < 0.01$) or GM-CSF-treated PMNs ($P < 0.01$) on per cent hyphal damage.



Conclusions: POS exerts synergistic activity in combination with PMNs, especially from GM-CSF-treated mice, against *S. prolificans* hyphae. Combined activity of POS and phagocytes may play an important role in the management of invasive *S. prolificans* infection.

P1791 Posaconazole as salvage therapy for *Pseudallescheria* infections: a case series

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Objectives: *Pseudallescheria boydii* (*Scedosporium apiospermum*) is a virulent fungal pathogen capable of infecting immunocompromised and immunocompetent patients. Immunocompetent persons typically acquire this disease after penetrating trauma or near-drowning in polluted water. *Pseudallescheria* infections are challenging to manage since the organism is relatively resistant to amphotericin B. In some reports, mortality associated with disseminated or central nervous system disease approaches 100%. Posaconazole (POS) is an investigational, broad-spectrum agent for the treatment of invasive fungal infections. We report the success of POS salvage therapy in the treatment of *Pseudallescheria* infections.
Methods: POS activity was evaluated in a large open-label, multicentre clinical trial conducted in patients intolerant of or with disease refractory to other antifungal therapy, of whom seven had *Pseudallescheria* infections. Patients received POS 800 mg/day in divided doses. Complete or partial response was considered a success; stable or persistent disease was considered a failure.
Results: Six patients had underlying disease, including haematologic malignancy (three), HIV infection (one), diabetes (two), solid organ or bone marrow transplantation (two) and graft vs.host disease (one); One patient had no identifiable underlying disease. POS therapy produced successful outcomes in three of seven (42%) patients. Sites of infection included lung, brain, bone, sinus, joints and skin. Cumulative duration of prior therapy with itraconazole, ketoconazole, or amphotericin B ranged from 1 month to 3 years. POS treatment duration ranged from 7 days to >1 year and was well tolerated. Importantly, POS demonstrated activity in bone and in the central nervous system. Additionally, two patients who were considered treatment failures had stable disease; one of these two patients responded after a second course of therapy.
Conclusions: POS resulted in successful outcomes in three of seven patients in whom other antifungal therapies had failed. POS was well-tolerated without any significant adverse events even with prolonged administration (>1 year). These cases suggest that POS is useful for the treatment of *Pseudallescheria* infections, for which there are few therapeutic options.

Progress in pharmacokinetics and pharmacodynamics - II

P1792 Serum protein binding of erythromycin as measured by ultrafiltration, dialysis filtration and ultracentrifugation

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Objectives: The protein-free, unbound fraction of antibiotic concentration is used for PK/PD calculations. Reliable and easy measurements of protein binding are therefore needed. Three methods are usually recommended: ultrafitration (F), dialysis (D) or ultracentrifugation (C), but we have not seen the methods compared simultaneously for any antibiotic. We studied the three methods for estimation of serum protein binding of erythromycin.
Methods: The same portion of pooled human serum was used for all experiments. Three concentrations of erythromycin (3, 1.5 and 1 mg/L, respectively), were subjected to the three protein binding methods. Erythromycin concentrations of serum or filtrate/dialysate/supernatant were measured by bioassay, with serum or PBS for standards. Protein binding was calculated from the formula (erythromycin concentrations): Serum–filtrate (dialysate, supernatant)/Serum, in per cent, corrected for binding of serum-free controls.

F: Amicon UF-vials, YM30 filter, were added one ml serum and centrifuged in a fixed-angle rotor Sorval RC-50 centrifuge at 3000 *g* for 8 min. This resulted in approx. 0.2 mL ultrafiltrate. D: Dialysis tubes (Spectra/por, MWCO 10.000) in pieces of 20 cm, were tied in both ends after adding 2 mL serum. With tubings placed in U-form in plastic tubes, these were centrifuged in a free angle rotor centrifuge at 339 *g* for 5 min, excess water was removed, and tubes centrifuged again at 339 *g* for 40 min. This resulted in approximately 0.12 mL dialysate. C: A Beckman ultracentrifuge type L-80, type 80 Ti rotor, was used. Beckman Ultra-Clear Centrifuge Tubes were filled with 13.4 mL serum samples and centrifuged at 175.000 *g* for 8 h at 30°C. The 0.5 mL supernatant was removed with a pipette.
Results: For the three samples with 3, 1.5 and 1 mg/L erythromycin the following serum protein binding was found (in %): F: 63, 65 and 65. D: 58, 70 and 72. C: 54, 58 and 60, respectively.
Conclusions: Within the variation of the methods we found slight concentration related binding, but little differences between methods. The results are comparable with published data of 41–74%. The ultrafiltration method was by far the easiest and least time consuming.

P1793 Pharmacokinetics of moxifloxacin in patients undergoing continuous ambulatory peritoneal dialysis

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Objectives: Moxifloxacin is a novel 8-methoxy-fluoroquinolone with high oral availability and considerable in vitro activity on Gram-positive cocci. The impact of end-stage chronic renal failure on pharmacokinetics of moxifloxacin was evaluated in four anuric patients on continuous ambulatory peritoneal dialysis (CAPD). This group of individuals is at high risk of presenting with infections by Gram-positive cocci, especially spontaneous bacterial peritonitis.

Methods: A single 400 mg dose was administered orally followed by blood and peritoneal fluid sampling in 0.5, 1, 2, 3, 4, 6, 12, 18 and 24 hours afterwards. Concentrations of moxifloxacin were estimated by a microbiological assay. Vital signs were observed and laboratory tests and ECG were performed every 24 h.
Results: Mean ± SD of moxifloxacin in serum 0.5, 1, 2, 3, 4, 6, 12, 18 and 24 h after oral intake were 2.48 ± 3.01, 4.91 ± 0.71, 4.11 ± 0.81, 3.80 ± 1.29, 3.05 ± 1.05, 2.44 ± 0.66, 1.52 ± 0.39, 1.12 ± 0.54 and 0.90 ± 0.28 μg/mL, respectively. Mean ± SD of moxifloxacin in peritoneal fluid 6, 12, 18 and 24 h after oral intake were 2.30 ± 0.60, 1.09 ± 0.44, 0.93 ± 0.33 and 0.78 ± 0.08 μg/mL, respectively. No adverse reactions were observed.
Conclusions: Oral intake of moxifloxacin in anuric patients on CAPD is accompanied by adequate penetration in the peritoneal cavity for 12 h after intake so as to be a promising agent for the therapy of spontaneous peritonitis.

P1794 An oral regimen to achieve steady serum levels of levofloxacin in patients undergoing haemofiltration

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Objectives: To evaluate the pharmacokinetics of levofloxacin in anuric patients undergoing chronic haemofiltration and to establish an oral regimen delivering therapeutic trough serum levels.
Methods: In the first phase of the study, 10 anuric patients were given a single oral dose of 500 mg of levofloxacin. Patients underwent haemofiltration after 2 h. In the second phase after 21 days, the same patients received 500 mg daily for 3 days consecutively. In that phase, haemofiltration was performed on days 1, 3 and 5. In both phases blood was sampled at regular time intervals. Concentrations of levofloxacin were estimated by a microbiological assay.
Results: In the first phase, estimated mean (±SE) concentrations were 0.97 ± 0.45, 2.67 ± 1.09, 3.37 ± 0.95, 4.22 ± 1.14, 7.41 ± 1.72, 4.25 ± 1.65 and 3.10 ± 2.23 mg/L at 0.25, 0.5, 0.75, 1.5, 24 and 48 h after intake, respectively. In that phase, estimated mean (±SE) respective concentrations at the arterial and the venous end of the fistula were 5.54 ± 1.18 and 3.08 ± 0.26 mg/L ($P = 0.014$) on the first hour of haemofiltration; 4.17 ± 0.87 and 2.50 ± 0.33 mg/L (*P*: NS) on the second hour of haemofiltration; and 3.90 ± 0.62 and 2.10 ± 0.29 mg/L ($P = 0.018$) on the fourth hour of haemofiltration. In the second phase, estimated mean (±SE) concentrations on day 1 after haemofiltration, on day 2, on day 3 before haemofiltration, on day 3 after haemofiltration, on day 4, on day 5 before haemofiltration and on day 5 after haemofiltration were 3.18 ± 0.34, 2.55 ± 0.34, 6.54 ± 0.73, 7.30 ± 0.95, 6.84 ± 0.86, 5.77 ± 0.76 and 2.97 ± 0.65 mg/L, respectively.
Conclusions: Levofloxacin is rapidly eliminated during haemofiltration. Oral administration at the proposed regimen of 500 mg daily for 3 days results in adequate therapeutic though serum levels for 5 days allowing its application in case of infections by Gram-positive cocci.

P1795 Multiple-dose pharmacokinetics of linezolid during continuous venovenous haemofiltration

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Objectives: Linezolid is a potent antimicrobial agent with a broad spectrum of activity against Gram-positive pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococcus (VRE) and penicillin-resistant *Streptococcus pneumoniae*. The aim of this study was to determine the pharmacokinetic characteristics of linezolid in critically ill patients undergoing continuous venovenous haemofiltration (CVVH).
Methods: A total of 20 patients was included. All patients received 600 mg linezolid intravenously every 12 h. CVVH was performed using highly permeable polysulphone membranes (PSHF 1200, Baxter, Germany and AV 400, Fresenius, Germany). Mean blood flow rate and ultrafiltration rate were 182 ± 15 mL/min and 40 ± 8 mL/min, respectively. Postdilution was performed. Linezolid concentrations in serum and ultrafiltrate were determined by high-performance liquid chromatography (HPLC).
Results: The mean linezolid serum concentration peak (Cmax) was 15.32 ± 3.98 μg/mL, the mean trough level (Cmin) was 1.87 ± 1.70 μg/mL. The elimination half-life (T1/2) was 4.30 ± 1.74 h. The total clearance (CLtot), haemofiltration clearance (CLhf) and volume of distribution (Vd) were 9.31 ± 3.48 L/h, 31.25 ± 12.77 mL/min and 51.30 ± 12.30 L, respectively.
Conclusions: Our results show that patients with severe Gram-positive infections undergoing CVVH can be treated effectively with a dosis of 600 mg linezolid every 12 h.

P1796 The breakpoint index – a new pharmacodynamic parameter for assessing antibiotic combinations

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Objectives: The standard method of assessing antibiotic combinations is the measurement of fractional inhibitory concentration indices (FICIs). The FIC however, gives no indication of the breakpoints (BPs) of the antibiotics. With the well established role of the MIC and BP in determining outcome of antibiotic therapy, we have developed a new measurement of antibiotic combinations, the breakpoint index (BPI), which takes into account both the minimum inhibitory concentrations (MICs) and BPs of antibiotics in combination.
Methods: FICIs for antibiotic combinations against Gram-negative isolates from cystic fibrosis patients, as measured by Etest, were compared against the MICs alone and in combination, expressed as a ratio to the relevant antibiotic BPs. The BPI = [(BP antibiotic 1/combination MIC of antibiotic 1) + (BP antibiotic 2/combination MIC of antibiotic 2)].
Results: A total of 120 strains of *Pseudomonas aeruginosa* (PA) were tested against 38 different antibiotic combinations, 69 strains of *Burkholderia cepacia* (BC) against 60 combinations and 27 strains of *Stenotrophomonas maltophilia* (SM) against 37 combinations. The number of organism/antibiotic combinations tested for PA was 740, for BC was 462 and for SM was 172. The BPI bore no relationship to the FICI ($r^2 = 6.9$ to -0.4). High BPIs were present in combination with low FICIs (synergistic combinations) and those with high FICIs (antagonistic combinations). The mean BPIs (±SD) for the synergistic combinations (FICI ≤ 0.5) for PA, BC and SM were 55.3 (±102), 25.5 (±55) and 29.1 (±41.1), respectively. The mean BPIs (±SD) for the additive combinations (FICI > 0.5 and ≤1.0) for PA, BC and SM were 8.7 (±132), 17.9 (±25.1) and 44.5 (±43.6) respectively. The mean BPIs (±SD) for the indifferent combinations (FICI 1.0 and ≤2.0) for PA, BC and SM were 68.1 (±112), 25.1 (±38.2) and 67.1 (±69.4), respectively. The mean BPIs for the antagonistic combinations of PA, BC and SM were 20 (±−20.6), 15.5 (±22.1) and 53.5 (±60.2), respectively.
Conclusions: The higher the BPI the greater the difference between the tissue levels and the MIC ensuring maximum clinical effect. It is apparent that the FICI can give quite misleading data, underestimating the pharmacodynamic benefits of additive and indifferent antibiotic combinations.

P1797 Influence of protein binding on the activity of β-lactam antibiotics on *Haemophilus influenzae*

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Objectives: Antibiotics are known to bind to plasma proteins, mainly to albumin. This leads to a change in the PK of the antibi-

otic and thus to a change in its pharmacology. It is supposed that protein binding (PB) only has to be taken into consideration if it exceeds 80% and that only the free fraction of an antibiotic is active. To examine these hypotheses, we performed kill kinetics of *H. influenzae* with four β-lactam antibiotics with diverse PB (meropenem (M) PB 2%, cefotaxim (CF) PB 40%, cefoxitin (CX) PB 70% and faropenem (F) PB 94%).

Methods: Kill kinetics of *H. influenzae* (MIC M: 0.06, CF: 0.06, CX: 4 and F: 0.5 mg/L) were carried out in batch cultures over 6 h with constant concentrations starting with 0.5 up to 128 times the MIC. Three types of kill kinetics were performed (i) without human albumin, (ii) with the addition of 40 g/L albumin and (iii) a dose resembling the free fraction. Kill kinetics of *H. influenzae* were performed in *in vitro* models over 24 h (M: 500 mg i.v., CF/CX: 2 g i.v., F: 300 mg p.o.). Again, albumin was added in different ways [s.a.(i) to (iii)]. The AAC values (area above the curve, as an indicator for the effect) were calculated. E-max and EC50 values were determined with the computer program GraFit®.

Results: Results derived from the batch cultures show that for M, CF and CX (PB <80%) the addition of albumin does not change the activity of the drugs. This is also seen in similar EC50 values. For CF and CX the simulation of only the free fraction slightly reduces the effect of the antibiotic at lower concentrations. For F the addition of albumin leads to a reduced effect and therefore to a higher EC50 value. However, this reduction is not as pronounced as in the experiment when only the free concentration is simulated (table). Above a concentration of 32-fold MIC, when E-max is obtained, the protein binding does not effect the activity of F at all.

Data derived from the in vitro models show for M, CF and CX that the addition of albumin does not cause a reduction of antibacterial activity. When adding albumin to F a marked reduction of the effect is observed. Moreover, the simulation of the free fraction of CX and F leads to a (further) reduced effect.

Table: EC50 and E_{max} values for faropenem against *H. influenza* in batch culture for the three different types of simulation

Type of simulation	EC50	E_{max}
(i) without albumin	1.06	17.07
(ii) with 4% albumin	9.88	17.56
(iii) free fraction (6%)	20.89	17.74

Conclusion: PB influences the activity of an antibiotic only in a narrow concentration range between no effect and the maximal effect. For drugs bound below 80%, an influence could not be observed. However, the hypothesis that only the free fraction is active could not be confirmed.

P1798 PK/ PD indices: good predictors for highly protein bound drugs?

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Objectives: Many authors use PK/PD indices to predict the efficacy of antibiotics. According to the International Society of Antiinfective Pharmacology (ISAP) these indices should be referred to the nonprotein bound fraction of the drug, as only the free fraction is active. This hypothesis was examined, as it is possible that not just the free fraction is active. Therefore, we performed kill kinetics with three different strains with faropenem, a highly protein bound antibiotic (94% protein binding).

Methods: Kill kinetics of *S. aureus* (MIC 0.125 mg/L), *H. influenzae* (MIC 0.5 mg/L) and *S. pneumoniae* (0.25 mg/L) were performed in a pharmacological in vitro model with faropenem (F). Three dosing schemes were simulated:

(i) 300 mg dose without human serum albumin (Cmax: 11.8 mg/L; AUC12: 28.125 mg h/L);

(ii) 300 mg dose with 40 g/L albumin(free Cmax: 0.708 mg/L, free AUC12: 1.7 mg h/L);

(iii) a dosing scheme resembling the free fraction (6% of 300 mg; Cmax: 0.708 mg/L, AUC12: 1.7 mg h/L). The PK/PD indices Cmax/MIC, AUC/MIC and T > MIC and the PD parameter AAC (area above the curve, as an indicator for the effect of F) were calculated.

Results: The table shows the calculated PK/PD indices based on the free fraction and the AAC values. If just the free fraction would be active, there should not be any difference between the AAC values of simulation (ii) and (iii). However, for *S. pneumoniae* and *S. aureus* the effects of (i) and (ii) are similar, whereas the effect of (iii) is much lower. For *H. influenzae*, whose MIC is in the range of the free Cmax, there is a difference between the effect of (i) and (ii). Again, the effect of (iii) is lower. These differences are not reflected in the PK/ PD indices, as they are the same for (ii) and (iii).

Table: PD parameters and PK/PD indices for faropenem and *S. aureus*, *S. pneumoniae* and *H. influenzae*

		Pharmacodynamic (PD) parameters		PK/PD indices		
Tested strain:	Simulated dosing scheme of faropenem:	MIC (mg/L)	Effect (AAC_{12}) (Delta cfu*h)	AUC_{12}/ MIC (h)	Cmax/ MIC	T>MIC (h)
S. aureus	(i) 300 mg without albumin	0,125	12,81	225	94,4	8
	(ii) 300 mg with 4% albumin		13,98	13,6	5,7	4
	(iii) 6% of 300 mg (free fraction)		3,65	13,6	5,7	4
S. pneumoniae	(i) 300 mg without albumin	0,25	26,33	112,5	47,2	7,5
	(ii) 300 mg with 4% albumin		20,95	6,8	2,8	2,5
	(iii) 6% of 300 mg (free fraction)		−0,83	6,8	2,8	2,5
H. influenzae	(i) 300 mg without albumin	0,5	11,29	56,25	23,6	6,5
	(ii) 300 mg with 4% albumin		−6,11	3,4	1,4	0
	(iii) 6% of 300 mg (free fraction)		−13,93	3,4	1,4	0

Conclusion: Not only the free fraction of F is active, the addition of albumin leads to a higher effect. Calculating the indices AUC/MIC, Cmax/MIC and T>MIC based on the free fraction does not reflect the actual conditions.

P1799 A new pharmacokinetic/pharmacodynamic model to characterise the antibacterial effect of antibiotics against *Escherichia coli*

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Objectives: The aim of the study was to develop a PK/PD model for the prediction of the effect of antibiotics on *Escherichia coli*. Therefore the long-term effect of constant ciprofloxacin (CIP) concentrations against *E. coli* was examined. Based on these experimental data, the model was developed and applied for the calculation of the effect of moxifloxacin (MOX), tobramycin (TOB) and ampicillin (AMP) on *E. coli*.

Methods: The efficacy of various constant CIP, MOX, TOB and AMP (0.5- to 32-fold MIC) concentrations against *E. coli* [MICCIP: 0.03125 mg/L, MICMOX: 0.0625 mg/L, MICTOB: 1 mg/L, MICAMP: 2 mg/L] was studied in batch cultures over 24 h. The experimental kill curves were used to develop the PK/PD model. The simulation and fitting of the model was performed with the computer program MatLab®.

Results: The antibacterial activity of CIP against *E. coli* showed an increasing effect of CIP up to 16-fold MIC. This or higher concentrations could not effect a total killing of the cultures as some bacteria persisted despite the presence of CIP. These data were fitted

to the following PK/PD model: The model assumes that the bacterial population (Ntotal) is heterogenous, consting of the subpopulations N1 and N2. N2 are persisting cells that cannot be killed by the antibiotic. The kill rate of N1 depends on the antibiotic concentration C and incorporates the Emax model. With time, N1 adapts to the antibiotic according to e-h·t. The growth rates of both N1 and N2 depend on the antibiotic concentration. The growth of the bacterial culture cannot exceed Nmax, the maximal density of bacteria in the culture (=logistic growth). Fitting the effect of CIP on *E. coli* resulted in a set of parameters, which allowed kill curve fitting of *E. coli* after the influence of MOX, TOB and AMP.

Conclusions: Based on the experimentally determined kill curves the model allows excellent curve fits of the activity of CIP. Furthermore, the variation of the parameters in the model enables the prediction of the antibacterial activity of MOX, TOB and AMP on *E. coli*. Thus, the new PK/PD model enables the prediction of the antibacterial effect of constant concentrations of various antibiotics on *E. coli*.

$$\frac{dN_1}{dt} = \left(\mu_{\max 1} \cdot e^{a_1 \cdot C} \cdot \left(1 - \frac{N_{total}}{N_{max}}\right) - \left(\frac{E_{\max} \cdot C \cdot e^{-h \cdot t}}{EC_{50} + C \cdot e^{-h \cdot t}}\right)\right) \cdot N_1$$

$$\frac{dN_2}{dt} = \left(\mu_{\max 2} \cdot e^{a_2 \cdot C} \cdot \left(1 - \frac{N_{total}}{N_{\max}}\right)\right) \cdot N_2$$

$$N_{total} = N_1 + N_2$$

P1800 Interaction of bacterial inoculum, strain mic and drug formulation on the antibacterial effect of co-amoxiclav on penicillin-resistant *S. pneumoniae*

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Objectives: Previous data has shown using *S. pneumoniae* (SP) strains with raised Co-a MICs that bacterial inoculum and strain MIC may impact significantly on the antibacterial effect measures (ABE). However, none of these data had been analysed collectively. The aim of this study was to perform ANOVA on the combined dataset to establish which of these factors were significant in determining the ABE for SP strains with raised Co-a MICs

Methods: An in vitro pharmacokinetic model was used to simulate pharmacokinetically enhanced (PE) [2000 mg amoxicillin (AMOX); 125 mg clavulanate] and standard formulation (SF) (875 mg AMOX:125 clav) co-amoxiclav (Co-a), against six SP strains with raised Co-a MICs (3–8 mg/L). The T > MIC for PE Co-a was 39–60% and SF 20–41%. All simulations were performed in triplicate using Brain Heart Infusion broth for 24 h. The initial bacterial inoculum was either 106 or 108 CFU/mL. ANOVA was used to exploit the factorial structure of the data to analyse the effect of MIC, drug formulation and initial bacterial inoculum on the ABE measure – the area under the bacterial kill curve (AUBKC).

Results: All three factors (inoculum, MIC and formulation) affected ABE as measured by ln AUBKC24. Significant interactions were noted between formulation and inoculum and formulation and MIC suggesting, formulation and MIC had different effects on ln (AUBKC24). For both inocula the ln AUBKC24 increased with increasing MIC, however the mean ln AUBKC24 increase for the standard formulation was greater than for PE Co-a. There was no interaction between inoculum and MIC.

Conclusions: PE Co-a is more likely to have a T > MIC of >40% against resistant SP than SF especially for strains with Co-a MIC >4 mg/L. These data confirm that MIC impacts upon on Co-a ABE and that drug formulations that have the greatest T>MIC are more effective.

P1801 High frequency of adverse effects to antibiotics in cystic fibrosis patients and fast protocol of intravenous desensitization

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Objective: Cystic fibrosis (CF) is a multisystem disease due to a defective gene on chromosome 7 which codifies for an altered cystic fibrosis transmembrane regulator protein. Median survival is improved thanks to aggressive treatments which include higher doses of antibiotics infused for 14–21 days. CF patients experience adverse effects (AE) from antibiotics more frequently than does the general population. We describe AE patterns in patients attending our CF Centre and the protocol for rapid intravenous (IV) antibiotic desensitisation employed when no alternative treatment is available.

Methods: Between January 1 1993 and December 31 2000, 71 of 145 patients (48.9%) were treated with IV antibiotic (cephalosporins, carbapenems, glycopeptides, fluoroquinolones in association with aminoglycosides). The CF patients' airways were colonised by: *Staphylococcus aureus* (*n*: 99, 68.2%), *Pseudomonas aeruginosa* (*n* 60, 41.3%), *Alcaligenes xylosoxidans* (*n*: 8, 5.5%), methicillin-resistant *S. aureus* (*n*: 8, 5.5%), *Burkolderia cepacia* (*n*: 6, 4.1%), *S. maltophilia*, (*n*: 2, 1.3%). Since some patients were colonised by more than one bacteria, the absolute number is higher than 71 and the overall proportion is over 100%. In patients with AE and no alternative antibiotic treatment, prick and intradermal skin tests with the native drug were carried out. Desensitisation begins by administering IV a 10-fold lower concentration of antibiotic than the concentration negative to the intradermal test. The doses are subsequently infused using a 10-min washout between doses. Each dose is increased from 2 up to 10 times until the therapeutic dose is reached and it takes about 4–5 h.

Results: Sixteen of 71 (22.5%) presented AE: 9 (56.2%) to ceftazidime, two to carbapenems, three to vancomycin, one to aztreonam, one to ciprofloxacin (Table 1). Eleven patients experienced AE after more than two courses with the same antibiotic. Desensitisation was carried out in nine patients in whom no alternative antibiotics therapy was available and was well tolerated in seven cases (Table 2).

Table 1. Adverse effects occuring in CF patients after intravenous antibiotic administration

Pt	adverse effect	i.v. antibiotic	occur AE after antibiotic courses (n°)	prick tests	intradermal tests	desensitization
1	urticaria	imipenem	2	negative (25 mg/ml)	negative (1 mg/ml)	no reaction
2	urticaria	ceftadizime	11	negative (50 mg/ml)	negative (1 mg/ml)	mild hand rash
3	angioedema	meropenem	1	=	=	=
4	urticaria	aztreonam	2	=	=	=
5	urticaria	ceftazidime	1	negative (50 mg/ml)	negative (1 mg/ml)	no reaction
6	angioedema	ceftadizime	6	=	=	=
7	urticaria	ceftazidime	4	negative (200 mg/ml)	negative (1 mg/ml)	no reaction
8	red men syndrome	vancomycin	1	negative (100 mg/ml)	negative (0.1 mg/ml)	mild urticaria
9	urticaria	ceftazidime		=	=	=
10	urticaria	vancomycin	2	=	negative (0.1 mg/ml)	no reaction
11	urticaria	vancomycin	2	positive (50 mg/ml)	positive (0.1 mg/ml)	no reaction
12	urticaria	ceftazidime	6	negative (250 mg/ml)	positive (1 mg/ml)	no reaction
13	urticaria angioedema	ceftazidime	6	negative (350 mg/ml)	negative (35 mg/ml)	no reaction
14	anaphylaxis	ceftazidime	3	negative (250 mg/ml)	positive (1 mg/ml)	=
15	urticaria	ceftazidime	2	=	=	=
16	urticaria	ciprofloxacin	1	=	=	=

Table 2. Ceftazidime desensitization schedule for CF patients

step	antibiotic concentration (mg/mL)	dose infused (mg)	time/vol infuse (min/mL sodium chloride 0.9%)	cumulative dose (mg)
1	0.0001*	0.005	20/50	0.005
2	0.001*	0.05	20/50	0.055
3	0.01*	0.5	20/50	0.555
4	0.1	5	20/50	5.555
5	1	50	20/50	55.555
6	5	250	20/50	305.555
7	10	500	20/50	805.555
8	20	1000	20/50	1805.555
9	22.5	2250	40/100	4055.555

*According to intradermal test

Conclusion: High dosage, long treatments, frequent infusions, and possibly altered pharmacokinetics could explain the high frequency of AE to antibiotics in CF patients. When no alternative treatment strategy is possible, desensitisation is helpful. This regimen is fast and allows the large majority of patients to tolerate essential antibiotic treatment.

P1802 Pharmacokinetics of gentacoll investigated by in vivo microdialysis

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Objectives: Antimicrobial agents exert their effect inside the interstitial space, which is the site of many infections. Recently, microdialysis was applied to cortical and cancellous bone for the evaluation of gentamicin. The principle of microdialysis is to introduce a semipermeable membrane into bone and perfuse it with liquid, thus enabling dynamic measurements to be made. The aim of this investigation was to measure pharmacokinetics of a Gentacoll sponge in bone tissue by the technique of microdialysis.
Methods: Nine pigs were randomised to either wet or dry application of a Gentacoll sponge (10 × 10 cm) into the bone marrow of tibia. Two catheters were inserted into cancellous bone tissue, one 1 cm (MD1 cm) and one 2 cm (MD2cm) apart from the aimed location of the sponge. Then, the Gentacoll sponge was implanted. Wet application was defined as; the sponge was wetted in 2 mL. blood. Dry application was defined as usual surgical procedure. Concentrations of gentamicin were measured in serum and microdialysates on an Abbott Drug Analyser. Data presented are median (range). A rank sum test was performed for statistical analysis. A *P*-value below 0.05 was considered significant. The pharmacokinetic measure presented is the area under the curve (AUC6h) and peak concentration (C peak).
Results: The AUC6h, serum wet was 92 (72–129) and AUC 6 h, serum dry was 196 (142–626) mg min/L ($P = 0.02$). The AUC6h, wet-group was12.631 mg min/L (5.155–152.855) and AUC6h, dry-group was 19.869 mg min/L (11.336–87.209) ($P = 0.45$). The Cpeak, wet-group was 120 mg/L (33–585) and Cpeak, dry-group 178 mg/L(59–1294), ($P = 0.31$). The overall ($n = 9$) AUCMD1 cm was 24431 mg min/L (5.155–152.855) and similar the AUCMD 2 cm 13759 mg min/L (6.351–74.573) ($P = 0.25$). The Cpeak, MD 1 cm was 209 (33–1294) and Cpeak, MD 2 cm was 106 (41–354) ($P = 0.21$).
Conclusions: This is the first study applying microdialysis for pharmacokinetic measurements of local implants. The distribution of local applied antibiotics into bone tissue is difficult to measure. The small sample size precludes a detailed analysis, but it is noteworthy that previous found variation on the distribution of gentamicin from a Gentacoll sponge is reproduced in this work. It seems that neither application nor distance had significant impact on the initial pharmacokinetic of Gentacoll in bone tissue.

P1803 Pharmacodynamics of levofloxacin (usual and high dose) and moxifloxacin vs. *Streptococcus pneumoniae* from the SENTRY antimicrobial surveillance programme (2002–2003) in elderly patients using Monte Carlo Analysis and population pharmacokinetics

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Objectives: *Streptococcus pneumoniae* (SP) is a leading cause of community-acquired infections worldwide. Decreased susceptibility to fluoroquinolones (FQ) has increased in parallel with FQ utility since 1997 and is more common in elderly vs. younger patients (Chen, 1999). Recent studies have correlated microbiologic outcomes with FQ pharmacokinetic-pharmacodynamic (PK-PD) target attainments (fAUC:MIC (0–24) ≥30) against SP (Ambrose, 2001). Monte Carlo (MC) analyses comparing FQ target attainment rates vs. SP have generally been conducted in the general population; however, none have focused on the group at highest risk for FQ SP resistance, the elderly. To evaluate and compare PK-PD target attainment rates for intravenous (IV) levofloxacin(L), including the high dose formulation (L750), and for IV moxifloxacin (M) against clinical isolates from elderly patients from the SENTRY Program in North America (2002–2003).
Methods: Mean parameter estimates and measures of dispersion derived from patient population PK models that included elderly patients were used to perform a 5000 patient MC simulation to determine the probability of PK-PD target attainment (based on free drug) for fAUC: MIC(0–24) ratios ≥30 for the three regimens. MIC distributions were obtained from clinical isolates from patients ≥65 years of age. AUC(0–24) values from population PK models for L500, L750, and M were mean (±SD): L500, 50.8 (35.8); L750, 76.2 (53.7); M, 15.1 (5.53) mg/mL × h. MIC data were characterised as follows: (L, MIC50/90: 1.0/1.0, range ≤0.03 to >4); M, MIC50/90: 0.125/0.25, range ≤0.03–4).
Results: The probabilities (mean %) of achieving a fAUC:MIC(0–24) ratio ≥30 against SP isolates were as follows: L500, 74.9; L750, 90.6; and M, 98%.
Conclusions: Although previous MC simulations have shown comparable results between L750 and M for reaching a fAUC:MIC(0–24) ≥30 in the general population, the target attainment for M is greater than L750 in the elderly population. Because optimising PK-PD target attainment results in a greater probability of microbiologic cure, selecting agents with the greatest PK-PD target attainment may improve not only outcomes but also minimize SP resistance development in specific patient populations (Bhavnani, 2003).

P1804 Pharmacokinetics and pharmacodynamics of ciprofloxacin in relation to the mutant prevention concentration for *E. coli*

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Objectives: Mutant prevention concentration (MPC) has been defined as the lowest antibiotic concentration at which resistant mutants do not arise on antibiotic-containing agar plates in a population of 10^{10} cells at 24–48 h. However, to become a clinically useful concept the MPC needs to be related to the pharmacokinetics and pharmacodynamics (PK/PD) of the drug. Our aim was to study, in an in vitro kinetic model, the relation between MPC and pharmacokinetic parameters needed to prevent selection of ciprofloxacin resistant mutants.
Methods: One wild-type (wt) *E. coli* and one gyrA mutant were used and their MIC and MPC on agar were measured. In the in vitro model a flask was inoculated with 10^8 bacterial cells and incubated for 3–4 h to obtain a total of 10^{10} cells. During the static experiments different concentrations of ciprofloxacin were maintained through a continuous flow of fresh antibiotic-containing medium. When using kinetics the antibiotic in the flask was diluted with a half-life of 4 h. In all experiments the number of remaining living cells, and their MIC values, were determined at intervals during 24 h. In addition the population was analysed on ciprofloxacin-containing agar plates.
Results: The MIC was 0.008 and 0.094 mg/L for the wt and mutant, respectively. The MPC of the wt using constant conc. in the model was 16 × MIC and corresponded well to the MPC on agar. In experiments with lower concentration resistant bacteria arose. In the kinetic experiments T>MPC varied between 2 and 24 h. Resistance appeared when T > MPC was <8 h (an AUC24/MIC ratio of <364 h). Population analysis revealed at least three subpopulations with different levels of resistance. In contrast, for the gyrA mutant the MPC was much lower (2 × MIC or less) and during the kinetic experiments no selection of further resistance could be detected.
Conclusions: Selection of resistant mutants from the wt could be prevented in these single dose experiments when the T > MPC exceeded 8 h (33% of the dosing interval). For the gyrA mutant, no further increase in resistance could be noticed. However, it is possible that a low-level resistant subpopulation (with an increase

of <4 × MIC) was selected. This model was shown to be useful in defining the mutant preventive pharmacodynamic index, e.g. T > MPC.

P1805 Pharmacodynamics of levofloxacin-imipenem combination against mutants of *Pseudomonas aeruginosa* resistant to one or both drugs

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Objectives: Previous studies demonstrated the ability of levofloxacin-imipenem to prevent the emergence of resistance among clinical isolates of *P. aeruginosa* in a two-compartment in vitro pharmacodynamic model (IVPM). This study was designed to further analyse levofloxacin-imipenem against strains of *P. aeruginosa* already lacking susceptibility to one or both drugs by different efflux- or OprD-associated mechanisms.
Methods: The strains used in this study included four levofloxacin-nonsusceptible strains overexpressing MexAB-OprM, MexCD-OprJ, MexEF-OprN or MexXY, one imipenem-resistant strain lacking OprD, and one dual-resistant strain overexpressing MexXY and lacking OprD. Log-phase cultures (10^7–10^8 CFU/mL) were inoculated into peripheral compartment of the IVPM and treated with simulated doses of 750 mg levofloxacin, 250 mg imipenem, or levofloxacin-imipenem. Levofloxacin was dosed at 0 and 24 h, and imipenem at 0, 12, and 24 h. Doses were adjusted to account for protein binding, and pharmacodynamics were evaluated over 30 h.
Results: Against the MexAB-OprM, MexCD-OprJ, and MexEF-OprN overexpressing mutants, levofloxacin-imipenem achieved a 5-log total kill of one strain and eradication of two strains . Furthermore, levofloxacin-imipenem prevented emergence of resistance observed with each drug alone. Levofloxacin-imipenem also prevented emergence of levofloxacin resistance and achieved eradication of the strain lacking OprD. Although levofloxacin-imipenem initially achieved 6- to 7 log kills of the two MexXY mutants, it could not prevent the emergence of resistance.
Conclusions: These data further support levofloxacin-imipenem as an effective combination for treating *P. aeruginosa* and preventing emergence of resistance during therapy. These data warrant further evaluation of levofloxacin-imipenem against *P. aeruginosa*.

P1806 Concentrations in plasma and urinary excretion of ciprofloxacin XR (1000 mg) vs. levofloxacin (500 mg) in healthy volunteers receiving a single oral dose

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Objectives: The new extended release formulation of ciprofloxacin (Cipro XR) was designed for once daily administration in the treatment of urinary tract infection (UTI). The aim of the study was to compare its plasma and urine concentrations and its pharmacokinetic parameters with those of levofloxacin (LVX).
Methods: In a randomised crossover study, 12 volunteers (six males, six females) received a single oral dose of 1000 mg of Cipro XR or 500 mg of LVX to assess the concentrations (by high-pressure liquid chromatography) in plasma and the urinary excretion at intervals up to 36 h. The following pharmacokinetic parameters were studied: Cmax, tmax, t1/2, AUC, Cltot, Clren, Vdß, Vdss, maximal urinary concentration (Umax), and urinary excretion (UE).
Results: Both fluoroquinolones were tolerated well. The mean pharmacokinetic parameters are shown in the table. The median cumulative levels of renal excretion of the administered dose of the parent drug were 43% for ciprofloxacin (range, 14–51%; mean ± SD, 41 ± 10%), and 80% for levofloxacin (range, 74–88%; mean ± SD, 80 ± 5%). Cmax, AUC and UE were statistically significantly higher in the LVX phase, whereas the other pharmacokinetic indices, except t1/2, were statistically significantly higher in the cipro XR phase.

Indices	Cmax	tmax	t1/2	AUC	Cltot
Unit	μg/mL	h	h	μh/mL	mL/min
CiproXR	3.28*	1.96*	8.17	19.5*	927.1*
LVX	6.17*	1.16*	6.40	47.8*	174.4*
Indices	Clren	Vdβ	Vdss	Umax	UE
Unit	mL/min	L	L	μg/mL	%
CiproXR	351.3*	856.4*	485.9*	892.5*	41*
LVX	142.9*	97.0*	95.5*	498.9*	80*

*t-test($P<0.05$)

Conclusions: After oral administration of Cipro XR 1000 mg and LVX 500 mg Cmax and AUC in plasma were significantly higher in the LVX phase. The renal excretion (in milligram) of cipro XR 1000 mg once daily, however, is equivalent to that of LVX 500 mg and overall comparable urinary concentrations and areas under the urinary concentrations are reached by both drugs. Therefore, it can be assumed that the two doses investigated can be considered equivalent for the treatment of UTI.

P1807 Failure to detect emergence of resistance to moxifloxacin at AUC/MIC ratios between 9 and 216 in *Bacteroides fragilis* (Bf), *Clostridium perfringens* (Cp) and Gram-positive anaerobic cocci (GPAC) in an *in vitro* pharmacokinetic (pK) model

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Objectives: Moxifloxacin (moxi) has in vitro activity against many anaerobes and human pK such that it may be clinically useful in anaerobic infection. We performed a dose ranging study to assess emergence of resistance (EoR) to moxi in Bf, Cp and GPAC strains with typical MICs – i.e. 0.25, 0.25, 0.12 mg/L, respectively.
Methods: An *in vitro*, dilutional, open, single compartment model was adapted to support anaerobic growth. N2 replaced air and tryptone, yeast extract, dithiothreitol, cysteine HCI broth was used. Moxi AUC/MICs from 9 to 216 were simulated with values of 72–144 equating to 400 mg/day doses. EoR was assessed by population analysis profiles before drug exposure and after 48 h (two doses) on recovery agar containing 0, ×0.5, ×1, ×2, ×4 MIC moxi.
Results: The PAPs were (mean of three experiments) – the three strains selected were nonheterogeneous in their moxi PAPs : no resistant subpopulations were recovered on MIC ×2 plates. The strains were not cleared from the model but EoR did not occur at any AUC/MIC ratio.

MIC multiple moxi in recovery media	pre exposure time	log reduction in count after 48hrs at AUC/MIC log.cfu/ml.h				
	0	9	18	36	72	216
B. fragilis						
0	6.9	5.1	3.2	3.4	2.6	2.7
x0.5	6.8	4.8	<2	3.3	<2	2.6
x1.0	4.1	<2	<2	<2	<2	<2
x2.0	<2	<2	<2	<2	<2	<2
C. perfringens						
0	6.5	4.3	2.9	<2	2.8	2.9
x0.5	6.3	3.9	2.9	<2	2.8	ND
x1.0	5.2	2.2	<2	<2	2.8	ND
x2.0	<2	<2	<2	<2	<2	ND
GPAC						
0	6.5	4.0	3.3	2.9	2.7	2.8
x0.5	6.4	3.2	3.3	2.9	3.1	2.6
x1.0	5.4	2.2	3.1	2.8	2.9	2.5
x2.0	<2	<2	<2	<2	<2	<2

Conclusions: Bf, Cp, GPAC do not have heterogeneous PAPs to moxi; EoR was not detected even at AUC/MIC ratios of 9–18.

P1808 Prevention of resistance development with levofloxacin in epithelial lining fluid demonstrated by simulation of the concentration time curves in an in vitro model

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Objectives: The epithelial lining fluid is the real site of infection for pulmunory infections. The concentration of Levofloxacin here is higher than its concentration in the serum of the same patient. Therefore these concentrations are responsible for the antibacterial effect. With *Pseudomonas aeruginosa* and *S. pneumoniae* as test organisms we wanted to test the killing activity of Levofloxacin on these organisms and the development of resistant subpopulations.
Methods: The concentration time curves of Levofloxacin doses of 500 and 750 mg were simulated in an open in vitro model as they were described by G. Mark *et al* (2001) in human volunteers. Brain–heart infusion broth was used as nutrient. Colony counts were done in appropriate time intervals. Concentration of Levofloxacin was determined by a bioassay using *K. pneumoniae* as test organism. All experiments were done in triplicate.
Results: Data of the three parallel experiments matched sufficiently, demonstrating the reproducibility of the in vitro model experiments. Simulating an oral dose of 750 mg Levofloxacin *P. aeruginosa* was reduced for five to six orders of magnitude within 4 h. Regrowth was seen after 8–12 h. The bacterial count after 24 h was still three orders of magnitude below the inoculum. Resistant subpopulations were seen in the order of the mutation rate of about 10^{-7} but were eliminated after 2 h and did not reappear. The 500 mg dose gave similar results. Reduction of the cell count was five orders of magnitude after 5 h. After 24 h the cell count was 1/10 of the inoculum. However, resistant mutants persisted and reached a cell count of five orders below the inoculum after 24 h. For *S. pneumoniae* the reduction of the bacterial count went continuously down for three orders of magnitude within 24 h. Resistant mutants did not arise. There was no real difference for both dosing schedules.
Conclusions: Results demonstrate, that Levofloxacin after sufficient dosing is able to reduce the actual bacterial count in the epithelial lining fluid for three to six orders of magnitude and to prevent selection of resistant mutants for Gram-positive and Gram-negative bacteria. Thus this drug seems useful for the treatment of nosocomial pneumonia.

P1809 Use of an in vitro pharmacodynamic model and Monte Carlo simulation to characterise the pharmacokinetics and pharmacodynamics of gatifloxacin vs. *Salmonella typhi*

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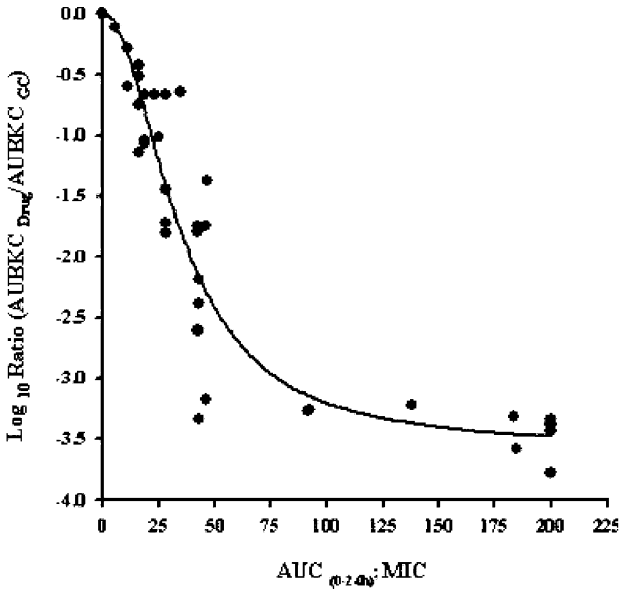
Background: *Salmonella* is an important public health concern, especially in third world countries. Ampicillin, TMP/SMX and chloramphenicol no longer provide reliable coverage in many areas and there are reports of resistance emerging to older quinolones. To date, no studies evaluating the pharmacokinetics-pharmacodynamics (PK-PD) of quinolones vs. *Salmonella typhi* have been conducted.
Methods: An in vitro PD model (IVPM) simulating human gatifloxacin (GAT) PK was used to determine which PK-PD measure is most strongly associated with drug response, and the relationship between exposure and response for *S. typhi* strains (GAT MIC 0.5–4 mg/L). Drug response was expressed as a log ratio of the 24 h area under the bacterial kill curve (AUBKC24) for drug and growth control (GC). Regimens (QD, BID, CI) that produced wide ranges of free (f) %T > MIC, peak:MIC and AUC:MIC ratios of GAT were studied. Using PK-PD targets derived from the IVPM and the current NCCLS susceptibility breakpoint for ciprofloxacin (CIP) vs. *S. typhi*, Monte Carlo simulation (MCS) was performed to assess the % PK-PD target attainment for recommended doses of selected quinolones. PK was obtained from normal healthy volunteers.
Results: Using nonlinear regression, a sigmoidal inhibitory E-max model was fit to log 10 AUCBK24 Drug/AUBKC24 GC for each PK-PD measure (see figure). fAUC:MIC correlated best with GAT efficacy ($R^2 = 0.96$). Peak:MIC also correlated with GAT efficacy ($R^2 = 0.93$) but %T>MIC did not ($R^2 = 0.68$). Model estimates of E-max, AUC:MIC50 and Hill's constant were −3.58, 34.7 and 2.04, respectively. Assuming that at least 90% of the IVPM Emax would translate to good activity *in vivo*, a GAT fAUC:MIC ratio of 105 mg/L × 24 h was identified as the PK-PD target. The per cent PK-PD target attainment for GAT, levofloxacin (LEV), ofloxacin (OFL) and CIP, based on simulations of 10 000 healthy subjects receiving standard dosing regimens for a range of *S. typhi* MICs (mg/L), is shown in the table below.



	0.03	0.06	0.125	0.25	0.5	1	2	4
LEV, 500 mg PO QD	100	100	99.2	92.1	<0.5	0	0	0
GAT, 400 mg PO QD	100	100	99.5	61.2	<0.43	0	0	0
OFL, 400 mg PO BID	100	100	100	99.54	64.1	<0.4	0	0
CIP, 500 mg PO BID	100	99.7	96.48	5.4	<0.48	0	0	0

Conclusions: A fAUC:MIC ratio of 105 mg/L × 24 h was associated with optimal effect for *S. typhi*. These findings are consistent with quinolone PK-PD target measures reported for other Gram-negative enterics. MCS results suggest a low per cent PK-PD target attainment for *S. typhi* strains with quinolone MICs ≥ 0.5 mg/L. Given the current NCCLS susceptibility breakpoint of 1 mg/L for CIP vs. *S. typhi*, these data suggest the need to reassess this susceptibility breakpoint.

P1810 Bactericidal activities of moxifloxacin in comparison to other substances used for the oral treatment of pulmonary infections

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Objective: This *in vitro* study investigated the bactericidal activity of moxifloxacin (MXF) when compared with amoxicillin (AM), coamoxiclavulanate (AMC), cefuroxime (CFR), cefixime (CFX) and clarithromycin (CLA) against *Streptococcus pneumoniae* (Sp), *Moraxella catarrhalis* (Mc), *Haemophilus influenzae* (Hi), and one strain of *Staphylococcus aureus* (Sa). Also, the influence of human serum on drug activities was determined.

Methods: Killing kinetics were performed under batch culture conditions . Two strains each (ATCC strain and a recent clinical isolate) of Sp, Mc, Hi and one clinical isolate of Sa were used. All strains were serum resistant and cultivated in BHI broth ±50% human serum in ambient air. The final inoculum was approximately 10 exp.6 CFU/mL. Multiples of the MICs were added to the cultures, drug concentrations ranging from 1 to 32× MIC. Samples were taken at 0.5, 1, 2, 4, 6, 8, and 24 h and subcultured quantitatively.
Results: Human serum promoted the growth of the drug-free controls by approximately 1–2 log CFU/mL and reduced the bactericidal activities of all agents studied . However, when the data were normalised by subtracting the effect of serum promoted growth, bactericidal activities of all agents against the gram + were enhanced in the presence of serum, irrespective of their % protein binding. Also, MXF exhibited a concentration dependant bactericidal effect against all pathogens and all ranges of concentrations studied; typically, the bactericidal effect of the β-lactams could not be increased beyond the optimally bactericidal concentration. CLA reduced the CFUs of the gram+ as well, but was ineffective against Hi as was CFX against Sa.
Conclusions: Despite the differences in protein binding between CLA (70%), MXF (39%), AM and CFR (30-40%), the addition of serum affected the bactericidal activities of the agents studied similarly. The augmented bactericidal activity in the presence of serum indicates that other yet unknown variables must have an impact on the bactericidal activities. Consequently, the effect of protein binding on the bactericidal activity cannot be mathematically corrected by exposing the bacteria to the calculated free fraction only. Compared with other agents widely used for the oral treatment of pulmonary infection, MXF exhibits the most pronounced bactericidal effect against all the pathogens and throughout the whole range of concentrations studied.

P1811 Pharmacodynamic studies of amoxicillin in an in vitro kinetic model against *Streptococcus pneumoniae* with different susceptibilities to amoxicillin: a comparison of different dosage regimens including a new pharmacokinetically enhanced formulation

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Objectives: To compare the pharmacodynamic effects of a new pharmacokinetically enhanced formulation of amoxicillin 2000 mg (XR), (component of Augmentin XR; amoxicillin/clavulanate 2000/125 mg) twice daily, with amoxicillin 875 mg twice daily, 875 mg three times daily and 500 mg three times daily against *Streptococcus pneumoniae* with different susceptibilities to amoxicillin in an *in vitro* kinetic model.
Methods: The strains of *S. pneumoniae* with MICs of 1, 2, 4 and 8 mg/L at an initial inoculum of approximately 105 CFU/mL were exposed to amoxicillin in an in vitro kinetic model with a concentration time-profile simulating the human serum profile of XR twice daily. All isolates were also exposed to amoxicillin with concentration time-profiles correlating to the human dosage of 875 mg twice, 875 mg three times daily and 500 mg three times daily with simulated half-life of 1 h. Repeated samples were taken regularly during 24 h and viable counts were performed.
Results: With the XR amoxicillin profile, bacterial eradication was noted for strains with a MIC of 1 and 2 mg/L and with a T > MIC of 73 and 60%. In the experiments with XR and the strain with a MIC of 4 mg/L (T > MIC of 47%) some regrowth occurred at the end of the dosing interval but there was a net reduction in bacterial counts at 24 h. The least-effective dose for all strains were 875 mg twice daily with T > MIC of 43, 35, 26 and 10%, respectively for strains with a MIC of 1, 2, 4 and 8 mg/L. None of the regimens were able to eradicate the strain with a MIC of 8 mg/L, although an initial substantial kill was noted with the enhanced XR profile after both doses.
Conclusions: The enhanced XR profile of amoxicillin was more effective in comparison to the other dosing regimens in eradicating *S. pneumoniae* with a MIC < 2 mg/L. For the strains with a MIC of 4 mg/L, there was a reduction of bacterial counts of approximately 1 log 10 CFU at 24 h with the XR regimen while all the other regimens resulted in static effect. Our in vitro system, like others, does not include the potential synergistic effects between the antibiotic and the immune defence system and may thus reflect the situation in immunocompromised hosts rather than in normal patients.

P1812 Strain-independent ratios of the 24-h area under the curve (AUC) to MIC of moxifloxacin (MXF) associating with the emergence of *Streptococcus pneumoniae* resistance: related changes in susceptibility and resistance frequency in an *in vitro* dynamic model

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Objective: According to the mutant selection window (MSW) hypothesis, resistant mutants are selected or enriched at antibiotic concentrations above the MIC but below the mutant prevention concentration (MPC). This hypothesis has been successfully tested in an in vitro dynamic model with MXF-exposed *S. pneumoniae* ATCC 49619 over a wide range of the AUC/MIC. The emergence of resistance was dependent on the simulated AUC/MIC ratio with most pronounced losses in the susceptibility and the highest resistance frequencies at AUC/MICs of 40–60 h. To explore if the AUC/MIC relationships of resistance are bacterial strain-independent, the development of resistance in a less susceptible strain of *S. pneumoniae* was tested using the same model.
Methods: Daily administration of MXF for 3 days consecutively was mimicked using a two-compartment dynamic model with peripheral units containing a starting inoculum of 108 CFU/mL of *S. pneumoniae* M231 (MIC 0.35 mg/L; MPC 6 mg/L). A series of monoexponential profiles of MXF was simulated to provide peak concentrations between the MIC and MPC (AUC/MIC 40–60 h). Changes in the susceptibility of *S. pneumoniae* to MXF were examined by repeated MIC determinations at 24, 48, 72 h and at the end of treatment. To determine the resistance frequency (f), the surviving organisms were counted by plating the specimens on agar containing 0×, 2×, 4×, 8 × IC of MXF.
Results: MXF concentrations determined by bioassay were close to the target values. The respective AUC/MICs varied from 40 to 58 h. Loss in the susceptibility of *S. pneumoniae* M231 to MXF was reflected by a six to 10-fold increase in MIC determined at the end of treatment and by an increased resistance frequency (f) (with specimens plated on agar containing 4 × MIC of MXF). The respective ratios of the elevated MIC to the initial value and the ratios of the final to initial f were concordant with those reported for *S. pneumoniae* ATCC 49619.
Conclusions: The present study demonstrates that *S. pneumoniae* resistance expressed by susceptibility testing and by population analysis relates to MXF AUC/MIC in a strain-independent fashion.

P1813 Pharmacodynamic activity of fluoroquinolones on different genotypes of *Escherichia coli*

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Objectives: The aim of the study was to assess the pharmacodynamic profile of ciprofloxacin (CIP) with regard to 11 strains of *E. coli* and on their first-step mutants.
Methods: We evaluated nine clinical strains of *E. coli* resistant to nalidixic acid but susceptible to CIP (antibiogram) and two reference strains of *E. coli* susceptible to nalidixic acid (NA). The MICs of nalidixic acid, ofloxacin, levofloxacin and CIP were evaluated using the Etest method. Mutant prevention concentra-

tions (MPC) were determined by applying 1011 CFU/mL of each organism to serially diluted antibiotic-concentration agar plates. All plates were incubated at 37°C for 2 days. The MPC of a drug is the lowest concentration capable of inhibiting growth of an organism. All procedures were performed in triplicate to ensure reproducibility. For the genotype, we sequenced the quinolone resistance-determining region on *gyrA* and *parC* from clinical strains and mutants. Bactericidal activity was determined by preparing time-killing curves for CIP with regard to three strains and the first-step resistant mutant for each strain.

Results: We noted three genotypes for these strains: two strains with no mutation (MIC < 0.025 mg/L of CIP and MIC < 4 mg/L of NA), six strains with a *gyrA* mutation at codon 83 or 87 (MIC = 0.06–0.5 mg/L of CIP and MIC > 12 mg/L of NA) and three strains with a *gyrA* mutation (codon 83) and a *parC* mutation at codon 80 (MIC = 1.5 mg/L of CIP and MIC > 12 mg/L of NA). MPCs ranged from 0.12 to 0.5 for the first genotype, 0.5 to 6 for the second genotype and 4–8 mg/L for the third genotype. For the wild strain with no mutation, concentration-dependent bactericidal activity of CIP was observed. For both clinical strains with *gyrA* mutation and first-step resistant mutants, we noted slow bactericidal activity of CIP (between 6 and 24 h). The minimum bactericidal activity of CIP ranged from 2–8 MICs.

Conclusions: CIP is less concentration-dependent against strains having a *gyrA* mutation.

P1814 Bone and serum concentrations of moxifloxacin in patients undergoing knee arthroplasty

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Objectives: Prosthetic joint infections represent an infrequent but feared complication of total joint arthroplasty, leading to a substantial postoperative morbidity, prosthesis failure and, in some cases, to death. Moxifloxacin is a new fluoroquinolone with a broad antibacterial spectrum and improved activity against gram positive microorganism and anaerobes; it shows an enhanced potency against methicillin-susceptible and -resistant isolates of *S. aureus* and *S. epidermidis*, if compared with the older fluoroquinolones. Penetration of moxifloxacin in bone has not still assessed. The main purpose of this study was to determine moxifloxacin levels in serum and bone following oral administration of single and multiple oral doses of 400 mg of the drug, to evaluate its potential use in perioperative orthopaedic prophylaxis.

Methods: Thirty consecutive patients, undergoing routine total knee arthroplasty were enrolled in this study. Three groups of 10 patients each were formed. Group A received moxifloxacin os 400 mg 2 h ± 30′ preoperatively. Group B received moxifloxacin os 400 mg 4 h ± 30′ preoperatively. Group C received moxifloxacin os 400 mg 14 h preoperatively, followed by a second dose 2 h± 30′ preoperatively. During surgery, at the time of bone removal, a blood sample and aliquots of cortical-cancellous bone were collected and moxifloxacin concentrations were measured by high performance liquid chromatography.

Results: The mean plasma, cancellous and cortical bone concentrations were respectively:

Group A: 3.45, 1.89 and 1.43 μg/mL.
Group B: 3.73, 1.81 and 1.56 μg/mL.
Group C: 6.26, 2.97 and 2.54 μg/mL.

Conclusions: These data show good penetration of moxifloxacin in both cancellous and cortical bone with higher serum and bone concentrations obtained after two doses of the drug (group C). Therefore, moxifloxacin may be considered in perioperative orthopaedic prophylaxis and repeated administrations should be preferred to achieve maximum antibacterial activity at the site of surgery.

P1815 Evaluation of the antibacterial activity of moxifloxacin vs. gatifloxacin vs. levofloxacin in serum and target tissues using in vitro and in silico PK/PD modelling

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Objective: Evaluation of the influence of PK-properties of moxifloxacin (MXF), gatifloxacin (GTF) and levofloxacin (LVF) in the various target tissues on the bactericidal activity (BA) of the drugs against causative pathogens encountered in RTIs using a PK/PD mapping technique. This new method provides a better view on their therapeutic use in the clinical situation than AUC/MIC or Cmax/MIC surrogates.

Methods: Time-kill studies were performed by exposing seven strains (3× *S. pneumo*, 1× *K. pneumo*, 3× *S. aureus*) with varying susceptibilities to fluctuating drug concentrations mimicking single oral doses of MXF (400 mg), GTF (400 mg) and LVF (500 mg) in serum and human target tissues (i.e. ELF, bronchial mucosa, macrophages). PK/PD surrogates such as AUBKCnorm (area under the curve normalised to the inoculum, and the bacterial elimination rate of from the test system, h) kel (h^{-1}) were used to map the in vitro BA as a function of the MIC of the pathogens and the concentrations represented by the PK in the target tissues. PK and PK/PD parameters were estimated using noncompartmental methods. Low AUBKCnorm <<24 h indicates high efficacy, while a value of 24 h indicates 'stasis'.

Results: AUBKC24 h,norm for MXF were consistently below 10–3 h indicating a good antimicrobial activity independent from the MIC and target tissue concentration. Similar results were obtained for GTF with the only exception that AUBKC tended to increase (AUBKCnorm = 1 h) when MIC was high (1 mg/L) and drug levels were low (i.e. in serum). For LVF AUBKCnorm consistently increased with increasing MIC of the pathogens and was >100-fold higher for less-susceptible bacteria when compared with GTX and MFX. This indicates an inferior BA which can not easily be overcome by increasing the dose. kel was in the range of 0.2–1.5 h^{-1} for GTF and 0.1–2.2 h^{-1} for MXF and independent from the tissue or the MIC, while kel tended to be higher for LVF (rate 0.5 – 6.9 h^{-1}).

Conclusions: PK/PD mapping showed good activity of the three FQs against RTI pathogens with the broadest therapeutic window for MXF > GTF > LVF, the latter being characterised by a shallow relationship between PK and PD indicating its inferior BA against pathogens with high MICs.

P1816 Population PK/PD analysis for moxifloxacin 400 mg once-daily to treat *Streptococcus pneumoniae* infections

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Objectives: For fluoroquinolones AUC/MIC ratios are known to correlate with clinical outcomes for patients suffering from respiratory tract infections (RTI). This paper describes the results of a population-PK/PD analysis followed by Monte Carlo simulations to estimate clinical outcome for a 400 mg once daily moxifloxacin (MFX)-treatment schedule.

Methods: Based on PK data from 416 subjects a noncompartmental population-PK model was developed first to describe the expected exposure (AUC) distribution in humans. Height and gender were the main population covariates with moderate influence on PK variability. Albumin, bilirubin and creatinine clearance (as derived from serum creatinine according to Cockroft and Gould) had a mild effect on AUC. Residual unexplained variability of AUC was low (13.1%). To describe the PD function the MIC distribution pattern of more than 3000 isolates of *S. pneumoniae* as a representative pathogen for RTI (MIC90, range: 0.125; 0.006–4 mg/L) was built into the population-PK/PD model. Simulations for 20000 subjects were performed to evaluate the AUC/MIC characteristics for MXF. Target hit rates were calculated using the AUC/MIC cut-off points for fluoroquinolones published in the literature.

Results: Overall, a target hit rate was approximately 99% when applying an AUC/MIC threshold of >30 predictive for community acquired pneumonia. A target hit rate of 93.6% was predicted when assuming that an AUC/MIC of >125 is indicative of clinical success (as shown for ciprofloxacin and severe RTIs due to Gram-negative infections). In clinical trials success rate was approximately 93.5%. Broken down according to MICs the data suggest that a the overall likelihood of getting an AUC/MIC <30 at an MIC = 1 mg/L is approximately 0.25% (for MIC = 2 mg/L: predicted likelihood approximately 0.5%) in case of *S. pneumoniae* infections. This finding is in line with the breakpoint definition of NCCLS for MXF.
Conclusion: The results of the investigation indicate that the non-compartmental PK/PD model for MXF is suitable to predict clinical outcomes in CAP.

P1817 Pharmacodynamics of moxifloxacin in combination with ceftazidime of *P. aeruginosa*

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Objective: Killing of ceftazidime susceptible *P. aeruginosa* (MIC CAZ 1 or 4 mg/l) was 2–3 log for MIC CAZ 1 mg/L and <1 log for MIC CAZ 4 mg/L when simulating serum pharmacokinetics of ceftazidime. We asked the question whether the ceftazidime-mediated killing can be affected in combination with a nonantipseudomonal agent (moxifloxacin).
Methods: Strains enrolled were *P. aeruginosa* ATCC 27853 and a nonmucoid *P. aeruginosa* isolate C7-2-2 from a cystic fibrosis patient. A pharmacodynamic *in vitro* model described previously [Bauernfeind, (1993) JAC **31**, 789–798] was used. The serum pharmacokinetic profiles of moxifloxacin 400 mg o.d. and ceftazidime 1 g o.d. were reproduced in bacterial cultures (Mueller–Hinton broth). The dynamics of killing were followed by colony counts at time intervals.
Results: For *P. aeruginosa* ATCC 27853 (MIC CAZ 1, MIC MOX 4 mg/l) exposure to the combination moxifloxacin plus ceftazidime strongly enhances killing in comparison with exposure to moxifloxacin alone by >1 log (3.5 to >4.5, sterilization) and to ceftazidime alone by >2.1 log (2.4 to >4.5, sterilization). Conversely dual combinations of moxifloxacin plus ceftazidime did not enhance the killing of *P. aeruginosa* C 7-2-2 (MIC CAZ 4, MIC MOX 16 mg/L) in comparison with single antibiotics significantly (0 log vs. moxifloxacin; 0.2 log vs. ceftazidime).
Conclusions: Enhanced killing of dual combinations of moxifloxacin plus ceftazidime was restricted to *P. aeruginosa* resistant to moxifloxacin and susceptible to ceftazidime while indifferent effects were seen against strains resistant to moxifloxacin and moderately susceptible to ceftazidime.

P1818 Comparative levofloxacin and ciprofloxacin mutant prevention concentrations in *Pseudomonas aeruginosa* populations from cystic fibrosis and ICU patients

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Background: Levofloxacin (LEVO) has been advocated to be at least as active as ciprofloxacin (CIP) against *P. aeruginosa* according to pharmacokinetic/pharmacodynamic parameters. The aims of this study were assess mutant prevention concentrations (MPCs) of both quinolones in two different *P. aeruginosa* populations and compare them with pharmacokinetic parameters in the respiratory compartment.
Methods: We studied 14 *P. aeruginosa* isolates recovered from respiratory secretions from patients with ventilator associated pneumonia admitted to an ICU in our hospital and 42 from cystic fibrosis (CF) patients. MPCs, defined as the lowest drug concentration precluding any bacterial growth at 48 h, were determined by applying 10^9–10^{10} CFU/mL of each organism to serially diluted CIP and LEVO containing plates. MICs were determined by the Etest.
Results: *P. aeruginosa* from ICU patients were both susceptible to LEVO (MIC range 0.12–0.5 μg/mL) and CIP (MIC range 0.03–0.12 μg/mL) whereas 69 and 76% isolates from CF patients were susceptible to LEVO (MIC ≤ 2 μg/mL) and CIP (MIC ≤ 1 μg/mL, respectively. In *P. aeruginosa* from ICU patients, LEVO-MPCs (range 0.5–8 μg/mL; geometric mean 5.1 μg/mL) were higher when compared with CIP-MPCs (range 0.25–8 μg/mL; geometric mean 2.0 μg/mL). MPCs were higher in CF populations but maintaining similar differences among both antibiotics: CIP-MPCs (range 2–64 μg/mL; geometric mean 12.9 μg/mL) and LEVO-MPCs (range 8–128 μg/mL; geometric mean 24.6 μg/mL).All isolates from ICU patients displayed MPCs below epithelial lining fluid (ELF) steady-state concentrations obtained at 4 h after once-daily dosing of LEVO-500 mg (Gotfried *et al*. *Chest* (2001), **119**, 1114–1122) whereas only 21.5% were below MPC values of CIP concentrations with similar dosing. The corresponding values with CF isolates were 9.5 and 0%, respectively. Interestingly, the percentage of MPC values below ELF concentrations increased to 47.6% with once-daily dosing of LEVO-750 mg.
Conclusions: Although higher MPC values for LEVO than CIP were observed in both *P. aeruginosa* isolates from ICU and CF patients, the risk for selection of fluoroquinolone resistant mutants may be higher with CIP monotherapy than with LEVO monotherapy in the respiratory tract compartment. Moreover, the risk for selection of fluoroquinolone resistant *P. aeruginosa* mutants may be lower with high scheduled regimens using LEVO-750 mg.

P1819 Comparative pharmacodynamics of telithromycin and clarithromycin against differentially susceptible *Streptococcus pneumoniae* in an in vitro dynamic model

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Objective: To compare the anti-pneumococcal effects of telithromycin (TEL) and clarithromycin (CLA), their pharmacodynamics with differentially susceptible *S. pneumoniae* were studied in an in vitro dynamic model that simulates their human pharmacokinetics.
Methods: Pharmacokinetic data reported in human studies with TEL and CLA were fitted by the two-compartment model with first-order absorption (ka, alpha and beta are 0.3; 1.9; 0.06 h^{-1}, respectively, for TEL and 0.5; 0.8 and 0.14 h^{-1}, respectively, for CLA). Based on this analysis, time courses of TEL and CLA concentrations were simulated. *S. pneumoniae* ATCC 49619 (MICTLR 0.001 and MICCLR 0.08 mg/L) and *S. pneumoniae* 1516 (MICTLR 0.01 and MICCLR 0.04 mg/L) were exposed to a single dose of TEL (800 or 1600 mg) vs. two 12-h doses of CLA (2 × 500 or 2 × 1000 mg). To quantitatively compare the antimicrobial effects, a 24-h area between the control curve and the time-kill curve (ABBC) was calculated.
Results: There was a distinct dose-dependent anti-pneumococcal effect with both TEL and CLA. In terms of the minimal number of surviving organisms, the effect of 800 mg TEL on *S. pneumoniae* ATCC 49619 was more pronounced than 2 × 500 mg CLA, but these differences were not seen with *S. pneumoniae* 1516. Similar results were obtained at higher doses: again, the advantages of TEL were seen with *S. pneumoniae* ATCC 49619 but not *S. pneumoniae* 1516. These findings were confirmed by the ABBC analysis. For the ATCC strain, a larger area under the curve (AUC) to MIC ratio of CLA may be needed to produce an ABBC similar to that of the smaller AUC/MIC of TEL.
Conclusions: These data pharmacodynamically support once-daily administration of 800 mg TEL in the treatment of infections due to *S. pneumoniae*.

P1820 **Bactericidal activity and postantibiotic effects of clarithromycin against *Streptococcus pneumoniae* with the efflux mechanism of macrolide resistance**

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Objective: This study assessed clinical isolates of *Streptococcus pneumoniae* (SPN) with the macrolide efflux mechanism to determine if the time kill (TK) kinetics and the postantibiotic effects (PAE) of clarithromycin-14-OH clarithromycin (CH-14OH, 3:1) and clarithromycin (CH) alone were comparable with macrolide susceptible isolates.
Methods: Four SPN isolates with the macrolide efflux pump were identified using PCR to detect the presence of the *mefE* gene. Four SPN isolates without the *mefE* gene were also identified. The minimum inhibitory concentration (MIC) for each antimicrobial agent was determined using broth dilution. TK studies were performed by exposing SPN in broth to 4× the antimicrobial concentrations of the MIC and assessing viable bacterial colony counts after 0, 2, 4, 6, 12, and 24 h of incubation. PAE studies were perfomed by exposing SPN isolates in broth to 4× the MIC for 2 h and removing the antimicrobial agent with washing. After suspended the bacteria in fresh, drug free media aliquots were withdrawn at hourly intervals for viable bacterial colonies. The PAE was calculated as T–C (where T is the time for viability counts of an experimental culture to increase by 1 log 10 above bacterial counts taken immediately after drug removal and C is the corresponding time for the growth control).
Results: The MICs for CH in susceptible SPN ranged from 0.06 to 0.25 mg/mL and MICs in SPN with the *mefE* gene were 2–16 mg/mL. TK assays of each drug showed similar kinetics between isolates with and without the mefE gene. The mean PAEs of CH-14OH, and CH with macrolide susceptible SPN were 6.1 and 7.4 h, respectively. The mean PAEs of CH-14OH, and CH with *mefE* positive SPN were 9.4 and 8.9 h, respectively.
Conclusions: In vitro SPN isolates with the macrolide efflux pump have MICs that are higher than the SPN isolates without the pump. However, when measuring the kinetics of antimicrobial activity the TK and PAE of SPN with the *mefE* gene are similar to SPN isolates fully susceptible to macrolides.

Molecular bacteriology: streptococcus, meningococcus and haemophilus

P1821 ***Cpn60* gene based PCR identification of streptococcal species**

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Objectives: *Streptococcus agalactiae*, *S. dysgalactiae* and *S. uberis* are the major streptococcal causative agents of bovine mastitis. The rapid simultaneous identification of these species is necessary for monitoring mastitis caused by streptococci. The 60 kDa chaperonin *cpn60* genes were recently discovered in all *S. agalactiae*, *S. dysgalactiae*, *S. uberis* strains and sequenced. The goal of the present study was to develop the *cpn60* gene based PCR approach for simultaneous identification of streptococcal species.
Methods: A total of 52 strains of *S. agalactiae*, *S. dysgalactiae* and *S. uberis* isolated from the dairy cows were tested. Human *S. agalactiae* strains were also analysed. Bacteria were grown in Todd-Hewitt broth. Sequences of *cpn60* genes were originally accessed through the GenBank database. DNA samples for PCR were prepared by boiling of one to two bacterial colonies for 5 min. PCR products were sequenced using ABI Prism 377 Perkin-Elmer Sequencer.
Results: Comparative analysis of nucleotide sequences of *S. agalactiae*, *S. dysgalactiae* and *S. uberis cpn60* genes revealed the certain differences between species. Based on these differences, three pairs of primers were designed and each pair was suggested to be the species-specific. After PCR analysis, amplification fragments of the expected sizes of 310, 192 and 400 bp were obtained for *S. agalactiae*, *S. dysgalactiae* and *S. uberis* strains, respectively. Both human and bovine *S. agalactiae* revealed PCR products of the same size. Nucleotide sequences of *S. agalactiae*, *S. dysgalactiae* and *S. uberis* amplification fragments were determined and an expected species-specificity of the primers was demonstrated. All the primers were used in multiplex-PCR for simultaneous identification of streptococcal species. Multiplex-PCR revealed the complete correlation with results of conventional PCR and neither false-positive no false-negative results were received.
Conclusions: These data demonstrate that *cpn60* gene based multiplex-PCR assay can be effectively used for simultaneous identification of *S. agalactiae*, *S. dysgalactiae* and *S. uberis* strains. This novel approach can be employed for analysis of the milk products and monitoring mastitis. This work was supported by Slovak Grant Agency VEGA 1-8021-01, Russian Grants MK-2782.2003.04, NSh-2206.2003.4 and RFBR 03-04-49760.

P1822 **Molecular epidemiology of nasopharyngeal penicillin-non-susceptible *Streptococcus pneumoniae* isolated from children in day-care centres and orphanages in Russia (SPARS study)**

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Objectives: *Streptococcus pneumoniae* (SP) is one of the leading bacterial pathogens causing a variety of community-acquired infections among children. Pneumococcal resistance represents a problem of high concern in many countries, including Russia. This study describes molecular typing of penicillin-nonsusceptible SP (MIC > 0.06 mg/L) isolated from children from day-care centres from Asian and Russian day-care centers (DCCs) and orphanages.
Methods: Fifty two penicillin-nonsusceptible SP isolates (MIC range 0.12–8 mg/L) from 14 Russian DCCs and orphanages situated in eight cities (Moscow, Kazan, St Petersburg, Voronezh, Ufa, Smolensk, Yakutsk, Khabarovsk) located in the areas of Western, Southern, Central Russia, Siberia and Far East during 2001–2002 underwent molecular typing by BOX-PCR with primer BOX-A (ATACTCTTCGAAAATCTCTTCAAAC) and RAPD-PCR with primer M13 (GAGGGTGGCGGTTCT). Cluster analysis of genetic fingerprints was performed by UPGMA algorithm with Pearson coefficient using the GelCompar software (AppliedMaths).
Results: The combined analysis of BOX- and RAPD-PCR patterns revealed 21 unique genetic types of which eight major groups comprised from three to six isolates each, and the other 13 included single isolates. Strains from the same orphanage in Moscow, Ufa and Khabarovsk belonged to one major clone suggesting their rapid transmission in closed communities. Two cases of clonal relatedness between the isolates from geographically distinct centers (Kazan and Moscow, Saint Petersburg and Voronezh) were also found. However, the multiplicity of genetic types indicated that resistance to penicillin was acquired by numerous strains.
Conclusions: Combination of BOX- and RAPD-PCR had a sufficient discriminatory power to allow distinguishing between penicillin-nonsusceptible clones. Penicillin-resistant SP isolated from Russian DCCs and orphanages mostly represent genetically diverse population of strains. Clonal transmission of penicillin-resistant clones was confirmed both within cities and also between geographical distinct areas.

P1823 Heterogeneity of PavA fibronectin-binding protein in *Streptococcus pneumoniae*

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Objectives: *Streptococcus pneumoniae* is an important human pathogen, causing life-threatening invasive diseases, such as pneumonia, meningitis and bacteraemia, and less serious but highly prevalent infections, such as otitis media and sinusitis. Fibronectin binding is an important factor in streptococcal colonisation of host tissue but the bacterial adhesins mediating these reactions are largely uncharacterised. Holmes *et al*. (*Mol. Microbiol*. (2001) **41**, 1395–1408.) have described a novel protein PavA in invasive isolates of pneumococci which binds fibronectin and is associated with pneumococcal adhesion and virulence. This is a potential candidate for novel pan-pneumococcal vaccines. The objectives of this study were to determine the prevalence and degree of sequence conservation of PavA among invasive and noninvasive isolates of *S. pneumoniae*.
Methods: Polymerase chain reaction (PCR) was used to determine the presence of pavA in 34 isolates from invasive disease and 32 isolates from throat swabs. PCR amplicons were then differentiated by digestion with frequent cutting endonucleases *Hin*f1 and *Alu*1 (PCR-RFLP). The nucleotide sequence of amplicons from four invasive and four noninvasive isolates.
Results: The presence of *pavA* gene was confirmed in both the invasive and noninvasive isolates. PCR-RFLP showed variation in pavA gene. *Hin*f-1 and *Alu*-1 digestion divided noninvasive isolates into five different groups and invasive isolates into 12 different groups. Sequence analysis showed that both the N- and C-terminal coding regions of PavA are conserved, but there were nucleotide polymorphisms at 21 locations predicted to result in 11 amino acid substitutions in the middle portion of the *pavA* gene.
Conclusions: Our study shows that *pavA* gene sequence is not completely conserved. The heterogeneity observed, although not substantial, could have functional or pathogenic implications. Since variation could potentially reduce cross-protection between different pneumococci, further work is needed to understand the implications of this variation on the potential of PavA as a vaccine component.

P1824 Evaluation of a LightCycler PCR assay for detection of group B streptococcus in pregnant women with rupture of membranes at term

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Objectives: To evaluate the effectiveness of a LightCycler PCR assay for the detection of Group B streptococcus (GBS) infection or colonisation in pregnant women with rupture of membranes (ROM) at term, and to compare it with conventional culture techniques.
Methods: A total of 100 high vaginal swabs (HVS) from women with ROM at 35 weeks gestation were incubated in nutrient broth. The first 50 HVS swabs were incubated for 5, 30, and 60 min. The remaining 50 HVS swabs were incubated for 1 and 18 h. Following incubation, DNA was extracted using the InstaGene matrix DNA kit (Bio-Rad) and real-time PCR was performed using a LightCycler instrument (Roche Diagnostics). Oligonucleotide primers and fluorescence-labelled hybridisation probes were used for the specific amplification and detection of a 153-bp fragment of the cfb gene. The HVS were also examined by conventional culture by plating out onto ISLAM agar and incubating overnight at 37°C aerobically with 5% CO_2. The sensitivity of the assay was examined by analysing dilutions of overnight broths of GBS, while specificity was studied by testing different species of *Streptococcus* with the assay.
Results: The LightCycler PCR assay was able to detect 10^3 GBS CFU/mL. All non-GBS isolates examined were negative when tested, thereby demonstrating the specificity of the assay. Of the first 50 HVS examined, four (8%) were positive at each time point (5, 30 and 60 min) by both the LightCycler assay and conventional culture. Of the second 50 HVS, eight (16%) were positive by LightCycler and six (12%) were positive by culture after 1 h incubation. Following 18 h incubation, 13 (26%) samples were positive by LightCycler, but only six (12%) were culture-positive. Three of these samples were only LightCycler-positive after overnight incubation. Four samples were negative by culture, but LightCycler-positive after 1 and 18 h incubation. Two culture-positive samples were LightCycler-positive only after 18 h incubation, indicating that these samples contained low numbers of GBS.
Conclusions: The LightCycler PCR assay is a specific, sensitive and rapid means of detecting GBS from HVS. The LightCycler assay was more sensitive than conventional culture, but overnight incubation increases the detection rate of GBS. However, whether this is clinically significant (i.e. is the woman infected or colonised) requires further investigation.

P1825 A PCR-RFLP assay for detection of *parC*, and *gyrA* mutations in fluoroquinolone-resistant *Streptococcus pyogenes*

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Objetives: Quinolone resistance in *S. pyogenes* is mainly due to mutations in the quinolone resistance-determining regions (QRDR) of either the ParC subunit of topoisomerase-IV or the GyrA subunit of DNA gyrase. We have developed a PCR-RFLP assay to detect mutations in the Ser79 and Asp83 codons of *parC*, and Ser81 and Glu85 of *gyrA* genes which lead to quinolone resistance.
Methods: *S. pyogenes* CIP56.41T and 5 *in vitro* and *in vivo*-resistant derivatives, with known mutations in the QRDR of the parC, and gyrA genes, were used to validate the assay. Mutations within parC were detected using restriction endonucleases *Hin*fI (Ser79) and *Lwe*I (Asp83), and those within *gyrA* by *Mbo*II (Ser81 and Glu85).
Results: The 339-bp *parC* product from CIP56.41T contained two *Hin*fI and one *Lwe*I cutting sites generating fragments of 212, 95 and 32 bp and of 229 and 110 bp, respectively. Loss of a *Hin*fI site following mutations at the Ser79 codon resulted in fragments of 127 and 212 bp. Mutations at the Asp83 codon suppressed the LweI site and a 339-bp fragment was observed. The 203-bp *gyrA* product from CIP56.41T contained a natural *Mbo*II site and an artificially created *Mbo*II site generating fragments of 126, 45 and 32 bp. Loss of the *Mbo*II site in resistant isolates due to mutations at the Ser81 codon led to 158 and 45 bp fragments. Mutations at the Glu85 codon were associated with loss of the artificially created *Mbo*II site leading to 126 and 77-bp fragments. Simultaneous mutations at Ser81 and Glu85 codons suppressed both MboII sites and a 203-bp fragment could be observed.

P1826 Cloning and expression of group B streptococcal gene – analogue of the streptococcal adherence related lipoproteins

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Objectives: In the genus of Streptococcaciae there exist a gene family: *papA* or *psaA* from *Streptococcus pneumoniae*, *ssaB* from *Streptococcus sanguis*, *fimA* from *S. parasanguis* and *scaA* from *S. gordonii*. All these genes encode for lipoproteins involved in metal transport and adherence, which are considered the possible virulence factors. We attempted cloning and expression analysis of the group B streptococcal (GBS) analogue of this gene family.
Methods: GBS strain O9OR (derivative of Ia serotype) used for cloning of the adherence genes analogue. PCR primers were designa-

ted according to the conserved regions of the gene family under study (*scaA* gene was used as a source of original sequence). DNA fragment of GBS *scaA* analogue was amplified and sequenced. Using this fragment as a probe the entire sequence of GBS *scaA* analogue was determined in the phage library. The gene was sequenced and expressed in *E. coli*.
Results: The entire sequence of *scaA* gene analogue of the GBS had been cloned, sequenced, and deposited into gene bank. GBS *scaA* regulon was found to be similar to one in group A streptococci where *scaA* gene is located in direct proximity to ATP binding protein *scaC* with similar orientation and negative regulator *scaR* which has an opposite orientation. The central portion of GBS *scaA* had been cloned into integration vector pT7Erm B. The resultant plasmid was used for GBS strain O9OR transformation. GBS transformants were unable to grow like parental strain in liquid media forming clots instead of typical chains and it expressed substantially different level of adherence. GBS *scaA* gene had been cloned in *E. coli* system and expressed employing pQE expression vector system. However, it was impossible to purify the entire protein using six His residues located in the N terminus because of processing of the recombinant streptococcal lipoprotein in the *E. coli*. In order obtain the mature protein we have cloned the GBS *scaA* starting from the region encoding the lipoprotein cleavage site LXXC. Purified mature protein of 35 kDa was obtained and used for immunisation experiments on mice and rabbits. The possible usage of GBS ScaA protein as vaccine against GBS infection is discussed.
Conclusions: A novel GBS gene encoding for putative metal transport adherence related lipoprotein was cloned and expressed in *E. coli*. The protein might be important for GBS adherence to human epithelium.

P1827 The relationship between the modular structure of eucaryotic-like serine/threonine protein kinase StkP of *Streptococcus pneumoniae* and its function

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Objectives: The main objective of this work was to determine the relationship between structure and function of eucaryotic-like Ser/Thr protein kinase StkP in *S. pneumoniae*. In the C-terminal part of StkP we identified four repeats with the PASTA signature (penicillin-binding protein and serine/threonine kinase associated domain). It has been proposed that this domain could bind unlinked peptidoglycan and act as its sensor. Thus, StkP might be involved in cell-wall biosynthesis through interaction of their PASTA domains with cell wall components. The PASTA domain is also an important antibiotic resistance determinant in the penicillin-binding proteins.
Methods: To determine cellular localisation StkP we prepared epitope-labelled protein kinase StkP. Both full-length and truncated forms of StkP were constructed and corresponding mutants were created by allelic exchange.
Results: The full-length form of StkP was detected in membrane fraction. Deletion of the C-terminal part of StkP including putative transmembrane domain resulted in the presence of mutant form of StkP exclusively in cytoplasmic fraction. These results clearly showed that StkP is a membrane-associated protein. We investigated further the influence of deletion of StkP C-terminal module on antibiotic susceptibility. *S. pneumoniae* mutant strain with cytoplasmic StkP showed increased sensitivity to penicillin G and vancomycin, antibiotics that inhibit cell-wall synthesis. In addition, the ability of mutant strain to develop a state of natural competence as measured by the efficiency of transformation was two orders of magnitude lower when compared with wild type strain.
Conclusions: These results indicate that the C-terminal part of StkP acts as a sensor that activates signalling pathway controlled by StkP protein kinase activity.

P1828 Identification of streptococci to the species level by pyrosequencing

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Objectives: The objective of this study was to develop a molecular method for inexpensive, reliable and simple identification of streptococcal species.
Methods: We developed a method for identification of streptococci based on pyrosequencing technique using PSQ 96MA System (BiotageAB, Uppsala, Sweden). Two hypervariable regions of the streptococcal 16S rDNA were analysed. These regions, called V1 and V2, locate close to the 5′ terminus of the 16S rRNA gene. In this study, the V1 and V2 signature sequences of 60 type strains of 54 different streptococcal species were determined. The first step in this method is to perform a PCR comprising both regions to be sequenced. In the PCR, heat-inactivated streptococcal suspension may be used as a sample. After checking the presence of the PCR products on an agarose gel, sequencing is carried out. Sequencing reactions of the V1 and V2 regions are performed separately using primers specific for V1 and V2 regions, respectively.
Results: By sequencing approximately 35 bases long signature sequences of both regions, all streptococcal species with a few exceptions can be differentiated. The two species groups that cannot be differentiated from each other are *S. salivarius*, *S. vestibularis* and *S. thermophilus* as well as *S. bovis* and *S. lutetiensis*.
Conclusions: A simple, reliable and fast method for identification of vast majority of streptococcal species was developed: the whole procedure can be completed during a single working day.

P1829 Multi-locus sequence typing of group A streptococci from a London hospital

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Objectives: Group A streptococci (GAS) were collected from a London Hospital and characterised by multi-locus sequence typing (MLST) to determine the identity and prevalence of clones circulating in this setting. London GAS were compared with strains from a global collection of GAS via the internet accessible MLST database (http://www.mlst.net).
Methods: An MLST sequence type (ST) was assigned to each isolate based on sequence of internal fragments of seven housekeeping loci. emm-type was defined by 160-bp of sequence at the 5′-end of the central emm gene. All isolates were tested for susceptibility to erythromycin and tetracycline.
Results: Between July and October 2003, 121 clinical isolates were collected from 121 persons, including hospitalised patients (9%) and outpatients attending emergency rooms (31%), speciality outpatient clinics (6%) and GPs (48%). Forty-one STs were identified of which 20 were represented by a single isolate. The eight most prevalent types among the 121 GAS were ST117/emm81 (15%), ST39/emm4 (9%), ST62/emm87 (7%), ST28/emm1 (6%), ST36/emm12 (6%), ST46/emm22 (5%), ST326/emm82 (5%) and ST101/emm89 (4%). When compared with the MLST database 14 (34%) of the 41 STs had not been previously identified, although six of these differed from recognised STs at only a single locus suggesting they were closely related. Resistance to erythromycin and tetracycline was seen in 5 and 18% of isolates, respectively, with three isolates resistant to both agents. Resistant isolates included strains with 12 distinct STs. Of these five STs were represented by both resistant and susceptible isolates.
Conclusions: GAS strains with higher emm types (>80) accounted for a significant proportion of GAS isolates collected during this study. This information may have important ramifications for emm-directed vaccine strategies. The appearance of resistant isolates was not associated with particular clones.

P1830 Microarray-based identification of genes and 2-D gel analysis of proteins regulated by Ser/Thr protein kinase of *Streptococcus pneumoniae*

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Objectives: Searching in the genome sequence of *S. pneumoniae* revealed the presence of a single eucaryotic-like Ser/Thr protein kinase gene *stkP*, associated with a gene encoding Ser/Thr protein phosphatase *phpP*. Eucaryotic-like protein kinases and phosphatases in procaryotes coordinate processes of differentiation and pathogenesis. Thus, StkP and PhpP may play a role in pneumococcal pathogenesis. The main objective of this work was to determine the targets of protein kinase StkP.
Methods: In order to determine the function of StkP we prepared deletion of the corresponding gene in *S. pneumoniae* by PCR ligation mutagenesis and allelic exchange. To identify the genes that are controlled by StkP we analysed the transcription profile of *stkP* loss-of-function mutant by DNA microarray technology.
Results: Measurement of transformation efficiency during natural competence development showed that deletion of *stkP* gene in *S. pneumoniae* resulted in the loss of genetic competence. The transcript analysis revealed the StkP-dependent expression of many potential target loci that were specifically and strongly up- and downregulated in *stkP* mutant suggesting the important regulatory role of StkP in *S. pneumoniae*. The data obtained from transcriptome mapping were further extended by proteomic studies. Mass spectrometric sequencing was used to identify putative targets of post-translational modification.
Conclusions: In conclusion, genes whose transcription was induced or repressed in *S. pneumoniae stkP* deletion mutant were identified. Proteomic studies and mass spectrometry were used to confirm some of the results.

P1831 Rapid identification of *Streptococcus* spp. by Pyrosequencing™ analysis of the *rnpB* gene

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Objective: The objective of this study was to investigate the use of pyrosequencing analysis of a variable region within the *rnpB* gene as species-specific target in identification of bacteria within the *Streptococcus* genus.
Methods: The *rnpB* gene is universally present in bacterial species and encodes a subunit of the RNaseP enzyme. Comparison of *rnpB* DNA sequences has been shown to be useful in phylogenetic studies of bacterial genera (Täpp *et al*., 2003). A short region of this gene was used as target in pyrosequencing, which is a rapid real-time method for sequencing-by-synthesis.
Results: The *rnpB* DNA sequences from 49 streptococcal species were aligned to identify target regions for PCR and DNA sequencing. The *rnpB* P3 region was chosen as the target, as this region is highly variable and is flanked by conserved DNA regions. The target region was amplified in PCR using DNA prepared from type strains as well as clinical isolates as templates. These amplicons were then analysed by pyrosequencing. Obtained DNA sequences 20–25 bases in length were found to be sufficiently informative for identification of most species in BLAST searches against a local database and GenBank. A limited number of closely related species could not be discriminated from each other in this analysis (e.g. *S. salivarius* and *S. vestibularis*). Most of these pairs could be separated to correct species level by sequence analysis of a second region in the same PCR fragment, the P9 region.
Conclusions: Pyrosequencing analysis of a short, variable region within the rnpB gene is a valuable tool in high-resolution species identification of streptococci.
Reference:

1. Täpp J., Thollesson M., Herrmann B. (2003) Phylogenetic relationships and genotyping of the genus *Streptococcus* by sequence determination of the RNase P RNA gene, rnpB. *Int J Syst Evol Microbiol* 531861–531871.

P1832 Multilocus sequence typing of *Neisseria meningitidis* directly from cerebrospinal fluid

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Objectives: The polymerase chain reaction (PCR) for nonculture diagnosis of invasive meningococcal disease was introduced in the Czech Republic in 2000. The multi-locus sequence typing (MLST) of PCR products was developed in our laboratory [*Epidemiol Infect* (2002) **128**: 157] to detect sequence types (STs) and ST-complexes from the culture-negative patients.
Methods: DNA was extracted from the cerebrospinal fluid (CSF) by the QIAamp kit (QIAGEN, Germany). MLST was carried out as described at the MLST website (http://neisseria.mlst.net). First amplification of seven alleles (abcZ, adk, aroE, fumC, gdh, pdhC and pgm) was performed in a Amplitrone II thermocycler using Hot start. Second amplification of the same alleles was performed from 1 μL of amplified products followed by purification of the product with 20% PEG. The sequencing reactions were performed in PCR tubes with the BigDye terminator cycle sequencing kit (PE Biosystems) and subsequently analysed with an ABI PRISM 377 automated DNA sequencer (Perkin Elmer). The final sequence of each locus was determined using the LASERGENE software package (DNASTAR, Madison, WI, USA).
Results: The PCR confirmation rate of meningococcal aetiology of invasive disease increased from 2% in 2000 to 13% in 2002. In last 2 years (2002 and 2003), the PCR investigation is used simultaneously with culture and gives quicker results. The MLST of the PCR products was performed only in culture-negative patients. It was performed directly from 21 samples of CSF and revealed STs in all PCR products mostly belonging to the hypervirulent meningococcal complexes: ST-11 (eight samples), ST-32 (four samples) and ST-18 (two samples).
Conclusions: MLST of *Neisseria meningitidis* directly from clinical specimens was introduced successfully and allows identification of STs and ST-complexes in culture-negative patients. It improves surveillance of invasive meningococcal disease.
Acknowledgements: This study was supported by research grants NI/6882-3 and NI/7109-3 of the Internal Grant Agency of Ministry of Health of the Czech Republic and made use of the Multi Locus Sequence Typing website (http://neisseria.mlst.net).

P1833 Clonal distribution of Czech invasive *Neisseria meningitidis* isolates

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Objectives: Clonal feature of 907 isolates, acquired from 70% of cases of invasive meningococcal disease in the Czech Republic since 1990 till 2003 (November), has been determined in order to monitor the spread of hypervirulent clonal lineages responsible for the most severe forms of the disease.
Methods: Multi-locus enzyme electrophoresis typing together with whole-cell ELISA phenotyping was used for routine screening of isolates, multi-locus sequence typing was adopted for further characterisation of isolates representative of electrophoretic types detected.
Results: Due to the increased disease incidence related to spread of the hypervirulent ET-15 strain of the ST-11/ET-37 complex, representation of serogroup C meningococci had prevailed since 1993 till 1999. In parallel to the decline of the ST-11/ET-37 complex, endemic occurrence of hypervirulent serogroup B lineages of the ST-32/ET-5 complex and the ST-18 complex of serotype 22-related strains increased for a temporary period. At present, the proportion of ST-11/ET-37 isolates increases again. Two other lineages showing hypervirulent nature, the ST-269 complex and the ST-23 complex/cluster A3, have been isolated to a small extent. Isolation of strains of the ST-41/44 complex/lineage III and the ST-8 complex/cluster A4, spread in western and southern European regions previously, was rare over the study period. In oppos-

ite to serogroup C strains, phenotyping of serogroup B isolates was frequently not reasonable for assessment of clonal relevance.
Conclusions: Partial replacement of the ST-11/ET-37 complex within *N. meningitidis* population in the Czech Republic was linked to the spread of serogroup B strains of the ST-32/ET-5 and ST-18 complexes. The detection of hypervirulent serogroup B lineages, for which a comprehensive vaccine is yet not available, underlines the need for assessment of clonal nature of clinical *N. meningitidis* isolates by multilocus genotyping.
Acknowledgements: The work was supported in part by the grants NJ/7458-3 and NI/7109-3 of the Internal Grant Agency of Ministry of Health of the Czech Republic and the MEN-Net project QLK2-CZ-2001-01436 of the European Commission and made use of the *Neisseria* Multi Locus Sequence Typing website (http://pubmlst.org/neisseria/), developed by Dr M.-S. Chan and Dr K. Jolley of the University of Oxford under funding of the Wellcome Trust and European Union.

P1834 Correlation of the latex agglutination and the PCR method in patients with invasive meningococcal disease

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Objectives: Invasive meningococcal disease is still life threatening. It is important to diagnose the infection rapidly in order to start appropriate treatment as soon as possible or continue treatment already initiated. Latex agglutination (LA) and PCR method are direct, quick and nonculture methods. LA is an immunological technique for detection of soluble antigens. The PCR method detects the presence of the DNA of pathogen. We focused our project on the correlation of the positive results of LA and PCR in various clinical materials and its time development.
Methods: We assessed 29 patients hospitalised at the clinic of infectious diseases with invasive meningococcal disease. The cerebrospinal fluid (CSF), serum and urine were collected from the first to the seventh day of hospitalisation. We used two kits for LA: Slidex méningite kit for CSF and Pasteur meningitis kit for other biological materials. The DNA was isolated by Qiagen kit and PCR method was used. PCR products were detected on the 2% gel electrophoresis. We tested 31 CSF, 94 serum and 99 urine samples.
Results: CSF: 54% of LA and 96% of PCR results were positive in the day of admission, 19% of them after the onset of antibiotic therapy (a.o. ATB). In 16 patients subsequent CSF testing was performed, six with positive and 10 with negative result of PCR. LA was negative in all the cases.
Serum: 31% of LA and 43% of PCR results were positive in the day of admission, 13% of them a.o. ATB. In samples collected for four and more days a.o. ATB the results of LA and PCR were negative. Urine: 21% of LA results were positive in the day of admission. In samples collected during next days the results were positive in a lower percentage.
Conclusions: The results of both methods depend on concentration of the pathogen in clinical material. LA is less sensitive than PCR. LA did not bring positive results in subsequent CSF testing, while 38% of PCR results were positive. There is correlation between the results of PCR and LA in serum.
Acknowledgement: This study was supported by research grant NI/7109-3 of the Internal Grant Agency of the Ministry of Health of the Czech Republic.

P1835 Design and evaluation of a CustomSeq microarray for genotypic characterisation of *Neisseria meningitidis*

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Objectives: CustomSeq is a resequencing microarray where up to 29.3 kbp of DNA sequence from multiple gene targets can be interrogated on a single array in a single hybridisation reaction. The technology is based on a 25-nucleotide probe 'tiling' strategy where four probes are used to interrogate the central nucleotide. The objectives were to design a CustomSeq microarray for the genotypic characterisation of *Neisseria meningitidis* using combined DNA sequences from four gene targets for the predication of genogroup, genotype and genosubtype.
Methods: The array was designed using the guidelines of Affymetrix Corporation. Genogrouping: alignments of available ctrA and siaD gene sequences were performed and eight fragments (total 0.91 kbp) identified that could predict genogroups A, B, C, 29E, H, W135, X, Y and Z. Genotyping is typically based on sequencing of four *porB* gene variable regions (VR). The encoded Outer Membrane Protein is divided into classes 2 and 3. The sequences to be included on the microarray were based on those in Sacchi *et al*. (1998). Multiple sequences for each class were aligned and new variants identified. There were 114 sequences (total 6.180 kbp). Genosubtyping is typically based on sequencing two *porA* VRs. All *porA* variant sequences (available at http://www.neisseria.org) within each VR were aligned and variant-defining sequences identified. There were 244 sequences (total 14.490 kbp). Seventy-five meningococcal isolates from diverse serogroups, serotypes, serosubtypes and genotypes were tested in the system. Data analysis was performed using GeneChip DNA Analysis Software.
Results: The CustomSeq microarray could be used to predict serogroup, serotype, serosubtype and genosubtype. There was 100% concordance with previous *porA* genosubtyping capillary-based sequence data. All samples were newly genotyped for *porB*.
Conclusions: This is the first application of CustomSeq technology to microbial typing. It provides DNA sequence data from a single PCR product for each target, an advantage over conventional capillary-based sequencing of *porA* and *porB* genes, which currently require multiple sequencing reactions for each. The genotyping data complements the current phenotypic typing scheme, where a proportion of isolates cannot be typed because of a limited number of monoclonal antibodies. This prototype could be applied to other organisms where extensive sequence data is required for typing.

P1836 Recent emergence of a virulent clone of *Neisseria meningitidis* C:2b:P1.5 with decreased susceptibility to penicillin in Italy

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Objectives: A great increase in cases due to *Neisseria meningitidis* with the phenotype C:2b:P1.5 with decreased susceptibility to penicillin (penI) has occurred in Italy since 2002. All the penI meningococci were analysed by MLST and PFGE to verify whether the increase in the number of cases with this phenotype correlates with the emergence of a single clone readily spreading in the country.
Methods: A phenotypic characterisation was obtained by defining the serogroup and sero/subtype, and the penicillin susceptibility by *E*test method. The molecular analysis of the *penA* gene was carried out in all the strains; in addition, the new approach by RealTime-PCR, recently set up, was applied. PFGE was performed by digesting chromosomal DNA with NheI and BglII and MLST by sequencing gene fragments of housekeeping genes as described (http://neisseria.org/nm/ typing/mlst/).
Results: Among serogroup C *meningococci* isolated since 2002, 30 (44.1%) belonged to phenotype C:2b:P1.5. Interestingly, the majority of them (80%) showed a decreased susceptibility to penicillin (MICs > 0.06 mcg/mL). The spread of these meningococci was more remarkable in the first 6 months of 2003 when twice as many were isolated compared with the previous year. According to MLST website data, all the C:2b:P1.5 *penI* meningococci were identical and assigned to the ST8/A4 cluster. DNA macrorestriction fragments generated with NheI and analysed by PFGE showed one main pulsetype (PTA) and two subclones with some minor differences, named PTA1 and PTA2. When BglII was used,

all the strains showed the same PT. The most frequent PT (PTA) seems to be identical to the fingerprint pattern 2 described by Arreaza et al. (*J Med Microbiol* 2000). It is possible to hypothesise that our clone is imported but, differently to the Spanish one, it is able to cause meningococcal invasive disease in all age groups.
Conclusions: All these findings unambiguosly confirm that the increase of meningitis caused in Italy by *N. meningitidis* C:2b:P1.5 is caused by the spreading of a single emergent clone belonging to the hypervirulent cluster A4. Moreover, it is also important to underline that there is a direct relationship between the increase in serogroup C strains in Italy and the eightfold increase of penI meningoccci as a result of spread of this single virulent clone.

P1837 Molecular characterisation by PFGE of phenotypically indistinguishable *Neisseria meningitidis* stains

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Objectives: Since October 2002, Portugal has a new laboratory surveillance system for Meningococcal Disease (VigLabMD) in which our Institute receives all *N. meningitidis* strains from the national hospital labs network. Between October 2002 and November 2003, the majority of cases of meningococcal disease were due to serogroup B of *N. meningitides*. Also, previous serotyping and subtyping of *N. meningitides* strains showed that phenotype C:2b:P1.5 was the most frequently isolated in strains from cerebrospinal fluid (CSF) and blood. In an epidemiological propose, we used molecular techniques to differentiate strains antigenically undistinguishable. Admitting the possibility of nonexpression of serosubtype-specific antigenic determinants we also included in this study strains NST (nonsero subtypable) and expressing any determinant from variable region VR2 of class 1 outer-membrane protein.
Methods: Strains with the same or potentially the same antigenic profile were submitted to a molecular characterisation by pulsed-field gel electrophoresis (PFGE), using the enzyme Bgl I. The restriction profiles were analysed by Bionumerics software application.
Results: We found 19 strains with the same, or potentially the same, antigenic type, which were submitted, to PFGE.

	Number of strains
C:2b:P1.5	11
C:2b:NST	4
C:2b:P1.2,5	3
C:2b:P1.5,15	1

Eleven different PFGE patterns were found, in the 19 strains studied.
Conclusions: Considering the diversity of pulsed field types of the studied strains and the geographic distribution of them, we did not identify any focus or outbreak during the period of this study.

P1838 Application of real-time PCR to *Meningococcus* detection in formalin-fixed tissues

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Objectives: Invasive meningococcal infection is a major cause of sudden death. *Neisseria meningitidis* has to be distinguished from other bacteria that can also be involved in Waterhouse-Friderichsen syndrome (WF). Due to the rapid development of such infections, antemortem cultures sometimes are not taken, and establishing the aetiology is needed. However, because of the unreliability of postmortem cultures a molecular diagnosis is needed. The aim of this study was to investigate the presence of meningococcus in formalin-fixed tissues from a legal sudden death by a real-time PCR assay.
Methods: The microbiological and histopathological analyses from a legal autopsy of an adult with a WF were performed. The possibility of a malpractice was being investigated. Formalin-fixed heart, liver, lung, kidney and supra-adrenal glands were the only available samples. DNA extraction from parafin-embeded sections of these tissues was performed with the Mini Kit Extraction System (QiaGen). Detection of *ctrA*, a gene target specific for *N. meningitidis*, was accomplished by a real-time PCR assay using a MGB-probe developed on the ABI 7000 Sequence Detection System. Posterior serogrouping was performed by amplification of the group specific *siaD B* and *C* genes. All real-time PCR assays were performed twice. Sensibility and specificity studies were also carried out to evaluate the PCR assays in postmortem samples.
Results: Positive real-time PCR results for *ctrA* were obtained in heart, liver, lung and kidney. The samples Ct (cycle number to reach the baseline threshold) values were between 34 and 39. serogroup B was detected by the *siaD* PCR assay. On the contrary, a postmortem haemoculture only yielded contaminants. The *ctrA* primer set amplified DNAs from meningococcal serogroups A, B, C, W135 and Y. There was no cross-reactivity with any of the other bacterial DNA extracts tested.
Conclusions: A reliable microbiological identification is particularly important in legal sudden deaths due to meningococcus, which has to be distinguished from other bacteria. We report the detection of meningococcus by real-time PCR in paraffin-embedded formalin-fixed tissues from a legal autopsy of a WF case. *Meningococcus* real-time PCR performed in formalin-fixed tissues may be of great help in the diagnosis of fulminant deaths when no other samples are available.

P1839 Amplification of capsule b locus in invasive *H. influenzae* strains isolated from children with Hib conjugate vaccine failures

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Objectives: *H. influenzae* type b (Hib) strains isolated from invasive disease generally possess a duplication of the capsule (cap b) locus. Amplification up to five copies has been reported and has been proposed to be a mechanism to evade host defence. To verify if amplification of cap b locus is involved in vaccine failure we determined the number of copies of the cap b locus in Hib isolates from true vaccine failures (TVFs) and from age-matched controls.
Methods: A total of 189 invasive Hib strains isolated from infants or children in the UK and the Republic of Ireland were tested. Of these, 95 were from patients with TVFs and 94 from age-matched controls. The copy number of cap b locus was determined by Southern blot analysis, using as a probe the 480 bp amplicon (capsule type b-specific) obtained by PCR of the Hib strain Eagan. As the KpnI and SmaI sites flank the cap b locus, the copy number can be estimated by the size of the restriction fragment obtained following digestion of the chromosome with these enzymes. The DNA fragment for a two-copy strain was expected to be approximately 45 kbp; strains with three or more copies of the locus featured fragments of increased size (63, 81 and 99 kbp).
Results: Most isolates both in the TVFs and the control group exhibited hybridisation signals at the expected position for a two-copy arrangement of cap b locus. However, besides the 45 kbp band, several isolates containing the two-copy arrangement showed strong hybridisation signals at molecular weights higher than 50 kbp, indicating the presence of multiple copies (three, four and five repeats) of the locus. A significantly greater proportion of isolates from patients with TVFs contained multiple copies compared with strains from controls. In fact, 24/95 strains harboured three or more copies of the locus in the TVF group, vs.

11/94 among controls ($P = 0.016$). Interestingly, the presence of multiple copies was significantly associated with a greater proportion of clinical presentations other than meningitis in children belonging to both the TVFs and control groups.
Conclusions: Our results show the number of multiple copy strains found among TVFs was significantly higher than in the control group. Although cases of invasive Hib disease in vaccinated children have been generally related to clinical or immunological conditions of the host or the use of less immunogenic vaccines, these data suggest that amplification of cap b locus may also be involved.

P1840 Comparison of conventional culture and PCR techniques in detection of *Haemophilus influenzae* in cerebrospinal fluids and middle ear effusions

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Objectives: Otitis media with effusion (OME) is one of the major causes of hearing loss in childhood, it is known that *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* are considered the chief pathogens of OME in children. Bacterial meningitis is still a disease with high morbidity and mortality rates despite the effective antimicrobial therapy. *H. influenzae*, *S. pneumoniae*, and *Neisseria meningitidis* are responsible for the most cases. These micro-organisms in both bacterial meningitis and OME have a fastidious characteristics in conventional culture, that have not been able to make clear cascade for identification, therefore, PCR has been applied to detect the bacterial DNA in cerebrospinal fluid (CSF) and middle ear effusion (MEE) specimens, and to evaluate the significant diagnostic value of PCR technique compared with conventional culture methods as 'gold standard'.
Methods: A total of 53 CSF and 22 MEE samples were collected from meningitis- and OME-suspected children, respectively, that were tested by both conventional culture and PCR methods.
Results: One of 53 CSF (*S. pneumoniae*) and three of 22 MEE (one *H. influenzae* non-b and two *M. catarrhalis*) samples were culture positive. The PCR revealed genomic DNA sequences of 5 (*S. pneumoniae*) from 53 CSF samples, and eight (three *H. influenzae*, three *M. catarrhalis* and two yielding both *S. pneumoniae* and *M. catarrhalis* with each others) from 22 MEE samples. The sensitivity, specificity, positive predictive value, negative predictive value, and the correlation rate for the total study samples from both CSF and MEE were 100, 87.3, 30.8, 100 and 88%, respectively.
Conclusions: It is concluded that PCR technique is a more sensitive, more rapid and appropriate method than conventional culture for detection of the most three common micro-organisms that lead to meningitis and OME in children.

Emerging infectious diseases

P1841 A prospective multicentre study on community-acquired pneumonia in the postoutbreak period of SARS in South Korea: a role of atypical pathogens including SARS-CoV

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Background: Since global threat of severe acute respiratory syndrome (SARS) outbreak, originally described as a severe atypical pneumonia in the Guangdong Province of China, has successfully been contained on early July, 2003, many countries remain vigilant for resurgence of SARS. We investigated the causative roles of SARS coronavirus (SARS-CoV) and atypical pathogens in community-acquired pneumonia (CAP) in the post-outbreak period.
Methods: From September to November 2003, a prospective multicentre study was performed to determine the atypical causes of CAP including SARS-CoV. Clinical samples collected from CAP patients between February and May, 2003 were also examined. To detect SARS-CoV, RT-PCR and Indirect IFA and rapid antibody tests were performed for respiratory specimens and paired sera, respectively. Tests for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Legionella* species, and respiratory viruses (influenza virus A and B, adenovirus, parainfluenza virus, respiratory syncytial virus) included PCR, urinary antigen test, indirect IFAs and virus culture.
Results: A total of 134 patients were enrolled in this study (103 patients prospectively and 29 patients retrospectively). SARS-CoV was not detected in any patients. Based on seroconversion, high antibody titre together with the presence of bacterial DNA in respiratory secretions (*M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila*) or bacterial antigen in urine (*L. pneumophila*), *M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila* were responsible for 12.2, 10.7 and 13.9% of cases, respectively. Respiratory viruses were not detected except for adenovirus in one patient.
Conclusions: Although SARS-CoV was not detected in this post-outbreak surveillance, we must remain vigilant for reemergence of SARS. Atypical bacterial pathogens appeared more prevalent than the previous reports in South Korea.

P1842 Detection of SARS-CoV by oligochromatography of RT-PCR amplicons

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The severe acute respiratory syndrome (SARS) is a new respiratory infection that has been reported in several countries around the world. It is recognised as a new type of atypical pneumonia that infects the lungs, caused by a new strain of coronavirus, SARS-CoV. SARS has killed at least 770 people and infected more than 8500 worldwide since the outbreak in China's Guangdong province late 2001. Molecular amplification has emerged as a powerful technology for the specific and sensitive detection of such a virus. However, the use of this new technology in routine laboratories requires a rapid, easy-to-perform and affordable detection method. Analysis on agarose gel is time-consuming and cannot exclude false-positive products while alternate specific detection methods are time and labour-consuming (ELISA) or require very expensive equipment (real-time amplification). In order to perform an easy and specific detection of amplified gene products, we set up and tested a new detection technology: oligochromatography. An internal probe to the nucleic acid sequence to be detected is conjugated to collodal gold particles. A conjugate pad containing this dried conjugate is placed overlapping the membrane of a chromatographic stick where anti-hapten antibodies are coated. Amplification is carried out with hapten-labelled primer(s). Detection is performed by dipping an oligochromatographic stick in the amplified solution. While migrating in the stick, amplicons react with the colloidal gold-conjuguated probe. The probe-amplicon complexes accumulate on the line where the anti-hapten antibody is coated, giving rise to a visible red line in <5 min. A highly sensitive RT-PCR for the detection of SARS-CoV was developed. It contains an internal control allowing to test for the absence of PCR inhibitors in the amplified material. Detectability of this RT-PCR coupled to oligochromatographic detection was compared with agarose gel electrophoresis with ethidium

bromide staining. Oligochromatography shall probably become the best way to specifically detect nucleic acid sequences after a molecular amplification process. It is rapid, specific, easy to handle, and does not require any specific equipment.

P1843 Experience in PCR diagnosis of SARS coronavirus in Taiwan

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Objectives: An outbreak of the severe acute respiratory syndrome (SARS) occurred in Taiwan in April, 2003, therefore we urgently established a nested RT-PCR and a real time RT-PCR to detect the SARS coronavirus.
Methods and Materials: From May 5th to August 31st, we examined 2585 specimens from 814 patients or medical workers. Viral RNA was extracted with the Blue Extract solution or the Qiagen viral kit from various clinical specimens. Each sample was tested in duplicate by both PCR methods.
Results: Eighty-eight of the 257 specimens from patients suspected to have SARS were positive for both PCR assays. These specimens included sputum (65%, 26/40), throat swabs (52%, 16/31), gargles (13%, 4/32), stool (35%, 17/48), urine (18%, 9/49), and blood (32%, 18/57).
Conclusions: Sputum and throat swabs were found to be the best sample types for the PCR assays, whereas gargle and urine samples were the least satisfactory. The first serum samples from 10 of the 30 SARS-probable patients were PCR positive, suggesting that serum samples can be used for early diagnosis of SARS.

P1844 Study of Q-fever on the territory of the Republic of Moldova

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Objective: Study of epidemiological and epizoological situations of Q-rickettsiosis, for determination the criteria of Q fever natural focus on the territory of the Republic of Moldova.
Methods: The research was carried out in the period 1997–2002. During the work were used standard zoological, parasitologic and bacteriologic methods. The caught small animals, gathered Ixodid ticks, people and farming animals blood serum were investigated in the laboratory of Extremely Dangerous Infection of the National Scientific-Practical Center of Preventive Medicine of the Republic of Moldova.
Results: During the investigation period the positive results of Q-rickettsiosis, from different carriers, were found in 30 areas. - Out of 3946 investigated persons suspected of Q fever, seropositive results were found at 2.46%. The cases of fever illnesses were registered since December till July in Southern and Central district of Moldova. It was established that most of the patients were related to farming animals' care or to processing of animal breeding products. The research into spontaneous contamination of *Coxiella burneti*, the dominant species of ticks in the republic (*Dermacentor reticulatus*, *D. marginatus*, *Haemaphysalis punctata*, *Ixodes ricinus*), gave the positive result in 62.19% of 6986 specimens. The study of epizoological situations among the feeders of all stages of ticks showed their presence in central and southern districts of the Republic of Moldova: among small animals were found antibodies of *C. burneti* at 0.17% of 2803 investigated specimens; in the blood of cattle at 1.9% of 207 and at 5% of sheep of 458 investigated animals.
Conclusions: Analysis of received materials allowed establishing the criteria of Q fever natural focus on the territory of the Republic of Moldova: (1) territorial coincidence at detection of Q-rickettsiosis signs at different natural objects, for example at farming animals and people; (2) presence of natural factors, especially in the foci of ticks increased number, among what it is a high percentage of *C. burneti* infection. According to these materials there were emphasised and described the most likely natural foci of Q-rickettsiosis on the territory of Moldova.

P1845 Human granulocytic Ehrlichiosis in Austria: epidemiological, clinical and laboratory findings in four patients

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Objective: The aim of our study was to determine, if *Anaplasma phagocytophilum* causes febrile illnesses after tick-bites in Western Austria.
Methods: Between May 2003 and October 2003, 167 patients with a nonspecific febrile illness, recent tick exposure and nonrevealing physical examination were tested for infection with *A. phagocytophilum* by a commercially available immunoflourescence assay and a real-time PCR targeting the *groEL*-gene.
Results: Four patients fulfilled the WHO-criteria for confirmed infection with *A. phagocytophilum* (human granulocytic Ehrlichiosis), i.e. PCR yielded a positive result with subsequent seroconversion (1 case) or they seroconverted against *A. phagocytophilum* with an at least fourfold titre rise within 2–4 weeks after onset of symptoms (three cases). All infections were acquired in the Inn valley between June and August 2003, three of four patients definitely recalled a tick-bite 1–2 weeks prior to onset of symptoms. Three patients required hospitalisation, one patient was treated as an outpatient. During the acute stage of illness laboratory findings included thrombocytopenia (100%), elevated CRP (100%), elevated neopterine (100%), elevated LDH (75%). Leucopenia, elevation of liver enzymes and elevated procalcitonin were less commonly observed. All patients were treated with tetracyclines, which lead to prompt improvement of clincal conditions.
Conclusions: Our findings demonstrate the presence of human granulocytic Ehrlichiosis in western Austria. It should be included in differential diagnosis of febrile illnesses after previous tick-exposure.

P1846 *Bartonella* infections in the British Isles: review of 7 years of diagnostic testing

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Objectives: To review microbiological data and basic demographics of *Bartonella* infections in the British Isles obtained during 7 years of routine laboratory testing.
Methods: Since January 1997, to aid the clinical diagnosis of *Bartonella* infections in the British Isles, RSIL has offered a service for the estimation of *Bartonella* antibodies using the Focus *Bartonella* IFA IgM and IgG kits. Serological data were reviewed for all patients from whom samples had been submitted during the 7 years to November 2003. All sera were examined in the indirect immunofluorescent antibody test according to the manufacturers' instructions: patient's age and sex were also recorded. Referral forms were reviewed for the first 1000 consecutive patients with positive serology and from the clinical details each was classified as a case of: Cat Scratch Disease (evidence of lymph node involvement and/or histological evidence), Bacillary Angiomatosis/peliosis, *Bartonella* endocarditis, PUO, other recognised manifestation (e.g. ophthalmic bartonellosis) or insufficient details. Serological results were interpreted as 'no evidence of recent infection', 'evidence of current/recent infection', 'suggestive of infection at an undetermined time' or 'evidence of past infection' as indicated by the manufacturers. Where available, biopsy samples from seropositive patients were examined by PCR to detect *Bartonella* DNA.
Results: Samples were received from 10507 patients. Overall 1759 (16.7%) patients had evidence of infection: 947 (9.0%) recent/

current infection, 740 (7.0%) infection at an undetermined time and 72 (0.7%) past infection. Analysis by age showed that rates of infection were highest in the decades 0–9 years (20.1%) and 10–19 years (18.7%), but they fell only a little in the next three (20–29: 17.2%, 30–39: 16.8%, 40–49: 14.9%). Of the first 1000 seropositive patients 449 had reported clinical features of CSD, 56 *Bartonella* endocarditis, 19 PUO, 3 BA, 40 other manifestations and 433 insufficient details. PCR analysis of excised heart valves from some of the endocarditis patients confirmed *B. henselae*, *B. quintana* and *B. vinsonii* as causative agents.
Conclusions: *Bartonella* spp. are a major cause of zoonotic infection in the British Isles in all age groups but particularly in children. *Bartonella* infection should always be considered as a possible aetiology in patients with endocarditis. Serology is an excellent and cost effective approach to the diagnosis of these infections.

P1847 Weil's disease in Northern Greece. Correlations between clinicoepidemiological aspects and laboratory parameters in 88 patients

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Objectives and aims: Leptospirosis has been described as a zoonosis of protean manifestations. This study was elaborated to search for associations between the multiform clinical course of the disease, on one hand, and selected epidemiological, clinical and laboratory parameters on the other.
Material and methods: During the period 1981–2000, 88 patients with Weil's disease, 85 males and three females (mean age 41.7 ± 14.2 years) were admitted to our hospital. Diagnosis was based on the clinical manifestations of the disease, and was subsequently confirmed serologically by ELISA method. All the clinical and laboratory data were assessed and multivariate analysis was performed.
Results: (a) Older patients (41–74 years, $n = 44$), compared with younger (16–40 yrs, $n = 44$) had: (1) longer duration of the disease (20.4 vs. 16.8 days, $P < 0.05$) and of antibiotic administration (9.1 vs. 6.7 days, $P = 0.007$), (2) twofold elevation of CPK levels (983 vs. 451.1 U/L, $P = 0.02$), (3) higher values of BUN (163.5 vs. 110 mg/dL, $P = 0.005$), total BIL (19.8 vs. 13.9 mg/dL, $P = 0.05$), as well as WBC (13.55 vs. 9.46×10^3/mL, $P < 0.0001$). (B) Patients with azotemia (BUN $\geq$ 90 mg/dL, $n = 48$), compared with those without azotemia ($n = 40$): (1) were older (44.6 vs. 38.3 years, $P < 0.05$), (2) had higher values of total BIL (22 vs. 10.5 mg/dL, $P < 0.0001$), (3) lower values of PLT (86.1 vs. 115×10^3/mL, $P = 0.04$) and Hb (10.8 vs. 12 g/dL, $P = 0.035$), (4) was the only patient group with complications (acute renal failure, myocarditis, pericarditis). (C) Patients with jaundice (total BIL > 3 mg/dL, $n = 69$), compared with those without jaundice ($n = 19$): (1) were older (43.1 vs. 34.4 years, $P < 0.05$), (2) had shorter symptomatic preadmission period (6.5 vs. 9.9 days, $P < 0.007$), (3) were administered antibiotics for longer time (8.8 vs. 5.4 days, $P < 0.02$), (4) had lower Hb levels (10.9 vs. 12.2 g/dL, $P < 0.003$), and higher WBC count (11.7×10^3/mL vs. 8.72×10^3/mL, $P < 0.01$).
Conclusions: The course of Weil's disease is more severe in older patients with azotemia and/or jaundice. Complications are related to the patients with azotemia.

P1848 Tick-borne pathogens in Danish roe deer

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Objectives: Roe deer-associated tick-borne infections form a large portion of emerging infections in Denmark. The aim of this study was to describe the seroprevalence of *Anaplasma phagocyophilium*, *Borrelia burgdorferi* s.l. and tick-borne encephalitis virus (TBE) in Danish roe deer (*Capreolus capreolus*) and at the same time to evaluate *Anaplasma* spp. infection by PCR.
Methods: Blood samples from 237 roe deer were collected during the hunting season (15/5–15/7 2002 and 1/10–15/1 2003). Samples were from 22 of 25 Danish State Forest districts. An indirect immunofluorescence assay was used to identify sera reactive against *A. phagocytophilium* and *B. burgdorferi*. For TBE serology haemagglutination inhibition (HI) test was used and positive samples were verified using neutralisation assay. Blood samples were tested for *A. phagocytophilium* genogroup 16S rRNA sequences by PCR. Positive samples were sequenced.
Results: *Anaplasma* seroprevalence was 87% with some variation between the two hunting periods (83%–91%). Overall a mean of 47% roe deer were found *Anaplasma* positive in PCR. Marked seasonal variation in PCR positive roe deer was found between the two hunting periods (71% vs. 26%). Sequencing revealed besides *A. phagocytophilium*, *A. platy* in two roe deer and a co-infection with an uncultured eubacterium (AJ 292457) *Borrelia* seroprevalence was 36% with even distribution between the two periods and no regional variation. TBE seroprevalence was 9% and positive animals were found in 10 forest districts.
Conclusion: These findings confirm that roe deer in Denmark are commonly infected with *Anaplasma* and provide evidence that roe deer are a major reservoir of *Anaplasma*. *Borrelia* seroprevalence in Denmark has not increased or changed in distribution over the last decade, whereas TBE distribution has increased and is now not only limited to the island of Bornholm.

P1849 Rapid PCR-based method for detection of orthopoxviruses and of other viruses important for differential diagnosis of smallpox

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Objective: Variola virus, belonging to Orthopoxviridae family, is one of the most dangerous biological agents that could be used as biological weapon. Problems associated with such an event are mainly due to high morbidity and mortality, absence of herd immunity in young population, and difficulty in the clinical diagnosis, due to the similarity with other exantematous diseases. Laboratory diagnosis is crucial to increase global preparedness to such an event. As culture-based methods are only applicable under maximum biosafety level, molecular methods are preferred for wide application, as they can be used in laboratories were BSL-3 or higher facilities are not available. To this aim, we established a rapid PCR-based protocol for the contemporary detection of Orthopoxviruses, VZV and HSV, that are relevant for differential diagnosis.
Methods: The target for detection of orthopoxvirus DNA is a region of the crmB gene, which is common to variola virus and to other old world orthopoxviruses pathogenic for humans. The VZV and HSV targets are ORF 29 and DNA polymerase, respectively. Suitability of the amplified fragments to RFLP or sequencing analysis was also tested, to recognise the involved viral species.
Results: Three primer set, for the three viruses, were selected, showing high sensitivity and specificity, and compatibility with common amplification conditions. The test conditions selected were validated by using biological samples from patients showing herpesviral infections, and, for orthopoxviruses, laboratory strains including vaccinia, camelpox, cawpox, monkeypox viruses. The results indicate that, by this method, it is possible to obtain rapid differentiation of herpesviral from orthopoxviral infections using a common amplification run. Furthermore, the amplicons obtained in this phase are suitable for further molecular analysis, allowing virus identification and definitive laboratory diagnosis.
Discussion: The PCR-based protocol here established for differential diagnosis of orthopoxvirus and herpesviral infections is rapid and specific. The amplification products are suitable for further molecular analysis, leading to definitive laboratory diagnosis under easily achievable laboratory conditions, without biological hazard for laboratory personnel. This method can be used also to detect orthopoxviral infections due to imported viruses, such as monkeypox. Work supported by grant by RF 2001-01.102.

P1850 Prevalance and impact of human meta pneumo virus among children and immunocompromised patients

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Objective: To determine the prevalence of Human Metapneumovirus (HMPV) infection among children and immunocompromised patients presenting with respiratory symptoms in the season November 2002 to March 2003.
Methods: Nasopharyngeal aspirates (NPAs) were collected from 55 patients and tested by direct immunofluorescence and virus culture for the presence of common respiratory viruses (CRVs) including respiratory syncytial virus (RSV), influenza viruses A and B, parainfluenza viruses (PIV), adenoviruses, enteroviruses and rhinoviruses. Specimens that tested negative for CRVs were submitted to a central laboratory and retrospectively tested for the presence of HMPV by PCR.
Results: The study included two groups. Group A, 29 children (M 12, F 17; mean age: 6 months, range 9 days–2 years) requiring hospitalisation for bronchiolitis, pneumonia or respiratory distress. Of these, 14 (48%) tested CRV positive (RSV 11, PIV-3 1, Enterovirus 1, Flu B 1) and 4 HPMV positive. The latter presented with disease ranging from mild-moderate wheeze, to pneumonia and respiratory failure. All four patients made a full recovery with supportive therapy. Group B, 26 patients (M 18, F 8; mean age: 39.1 years, range 2–80 years) who had either received stem cell transplantation (SCT, $n = 16$) or were receiving myelotoxic chemotherapy for haematological ($n = 15$) or other ($n = 1$) malignancies. Of these, 8 (31%) tested CRV positive (Flu A 2, RSV 6). One recipient of allogeneic STC was HMPV positive. The 32 year old patient was admitted on day 28+ after Sibling HLA-Identical T-cell depleted BMT with fever and bilateral lung shadowing. A bronchoalveolar lavage (BAL) was negative for fungal and bacterial pathogens. Both a NPA and the BAL were negative for viral pathogens. He received therapy with broad spectrum antibiotics and antifungals, but progressed to respiratory failure and died at day 57+. The diagnosis of HMPV pneumonia was obtained retrospectively.
Conclusions: These data are minimal estimates of the prevalence of HMPV, as dual CRV HMPV infections were not excluded. HMPV was common among children hospitalised with RSV-like disease, accounting for 14% of such presentations (RSV 38%). Although HMPV was less common among immunocompromised patients than RSV (4% vs. 23%), it was associated with fatal pneumonia in the post-SCT setting. These data highlight the potential for significant morbidity and mortality associated with HMPV infection.

P1851 Isolation and characterisation of spotted fever group rickettsiae in Taiwan

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Objective: Spotted fever group (SFG) rickettsioses have been recognised as important emerging vector-borne human diseases worldwide. Several distinct SFG rickettsiae had been reported just near by Taiwan, such as *Rickettsia sibirica* (in China), *R. australia* (in Australia) and *R. japonica* (in Japan). This study therefore sought to isolate and characterise the SFG rickettsiae from arthropods in Taiwan.
Methods: All arthropods were collected from rodents captured in Kinmen, Matsu and Taiwan main-island. The citrate synthase (gltA) and 17 kDa protein genes were partially or completely amplified and sequenced for determining the phylogenetic relationship between Taiwan strains and other SFG rickettsiae.
Results: After BLAST comparison, three SFG rickettsia strains named TwKM01, TwKM02 and TwKM03 were identified and were most similar to *R. rhipicephalis*, *R. australisi* and *Rickettsia* spp. California, respectively. Sera from TwKM01, TwKM02 and TwKM03 infected rodents were positive to *R. rickettsii* by indirect immunofluorescent assay indicated that SFG rickettsia might widely distributed in wild rodents of Taiwan. TwKm01 and TwKm02 were first detected from *Rhipicephalus haemophysaloides* tick and Leptrombidial chiggers. A recombinant 17 kDa protein had been expressed and purified by the His-tagged affinity column, in order to further evaluate the prevalence of SFG rickettsial infection in humans and wild rodents.
Conclusions: Although the infectivity and pathogenicity of these three SFG rickettsia for vertebrates are unknown, this finding raises the possibility that bacteria other than *Orientia tsutsugamushi*, *Coxiella burnetii* and *R. typhus* may be involved in rickettsial diseases in Taiwan.

P1852 Fluoroquinolones vs. macrolides in the treatment of Legionnaires' disease

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Objectives: Erythromycin has been the treatment of choice for Legionnaires' disease (LD). However, new drugs such as fluoroquinolones are currently under evaluation. In a comparative study in 2001 we found fluoroquinolones to be as effective as erythromycin in LD treatment and time to apyrexia and hospital stay tended to be shorter in patients on quinolones, although the small number of patients included did not allow significant differences to be found. We have now included cases of LD from 2 other centres in Spain, which use both of these antibiotics. The present study compares the evolution of patients with LD treated with macrolides or fluoroquinolones.
Methods: We performed a prospective observational study in 130 patients diagnosed by *Legionella* urinary antigen. Patients receiving any antibiotic more than 36 h before starting the study therapy were excluded. The patients were divided into two groups: 76 in group 1 receiving macrolides (erythromycin/clarithromycin) and 54 in group 2 receiving fluoroquinolones (ofloxacin/levofloxacin).
Results: No significant differences were seen between the two groups regarding age, sex, smoking, alcohol intake, underlying diseases or community/hospital acquisition. Time from onset of LD symptoms until starting antibiotic treatment was 78.5 and 92.7 h in groups 1 and 2, respectively ($P = 0.1$). Time to apyrexia was significantly longer in the macrolide group (77.1 vs. 48 h for groups 1 and 2, respectively) ($P = 0.000$). There were no differences in radiologic, clinical complications or mortality. However, a trend to longer hospital stay was observed in Group 1 (9.9 vs. 7.6 days in Groups 1 and 2, respectively) ($P = 0.09$).
Conclusions: Fluoroquinolones were found to be as effective as erythromycin in the treatment of LD. Time to apyrexia was significantly shorter and hospital stay tended to be shorter in patients on fluoroquinolones.

P1853 The characteristics of Crimean Congo haemorrhagic fever in a recent outbreak in Turkey and the impact of oral ribavirin therapy

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Objective: The epidemiological, clinical, and laboratory findings of the patients diagnosed as Crimean-Congo haemorrhagic fever (CCHF), and the role of ribavirin therapy were described.
Methods: The patients infected with CCHF virus in 2002 and 2003, and hospitalised in Ankara Numune Education and Research Hospital were studied. All the patients had positive results of IgM, and/or IgG, and/or PCR results for CCHF virus in blood or tissue. The patients were grouped as mild-moderate or severe. The severity was defined as having thrombocytopenia <20 000, active bleeding, and somnolence.

Results: Thirty-five cases were eligible, and all were from the northeastern Anatolia and the southern parts of Black sea region; all of them were dealing with husbandry. The mean age was 43.4 (+17.3). Fifty-three per cent of the cases had the history of tick bite. The clinical status of 31% of the cases was defined as severe. The age, geographic residency, history of tick bite, mean number of days before hospitalisation, the complaints were not different between mild to moderate cases and severe cases ($P > 0.05$). The mean length of stay was longer among severe cases ($P = 0.040$). Females were more severely affected than males (RR, 1.8; CI, 0.2–1.5, $P = 0.146$). The decrease in haemoglobin level ($p = 0.042$), elevated AST ($P = 0.002$), ALT ($P = 0.007$), LDH ($P = 0.042$), and AST/ALT ratio ($P = 0.044$) were more common among severe cases. Five out of 11 severe cases were given ribavirin therapy according to WHO recommendations, and all survived. Six of the severe cases did not receive ribavirin therapy, one of them died. The overall case fatality rate (CFR) was 2.8%, which was the lowest rate in the literature. The CFR was increased to 17% among severe cases, who did not receive ribavirin. The cost-effectiveness assessment was limited to the severe cases, which includes the drug, laboratory, and hospital expenses. The cost of the infection in ribavirin group was 3661 per patient after excluding the outlier, whereas it was 4860 per patient among nonribavirin group.
Conclusions: CCHF was not reported before in Turkey, although epidemics were reported from the neighbouring countries. In conclusion, oral ribavirin should be administered to the severe cases, which have been suspected of having CCHF virus infection.

P1854 Phenotypic characters of *S. aureus* strains associated with 79 cases of toxic shock syndrome in the Czech Republic

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Objectives: The Czech reference laboratory for staphylococci has been paying attention to the Toxic Shock Syndrome (TSS) since 1983 when the disease emerged. Seventy-nine cases with required clinical records and diagnosis confirmed by toxinogenicity screening of *S. aureus* isolates have been documented in our laboratory to date. The aim of this study was to investigate relevant characteristics of strains.
Methods: The production of TSST-1 and enterotoxins A, B, C, D and E was detected by microslide-gel diffusion test, using Bergdoll's antisera until 1997. The RPLA method (Denka Seiken kit for types A, B, C, and D) has been used since 1998. The production of alpha, beta, and delta-haemolysin was detected based on either synergy or antagonism with beta-haemolysin of the *S. intermedius* standard strain on blood agar plates in mixed atmosphere. Phage typing was performed by the standard method using the international set of phages (PHLS London). Resistance to 12 antibiotics was tested by the disk diffusion method (Oxoid).
Results: The aetiological agents of eight cases of the menstrual form of TSS were *S. aureus* strains with TSST-1 production, mostly with parallel enterotoxin A toxigenicity. All of the patients survived. Other 71 cases of TSS were complications of the following staphylococcal infections: infected injury wounds (13 cases), postoperative hospital infections (11), and infected scaldings and burns (9). Fatal outcomes were reported in 17 cases, i.e. in three patients aged over 75 years, two patients with TSS-like complication of staphylococcal endocarditis and 12 other patients accounting for a significant lethality rate of 17%. As many as 43 (60%) strains produced TSST-1 and 34 of them in combination with enterotoxin of any type. The other 28 (40%) strains produced considerable amounts of the following enterotoxins: A (5 strains), B (9), C (10), D (2), and two strains produced types A + C or B + C.
Conclusions: TSS morbidity in the Czech Republic is evidently under-reported. TSS should be taken into account in differential diagnosis. Two of our patients with misdiagnosed streptococcal infection unresponsive to treatment with penicillin recovered when given oxacillin. TSS is a life-threatening illness. The work was partially supported by grant GACR 301/02/1505 from Grant Agency of the Czech Republic.

P1855 The recognition of two tentative novel species among haemolytic *Acinetobacter* strains

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Objectives: The genus *Acinetobacter* currently consists of 32 (genomic) species and a number of as yet unclassified strains. The aim of the present study was to classify haemolytic *Acinetobacter* strains of unknown taxonomic status isolated mostly from human clinical specimens.
Methods: Twenty-eight haemolytic strains that could not be identified as belonging to any known (genomic) species were studied by phenotypic analysis, amplified rDNA restriction analysis (ARDRA), AFLP fingerprinting and 16S rDNA comparative sequence analysis. Type or reference strains of all known *Acinetobacter* (genomic) species were included.
Results: Using a polyphasic approach, the investigated strains were classified into two well-separated phenetic groups, termed phenon 3 ($n = 8$) and phenon 7 ($n = 15$), five isolates remaining ungrouped. Each of the two phenons contained nonglucose-acidifying strains that showed identical or highly similar phenotypic properties and ARDRA profiles, and formed distinct AFLP clusters at a similarity level of >50% which is generally the species delineation level. In addition, 16S rDNA sequence analysis of three and two strains of phenons 3 and 7, respectively, indicated that these groups formed two separate lineages within the genus *Acinetobacter*. The phenons could be distinguished phenotypically from each other and from all known (genomic) species. The strains of phenon 3 were isolated exclusively from human clinical specimens whereas the phenon 7 strains originated from human ($n = 8$) and equine ($n = 2$) clinical specimens, hospital environment and staff ($n = 2$), or from soil ($n = 2$). The human clinical specimens were mostly represented by tracheal aspirate of hospitalised patients.
Conclusions: This study has shown that most of the unclassified haemolytic strains belong to two phenons distinct from all described (genomic) species of the genus *Acinetobacter*. These phenons probably represent two novel species as indicated by the presented results and by partial DNA-DNA reassociation data available from the previous taxonomic studies. The available epidemiological and clinical data suggest that the strains of both phenons may play a role in infections of hospitalised patients.

P1856 First Austrian case of a severe infection with a sorbitol-fermenting, Shiga toxin-producing *E. coli* O157 and first case of a documented transmission of such a strain by animal contact

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Objectives: Vero or shiga toxin (Stx)-producing *Escherichia coli* (VTEC) O157:H7 is a major cause of haemorrhagic colitis and haemolytic-uraemic syndrome (HUS) worldwide. Human infections with VTEC serotype O157 have been linked to transmission from animals. Cattle act as domestic animal reservoir for VTEC O157. The aim of this study was to investigate an outbreak of sorbitol-fermenting (SF) VTEC O157 infection affecting a farmer family during June 2003 in the Austrian province Salzburg.
Methods and results: The investigations started when a 10-month-old boy was admitted to the Childrens' Hospital in Salzburg on 07 June 2003 because of gastroenteritis accompanied by fever and jaundice. In the hospital the child developed a HUS. A stool specimen yielded SF VTEC O157. Isolate identification was confirmed by conventional biochemical tests and serotyping by latex test (Murex). Production of toxins by the isolates was assayed by EIA (Premier EHEC; Meridian, Milano, Italy) and presence of *stx*, *eaeA* and *hlyA* genes were confirmed by PCR. β-Glucuronidase activity and motility were also tested. As a part

of the case–control study conducted at the ARLV faecal specimens of the rest of the farmer family and their cattle were collected. An investigation was initiated to identify a source and contributing factors and to determine the extent of the outbreak. A stool specimen of the 2-year-old brother of the patient and two faecal samples of two cattle (one calf and one bull) also identified SF STEC O157. All four STEC isolates carried the virulence genes *stx* 2, *eaeA* and *hlyA*, and were characterised by automated ribotyping (using EcoRI as restriction enzyme) and pulsed-field gel electrophoresis (PFGE; with XbaI as restriction enzyme). The patterns of all four strains were indistinguishable from each other by both typing methods. SF VTEC O157 strains have been previously discovered in Germany and the Czech Republic.
Conclusions: This is the genuine Austrian case of a VTEC O157 and furthermore resembles the first case of such a strain with documented transmission by animal contact. It illustrates the hazards associated with animal contact and underlines the importance of microbiological diagnostic approaches designed to detect SF VTEC O157. These strains are normally missed by methods solely relying on sorbitol–Mac Conkey agar plates. Thus, stool specimens not only from patients with HUS but also from young children should at least be tested for the presence of Shiga toxins.

P1857 The molecular epidemiology of selected food-borne pathogens recovered from paediatric patients, indigenous peoples, and food animals in northern Thailand

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Objectives: Prospective epidemiological studies were conducted to determine the molecular relatedness of *Campylobacter* and *Salmonella* isolates from paediatric patients, indigenous peoples (farm and nonfarm workers), and food animals in northern Thailand.
Methods: Faecal samples were collected from food animals at farms, farm workers and their nonfarming neighbours (controls), animals and workers at slaughterhouses from 2000 to 2002, and from paediatric patients from three hospitals in Thailand in 2003. Multiplex polymerase chain reaction (M-PCR) assay of various genes was used to determine the species of *Campylobacter*. Single-stranded conformation polymorphism (SSCP) was used to determine the molecular profile of flaA gene of *Campylobacter*.
Results: A total of 369 *Campylobacter* and 221 isolates of *Salmonella* were processed during 2000–2002 from farm workers, nonfarm workers and food animals. Samples were also collected from three paediatric hospitals yielding 36 *Campylobacter* and 136 *Salmonella* isolates from 205 children <5 years of age with acute diarrhoea. The risk factors for *Campylobacter* infections in children age <5-year-old include consumption of pork (OR 2.86), of chicken (OR 5.27) and of milk products (OR 1.05). Comparison of SSCP profile showed that all *Campylobacter* isolated from paediatric diarrhoea cases shared similar molecular profiles with isolates from food animals at the farm. Ninety-four and 98% of *Campylobacter* isolated from the market and slaughterhouse share similar profile with *Campylobacter* isolated at the farm. Ninety per cent of *Salmonella* isolated from the paediatric diarrhoea patients had similar profiles with the *Salmonella* isolated at the farms, slaughterhouse or market.
Conclusions: Analysis of risk factors for acute diarrhoea showed a positive association with the consumption of pork and milk, but the association was not statistically significant, except for chicken (OR 5.27, $P = 0.043$) in relation to the *Campylobacter* infections in children <5 years old. There were also positive associations between the consumption of chicken and pork with *Salmonella* infection, but the association was not statistically significant. *Campylobacter* and *Salmonella* from food animals at the farm may be transmitted through the food chain and cause diarrhoea in children in Northern Thailand.

P1858 *Chlamydia pneumoniae* infection in carotid artery disease (CAD) patients and its clinical impact

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Objectives: The aim of our study was to assess the presence of *Chlamydia pneumoniae* infection in CAD patients and to evaluate its association with clinical symptoms.
Material and methods: Twenty-eight patients operated for CAD (eight females, 20 males; mean age 64.5 years) and 20 healthy controls took part in our study. Microimmunofluorescence method was applied to evaluate the level of anti-*C. pneumoniae* IgG, IgA and IgM. *C. pneumoniae* micro-IF test (Labsystem) was used. PCR method was applied for *C. pneumoniae* DNA detection in atherosclerotic plaques, obtained during carotid endartherectomy.
Results: Serological markers of chronic *C. pneumoniae* infection were detected in 22 of 28 (78.6%) patients and in six of 20 (30%) healthy controls. In 36.4% (eight of 22) of patients with serological markers of chronic *C. pneumoniae* infection high titres of specific IgG and IgA were noted. Interestingly, all patients in high serology group proved to have transient ischaemic attacks (TIA). *C. pneumoniae* DNA was present in carotid atherosclerotic plaques obtained from 17 (60.7%) patients.
Conclusions: Serological signs of chronic *C. pneumoniae* infection occur statistically more frequent in CAD patients in comparison with healthy controls. High titres of IgG and IgA against *C. pneumoniae* are associated with TIA symptoms.

P1859 Extraintestinal salmonellosis

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Objectives: To explore the profile and frequency of nontyphoid extraintestinal salmonellosis at the regional General Hospital Uzice.
Methods: We retrospectively surveyed (1972–2001) microbiology records of 360 patients, with primary nontyphoid *Salmonella* infections. Our strains were obtained from blood, cerebro-spinal fluid, burns, wounds, urines and sputa. We compared the number of these isolates with the total number of *Salmonella* isolates.
Results: Although we found 26 serotypes of all *Salmonella* isolates, only 16 serotypes and 360 strains were recovered from nonfaecal material. *S. wien* was the most prevalent isolate (77.30%), then *S. abony* (9.72%) and *S. enteritidis* (5.67%). In our hospital, the number of *Salmonella* isolates per year persists (average 93.8, range 35–261), but the number of extraintestinal isolates decreases, so in the last 10 years their total number was 10 and no one specimen yielded *S. wien*. Children and neonates were 46.21% of studied populaton.
Conclusions: Our findings strongly support the fact that *S. wien* is known to have a propensity for causing extraintestinal infections. But, nowadays it's rare in faecal and nonfaecal isolates and *S. enteritidis* prevails. As *S. enteritidis* is moderately invasive serovar, the total number of extraintestinal isolates is decreasing.

P1860 Extended-spectrum beta-lactamase producing *Salmonella isangi*, an emerging pathogen in South Africa

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Salmonella isangi (group C1) is a poorly known enteric pathogen peculiar to Africa. Since the national surveillance was introduced in 1999, isolates have been identified in South Africa. It is unusual in its presentation as it predominantly affects extreme ages, has a propensity to invade the blood stream and most importantly its multiple-antibiotic resistant which poses serious problems for management.

Objective: To determine the size of the problem in children less than 5 years of age with diarrhoea admitted to Tygerberg Hospital (Cape Town).
Method: The study covered the period from 01 October 2002 to 31 May 2003 where all cases of diarrhoea under the age of 5 years had a stool sample sent for bacteriological examination for *S. isangi*. Antibiotic sensitivity was performed on all isolates. Demographic data, which included age, sex, location and HIV status, where available, was collected on confirmed cases below the age of 5 years admitted to hospital with diarrhoea.
Results: There were 21 cases of ESBL producing *S. isangi* identified from stool culture (and blood culture). Sixteen (76.2%) of the 21 cases were nalidixic acid resistant. Three children died two of whom were HIV+. The median hospital stay (excluding the deceased) was 31.5 days. The ESBL producing *S. isangi* was community-acquired bacterium but was highly resistant to other antibiotics including nalidixic acid.
Conclusions: ESBL producing *S. isangi*, which is also nalidixic acid resistant, affects mostly the very young children from poor socioeconomic conditions. Further surveillance is warranted into reasons for the development of multiply-antibiotic resistance developing in this species.

P1861 Meningococcal carriage in an urban population in Burkina Faso in 2003

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Objectives: Recurrent epidemics of meningitis in Burkina Faso usually are caused by serogroup A meningococci, but a large W135 epidemic occurred in 2002. In order to define an appropriate future vaccination strategy, the propagation of this new epidemic strain in the population needs to be better known. This longitudinal study describes meningococcal carriage in an urban population of Burkina Faso during the 2003 meningitis season.
Methods: In a representative sample of residents between 4 and 29 years of age in Bobo-Dioulasso, Burkina Faso, oro-pharyngeal swabs were taken from each participant at five study visits between February and June 2003. The swabs were analysed by culture and all isolated *N. meningitidis* were serogrouped by PCR [A, B, C, W135, Y; nonserogroupable (ns)].
Results: Of 491 participants included, 407 came to all five visits without use of any relevant antibiotic treatment during the 4 weeks before the visits. The 5-months carriage prevalence was 7% (95% confidence interval 5, 11) for serogroup W135, 0.3% (0.03, 2) for A, 0.7% (0.2, 4) for Y, 14% (11, 18) for ns, and 14% (10, 17) for *N. lactamica*. No serogroup B or C was found in carriage. A significant difference between the age strata was found only for carriage of *N. lactamica*: 21% in 4–14 years vs. 6% in 15–29 years ($P = 0.002$). Recent meningococcal vaccination status did not have any impact on carriage. During the observation time, only ns meningococci demonstrated an increase in the point carriage prevalence (from 1 to 7%, $P < 0.001$); for serogroup W135, the point prevalence at each visit was approximately 2%. Antibiotical resistance was found against oxacillin (36% of *N. meningitidis*. strains) and cotrimoxazole (11%), but not for chloramphenicol, ciprofloxacine, ceftriaxone, or penicillin (resistance in <1% of strains). ns meningococci are currently being analysed by immunsera.
Conclusions: Concurrent meningitis surveillance during the study season in Bobo-Dioulasso found that W135 and A caused an approximately equal number of meningitis cases. Nevertheless, we found that W135 was the most commonly identified *N. meningitidis*. Serogroup among healthy carriers with a 5-month prevalence over 20-fold higher than serogroup A. Combined, these findings suggest that serogroup W135 may require a substantial carriage burden for the propagation of epidemic disease while serogroup A may have a higher virulence.

ESBL and metallo-beta-lactamases - II

P1862 Evaluation of extended-spectrum beta-lactamase (ESBL) among *K. pneumoniae* and its susceptibility pattern among infection isolates from a university hospital in So Paulo, Brazil

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K. pneumoniae (Kp) ESBL are also a growing concern in our University Hospital in So Paulo, Brazil. When producing ESBLs those strains become highly efficient at inactivating third and fourth-generation cephalosporins and in addition may frequently be resistant to other classes of antibiotics, resulting in difficulties-to-choose adequate empiric therapy before antibiogram results become available.
Objective: Evaluation of ESBL prevalence among Kp from blood and urine nosocomial isolates during 2002 and analysis of its specific antimicrobial susceptibility pattern.
Methods: The 624 Kp strains (no duplicates) were tested for ESBL by three-disc-combination methodology (ceftazidime, ceftriaxone and cefpodoxime with and without clavulanic acid; Oxoid). Susceptibility tests were performed by the Vitek System (GNS-650 and GNS-204) and interpretations were made according to current NCCLS recommendations. QC strains achieved properly results. Data analyses were performed before any report susceptibility change due to ESBL detection.
Results: 57% from 189 blood isolates were ESBL positive and 55% from 455 urine isolates.

Susceptible patterns were as follow from blood and urine respectively: See Table below.

Antimicrobial agent	Blood	Urine
Amikacin	67%	59%
Gentamicin	49%	44%
Aztreonam	4%	7%
Cefepime	27%	30%
Ceftazidime	58%	38%
Cefotaxime	11%	21%
Cefoxitin	98%	87%
Cephalothin	3%	2%
Ciprofloxacin	43%	37%
Imipenem	100%	100%
Piperacillin/taz	43%	42%
Trimethoprim-sulfa	20%	15%
Nalidixic acid	NA	31%
Nitrofurantoin	NA	27%
Norfloxacin	NA	40%

Conclusion: Kp ESBL susceptibility data from both clinical sources were similar. Amikacin was the most active agent after imipenem witch narrows therapeutical choices. Cefotaxime was more affec-

ted than ceftazidime and this resistance phenotype can be considered an important preliminary indicator of CTX-M producing strains, even though a genotyping confirmation of this enzyme is essential in this study. Epidemiological and molecular studies will be conducted to establish the relationship between ESBL producing isolates and to characterise the different types of ESBL in our hospital.

P1863 Risk factors for acquisition of carbapenem-resistant *Klebsiella pneumoniae* among hospitalised patients

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Objectives: Carbapenem has been therapeutically used for patients infected with organisms that are resistant to multiple classes of antibiotics, including third-generation cephalosporins. The use of carbapenem has resulted in the emergence of carbapenem-resistant mutants and *K. pneumoniae* has been included in the class of bacterial species that are likely to be resistant to carbapanem since 1997. This study aimed to identify risk factors for the nosocomial isolation of carbapenem-resistant *K. pneumoniae* from clinical specimens.
Methods: A case–control study was conducted for the intended objective. Case patients were defined as patients from whom carbapenem-resistant *K. pneumoniae* was isolated in clinical cultures. Control patients were randomly selected by the ratio of 4:1 from the same medical or surgical services that hospitalised case patients at the time of isolating carbapenem-resistant *K. pneumoniae*. Risk factors that were investigated included antimicrobial drug exposures, demographic variables, comorbid conditions, and variables related to hospitalisation.
Results: Carbapenem-resistant *K. pneumoniae* was isolated from 30 patients during the period of January 1997 to August 2003. 93.3% of carbapenem-resistant *K. pneumoniae* was analysed as ESBL(extended-spectrum beta-lactamase)-producing organisms. Carbapenem-resistant *K. pneumoniae* was recovered from bile (36.7%), most frequently. The rest was recovered from clinical culture of wound specimens (23.4%), ascitic fluid (16.7%), and blood (13.3%). Logistic regression analysis demonstrated the previous use of carbapenem (odds ratio 28.68; $P < 0.001$) and cephalosporin (odds ratio 4.10; $P = 0.013$) as significant risk factors for the acquisition of carbapenem-resistant *K. pneumoniae*. The use of quinolone appeared to be protective (odds ratio 0.26; $P = 0.045$).
Conclusions: Carbapenem resistance in *K. pneumoniae* is a particularly disturbing development in the world of antibiotic resistance. This study identified carbapenem and cephalosporin as risk factors. The protective effect of quinolone against isolation of carbapenem-resistant *K. pneumoniae* is issue for further investigation.

P1864 Incidence rate and detection of *Klebsiella pneumoniae* producing extended-spectrum beta-lactamase in a department of anaesthesiology and resuscitation, Ceske Budejovice

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Introduction: *Klebsiella pneumoniae* is the first pathogen to be found producing extended-spectrum beta-lactamases (ESBLs), mainly due to TEM and SHV enzymes gene mutation. The mutation development is facilitated by frequent empiric use of third generation cephalosporins in the ICU setting.
Methods: To detect the presence of ESBL, we use Double Disc Synergy Test, utilising the following discs: amoxicillin/clavulanic acid (AMC)-disc contents 20/10 μg, ceftazidime (CTZ) – 30 μg, cefpodoxime (CPD) – 10 μg, aztreonam (AZT) – 30 μg. The test is based upon the susceptibility of extended spectrum beta-lactamases to clavulanic acid. A positive result is manifested by characteristic extension of inhibition zone between the discs with AMC and a third generation cephalosporin (or AZT). The test is performed in *K. pneumoniae* strains that show intermediate susceptibility or resistance to second and third generation cephalosporins.
Results: From 2000 till 2003, the number of patients who tested positive for ESBL producing strains of *K. pneumoniae* dropped from 80 (17%) down to 2 (0.4%). Concurrently, the use of third generation cephalosporins (ceftazidime, cefotaxime, ceftriaxone, cefoperazone) was reduced from 3.5 g down to 2 g per one hospitalised patient. A certain contribution should also be attributed to a more strict adherence to hygienic guidelines (patient isolation, handwashing, single-use devices, etc.).
Summary: The incidence rate of multiresistant, ESBL-producing strains of *K. pneumoniae* may be mitigated by controlled broad-spectrum antibiotic use and a stricter adherence to hygienic guidelines.

P1865 Extended-spectrum beta-lactamases in *Klebsiella pneumoniae* isolates from a Madrid hospital: an updated complex situation (2001–2003)

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Background: Although ESBL producing *Escherichia coli* isolates have rising to prominence during the last years, *K. pneumoniae* (Kp) is maintained as an important reservoir of these enzymes. The aim of this study was to analyse ESBL producing Kp isolates recovered in our institution January 2001 to June 2003 and compare current situation with that previously found (1988–2000) (Coque *et al.* (2002) AAC **46**, 500–510).
Methods: All Kp isolates recovered during the studied period were screened for ESBL production. One isolate per patient was selected for clonal typing (XbaI-PFGE) and ESBL characterisation (IEF, PCR, and sequencing).
Results: A total of 56 ESBL producing Kp (3.7%) isolates recovered from 35 patients (43%, surgical wards; 23%. ICU; 14%, medical wards; 20%, outpatients) were identified. The proportion of outpatients with ESBL-Kp isolates significantly increased ($P < 0.01$) during the studied period. Population analysis revealed a complex situation with the absence of outbreaks and the presence of a polyclonal structure. Moreover, the variety of different ESBL significantly increased, with persistence of previously identified ESBL types (TEM-4, SHV-2, CTX-M-9, CTX-M-10) and the emergence of new ESBL (SHV-11, SHV-12, CTX-M-14). Co-resistance analysis revealed an increment of resistance to nalidixic acid (30%), ciprofloxacin (30%), trimethoprim (48%), and sulphonamide (62%), most of these antibiotics widely used in the community. Changes in the prevalence of different ESBLs may justify associated changes in the frequency of gentamicin and tobramycin resistance that were reduced to 23 and 37%, respectively.
Conclusions: Although epidemics involving ESBL-Kp were not observed in our institution during 2001–2003, this organism actively participates in global dissemination and maintenance of ESBLs. Moreover, the significant increase in the entrance of ESBL-Kp from the community to the hospital setting represent a new concern for nosocomial infections.

P1866 The occurrence and relatedness of extended-spectrum beta-lactamases (ESBLs) among *Enterobacter* spp. isolates in Tel Aviv

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Background: *Enterobacter* are leading nosocomial pathogens. ESBL-producing phenotype (PP) was found among 22% of *Enterobacter* clinical isolates in our institution. We examined the clonal relatedness, and characterised the ESBL enzymes of these strains.

Methods: Seventeen *Enterobacter* spp. with ESBL PP were studied. Clonal relatedness determined by PFGE. Enzymes were characterised using Isoelectric focusing (IEF) staining with nitrocefin ± clavulanate. Transconjugation experiments were performed with *E. coli* HB101 selected on ceftazidime and streptomycin. ESBL gene families were screened by colony hybridisation. PCR for TEM, SHV, CTX-M genes was performed and products were sequenced.
Results: Seventeen *Enterobacter* isolates [*E. cloacae* (13), *E. aerogenes* (3), and not identified (1)] belonged to 15 distinct PFGE types. All isolates had at least one IEF band that hydrolysed nitrocephin and whose activity was inhibited by clavulanate (range 1–5 bands); pI ranged between 5.4 and ≥7.8. All 17 strains had a beta-lactamase with a pI of 5.4, and 11 strains had a beta-lactamase with pI of ≥7.8. Colony hybridisation identified in 16 isolates at least one family of beta-lactamase gene: two harboured only a TEM gene, four CTX-M only, seven TEM and SHV, two SHV and CTX-M, and one Enterobacter isolate harboured all three. PCR screening for TEM and SHV yielded similar results. Seven isolates were labelled with the CTX-M specific probe, but only one was positive according to PCR. One isolate not labelled with the CTX-M probe gave a CTX-M gene PCR product. Sequence analysis of PCR products identified all the TEM genes as TEM-1 (11 isolates), all the SHV genes as SHV-12 (seven isolates), except one identified as SHV-1. CTX-M PCR product sequencing identified one of the two CTX-M genes as similar to CTX-M-2, and the second as similar to CTX-M-26. In trans-conjugation experiment with four isolates harbouring TEM-1 and SHV-12, both genes were simultaneously transferred to *E. coli* HB101 recipient; trans-conjugants exhibited ESBL PP, had two distinct IEF bands (pI 5.4 and ≥7.8) similar to their parental *Enterobacter* isolates, and PCR and sequencing, confirmed that all carried the same beta-lactamases as their parental strains.
Conclusions: The widespread of ESBL among *Enterobacter* isolates in Tel Aviv is not due to clonal spread but rather by multiple clones carrying various and multiple enzyme families, some of which are transferred simultaneously. The dominant identified ESBL was SHV-12.

P1867 Outbreaks of SHV and CTX-M co-producing *Enterobacter cloacae* in the intensive care unit of a Belgian hospital

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Objectives: The rapid increase of extended-spectrum beta-lactamases (ESBLs) producing Enterobacteriaceae has become a serious problem in European hospitals. The purpose of this study was to assess the molecular epidemiology of two successive outbreaks of *E. cloacae* (EC) resistant to third generation cephalosporins (CIII-R), which occurred during periods from June 2000 to March 2001 and July 2001 to December 2002, respectively.
Methods: A total of 75 CIII-R *E. cloacae* clinical isolates from patients admitted in ICU were included. ESBL screening was performed by double disk (DD) synergy test using cefepime, ceftazidime and amoxicillin–clavulanate. A multiplex PCR was used to detect blaTEM, blaSHV and blaCTX-M gene families. Genotyping was performed by pulsed field gel electrophoresis (PFGE) with XbaI restriction.
Results: From the 75 CIII-R *E. cloacae* strains, ESBL was detected in 58 (77%) by DD synergy test. Co-resistance to sulphamethoxazole was also found in 81%, to ciprofloxacin in 18%, to gentamicin in 48%, amikacin in 1%, to cefepime in 18% and to meropenem in 1%. PFGE analysis of ESBL producing strains revealed 20 different clonal types including three major ones: A ($n = 28$), L ($n = 14$) and K ($n = 6$). During the first outbreak, clone A was recovered in 22 of 30 strains, while the second outbreak was characterised by 14 of 45 strains from clone L, six of 45 from clone K and six of 45 from clone A. The three epidemic clones and 10 sporadic isolates harboured both SHV and CTX-M ESBL, representing 83% of ESBL-producing EC. Among 11 ESBL environmental isolates, seven also carried both SHV and CTX-M genes. ESBL-carrying Enterobacteriaceae strains of four species other than EC were co-isolated during this period in 15 patients, of which 8 had both SHV and CTX-M genes (two *E. coli*, one *E. aerogenes*, four *K. pneumoniae*, one *M. morganii*). DNA sequencing of representative strains revealed SHV-12 ESBL. CTX-M identification and plasmid profile analysis are still under investigation.
Conclusions: These successive polyclonal ICU outbreaks of CIII-R *E. cloacae* are highly suggestive of horizontal co-transfer of SHV and CTX-M ESBL-carrying plasmid. However, further investigation is necessary to determine if SHV and CTX-M genes are carried by the same plasmid. Our study demonstrates that ESBL-producing EC is an emerging threat causing nosocomial outbreaks and underlines its probable role as a reservoir of ESBL gene interspecies transmission.

P1868 The first identification of VIM-1 in an *Enterobacter cloacae* isolate from a university hospital in Greece

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Objectives: Carbapenem resistance in *E. cloacae* is unusual and has been described in strains with porin alterations combined with hyperproduction of chromosomal cephalosporinase, in strains producing class A carbapenem-hydrolysing nonmetallo-beta-lactamases, such as NmcA beta-lactamase and in strains producing IMI-1 beta-lactamase. Recently a class B metallo-beta-lactamase (MBL) has been reported in an imipenem-resistant *E. cloacae* strain, isolated in Korea, which carried a blaVIM-2-containing integron. In 2003, an *E. cloacae* strain with an imipenem MIC of 1 mg/L was isolated in a tertiary care hospital in Athens (Greece), from the blood culture of a hospitalised patient. The strain demonstrated a positive EDTA-disc synergy test and it was studied for carbapenemase production.
Methods: *E. cloacae* was isolated from the blood culture of a patient suffering from Fournier's gangrene. Susceptibility testing was performed by the disk diffusion technique and MICs were determined by the broth microdilution method (Sensititre Ltd, West Sussex, UK), according to NCCLS guidelines. EDTA-disc synergy test, was used to screen for MBL production. Beta-lactamases were detected by isoelectric focusing (IEF) and the presence of a MBL gene was determined by PCR with the following set of primers: VIM-F (5′-ATGGTGTTTGGTCGCATATC-3′) and VIM-B (5′-TGGGCCATTCAGCCAGATC-3′). Sequencing of cloned PCR products was performed by MWG- THE Genomic Company.
Results: The isolate was resistant to aminopenicillins, cephalosporins and monobactams and had reduced susceptibility to imipenem but not to meropenem (MICs; 1 and 0.25 mg/L, respectively). It demonstrated a positive EDTA-disc synergy test. IEF identified a beta-lactamase with pI of 9 probably corresponding to the chromosomal AmpC cephalosporinase of *E. cloacae*. No band of pI 5.3 was visible. Sequencing of the cloned PCR product identified blaVIM-1.
Conclusions: To the best of our knowledge, this is the first report of the presence of blaVIM-1 MBL in *E. cloacae*. The spread of MBLs in Enterobacteriaceae is becoming a grate concern in Greece as VIM-1 has also been reported in *E. coli*, and *K. pneumoniae* isolates. The spread of blaVIM-1 could compromise the future usefulness of carbapenems for the treatment of serious infections caused by Gram-negative bacilli and requires more attention than ever before.

P1869 Prevalence of extended-spectrum beta-lactamases and metallo-beta-lactamases in clinical isolates of *Enterobacter cloacae* from Northern Italy

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Objectives: Resistance to broad-spectrum cephalosporins in *Enterobacter cloacae* may be a consequence of the selection of stably derepressed mutants producing a chromosomal group-1 beta-lactamase. This species can also acquire and express genes

encoding extended-spectrum beta-lactamases (ESBL). So far, isolates that produce metallo-beta-lactamases (MBL) have been reported from Taiwan, Korea, and recently from Italy. This study was initiated to investigate the prevalence of ESBL and MBL in clinical isolates of *E. cloacae* collected at our Institution (Varese, Italy) in the year 2002.
Methods: All consecutive *E. cloacae* isolates showing reduced susceptibility to broad-spectrum cephalosporins (MIC > 1 mg/L) were collected. The double-disk synergy test was used to evaluate ESBL production. The *E*test method (AB Biodisk, Solna, Sweden) was used to assess MIC values of several beta-lactams, including last-generation cephalosporins, carbapenems, and aztreonam. DNA probes specific for TEM, SHV, CTX-M and PER-1/2 genes were used to assess the presence of these resistance determinants. ESBL-positive isolates and those showing MIC for carbapenems >1 mg/L were also investigated for MBL production with molecular methods (RFLP-multiplex PCR) and enzymatic assays. The plasmid location of ESBL and MBL determinants was investigated through conjugation experiments and Southern blot hybridisation.
Results: We studied 384 consecutive nonduplicate *E. cloacae* isolates (252 from inpatients, 132 from outpatients). Overall, 86 isolates (22.4%) were resistant to broad-spectrum cephalosporins (27.9% in inpatients, 7.6% in outpatients). On the basis of the double-disk synergy test, 28/384 (7.3%) isolates were found to produce ESBL. Molecular analysis showed that six isolates carried a CTX-M-type gene, whereas most of the remaining 22 carried the SHV gene. Notably, two isolates were suspected to produce MBL since they had EDTA-inhibitable carbapenemase activity. In the latter strains, RFLP-multiplex PCR indicated that a VIM-1-like gene was present. CTX-M and VIM determinants were carried on plasmids that were transferred to *E. coli* recipients by conjugation.
Conclusions: In addition to classical TEM-type and SHV-type ESBL, CTX-M-type enzymes and MBL appear to emerge among clinical isolates of *E. cloacae*. Monitoring ESBL prevalence in this species may be of value to control the spread of these worrisome resistance determinants.

P1870 Drug resistance and molecular epidemiology of beta-lactamase-producing *Enterobacter cloacae* strains isolated from hospital and aquatic environments

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Introduction: Clinical and environmental *E. cloacae* isolates exhibit high resistance to third generation cephems.
Objective: To compare antibiotic resistance patterns and to delineate the clonal diversity and transmission patterns by plasmid profile, rep-PCR, PFGE and RAPD analysis between *Enterobacter cloacae* strains isolated from different environments.
Methods: Sixteen *E. cloacae* strains from cardiovascular devices inserted patients and seven *E. cloacae* strains from fresh and polluted waters were studied for: antimicrobial susceptibility (NCCLS) by disc diffusion method; double-disc and inductibility disc diffusion tests, plasmid DNA profiles; Rep-PCR fingerprinting; DNA macrorestriction with XbaI endonuclease; RAPD using primers HLWL74, AP4 and R108. PFGE and RAPD patterns were visually compared into clonal groups and variants using UPGMA algorithms and computational NTSYS program.
Results: A 62% of hospital isolates are ESBLs producers, with 7% susceptibility to inhibitors; 20% intermediate susceptible to third generation cephalosporins; 62% susceptible to quinolones, 40% to aminoglicosides and 100% to imipenem. All aquatic strains expressed inducible beta-lactamase phenotypes, with 100% resistance to ampicillin, amoxicillin/clavulanic acid, chloramphenicol and tetracycline, 80% to cephalosporins, 60% to aminoglycosides, 21% to quinolones. Plasmid DNA profile analysis showed variable number of plasmids (ranging from 2.5 to 30 kpb) for both clinical and aquatic isolates. Chromosomal DNA digested with XbaI produced an average of 20 fragments ranging between 40 and 700 kb. Nine of epidemiologically related strains were classified in one cluster by rep-PCR and RAPD. Most informative profiles were obtained with PFGE, six of these nine strains were classified in one cluster whereas the remaining three strains were not related to clonal strains and differed from each other. We detected two clusters for aquatic strains: cluster B (five subtypes) and cluster C (two subtypes), according to UPGMA criteria and rep-PCR, RAPD and PFGE profiles.
Conclusions: Most of ESBL strains isolated from hospitalised patients belong to the same clone. All inducible beta-lactamase producing strains isolated from different aquatic sources belong to two clones. Concluding, we found three spatial clusters and 10 unrelated isolates with possible clonal relatedness among them. Both RAPD and PFGE are suitable for molecular typing *E. cloacae* isolates with intra- and extra-hospital origins.

P1871 Spread of OXA-23-producing *Acinetobacter baumannii* clones in England

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Introduction: *Acinetobacter baumannii* is an important nosocomial pathogen, causing infections in immunocompromised patients and those under artificial ventilation. Treatment is often complicated by multi-resistance. Carbapenems have become the drugs of choice, but reports of resistance are increasing. Target modification and/or porin loss can cause this resistance, but carbapenemases of molecular classes B (IMP and VIM) and D (OXA-23 and -24-related) seem more frequent. We report here the spread of two clones of OXA-23-producing *A. baumannii* across southern England.
Methods: Carbapenem-resistant *Acinetobacter* were received for MIC confirmation and typing by the reference laboratories during 2002/2003. They were identified by standard methods, and typed by PFGE. MICs were determined by *E*test or agar dilution. Genes encoding carbapenemases were sought by PCR with primers for blaIMP, blaVIM, blaOXA-23-like and blaOXA-24-like; PCR products were sequenced on both strands. Chromosomal DNA was digested with EcoRI and EcoRV restriction endonucleases, and an OXA-23 probe was used in DNA-DNA hybridisations.
Results: blaOXA-23-like genes were detected by PCR in representatives of the two clones (1 and 2), and sequencing indicated that the enzyme was classical OXA-23. DNA–DNA hybridisation indicated that the OXA-23 enzyme was chromosomally encoded in both clones. Clones 1 and 2 had distinct PFGE profiles, with both containing several PFGE variants. Both clones differed from the original OXA-23-producing *A. baumannii* reported in Scotland in 1985. Eleven hospitals submitted samples from clone 1 only; three submitted samples from clone 2 only; and one submitted isolates belonging to both clones. All affected hospitals were in southern England. All isolates were carbapenem-resistant and were also multi-resistant to ciprofloxacin, aminoglycosides and other beta-lactams including sulbactam, and consistently sensitive only to polymyxins (MICs $\leq$ 0.5 mg/L) and minocycline (MICs 1–8 mg/L).
Conclusions: Two *A. baumannii* clones producing OXA-23 beta-lactamase have spread in multiple hospitals in southern England. This simultaneous spread of unrelated, but similarly resistant, clones is disturbing.

P1872 The emergence of VIM-2 in Latin American hospitals: report from the SENTRY Antimicrobial Surveillance Programme

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Background: The VIM metallo-β-lactamases (MBL) are emerging mobile resistance determinants recently described and reported in various European countries and in the Far East. We report the

detection and characterisation of *Pseudomonas* isolates producing VIM-2 causing infections in Latin American (LA) hospitals.
Methods: As part of the SENTRY Programme, MBL production was screened by Etest (AB BIODISK) MBL strip, then confirmed by hydrolyses assays and PCR for blaIMP, blaVIM and blaSPM followed by gene sequencing. Integrons were detected with primer sets specific to the 5′CS and 3′CS of class 1 integrons. The PCR products were sequenced bidirectionally using DuPont Automated systems and the sequences analysed by DNAstar.
Results: Two isolates were selected for characterisation. Strain 43-14926A was a *P. fluorescens-putida* isolated in Chile from bloodstream infection in a 19-year-old female who was underwent bone marrow transplantation. Strain 49.4597C was a *P. aeruginosa* isolated in Venezuela from a 52-year-old male who developed sepsis and nosocomial pneumonia as a complication from a surgical abdominal infection. Both patients received imipenem (IMP) therapy and other β-lactams previous to the isolation of the carbapenem-resistant (R) strains. Both isolates were R to IMP, meropenem and ceftazidime. Sequence analysis revealed a MBL gene blaVIM-2 in both isolates. Upstream from the MBL (43-14926A) blaVIM-2, lies a class 1 integron. Downstream of blaVIM-2 was found a gene cassette aacA4 followed by aadA1 and aadA2.
Conclusions: We documented the emergence of VIM-2 in two LA hospitals. Global surveillance programmes, such as SENTRY, play an important role in detecting the regional emergence and dissemination of novel MBL mechanisms.

P1873 Endemic and epidemic occurrences of metallo-beta-lactamases in Japanese medical centres (1998–2002): report from the SENTRY Antimicrobial Surveillance Programme

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Background: Metallo-beta-lactamases (MBL) were initially characterised in Japan, usually of the IMP-type and found in *P. aeruginosa* (PSA), *Acinetobacter* spp. (ACB) or *S. marcescens* (SM). The number of MBL types has increased worldwide, but geographical dissemination in Japan has appeared limited. This study compares baseline levels of MBL-resistance (R) from two, 22 centre studies (1996–97) to the longitudinal sample (three sites) of Japanese isolates from the SENTRY Programme (1998–2002).
Methods: All MICs were determined by reference NCCLS methods. 84 of 456 (18.4%) PSA, three of 88 (3.4%) ACB, and six of 258 (2.3%) Enterobacteriaceae (enterobacters and SM) with R to both monitored carbapenems (CARB; MIC $\geq$ 8 mg/L) were screened for MBL by disk approximation (EDTA and 2MPA inhibitors), CARB hydrolysis by enzyme extracts and selected PCR primers for all known MBLs. All MBL-positive strains (10) were sequenced to determine type. Clonality in each centre was determined by automated ribotyping and PFGE, where needed.
Results: The CARB-R rates in PSA (15.5–28.0%) appear to be increasing over the monitored interval (1998–2002), but varied by medical centre location. Among CARB-R isolates, 2.2% were attributed to MBL strains (1.1% of all PSA tested). MBL identification showed: 5 PSA (3 IMP-1; 2 IMP-2), 4 SM (1 IMP-1; 2 IMP-1 + OXA-1, and 1 novel MBL being characterised). The sequence of the new MBL most closely resembles IMP-8 and has a complex integron (integrase-MBL-aacA4-ORF1-QAC/SUL). Also a single ACB had an IMP-1. Eight of 10 MBLs occurred between 2000 and 2002; four in 2002 (ACB, PSA, 2 SM). BRL42715, an AMP-C inhibitor confirmed AMP-C-mediated R in 87.3% of PSA and OMP changes were also discovered by membrane preparations. Prior 1997–98 (22 sites) results showed CARB-R at 22.4–25.6% and 0.5–0.9% MBLs (IMP-1) overall.
Conclusions: MBL-producing strains from several species persist in Japan, but represent a minority of all CARB-R isolates (1998–2002). Epidemic (SM 196-3 ribotype in 2002) or endemic dissemination was observed; and some novel beta-lactamase combinations and MBL-types were discovered. MBL rates appear generally stable in Japan. Continued global surveillance for these R mechanisms appears to be a prudent practice due to the mobility of the genetic determinants (plasmids or integrons) and the emergence of novel enzyme types, especially in SM and other non-PSA species.

P1874 Resistance to imipenem and detection of class 1 integrons on *Ps. aeruginosa* and *A. baumannii* clinical isolates

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Objectives: The aim of this study was to analyse the epidemiological relationships among imipenem resistant (IR) clinical isolates of *Ps. aeruginosa* and *A. baumannii* and to detect the presence of class 1 integrons in these isolates.
Methods: The study included all isolates obtained at a Hospital from Bilbao (Northern Spain) during 2002 (226 and 88 isolates of *Ps. aeruginosa* and *A. baumannii*, respectively). Susceptibility to antimicrobial agents was determined by the agar dilution method following the NCCLS recomendations. The antibiotics tested were imipenem, meropenem, cefotaxime, ceftazidime, amikacin and gentamicin. Total DNA was used as target for PCR-fingerprinting experiments with primers M13 and ERIC2. To detect class 1 integrons, primers 3′CS and 5′CS were used in amplification experiments.
Results: Fifty-nine isolates (67.4%) of *A. baumannii* and 33 (14.6%) of *Ps. aeruginosa* were resistant to imipenem. All imipenem resistant *A. baumannii* isolates (IR-Ab) were also resistant to meropenem, 98.3% to gentamicin and 91.5% to cefotaxime, ceftazidime and amikacin. A 72% of the imipenem resistant *Ps. aeruginosa* isolates (IR-Ps) were resistant to gentamicin, 57.5% to cefotaxime, 54.5% to ceftazidime, 30% to meropenem, and 9% to amikacin. Using the RAPD technique, four distinct genotypes were identified among IR-Ab isolates. Clone I accounting for 69.5% (41 isolates), clone II 23.7% (14 isolates) and Clone III 5,1% (three isolates). Among IR-Ps isolates 22 distinct genotypes were recognised; one of them (named 1) accounting for 27.3% (nine isolates) and two of them (named 2 and 3) accounting for 6.1% (two isolates respectively). All the isolates of IR-Ab bored integrons ranging in size from 550 to 1600 bp. Integron named a (760 bp) was the predominant accounting for 52 isolates (88%). Most of the isolates bored combinations of three bands. Class 1 integrons were present in most of the IR-Ps isolates, ranging in size from 600 to 1700 pb.
Conclusions: Resistance to imipenem in *A. baumannii* isolates was: (a) higher than the one showed by *Ps. Aeruginosa*, (b) always combined with meropenem resistance and (c) most of the isolates were also resistant to all antibiotics tested. PCR-typing showed that the majority of *A. baumannii* isolates belonged to few clones but in *Ps. aeruginosa* several clones were identificated. All isolates bored several class 1 integrons which means a rapid spread of the resistant genes in nosocomial envirorment.

P1875 Metallo-beta-lactamase-mediated carbapenem resistance in *Pseudomonas aeruginosa* in an Indian intensive care unit

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Objectives: Metallo beta lactamase mediated resistance is being increasingly reported in *Pseudomonas aeruginosa* especially in the far east Asian countries.The use of carbapenems has increased over the last 2 years in our Intensive care unit (ICU) due to increased prevalence of ESBL producing strains. This study was performed in 2003 to determine the prevalence of metallo betalactamase medited (MBL) carbapenem resistance in *P. areuginosa* in ICU patients.
Methods: One hundred clinically significant isolates of *P. aeruginosa* from ICU patients were taken for the study. Susceptibility to all anti pseudomonal drugs including imipenem and aztronam was performed by disc diffusion technique and minimal inhibi-

tory concentratrion (MIC) of imipenem and imipenem/EDTA combination by agar dilution. For the detection of MBL two discs method of imipenem 10 micrograms, imipenem EDTA 750 micrograms and ceftazidime 30 micrograms, ceftazidime EDTA 750 micrograms were used.
Results: Fourteen of the hundred strains were MBL producers. Their MIC to imipenem was 16–128 micrograms. They exhibited eight–128-fold decrease in MIC with imipenem EDTA combination. There was significant difference in the inhibitory zone diameters with EDTA as compared with MBL nonproducers. Two strains exhibited this difference only with ceftazidime EDTA and another only with imipenem EDTA. One isolate had high MIC values to imipenem but was not an MBL producer reflecting other mechanisms of resistance. All MBL producers had coresistance to aminoglycosides and quinolones.
Conclusions: MBL mediated carbapenem resistance is present in 14% of pseudomonas strains in our ICU. With increased use of carbapenems in the critically ill patients this is bound to increase. This is to be addressed with judicious use of carbapenems and a continuous monitoring programme. Use of both ceftazidime and imipenem with EDTA will increase the sensitivity of MBL detection by the two discs method.

P1876 Outbreak of multiresistant *Pseudomonas aeruginosa* producing the metallo beta-lactamase IMP-13 in a general intensive care unit in southern Italy

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Background and Objectives: Metallo-beta-lactamases (MBLs) of the IMP and VIM type are emerging resistance determinants in Gram-negative nosocomial pathogens. Their spreading is of considerable concern for antimicrobial chemotherapy, due to their carbapenemase activity and resistance to conventional beta-lactamase inhibitors. Although epidemiology of these enzymes remains largely unknown, in Europe IMP-type producers have been reported less frequently than VIM-type producers, and only in sporadic cases. In this work we describe an outbreak due to *P. aeruginosa* producing an IMP-type MBL (IMP-13) in an Intensive Care Unit of an Italian Hospital.
Methods: Twenty-seven nonreplicate carbapenem-resistant isolates of *P. aeruginosa* were collected from 27 inpatients at the Intensive Care Unit of the S. Giovanni Rotondo Hospital (southern Italy) during the period October 2002 to June 2003. Most isolates (25 to 27) were from the lower respiratory tract. *In vitro* susceptibility tests were carried out by a microdilution method as recommended by the NCCLS. The Etest and a broth microdilution method (EPI test) were used for phenotypic detection of MBL producers. PCR and sequencing were carried out to identify the MBL determinants. Pulsed Field Gel Electrophoresis (PFGE), using the SpeI restriction enzyme, was performed to evaluate the clonal relationships between the imipenem-resistant clinical isolates.
Results: Six carbapenem-resistant *P. aeruginosa* isolates were shown to produce an MBL activity by the EPI test, while none of them resulted positive with Etest. In all cases the MBL was identified as IMP-13 by molecular methods. The IMP-13 producers were resistant to imipenem, meropenem and ceftazidime, while 50% retained susceptibility to piperacillin/tazobactam and 83% intermediate susceptibility to aztreonam. PFGE analysis showed that all the MBL producers were clonally related suggesting a clonal spread within the ward. In the remaining isolates, which belonged in clonal lineages different from that of the IMP-13 producers, carbapenem resistance was due to mechanisms other than MBL production.
Conclusions: To our best knowledge this is the first report of a nosocomial outbreak caused by *P. aeruginosa* producing the IMP-13 MBL. Concerning phenotypic detection, the EPI test could correctly detect all the IMP-13 producers, while the Etest failed in revealing them.

P1877 Characterisation of integrons carrying blaVIM type metallo-beta-lactamase genes from carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates in Hungary

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Objectives: The aim of this study was to investigate the presence and frequency of occurrence for acquired metallo-beta-lactamase (MBL) producing *Pseudomonas aeruginosa* clinical isolates in Hungary.
Methods: We screened the isolates by EDTA-inhibition based phenotypic tests together with PCR using integron, blaVIM and blaIMP specific primers to identify acquired MBL-producers.
Results: We recently isolated the first integron-borne MBL gene in Hungary from the urine sample of a Greek citizen treated in August 2002 in Budapest. DNA sequencing revealed the presence of a Class 1 integron carrying three gene casettes: an OXA-type lactamase, an aminoglycoside 6′-N-acetyltransferase and a blaVIM-4 type MBL gene cassette. VIM-4 has been reported for the first time from Greece in 2002. These observations raise the possibility that this *P. aeruginosa* strain originates in Greece, and has been imported to Hungary by a human carrier. In October 2003 another VIM-producing *P. aeruginosa* strain has been isolated in Southern Hungary. PCR mapping experiments suggest that this strain carries a class 1 integron with only two resistance gene cassettes in its variable region. The blaVIM type MBL gene cassette is right upstream of the 3′ conserved sequence. The structural diagram of the two integrons is presented with implications for their possible phylogenetic relationship.
Conclusions: With the isolation of a second VIM-producing strain from an apparently independent case the repeated appearance of MBL producing clinical isolates can be anticipated in Hungary. A regular screening/monitoring system should be set up to prevent the wider spread of these resistance determinants.

P1878 Metallo-beta-lactamase-producing Gram-negative bacilli in KONSAR group hospitals: continued prevalence of VIM-2-producing *Pseudomonas* spp. and increase of IMP-1-producing *Acinetobacter* spp. in 2002

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Objectives: Significant proportion of carbapenem-resistant isolates of *P. aeruginosa* and *Acinetobacter* spp. is due to acquired MBL production, which renders all beta-lactams inactive. Since the first report of acquired MBLs, IMP-1 and VIM-1, MBL-producing Gram-negative bacilli have been increasingly reported in many countries. Previous study showed high prevalence of VIM-2-producing *Pseudomonas* spp. and emergence of IMP-1-producing *Acinetobacter* spp. in KONSAR hospitals. The aim of this study was to determine any change of prevalence of VIM-2-producing *Pseudomonas* spp. and IMP-1-poducing *Acinetobacter* spp. among isolates collected from KONSAR hospitals in 2002.
Methods: Nonduplicate, imipenem nonsusceptible isolates of *Pseudomonas* spp. and *Acinetobacter* spp. were collected in 2002, from 27 KONSAR group hospitals. MBL production was screened by the Hodge test and imipenem-EDTA + SMA double disk synergy test. blaIMP-1 and blaVIM-2 alleles were detected by PCR using heat-extracted DNA template. Xba I- and Sma I-digested genomic DNAs of *P. aeruginosa* and *Acinetobacter* isolates, respectively, were separated by PFGE and the patterns were compared.
Results: MBL-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. were detected in 19 of 27 (70.3%) and 10 of 20 (50.0%) hospitals, respectively, which are located in five of seven city/province in Korea. Among the imipenem-nonsusceptible isolates, 53 of 459 (11.5%) Pseudomonas spp. and 31 of 212 (14.6%) *Acinetobacter* spp. were MBL producers. The proportions were similar to those in 2000–2001, 11.4 and 14.2%, respectively. The species of MBL-producing isolates were: 46 *P. aeruginosa*, seven *P. putida*, 30 saccharolytic *Acinetobacter* spp., and one *A. lwoffii*. Among the

MBL-producing isolates, all isolates of *Pseudomonas* spp. had blaVIM-2 alleles, while 13 (41.9%) and 18 (58.1%) of *Acinetobacter* spp., respectively, had blaVIM-2 and blaIMP-1 alleles. The proportion of blaIMP-1-positive isolates was 28.9% in 2000–2001. The source of isolation of MBL-producing strains were: 32.9% from ICU patients and 64.7% from other inpatients; 36.9% from sputum and 38.1% from urine.
Conclusions: High prevalence of blaVIM-2 allele-positive *P. aeruginosa* remained similar. The proportion of blaIMP-1 allele-positive *Acinetobacter* spp. became higher. This study suggests clonal spread is a more important factor than horizontal transfer in dissemination of the resistance.

P1879 Characteristics of ESBL-producing *Proteus mirabilis* isolates in Korea

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Objectives: *Proteus mirabilis* strains were usually susceptible to ampicillin and other beta-lactams. However, a progressive increase of beta-lactam resistance has been reported in this species. Recently, *P. mirabilis* is the one of the common extended-spectrum beta-lactamase producing organisms in Europe. However, ESBL-producing *P. mirabilis* has not been reported in Korea. In this study, we performed the screening and confirmation test to detect ESBL-producers in *P. mirabilis* isolates in a Korean teaching hospital, and characterised the ESBL types.
Methods: Consecutive 105 isolates of *P. mirabilis* were collected from December 2002 to September 2003. The susceptibility was tested by the NCCLS disk diffusion test. Double-disk synergy test (DDST) with amoxicillin–clavulanate, cefotaxime, and ceftazidime disks at a distance of 15 mm (edge to edge) was performed to detect the ESBL-producers. Conjugations were carried out in some of the representative isolates. blaTEM, blaSHV, and blaCTX-M were detected by PCR. PCR-products of blaCTX-M were sequenced by dideoxynucleotide-chain termination method.
Results: Disk diffusion test showed 46 (43.8%) and 10 (9.5%) isolates were resistant to ampicillin and to cefotaxime, respectively. None of the isolates were resistant to ceftazidime, cefepime, and cefoxitin. Twenty-one isolates suspected to have ESBLs according to NCCLS criteria were tested by DDST. Among them, 10 isolates with cefotaxime-resistance were positive in DDST with amoxicillin–clavulanate and cefotaxime only. All of the 10 isolates had blaCTX-M alleles. Sequencing showed that nine had blaCTX-M-14 and one had blaCTX-M-2 type. Among the 36 ampicillin-resistant, cefotaxime-susceptible isolates, 31 strains were blaTEM positive, one strain was both blaTEM and blaSHV positive, and four strains were both blaTEM and blaSHV negative. Conjugations were not successful in blaCTX-M alleles-positive strains.
Conclusions: This is the first report on the presence of ESBL-producing *P. mirabilis* isolates in Korea. It is very interesting that the ESBLs detected in all *P. mirabilis* isolates were only CTX-M type. The prevalence of CTX-M in *P. mirabilis* was very high, almost 10%. Further study on the transferability of CTX-M gene in *P. mirabilis* isolates will be needed.

P1880 Clonal diversity and carbapenemase production in carbapenem-resistant *Acinetobacter baumannii* isolated in a Portuguese hospital, during a 3-year period

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Objectives: Carbapenemase production has been observed in Portuguese clinical isolates of imipenem-resistant *Acinetobacter baumannii* (IMRAb), associated to IMP-5 and OXA-40 enzymes. In this work, we investigated the clonal relatedness of carbapenem-resistant *A. baumannii* isolated from a University Hospital with a high rate of imipenem resistance, together with the relative contribution of carbapenemase production.
Methods: Carbapenem-resistant *A. baumannii* ($n = 108$) were isolated between March 2001 and March 2003, from patients attending the Hospital de Santo António, Porto. Genomic macrorestriction analysis was obtained after digestion with ApaI restriction enzyme. MICs were determined by Etest. Susceptibility to non-beta-lactam antibiotics was performed by the disk diffusion method. Carbapenemase producing strains were detected by a bioassay. Genes were sought by PCR with primers specific for blaIMP, blaVIM and blaOXA-24-like. Obtained products were sequenced on both strands.
Results: Imipenem resistance in *A. baumannii* increased from 32% ($n = 47$) in 2001 to 53% ($n = 31$) in 2002, and all isolates ($n = 30$) obtained during the first 3 months of 2003 showed imipenem resistance. IMRAb were isolated from different hospital units, mainly from intensive care units. No PCR evidence suggested the presence of blaIMP or blaVIM type genes in all the isolates. A total of 44 carbapenem-resistant isolates yielded a PCR product with primers specific for blaOXA-24-like. Sequencing showed the presence of an OXA-40 enzyme and analysis of PFGE revealed that these isolates belong to a single clone. Resistance to all beta-lactams and variable susceptibility to amikacin and tobramycin was a common feature of this clone. Macrorestriction analysis of other 57 imipenem-resistant isolates demonstrated the simultaneous dissemination of another clonal type, which differs also in the susceptibility patterns. Apart from the resistance to almost beta-lactams, isolates from this second clone were susceptible to minocicline and presented variable behaviour to ceftazidime and to aminoglycosides.
Conclusions: The high rate of imipenem-resistant *A. baumannii* in this hospital is associated to the presence of two major clones. Also, the results suggest that the spreading of OXA-40 enzyme, in Portugal, was mainly because of the progressive dissemination of a single clone that persists until 2003.

P1881 Communitiy-acquired urinary tract infections by extended-spectrum beta-lactamase-producing *Escherichia coli* in children: clinical and molecular characteristics

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Objectives: Recently our institution noted a marked increase in the number of ESBL (extended-spectrum beta-lactamase)-producing *E. coli* isolated from children with urinary tract infections (UTI). The children were ambulatory patients or had been seen in the emergency department. The purpose of this study was to investigate clinical and molecular characteristics of ESBL-producing *E. coli* from community-acquired UTI in children.
Methods: We analysed 36 children with UTI due to ESBL-producing *E. coli* (cases) and 58 children with UTI due to non-ESBL-producing *E. coli* (controls) in Ewha Womans University Hospital from July 2001 to June 2002.
Results: Of the total 1136 *E. coli* isolates from urine, 119 (10.5%) produced ESBL and the prevalence of UTI due to ESBL-producing *E. coli* was higher in children (19.3%) than in adults (4.6%). Case patients had significantly higher resistance to aztreonam, ceftriaxone, cefotaxime, cefepime, and ceftazidime than control patients ($P < 0.05$). Case patients were younger (4 ± 1 months) than control patients (24 ± 76 months) and were more frequently male (30of 36) than control patients (39 of 58). No significant differences were noted in prior UTI, prior antibiotic use, genitourinary abnormality, vesicoureteral reflux, urinary catheter, pyelonephritis or underlying diseases between cases and controls ($P < 0.05$). No significant difference in cure rate was noted between both groups, but case patients had a significantly higher relapse rate (41.7%) than control patients (2.1%, $P < 0.05$). Of the 27 strains analysed by PCR, 23 strains produced TEM, three produced TEM and SHV, and one produced SHV. Pulsed-field gel electrophoresis of 24 ESBL-producing organisms showed 18 distinct genotypes including five clusters.
Conclusions: ESBL-producing *E. coli* may be a causative agent of community-acquired UTI in children without any specific risk factors. Most strains were genetically unrelated and these findings suggested as community-acquired infection through dissemination of plasmids rather than the clonal spread.

Susceptibility to miscellaneous antimicrobial agents

P1882 Comparison of in vitro activity of gatifloxacin, moxifloxacin, trovafloxacin and clindamycin against clinically significant anaerobes

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Objectives: Newer fluoroquinolone antimicrobials have increasingly broad-spectrum potency and activity against aerobic Gram-positive and Gram-negative bacteria while their anaerobic coverage varies widely. This study was performed to provide contemporary in vitro data on the anti-anaerobe spectrum of three quinolone agents: two respiratory fluoroquinolones gatifloxacin (GAT) and moxifloxacin (MXF); and trovafloxacin (TRV), a previously FDA-approved fluoroquinolone with good anti-anaerobe activity, but limited use due to toxicity, and clindamycin (CLI), a long-time used anti-anaerobic lincosamide.
Methods: Test isolates composed the *B. fragilis* group (no. 457); *Clostridium* (no. 80); *Fusobacterium* (no. 22); *Prevotella* (no. 38); and *Peptostreptococcus* (no. 89) isolated from wounds, abscesses, body fluids, blood, and Ob-Gyn specimens. The agents were tested in twofold dilutions (0.06–64 mg/L) using the NCCLS-recommended broth microdilution method with MIC determinations performed at 48 h postinoculation.
Results: The table below compares the *in vitro* activity of each fluoroquinolone agent indicating the mode MIC (M) and percentage of isolates inhibited at 1, 2 and 4 mg/L which brackets the approved NCCLS breakpoint of 2 mg/L for TRV. CLI activity was determined at the following NCCLS breakpoints: 2 (S), 4 (I), 8 (R).

	GAT				MXF				TRV				CLI			
	M	1	2	4	M	1	2	4	M	1	2	4	M	s (2)	I (4)	R (8)
B.f.g	1	44	56	63	.5	32	44	61	.25	64	77	94	64	62	69	100
Clos.	.5	66	90	94	.5	66	84	89	.25	85	93	96	.06	66	74	100
Fuso	.25	68	82	91	.2	64	82	91	.5	82	91	96	.06	91	91	100
Prevo.	4	45	45	66	.5	45	48	69	.5	58	79	92	.06	76	0	24
Peptostr.	42	82	80	90	25	83	89	90	.25	88	80	96	.06	81	84	100
All	.5	53	65	72	.5	53	67	74	.25	70	81	95	64	67	73	100

Conclusions: Based on the present results, TRV was the most active fluoroquinolone tested while GAT and MXF had comparable with CLI overall and superior activity against gram-positive anaerobes. Both MOX and GAT have no established breakpoints for anaerobes and their spectrum of anti-anaerobic activity awaits appropriate pharmacokinetic and clinical studies.

P1883 Antibiotic susceptibility of bacterial strains isolated from community-acquired respiratory tract infections in Austria 2002

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Objective: The aim of this study was to determine the susceptibility of the most common bacterial pathogens in respiratory tract infections.
Material and Methods: Between January 2002 and December 2002 a total of 658 strains were collected from 361 adults and 297 children with community acquired respiratory tract infections from different Austrian regions. A total of 282 *Staphylococcus aureus*, 150 *Streptococcus pyogenes*, 120 *Haemophilus influenzae* and 106 *S. pneumoniae* were identified according to standard laboratory techniques. Antimicrobial susceptibility testing was performed by broth microdilution method according to NCCLS guidelines. The following antibiotics were tested: erythromycin (ERY), clarithromycin (CLA), azithromycin (AZI), telithromycin (TEL), penicillin (PEN), amoxicillin–clavulanic acid (AUG), ampicillin (AMP), cefotaxime (CTX), cefpodoxime (CPD), ciprofloxacin (CIP), levofloxacin (LEV), moxifloxacin (MXF), gatifloxacin (GAT), tetracyclin (TET), quinupristin–dalfopristin (SYN), linezolid (LNZ), vancomycin (VAN), gentamicin (GEN) and fusidic acid (FUS).
Results: A 72% ($n = 475$) of the strains were isolated from upper respiratory tract and 28% ($n = 183$) from lower respiratory tract. Against *S. aureus* the most active agents were LNZ (100%), VAN (100%), LEV (99%), MXF (99%), and FUS (99%). PEN, LNZ, MXF, GAT, LEV, CTX and CPD showed the best activity against *S. pyogenes* (from 100 to 99%). The most active agents against *S. pneumoniae* were AUG, LNZ, MXF, TEL, CTX (100% susceptible). Among *H. influenzae* 100% were susceptible among CIP, LEV, MXF, GAT, TEL, CTX and CPD.
Conclusions: The results demonstrate that the resistance rates of the respiratory tract pathogens among most antibiotics were less than 10%.

P1884 A comparison of the bactericidal activities of penems, a penam, a cephem and a fluoroquinolone against common respiratory tract pathogens

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Objective: Evaluation of the concentration dependent or independent bactericidal effects of penems (imipenem, meropenem and ertapenem) as compared with a representative penam (amoxicillin), a cephem (cefixime) and a fluoroquinolone (moxifloxacin).
Methods: Time-kill studies were performed by exposing *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae*, *Escherichia coli* and *Klebsiella pneumoniae* to constant drug concentrations ranging from 1 to 32x their respective MICs. Samples were taken at 0.5, 1, 2, 4, 6, 8, and 24 h and subcultured quantitatively.
Results: In general the penems exhibited the most pronounced effects against all the bacterial species. In contrast to amoxicillin and cefixime, all the penems exhibited a concentration-dependent bactericidal effect at all concentrations tested. Amongst the penems, imipenem tended to be the most bactericidal agent, followed by ertapenem and meropenem; however, the differences were not significant. The bactericidal effect of amoxicillin and cefixime could not be enhanced beyond the concentration producing a maximal effect. Moxifloxacin was also bactericidal throughout the concentration range studied. The bactericidal activity of moxifloxacin was similar to that of imipenem. However, moxifloxacin was more active against *H. influenzae* compared with all the penems.
Conclusions: The penems and moxifloxacin exhibit concentration dependent killing against the major RTI pathogens, whereas the penams and cephems do not. Unlike the penems, moxifloxacin has the advantage of being available as both an oral or parenteral therapy. Thus, moxifloxacin combines pronounced bactericidal activity with the convenience of sequential therapy.

P1885 Antibiotic susceptibility of isolates in the intensive care unit in Greece: 3 years of the Meronem Yearly Susceptibility Test Information Collection (MYSTIC) programme

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Objective: To determine the in vitro activity of meronem (MEM) and seven other antimicrobial agents against isolates from inten-

sive care units (ICUs) in two Greek university hospitals the last 3 years. This is part of the MYSTIC (Meronem Yearly Susceptibility Test Information Collection) program.
Methods: Minimum inhibitory concentrations (MICs) for MEM, imipenem (IPM), ceftazidime (CAZ), ceftazidime plus clavulanic acid, piperacillin plus tazobactam (TAZ), ciprofloxacin (CIP), gentamicin (GM) and tobramycin (TOB) were determined for 861 isolates using NCCLS agar dilution method.
Results: During the period 2000–2002, a total of 538 Gram-negative strains and 323 methicillin-susceptible staphylococci were collected and tested at the ICUs of two Greek university hospitals. The most common Gram-negative species tested were *Escherichia coli* (25.8%), *Pseudomonas aeruginosa* (23.1%), and *Klebsiella pneumoniae* (15.2%), followed by *Proteus mirabilis* (11.3%), *Enterobacter cloacae* (10.4%), *Acinetobacter baumannii* (7.1%), *Serratia marcescens* (3.9%) and *Klebsiella oxytoca* (3.2%). The percentages of susceptibility to MEM were the same as or higher than IPM against every organism tested. The carbapenems were highly active against the Enterobacteriaceae (>98%). All the other tested agents also showed a high potency against Enterobacteriaceae (80.9–92.8% susceptibility). Especially *K. pneumoniae* demonstrated relatively lower susceptibility to CAZ (70.7%), TAZ (73.2%) and CIP (75.6%) when compared with MEM (96.3%) and IPM (91.5%). Against *P. aeruginosa*, a higher proportion of isolates were susceptible to TAZ (85.5%) and to CAZ (80.6%) than to the carbapenemes (76.6 and 72.3% for MEM and IPM, respectively). MEM and IPM were the most active agents against *A. baumannii* (86.8 and 71.1% susceptibility, respectively), when compared with CAZ (13.2%), TAZ (52.6%), CIP (5.3%) and aminoglycosides (25.8%). All the tested agents showed a high potency against methicillin-S-staphylococci (88.9–93.5% susceptibility) with the exception of CAZ and CIP (59.1 and 78.3%, respectively).
Conclusions: Carbapenems (MEM > IPM) were the most active of the antimicrobials tested overall against the commonly isolated ICU organisms in two Greek university hospitals, over the last 3 years.

P1886 Comparative in vitro activity of ertapenem against respiratory pathogens circulating in Italy

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Objective: To evaluate the in vitro activity of ertapenem, a new parenteral carbapenem, against recently isolated community-acquired typical respiratory pathogens.
Methods: A total of 550 respiratory pathogens displaying different resistance phenotypes have been studied. Minimal inhibitory concentrations of ertapenem and of 15 other comparative drugs have been determined by the broth microdilution method (NCCLS, M7-A5) and results interpreted according to NCCLS (2003) approved breakpoints.
Results: Percentages of susceptible strains are depicted in the Table.

Antibiotic/ (no of strains)	*S. pneumoniae*			*S. pyogenes*		*S. aureus*
	Pen-S (100)	Pen-I (50)	Pen-R (30)	Ery-S (40)	Ery-R (60)	Met-S (100)
Ertapenem	100	100	96.7	100	100	100
Ampicillin	–	–	–	–	–	18
Amoxicillin/ clavulanate	100	100	63.3	100	100	100
Cefuroxime	100	42	0	–	–	100
Cefotaxime	100	100	83.4	100	100	100
Imipenem	100	94	20	100	100	100
Clarithromycin	78	36	40	100	0	69
Chloramphenicol	94	54	53.3	100	100	98
Clindamycin	78	36	43	100	66.6	61
Tetracyclin	74	32	23.3	87.5	51.6	97
Ciprofloxacin	–	–	–	–	–	99
Levofloxacin	100	100	100	100	100	100
Gentamicin	–	–	–	–	–	91
Amikacin	–	–	–	–	–	98
Vancomycin	100	100	100	100	100	100

Antibiotic/ (no of strains)	*H. influenzae*		*M. catarrhalis**		*K. pnenumoniae*	
	bla+ (20)	bla+ (50)	bla+ (50)	bla− (20)	ESBL+ (10)	ESBL− (20)
Ertapenem	100	100	100	100	100	100
Ampicillin	0	100	0	100	0	0
Amoxicillin/ clavulanate	100	100	100	100	100	100
Cefuroxime	100	100	100	100	0	100
Cefotaxime	100	100	100	100	0	100
Ceftriaxone	100	100	100	100	0	100
Imipenem	100	100	100	100	100	100
Clarithromycin	100	88	100	100	–	–
Chloramphenicol	100	86	100	100	10	80
Tetracycline	80	96	100	100	80	95
Ciprofloxacin	100	100	100	100	30	100
Levofloxacin	100	100	100	100	–	–
Gentamicin	–	–	–	–	0	100
Amikacin	–	–	–	–	100	100

*For interpretation of *M. catarrhalis* results, *H. influenzae* breakpoints have been adopted
–: not tested; Pen-S, Pen-I, Pen-R: penicillin-susceptible, -intermediate, -resistant; Ery-S and Ery-R erythromycin - suscptible and resistent; Met-S: methicillin-susceptible; bla+ and bla−: beta-lactamase-positive and -negative; ESBL+ and ESBL: extended spectrum beta-lactamase positive and negative.

Conclusions: Ertapenem, because of its in vitro activity encompassing the most important respiratory pathogens, and its ability to overcome different antibiotic resistance traits can be considered a useful therapeutic option in the treatment of community-acquired respiratory infections.

P1887 In vitro activity of ertapenem in comparison with penicillin, piperacillin/tazobactam, clindamycin, and metronidazole against clinical isolates of anaerobic bacteria

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Objectives: Ertapenem is a new carbapenem with high activity especially against enterobacteria. This antibiotic has no or only weak effect on *P. aeruginosa*, other nonfermenters, enterococci, and methicillin-resistant staphylococci. There are only a few reports about activity of ertapenem to anaerobes.The aim of the study was therefore to evaluate the in vitro activities of ertapenem (ERT) in comparison with penicillin (PEN), piperacillin/tazobactam (PIT), clindamycin (CLI), and metronidazole (MET) against anaerobic bacteria.
Methods: A total of 370 clinical isolates of anaerobic bacteria from patients of an university hospital were investigated in a prospective study over a time period of 6 months. They included 90 *Prevotella* spp., 80 *Bacteroides* spp. including 38 *B. fragilis* and 18. *B. thetaiotaomicron*, 55 *Peptostreptococcus* spp., 55 *Veillonella* spp., 46 *Fusobacterium* spp., 25 *Porphyromonas* spp., and 19 *Clostridium* spp. (16 *C. perfringens*, three *C. fallax*). The MIC-values were determined by use of Etest on *Brucella*-Blood-Agar (AB Biodisk, Solna, Sweden).
Results: The ranges of the MIC-values (mg/L) of ERT against the anaerobic strains tested were: *B. fragilis* 0.032–0.5; *B. thetaiotaomicron* 0.064–2; *Bacteroides* spp. 0.016–0.5; *Prevotella* spp. 0.008–0.125; *Porphyromonas* spp. 0.008–0,016; *Fusobacterium* spp. 0.008–16; *Veillonella* spp. 0.016–0.5; *Peptostreptococcus* spp. 0.016–1; *Clostridium* spp. 0.016–1. With the exception of one strain of *Fusobacterium varium* (MIC 16 mg/L) all other anaerobic strains were sensitive to ertapenem. The number of resistant strains to the other antibiotics tested was: *B. fragilis* (38) PEN 38 PIT 21 CLI 12 MET 1 *B. thetaiota* (18) PEN 18 PIT 9 CLI 7 MET – *Bacteroides* sp.(24) PEN 18 PIT 8 CLI 2 MET 1 *Prevotella* sp. (90) PEN 23 PIT 1 CLI 5 MET 4 Fusobact. sp. (46) PEN 11 PIT 11 CLI 1 MET 2 Porphyrom. sp. (25) PEN - PIT - CLI - MET 1 *Veillonella* sp.(55) PEN 19 PIT 19 CLI 3 MET 11 Peptostr. sp. (55) PEN 2 PIT - CLI 2 MET 55 *Clostridium* sp.(19) PEN 3 PIT 1 CLI 3 MET 1.
Conclusions: Ertapenem was *in vitro* the most active antibiotic against the tested anaerobic clinical isolates. Only one strain of 370 exhibited resistance in this in vitro study.

P1888 Is antibiotic combination more effective than single drug therapy in anthrax?

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Objectives: Antibiotic combinations are used to enhance the antibacterial effect and to prevent the development of bacterial resistance. We have tested possible synergistic effect of several antibacterial combinations in *Bacillus anthracis*.
Methods: The in vitro activity of antibiotic combinations against two strains of *B. anthracis*, the Sterne strain and the Russian anthrax Vaccine strain ST-1 were tested by calculating the fractional inhibitory concentration derived from the MIC's of the different agents and by measuring the rate of bacterial kill by antibiotic combinations.
Results: The FIC calculations rendered the results shown in the table:

Antibiotic combination	ST-1 strain	Sterne strain
Ciprofloxacin+tetracycline	Indifference	Indifference
Ciprofloxacin+penicillin	Indifference	Indifference
Ciprofloxacin+clarithromycin	Antagonism	Antagonism
Ciprofloxacin+clindamycin	Antagonism	Indifference
Penicillin+quinup/dalfop	Antagonism	Antagonism
Penicillin+tetracycline	Antagonism	Antagonism
Penicillin+clindamycin	Antagonism	Indifference
Penicillin+vancomycin	Antagonism	Antagonism
Penicillin+rifampicin	Antagonism	Indifference
Penicillin+linezolid	Antagonism	Antagonism
Penicillin+telithromycin	Antagonism	Indifference
Rifampicin+clindamycin	Synergism	Synergism

The results of the bacterial time-kill demonstrated the same trend, i.e combinations that exhibited antagonism had slower kill rate than any of the members of the pair.
Conclusions: These in vitro results demonstrate the difficulties in obtaining synergistic combinations of antibiotics against *B. anthracis* and suggest that the recommendations for therapy with antibiotic combinations are unfounded.

P1889 Combination effect of ertapenem (MK 826) with aminoglycosides against Gram-positive and Gram-negative community-acquired pathogens

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Introduction: Combination of beta-lactam and aminoglycoside antibiotics broadens the spectrum of bacterial coverage and often achieves synergistic inhibition of the infecting pathogen. Ertapenem is a new carbapenem, which exhibits activity against gram-negative and gram-positive aerobes and anaerobes.
Objectives: To provide insight into the optimal synergistic combination of ertapenem and an aminoglycoside.
Materials and Methods: During a 12-month period, bacterial strains were prospectively isolated from patients with community-acquired infections. The in vitro activity of ertapenem was evaluated in combination with amikacin, netilmicin and tobramycin against 100 isolates including *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae*, methicillin susceptible and resistant coagulase-negative staphylococci and *S. aureus* (MSSA, MRSA), *E. faecalis* and *E. faecium*. Susceptibility testing was carried out according to NCCLS-Guidelines. The combination effect was determined using the checkerboard technique.
Results: Ertapenem exhibited a synergistic effect in 17% of all the strains tested when combined with amikacin, but only in 3% when combined with gentamicin. There were noticeable variations between the different species tested. In combination with amikacin (MIC 4–16 μg/mL), ertapenem (MIC 2–32 μg/mL) was found to be synergistic against enterococci (45%). The same combination (ertapenem MIC 0.125–0.5 μg/mL; amikacin (MIC 2 μg/mL) was also synergistic against one-third of methicillin-resistant CNS isolates (33%) There was hardly any synergism against the other species tested.
Conclusions: Ertapenem in combination with aminoglycoside antibiotics exhibited a marked synergistic effect against certain pathogens, including *E. faecalis*, *E. faecium*, and coagulase-negative staphylococci. Based on these findings a combination therapy could be beneficial for the treatment of infectious diseases caused by these organisms.

P1890 QC study evaluating the performance of daptomycin and combination daptomycin/calcium disks and Etest

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Background: In vitro susceptibility testing of daptomycin, a cyclic lipopeptide antibiotic with good antimicrobial activity against most Gram-positive bacteria, requires the presence of calcium ions. Previous studies have shown that agar calcium levels as low as 25 mcg/mL have been adequate for disk diffusion testing. This study was performed to evaluate five commercial agars and to determine the efficacy of both disks and Etest strips containing a combination of daptomycin and calcium.
Methods: We tested each of the QC strains, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212, on multiple days using Mueller–Hinton agar (MHA) from both BD and Hardy, Mast Isotonic agar supplemented with 50 mcg/mL of calcium (ISTA), and Oxoid IsoSensitest agar (ISOA). We studied each of the strains on multiple days using a different lot of MHA from BD and Remel. Thirty-microgram daptomycin disks/tablets from each of the four different manufacturers, BBL (BD), Oxoid (Remel), Mast (Hardy) and Rosco were tested. In addition, Mast disks with 20, 40, 60, 80 and 100 mcg calcium and Etest strips with daptomycin, daptomycin + 40 mcg/mL calcium and daptomycin + 50 mcg/mL calcium were evaluated. Calcium testing of each media was performed using an ion selective electrode.
Results: Mean zone diameters (mms) for *S. aureus* 25923 and mean Etest MICs (mcg/mL) for *S. aureus* 29213 were:

	Study 2		Study 1			
	BBL MHA	Remel MHA	BBL MHA	Hardy MHA	Oxoid ISOA	Mast ISTA
Disk - *S. aureus* 25923 (expected=18–23 mm)						
Oxoid	18.6	20.75	–	–	–	–
BBL	18.75	21.05	20.19	21.09	15.28	22.17
Rosco	17.75	19.5	19.84	21.5	12.91	25
Mast	17.5	19.4	19.25	20.06	12.38	21.33
Mast+100	19.5	20.75	21.19	21.03	19.56	25.53
Rosco+	20	20.8	22.06	22.44	20.94	27.42
Etest-*S. aureus* 29213 (expected =0.25–1 mcg/mL)						
Dap	1.68	0.84	1.46	0.88	8.95	0.18
Dap+40	0.74	0.47	–	–	–	–
Dap+50	0.35	0.23	0.35	0.19	0.4	0.08

Conclusions: Disks containing daptomycin alone from BBL, Rosco, and Mast resulted in QC values in range for all media except ISOA. With the exception of ISTA, the addition of 100 mcg of calcium to daptomycin Mast disks and Rosco tablets provides for QC results within range for all media including ISOA. The two different lots of BBL affected the MICs and zone diameters. *E*-test containing daptomycin and 40 mcg of calcium provided optimal QC results.

P1891 Impedimetry – test for determination of bactericidal activity of antiseptics

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Objectives: All products for chemical disinfection and antisepsis should meet European Standards requirements. Normative documents describe a suspension test method for establishing bactericidal activity under the specific laboratory conditions. According to those methods suspension of bacterial cells is added to portion of the diluted product followed by collection of samples at specified contact time and finally mixed with agar medium and incubated up to 48 h. New alternative technique based on impedimetric procedure (Bactometer BioMerieux, Vitek System, USA), provide possibilities to reduce this time to several hours. The procedure utilises the capability of an instrument to detect bacterial growth in sample by measurement of media conductivity changes. The impedance detection time is inversely proportional to the number of micro-organisms present at initial inoculum and it correlates with the original bacteria count in the sample as determined by standard plate count method. The aim of the study was to adapt the impedimetric method to microbiological control of antiseptics used for skin treatment.
Methods: Eight different products were analysed throughout the study as following: Kodan Tinktur Forte, Sagrosept and Octeniderm produced by Schulke & Mayer, Manorapid and Dermorapid produced by Antiseptica GmbH, Hospidermin from Lysoform, Frekasept 80 and Frekaderm produced by Fresenius Kabi Deutschland GmbH. The bactericidal activities were evaluated using four strains recommended by standards EN 1040 and EN 12054. Activity was measured by dilution–neutralisation method described in normative documents and impedimetric method. The samples for both methods were prepared in the same way. For impedimetric assay curve was prepared for each strain. The contact time of bacterial strains with antiseptics varied from 30 s to 1 min. The samples were neutralised and mixed with media. In standard plate method the colonies were counted after incubation whereas in Bactometer system the number of bacterial cells was calculated automatically during incubation.
Results: The high correlation was observed between results obtained by normative method and impedimetric measurement. The neutralisation solution was effective and all tested products meet standard requirements. The procedure utilising the Bactometer, provided a rapid and accurate system for bacteria count in bactericidal test.

P1892 In vitro synergism between linezolid and UV radiation against biofilms of *Staphylococcus aureus*

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Objective: Linezolid (LNZ) is an effective antibiotic against gram-positive bacteria including those that are resistant to vancomycin. However, the bacteriostatic activity of the antibiotic may limit its usefulness. A good example is infections related to indwelling medical devices where the micro-organisms exist within the biofilms and bactericidal antibiotics may be more effective. Our objective was to study the effect of combination of linezolid (LNZ) and UV radiation on viability of *S. aureus* (SA) within the biofilms.
Methods: We tested LNZ against 10 clinical isolates of *S. aureus*. The MICs of the antibiotic were determined by using the broth microdilution technique (NCCLS M7-A5). To form biofilms, 100 ml portions of TSB medium containing 1×106 CFU/mL of the micro-organism were delivered to flat bottom 96 micro plates. After 24 h incubation at 37°C, the supernatants were aspirated and the remaining biofilms were washed twice with distilled water. Plates used to study the effects of UV radiation alone or in combination were then exposed for short UV radiation for 5 min. TSB containing the antibiotics at MIC90 value was added to the wells and plates were incubated again for 24 h. Plates were then aspirated followed by addition of 100 microliter Lactate Ringer solution containing XTT (0.5 gm/L) and menadione (1 micromol). Plates were placed in plate shaker for 5 min followed by incubation for 1 h at 37°C and then measured in microplate reader at 490 nm.
Results: When used alone LNZ, percentage viability range 74.33–100, median 93.66 and mean 91.79, or UV radiation, percentage viability range 85.40–100, median 95.52, and mean 94.33, showed very low activity against the biofilms. However in combination, percent viability range 57.38–100, median 80.52 and mean 79, the activity of LNZ was promoted. The killing effect when combined the two agents was significantly increased compared with LNZ alone ($P < 0.05$) or UV radiation alone ($P < 0.01$).
Conclusions: Short time exposure of the biofilms of SA to short wave UV radiation promoted the killing effect of LNZ. This approach to improving the activity of LNZ against biofilm associated bacteria needs to be studied further.

P1893 Antimicrobial activity of amniotic membrane soaked in antibiotics

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Objectives: Amniotic membrane transplantation (AMT) is carried out to accelerate or improve re-epithelisation and reduce inflammation. It has been suggested that AM could be used to deliver anti-infective drugs similar to collagen shields and a variety of prosthetic devices. The aim of our study was therefore to evaluate such hypothesis. We report an in vitro study to assess the antibacterial activity of amniotic-treated AM.
Methods: Aminoglycosides (netilmicin and gentamicin) and quinolones (ofloxacin and ciprofloxacin) were used. The AM fragments were washed in saline, drained and immersed into the antibiotic solution; after incubation at various times at 37°C on a shaker, the washed and drained AM fragments were either tested for antibacterial activity or further incubated in antibiotic-free medium, to evaluate then the activity of both AM and elution media. Antibacterial activity was carried out by the Kirby–Bauer method, measuring the inhibition zone after overnight incubation on *S. epidermidis*.
Results: The AM fragments soaked in antibiotics inhibited bacterial growth: Antibiotic uptake was dose-dependent, and occurred rapidly. Most of the drug was released from the membrane, and the antibacterial effect was present in the elution media at least three days after treatment.
Conclusions: Our preliminary in vitro data show that AM might be used to deliver antibiotics, as reported for collagen shields and other medical prosthetic devices like heart valves.
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P1894 Mechanism of the inoculum effect of *Providencia stuartii* with beta-lactam antibiotics

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Objectives: The inoculum effect (IE) is an increase in the MIC of an antibiotic when the inoculum is increased. Most Enterobacteriaceae possess a chromosomally encoded AmpC beta-lactamase that is expressed at low level in the absence of an inducer. Mutations in regulatory genes result in high-level production. An IE could be due to selection of derepressed mutants or cells with decreased outer membrane permeability. IE might be regulated by a quorum sensing mechanism via an extracellular signal accumu-

lating with increasing cell count. Many studies referred to the IE in relation to the quantity of beta-lactamase, but this was not quantitatively proven for AmpC enzymes. The aim of this work is to evidence that the IE of *P. stuartii* with beta-lactams is due to the quantity of AmpC.
Methods: The MICs of beta-lactams against four *P. stuartii* strains using different initial inocula (104–107 CFU/mL) were determined by a microdilution procedure. Cefotiam MICs were determined using 38 different inocula. To test for the presence of an extracellular signal MIC determination was performed in a conditioned medium according to Rather *et al.* [*J Bac* (1999) **181**, 7185–7191). Spontaneous cefotiam-resistant mutants of strain 34-33 were selected on MH agar plates. Hydrolysis of cefotiam in broth cultures of *P. stuartii* 34-33 with initial inocula of 105–108 CFU/mL was bioassayed using *K. pneumoniae* as indicator organism. Cefotiam degradation by serially diluted crude beta-lactamase extracts from *P. stuartii* 34-33 was tested similarly and the results were compared using a computer program (GraFit, Erithacus software) based on a linear equation.
Results: A strong inoculum effect was seen with all tested *P. stuartii* strains. Cefotiam MICs increased 2048-fold (from 0.125 to 256 microg/mL) with a 10-fold increase in the inoculum of *P. stuartii* 34-33 from 1.2×10^5 to 1.2×10^6 CFU/mL. Quorum sensing was excluded as MICs were not influenced by the use of a conditioned medium. Cefotiam selected resistant mutants at a frequency of 10–7. Hydrolysis of cefotiam with increasing cell counts was parallel to the breakdown of the antibiotic by the crude enzyme extract and correlated with the MIC values.
Conclusions: A small increase in inoculum size converts *P. stuartii* from a beta-lactam sensitive bacterium to a resistant one. This is solely due to the cumulative beta-lactamase production as the cell count increases. Selection of mutants and quorum sensing were excluded to be the basis of the inoculum effect.

P1895 Evaluation of the antimicrobial activity of chlorhexidine gluconate adjunctive zinc polycarboxylate cement in fixed prosthodontics

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Objectives: Dental cement is used as an adhesive material that protects, seals, and insulates the tooth in fixed prosthodontics. The purpose of this study is to examine the feasibility of adding antimicrobial activity to zinc polycarboxylate cement (DURELON â) by the addition of the antiseptic chlorhexidine gluconate (CHx).
Methods: A total of 36 teeth with 84 sites and samples were evaluated. Sites were randomly assigned to either the test group (CHx and zinc polycarboxylate cement) or the control group (conventional zinc polycarboxylate cement). In the test group, CHx was used instead of water. The bacteriological evaluations were done at the begining and in 5th and 13th weeks after the restorations were cemented. Subgingival microbiological samples were obtained by inserting a sterile paper point for 30 s into the gingival sulcus subjacent to the restoration. Standardised paper strips were placed at six locations of each restored individual tooth after isolating the quadrant from salivary contamination. All bridges were made in porcelain and cemented with zinc-poly carboxylate phosphate following standard procedures. The samples were cultured both aerobically and anaerobically by conventional methods. Isolated bacteria were identified by using API Automated System (BioMerieux, France).
Results: The restorations with only zinc polycarboxylate cement resulted in changes in the associated microflora. The altered flora resembled in some ways that which has been observed in adult chronic periodontitis. Increased proportions of Gram-negative anaerobic rods especially *Provotella intermedia* and *Fusobacterium nucleatum* were observed. The restorations with CHx and zinc polycarboxylate cement resulted in only relatively few changes in the associated subgingival microflora relative to pre-experimental findings.
Conclusions: The addition of CHx subscantially increased the antimicrobial action of zinc-polycarboxylate luting agent without interfering with its physical properties.

P1896 Reproducibility of mycobacterial QC strains in the Sensititre rapid growing and slowly growing nontuberculosis mycobacteria broth microdilution susceptibility procedure

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Objective: Presently there are no NCCLS QC ranges for *M. avium* and only a few for rapid growing mycobacteria. The purpose of this study was to measure reproducibility with *M. peregrinum* ATCC 700686, *M. smegmatis* ATCC 19420 and *M. avium* ATCC 700898 on commercial Sensititre dried susceptibility panels. For rapid growers, the effect of incubation time and the addition of saline/0.2% Tween with or without glass beads was also investigated. The antimicrobials evaluated were: amikacin, ofloxacin, ansamycin, streptomycin, clofazimine, kanamycin, capreomycin, rifampicin, ethambutol and isoniazid.
Method: Rapid growers: A 0.5 McFarland standard was prepared from colonies on a 48-h-old TSA/blood plate. Suspensions were vortexed for 5 min allowing large clumps to settle. 50 μL was transferred to 10 mL Sensititre Mueller–Hinton broth. Hundred microlitres were dosed into each well in the plate. Plates were sealed and incubated at 30 degrees. Plates were examined for growth after 72, 96 and 120 h. Two sites tested 25 and 50 replicates MICs for each isolate. An additional 10 results were collected for each of the three suspending media.
Slow growers: The set up procedure was the same except that 7H9 or Sensititre Mueller Hinton broth were supplemented with OADC. Plates were sealed and incubated at 35 degrees and read at 10–14 days depending upon the extent of growth. A total of 50 replicates MICs were collected
Rapid growers: All onscale MICs fell within one doubling dilution of the mode. The maximum shift in MIC over time was one dilution. The presence of saline/Tween resulted in a one well shift in MIC for ansamycin and clofazimine with ATCC 19420 and clofazimine with ATCC 700686
Slow growers: All MICs fell within four dilutions with the exception of Ofloxacin. Weak growth requiring 14 days incubation tended to give lower MICs but fell with four well range.
Conclusions: MICs were highly reproducible allowing tentative QC ranges to be set for testing Sensititre plates. MICs were little affected by the length of incubation. Saline/Tween showed notable effects on the MICs with two of the drugs with rapid growers.