Automated methods for antibiotic susceptibility testing

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Vancomycin reformulation in a Microscan Dried Overnight Panel: a multicentre evaluation with Gram-positive cocci including VRSA

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Objectives: Vancomycin-resistant *Staphylococcus aureus* (VRSA) (MIC >16 μg/ml) were first described in 2002 with four documented VRSA by 2005. Investigations showed inconsistent detection of VRSA by commercial systems including MicroScan panels. This study assessed performance of a revised formulation of vancomycin (Va) on MicroScan Dried Overnight Panels for ability to detect Va resistance in VRSA and other gram-positive cocci (GPC).
Methods: Accuracy was evaluated by comparing the test panel to CLSI reference (REF) panel. Strains tested included staphylococci obtained through NARSA (53 strains with reduced susceptibility to Va and 3 VRSA) and 50 *Enterococci* (CDC challenge set with 25 VRE). An additional 172 isolates of *S. agalactiae*, *S. bovis*, and recent *Staphylococci* and *Enterococci* were evaluated. All testing included turbidity and Prompt inocula, and three read methods: WalkAway and autoSCAN-4 instruments, and manual. In addition, the three NARSA VRSAs and one additional VRSA were tested at CDC and MicroScan panels read manually.
Results: Testing with revised Va formulation showed 3 VRSA to have MICs >16 μg/ml at 16 h with WalkAway or manual read, and >16 μg/ml at 18 h with the autoSCAN-4. Testing of the fourth VRSA at CDC showed a stably resistant morphotype giving MICs of >16 μg/ml with manual read. Results obtained were equivalent to REF for 100% sensitivity in detection of VRSA isolates. Combined results for all strains tested were >97% in essential agreement and >93% in categorical agreement (M100-S15) with REF. There were no very major or major errors.
Conclusion: The revised formulation of Va on MicroScan Dried Overnight Panels showed excellent sensitivity and specificity. Va resistance was detected in all VRSA available at the time of comparative testing and VRE without overcalling resistance in Va-susceptible GPC.

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Direct identification and susceptibility testing of Gram-positive cocci from positive Bactec blood cultures with BD Phoenix Automated Microbiology System

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Aim of the study: Rapid identification and antibacterial susceptibility testing of micro-organisms isolated in blood cultures (BC) can be valuable for clinical management of sepsis. In the present study, we evaluated the performance of a scheme based on direct identification and susceptibility testing of gram-positive cocci by direct inoculation of specially treated fluid collected from positive Bactec 9240 culture bottles into BD Phoenix Automated Microbiology System (PHX system; BD). Results were compared to those obtained by usual standard procedures.
Materials and methods: 100 positive monomicrobic blood cultures showing gram-positive cocci (*Streptococcus* spp. excluded) collected between March 2005 and August 2005 were assessed in the study. Shortly, a Serum Separator Tube (Vacutainer SST-II; BD) filled with fluid aspirated from a positive BC bottle was centrifuged in a swinging bucket rotor. After discarding the supernatant, the bacterial layer was resuspended and then inoculated dropwise into a PHX system ID broth in order to obtain a suspension matching a McFarland 0.5 standard. The remaining of the panels setup and loading was performed according to the manufacturer's instructions.
Results: By comparison with the identification obtained by standard procedure, direct method by Phoenix system correctly identified 27/36 (75%) *Staphylococcus aureus*, 12/20 (60%) *Staphylococcus epidermidis*, 18/30 (60%) coagulase-negative *Staphylococcus* other than *S. epidermidis* and 9/14 (65%) *Enterococcus* spp. Among the 1378 antibiotic-isolate combinations of antimicrobial susceptibility tested, 97.7% showed category agreement. The number of very major, major and minor error was 4 (0.3% of false susceptibility), 4 (0.3% of false resistance) and 21 (1.5% of all antibiotic-organism combinations) respectively.
Conclusion: For gram-positive cocci, the rapid direct method combining BD Phoenix System and Bactec cultures does not provide acceptable bacterial identification, but the susceptibility results obtained more rapidly are accurate for routine use and can be valuable in the clinical management of sepsis.

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Evaluation of MicroScan WalkAway 96 for susceptibility testing of Gram-negative bacilli to quinolones

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Objectives: To evaluate the reliability of the MicroScan WalkAway 96 system (W/A) for determining susceptibility testing of gram-negative bacteria isolated from clinical samples to quinolones, including organisms totally resistant or with low level resistance.
Methods: We evaluated 278 clinical isolates (1 per patient) with different phenotypes of resistance to quinolones, including 108 *E. coli*, 43 *P. mirabilis*, 19 *K. pneumoniae*, 15 *M. morganii*, 11 *C. freundii*, 20 other enterobacteria, 47 *P. aeruginosa*, 11 *A. baumannii* and 4 other non fermenters. Enterobacteria were selected for including consecutive isolates with the following phenotypes (CLSI definitions): Group 1 (54 *E. coli* and 52 non-*E. coli*): susceptible to nalidixic acid (NAL) and to ciprofloxacin (CIP); Group 2 (26 *E. coli* and 34 non-*E. coli*): NAL-resistant (R), and with MIC of CIP ranging 0.5—2 mg/l; Group 3: (28 *E. coli* and 22 non-*E. coli*): NAL-R and CIP-R. W/A testing was done with Combo Urine 1S panels, containing NAL (4, 16 mg/l), CIP (0.12, 0.5, 1, 2 mg/l), norfloxacin (NOR: 8, 16 mg/l) and ofloxacin (OFL: 2, 4 mg/l). The reference method was microdilution (MD, CLSI; 128–0.06 mg/l for NAL, 32–0.015 mg/l for others). Results of W/A and MD were compared by calculating agreement in clinical categories, number of minor, major (ME, false resistance) and very major errors (VME, false susceptibility), and essential agreement (that in ±1 dilution, considering no disagreement when both MICs by W/A and MD were under or over the limit

concentrations in W/A panels). NAL was evaluated only for enterobacteria.
Results: Agreements in clinical categories and essential agreements were: 98.2% and 97.8% (NAL), 93.5% and 92.8% (CIP), 97.5% and 99.6% (NOR) and 95.3% and 99.0% (OFL). For the 1050 organism-quinolone combinations there were 3 (0.3%) VME: 1 each for CIP, NOR and OFL, all in *P. aeruginosa*; 6 (0.6%) ME: 2 for NAL (1 *E. coli*, 1 *P. mirabilis*), 2 for CIP (1 *P. mirabilis*, 1. *S. maltophilia*) and 2 for OFL (2 *S. marcescens*); and 31 (2.9%) minor errors: 15 for CIP (7 *P. mirabilis*, 2 *P. aeruginosa*, 2 *S. marcescens*, 4 others), 6 for NOR (2 *P. aeruginosa*, 2 *S. marcescens*, 2 others) and 10 for OFL (4 *P. mirabilis*, 3 *M. morganii*, 3 others).
Conclusions: The W/A system can be reliably used for testing gram-negative bacteria to quinolones, including isolates resistant or with decreased susceptibility to these antimicrobial agents.

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False resistance to amikacin in *Pseudomonas aeruginosa* with the MicroScan WalkAway 96 system

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Objectives: To determine the reliability of susceptibility testing results of *P. aeruginosa* strains reported as resistant to amikacin (AMK) with the MicroScan WalkAway 96 system (W/A).
Methods: We evaluated 131 consecutive non duplicated *P. aeruginosa* strains isolated during February 2004–January 2005, resistant to AMK according to the W/A system. Identification and susceptibility testing was performed according to manufacturer's instructions. Two type of panels with the following concentrations (mg/L) of aminoglycosides were used: Negative Combo 1S panel [gentamicin (GEN): 4 and 8; tobramycin (TOB): 4 and 8, AMK: 8 and 16] and Urine Combo 1S panel (GEN 4 and 8; TOB 4 and 8; AMK 16 and 32). The results were compared with those obtained by reference microdilution (CLSI guidelines). MICs were also obtained with Etest. For comparisons, MICs by Etest within two values of the 2-log dilution scale were rounded up to the next higher dilution. Percentages of agreements in clinical categories were calculated. Disagreements were defined as very major errors (VME: resistant by microdilution and susceptible by W/A or Etest), major errors (ME: susceptible by microdiluiton and resistant by W/A or Etest) and minor errors (mE: intermediate by one method and susceptible or resistant by the other one). Agreements of MICs by Etest within one and within two dilution steps of those obtained by microdilution were also calculated.
Results: Percentages of agreement in clinical categories between W/A and microdilution for GEN, TOB and AMK were 81.7%, 92.4% and 3.0%, while for Etest and microdilution the corresponding values were 97.7%, 100% and 99.2%. No VME were obtained with W/A, but ME were observed for 18/131 (13.7%), 9/131 (6.9%) and 120/131 (91.6%) of the strains. mE with W/A were obtained in 6/131 (4.58%), 1/131 (0.7%) and 7/131 (5.3%) of the strains. Only mE were obtained with the Etest: 3/131 (2.3%) for GEN and 1/131 (0.7%) for AMK. Agreement within one and within two dilution step of MICs by Etest and by microdilution were 91.6% and 98.4% (AMK), 81.6% and 94.6% (TOB) and 87.0% and 97.7% (GEN).
Conclusions: In his study, the W/A system was not reliable for susceptibility testing of *P. aeruginosa* to AMK, as most strains reported as AMK-resistant actually were susceptible to this antibiotic. To a lesser extent, major errors were also noted for GEN and TOB. On the other hand the Etest agreed very well with reference microdilution for all three aminoglycosides.

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Evaluation of identification and antimicrobial susceptibility testing of bacterial pathogens by VITEK 2 Compact System

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Objectives: The aim of this study was the evaluation of VITEK 2 Compact System for identification and antimicrobials susceptibility determination of the most important bacterial clinical pathogens. The results of the analyses were compared with those obtained by other microbiological methods.
Methods: A total of 279 clinically significant gram-negative (n = 158) and gram-positive (n = 121) bacterial pathogens were included in the study. The isolates were collected from patients hospitalised in different medical centres in Poland and were not epidemiologically related. They were identified to the species level according to standard methods. These results were compared with identification data using VITEK 2 Compact GNI and DPI cards. MICs of various antimicrobials were evaluated by agar or broth dilution methods in accordance with the CLSI guidelines. A wide variety of clinically important antimicrobial resistance mechanisms were represented by the isolates. These included extended-spectrum beta-lactamases in *Enterobacteriaceae*, methicillin resistance in *Staphylococci*, decreased pneumococcal susceptibility to penicillin and resistance to vancomycin and to high concentrations of aminoglycosides in *Enterococci*. The same antimicrobial agents were tested as those that are present in the VITEK 2 Compact cards used in the study. Characteristic features of the automatic method of identification and drug susceptibility determination were defined: total accordance of resistance categories, accuracy, sensitivity, specificity and errors.
Results: A high rate of ID agreement (96,5%) between VITEK 2 Compact and the conventional methods was observed. It ranged from 95% for gram-positive cocci to 98,1% for gram-negative rods. The study has shown a high correlation of results (n = 1118) obtained by aid of VITEK 2 Compact drug sensitivity tests and the results obtained by the aid of reference methods. The total accordance of resistance categories has been estimated at the level of 99,1%. A high concordance (99%) between the two methods was also achieved in the respect to the detection of bacterial resistance mechanisms
Conclusions: VITEK 2 Compact System is an esthetical, functional and easy to use device. The VITEK 2 Compact System appears a reliable tool for the detection and interpretive reading of clinically important mechanisms of resistance and can be recommended for routine work.

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Evaluation of the Vitek-2 system using AST-N020 y AST-N041 cards for susceptibility testing of beta-lactam-resistant *Escherichia coli* and *Klebsiella pneumoniae* strains of clinical origin

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Objectives: To evaluate the Vitek-2 (V2) system (bioMérieux, France) using AST-N020 or AST-N041 cards for susceptibility

testing and recognition of phenotypes of resistance of clinical isolates of *Escherichia coli* (Eco) and *Klebsiella pneumoniae* (Kpn) resistant to beta-lactams. AST-N041 contains specific test for extended-spectrum beta-lactamase (ESBL) detection.

Methods: We tested 136 organisms: 44 Eco and 20 Kpn producing TEM, SHV or CTX-M ESBL, 39 Eco hyperproducing chromosomal AmpC (HAmpC), 11 Kpn ESBL(+) and lacking porins [POR(-)] and 22 Kpn with plasmid-mediated AmpC beta-lactamase (pACBL). MICs were determined by microdilution (MD, CLSI guidelines); resistance mechanisms were studied by beta-lactamase characterization (isoelectric focusing and gene sequencing) and SDS-PAGE of outer membrane proteins. MICs of amoxicillin-CLV (AMC), piperacillin-tazobactam (PTZ), cefoxitin (FOX), cefotaxime (CTX), ceftazidime (CAZ) and cefepime (FEP) determined with N020 and N041 cards were compared between them and with the reference MD. Presumed phenotypes of resistance by the V2 advanced expert system (AES), and suggested changes in clinical categories were also compared for both cards.

Results: For the 136 strains, MICs of AMC, PTZ, and FOX with each card were within one dilution step for 135 strains; MICs of CTX, CAZ, and FEP with N020 were ≥2 dilutions higher than with N041 for 7, 2 and 0 strains, while the reverse was noted for 8, 6 and 3 strains. MICs of FEP with N020 and N041 were ≥2 dilutions lower than with reference MD for 28/64 ESBL organisms, and for 10/33 Kpn POR (-) or pACBL (+), but agreed for all Eco HAmpC. Phenotypes suggested by the AES with N020 card agreed with reference results for 59/64 ESBL (+) strains, 9/11 ESBL (+)/POR(-) and 19/22 pACBL. With N041 these values were 61/64, 11/11 and 17/22, respectively. For 31/39 Eco HAmpC the AES with N020 suggested several phenotypes, including an ESBL, reporting them as resistant to CTX, CAZ and FEP; the N041 card correctly discarded the presence of an ESBL in all 39 strains.

Conclusions: Differences in MICs of CTX, CAZ and FEP obtained with AST-N020 and AST-N041 cards for organisms ESBL(+) or pACBL(+) were noted in comparison with reference results, but this usually did not translate into changes in clinical categories after AES suggestions. The AES shows excellent agreement with underlying mechanisms of resistance with card N041, but with N020 it suggests false presence of ESBLin most Eco HAmpC.

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Susceptibility testing of unusual species of *Enterobacteriaceae*: comparison of disk diffusion, Vitek 2, and broth microdilution

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Objective: Unusual species of *Enterobacteriaceae* can cause clinical infections in humans on rare occasions. However, there have been few studies evaluating the accuracy of susceptibility testing methods for these organisms. The objective of this study was to determine whether disk diffusion (DD) and Vitek 2 gave accurate susceptibility test results for these unusual isolates when compared to the Clinical and Laboratory Standards Institute (CLSI) broth microdilution (BMD) reference method.

Methods: Sixty-one isolates representing 15 genera and 25 different species including *Buttiauxella*, *Cedecea*, *Ewingella*, *Kluyvera*, *Leminorella*, *Rahnella*, and *Yokenella* from the strain collection of the Centers for Disease Control and Prevention were tested by the DD and BMD reference methods using Mueller-Hinton (MH) agar and cation-adjusted MH broth at 35C for 18–20 h. Antimicrobial agents included aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, penicillins, and trimethoprim-sulfamethoxazole. CLSI interpretative criteria for *Enterobacteriaceae* were used. Identification and susceptibility of the isolates were also tested by Vitek 2 as described by the manufacturer; identification results were compared to conventional biochemical tests.

Results: Of the 12 drugs tested by BMD and DD, 10 showed >95% categorical agreement (CA). CA was lower for ampicillin (80.3%) and cefazolin (77%). There were three very major errors (all with cefazolin, one resolved on repeat testing), one major error (also with cefazolin), and 22 minor errors. Thirty-four of 40 isolates (covering 12 species) that were in the Vitek 2 database were identified correctly to species level, 1 was correct to genus level, and five were reported as unidentified. Vitek 2 generated MIC results for 41 (67.2%) of 61 isolates but categorical interpretations (S, I, R) were provided only for 24. For the 17 drugs tested by both BMD and Vitek 2, essential agreement (41 isolates) ranged from 80.5–100% and CA (24 isolates) ranged from 66.7% (ampicillin) to 100%; twelve drugs exhibited 100% CA.

Conclusions: DD, which is often the backup method for laboratories with automated systems, provided accurate susceptibility test results for these unusual organisms. The Vitek 2 identified 85% of a subset of isolates correctly, but only provided MIC interpretations for 58.5%. Thus, DD provides a reliable alternative to BMD for testing unusual *Enterobacteriaceae*, some of which cannot be tested with automated methods.

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Evaluation of new VITEK 2 AST cards for detection of ESBL mediated resistance in *E. coli* and *Klebsiella* spp.

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Objective: VITEK 2 (BioMerieux) has developed novel gram-negative susceptibility cards (AST-045 and AST-046) to improve the accuracy of ESBL detection among *E. coli* and *Klebsiella* spp. These cards contain a specific ESBL test combining cefotaxime, cefepime and ceftazidime alone and with clavulanate. We evaluated these cards and Advanced Expert System (AES) ability to detect ESBL mediated resistance in a well characterized collection of ESBL-producing *E. coli* and *Klebsiella* spp clinical isolates.

Methods: A total of 100 *E. coli* strains (83 ESBL and 16 AmpC producing *E. coli*) and 26 *Klebsiella* spp isolates (26 ESBL strains) were included. The presence of ESBL was confirmed by double disc synergy test and combined disc with three substrates and PCR for bla TEM, bla SHV and bla CTX-M genes. ESBL were characterized by isoelectric-focusing and/or DNA sequencing. ESBL strains included *E. coli* harbouring CTX-M group 1 enzymes in 50%, CTX-M group 2 in 11%, TEM enzymes in 13% and SHV enzymes in 6%. All ESBL producing *Klebsiella* spp harboured CTX-M enzymes in combination with TEM or SHV beta-lactamases. Organisms were subcultured twice on blood-sheep agar before loading the AS045 and AS046 cards according to the manufacturer recommendations.

Results: No discordances were observed between the two cards. The mean time to obtain results was 7.5 h (range 5.75–9.25). Overall agreement for enzyme detection was 97 %. ESBL VITEK 2 test was positive in all ESBL-producing strains. Sensitivity and specificity were: *E. coli*, 99% and 100% respectively, *K. pneumoniae* 100% for both and *K. oxytoca* 71%

and 100% respectively. Disagreements concerned mainly CTX-M producing *K. oxytoca* in which the ESBL positive card test was systematically interpreted by AES as high-level penicillinase production. In 33% of ESBL-producing *K. pneumoniae* AES inferred the expression of impermeability mechanism in addition to ESBL production.
Conclusion: These new AST VITEK 2 cards showed accurate and rapid detection of ESBL-producing *E. coli* and *K.pneumoniae*, including strains harbouring CTX-M enzymes. The lower sensitivity of AES to detect CTX-M producing *K. oxytoca* indicates the difficulty to detect this emerging phenotype in this organism.

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Detection of ESBL mediated resistance in *Enterobacter* spp. by using new VITEK2 AST cards and advanced expert system

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Objective: VITEK 2 AST-045 and AST-046 cards (BioMerieux) for Gram-negative susceptibility testing include a specific ESBL detection test combining cefotaxime, cefepime and ceftazidime alone and with clavulanate. Because this test has been validated by the manufacturer only for *E. coli* and *Klebsiella* spp , the result is reported only for these species. We determined the ability of VITEK 2 and Advanced Expert System (AES) to detect ESBL mediated resistance in *Enterobacter* clinical isolates using these cards.
Methods: A total of 76 *Enterobacter* isolates : 63 ESBL producing strains (32 *E. aerogenes* and 31 *E. cloacae*) and 13 non ESBL producing strains (7 *E. aerogenes* and 6 *E. cloacae*) were inoculated into the VITEK 2 AST-045 and AST-046 cards according to the manufacturer recommendations. Production of ESBL was confirmed by double disc synergy test (with ceftazidime, cefotaxime and cefepime), combined disc method (Oxoid) and PCR for bla TEM, bla SHV and bla CTX-M genes. *E. cloacae* harboured SHV12 in combination with CTX-M9 in 94%. *E. aerogenes* ESBLs included: TEM enzymes in 59%, SHV in 9% or other combinations of enzymes in 32%. Results were graded as ''agreement'' when AES inferred ESBL mechanism, ''partial agreement'' when AES suggested several mechanisms including ESBL and ''disagreement'' when AES concluded to different mechanism(s).
Results: Results are summarized in the table.Overall sensitivity was 92% (91% for *E. aerogenes* and 94% for *E. cloacae*) and specificity 23.1% (14.3% for *E. aerogenes* and 33.3% for *E. cloacae*). The mean time to obtain results was 8.2 h (range 6.25–11).

Species (No ESBL producing strains)	Agreement N (%)	Partial agreement N (%)	Disagreement N (%)
E.aerogenes (32)	18 (56.2)	11 (34.3)	3 (9.4)
E. cloacae (31)	15 (48.4)	14 (45.2)	2 (6.4)

Conclusion: This study shows that VITEK2 AES provides a rapid and sensitive tool to detect possible ESBL production in *Enterobacter* spp by using AST-045 or AST-046 cards but confirmatory analysis is frequently necessary. More efficient automated methods should be developed to distinguish ESBL production from AmpC hyperproduction in this genus.

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Comparison of the E-test method with the VITEK 2 antimicrobial susceptibility detection system for screening of extended-spectrum beta-lactamase *Klebsiella* and *E. coli* strains in a Greek university hospital

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Objectives: Extended-spectrum beta-lactamases (ESBLs) are a rapidly evolving group of beta-lactamases which share the ability to hydrolyze third-generation cephalosporins and aztreonam yet are inhibited by clavulanic acid. At present the detection of ESBLs, which frequently are plasmid encoded, in *Klebsiellae* and *Escherichia coli* strains carries trumendous clinical significance which relates to the extremely limited antibiotic options in the treatment.
Methods: In our study, a total of 782 clinical isolates (553 *E. coli*, 229 *Klebsiella* strains) were examined for the presumptive detection of extended-spectrum beta-lactamase (ESBL) production by tow methods: the VITEK 2 automated detection system (BioMerieux) and the commercially available ESBL screening E-test strip (AB Biodisk, Solna, Sweden).
Results: The percentage of the ESBL-producing *Klebsiella* and *E. coli* strains identified by VITEK 2 system was 11,7% (27 from 229) for *Klebsiella* strains and 4.88% for *E. coli* strains (27 from 553).The most common infection associated with ESBL-producing pathogens was urinary tract infection (44%), followed by wound infection (31%) and bloodstream infection (25%). On the contrary using the E-test strips, which based on the evaluation of the difference between the antimicrobial activity of ceftazidime alone compared to that of ceftazidime plus clavulanic acid, ESBL production was detected in only 16 *E. coli* strains and in 22 *Klebsiella* strains. These data demonstrate that only 59% of *E. coli* and 81% *Klebsiella* strains, that were alerted as ESBL positive with the VITEK 2 system, were also positive with the E-test method.
Conclusion: These data indicate that even if Vitek 2 ESBL test is reliable for the detection of ESBLs in *E. coli* and *K. pneumoniae*, it is precarious to be directly assumed as the sole criterion for defining ESBL production in these two bacterias. As ESBLs become more complex, diverse and widespread, the likelihood of any single test being universally appropriate for their detection must diminish. Conclusively we can assume that clinical microbiology laboratories not only should rely on these rapid automated systems but also use another method for screening ESBL producers, such as the E-tests.

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Evaluation of the VITEK 2® ESBL test in detecting ESBL-positive clinical *Escherichia coli* and *Klebsiella pneumoniae* isolates

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Objectives: Rapid and accurate detection of ESBL-positive *Enterobacteriaceae* is an absolute must as antimicrobial therapy is guided by the susceptibility results of the organism. In the present study we evaluated the VITEK 2 ESBL test, consisting of indicator antibiotics with and without clavulanic acid, on a set of ESBL-positive clinical isolates.
Methods: Eighty *E. coli* and 85 *K. pneumoniae* strains were obtained at the Dr Soetomo Hospital in Surabaya, Indonesia. Strains originated mainly from urine (n = 104) and blood specimens (n = 18). All isolates were ESBL-positive by the

double disc test method and by ESBL combination discs (Oxoid). Of all isolates, β-lactamase enzymes were isolated for Iso-electric point determination. Strains were tested in the VITEK 2 AST-N041 panel containing cefepime, ceftazidime, and cefotaxime alone and in combination with clavulanic acid.
Results: The VITEK 2 ESBL test confirmed 72 of the *E. coli* (90%) and 73 of the *K. pneumoniae* (86%) as ESBL positive. For two *E. coli* strains VITEK 2 gave an indeterminate result. The six *E. coli* ESBL-negative strains consisted out of isolates containing TEM-like enzymes (n = 4), TEM + chromosomal beta-lactamase (n = 1) and SHV + chromosomal beta-lactamase (n = 1). TEM-type beta-lactamases (n = 72) were most prevalent in *E. coli* ESBL-positive isolates. The 12 K. pneumoniae ESBL-negative isolates consisted out of isolates containing no plasmid encoded beta-lactamases (n = 6), SHV-like enzymes (n = 3), TEM-like enzymes (n = 2), CTX-M + SHV-like (n = 1). In the ESBL-positive isolates (n = 73) CTX-M like enzymes were demonstrable in 33 isolates.
Conclusions: The ESBL VITEK 2"R" test gives rapid and conclusive results. However, the discrepant results have to be resolved in order to give the ''true'' accuracy of the VITEK 2 ESBL test.

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Overestimation of extended-spectrum beta-lactamases by VITEK-2 and possible consequences for antibiotics policy

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Objective: We evaluated identification of Extended-Spectrum Beta-Lactamases (ESBLs) with the VITEK-2 system (bioMérieux, Marcy l'Etoile, France) by comparing it to ESBL detection using Etest (AB-Biodisk, Solna, Sweden) in clinical isolates of Intensive Care Unit (ICU). Furthermore we evaluated the possible impact of these data on the antibiotic policy in our hospital.
Methods: We have collected prospectively 100 consecutive *Enterobacteriaceae* strains (no duplicates) from patients hospitalised in ICU for more than four days. The following samples were included: blood, sputum, broncho-alveolar lavage fluid or tracheal aspirates and pus. Identification as well as susceptibility-profile were determined with the VITEK-2 system. *Escherichia coli* and *Klebsiella pneumoniae* growing on screening plates containing ceftazidime 0.5 μg/ mL or cefotaxime 0.5 μg/ mL were further tested with following Etest ESBLs strips: ceftazidime ± clavulanate (TZ/TZL) and cefotaxime ± clavulanate (CT/CTL). *Klebsiella oxytoca* and all other AmpC producers were tested using Etest ceftazidime. Only strains with MIC's for TZ > = 4 μg/ mL were further tested: Etest ESBLs strips (TZ/TZL) for *K. oxytoca* and cefepime ± clavulanate (PM/PML) for AmpC producers.
Results: The VITEK-2 expert system identified 16 possible ESBLs. Out of those 16 strains, the expert system reported 10 strains as 'ESBL or high level cephalosporinase'. For three strains VITEK-2 suggested a number of resistance mechanisms ('high level natural penicillinase', 'impermeability' and 'acquired penicillinase') and three strains were determined as 'ESBL' by VITEK-2. Only three strains were confirmed by Etest as ESBL: two of the isolates were identified by VITEK-2 as 'ESBL' and one as 'ESBL and impermeability'. Thus one of the three strains determined by VITEK-2 as 'ESBL' wasn't confirmed by our Etest.
Conclusion: VITEK-2 overestimates the number of ESBLs among *Enterobacteriaceae*. The VITEK-2 report of 'high level cephalosporinase or ESBL' was systematically false-positive for ESBL. Automatic validation of VITEK-2 results may lead to unnecessary use of carbapenems. Rechecking of possible ESBL positive identifications on VITEK-2 could lead to alternative therapeutic options for these strains and have a substantial impact on antibiotic policy.

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Detection of decreased susceptibility of *Staphylococcus aureus* to vancomycin with the VITEK 2 System

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Objective: The purpose of this study was to determine if the combination of the current vancomycin VITEK 2 MIC test and a new vancomycin resistant *Staphylococcus aureus* (VRSA) screen test* could accurately detect strains of *S. aureus* with decreased susceptibility to vancomycin. Recently, four VRSA strains were detected in the United States. These strains were not reliably detected with the current vancomycin MIC test on VITEK 2. Due to the acquisition of vanA, these VRSA strains grow very differently than the vancomycin intermediate *S. aureus* (VISA) or the hetero-resistant *S. aureus* (hVISA). A new VRSA screen test for *S. aureus* was developed to specifically flag VRSA strains. This screen test uses a different medium formulation to increase expression of resistance in these VRSA strains.
Methods: Seventy-two *S. aureus* isolates (including strains received from CDC and NARSA) with vancomcyin MICs of 2–8 μg/ml were tested on VITEK 2 AST-P541 cards. Reference MICs were determined by broth microdilution according to Clinical Laboratory Standards Institute (CLSI) guidelines. VITEK 2 vancomycin MIC results, along with corresponding Advanced Expert System (AES) findings were evaluated. The current CLSI breakpoints for vancomycin are ≤4 S, 8–16 I, ≥32 R (with a note stating that any strain with a result of ≥4 be sent to a reference laboratory).
Results: The essential agreement was 95.8% (69/72), category agreement was 90.3% (65/72), with 9.7% (7/72) minor errors, and no major errors. Six of the seven minor errors were one-dilution errors with a reference MIC of 8 μg/ml calling 4 μg/ml by VITEK 2. Each of the six strains was flagged by AES as a VISA phenotype. Only the known VRSA strains gave a positive VRSA screen result. Susceptible, VISA, and hVISA strains gave negative VRSA screen results. A positive screen test strongly suggests that a VRSA may be present, however, resistance to vancomycin must be confirmed by performing an offline test as recommended by CLSI (M100-S15, vol. 25 no.1, January 2005) or as recommended by the local authorities.
Conclusion: These data indicate that the VITEK 2 can accurately determine decreased susceptibility to vancomycin by using the combination of the current MIC test (for susceptible, VISA, and hVISA strains) and the VRSA screen test (for VRSA).*The new VRSA screen test for *S. aureus* has not been cleared for use with the VITEK 2 system by the United States FDA and is not yet available for commercial use.

P454

Bench validation of methicillin-resistance detection by Vitek 2 AST P-536 card combined with cefoxitin disc diffusion in clinical staphylococci isolates

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Objectives: Test nonselected consecutive clinical strains of *Staphylococci* for methicillin resistance (MR) with Vitek2 (VT2)

instrument combined with cefoxitin disc diffusion by Kirby-Bauer (FOXKB) and devise a strategy to solve discrepancies.
Methods: During a 6 months period (January 2005–June 2005) all clinically significant *Staphylococci* isolated in our laboratory, except blood culture isolates, were tested simultaneously by VT2 AST P-536 card and Advanced Expert System (AES) (R04.00D) and by FOXKB using CLSI guide lines and M100-S14 interpretive criteria. Discrepancies between the three tests [OX calculated MIC (OXMIC); growth in the presence of 3 mg/L OX + 2% NaCl (OXSALT) (for SA); disc diffusion (FOXKB)] were resolved by the detection of mecA gene by PCR and phenotypic identification to the species level for coagulase-negative *Staphylococci* (CNS).
Results: 787 *S. aureus* (SA) (82 MRSA) and 290 CNS (175 MR CNS) were tested. Of these, 45 (4%) presented discrepant results: 15 SA and 30 CNS [*S. saprophyticus* (11), *S. lugdunensis* (10), *S. epidermidis* (4), *S. hominis* (2), *S. haemolyticus* (1), *S. warneri* (1) and *S. xylosus*(1)]. Of the 15 SA, three were false MSSA by VT2 and 12 (2%) were MSSA flagged by the AES because of discordances between OXMIC and OXSALT. For all strains, FOXKB interpretation correlated with mecA gene determination. Of the 30 CNS, there were 25 false MR and 1 false MS by OXMIC. By comparison, FOXKB missed four MR [*S. epidermidis* (2); *S. hominis* (1); *S. saprophyticus* (1)]. Thus, after exclusion of results flagged by the AES, the PPV/NPV of VT2 for detecting MR in SA vs CNS were 100%/99% vs 87%/99%. By comparison these figures for FOXKB were 100%/100% vs. 100%/97%.
Conclusions: MR results obtained by VT2 AST P536-card combined with FOXKB correlated in 96% of *Staphylococci* analysed. Among the 4% discordant results, FOXKB interpretive criteria could replace mecA gene determination for SA; for CNS, OXMIC by VT2 overcalled MR in *S. saprophyticus* and *S. lugdunensis* mainly. For them, mecA gene determination is mandatory to solve discrepant results. VT2 AST card for *Staphylococci* should include FOX.

P455

Capability of the VITEK®2 System to screen the beta-lactamase producing *Enterococcus faecalis*

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Objective: The first reported beta-lactamase-producing (Bla+) enterococcal isolate was recognized in the early 1980s. Since that time, nearly all other Bla+ *Enterococci* reported have been *Enterococcus faecalis*. Beta-lactamase detection using dilution methods is known to be difficult. A nitrocephin-based beta-lactamase test is generally recommended. The purpose of this study was to evaluate the capability of the VITEK® 2 system to screen the Bla+ *E. faecalis* strains using the MIC of benzylpenicillin.
Methods: The nitrocefin-based beta-lactamase test was performed on 53 *E. faecalis* isolates. These strains were tested using three lots of VITEK 2 cards (two lots of AST-P532 and one lot of AST-GP61), on three VITEK 2 instruments, from three subcultures. Nine benzylpenicillin VITEK 2 MICs were obtained for each strain. The reference MIC was determined by broth microdilution according to Clinical Laboratory Standards Institute (CLSI) guidelines. Based on the results of the nitrocefin test, a cut-off for differentiation of Bla+ and Bla- (beta-lactamase non-producing) *E. faecalis* strains was set for both MIC determination methods.
Results: The nitrocefin test was positive for 17 strains. The modal (or median) benzylpenicillin VITEK 2 MICs were determined for each of the 53 strains and compared to broth microdilution. The essential agreement was 94.3% (50/53). A slight overestimation of the modal VITEK 2 MICs compared to the reference MICs was observed. By choosing a benzylpenicillin VITEK 2 MIC cut-off of ≥8 μg/mL for the Bla+ strains (and < 8 for the Bla- strains), sensitivity and specificity were 99.3% (152/153) and 88.3% (286/324) respectively. By comparison, a sensitivity of 100% (17/17) and a specificity of 83.3% (30/36) were obtained with a benzylpenicillin microdilution MIC cut-off of ≥4 μg/mL.
Conclusion: The benzylpenicillin VITEK 2 MIC can help to screen Bla+ *E. faecalis* strains. A benzylpenicillin MIC ≥8 μg/mL indicates a presumptive Bla+ *E. faecalis* which has to be confirmed by a nitrocefin test. Furthermore, with optimized benzylpenicillin MIC ranges for the wild and acquired penicillinase resistance phenotypes, the VITEK 2 Advanced Expert SystemTM (AES) would increase the discrimination between both phenotypes. The nitrocefin test would be then performed only when acquired penicillinase phenotype is proposed. Consequently, AES would allow labs to drastically decrease the number of off-line nitrocefin tests performed on *E. faecalis*.

P456

Performance evaluation of the *Streptococcus pneumoniae* antimicrobial susceptibility investigative use only SPCA3 card on the VITEK® 2 system

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Objective: The purpose of the study was to demonstrate the effectiveness of *S. pneumoniae* (SPN) antimicrobial susceptibility tests for ertapenem (ERT), garenoxacin (GAR), and telithromycin (TEL) on the VITEK®2 platform in a clinical setting.
Methods: This multicentre trial tested SPN strains using an investigative use only (IUO) card (SPCA3) containing ERT, GAR, and TEL. Test cards were inoculated from a standard inoculum for each SPN strain. Challenge (80 isolates), reproducibility (34 isolates), and quality control (SPN ATCC 49619) isolates were tested by automatic and manual dilution using the SPCA3 IUO card. The 311 clinical isolates were tested by automatic dilution. Broth microdilution reference testing was assayed on each challenge, quality control and clinical isolate from the same standard inoculum used for the SPCA3 cards. Reproducibility tests were performed in triplicate daily for three days. Twenty replicate tests of SPN ATCC 49619 were performed at each site for quality control.
Results: Essential agreement for all 311 clinical isolates was >99.7%, while category agreement was 95.5%, 98.7%, and 99.7% for ERT, GAR, and TEL, respectively. Best-case reproducibility results revealed an essential agreement of 100% and >99.6% for the automatic and manual dilution, respectively, of ERT, GAR, and TEL. QC in-range results for the SPCA3 card were >98.5% for all three antibiotics. Essential agreement for automatic and manual dilution method challenge experiments was >97.5% for ERT, GAR, and TEL. No very major errors were observed.
Conclusions: This evaluation provides convincing evidence that the performance of antimicrobial susceptibility tests for ERT, GAR, and TEL on the VITEK®2 platform is comparable to conventional testing in a clinical laboratory.

Epidemiology of MRSA

P457

Molecular epidemiology of community-associated methicillin-resistant *Staphylococcus aureus*

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Objective: The purpose of this study is to gain a better understanding of the molecular epidemiology of CA-MRSA.
Methods: Isolates were prospectively collected from patients with documented CA-MRSA.
Results: Over a period of 2 years (October 2003–2005) prevalence of CA-MRSA was 11.0%. It has increased from 5.3% in 2003 to 11.0% in 2005. The sites of culture were skin/soft tissue (75%), respiratory tract (3%), blood (4%), urine (3%), and nares (3%). Isolates were susceptible *in vitro* to multiple antimicrobial agents with the exception of beta-lactams, clindamycin, and erythromycin. The MIC90 were < 2/38 mg/L for trimethoprim/sulfamethoxazole, 8 mg/L for tetracycline, >2 mg/L for clindamycin, < 1.0 mg/L for gentamicin, and 2.0 mg/L for linezolid. There was no resistance increase noted over the period of study. The results of molecular typing are listed on Table 1.

Table 1. Molecular Typing of Community-Associated *Staphylococcus aureus* Isolates

SCC*mec* Type	N	PVL		*Agr* Type				
		Positive	Negative	I	II	III	IV	unknown
II	30	0	30	1	29	0	0	0
IV a	57	53	4	52	1	4	0	0
IV b	1	1	0	1	0	0	0	0
IV (subtype unknown)	13	0	13	5	7	0	0	1
Unknown (not I-IV)	1	1	0	0	0	0	1	0
Total	102	55	47	59	37	4	1	1

Conclusion: Strains of *S. aureus* from patients documented to have community-associated acquisition demonstrated more molecular diversity than in earlier studies with variability in PVL presence, SCCmec, and PFGE types.

P458

Clinical epidemiology of community-associated methicillin-resistant *Staphylococcus aureus*

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Background: The purpose of this study is to gain a better understanding of the epidemiology of community associated MRSA (CA-MRSA).
Methods: Patients with CA-MRSA were prospectively compared with patients with health care associated MRSA (HA-MRSA) and with community associated MSSA (CA-MSSA).
Results: Over a 2-year period (October, 2003–2005) overall prevalence of MRSA in the community was 46%; CA-MRSA was 11% (increase from 5.3% in 2003 to 11% in 2005), HA-MRSA 35%. The remaining 54% had CA-MSSA. Patients with CA-MRSA (n 102), HA-MRSA (n 102) and CA-MSSA (n 102) had a median age of 46, 62 and 52 respectively (p < 0.001). Sites of culture were skin/soft tissue (75, 74 and 92 %), respiratory tract (3, 4, and 3 %), blood (4, 7 and 1%), urine (3, 11 and 0%), and nares (3, 2 and 2%). Isolates of CA-MRSA were susceptible *in-vitro* to multiple antimicrobial agents with the exception of beta-lactams, clindamycin and erythromycin. The MIC 90's were < 2/38 μg/ml for TMP/SMX, 8 μg/ml for tetracycline, >2 μg/ml clindamycin, < 1.0 μg/ml for gentamicin and 2.0 μg/ml for linezolid; resistance did not increase over the period of study. 55, 62 and 46% (p = NS) had received previous antibiotics in 3 months prior to infection; 60, 74 and 65% (p = 0.111) had multiple outpatient visits; 45, 31 and 50% (p = 0.020) travel; 13, 0 and 0% (p < 0.001) health club; 50, 29 and 53% (p = 0.001) pets; 5, 0 and 0% (p = 0.006) MSM; 19, 16 and 21% (p = NS) with family members of HCW; 30, 57 and 33% (p < 0.001) had recent contact with a hospitalized patient; 18, 5 and 30% (p < 0.001) with a history of sports activity and 3, 3 and 0% (p = NS) respectively incarcerated within last year. Demographics and comorbidities are listed in Table 1.

Table 1. Univariate Comparison of Characteristics of Patients with *S. aureus* Infections Originating in the Community

Characteristics		CA-MRSA (n=102)	HA-MRSA (n=102)	CA-MSSA (n=102)	*P*
Age (median, range)		46 (3-88)	62 (19-90)	53 (3-82)	<.001
Gender	Male	58%	57%	48%	NS
	Female	42	43	52	
# Antibiotic classes	0	45%	38%	54%	.132
received in	1	40	49	42	
past year	2	10	7	4	
	>=3	5	6	0	
Comorbid conditions					
Injection drug use		0	3	1	.170
Cardiovascular disease		14	43	13	<.001
Diabetes mellitus		14	42	22	<.001
Chronic skin disease		7	2	9	.100
Pulmonary disease		19	17	4	.003
Renal disease		4	16	1	<.001
Malignancy		6	16	4	.005
HIV		4	0	0	.017
Neurological disease		3	7	3	NS
Prior endocarditis		0	1	1	NS
Prior influenza		3	1	5	NS
Concurrent infection		19	4	14	.005

Conclusion: When compared to patients with HA-MRSA patients with CA-MRSA were younger and less commonly had underlying disease. Other risk factors were similar among the groups studied.

P459

Comparison of epidemic MRSA isolated in the three Nordic countries Denmark, Finland and Sweden during 2003–2004 – how similar are they?

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Objectives: To compare the most frequently found methicillin-resistant *Staphylococcus aureus* (MRSA) in the Nordic countries Denmark, Finland and Sweden during 2003–2004.
Methods: MRSA types isolated from at least 10 patients/carriers during the 2-year study period were identified in the respective countries (in this study, only isolates with indistinguishable PFGE patterns were included in a type). One isolate of each frequently found MRSA type was exchanged between the laboratories, and PFGE was performed. In addition, the isolates were subjected to spa typing, multilocus sequence

typing (MLST), SCCmec typing, antimicrobial susceptibility testing, and PCR for detection of Panton-Valentine leukocidin (PVL) genes.
Results: The number of frequently found MRSA in Denmark, Finland and Sweden were 8, 31 and 20, respectively. PVL genes were detected in ten of the isolates (3/8, 1/31, 6/20). The 59 isolates gave 54 indistinguishable PFGE patterns, of which the following five were found in two countries each: (i) UK E15 (UK EMRSA-15, t032, ST22, PVL negative), in Finland and Sweden; (ii) SE98-6a (UK EMRSA-15 variant, t032, ST22, PVL negative), in Finland and Sweden; (iii) SE00-3 (UK EMRSA-16 variant, t019, ST30, PVL positive), in Denmark and Sweden; (iv) SE97-3 (Berlin IV variant, t015, ST45, PVL negative), in Finland and Sweden; (v) SE00-7 (France B variant, t008, ST8, PVL positive) in Denmark and Sweden. Although not indistinguishable, many of the other PFGE patterns were closely related, and most of them belonged to internationally well-known MRSA clones.
Conclusion: Many of the frequently found MRSA types in the three Nordic countries were clonally related. However, no single MRSA type was seen in all three countries. The overall result showed national differences and a rather high diversity among the MRSA isolated within this limited geographical region.

P460

Genotyping as a tool for naming epidemic methicillin-resistant *Staphylococcus aureus* strains in Finland

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Objectives: We used several molecular genotyping methods of *S. aureus*to analyze clonal grouping of the EMRSA strains in Finland with a purpose of renewing their nomenclature. The other objective was to refine the genotyping scheme to better support local outbreak investigations.
Methods: Finnish epidemic MRSA strains from years 1991 to 2004 were tested with several molecular genotyping methods. Pulsed field gel electrophoresis (PFGE) was used as the primary method to distinguish different EMRSA strains. In addition, multi locus sequence typing (MLST) of seven housekeeping gene alleles, staphylococcal cassette chromosome mec (SCCmec) analysis of mobile methicillin-resistance genetic element (I-V), and spa-typing to identify the polymorphic X-region of protein A of *S. aureus* were used. The clonal complex analysis based on spa and MLST results was performed by BURP and eBURST programs, respectively.
Results: PFGE identified 44 different EMRSA strains. These strains represented all SCCmec elements (I-V). Spa-typing found 27 different spa-types among the 44 EMRSA strains, six of them being new spa-types that were not found previously from the Ridom SpaServer. The spa-types divided into four different clonal complexes and into six singletons. With MLST, we were able to find 20 different allelic profiles and those divided into 15 different clonal complexes. When all typing results were combined, the EMRSA strains clustered into 26 more closely related groups (FIN-1 to FIN-26) based on PFGE, SCCmec, spa-typing and MLST results. MLST and spa-typing results supported and specified clonal groupings of the Finnish EMRSA strains.
Conclusion: PFGE can be used as the primary molecular method for naming of MRSA strains in Finland. SSCmec, MLST and spa-typing are needed to verify clonal complexes. Usage of several different molecular typing methods is necessary for accurate nomenclature of MRSA, also in outbreak situations.

P461

Hospital- and community-acquired methicillin-resistant *Staphylococcus aureus* in Germany

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Objectives: The ongoing emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in Germany has generated considerable concern among medical and public health professionals. In addition, community-acquired MRSA (CA-MRSA) is becoming an important public health problem. However, representative nation-wide data on prevalent MRSA clones, their pathogenicity potential as well as on the prevalence of CA-MRSA are not available.
Methods: To learn about the prevalence and the characteristics of MRSA including CA-MRSA, 36 centres throughout Germany (laboratories associated with university and general hospitals, outpatient clinics) were enrolled to collect 50 consecutive MRSA isolates (02/2004 – 01/2005) and to respond to a questionnaire. Only one isolate per patient was included. All isolates were spa-genotyped. Among others, staphylococcal cassette chromosome mec (SCCmec), accessory gene regulator (agr) alleles, and the possession of exotoxin genes were determined.
Results: Overall, MRSA of 1753 patients (56% male, 44% female) were included. Of these, 41.9% were infection-associated, while 35.9% of the MRSA represented colonization. Analyzing isolates recovered from 1423 in-patients and 323 out-patients and confirmed as MRSA by the detection of the mecA gene and the *S. aureus*-specific nuc gene, the following distribution of SCCmec types and agr alleles were found: SCCmec I, 267 (15.2%); II, 762 (43.5%); III, 8 (0.5%); IV, 564 (32.2%);V, 1 (0.1%); and non-typeable, 151 (8.6%); agr I, 677 (38.6%); agr II, 1012 (57.7%); agr III, 33 (1.9%); agr IV, 19 (1.1%); and non-typeable, 12 (0.7%). Altogether, only 18 (1.0%) isolates were found to be positive for the genes (lukS-PV-lukF-PV) encoding the Panton Valentine leukocidin (PVL). Superantigen genes encoding staphylococcal enterotoxins and toxic shock syndrome toxin-1 were found in 12.9% (sea), 0.5% (seb), 16.4% (sec), 51.8% (sed), 0% (sec), 89.3% (seg), 0.3% (seh), 90.8% (sei), 41.6% (sej), and 9.2% (tst) of MRSA isolates. Exfoliative-encoding genes were detected as follows: 0.4% (eta), 0.1% (etb), and 1.2% (etd).
Conclusion: These data on MRSA collected throughout the country offer an overview on strains currently circulating in Germany. According to the prevalence of PVL-positive isolates, CA-MRSA isolates seem to be still rare in Germany. Further analyses of the data given in the questionnaires will allow a more detailed view on demographical and clinical backgrounds.

P462

Molecular spa and SCCmec typing of MRSA isolates from northern Germany

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Objectives: In the past, the MRSA epidemic was known as a primarily clinical problem. In the last years there are dramatically increasing numbers of community-associated MRSA infections. These cMRSA isolates where mostly associated with the small SCCmec type IV and atypical resistance profiles. Recent data indicate, that the SCCmec type IV may infiltrate clinical settings.
Aim: The aim of the present study was the molecular characterisation of MRSA isolates from clinical and non-clinical settings in northern Germany during a 1 year period

2004–2005. Epidemiology data was generated by spa-typing and correlated with results from multiplex PCR SCCmec typing.
Methods: 252 clinical and 18 community-aquired MRSA isolates from different sources in northern Germany were collected. Of these strains the repeat region of *S.aureus* protein A was sequenced on a ABI 3100 sequencer and analysed with RIDOM Staph software. The SCCmec typing was characterised by an multiplex PCR assay as described before.
Results: The dominating spa-types were t032 and t022 (both EMRSA-15) with frequencies of 40,3% and 6,7% and both always harboured the type IV SCCmec. Isolates had been present both in clinical and community patients. Isolates with spa-types t044 and t021, previously categorised as typical cMRSA isolates, were detected only in clinical cases and harboured mostly SCCmec type II. Overall 40 different spa-types and and very hererogeneous multiplex SCCmec type patterns were observed. However, no isolate harboured type V and type I SCCmec cassettes.
Conclusion: Typing of MRSA with molecular methods revealed a complex pattern of spa-types and different SCCmec cassettes. No typical association for hMRSA and cMRSA was observable. The vast majority of isolates had the spa-type t032, which corresponds with EMSA-15 / Barnim epidemic clone, and integrated the small SCCmecIV, which is the classical cMRSA cassette. This may indicate a spread of successful epidemic clones into the community and vice versa adaptation of cMRSA characteristics in the clinical setting.

P463

Methicillin-resistant *S. aureus* in German university hospitals: changes in resistance 2002–2005

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Objectives: During the 1990s the prevalence of methicillin resistant *S. aureus* (MRSA) increased in German hospitals while resistance phenotypes changed with a decrease in the number of resistance markers. We want to examine the changes in resistance in MRSA during recent years using the dataset of the GENARS-project (German Network for Antimicrobial Resistance Surveillance), a prospective multi-centre surveillance study designed to provide epidemiological data for German university hospitals.
Methods: Analysis was based on non-duplicate isolates of MRSA from five laboratories with continuous data collection from January 2002 to June 2005. Antimicrobial susceptibility was determined as minimal inhibitory concentrations by broth microdilution method performed by automated quality controlled test systems for antibiotics of various classes. Resistance rates were evaluated by using break-points according to DIN guidelines.
Results: The percentage of *S. aureus* isolates (n = 22,999) tested as resistant to oxacillin increased from 9.4% in 2002 to 15.2% in the first half of 2005 with considerable variation between hospitals. A total of 2,680 MRSA isolates was analysed. Resistance rates to ciprofloxacin (CIP), erythromycin (ERY) and clindamycin (CLI) remained on a very high level with little fluctuation, whereas the already low rates for doxycycline (DOX), rifampicin (RAM) and quinupristin/dalfopristin (SYN) tended to decline. For gentamicin (GEN) there was a significant decrease from 31.4% in 2002 to 17.8% in 2005 with extreme variation between hospitals. No resistance was observed against teicoplanin, vancomycin and linezolid. Analysis of resistance patterns including CIP, ERY, CLI, DOX, RAM and SYN resulted in six main patterns accounting for about 90 percent of the strains. The two most frequent patterns showed a reverse trend: While the frequency of OXA-CIP-CLI-ERY increased (37.3%/2002 to 52.4%/2005) OXA-CIP-CLI-ERY-GEN decreased (21.3%/2002 to 12.8%/2005). On the hospital level results are heterogeneous: In two centres the pattern OXA-CIP-CLI-ERY-GEN is still dominating with a proportion of more than 40% of all MRSA strains in 2005.

Resistance of MRSA strains against selected drugs and resistance patterns in percent by year

Antibiotic	2002	2003	2004	2005
	(n = 523)	*(n = 727)*	*(n = 904)*	*(n = 526)*
Ciprofloxacin (CIP)	86.8	90.9	90.9	92.0
Erythromycin (ERY)	71.1	73.7	74.4	73.3
Clindamycin (CLI)	66.3	67.4	70.7	68.3
Gentamicin (GEN)	31.4	31.6	20.4	17.8
Doxycycline (DOX)	6.1	4.0	3.2	1.1
Rifampicin (RAM)	4.4	3.2	2.5	2.9
Quinupristin/Dalfopristin (SYN)	2.5	1.0	0.7	0.9
Linezolid (LIZ) *	0.2	0.3	0.0	0.4
Teicoplanin (TPL)	0.0	0.0	0.0	0.0
Vancomycin (VAN)	0.0	0.0	0.0	0.0
Resistance pattern				
OXA CIP CLI ERY	37.3	39.8	49.9	52.4
OXA CIP CLI ERY GEN	21.3	22.7	15.2	12.8
OXA CIP	16.1	17.2	18.9	19.0
OXA	8.6	5.8	4.3	6.0
OXA CIP ERY	2.4	3.1	1.1	2.5
OXA CIP ERY GEN	1.8	1.4	0.5	1.0
others	12.4	9.9	10.0	6.2

* retests did not confirm resistance to LIZ

Conclusions: Data from the GENARS-project show that changes in resistance phenotypes of MRSA reported for the 1990s continue in the observed period from 2002 to 2005 with a remarkable decrease of resistance to gentamicin as the main feature. However, findings from pooled data mask substantial diversity on the local level.

P464

Methicillin-resistant *Staphylococcus aureus* epidemiological setting in the emergency ward of a large French teaching hospital

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Objective: The university hospital of Nantes is a 3200 bed tertiary care teaching hospital offering medical and surgical acute-care services (1800 beds) to a population of 600,000. Approximately 30,000 adult, non traumatized patients are admitted to the medical emergency ward (MEW) each year. The purpose of this study was to determine the proportion of community and hospital-acquired MRSA.
Methods: A case patient was defined as any patient admitted to the MEW from whom MRSA was isolated from clinical samples between 1 January 2003 and 31 December 2004. Only one strain per patient was included and no systematic screening for colonization was done during the study period.
Results: Among the 776 patients with MRSA identified in the hospital during the study period, 41 were isolated at the admission in the MEW (5%). The proportion of *S. aureus* resistant to methicillin was 27% both at hospital and MEW. The resistance to antibiotics tested is reported in table 1. Nineteen patients were male and the mean age was 74 years (range 41–94). Only 3% of the patients were transferred from another hospital. Most of them came from their house (85%) or nursing home (12%); 88% were hospitalized in the last year with a mean delay of 78 days (range 2–299); only 5% of them had a prior MRSA isolation.

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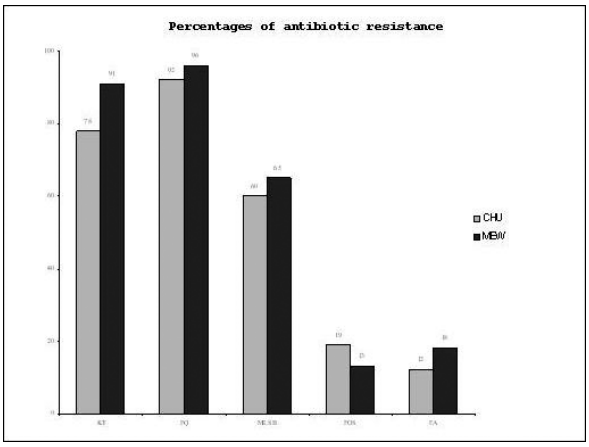

Conclusion: Our study suggest MRSA carriage in patients discharged from hospital and MRSA isolation by a clinical sample upon hospital readmission from the community. Early discharge, prolonged portage and low virulence can explain the spread of hospital MRSA in the community. Our findings indicate that MRSA isolated at the admission to MEW were not community-acquired MRSA and that acquisition could be linked to a prior hospitalisation. Usual epidemiological definition of community or hospital MRSA acquisition is not clear without systematic screening for colonization

P465

Molecular characterisation of *Staphylococcus aureus* isolated in a nationwide prevalence study in Spain

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Objectives: In a Spanish multicenter study (143 hospitals) performed in 2002 we analysed a total of 439 clinical isolates of *S. aureus*, and 30.5% were resistant to methicillin (MRSA). In this study we analyze the presence of virulence factors (Panton-Valentine leukocidin -PVL- and accesory gen regulator – agr-) among the isolates as well as the clones and SCCmec types involved.
Methods: Methicillin-resistance was confirmed by the detection of the mecA gene by PCR. SCCmec types and agr allele types were determined by previously described multiplex PCR strategies; pvl genes were detected by PCR amplification of the lukS-PV and lukF-PV genes. To assess the specificity of the amplification, PCR products were subjected to DNA sequencing. The isolates were analysed by PFGE after DNA digestion with SmaI.
Results: Among the 439 isolates, only 5 isolates (1.14%) were PVL positive (all mecA negative) and corresponded to children and young adults with skin and soft-tissue infections (abscesses and conjunctivitis). The presence of the agr gene was evaluated in 54 MRSA isolates and in 103 methicillin-susceptible *S. aureus* (MSSA) isolates. The agr alleles of the MRSA isolates were: type 1 (9.2%); type 2 (85.2%); type 3 (5.6%). The agr alleles of the MSSA isolates were: type 1 (29.1%); type 2 (34.0%); type 3 (28.2%); and type 4 (8.7%). The 5 PVL positive isolates showed different agr types (one isolate type 2; two isolates type 3; and two isolates type 4) and five different PFGE patterns. PFGE analysis of the MRSA isolates demonstrated the presence of 32 electrophoretic profiles (10 majoritary clones, two dominant). SCCmec types were: type I (20.6%), II (6.9%), IIIA (0.8%), IVA (53.4%), and IV (18.3%).
Conclusions: In Spain we show a low prevalence of PVL positive *S. aureus* isolates (1.14%) belonging to different genotypes. We identified the presence of two dominant clones of MRSA carrying types IVA and IV SCCmec, and mainly agr type 2. A high variability in the agr alleles was observed among MSSA. (This study was financed by the Red Española de Investigación en Patología Infecciosa-REIPI).

P466

First report of infection with community-acquired methicillin-resistant *Staphylococcus aureus* in Spain

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Objectives: The presence of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) producing the Panton-Valentine leukocidin (PVL) has never been reported before in Spain. We describe the first four cases of infection due to typical CA-MRSA in our institution in Madrid.
Methods: From September 2004 to October 2005 we detected in our institution four MRSA isolates from four patients from the community. They had no history of previous MRSA isolation, hospitalization, or surgery before the MRSA isolation. Susceptibility testing was performed by the disk-diffusion method. Methicillin-resistance was confirmed by the detection of the mecA gene by PCR. SCCmec types and agr allele types were determined by previously described multiplex PCR strategies. pvl genes were detected by PCR amplification of the lukS-PV and lukF-PV genes. To assess the specificity of the amplification, PCR products were subjected to DNA sequencing. The isolates were analysed by PFGE after DNA digestion with SmaI.
Results: The four isolates were CA-MRSA, showed resistance only to beta-lactams (one was also resistant to erythromycin) and were heteroresistant to oxacillin. The isolates corresponded to four children (all males; from 2 months to 11 years) presenting with skin- and soft-tissue infections (pyogenic abscesses). All patients needed surgical drainage and antimicrobial treatment for recovery. Three isolates contained type IV SCCmec and agr1, and one case presented type I SCCmec and agr2. PFGE analysis showed the presence of two different patterns (one including three cases with three different subtypes), that were different from nosocomial clones. All isolates were PVL-positive.
Conclusions: We detected PVL-positive CA-MRSA in Spain. Although three isolates presented type IV SCCmec, the presence of type I SCCmec in one of the isolates indicates the spread of different SCCmec types in the community.(This study was financed by the ''Red Española de Investigación en Patología Infecciosa'').

P467

Epidemiology of methicillin-resistant *Staphylococcus aureus* in Bilbao

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Objective: To assess the epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in Bilbao.
Materials and methods: Records from our hospital were reviewed to identify cases of MRSA infection that occurred during January 2004–September 2005 and to identify which cases were community acquired (CA-MRSA).

Results: A total of 391 isolates belonged to 226 patients were identified during the period of the study. The distribution of isolates recovered were 263 samples (67.3%) in 2004 as well as 128 samples (32.7%) in 2005. CA-MRSA infection was diagnosed in 126 patients with 186 isolates while, hospital acquired methicillin-resistant *Staphylococcus aureus* (HA-MRSA) were 100 patients and 205 samples. The MRSA isolates were resistant to fluoroquinolones (78.4%), erythromycin (38.4%), clindamycin (14.2%) and gentamycin (7.6%).
HAMRSA: The main samples in (H-MRSA) acquired infections were from skin and soft infections (60.9%), respiratory tract infections (17.6%), blood (7.8%), and arthritis (5.4%), and the prevalence was highest in medical ward (23%), orthopaedic surgery ward (14.6%), followed by the otorhinolaryngology ward (9.8%), plastic surgery ward (6.8%), vascular surgery ward (6.8%) and the intensive care units 5.9%. The proportion male/female was 56/44 mean age 59.26 y. The study of colonization of the nasophariynx, perineum or skin between (H-MRSA) was positive in 57 patients. Carriage rate was: 57/100 (57%).
CA-MRSA: The origin most frequent of the samples were: outpatient department of hospital and out of hospital 156 (73.1%) and from emergency services 30 (16.3%). The proportion male/female was 60/66, mean age 69.3 y. CA-MRSA samples were recovered more frequently from outpatients department that were situated in Casco Viejo (19.6%) and Txurdinaga (7.6%) and the out patient department of hospital (9.7%). Skin infections and soft infections 135 (76.4%) were the most frequent (wounds), urinary tract infections 15 (8.2%) and otitis 15 (8.2%). Invasive disease occurs in 4.3% of cases (hemoculture positives).
Conclusions: The epidemiology of MRSA in Bilbao is changing rapidly, with increases in both the numbers of notification and the number of CA-MRSA. Urinary tract infection and otitis by MRSA are very frequent in community and occurred in all age groups including paediatrics infections.

P468

Identification epidemic methicillin-resistant *S. aureus* in Russian hospitals: multicentre study 1998–2002

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Objectives: Over the last years there has been a dramatic increase in the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in some hospitals in Russia. A multicentric study was done to investigate epidemic situation.
Methods: 1500 *S.aureus* isolates collected in hospitals in different region of Russia in 1998–2002 and 10 MRSA isolates from hospitalized patients over period 1973–1990 selected from the laboratory collection were studied. Antibiograms and phage typing with phages of International Typing Set (ITS) were used for phenotyping analysis. Molecular features of MRSA isolates were analysed by using coa-, spa- and SCCmec typing. Presence of sea, seb, sec and tst-H was investigated by PCR method.
Results: MRSA were identified in 15 from 22 hospitals with the rate from 2 to 80%. They were recovered from pus (37,7%), respiratory tract (30,1%), blood cultures (19%). 82% of them were multiresistant, about 80% were typable with phages of ITS at 100 RTD. We discovered two epidemic genotypes and every one was circulating in more than 10 hospitals. One of these genotype was similar to EMRSA-1, had the same coa type, spa type t037, carried SCCmec III. But only few isolates of these epidemic strain carried sea. We discovered resembles isolates among laboratory collection of MRSA strains. Another epidemic strain had spa type t008. All isolates of this strain were produced PCR products with the primers for identification ccr type 2, mec complex class B and region J1(4b1, 4b2) and with the primers set for identification ccr type 1. So we proposed that they carried two different copies SCCmec. All isolates sea and some isolates carried sec. MRSA isolates from laboratory collection with the same coa type carried only SCCmec type IVb, but sea and seb.
Conclusion: A progressive increase of the proportion of the MRSA isolates in hospitals in Russia is a result of dissemination as much as two epidemic strains resembles to international. One of them was circulated since 1986, but the second appeared after 1990. New epidemic strain carried two different copies SCCmec probably.

P469

Methicillin-resistant *Staphylococcus aureus* nasal colonisation in a rehabilitating / nursing home in Italy

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Objectives: Evaluation of the prevalence of MRSA nasal colonization in a nursing home with rehabilitation activity in Italy.
Methods: The study was conducted at ''Azienda Cremona Solidale'', a nursing home located in Cremona, Italy with 350 beds. The prevalence of MRSA nasal colonization was evaluated by performing a nasal swab to all patients. The swabs were cultivated on Columbia CNA 5% mutton blood Agar and put into incubation at 37°C for 18–24 hours. The colonies with a probable *Staphylococcus aureus* growth were further cultivated on Mannitol Salt Agar and Oxacillin Screen Agar for final identification and sensitivity test with Break point panel and ID for *Staphylococci* performed. Positive patients were treated with the application of nasal mupirocine and baths / shampoo with chlorexidine. Three microbiological controls were performed after one week from the end of the treatment. Data were matched with MRSA data-base of the hospital of Cremona, which holds data regarding over 1300 MRSA carriers identifies from 1997 onwards, in order to identify previously known MRSA subjects.
Results: 331 patients were involved in the study and 69 (20.8%) among them had a nasal *Staphylococcus aureus* colonization, in particular 53.6% were MRSA while 46.4% were methicillin-sensitive *Staphylococcus aureus*. The MRSA nasal colonization rate on the overall population was 11.2%. The specific treatment permitted the germ eradication in 33 over 37 patients (89.2%). Only two of the 37 MRSA colonized patients were identified within the Cremona Hospital database.
Conclusions: The MRSA nasal carriage rate in nursing home has shown to be significant (11.2%); these patients seem to be a different population if compared to the hospitalized one. The treatment has permitted to eradicate the colonization in a high percentage of cases.

P470

Methicillin-resistant *Staphylococcus aureus* from airway secretions from patients with cystic fibrosis

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MRSA colonization/infection is increasingly found in patients with CF during last years. Although there is no clear evidence

that it affects significantly the pulmonary function, MRSA colonization/infection is an emerging problem resulting to the burden of glycopeptides and consisting a relative contraindication to lung transplantation.
Objectives: The aim of our study was to determine some epidemiological features of MRSA airway colonization/infection in patients with CF of our institution. We reviewed retrospectively the clinical, demographic and laboratory data of the 388 patients with CF who have been attended our hospital between 01/2000–11/2005 for at least one year. During this period 6100 sputum or deep throat specimens were cultured. The identification was performed by the Standard methodology, (catalase and Dnase production, API Staph, Biomerieux). The antibiotic resistance was evaluated by the Vitek II automated system (Biomerieux) and by the disk diffusion method (Kirby-Bauer) according to NCCLS recommendations.
Results: 1) MRSA positive were 267 cultures, corresponding to 125 patients (32.2 %) (55 males and 70 females). 2) The mean age of patients at the 1st isolation of MRSA was 7.2 y (SD: 3.8 y). 3) Eleven patients (8.8 %) were persistently colonized/infected with MRSA (>3 years) , 82 patients (65.6 %) had contemporary colonization (<8 months), while 32 (25.6) had short time colonization. 4) Eight different antibiotic resistance phenotypes were observed. The most prevalent phenotype was the one with resistance only to oxacillin (59.2 %), followed by resistance to oxacillin plus aminoglycosides (15.5 %) and oxacillin plus erythromycin (8.5 %). Three patients (2.4%) were colonized/infected with multidrug resistant phenotype (susceptible only to linezolid and dalfopristin/ quinopristin).
Conclusions: 1) The mean annual incidence rate during this period showed no changes, but the mean age of 1st acquisition seems to decrease by time. 2) The multidrug-resistant phenotypes are rare, for the time being.3) Although MRSA strains are often isolated from patients with CF, only a small proportion of this population remains chronically colonized.

P471

A new computer software for methicillin-resistant *Staphylococcus aureus* typing based on spa-sequencing: comparison to MLST

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Objectives: The worldwide increase in the number of MRSA infections has enhanced the need for fast and reliable typing methods in order to investigate local outbreaks as well as global spread and phylogeny. Two molecular approaches have proven especially useful for *S. aureus* typing, multilocus sequence typing (MLST), and sequencing of the repeat region of the variable. X-region of the *Staphylococcus* protein A (spa-typing). Computer software has for some time been available for phylogenetic analysis based on MLST data, but only recently a software has been developed for phylogenetic interpretation of spa-typing. We have applied both softwares to a collection of 350 clinical MRSA isolates and compared the results.
Methods: The 350 MRSA isolates were collected in the Copenhagen area from 2003–2005. The spa-sequences from all isolates were amplified using AmpliTaq. For each spa-type, one or two isolates were selected for MLST. The seven MLST gene sequences were amplified using AmpliTaq Gold. After purification, the PCR products were sequenced on an ABI 3100 sequencer. For MLST analysis, the eBURST v. 2 software was applied (http://saureus.mlst.net/eburst/). For spa analysis, the newly developed BURP software (www.ridom.de/staphtype/support/) was used.
Results: The 350 MRSA isolates exhibited 46 different spa-types and 19 different MLST sequence types (STs). The spa-based BURP software grouped the isolates into six clonal complexes and a six singletons. By MLST the isolates fell into nine well-know clonal complexes and five singletons. In general, very good agreements between the two methods were seen. MLST clonal complexes CC5, CC8, CC22 and CC45 were identified as spa-based clonal complexes as well with only a few cases of mismatches. However, discrepancy was seen for MLST CC1 and CC80. Based on STs, the best connection between these isolates was a triple locus variant. The spa-based BURP placed the isolates together in one extended clonal complex. Manual inspection of the spa-types involved confirmed a connection between the sequences.
Conclusion: The single locus spa seems to be as useful and reliable for MRSA typing as the seven-genes based MLST. Sequencing of one gene is faster and cheaper than sequencing of seven genes, and spa-typing might replace MLST as the typing method of choice. The new software BURP for spa-based analysis of the relationship between MRSA types performs well and is easy to use.

P472

A multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCCmec types I to V

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Objectives: The worldwide increase in the number of MRSA infections has enhanced the need for fast and reliable typing methods. One of the typing systems for MRSA is determination of SCCmec-type (Staphylococcal Chromosomal Cassette containing the resistance gene mecA). The "gold standard" for SCCmec-typing has been the multiplex PCR developed by Oliveira and de Lencastre (1). However, this multiplex was primarily designed to characterize the hospital-acquired types I-III. The current increase in MRSA infections is caused by community-onset MRSA carrying the smaller SCCmec types IV and V. We have created a multiplex PCR-detection method for routine screening of MRSA isolates by which the five SCCmec types I-V can be detected.
Methods: Based on the literature and sequences found in GenBank/EMBL, primers were designed for detection of SCCmec-specific sequences. The multiplex PCR was designed to ensure that SCCmec types IV and V, the two most common MRSA types in Copenhagen, showed two bands when analysed by gel electrophoresis. The more seldom types I, II and III only showed one band, and were confirmed by an additional PCR. A collection of 255 clinical MRSA isolates was used to test the PCR. The isolates had previously been tested mecA-positive by PCR and had been characterized by spa-typing. As a control, 50 of the isolates were also SCCmec-typed using the Oliveira multiplex PCR.
Results: Of the 255 tested MRSA isolates, 14 were SCCmec type I (5.5%), 9 were type II (3.5%), 3 were type III (1.2%), 213 were type IV (83%) and 16 were type V (6.3%). Three (1.2%) isolates were non-typable and will be analysed further. The Oliveira PCR gave identical results, except that type V is untypable by this PCR. More than half of type IV represented just three MRSA clones: ST8-IV (spa-types t008 and t024;

121 isolates), ST30-IV (t019; 27 isolates), ST80-IV (t044; 23 isolates).
Conclusion: We have developed a multiplex PCR that can be used as a fast screening to identify MRSA SCCmec types IV and V by the presence of two PCR-bands and types I-III by the presence of one PCR-band. The method identifies main types, but sub-typing needs further analysis. 252 of 255 isolates (98.8%) were typable, and the results confirmed by additional PCR.(1): Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2002; 46:2155–61.

P473

Evaluation of real-time PCR as a rapid method to detect carriage of methicillin-resistant *S. aureus* in the investigation of a ST30 outbreak

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Objectives: To compare detection of MRSA carriage by standard culture and real-time PCR and to describe the epidemiology of a MRSA clone in Copenhagen.
Methods: 27 patients in Copenhagen that have been infected with MRSA spa type t019, ST30 in the period 1. November 2003 until 1. November 2005 were contacted. Swabs were taken from the nose, throat and perineum of all persons living in the households and were inoculated into an enrichment broth. The broth incubated overnight at 37°C and was then cultured and used for PCR. Real-time PCR was performed as described by Huletsky et al, except that we used only one specific primerset and a Taqman probe. MRSA colonies were tested by duplex PCR for the presence of the spa and mecA genes. Patients were interviewed to find a possible link between them.
Results: 25 patients agreed to participate, 2 had left Denmark. 187 swabs were taken from 62 persons in 18 households. Seventeen samples were MRSA positive from thirteen persons, six were new patients. Eight households were found to be epidemiologically connected. Three of the eight families had been to the Philippines and via their children had contact to the remaining five families. Another five households had connection to the Philippines, one household to Australia and one to Spain. Thirteen (76 %) specimens were found culture and PCR positive. Three specimens were MRSA positive by culture only. PCR was repeatedly negative from the enrichment broth but was positive on the pure colonies in two of three cases. One isolate was PCR positive but culture negative twice. It became culture positive after repeated culture from the enrichment broth. Three samples were PCR positive and culture negative. Spa and mecA PCR on the enrichment broth found MSSA with spa types t091 (2) and t230.
Conclusion: Interviews gave very useful epidemiological information. ST30 is known as the South Pacific clone and we found a strong initial connection to the Philippines and between households. Eradication is now ongoing. Culture had a sensitivity of 94% compared to 82% for the PCR. The three culture positive and PCR negative samples could be caused by a very low number of MRSA or in one case variability in the sequences amplified by the primer set. The PPV for culture was 100 % and for PCR 82 %. By further PCR optimizing it could be possible to achieve a higher PPV. Our results show that culture is still the best method for detecting MRSA carriage.

P474

Methicillin-resistant *Staphylococcus aureus* and pig-farming

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Objectives: Sporadic cases of CA-MRSA in persons without risk-factors for MRSA carriage are increasing. We report a MRSA cluster among family members of a pig-farmer, his co-workers and his pigs. Initially a young mother was seen with mastitis due to MRSA. Six months later her baby daughter was admitted to the hospital with *Pneumococcal otitis*. After staying 5 days in hospital, the baby was found to be MRSA positive. At that point it was decided to look for a possible source, such as other family members and house-hold animals, including pigs on the farm, since those were reported as a possible source of MRSA earlier.
Methods: Swabs were taken from the throat and nares of family members and co-workers. A veterinarian obtained swabs from the nares, throat and perineum of 10 pigs. Swabs were cultured following a national protocol to detect MRSA that included the use of an enrichment broth. Animal and human strains were sent to the national MRSA reference centre (RIVM) for typing and genetic analysis, including PFGE, spa-typing, and MLST analysis.
Results: Three of the four family members, three co-workers, and 8 of the 10 pigs were MRSA positive. With the exception of the initial case (the mother), all persons had no signs of clinical infections but were only colonized.After digestion with SmaI, none of the strains showed any bands using PFGE. All isolates belonged to spa type t108. The isolates from the farmer and his wife had MLST type 398.
Conclusion: 1. This report clearly shows clonal spread and transmission between humans and pigs in the Netherlands. 2. MLST type 398 might be of international importance as pig-MRSA, since this type was shown earlier to be present in epidemiologically unrelated French pigs and pig-farmers. 3. Research is needed to evaluate whether this is a local problem or a new source of MRSA, that puts the until now successful search and destroy policy of the Netherlands at risk.

P475

Methicillin-resistant coagulase-negative *Staphylococci* isolated from horses

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Objectives: Methicillin-Resistant Coagulase-Negative *Staphylococci* (MRCNS) were isolated from a horse with osteolysis and from the nares of healthy horses. The isolates were characterized by genotypic analysis and by evaluation of antimicrobial susceptibility.
Methods: A saddle horse affected by osteolysis (horse A) of the 3rd metacarpal bone was subjected to surgery. From the removed bone fragment a strain of *Staphylococcus epidermidis* was isolated that was found to be MR by a PCR specific for the mecA gene. Nares and skin swabs of horse A and nares swabs from 12 healthy horses housed in the same stable were collected. In addition, nares swabs from the personnel of the stable were sampled. All the swabs were inoculated on Mannitol Salt Agar (MSA) plus oxacillin. The isolates grown on MSA were identified by biochemical methods, tested by the mecA PCR and evaluated for susceptibility to non beta-lactam drugs. Also, all the strains were tested for the presence of virulence markers such as the icaA and IS256 genes and were

typed by a multiplex PCR specific for the SCCmec type and for the ccrB subtype.
Results: Horse A was found to be colonised by MRCNS in the nares but not on the skin. The nares isolate exhibited the same phenotypic and genotypic features as the bone tissue isolate, i.e. both the strains were characterised as *S. epidermidis*, were resistant to nalidixic acid, erythromycin and cotrimoxazole, were Not Typeable (NT) by the SCCmec PCR assay, were of ccrB type 2, and positive to icaA and IS256 genes. A total of 14 MRCNS were isolated from the nares swabs of 10 out of 12 horses tested. As for the SCCmec type, 3 strains were type II, 1 strain was type I,1 strain with type IV and 9 isolates were NT. Thirteen strains were of ccrB subtype 2, 1 of subtype 1. All the strains but one were resistant to at least 2 classes of non beta-lactam drugs. Four out of 14 strains were positive to PCR for the IS256 gene. No MRS were found in the nares swabs of human origin.
Conclusion: Infection by MRS is considered an emerging problem in animals. In this survey most horses tested were found to be carriers of MRCNS, with some strains displaying invasive potential. As in the stable personnel there were not MRS carriers, the human origin of the MRCNS strains may be ruled out. The detection of new SCCmec types, and of the ccrB type 2 as well, supports the hypothesis that horse MRCNS represent a potential source of resistance genes that may be transmitted to *S. aureus*.

P476

First report of non-prototypic Tn1546 among vanA vancomycin-resistant-*Enterococci* isolated from domestic chickens in Korea: evidence of communication between VRE niche of human and those of chicken?

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Background: VRE rate has abruptly been increased up to 20% in tertiary-care- hospitals since late 1990 even though avoparcin was banned in 1997 in Korea. This study was performed to estimate the prevalence of vanA vancomycin-resistant-enterococci (VRE) among domestic chickens and characterize epidemiologically the vanA VRE strains using transposon typing of Tn1546-like elements and pulsed field gel electrophoresis (PFGE) to elucidate the epidemiology of dissemination of VRE in Korea.
Methods: From January to February 2005, fresh faeces were collected from chickens and workers in free-range-chicken farms (FRCF) and caged-chicken-farm (CCF) located at Kyunggi province and inoculated on bile esculine azide agar containing 6 μg/mL of vancomycin. Identification and susceptibility tests for screen-positive colonies were performed by MicroScan Pos Combo Panel (Dade Behring, US) and vanA VRE were confirmed by vancomycin and teicoplanin MIC determined by Clinical Laboratory Standard Institute agar dilution method and multiplex PCR for van gene. For epidemiologic typing of vanA VRE, sequence-based transposon typing of Tn1546 and PFGE of Sma I digested chromosomal DNA were carried on.
Results: Three (0.6%) of 492 chickens of FRCF, 34 (4.3%) of CCF and 0 of 47 workers yielded vanA VR E. faecium. 30 (81%) belonged to single cluster (type A) showed identical and closely-related PFGE patterns and the other 7 showed various PFGE types unrelated with type A and each other. While all type A vanA VRE were found to harbor prototypic Tn1546, non-typeA vanA VRE revealed insertion of IS1216V in vanX-vanY intergenic region in six strains, IS1542 in orf2 in four, and deletion of vanY and vanZ in one, which was previously reported only in clinical isolates. Non-type A VRE isolated from two CCFs and showed resistant MIC against teicoplanin, high-level gentamicin, and erythromycin.
Conclusions: Clonal vanA VRE contaminated chicken farms, especially caged-type, located at Kyunggi province. In Korea, nonprototypic Tn1546 was first found from the vanA VRE isolates of domestic chicken, which suggest communication between VRE niche of human and those of chicken.

P477

Identification and genotyping of vancomycin-resistant enterococci isolated from slaughtered poultry in Hungary from 2001 to 2004

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Objectives: To identify the presence of the vanA gene in *Enterococci* from poultry, originating from the Hungarian resistance monitoring system from 2001 to 2004, the species of the isolates that carry the gene and their genetic relatedness.
Methods: *Enterococcus* spp. were collected from January 2001 to December 2004 from intestinal samples of slaughtered poultry and the vancomycin sensitivity was checked by disk diffusion. The presence of the van genes in vancomycin-resistant enterococci (VRE) was detected by PCR. The origins of the samples were grouped according to the county of isolation. The identities of the vanA gene carrier strains were determined by PCR using genus-specific and species-specific primers. The relationship of these strains was determined by PFGE (digesting with SmaI) and dendrograms were created by the Diversity Database software.
Results: The VRE strains carried only the vanA gene. In 2001 25 strains, from a total of 289, were vanA carriers (1 *E. casseliflavus*, 13 *E. durans*, and 11 *E. faecium*). In 2002, 21 strains of 87 were vanA positive (11 *E. durans* and 10 *E. faecium*) and in 2003 and 2004 none of the strains (n = 95 and 91, respectively) were positive for the most common van genes, although some strains showed higher MIC values (4–8 mg/L for vancomycin and teicoplanin).
Conclusion: Surprisingly the most common species in poultry were *E. durans* and *E. faecium*, although *E. casseliflavus* and *E. avium* had been expected. Avoparcin had been permitted in Hungary as a growth promoter for broiler chickens from 1989 until it was banned in 1998. Despite prohibition of avoparcin, the VRE strains disappeared only in 2003. It is alarming to note that five years were required for vancomycin resistance to disappear from *Enterococci* in this population.

P478

Antimicrobial susceptibility of enterococcal strains isolated from slaughter animals in Hungary from 2001 to 2004

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Objectives: We determined the antibiotic sensitivity of *Enterococci* from caecal samples of slaughtered animals in Hungary.
Methods: *Enterococci* (n = 562) were collected from January 2001 to December 2004 from intestinal samples of slaughtered poultry, swine and cattle in Hungary. The isolates were identified by biochemical tests and PCR. The antibiotic susceptibility was tested with disk diffusion to ampicillin, gentamicin, streptomycin, tetracycline, erythromycin and vancomycin.

Results: Almost all isolates were sensitive to ampicillin. Strains showing high-level gentamicin resistance were isolated in two cases, one from a pig and one from a cattle in 2001. Similarly low rate of streptomycin resistance was observed in cattle, and somewhat higher in the other animals. The number of strains with reduced susceptibility to erythromycin was nearly as high as the number of tetracycline resistant strains and the non-sensitivity was still retained in 2003 and 2004 in all animal species. Resistance to tetracycline was highest in chicken (ranging between 40–80 %) around 60% in pigs and only around 18–30 % in cattle. Interestingly the lowest resistance rates were observed in 2002. In addition, 434 of the total 562 isolates (= 77.2%) were resistant to both tetracycline and erythromycin. Vancomycin resistance increased steadily in chickens (from 23.5 % in 2001 to 2.2 % in 2004), and disappeared totally in pigs by 2003 and 2004. In cattle, there were 5 intermediate resistant and 11 fully resistant strains in 2001, 6 and 1 in 2002, 4 and 7 in 2003 and 5 and 0 in 2004. Among the 121 vancomycin and tetracycline non-susceptible isolates, 74 strains were resistant to erythromycin as well.
Conclusion: Although ampicillin and amoxicillin are often used in veterinary practice, the resistance rate to these was relatively low. High resistance was found to tetracyclines and macrolides, which increased by 2003 and 2004 in all animal species. This may be due to the higher rate of consumption of these drugs in the animal treatment after the ban of other growth promoters. The annual data of vancomycin resistance point to an association between the recovery of vancomycin-resistant enterococci (VRE) from tested animals and the use of avoparcin. This study indicates that it could be possible to reduce antimicrobial resistance in food animals by reducing the use of antibiotics, although variations can occur with different strains.

Sepsis

P479

Analysis of mortality in septic shock patients

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Objectives: Septic shock is a common cause of death in Intensive Care Units and mortality prediction may help to identify patients who may benefit from a given treatment strategy. The aim of this work was to assess the mortality in septic shock and the performance of Sequential Organ Failure Assessment (SOFA) score to predict this mortality.
Methods: Prospective observational study of 182 patients who meet the criteria for septic shock defined by the American College of Chest Physicians and Society of Critical Care Medicine Consensus Conference. Clinical and physiologic data for the model were prospectively collected applying the criteria described by the developers. Statistical analyses were performed using SPSS (SPSS 11.0 inc. Chicago IL). Predicted hospital mortality was calculated and was compared with the actual mortality. Performance was assessed by evaluating calibration with the Hosmer-Lemeshow goodness-of-fit test, and discrimination with the area under the receiver operating characteristic (AUROC) curve.
Results: Sex ratio was 61.5% men and 38.5% women. Mean age was 59.8 years. Mean SOFA score was 12 (range 7–21). Mortality rate was 42.3%. Lemeshow-Hosmer chi-square was 3.725. AUROC curve was 0.901 (CI 95%: 0.862–0.935).
Conclusion: In our experience mortality in septic shock patients remains high and SOFA score perform very well, with calibration and discrimination very high, and it is an appropriate tool to assess and to predict this mortality.

P480

The significance, clinical presentation and evolution of recurrent *E. coli* bloodstream infections

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Background: Knowledge on the incidence and clinical significance of recurrent *Escherichia coli* bloodstream infections (REC-BSI) is sparse. We assessed both aspects by reviewing the experience of our institution during the last 20 years.
Methods: From 1984 to 2004 our institution had 23,694 episodes of BSI. Of them 4339 were due to *E. coli*. Overall, we selected as REC-BSI those episodes separated at least 30 days. In order to assess risk factors for REC-BSI we performed a case-control study of a randomly selected population that was analysed with a multivariable logistic regression test.
Results: Out of the 4339 episodes of *E. coli* BSI (EC-BSI), 568 (13 %) had at least a recurrent episode. For the assessment of risk factors for recurrence we randomly selected 79 cases and 79 controls. Patients with RCE-BSI were predominantly males (67%) with a median age of 66 years. Of them, 51% had a rapidly or ultimately fatal underlying condition (McCabe I and II) and 30.4% were immunosupressed (12.7% had an haematological malignancy). Origin of the RCE-BSI was UTI (40%), intra-abdominal (20.8%) biliary (11.7%), primary bacteremia (15.6%) and catheter-related (5%). Median time to recurrence was 157 days (IQR 62–581). Mortality among REC-BSI was 15.4% (attributable 14.3%). Survivors had more episodes in 19.4% of the cases. In the univariate analysis, factors significantly associated with REC-BSI were: male sex (OR 3.15;1.64–6.06), immunosupression (OR 4.48;1.8–11.23), haematological malignancy (OR 11.63;1.41–90.91), non-urinary origin (OR 5.36;2.66–10.79) and McCabe I and II (OR 2.35;1.23–4.54). Factors not significantly different between both populations were: age, presence of indwelling devices, adequacy of antibiotic therapy and place of acquisition of the first episode. The multivariable analysis showed as independent factors predicting RCE-BSI the following: male sex (OR 2.78; 1.34–5.78), immunosupression (OR 2.84;1.06–7.63) and non-urinary origin (OR 4.26;2.01–9.06).
Conclusions: RCE-BSI is a relatively frequent disease complicating mainly immunosupression particularly haematologic malignancies. We were not able to show a relationship of RCE-BSI with inadequacy of antimicrobial therapy during the first episode.

P481

The influence of SIRS criteria on outcome in Gram-negative versus Gram-positive sepsis

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The clinical significance of the systemic inflammatory response in septic patients remains unclear. The aim of the study is to compare the outcome in patients with documented gram-positive versus gram-negative sepsis with 2, 3 or 4 criteria of SIRS.

Material and method: We performed a retrospective study based on case records over a 9 year period (1996–2004). We selected adult patients having at admittance in ICU Teaching Hospital of Infectious Diseases the diagnosis criteria of sepsis, according to ACCP/SCCM expert panel recommendation. We compared the short term outcome (till hospital discharge) of confirmed bacterial septic patients, regarding on number of SIRS criteria, using EpiInfo 6, Excel statistical analysis and SPSS10.
Results: 316 adult patients non HIV, median age 57,1 years, 187 males (59%), 227 with community acquired and 89 with hospital acquired sepsis. The etiology was: 61 gram-positives (19%), 87 gram-negatives (28%), 2 anaerobes (1%), 2 fungi (2%), 15 polymicrobial (4%) and 149 cases without documented etiology (47%). The most frequent isolated strains were: *S. aureus* between gram-positives (67%) and *E. coli* between gram-negatives (51%). The percent of patients with gram-positive sepsis was: 25% for 2 SIRS criteria, 34% for 3 SIRS criteria and 10% for 4 SIRS criteria. Patients with gram-negative sepsis met: 2 criteria of SIRS 28%; 3 criteria 31% and 4 criteria 11%. The hospital mortality of sepsis was 42%: 34% for gram-positive (32% *S. aureus*) and 30% for gram-negative (34% *E. coli*) sepsis. The outcome was unaffected by the number of inflammatory response criteria in gram-positive versus gram-negative sepsis, $p = 0.53$ for two criteria, $p = 0.45$ for three criteria, $p = 0.65$ for four criteria. There are no significant differences between
S. aureus versus *E. coli* sepsis outcome for 2, 3 or 4 SIRS criteria.
Conclusions: Characterization of septic patients by the presence of SIRS criteria has no prognostic implication. There are no differences in outcome in gram-positive versus gram negative sepsis sorted by SIRS criteria.

P482

Haemophilus influenzae bloodstream infections: a reappraisal

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Introduction: *H. influenzae* (HI) is a well-known cause of disease in children and adults with local impairment of the host defence mechanisms. COPD is the first predisposing condition in immunocompetent adults, and the most common clinical presentation is pneumonia. However, bloodstream infections (BSI) due to HI are uncommon. The aim of our study was to review our experience on HI BSI in the last 18 years.
Material and methods: From Jan 1988 to April 2005 all patients with BSI due to HI were identified through records of the Clinical Microbiology Laboratory of the hospital. Demographics, comorbidities, clinical presentation, antimicrobial resistance, serotypes and outcome were reviewed from medical charts.
Results: 58 HI BSI out of 5178 BSI (1.1%) were identified. 59% were male. 46 (79%) were adults with a median age of 64 y (range, 25–89) and 91% were community-acquired. Globally, the most common clinical presentations were pneumonia (41.4%), biliary tract infection (8.6%), primary bacteraemia (PB) (8.6%), meningitis (8.6%) and gynecologic infections (5.2%). 60% of adults had comorbidities. COPD, smoking habit, enolism and HIV were present in 30%, 27%, 15% and 12%, respectively. 21% were beta-lactamase producers. Serotype b was identified in 8.6% and 41.6% of adults and children, respectively. The last case of serotype b was diagnosed in 1996. Mortality was 27% in adults and 9% in children. The highest fatality rate was found in adults with PB (75%). 592 *Streptococcus pneumoniae* BSI (11.4%) were documented during the same time period.
Conclusions: HI BSI is uncommon in our community, 10-fold less frequent than invasive pneumococcal disease. Adults, most with comorbidities, represent three quarters of all cases of HI bloodstream infections (which are rarely caused by HI type B). Mortality remains high, especially in PB cases.

P483

Protein C levels in plasma and APACHE II scoring system for risk evaluation in patients with sepsis

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Objective: Concentrations of protein C reduces in septic patients; this reduction is associated with poor outcome. The aim of this study was to investigate the relation of protein C levels and APACHE II score with prognosis in patients with sepsis.
Methods: A prospective study was conducted in 73 patients. Included patients were classified as having sepsis (n = 41), severe sepsis or septic shock (n = 19), according to established concensus definitions. Patients with only infection were selected as control group (n = 13). Acute Physiology and Chronic Health Evaluation (APACHE) II score were determined at the beginning of the study. Blood samples were obtained from all patients for protein C levels at the beginning and 48 hours after initiation of study. Protein C were measured in plasma by Enzyme-Linked Immunoabsorbent Assay (ELISA). Patients were observed for clinical outcomes, with 28-day all-cause mortality, duration of ICU stay and the presence or absence of mechanical ventilation.
Results: APACHE II score was 14.21 in patients with sepsis, 22.12 in severe sepsis and septic shock and 7.5 in control group. Protein C levels at the beginning and 48 hours after initiation of study were seen in Table 1.Initial protein C levels were lower in patients who died than survived ones. However protein C levels at 48 hours after initiation of study were not associated with mortality. There were negative correlation with APACHE II score to initial and 48 hours after initiation of study protein C levels.

Table 1 APACHE II score and Protein C levels initial and 48 hours after initiation of study in patients with sepsis, severe sepsis or setic shock and only infection

	Protein C levels initial %*	Protein C levels 48 h %*	APACHE II score	n
Sepsis	106.00	67.77	14.21	41
Severe sepsis or septic shock	84.57	56.74	22.12	19
Only infection	157.12	82.25	7.5	13

*% of level of protein C in pooled normal human plasma

Conclusion: Patients who have protein C concentration below 70% at the beginning of the study was correlated with poor prognosis in septic patients. This reduction is related with poor outcome in septic patients. Initial protein C levels were significantly lower and APACHE II score was significantly higher in the patients who died compared with the survived ones. APACHE II scoring system and measure of initial protein C levels were significant prognostic factors in septic patients.

P484

The role of plasma BNP levels in the early prognostic stratification of septic patients at the emergency department

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Myocardial dysfunction is observed in 44% of patient with severe sepsis or septic shock and high plasma BNP levels could

be associated with poor outcome of sepsis. To investigate whether the plasma BNP level could represent a prognostic marker for septic patients, we started a two phases project. First, a retrospective study on factors influencing the poor clinical outcome in septic patients was conducted reviewing the clinical records of 234 patients. The age >65 years, the Karnofsky score < 60, the SaO2 < 90% , the PT < 70% and the presence of ''pulmonary edema'' at the traditional chest X ray resulted to be independently correlated with death. Then, a prospective study was started in December 2003 enrolling all the adult patients presenting to our ED with SIRS; the esclusion criteria are the presence of other causes of SIRS, persistent immunodeficiency and the presence of preexisting conditions known to increase plasma BNP levels. For all the enrolled patients a complete clinical, laboratory and instrumental examination including BNP and cardiac Troponin I l plasma levels are carried out. We use a non-radiometric bed-side test (Triage BNP Test Panel Biosite) with a cut off value of 100 pg/ml (4). At today, 30 patients were enrolled in the study.

Results: Survivors showed a value of BNP plasma level of 120.6 pg/ml (95% CI 60, 12–181, 21) when compared to non-survivors 1468.25 pg/ml (95% CI of 200, 7–2735.78). Such a difference is statistically highly significant (p < 0,0001), despite the small number of patients. We are progressing with our study to reach larger figures in order to evaluate the independent influence of BNP on prognosis. It is generally accepted that in the pathogenesis of sepsis and the evolution to septic shock there is an abrupt passage from a reversible disease to an irreversible condition that leads to death. Left ventricular failure might represent one of the underlying events leading to multi-organ failure. Thus BNP plasma levels as an early marker may consistently contribute to predict unfavourable clinical evolution before the myocardial failure is evident. Should this hypothesis be confirmed, the early aggressive therapy recommended in patients with severe sepsis/septic shock could be properly set up without any delay but targeted to high risk patients, allowing an appropriate clinical management together with an improved human and economic resources allocation.

Tigecycline *in vitro* studies

P485

Monocyte CD14 and soluble CD14 in predicting the mortality of patients with severe community-acquired infection

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Objectives: In patients with sepsis, low membrane-bound monocyte CD14 (mCD14) expression level and high soluble CD14 (sCD14) concentration level have been reported separately as predictors of poor outcome. However, no results about the mutual relationship between mCD14 and sCD14 have been published so far. We measured both markers in patients with severe community-acquired bacterial infection, and studied their predictive value for 28 day mortality.

Methods: The study comprised 142 acutely ill patients (83 male, 59 female) with community-acquired pneumonia and/or blood culture-positive sepsis. Expression level of mCD14 by whole blood flow cytometry and sCD14 by ELISA were measured in blood sample obtained on admission to hospital. Clinical data were collected retrospectively from the medical records. 28-day mortality enquiry was made from the National Population Register Centre.

Results: There was no significant correlation between mCD14 expression and sCD14 levels. The survival rate in the lowest tertile of mCD14 expression was significantly lower than that in the middle/highest tertiles. The survival rates in the highest and middle/lowest tertiles of sCD14 levels were comparable. The hazard ratios of mCD14 and sCD14 were 9.79 (95% confidence interval (CI) 1.31 to >50, p = 0.006) and 1.22 (95% CI 0.2–5.42, p = 0.77), respectively. The hazard ratio of mCD14 appeared to improve after stratification by sCD14. A significant positive correlation was detected between C-reactive protein and sCD14 levels, providing evidence that sCD14 may serve as an acute phase reactant.

Conclusion: Low level of mCD14 on monocytes, but not the concurrent circulating level of sCD14, predicts 28-day mortality in patients with severe community-acquired infection.

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Tigecycline *in vitro* activity in an outpatient vs. inpatient global population

B. Johnson, S. Bouchillon, T. Stevens, J. Johnson, D. Hoban, M. Dowzicky *(Schaumburg, Collegeville, US)*

Background: Tigecycline, a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the *in vitro* activity of tigecycline compared to most commonly prescribed broad spectrum antimicrobials against gram-negative and gram-positive species collected from hospitals globally throughout 2004–2006.

Methods: A total of 19,158 clinical isolates were identified to the species level at each site and confirmed by the central laboratory. Minimum Inhibitory Concentration (MICs) were determined by each site using supplied broth microdilution panels and interpreted according to CLSI guidelines.

Results: Results are in the table as follows*:*Tigecycline susceptibility defined according to FDA package insert (Tygacil ®, 2005) where available. Tigecycline Acinetobacter

	Enterobacteriaceae				*Acinetobacter* spp.			
	In patients (n=6,680)		Out patients (n=1,688)		In patients (n=1,161)		Out patients (n=223)	
	%S	MIC90	%S	MIC90	%S	MIC90	%S	MIC90
Tigecycline	96.3	1	96.3	1	98.4	1	99.1	1
Amikacin	98.9	4	99.3	4	77.9	>64	89.2	32
Cefepime	94.5	4	97.4	1	45.5	>32	66.8	>32
Ceftazidime	83.9	32	90.8	8	46.9	>32	66.4	>32
Imipenem	98.6	1	99	1	83	16	93.7	2
Levofloxacin	85.6	8	88.7	4	49.4	>8	67.3	>8
Minocycline	85.1	8	95.9	8	89.5	8	91	4
Pip-Tazo	88.3	32	89.9	8	70.1	>128	86.5	128

	S. aureus				*Enterococcus* spp.			
	In patients (n=1,919)		Out patients (n=526)		In patients (n=1,302)		Out patients (n=221)	
	%S	MIC90	%S	MIC90	%S	MIC90	%S	MIC90
Tigecycline	100	0.25	100	0.25	98.6	0.12	97.3	0.12
Levofloxacin	56.3	32	66.9	32	44.8	>32	52	>32
Linezolid	100	4	100	2	96.6	2	96.8	2
Minocycline	98	0.5	99.4	0.5	50.2	>8	42.5	>8
Vancomycin	100	1	100	1	81.7	>32	86.6	>32

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susceptibility breakpoint defined as ≤2 μg/mL for comparative purposes only.
Conclusion: Tigecycline's *in vitro* activity was comparable to or greater than most commonly prescribed antimicrobials without any demonstrable change in activity between in- and out-patient bacterial study strains. The presented data suggest that tigecycline may be an effective and reliable therapeutic option against nosocomial or community pathogens.

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Tigecycline antibacterial activity in current (2004–2006) U.S. pathogen population

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Background: Tigecycline (TIG) possesses potent bacteriostatic/bactericidal activity against a variety of bacterial species. The T.E.S.T. program compares TIG and comparative agents to both gram positive/negative community and hospital pathogens.
Methods: Between 2004–2006, 77 hospital sites in the United States collected 13,291 clinically significant isolates from various infection sites. Following isolate identification, MICs were performed and interpreted using CLSI guidelines and supplied broth microdilution panels.
Results: Selected U.S. pathogens tested against tigecycline are shown below:

	Tigecycline		% inhibited at MIC				
Organism (#)	MIC_{50}	MIC_{90}	≤0.25	0.5	1	2	4
Acinetobacter spp. (921)	0.25	1	50	66.8	91	98.4	99.9
E. faecalis/faecium (1,034)	0.06	0.12	99.6	100	-	-	-
VRE[a] (232)	0.06	0.12	99.6	100	-	-	-
EC, KO, KP[b] (3,506)	0.25	1	64.5	86.9	94.7	97.6	99.7
ESBL[c] (166)	1	2	26.5	48.8	83.1	93.4	98.8
H. influenzae (860)	0.12	0.25	94.7	97.7	99.9	100	-
P. aeruginosa (1,338)	8	>16	0.7	1.3	2.5	5	16.9
S. aureus (MS) (826)	0.12	0.12	99.6	100	-	-	-
S. aureus (MR) (870)	0.12	0.25	99.6	100	-	-	-
S. pneumoniae (972)	0.06	0.5	83	93.7	99.9	100	-

[a] VRE, *E. faecium/faecalis* phenotypes
[b] EC, *E. coli*; KO, *K. oxytoca*; KP, *K. pneumoniae*
[c] ESBL, producing EC, KO and KP

Conclusions: TIG is described as expanded broad-spectrum because it demonstrated excellent activity against both gram-positive/negative pathogens representing a variety of resistant phenotypes. MIC90 of ≤2 μg/ml and ≥97% inhibited at ≤2 μg/ml of all non-pseudomonal isolates clearly validate the potent activity of TIG against U.S. pathogens encountered in community/hospital settings.

P488

In vitro evaluation of tigecycline against 261 recent isolates of vancomycin-resistant *Enterococci* C T.E.S.T. Program 2006

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Background: Tigecycline (TIG), a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the *in vitro* activity of tigecycline compared to amoxicillin-clavulanic acid, piperacillin-tazobactam, levofloxacin, ceftriaxone, linezolid (LZD), minocycline, vancomycin (VAN), ampicillin (AM), penicillin, and imipenem against VRE collected from hospitals globally throughout 2004–2006.
Methods: 261 VRE (52 *Enterococcus faecalis*, 209 *E. faecium*) clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI guidelines with tigecycline susceptible breakpoint defined as < 0.25 μg/mL.
Results: Percentage susceptible of all VRE to TIG, LZD, and MIN were 99.2, 94.6, and 62.9, respectively. For *E. faecalis* strains, the three most active drugs were TIG (98.1%), LZD (97.1%), and AM (96.7%). For *E. faecium*, the three most active drugs were TIG (99.1%), LZD (95.6%), and MIN (69.1%). There were significant differences in VRE rates between North America (*E. faecalis* 5.2%, *E. faecium* 66.7%), Europe (*E. faecalis* 2.4%, *E. faecium* 12.5%), Middle East (*E. faecalis* 0%, *E. faecium* 9.1%), Latin America (*E. faecalis* 20%, no *E. faecium*) and Asia (*E. faecalis* 0%, *E. faecium* 9.1%).
Conclusions: TIG exhibited outstanding activity against VRE, inhibiting 100% of strains with MICs ≤0.5 μg/ml (MIC90 = 0.12), surpassing LZD as the most active drug in this study. The exceptionally broad spectrum of TIG, which includes many other multi-resistant gram-positive and gram-negative bacteria in addition to VRE, will make it an attractive addition to hospital formularies.

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Evaluating tigecycline against cross-resistant *Enterobacteriaceae* in the Global T.E.S.T. Program

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Background: Tigecycline, the first member of a new class of glycylcyclines antimicrobial class to reach clinical trials, has been shown to have potent broad spectrum activity against most commonly encountered species responsible for hospital acquired infections. Cross-resistance to several classes of antimicrobials is often seen in nosocomial pathogens. The T.E.S.T. program determined the *in vitro* activity of tigecycline against strains of *Enterobacteriaceae* cross-resistant to one or more of the following antimicrobials: amoxicillin-clavulanic acid, piperacillin-tazobactam, levofloxacin, ceftriaxone, cefepime, ampicillin, amikacin, minocycline, ceftazidime and imipenem. The isolates were collected from 80 investigational sties in 19 countries throughout 2004–2006.
Methods: A total of 9231 clinical *Enterobacteriaceae* were identified to the species level at each site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using broth microdilution panels. Antimicrobial resistance was interpreted according to CLSI breakpoints with TIG's FDA susceptible and resistant breakpoints defined as < 2μg/ml and >8 μg/ml, respectively.
Results: Of 5574 *E. coli* and *Klebsiella* spp collected and tested for ESBL production, 52 isolates were found to be ESBL producers and resistance to all cephalosporins, beta-lactam, and beta-lactam/beta-lactamase inhibitor combinations. Of these strains, 90.4% presented cross resistance to levofloxacin, 15.4% to minocycline, 0% to amikacin, 21.2% to imipenem, and 1.9% to tigecycline. Of the 3623 *Enterobacter* spp. and *S. marcescens* collected, 206 presented resistance against ceftriaxone and ceftazidime but susceptible to cefepime suggestive of AmpC phenotype. Only 38 of these 206 isolates showed any degree of non-susceptibility against tigecycline with

MICs ranging from 0.6 to 16 μg/mL. Tigecycline also showed excellent inhibitory activity against members of *Enterobacteriaceae* that were resistant to amikacin, levofloxacin, and imipenem inhibiting 100%, 91.3%, 95.2% of isolates, respectively, at 2 μg/mL.

Conclusion: The presented data suggest that tigecycline is little affected by cross-resistance mechanisms present in these selected strains of *Enterobacteriaceae*. Tigecycline may be an effective therapeutic option against *Enterobacteriaceae* regardless of the resistance patterns to commonly used antimicrobial agents.

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Tigecycline *in vitro* activity in current European pathogens – T.E.S.T. Program 2006

D. Hoban, S. Bouchillon, B. Johnson, T. Stevens, J. Johnson, M. Dowzicky *(Schaumburg, Collegeville, US)*

Background: Enhanced activity agents such as TIG may offer antibacterial coverage of common pathogens including multi-drug resistant gram-negatives/positives. The T.E.S.T. program monitored the *in vitro* activity of TIG and comparators against current European pathogens.

Methods: Thirty-three hospital sites in 15 European countries collected over 6215 significant isolates from community/hospital infection sites. MICs were determined to TIG and comparators using broth microdilution panels (CLSI specified and interpreted).

Results: Selected European pathogens tested against TIG are shown below:

	TIG		% inhibited at MIC				
Organism (#)	MIC_{50}	MIC_{90}	≤0.25	0.5	1	2	4
A. baumannii (349)	0.25	1	57.3	77.1	95.7	99.1	100
E. faecalis/faecium (482)	0.12	0.25	97.9	99.4	100	-	-
EC, KO, KP[a] (1588)	0.25	0.5	69.3	90	94.1	96.7	99.7
ESBL[b] (126)	0.5	4	43.7	72.2	81.7	89.7	99.2
Enterobacter spp. (772)	0.5	1	25	78	90.7	94.8	99.1
H. influenzae (461)	0.12	0.25	97	99.6	99.8	100	-
P. aeruginosa (582)	8	>16	0.5	1.5	2.7	6.9	25.4
S. agalactiae (292)	0.03	0.25	99	100	-	-	-
S. aureus (MS) (585)	0.12	0.25	99.7	100	-	-	-
S. aureus (MR) (199)	0.12	0.25	98	100	-	-	-
S. pneumoniae (482)	0.12	0.5	71	90	100	-	-

[a] EC, *E. coli*; KO, *K. oxytoca*; KP, *K. pneumoniae*
[b] ESBL, producing EC, KO and KP

Conclusions: European isolates of both gram-positive/negative community/hospital pathogens demonstrated excellent TIG MIC90s, excluding *P. aeruginosa*. For most resistant phenotypes, TIG MIC90s were ≤1 μg/ml with >96% inhibited at ≤ 2μg/ml. TIG activity was reduced against European ESBL producing *Enterobacteriaceae* (MIC90 = 4; % Susceptible = 87.8). TIG promises expanded broad spectrum activity against multiply resistant European pathogens.

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Tigecycline *in vitro* activity against multidrug-resistant *Acinetobacter* spp. in the T.E.S.T. Program Global Data

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Background: Tigecycline is a glycylcycline, a new generation of tetracyclines significantly different to be classified as a separate antimicrobial class. Glycylcyclines are being developed to overcome the problem of bacterial resistance to tetracyclines and other antimicrobials. Tigecycline is better tolerated and is more active than tetracyclines against a wide variety of gram-positive and gram-negative bacteria including *Acinetobacter* spp. The T.E.S.T. program determined the *in vitro* activity of TIG against *Acinetobacter* resistant to one or more of piperacillin-tazobactam (PT), levofloxacin (LVX), ceftriaxone (CAX), cefepime (CPE), amikacin (AK), minocycline (MIN), ceftazidime (CAZ) and imipenem (IMP). Study strains were collected from hospitals globally throughout 2004–2006.

Methods: A total of 1491 clinical isolates of *Acinetobacter* spp. were identified to species level from participating sites and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI guidelines, with tigecycline susceptible breakpoint defined as < 2 μg/ml.

Results: TIG inhibited 98% of Acinetobacter resistant to two or more drug classes and inhibited 145/153 (94.7%) and 33/35 (94.3%) or imipenem- and minocycline-resistant strains, respectively. Resistance rates for comparator drugs against all Acinetobacter were CAX 43%, CAZ 44%, LVX 40%, CPE 34%, PT 27%, AK 14%, IMP 10%, and MIN 2.3%. Only one strain had a MIC of 8 μg/mL against TIG. The modal TIG MIC for strains resistant to two or more drug classes was 1 μg/ml compared to 0.12 μg/ml strains with no resistant parameters, indicating an eight-fold diminishment of activity.

Conclusions: It has been seen in some species that existing multi-drug efflux pumps may also pump TIG. In spite of this, TIG remained effective and inhibited most *Acinetobacter* strains resistant to two or more other drugs in this study, although the higher TIG MICs seen for these strains suggests some linkage to resistance mechanisms for other drugs. TIG remained effective in inhibiting multi-drug resistant *Acinetobacter* spp., further broadening its wide spectrum of activity vs. drug-resistant bacteria.

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Evaluating cross-resistance for *Enterobacteriaceae* as compared to tigecycline for the T.E.S.T. Program in Europe

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Background: Tigecycline (TIG), the first of the glycylcyclines to enter clinical trials, has been shown to have potent broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. Cross-resistance to several classes of antimicrobials is often seen in nosocomial pathogens. The T.E.S.T. program determined the *in vitro* activity of tigecycline compared to amoxicillin-clavulanic acid (AC), piperacillin-tazobactam (PT), levofloxacin (LV), ceftriaxone (CX), cefepime (CP), ampicillin (AMP), amikacin (AK), minocycline (MN), ceftazidime (CZ) and imipenem (IMP) against multiple-drug resistant (2 or more drug classes) *Enterobacteriaceae*. The isolates were collected from 33 investigational sties in 15 countries throughout Europe during 2004–2006.

Methods: A total of 2686 clinical *Enterobacteriaceae* were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentration (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI guidelines. Antimicrobial resistance was interpreted according to CLSI breakpoints with TIG's FDA

susceptible and resistant breakpoints defined as < 2 μg/ml and >8 μg/ml, respectively.
Results: 163/2686 *Enterobacteriaceae* were multi-resistant to two or more drug classes. Of these resistant strains, 4.9% were resistant to TIG (MIC >8) compared to AMP 99%, AC 47%, CZ 39%, LV 71%, MN 46%, CX 33%, PT 22%, CP 16%, IMP 2.5% and AK 0.6%. Of the 1,082 *Enterobacter* spp. and *S. marcescens* collected, 75 presented resistance against CX and CZ but susceptible to CP suggestive of AmpC phenotype. 61/75 (81%) of these isolates demonstrated MICs < 2 μg/mL against TIG. TIG showed excellent inhibitory activity against members of *Enterobacteriaceae* that were resistant to AK (n = 1), LV (n = 274), and IMP (n = 15) inhibiting 100%, 85.8%, 93.3% of isolates, respectively at < 2 μg/mL.
Conclusion: The presented data suggest that TIG is little affected by cross-resistance mechanisms present in these selected strains of *Enterobacteriaceae*. TIG may be an effective therapeutic option against *Enterobacteriaceae* regardless of the resistance patterns to commonly used antimicrobial agents.

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Tigecycline in vitro activity against inpatient and outpatient pathogens collected from centres in Europe – T.E.S.T. Program 2006

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Background: The T.E.S.T. program determined the *in vitro* activity of tigecycline compared to most commonly prescribed broad spectrum antimicrobials against gram-negative and gram-positive species collected from patients in European centers throughout 2004–2006. Tigecycline, a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections.
Methods: A total of 6215 clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentration (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI guidelines.
Results: Results are in the table as follows*:

	Enterobacteriaceae				*Acinetobacter* spp.			
	In patients (n=1,920)		Out patients (n=360)		In patients (n=345)		Out patients (n=39)	
	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}
Tigecycline	95.9	1	98.3	1	99.7	1	99.4	2
Amikacin	99.5	4	98.6	4	74.5	>64	87.2	32
Cefepime	94.2	4	94.2	4	55.7	32	66.7	32
Ceftazidime	83.4	32	86.4	32	55.9	>32	71.8	>32
Imipenem	99.3	1	100	1	82.6	16	94.9	4
Levofloxacin	86.2	8	86.4	4	58.3	>8	76.9	8
Minocycline	83.5	8	85.8	8	89.6	8	94.9	2
Pip-Tazo	86.5	64	90.3	16	68.1	>128	84.6	>128
	S. aureus				*Enterococcus* spp.			
	In patients (n=514)		Out patients (n=146)		In patients (n=360)		Out patients (n=49)	
	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}
Tigecycline	100	0.25	100	0.25	95.9	0.25	100	0.12
Levofloxacin	67.5	16	76	8	69.4	>32	63	>32
Linezolid	100	4	100	2	98	2	100	2
Minocycline	99.4	0.5	100	1	42.9	>8	37	>8
Vancomycin	100	1	100	1	98	2	100	2

*Tigecycline susceptibility defined according to FDA package insert (Tygacil ®, 2005) where available. Tigecycline *Acinetobacter* susceptibility breakpoint are defined as ≤2 μg/mL for comparative purposes only.

Conclusions: Tigecycline's *in vitro* activity was comparable to or greater than most commonly prescribed antimicrobials against clinical pathogens from both out-patient and in-patient settings. The presented data suggest that tigecycline as an expanded broad-spectrum antimicrobial may be an effective empiric therapeutic option against nosocomial or community pathogens.

P494

Tigecycline in vitro activity against often difficult to treat pathogens from centres in Asia/Pacific Rim – T.E.S.T. Program 2006

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Background: Tigecycline (TIG), a new glycylcycline, promises enhanced activity against many multi-drug resistant phenotypes of community and nosocomial pathogens causing serious disease. The T.E.S.T. program was designed to elucidate the activity of TIG vs. comparators in clinical use to organisms in Asia/Pacific Rim.
Methods: Between 2004–2006, over 1059 organisms isolated from both inpatients and outpatients in Asia/Pacific Rim were collected by six centres and deemed clinically significant. They underwent site directed CLSI specified MIC testing utilizing supplied broth microdilution panels.
Results: Selected Asia/Pacific Rim pathogens tested against TIG are shown below:

	Tigecycline		Cumulative % Inhibited at MIC				
Organism (#)	MIC_{50}	MIC_{90}	≤0.25	0.5	1	2	4
Acinetobacter spp. (75)	0.25	1	64	98.3	97.3	100	-
Enterobacter spp. (120)	0.5	1	15.8	82.5	92.5	97.5	98.3
E. faecalis/faecium (94)	0.12	0.25	95.7	98.9	100	-	-
EC, KO and KP (280)	0.25	1	60	85	93.2	98.6	100
ESBL[a] (61)	0.5	2	34.4	68.9	85.2	96.7	100
H. influenzae (82)	0.12	0.25	97.6	100	-	-	-
P. aeruginosa (108)	8	>16	0	2.8	4.6	5.6	15.7
S. agalactiae (35)	0.03	0.12	100	-	-	-	-
S. aureus (MR)(46)	0.25	0.5	80.5	100	-	-	-
S. aureus (MS)(88)	0.12	0.25	97.7	100	-	-	-
S. pneumoniae (70)	0.25	0.5	61.4	91.4	100	-	-

[a] EC, *E. coli*; KO, *K. oxytoca*; KP, *K. pneumoniaea*; ESBL producing EC, KO and KP

Conclusions: Tigecycline MIC90 of ≤0.5 μg/ml for gram-positive pathogens (including resistant phenotypes) and MIC90 of ≤2 μg/ml of gram-negative pathogens (excluding *P. aeruginosa*) validate the potent activity of TIG against community/hospital pathogens isolated in six Asia/Pacific Rim countries. TIG may add value to this therapeutically challenged geographical region.

P495

Acinetobacter resistance in clinical isolates from the United States evaluated against tigecycline and 10 comparators

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Background: Tigecycline (TIG), a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the *in vitro* activity of TIG against *Acinetobacter* resistant to one or

more of piperacillin-tazobactam (PT), levofloxacin (LVX), ceftriaxone (CAX), cefepime (CPE), amikacin (AK), minocycline (MIN), ceftazidime (CAZ), and imipenem (IMP). Study strains were collected from hospitals in the United States throughout 2004–2006.
Methods: A total of 921 clinical isolates were identified to species level from participating sites and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI guidelines, with TIG resistant breakpoint defined as >8 μ/ml.
Results: Resistance rates for comparator drugs were CAZ 45%, CAX 45%, LVX 45%, CPE 37%, PT 24%, AK 8%, IMP 6%, and MIN 2%. TIG inhibited 96.7% of all multi-drug resistant (two or more drug classes) strains at a MIC of 2 μg/mL. There were no TIG-resistant (>8 μg/mL) strains found. TIG MIC50/90 for all multi-drug resistant strains was 1/2 μg/ml including AK- and IMP-resistant strains. The modal TIG MIC for the multi-drug resistant strains was 1 μg/ml compared to 0.12 μg/ml for strains without any resistant parameters, indicating an 8-fold diminishment of activity.
Conclusions: It has been seen in some species that existing multi-drug efflux pumps may also pump TIG. In spite of this, TIG remained effective and inhibited most *Acinetobacter* strains resistant to one or more other drugs in this study, although the higher TIG MICs seen for these strains suggests some linkage to resistance mechanisms for other drugs. TIG remained effective in inhibiting multi-drug resistant *Acinetobacter* spp., further broadening its wide spectrum of activity vs. drug-resistant bacteria.

P496

The *in vitro* activity of tigecycline and 10 comparators in a global population – T.E.S.T. Program 2006

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Background: Tigecycline (TIG), a new glycylcycline, has been shown to have potent broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the *in vitro* activity of TIG and 10 comparators against respective gram-positive/negative species. Isolates were collected from 121 hospital sites in 25 countries throughout 2004–2006.
Methods: Over 21,000 clinically significant isolates were identified to the species level at participating sites and confirmed by the central laboratory. MICs were determined by each site using supplied broth microdilution panels and interpreted according to NCCLS guidelines.
Results: Selected global pathogens tested against tigecycline are shown in the table below:

	Tigecycline		% inhibited at MIC				
Organism (#)	MIC_{50}	MIC_{90}	≤0.25	0.5	1	2	4
Acinetobacter spp. (1,065)	0.25	1	53.6	71.9	94	98.9	100
E. faecalis/faecium (1,676)	0.06	0.12		99.6	100	-	-
VREs (261)	0.06	0.12	99.2	100	-	-	-
Enterobacteriaceae (9,231)	0.5	1	47	79.2	92.2	96.5	99.3
ESBLs (228)	0.5	2	33.7	61.9	84.1	93.5	99
P. aeruginosa (2,096)	8	>16	0.6	1.5	2.6	5.5	18.9
S. aureus (2,706)	0.12	0.25	97.4	100	-	-	-
MRSAs (1,145)	0.12	0.25	95.3	100	-	-	-
S. pneumoniae (1,569)	0.06	0.5	78.1	92.1	99.9	100	-

*Tigecycline susceptibility defined according to FDA package insert (Tygacil ®, 2005) where available. Tigecycline *Acinetobacter* susceptibility breakpoint is defined as ≤2 μg/mL for comparative purposes only.

Conclusions: TIG demonstrated broad spectrum antimicrobial because of its consistent activity against *Enterobacteriaceae* including ESBL phenotypes, *S. aureus* including MR strains, *S. pneumoniae* (all phenotypes) and both VS and VR *Enterococcus* spp. TIG wide spectrum of activity promises to provide enhanced antimicrobial coverage of serious nosocomial/ community pathogens.

P497

Tigecycline *in vitro* evaluation of 1,145 methicillin-resistant *Staphylococcus aureus*

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Background: Tigecycline (TIG), a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the *in vitro* activity of TIG compared to amoxicillin-clavulanic acid, piperacillin-tazobactam, levofloxacin, ceftriaxone, linezolid (LZD), minocycline (MIN), vancomycin (VAN), ampicillin, penicillin and imipenem against methicillin-resistant *S. aureus* (MRSA) isolates collected from hospitals globally throughout 2004–2006.
Methods: A total of 1145 clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentration (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI guidelines with tigecycline susceptible FDA breakpoint defined as < 0.5 μg/mL.
Results: Of the study drugs with MRSA activity, the %S for TIG, VAN, LZD, and MIN was 100, 100, 100, and 96.5 respectively. There were no differences among geographic regions, except for lower activity of MIN in Asia and Middle East (%S = 60.5 and 14.3, respectively) compared to Europe (98.0%) and North America (99.3%), whereas TIG inhibited 100% of strains in all regions. MIC50/90 (μg/ml) for TIG, VAN, LZD, and MIN were 0.12/0.25, 1/1, 2/4, and 0.25/2, respectively.
Conclusions: TIG was the most potent anti-MRSA drug in this study, inhibiting all 1145 MRSA strains at an MIC value of 0.5 μg/mL. TIG's excellent expanded broad spectrum of activity against MRSA and other gram-positive and negative resistant bacteria should make it a very useful drug in treatment of difficult Staphylococcal infections.

P498

Antibiotic cross-resistance for *Staphylococcus aureus* and *Enterococcus* spp. among tigecycline and 10 comparator antimicrobial agents - T.E.S.T. Program in Europe 2006

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Background: Tigecycline (TIG) is a new first-in-class antimicrobial agent with expanded broad-spectrum activity against gram-negative and -positive aerobes and anaerobes responsible for community and hospital acquired infections. The T.E.S.T. program determined the *in vitro* activity of tigecycline

compared to amoxicillin-clavulanic acid, piperacillin-tazobactam, levofloxacin, ceftriaxone, linezolid, minocycline, vancomycin, ampicillin, penicillin and imipenem against *Enterococcus* spp. and *Staphylococcus aureus* isolates. Isolates were collected from hospitals within Europe throughout 2004–2006.

Methods: A total of 1276 clinical isolates (492 *Enterococci*, 784 *S. aureus*) from 33 labs from 15 countries in the Europe were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using broth microdilution panels. Antimicrobial resistance was interpreted according to CLSI breakpoints with TIG susceptible breakpoints (FDA, 2005) defined as < 0.25 μg/ml for *Enterococci* and < 0.5 μg/ml for *Staphylococci*.

Results: 114/492 (23%) *Enterococci* and 190/784 (24%) *S. aureus* (including MR + MS strains) were resistant to two or more drug classes. Among the multi-drug resistant (MDR) *Enterococci*, resistance rates were LVX 91%, P 75%, AMP 68%, VAN 20%, MIN 33%, and LZD 2.6%. Resistant rates for MDR *S. aureus* were P 100%, AMP 100%, AUG 67%, LVX 79%, PT 64%, CAX 62%, IMP 45%, LZD 0%, MIN 0% and VAN 0%. TIG inhibited 95.6% of the MDR *Enterococci* and 100% of the MDR *S. aureus* at 0.25 and 0.5 μg/mL, respectively. Modal TIG MICs were 0.06 and 0.12 μg/ml for *Enterococci* and *S. aureus*, respectively, against strains with or without resistant determinants.

Conclusions: TIG retained potent activity against MDR *S. aureus* and *Enterococci*, inhibiting >97% of all strains at the respective breakpoints. TIG should prove to be a useful empiric agent against these gram-positive pathogens whether they are determined to be resistant to other drugs or not.

P499

Asia and Pacific Rim community vs. hospital-acquired infections: *in vitro* activity against tigecycline and comparators

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Background: Tigecycline, a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the *in vitro* activity of tigecycline compared to most commonly prescribed broad spectrum antimicrobials against gram negative and gram- positive species collected from hospitals within Asia and the Pacific Rim countries throughout 2004–2006.

Methods: A total of 1059 clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentration (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI guidelines.

Results: Results are in the table as follows:

	Enterobacteriaceae				*Acinetobacter* spp.			
	Hospital		Community		Hospital		Community	
	(n=329)		(n=50)		(n=67)		(n=5)	
	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}
Tigecycline	98.8	1	100	1	97	1	100	0.5
Amikacin	99.4	4	94	8	62.7	>64	60.5	>64
Cefepime	89.1	16	92	8	47.8	>32	40	>32
Ceftazidime	75.1	>32	86	32	46.3	>32	40	>32
Imipenem	98.8	1	98	1	67.2	>16	80	>16
Levofloxacin	79.3	>8	88	>8	58.2	8	40	8
Minocycline	79.9	16	88	8	97	4	100	2
Pip-Tazo	87.5	32	96	8	67.2	>128	60	>128
	S. aureus				*Enterococcus* spp.			
	Hospital		Community		Hospital		Community	
	(n=85)		(n=13)		(n=57)		(n=11)	
	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}
Tigecycline	100	0.5	100	0.25	98.2	0.25	100	0.12
Levofloxacin	56.5	16	92.3	0.25	49.1	>32	54.5	32
Linezolid	100	4	100	4	100	2	90.9	2
Minocycline	85.9	8	100	0.5	35.1	>8	36.4	8
Vancomycin	100	1	100	1	96.5	2	100	2

*Tigecycline susceptibility defined according to FDA package insert (Tygacil ®, 2005) where available. Tigecycline *Acinetobacter* susceptibility breakpoint are defined as ≤2 μg/mL for comparative purposes only.

Conclusions: Tigecycline's *in vitro* activity was comparable to or greater than most commonly prescribed antimicrobials. The presented data suggest that tigecycline may be an effective and reliable therapeutic option against nosocomial or community pathogens in both in-patient and out-patient clinical settings.

P500

Extended-spectrum beta-lactamase producing *Enterobacteriaceae* evaluated *in vitro* against tigecycline and comparator agents – T.E.S.T. Program 2006

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Background: Tigecycline (TIG), a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the *in vitro* activity of TIG compared to amoxicillin-clavulanic acid, piperacillin-tazobactam (PT), levofloxacin, ceftriaxone, cefepime, ampicillin (AMP), amikacin (AK), minocycline, ceftazidime and imipenem (IMP) against ESBL isolates collected from hospitals globally throughout 2004–2006.

Methods: A total of 415 ESBL producing clinical *Enterobacteriaceae* were identified to the species level by 120 labs in 25 countries and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using custom supplied broth microdilution panels and interpreted according to CLSI guidelines with tigecycline susceptible FDA breakpoint defined as < 2 μg/mL.

Results: Percentage Sus for all ESBL-producing isolates vs. TIG, IMP, and AK was 93.5, 91.3, and 89.4%, respectively; %Sus for other comparators ranged from a high of 65.1% (PT) to a low of 0.5% (AMP). TIG had the lowest percentage of resistant strains, 1.0%, compared to 3.4% for IMP and 1.4% for AK. MIC50/90 for TIG, IMP, and AK were 0.5/2, 0.5/2 and 4/32 μg/mL; the MIC90 for all other drugs was in the resistant range. There were minor regional differences in levels of activity, with either TIG (North America) or IMP (Europe, Asia/Pacific) being the most active.

Conclusions: TIG is as active *in vitro* as IMP against ESBL producing strains of Enterobacteriaceae. TIG's expanded broad spectrum of activity, including strains resistant or multiple-

resistant to other agents, should make it a very useful treatment option for this difficult to treat group of gram-negative pathogens.

P501

Activity of tigecycline against well-characterised multi-resistant Gram-positive strains

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Objective: Tigecycline, the first glycylcycline, is a novel compound that provides the medical community with a potent new antimicrobial for treating infections caused by a broad spectrum of clinically relevant pathogens for which limited therapeutic options exist. This study was undertaken to: i) assess the *in vitro* activity of tigecycline against n.13 *S. agalactiae*, 10 *S. pyogenes*, 11 MRSA, 7 *E. faecalis*, and 3 *E. faecium*, isolated from SSSI; and ii) identify their resistance genes.
Methods: The strains were tested against a panel of antimicrobial agents, including tigecycline, by the broth microdilution method performed according to CLSI guidelines. Furthermore, vancomycin (vanA and vanB), tetracycline [tet(O), tet(M), tet(K)], macrolide resistance [erm(B), erm(A), mef(A)] genes were analysed by PCR and the mec-complex was characterized.
Results: Overall tigecycline was very active against the 44 gram-positive cocci, showing a MIC range 0.03–0.25 mg/L, able to potently inhibit all *Streptococci*, *Enterococci* and MR-*S. aureus*. Tigecycline was the most active antibiotic against MRSA and *Enterococci* with respect to linezolid (MIC 2 and 0.5 mg/L respectively) and quinopristin/dalfopristin (MIC 0.25 and 0.5 mg/L respectively), and with respect to macrolides, amoxicillin and tetracycline for all *Streptococci*. The molecular characterization of resistance determinants demonstrated a concomitant presence of different classes of genes: in particular in the 13 *S. agalactiae* the predominant genes were tet(M) in eight strains, erm(B) in three strains and mef(A) in two strains. In *S. pyogenes* tet(O) and tet(M) were equally distributed, while the three most frequent classes of erythromycin genes were found. VanA- mediated resistant *E. faecalis* possessed tet(M) in all strains and erm(B) in four strains. The 11 strains of MRSA harboured four types of SCCmec DNA, namely I and IA (n.6 strains), IIIA (n. 4) and IV (n.1). All strains were erythromycin resistant – in six strains erm(A) gene was found and six strains had the tet(K) gene responsible for tetracycline but not minocycline resistance.
Conclusion: Our results clearly confirm that tigecycline has an excellent activity against multi-resistant gram-positive, both tetracycline-susceptible and resistant isolates, possessing three different tetracycline-resistant genes and other determinants including mec and VanA, B together and suggesting that this drug may play an important role in the treatment of infections caused by gram-positive pathogens.

ESBL and cephalosporins

P502

Prevalence of extended-spectrum beta-lactamases among *Enterobacteriaceae*: a Bulgarian survey

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Objectives: 1. To identify and characterize the type of ESBLs among clinically significant isolates of *Enterobacteriaceae* from Bulgarian hospitals. 2. To investigate their distribution in different genera of *Enterobacteriaceae* and the participating centres.
Methods: Antibiotic susceptibility was determined by disc diffusion method (CLSI standards 2002). The ESBL production was confirmed by CLSI double disc ESBL confirmatory method. Conjugation was performed on solid medium. Isoelectric focusing, followed by bioassay, ESBL-group specific PCR and sequencing of ESBL genes were carried out. RAPD with ERIC-1A and ERIC 2 primers and plasmid fingerprinting with Pst I restriction were performed with representative strains.
Results: Seven medical institutions, five in Sofia, one in Pleven and one in Stara Zagora participated in the study. 451 strains positive in double disk synergy tests were collected during 8 years (1996–2003). ESBL-producing were: *K. pneumoniae* 239, *E. coli* 155, *Enterobacter* spp. 21, *S. marcescens* 16, *C. freundii* 11, *K. oxytoca* 8, *Salmonella Corvallis* 1. Five different beta-lactamases were identified among the Bulgarian isolates, namely SHV-2, -5, -12; CTX-M-3 and -15. The most widespread enzymes were SHV-12 and CTX-M-15 (all centres), followed by CTX-M-3 (six centres). SHV-2 and -5 were found only once. The rate of CTX-M enzyme harbouring strains have increased rapidly since 2001, after introducing ceftriaxone widely in Bulgaria. The RAPD and plasmid fingerprinting data suggest that plasmid transfer is the main mechanism of CTX-M-3 spread, while CTX-M-15 shows a clonal dissemination in one centre.
Conclusions: We detected a remarkable variety both of ESBLs (SHV-2, -5, -12; CTX-M-3, -15) and producing pathogens (*K. pneumoniae*, *E. coli*, *Enterobacter* spp., *S. marcescens*, *C. freundii*, *K. oxytoca*, *Salmonella Corvallis*). Our data show the importance of monitoring of the epidemiology of resistance and need of rational antibiotic policy.

P503

Surveillance of PER-1 producers among Gram-negative bacilli isolated at a tertiary care hospital over a 5-year period and analysis of the genetic location of blaper-1

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Objectives: To investigate the prevalence of PER-1 beta-lactamase over a 5-year period among ceftazidime resistant Gram-negative bacteria isolated at a tertiary care hospital in Izmir, Turkey and to determine whether blaPER-1 was associated with class-1 integrons in any of the isolates.
Methods: PER-1 beta-lactamase production was sought in 289 ceftazidime resistant Gram-negative bacteria isolated between 1998 and 2003. The isolates consisted of 116 *Pseudomoas aeruginosa*, 91 *Acinetobacter baumanii* and 82 member of *Enterobacteriaceae*. blaPER-1 presence was determined by PCR using specific primers and clonal relationship of PER-1 producers was determined by ERIC-PCR. Integron-location of blaPER-1 was analysed by PCR combining primers specific for conserved region of class-1 integrons and blaPER-1, in representatives of each ERIC-PCR pattern. Direct sequencing of PCR-products was performed on both strands.
Results and Conclusion: PER-1 production rates were 32.3%, 33.9%, 14.9% and 37.9% in 1998–2000 period, 2001, 2002 and 2003 respectively. Percentages of PER-1-producer *P. aeruginosa* strains were always higher than of *A. baumanii* strains. All

members of *Enterobacteriaceae* were blaPER-1 negative. ERIC-PCR results revealed dissemination of two endemic clones for both *P. aeruginosa* (pattern X and Y) and *A. baumanii* (pattern A and B) was responsible for the high prevalence. Conjugation experiments were negative in all representative strains. A 2300 bp PCR-product was obtained by combined class-1integron and blaPER-1 specific primers in three *P. aeruginosa* isolates. Standard PER-PCR was performed using 2300 bp PCR-products of previous reaction as template. A 930 bp PCR-product specific for blaPER-1 was detected in a *P. aeruginosa* strain showing a different ERIC-PCR pattern (Z) from others. Direct sequencing of the PCR-product on both strands confirmed the blaPER-1 presence.To our knowledge, this is the first report which shows association of blaPER-1 with a class-1 integron.

P504

Plasmid-mediated carbapenem-hydrolysing oxacillinase OXA-58 in *Acinetobacter baumannii* in Italy

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Objective: Emergence of carbapenem resistance in *Acinetobacter baumannii* has reported worldwide. An outbreak by *A. baumannii* occurred during the summer 2004 at the intensive care unit (ICU) at the Policlinico Umberto I of Rome. The aim of this study was to investigate the genotype and antibiotic resistance mechanism of epidemic *A. baumannii* isolated at the ICU during the outbreak, comparing them with sporadic *A. baumannii* isolated in the same hospital and in a different hospital of the town.
Methods: The molecular epidemiology of 66 epidemic and sporadic isolates of *A. baumannii* was investigated by plasmid analysis, PFGE- and RAPD-based genotyping and integron identification. Fifty-two isolates showed carbapenem resistance (imipenem MIC > 16 μg/ml, intermediate meropenem MIC = 8 μg/ml). The occurrence of the plasmid-mediated carbapenem-hydrolyzing oxacillinase OXA-58 was detected by PCR and Southern blot analysis.
Results: The outbreak involved 14 cases of infection by multidrug-resistant *A. baumannii* and 28 isolates were collected over a 3-month period. A unique RAPD profile was observed among these epidemic isolates. Twenty-four isolates showing the same RAPD-profile of the epidemic clone were also detected among sporadic strains isolated from different wards of the same hospital or in another hospital of the town, during the period of the outbreak and in the following months. This analysis also showed a second RAPD profile, highly represented among the 14 carbapenem susceptible isolates. The different genotypes observed by RAPD were also confirmed by PFGE analysis. PCR identification and characterization of integrons provided a further characterization of these isolates, identifying two integrons, carrying 2.2 kb (aacA4-orfO-blaOXA20) and 2.5 kb (aacC1-orfX-orfX′-aadA1) variable regions, respectively associated to the two prevalent RAPD profiles. Interestingly, all the 52 carbapenem resistant isolates were found positive to the blaOXA-58 gene. This gene was located on different plasmid variants.
Conclusion: The detection of a plasmid-mediated oxacillinase conferring imipenem-resistance located on different plasmid variants, suggests that this resistance may spread horizontally in *A. baumannii*, a finding with serious implications for patient management in the hospital. Molecular typing of the isolates was very useful to monitor the spread of resistance within the hospital, helping to detect the genotype and the resistance pattern of the emerging clone.

P505

Outbreak of OXA-58-producing carbapenem-resistant *Acinetobacter baumannii* isolates in a paediatric university hospital in Athens, Greece

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Purpose: To characterize the clonal relationship and the beta-lactamase content of carbapenem-resistant *Acinetobacter baumannii* isolates recovered in a Pediatric Hospital in Athens, Greece.
Material and methods: Twelve non-repetitive carbapenem-resistant *Acinetobacter baumannii* isolates were recovered during the November 2003–May 2005 period at the P. & A. Kyriakou Children's Hospital in Athens, Greece. Species identification was done by the biochemical API32 GN test (bio-Mérieux, France) and by 16S rRNA sequencing. Sensitivity testing was done by disk diffusion method and Minimum Inhibitory Concentration (MICs) were determined by agar dilution. The presence of oxacillinase or metallo beta-lactamase genes was performed by PCR. In particular, genes coding for the carbapenem-hydrolyzing OXA-23, OXA-40 and OXA-58 subgroups were searched. Genotyping was done by pulsed field gel electrophoresis (PFGE) after digestion by ApaI.
Results: All isolates were identified as *A. baumannii*. They all possessed the blaOXA-58 gene. Genotyping revealed that eight out of ten OXA-58-positive *A. baumannii* isolates corresponded to a single clone. The blaOXA-58 gene was plasmid-located.
Conclusion: This study is the first description of the blaOXA-58 gene in Greece after that identifying this carbapenemase gene in other European countries. As observed in France and Romania, this gene was identified in *A. baumannii* strains as a source of an outbreak period.

P506

Characterisation of conjugative plasmids encoding CTX-M-type extended-spectrum beta-lactamases in Italian clinical isolates of *Escherichia coli*

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Objectives: Extended-spectrum beta-lactamases (ESBLs) are among the most important emerging resistance determinants in *Enterobacteriaceae*, and their dissemination is mostly mediated conjugative plasmids. The CTX-M-type ESBLs are among the most widespread ESBLs worldwide, and have recently shown a rapid dissemination in some European countries. Current knowledge on plasmids encoding these resistance determinants are still very limited. Here we report the characterization of CTX-M-encoding conjugative plasmids from *Escherichia coli* clinical isolates collected during a nationwide survey carried out in Italy.
Methods: Representatives of all clonal lineages of *E. coli* producing CTX-M-type ESBLs, obtained from 10 hospital-associated clinical microbiology laboratories representative of different Italian regions during a cross-sectional nationwide survey, were assayed for transferability of the CTX-M

determinants by mating experiments. Plasmid extraction was conducted by alkaline lysis. The presence of blaCTX-M determinants in transconjugants was confirmed by PCR. Plasmid profiles of the tranconjugants were determined after digestion with the *Pst*I and *Eco*RV endonucleases. A PCR-based method was used to assign the incompatibility group.

Results: Of 53 clonal lineages of *E. coli* producing CTX-M-type enzymes (31 producing CTX-M-1, 21 producing CTX-M-15, and one producing CTX-M-32) detected across the Italian national territory, 23 (43%) were found to harbour the blaCTX-M gene on a conjugative plasmid. Transfer frequencies in the order of 10^{-2}–10^{-5} transconjugants per recipient were observed. Plasmid analysis revealed that 20 strains from seven different centres carried an apparently identical plasmid encoding CTX-M-1 and belonging to the incompatibility group IncN. The plasmid from the CTX-M-32-encoding strain was also of the IncN group, while those from the remaining two strains were of the IncF group (IncFIA, blaCTX-M-15; IncFIB, blaCTX-M-1).

Conclusion: The epidemic dissemination of a single IncN plasmid encoding CTX-M-1 was apparently the major responsible for the spread of this ESBL recently observed among *E. coli* circulating in Italy, although other plasmids could also be involved in the dissemination of CTX-M-type ESBL determinants.

P507

Unusual CTX-M enzyme variants in the United Kingdom

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Objectives: Since 2003, *Escherichia coli* with CTX-M extended-spectrum beta-lactamases (ESBLs) have become widespread in the UK. Most have CTX-M-15, but *c.* 5% have group 9 CTX-Ms, predominantly CTX-M-9 and -14, whilst a few have other types. We investigated these rare CTX-M types.

Method: MICs were determined by the British Society for Antimicrobial Chemotherapy method. blaCTX-M genes were phylogenetically grouped by multiplex PCR, then cloned into the vector pCR®2.1 and sequenced. XbaI-digested genomic DNA profiles were compared by pulsed-field gel electrophoresis. Plasmids were extracted by alkaline lysis, electrophoresed, and then hybridised with a blaCTX-M probe. Plasmids were electroporated into *E. coli* DH5alfa.

Results: Three group 2 and one group 8 blaCTX-M genes were detected among 1,123 blaCTX-M-positive clinical isolates of *E. coli* collected in the UK since 2003. Sequencing revealed that all three group 2 blaCTX-M alleles encoded classical CTX-M-2 and that the group 8 blaCTX-M gene encoded CTX-M-40. One other unusual *E. coli* isolate had both blaCTX-M-14 and blaCTX-M-15. All five isolates required MICs consistent with the production of CTX-M ESBLs and remained susceptible to carbapenems. All, except the CTX-M-40 producer had reduced susceptibility or resistance to aminoglycosides. Two CTX-M-2 producers remained susceptible to ciprofloxacin. The three CTX-M-2-producers, all from different hospitals, were unrelated by PFGE, and the isolate with both CTX-M-14 and CTX-M-15 was distinct from major CTX-M-15-producing clones; its CTX-M-15-harbouring plasmid was successfully transformed to DH5alfa, but did not encode CTX-M-14. Hybridisation suggested that CTX-M-2, -14 and -40 were chromosomally-encoded or mediated by large plasmids (>200 kb).

Conclusion: We report here the first CTX-M-2-producers from the UK. This is also the first report from the UK of the production of two CTX-M enzymes by a single clinical isolate.

P508

Differential expression of CTX-M-15 beta-lactamase between two major *Escherichia coli* strains in the UK

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Objectives: *E. coli* with CTX-M beta-lactamases are a major problem in the UK, with several CTX-M-15-producing outbreak strains as well as many sporadic producers. One outbreak strain (A) is widespread across England, and a second (D) is prevalent only at a single centre, where A is also frequent. Strain A generally has lower-level cephalosporin resistance than strain D and we investigated the basis of this difference.

Method: Three representatives each of strains A and D were investigated; each strain A representative was from a different centre. Plasmids were transformed into *E. coli* DH5alfa. MICs were determined by the British Society for Antimicrobial Chemotherapy method. Culture sonicates were used for isoelectric focusing and to assay cefotaximase specific activity. RNA was extracted from clinical isolates and used for reverse-transcriptase (RT) PCR to assess expression of blaCTX-M-15. The region upstream of blaCTX-M-15 was sequenced in strain A representatives.

Results: Two typical strain A isolates (A1 and A3) required lower cefotaxime (16–32 mg/L vs. >64 mg/L) and ceftazidime (2–4 mg/L vs. 32–64 mg/L) MICs compared with the three strain D isolates (D1–D3) and with the third strain A representative (A2). Cefotaximase specific activity was up to 15-fold lower in strain A1 and A3 than in D1, but only 2-fold lower in the case of A2. CTX-M-15-producing transformants derived from all three strain A representatives (including A2) required lower cephalosporin MICs compared with those derived from the strain D representatives, and all had significantly lower cefotaximase specific activity. CTX-M-15 was hardly detectable by electrofocusing in isolates A1 and A3 but was obvious in A2 and in D1–D3; an OXA-1 band was equally intense in all six isolates. Similarly, RT-PCR showed a blaCTX-M-15 band of lower intensity for isolates A1 and A3 than for A2 or D1–D3, whilst the blaOXA-1 band was equally intense in all. All three strain A isolates had an IS26 element between blaCTX-M-15 and its normal promoter, provided by ISEcp1; DNA sequencing indicated no differences immediately upstream of blaCTX-M-15 in A1–A3.

Conclusion: Expression of CTX-M-15 beta-lactamase in strain A was generally lower than in strain D, explaining the lower cephalosporin resistance. IS26, between blaCTX-M-15 and its normal promoter, may be responsible for this lower expression but, it also appears that trans-acting factors may up-regulate CTX-M-15 expression, as in isolate A2.

P509

Dominance of CTX-M beta-lactamases among *Escherichia coli* isolates in an Algerian hospital

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Objectives: Class A extended-spectrum beta-lactamases (ESBLs), as CTX-M, are rapidly expanding worldwide. The aim of this study was to establish the frequency of resistance to broad-spectrum cephalosporins of *E. coli* strains collected in Algeria, and to characterize the types of ESBL produced.

Methods: During January–June 2005, 279 *E. coli* strains were recovered consecutively from separate patients at the Mustapha Pacha Hospital of Algiers, Algeria. ESBL producing enzymes

were detected by disc diffusion method; E-test ESBL with cefotaxime (CTX) and ceftazidime (CAZ) plus clavulanate was used to confirm strains as ESBL producers. MICs of 23 antibiotics were performed by microdilution broth method against ESBL-positive strains. Isoelectric focusing was used to characterise pI of beta-lactamases. PCR was performed with specific primers to type beta-lactamase genes in ESBL producer strains: blaTEM, blaOXA, blaSHV, blaCTX-M and ampC. Sequencing identified ESBL enzymes and pulsed-field gel electrophoresis (PFGE) with XbaI-digested genomic DNA established the diversity of ESBL-positive clones. Specific primers were used to screen for the presence of ISEcp1 upstream from blaCTX-M.

Results: Sixteen of 279 (5.7%) strains were confirmed as ESBL producers among the following biological products: urine (n = 5), pus (n = 5), CSF (n = 1), blood (n = 4) and sputum (n = 1). All strains had the ubiquitary ampC gene plus blaTEM and blaCTX-M genes. Sequencing of blaTEM and blaCTX-M amplicons identified that all strains encode the TEM-1A enzyme, 13 the CTX-M-15 and 3 the CTX-M-3; these enzymes were characterized with pIs of 5.4, 8.9 and 8.0 respectively. ISEcp1 was detected in all *E. coli* strains producing CTX-M enzymes; and PFGE profiles of these strains indicated that only five clones were related. CTX-M producers showed diminished susceptibility to different antibiotics, such as CTX (94%), ceftriaxone (94%), CAZ (75%), aztreonam (94%), trimethoprim/sulfamethoxazole (88%), gentamicin (94%), amikacin (25%), ciprofloxacin (19%) among others; 81% of strains CTX-M producers were multidrug-resistant.

Conclusions: We showed a high frequency of policlonal dissemination of resistance to broad-spectrum beta-lactams in a hospital in Algeria through CTX-M ESBL, probably facilitated by mobile elements. Our results suggest that therapeutic options may be dramatically diminished if CTX-M enzymes continue spreading in hospital environment, as multidrug-resistance was demonstrated in a high frequency.

P510

Extended-spectrum beta-lactamases and plasmid-mediated AmpC enzymes among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*, Taiwan

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A total of 291 *Escherichia coli* and 282 *Klebsiella pneumoniae* isolates that showed decreased susceptibilities to extended-spectrum cephalosporins were collected between March and August 2003 from seven medical centres and were examined to evaluate the distribution of extended-spectrum B-lactamases (ESBLs) and plasmid-mediated AmpC enzymes in *E. coli* and *K. pneumoniae* in Taiwan. Overall, ESBL production was detected in 60.5% of the *E. coli* isolates and 94.0% of the *K. pneumoniae* isolates, and 43.6% of the *E. coli* isolates and 14.5% of the *K. pneumoniae* isolates exhibited plasmid-mediated AmpC enzymes. In *E. coli*, CTX-M (54.3%), SHV ESBLs (9.3%), and CMY-2-related AmpC enzymes (43.6%) were detected; in *K. pneumoniae*, CTX-M (55.0%), SHV ESBLs (47.9%), CMY-2-related enzymes (3.5%), and DHA-1-related enzymes (11.0%) were detected. Thirty-five (12.0%) of the 291 *E. coli* isolates and 46 (16.3%) of the 282 *K. pneumoniae* isolates harboured two or three B-lactamases involved in resistance to extended-spectrum cephalosporins. Nucleotide sequencing of blaCTX-M genes revealed the presence of seven subtypes, which were blaCTX-M-3, blaCTX-M-9, blaCTX-M-14, blaCTX-M-15, blaCTX-M-17, blaCTX-M-19, and a novel blaCTX-M-9-related gene. Among the seven blaCTX-M subtypes, blaCTX-M-3 and blaCTX-M-14 were predominant in *K. pneumoniae* and *E. coli*, respectively, and blaCTX-M-17, blaCTX-M-19, and the novel blaCTX-M gene were firstly identified in Taiwan. Randomly amplified polymorphic DNA analysis revealed genetic diversity among the isolates with blaCMY-2, blaCTX-M-3, or blaCTX-M-14 randomly selected from different hospitals, and conjugation experiments and plasmid analysis suggest the interhospital spread of similar resistance plasmids.

P511

Salmonella enterica serovar Enteritidis producing a TEM-52 B-lactamase. First report in Spain

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Objectives: To report the first finding of a TEM-52-producing *S. enterica* serovar Enteritidis in Spain.

Materials and methods: The isolate was obtained from a stool sample from a 9 year old girl with gastroenteritis. One month before she had received amoxicillin for a respiratory infection. The isolate was identified by using the Wider System (Dade-Behring, California), and the serotype by using Difco antisera (Becton Dickinson, USA). The MICs were determined by the agar microdilution method. ESBL phenotype was determined according CLSI guidelines. Plasmid DNA was obtained and amplified by PCR by using primers specific for TEM, SHV and CTX-M β-lactamases. *E. coli* XL1-Blue MRF′ Kan, β-lactam-susceptible, kanamycin-resistant, was used as the recipient for conjugation experiments. MacConkey agar plates containing 2 mg/l cefotaxime and 20 mg/l kanamycin were used for selection. PCR and sequencing of transconjugants was performed. Since TEM-52 has been described to be associated to a Tn3 transposon in *Salmonella*, PCR with primers specific for this transposon was performed on plasmid DNA.

Results: The main MICs obtained from this *Salmonella* isolate were as follows: amoxicillin, >128 mg/l; amoxicillin/clavulanic acid, >4/2 mg/l; cefuroxime, 64 mg/l; cefoxitin, >16 mg/l; ceftazidime, 64 mg/l; ceftazidime/clavulanic acid, 1/4 mg/l; cefotaxime, 8 mg/l; cefepime, 4 mg/l; amikacin, 4 mg/l. Only primers for TEM yield an 800-bp fragment, whose sequence was 100% identical to the TEM-52 sequence available in GeneBank. The same amplification for the transconjugant strain showed it harboured the TEM-52 gene. Both, *Salmonella enteritidis* isolate and transconjugants, gave the expected 500 bp PCR product for Tn3, suggesting the presence of TEM-52 gene in a Tn3-like structure, carried by a conjugative plasmid.

Conclusions: TEM-52 was first described in 1998. It has been reported in different *Salmonella* serotypes in Greece and Korea and reported in *Salmonella* in the UK for the first time in 2004. It has also been found to be the predominant ESBL gene in a study on ESBL-resistant *Salmonella* obtained from humans and poultry in The Netherlands. Nevertheless, TEM-52 is infrequent in Spain. Only two *E. coli* isolates producing TEM-52 have been found in our Department among 156 ESBL-producing isolates (unpublished data), and had not been found before in *Salmonella* in our country. As in previous studies, Tn3-like structures seem to be the genetic element in which TEM-52 is usually included.

P512

Biochemical characterisation of the plasmid-encoded class C β-lactamase FOX-7

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Objectives: Plasmid-mediated class C β-lactamases (e.g. CMY-, FOX-, MOX- or ACT-type enzymes) are characterized by their ability to hydrolyse most cephalosporins (including cephamycins) and are poorly inhibited by conventional β-lactamase inhibitors (e.g. clavulanate, tazobactam). FOX-7, a plasmid-encoded class C β-lactamase originally identified in clinical isolates of *Enterobacter cloacae* and *Klebsiella pneumoniae* which caused an outbreak at the Neonatal Intensive Care Unit of the Teaching University Hospital of Siena, Italy, was purified and its biochemical properties investigated.
Methods: The blaFOX-7 ORF was amplified by PCR and cloned into vector pET-9a, yielding plasmid pET-FOX-7. The latter was used to transform *E. coli* BL21(DE3) for high-level production and grown in ZYP-5052 auto-inducing medium. The FOX-7 enzyme was purified (purity, 95%) by means of two chromatographic steps, a cation-exchange at pH 6.0 followed by an anion-exchange at pH 7.2. Steady-state kinetic parameters for the hydrolysis of β-lactam antibiotics were determined by measuring spectrophotometrically the initial reaction rates. Low Km values were measured as Ki using a competitive inhibition model. Inactivation by imipenem, meropenem, aztreonam was investigated using cephalothin as the reporter substrate.
Results: Using *E. coli* BL21(DE3) [pET-FOX-7], the β-lactamase was purified with an overall yield of 3 mg per litre of culture. High catalytic efficiencies were measured with most tested substrates (kcat/Km > 106 M^{-1} s^{-1} measured with e.g. ampicillin, benzylpenicillin, cephalothin, cefazoline, cephalexin, cefamandole and cefotaxime) while ceftazidime and cepefime were hydrolysed less efficiently (kcat/Km, 1.8 x 104 and 4.6 x 104 M^{-1} s^{-1}, respectively). Km values ranged from 370 μM (cefazoline) to 0.04 μM (cefotaxime). In comparison with other FOX-type enzymes (including CAV-1), FOX-7 exhibited overall higher turnover rates, lower Km values for penicillins, cephaloridine and cefotaxime, accounting for the overall high catalytic efficiencies observed. FOX-7 was inhibited by aztreonam, imipenem and meropenem, the latter exhibiting the best inactivation efficiency.
Conclusion: A detailed biochemical analysis carried on the FOX-7 β-lactamase revealed that the enzyme exhibits a broad-spectrum of activity and was particularly active against cefotaxime, in comparison with other FOX-type enzymes.
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P513

Analysis of the promoter/attenuator region of the ampC gene in clinical isolates of *Escherichia coli* with ''AmpC phenotype''

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Objective: To analyse the DNA sequences upstream of the ampC gene in clinical isolates of *Escherichia coli* with a phenotype compatible with AmpC-hyperproduction, to determine the frequency with which promoter and attenuator mutations occur and to determine the possibility of clonal spread of these organisms.
Methods: *Escherichia coli* from clinical samples obtained at the Service of Microbiology, Univ. H.V. Macarena, Seville, Spain during 1999–2001 were evaluated. Reference susceptibility testing was performed by microdilution (NCCLS guidelines). Seventy-seven organisms resistant to amoxicillin plus clavulanate (AMC), cephalotin and cefoxitin (FOX) and lacking extended-spectrum beta-lactamases (no synergy of cefotaxime or ceftazidime with clavulanic acid) were selected. Clonal relationship was determined by REP-PCR, with primer 5′-III GCG CCG ICA TCA GGC-3′. PCR amplification of the promoter/attenuator region of the *E. coli* ampC gene was performed using primers AB1 (5′-GATCGTTCTGCCGCTGTG-3′) and ampC2 (5′-GGGCAGCAAATGTGGAGC-3′). Amplicons were purified and sequenced in both directions. Nine *E. coli* clinical isolates fully susceptible to AMC and FOX were also analysed.
Results: Fifty-seven REP-PCR profiles were observed. PCR and sequence analysis of the ampC promoter/attenuator region revealed 14 different variants among *E. coli* isolates with an AmpC phenotype. The mutations most frequently found (67/77 isolates) were located at positions -42, and -18, resulting in a new displaced -35 perfect consensus sequence. These mutations were always associated with mutations at positions -88, -82, -1 and +58. In addition to these mutations a deletion of 30 nucleotides between +16 and +45 containing the dyad symmetry region of the ampC attenuator was also observed in three strains. Two isolates did not presented mutations. The isolates with higher level of resistance to β-lactams were those that presented the 30 bp deletion in the attenuator region of ampC and those without mutations. Among the nine fully susceptible isolates only two presented the wild-type promoter/attenuator region.
Conclusions: Most clinical isolates of *E. coli* with ''AmpC hyperproduction phenotype'' in our area presents mutations in the promoter/attenuator region of the ampC gene that create a strong consensus – 35 promoter box. There is considerable clonal variability among the isolates. The contribution of acquired AmpC-type β-lactamases to this resistance phenotype does not seem very relevant.

P514

Evaluation of an AmpC disk test and genetic detection of imported ampC-genes in Norwegian clinical strains of *E. coli*

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Background: Convenient phenotypic tests to detect clinical significant AmpC-production in *E. coli* are important for diagnostic purposes, infection control and to ensure effective therapy.
Objectives: The aims were to evaluate an EDTA-based disk test for detection of AmpC-production and examine for imported ampC-genes in Norwegian clinical strains of *E. coli* with an AmpC-susceptibility profile.
Methods: We included 23 clinical strains of *E. coli* collected during 2003–2005 from 11 Norwegian laboratories with an AmpC-susceptibility profile: resistance to aminopenicillins (ampicillin MIC > 128 mg/L), and oxyimino cephalosporins without clavulanic acid synergy (cefpodoxime and cefotaxime and ceftazidime MIC > 4 mg/L), and cephamycins (cefoxitin > 16 mg/L), and susceptibility to cefepime and carbapenems. β-lactam susceptibility testing was performed by E-test. Extended susceptibility testing was done by VITEK II. The strains were negative for blaSHV and blaCTX-M by consensus PCRs. The strains were examined for β-lactamase production by IEF. Phenotypic detection of AmpC-expressional was performed by a recently described disk test

based on the use of Tris-EDTA to permeabilize a bacterial cell causing extracellular release of β-lactamases (Black et al., J Clin Microbiol 2005;40:3110). The strains were examined by a multiplex PCR covering six families of ampC-specific genes (ACC, CIT, DHA, EBC, FOX, and MOX) as well as a specific CMY-PCR. Control strains included: ampC-positive strains (N = 6), susceptible *E. coli* ATCC reference strains (N = 3), clinical strains of *E. coli* (N = 10) susceptible for oxyimino-cephalosporins (MIC < 1 mg/L) and cefoxitin (MIC < 4 mg/L).
Results: All 23 clinical strains with an AmpC-profile produced a β-lactamase with a pI between 8.5 and 9.0 corresponding to pIs of AmpC-β-lactamases. CMY-genes were detected in 10/23 strains as the only imported ampC gene. Sequence typing revealed both CMY-2 and CMY-7. A total of 21 out of 23 clinical strains were positive in the AmpC-disk test including all ten CMY-positive strains. The AmpC-profile negative control strains were negative in the AmpC disk test. There was a trend towards a multiresistance phenotype in the AmpC-positive *E. coli* strains.
Conclusions: (i) The EDTA-based AmpC disk test is a potential useful, convenient test to detect clinical significant AmpC-production in clinical strains of *E. coli*. (ii) Plasmid-mediated ampC-genes of CMY-type are detected in Norwegian clinical strains of *E. coli*.

P515

Detection of extended-spectrum β-lactamase in *Enterobacteriaceae* with AmpC β-lactamase type of inducible resistance

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Objectives: ESBL production in *Enterobacteriaceae* with inducible AmpC chromosomal enzymes is rare, while its detection is problematic. In this study, the prevalence of the concurrent production of these enzymes in clinical *Enterobacteriaceae* strains was determined and three phenotypic confirmatory methods were also compared.
Methods: Fifty-five nonrepetitive clinical strains of *Enterobacter* spp. (n = 35), *Citrobacter freundii* (n = 10), *Morganella morganii* (n = 7), and *Providencia stuartii* (n = 3) exhibited an inducible AmpC β-lactamase type of resistance were isolated from urine, blood and pus over a 15-month period. Cefoxitin resistance, identification to species level and the cefoxitin/cefotaxime (CTX) disk antagonism test were used to screen for likely inducible AmpC producers. Three confirmatory phenotypic methodologies to address the detection of ESBLs were used: (a) combination disk tests CTX ± clavulanic acid (CA), ceftazidime (CAZ) ± CA, and cefepime (CEF) ± CA, (b) double-disk tests CTX/CA, CAZ/CA, and CEF/CA, and (c) modified double-disk test with CEF/piperacillin + tazobactam (TZP). AmpC production was confirmed by the three-dimensional test.
Results: Of the 55 isolates that were tested, 53 were found to be ESBL non-producers; only two (3.6%) strains (*E. aerogenes* and *E. cloacae*) were resistant to cefotaxime and appeared to be co-producers of ESBL and AmpC β-lactamases enzymes. Combination disk test with CEF ± CA, double-disk test with CEF/CA and the modified double-disk test with CEF/TZP were capable of detecting the ESBL positive strains while the others tests failed to confirm the concomitant ESBL production.
Conclusions: In *Enterobacteriaceae*, production of inducible AmpC-beta lactamases can mask ESBL activity making its detection a challenge. The use of cefepime instead of cefotaxime or ceftazidime in inhibitor-based confirmatory tests seems to be preferable. In our institution, the occurrence of co-production of extended-spectrum and inducible chromosomal AmpC beta-lactamases, in the selected isolates we studied, was too low (3.6%) for justifying the routine use of detection methods. These procedures can be carried out in strains isolated from serious infections and to survey for complex resistance mechanisms.

P516

Modelling the effect of copy number of plasmids carrying ESBL genes on resistance levels to β-lactam antibiotics

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Objectives: Variable resistance to β-lactams among ESBL-producing strains has been widely discussed in the literature and has been attributed to many factors. Some of them, as different substrate preference of ESBLs, efficiency of ESBL gene promoters, or outer membrane permeability of host strains, have been studied extensively using both clinical and laboratory strains. This study aimed to demonstrate the effect of copy number of plasmids carrying ESBL genes on resistance levels to β-lactams in the isogenic *E. coli* strains.
Methods: The genes for SHV-3, CTX-M-3 and its P167T-mutation variant, CTX-M-42, were cloned under their natural promoters in the pCC1 vector which contains a single copy F factor origin of replication and a high copy oriV origin of replication. The plasmids were introduced into the *E. coli* EPI300 strain in which they were maintained at single copy number per cell under standard growth conditions or at multiple copy number upon induction with L-arabinose. The MICs of ampicillin (AMP), ceftazidime (CAZ), cefotaxime (CTX), cefepime (FEP) and aztreonam (AZT) were determined for the strains carrying recombinant plasmids or the pCC1 vector without insertion using the broth microdilution method as recommended by NCCLS except that LB broth with or without arabinose at 0.02% final concentration was used as testing medium. The plasmid copy number was determined by real-time qPCR with plasmid-specific primers and SYBR Green I and related to the total number of viable cells to yield the plasmid copy number per cell.
Results: An induction with arabinose resulted in increase in the copy number of plasmids from 1 to ~10 copies per cell. As shown in the table, the multiplication of ESBL-coding plasmids was paralleled by significant increase in resistance of the host strain to all the antibiotics, including those known to be weak substrates for particular ESBLs (e.g. CAZ for SHV-3 and CTX-M-3, or CTX for CTX-M-42). Resistance levels to oxyimino-beta-lactams raised by 4–5 log2 MIC dilutions for

Table 1. Susceptibilities of *E. coli* EPI300 strains containing recombinant plasmids and pCC1 at different copy numbers.

Plasmid	Copy number per cell	MICs, mg/L				
		AMP	CTX	CAZ	FEP	AZT
pCC-*bla*$_{SHV-3}$	1	1024	2	1	0.25	0.5
	10	≥2048	64	16	4	8
pCC-*bla*$_{CTX-M-3}$	1	512	16	1	1	2
	10	≥2048	256	4	4	16
pCC-*bla*$_{CTX-M-42}$	1	256	1	32	0.5	1
	10	≥2048	4	128	1	4
pCC1	1	2	0.25	0.5	≤0.125	0.25
(*bla* negative)	>10	2	0.25	0.5	≤0.125	0.25

the SHV-3-producing strain, and by 1–4 log2 MIC dilutions for the CTX-M producers. At the same time, induction with arabinose did not affect the MICs for the beta-lactamase negative strain.

Conclusion: In our model experiment, a strong correlation between the ESBL genes copy number and the MICs of oxyimino-beta-lactams was demonstrated. This observation raises the importance of accurate detection of ESBL-mediated resistance in clinical isolates.

Molecular typing

P517

Genetic relatedness between sporadic and epidemic methicillin-resistant *Staphylococcus aureus* in Belgian hospitals

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Objective: The objective was to compare the genetic backgrounds of sporadic MRSA isolates with those of successful epidemic MRSA clones.
Material and methods: 30 strain representative of the nine most frequent Belgian epidemic clones and 20 sporadic strains were selected from the ULB-Staphylococcus Reference Laboratory collection (MRSA isolates recovered from patients hospitalised in Belgian acute-care hospitals from 1992 to 2003) based on their pulsed field gel electrophoresis (PFGE) type after *Sma*I macrorestriction. A PFGE pattern was considered as sporadic if there was a difference of >6 DNA fragment with any other PFGE pattern in the entire database (~2500 strains). Strains were further characterised by Multilocus Sequence Typing (MLST), spa typing and SCCmec typing.
Results (See table): The 20 sporadic isolates were classified by MLST in 9 Sequence Types (ST) all but one belonging to the 5 same Clonal Complexes as epidemic clones (CC5, 8, 45, 22 and 30). SCCmec type distribution among sporadic strains was as follow: type I: 9 isolates, type IV: 7, type III: 2, type II and V: 1 isolate each. SCCmec type IV was associated with the greatest number of different STs (5). Based on MLST combined with SCCmec typing, 12 clonal types were identified, only 5 of which were identical to those of major Belgian epidemic clones. The 20 sporadic isolates belonged to 16 spa types of which up to 6 were shared with epidemic strains.

Distribution of MLST-ST, SCC*mec* types and *spa* types among 30 epidemic and 20 sporadic Belgian MRSA strains.

CC	ST	SCC*mec*	Epidemic strains (n=30)			Sporadic strains (n=20)	
			PFGE type	n°	*spa* type	n°	*spa* type
8	8	III	-	-	-	1	t064
		IV	A20	4	t008	2	t008
			A22	1	t008		
	247	I	A1	9	t051, t052, t054, t770	5	t051, t052, t303
	250	IV	-	-	-	1	190
5	5	I	-	-	-	2	t001
		II	G10	2	t041, t002	-	-
		III	-	-	-	1	t045
		IV	C3	2	t002, t447	1	t769
	228	I	D8	1	t041	2	t001, t041
		II	C1	1	t003	-	-
	231	II	-	-	-	1	t002
45	45	IV	B2	8	t038, t247, t598,t739, t740	2	t038, t655
22	22	IV	L1	2	t032	-	-
30	30	IV	-	-	-	1	t019
?	377	V	-	-	-	1	t355

Conclusion: The majority of sporadic and epidemic MRSA strains identified over a 11-year period in Belgian hospitals share common genetic background. Although, only half of sporadic MRSA strains belong to the same ST-SCCmec type as epidemic strains, suggesting that resistance cassette type and other epigenetic markers are involved in conferring the epidemic behaviour of MRSA.

P518

Analysis of polymorphisms within mid-region inserts of vacuolating cytotoxin alleles of *Helicobacter pylori* to identify genotypic markers of geographic adaptation

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Objectives: The presence or absence of inserts in the vacuolating cytotoxin (VacA) gene that encodes the VacA toxin, a key pathogenicity factor of H. pylori, provides the basis of a widely used scheme for genotyping. The aim of the present study was to determine diversity of insert sequences in the mid-region (m) of vacA, that includes a toxin subunit coding region (cell binding domain), in order to find genotypic markers indicative of geographic adaptation.
Methods: Mid-region sequences of vacA (n = 408, from new and from public databases) from 27 countries were analysed. The dataset included 255 sequences of the m2-allelic family of which 179 sequences were determined in house by standard procedures from isolates in the laboratory collection, and comparisons and multiple alignments were implemented in BioEdit.
Results: The 255-m region inserts (MRIs) were highly conserved in size (75 bp) and constituted 12% of the region. Base compositions were typically 37–40% G+C. Sequence diversity was evident from alignments with reference strain Tx30a for the m2 allelic family (MRI-1). A total of 23 amino acid variants were defined by one or more differences from the type 1 reference. The commonest types were MRI-4 and MRI-2 present in 62% and 14% respectively of geographically diverse isolates of the m2 allelic family. The MRI inserts characteristically contained a SDNGLN motif except for inserts from 16 Chinese strain sequences (MRI-21 and 22) with a unique GRNGID motif.
Conclusion: Our results indicated that despite conservation at the predicted amino acid level, sequences of vacA mid-region inserts were sufficiently diverse to provide additional genotypic markers for strain discrimination within the m2 allelic family, and to indicate possible host-associated adaptations in a Chinese subgroup.

P519

Differences in group A *Streptococcus* clinical and molecular epidemiology between Belgian and Brazilian children

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Objectives: To compare the epidemiology of Group A *Streptococcus* (GAS) colonisation and infection among children from Brussels (Belgium) and Brasília (Brazil).

Methods: We prospectively included Belgian and Brazilian children attending 4 public hospitals from February 1 to October 31, 2004. Inclusion criteria were any clinical suspicion of GAS infection or microbiological GAS isolation. We excluded children under current antimicrobial treatment. A pre-designed clinical form was filled and microbiological sampling was performed for each child. GAS strains were identified by standard laboratory methods (CLSI) and stored at −80°C. The GAS were emm-typed using the CDC sequencing method.
Results: 334 GAS strains (Brussels: n = 204, Brasília: n = 130) were isolated from the 706 children (Brussels: n = 360, Brasília: n = 346). In Brussels, pharyngitis was the most common GAS infection (83%). In Brasília, cutaneous infections (48%) and pharyngitis (44%) were evenly associated with GAS isolation. The mean age of children with GAS pharyngitis in Brussels was much lower than in Brasília (65 versus 92 months; $t = -3.99$, $p < 0.001$). emm-typing revealed striking differences between Brazilian and Belgian GAS strains. 20 different emm-types were identified among the 200 Belgian strains, whereas 48 different emm-types were found among the 129 Brazilian strains. Moreover, the Belgian strains belonged to the classical emm-types recovered in children from developed countries. The Brazilian emm-types have been rarely or not yet reported. 9 of the Brazilian strains presented a novel emm gene. Additionally, the pathologies associated with the Brazilian emm-types were different from those usually described in developed countries.
Conclusions: Our study highlighted important differences between developing and developed countries in terms of GAS clinical and molecular epidemiology. Besides the mean age of children with GAS pharyngitis, the differences in clinical distribution that we observed were expected. Unexpectedly, the emm-types distribution was totally different: oligoclonal in Brussels and polyclonal in Brasília. Correlation between pathology and emm-types might be less stringent than previously thought. Vaccinal strategies should take into account these major variations.

P520

AFLP analysis of international *Clostridium difficile* strains of different toxin type reveals major toxin-dependent clonal clusters

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Objectives: *C. difficile* isolated from patients with AAD or PMC usually produce toxin A and toxin B (TcdA+TcdB+). An increasing number of reports mention infections due to toxin A-negative *C. difficile* (TcdA-TcdB+). Strain carrying binary toxin genes (cdtA and cdtB) and producing toxin A and B (TcdA+TcdB+CDT+) were identified as well. Many molecular typing methods have been used to investigate their genetic relationship. We here describe the population structure of 89 *C. difficile* strains belonging to different toxin types isolated from patients with AAD in Poland and Japan using AFLP method.
Methods: We obtained 83 *C. difficile* strains from Polish patients and six Japanese isolates. Four reference strains of *C. difficile* belonging to different toxin profile types were included. Toxin types were determined by commercial test for toxin A and cytotoxicity test for toxin B. TcdA, tcdB and binary toxin (cdtA and cdtB) were detected by PCR. Analysis with these two primer combinations resulted in 78 reliable markers. All markers were used as the input for the TREECON software.
Results: The phylogenetic tree showed highly distinctive groups. Seven Polish *C. difficile* strains belonged to TcdA+TcdB+CDT+ and were clearly distinct from the other strains. The other strains could be divided into several toxin type-specific subgroups. The Japanese strains were included in the clonal groups that shared the same toxin type. This suggests a common evolutionary origin. Among the Polish TcdA-TcdB+ strains 22 strains showed an identical AFLP profile. In some case Japanese and Polish AFLP patterns were identical.
Conclusion: Polish and Japanese *C. difficile* with differing toxin profiles clustered in the AFLP derived phylogram. This suggests highly clonal dissemination and single events in which both toxin A and B genes were lost simultaneously. Apparently, successful *C. difficile* toxin types disseminate worldwide. This has important epidemiological and population structure consequences.
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P521

Molecular epidemiology and genetic diversity of *Mycobacterium tuberculosis* clinical isolates in Okinawa, Ryu-Kyu islands, Japan

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A collection of 101 clinical isolates of *M. tuberculosis* from pulmonary tuberculosis patients living in Okinawa, collected between 2003 and 2005, was studied by spoligotyping, a reverse-line-based hybridisation assay that allows to study the genetic diversity of the Direct-Repeat locus, the infra-species classification of *M. tuberculosis*. Results on all isolates were available. Seventy-two clinical isolates (71.3%) belong to the Beijing type, the predominant clade in Okinawa. 5 clinical isolates belong the previously described East-African-Indian (EAI)-2-Manilla clade. A total of 18 clusters (from 2 to 69 isolates) and 4 unique spoligotypes are described. Comparison to the latest available international-spoligotyping database (SpolDB4) allowed to detect two identical and one similar isolates between this study and previous studies performed in Okayama and Osaka, thereby characterizing the spoligotyping-international-type SIT627 as representative of a new, low IS6110-copy, genetic family of *M. tuberculosis*, which was baptised the Osaka-type family (T. Matsumoto). Further characterization of this genotype by an independent genotyping method, VNTR-MIRU-typing, provided classical 5-VNTR and 12 MIRU allelic values of 32423 and 215125113322 respectively (VNTR-MIRU international type, VIT310). VIT310 had previously been detected in Turkey, which raises interesting hypothesis about the genetic link between these two bacterial populations. The 72 clinical isolates of the Beijing type were further discriminated using QUB (Queen-University-Belfast) markers and a set of four epidemiologically-informative MIRU markers to search for epidemiologically-linked clusters within the Beijing group of strains.

P522

A view of the *Neisseria gonorrhoeae* population transmitted in Arkhangelsk, Russia: phenotypic and genetic characteristics

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In Arkhangelsk region, Russia the *gonorrhoeae* incidence was estimated to 135.9 cases per 100 000 inhabitants in 2004.

However, as in many East-European countries reliable incidence figures are still lacking mainly due to suboptimal diagnostics and incomplete case reporting. Regarding antibiotic resistance and molecular epidemiological characteristics of the *N. gonorrhoeae* strains that circulate in Russia, no thorough knowledge has yet been published.

Objectives: To phenotypically and genetically characterize clinical *N. gonorrhoeae* isolates from Arkhangelsk, Russia. This in order to describe the *N. gonorrhoeae* population transmitted in Arkhangelsk and to compare with characteristics of the *N. gonorrhoeae* populations in some West-European countries.

Materials and methods: *N. gonorrhoeae* isolates (n = 76), cultured between June–November 2004, from 76 mainly consecutive patients in Arkhangelsk, Russia were included. The isolates were characterized using antibiograms (see presentation by Vorobieva et al.), serovar determination, sequencing of the entire porB gene, and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST). Phylogenetic trees were constructed with TREECON v1.3b by using Neighbour-joining method.

Results: The *N. gonorrhoeae* isolates were assigned serovar IA-1.2 (n = 1), IA-6 (n = 17), IA-25 (n = 2), IB-1 (n = 23), IB-2 (n = 3), IB-3 (n = 12), IB-5 (n = 7), IB-14 (n = 5), IB-21 (n = 1), IB-23 (n = 1), IB-26 (n = 1), and IB-31 (n = 3). Complete results of the correlations with the antibiotic susceptibility testing, porB gene sequencing, and NG-MAST will be included at the presentation.

Conclusions: According to previous studies, the diagnosis of *N. gonorrhoeae* needs to be optimised and quality assured in several East-European countries. Furthermore, thorough knowledge of the gonorrhoea incidences, antibiotic resistance and genetic characteristics of the *N. gonorrhoeae* strains circulating in East-European countries is crucial.

P523

Discrimination between *E. coli* O157 isolates using PCR-mediated fingerprinting based on SER element

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Objectives: Due to close relationship between *E. coli* O157 strains so far the pulsed-field gel electrophoresis (PFGE) was found to be the only method with sufficient discriminatory power As PFGE is time-consuming and not suited to handling high sample numbers, there is a need for a fast, high-throughput PCR-based method. We used a *Salmonella Enteritidis* Repetitive Element (SERE)-based PCR approach for typing 46 Hungarian bovine *E. coli* O157 isolates (31 EPEC, 8 EHEC, 1 STEC and 6 toxin and intimin negative strains), 11 from the same, while 35 from different herds. We also examined 4 rabbit and 1 porcine EPEC isolates. Furthermore, we included 5 porcine ETEC O157 strains isolated in Hungary or Austria as well as 5 human EHEC strains isolated in different countries as epidemiologically independent controls. SERE results were compared to that of PFGE.

Methods: For SERE-PCR we used primers and annealing described earlier (Alam et al. J Clin. Microbiol 37(9):2772–6). PFGE separation of fragments was performed in a CHEF DRIII apparatus, at 6 V/cm for 20 h with a ramped switch time of 2–64 s. Patterns were analysed using the Fingerprinting II Software.

Results: Both methods found epidemiologically independent strains unrelated. PFGE revealed the presence of four clusters and 27 unrelated isolates. The first three included exclusively bovine isolates, Cluster P1 consisted of 20 EPEC isolates, cluster P2 included six EHEC isolates and the STEC isolate, while cluster P3 was comprised of four milk-derived isolates from the same herd. Cluster P4 comprised of three rabbit isolates. SERE-PCR showed 16 unrelated isolates and three clusters. Cluster S1 included eight bovine strains from the same herd and contained Cluster P3, cluster S2 was identical to cluster P4 of rabbit-derived strains and cluster S3 consisted of bovine clusters P1 and P2 also including several bovine isolates found to be unrelated by PFGE. Interestingly, SERE cluster S1 contained eight of eleven isolates of the same herd, while PFGE assigned four of them to cluster P3 finding the other four independent of clusters, suggesting that SERE-PCR may be less sensitive to microevolutionary changes.

Conclusion: SERE-PCR proved to be a useful alternative of PFGE, though we found it slightly less discriminatory. Thus, SERE-PCR may fulfil the need for PCR-based typing of *E. coli* O157.

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P524

Application of amplified fragment length polymorphism as an epidemiological tool for infections due to *Mycobacterium haemophilum*

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Objectives: *Mycobacterium haemophilum* was previously rarely recognized as a pathogen in the Netherlands. However, with the application of specific culturing methods and real-time PCR methods, this species has been identified as the involved pathogen in several diseases like skin inflammation, lymphadenitis and arthritis.In 2003–2004 a sudden increase of patients with cervicofacial lymphadenitis caused by *M. haemophilum* was observed in the Amsterdam region. As a part of an epidemiological study to investigate the possibility of a common source for these infections, the genetic diversity of the strains was investigated and compared to unrelated strains.

Methods: In total, 130 *M. haemophilum* isolates were collected: 30 European strains (of which 20 from the Amsterdam region) and 100 strains from different continents (among which 43 Australian strains 40 USA strains). Genome comparison was carried out with Amplified Fragment Length Polymorphism (AFLP) methodology to detect intraspecies variation. DNA was extracted using the MoBio® UltraClean Microbial DNA kit. An enzyme combination of *Eco*RI and *Mse*I with selective priming was used to obtain a high discriminatory power. Results were analysed by Dice calculation.

Results: The AFLP method enabled differentiation between *M. haemophilum* and closely related species as well as strain differentiation within the speciesIn general, strains belonging to a certain continent showed a specific AFLP pattern. The 43 Australian strains represented 2 separate clusters, encompassing 21 and 14 strains. Among the 40 strains from USA, 36 were from New York area. Within these 36 strains, AFLP discriminated 5 types, including 1 large cluster of 23 strains. No differences were observed in the AFLP patterns of the 20 Amsterdam strains while genetic diversity was present in 10 other European *M. haemophilum* strains.

Conclusion: While *M. haemophilum* seems highly conserved as a species, geographical distances appear to be correlated with genetic diversity. Application of AFLP demonstrated a

clustering of 20 children with *M. haemophilum* lymphadenitis in a region around Amsterdam.

P525

Distribution of toxinotypes and detection of binary toxin in 199 *Clostridium difficile* strains at a university hospital, Montpellier, France

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Objectives: *Clostridium difficile* is the most common agent of nosocomial diarrhoea. The aim of this study was to characterize the clinical strains of C. difficile isolated in our hospital over a 18-months period by toxinotyping and binary toxin gene detection.
Methods: Traditional methods included direct detection of toxin A on stool samples using the immunological kit *C. difficile* Toxin A test, Oxoid® and anaerobic culture on cycloserine-cefoxitin-fructose agar incubated during 7 days. All *C. difficile* strains were tested for toxin A production with the immunological method. Toxinotyping was performed as described by Rupnik et al. (A3 and B1 fragments) and binary toxin gene detection was done by PCR using cdtB pos and cdtB rev primers.
Results: A total of 199 *C. difficile* strains were collected from 159 patients between January 2004 and June 2005. Detection of toxin A was positive for 145 isolates and only for 53 stool specimens. Among the 199 strains, toxinotyping revealed that 156 strains were phenotypically A+/B+ (78.5%), 37 A-/B- (18.5%) and 6 (3%) A-/B+. Strains A+/B+ mainly belonged to toxinotype 0 (n = 127, 63,5% of the total strains), the 29 other were identified as variant toxinotypes III, IV, V, VI, IX, XII, XXI and XXII. The production of toxin A could not be detected with the commercial kit in 11 strains with toxinotypes 0 (n = 9), V (n = 1), and XXI (n = 1). Among the 37 strains A-/B-, 31 isolates were non-toxigenic and 6 were toxinotype XIb. The 6 isolates A-/B+ belonged to toxinotypes VIII (n = 4) and X (n = 2). Detection of binary toxin gene was positive for 31 strains (15.4%), which grouped into 8 variant toxinotypes (0, III, IV, V, VI, IX, X and XXII). Identical toxinotypes were observed in 19 of the 29 patients with successive *C. difficile* isolates whereas different toxinotypes were found for the 10 remaining patients.
Conclusion: These data confirmed that traditional methods are less effective than molecular methods to detect *C. difficile* toxins. A total of 12 toxinotypes were identified among the 199 *C. difficile* strains, with high prevalence of toxinotype 0 as previously observed. More variant toxinotypes were detected in comparison with previous European studies. We found 3% of virulent A-/B+ *C. difficile* strains. A relatively high level of strains with binary toxin gene (15.4%) was observed in comparison with Asian data (1.6%) and French data (6%). Toxinotyping showed that relapse occurred in 19 cases and reinfection in 10 cases.

P526

Molecular analysis of *Pneumococci* isolated from invasive infections of children in Hungary

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Objectives: to type *Streptococcus pneumoniae* strains isolated from invasive infections of children under 5 year in Hungary by molecular techniques; to establish the genetic mechanisms for erythromycin resistance; to analyse and relate the data to the serotype and antibiotic resistance profile of the isolates determined earlier.
Methods: 66 isolates from all over the country were tested with pulsed-field gel electrophoresis/PFGE/; 18 strains were analysed by multi locus sequence typing/MLST/; all erythromycin resistant isolates were tested for the presence of *mef* and *erm* genes by PCR
Results: of the 66 isolates 11 and 9 strains belonged or were genetically related to the England 14-9 and the ST156 clones, respectively. All of these isolates carried serotype 14 capsules (altogether 23 isolates belonged to serotype 14) and most of them showed resistance to erythromycin. The genetic mechanisms for erythromycin resistance proved characteristic for the two groups: 9 of the 11 England 14-9-related strains harboured the *mef*(A) gene, while in all of the erythromycin resistant ST156-related isolates (6 strains) the *erm*(B) gene could be demonstrated. These two international clones contributed substantially to the high rate (47%) of erythromycin resistance in our strainsTwo of our 6 serogroup B6 strains belonged to ST 473 and another to ST176.
Five of the 6B strains proved resistant to erythromycin and all of them carried the *erm*(B) gene.
S. pneumoniae strains with ST types characteristic for Hungary have also been detected. All of our serotype 3 strains (6) showed close genetic relatedness by PFGE. One isolate was tested by MLST; it belonged to a novel sequence type which was, however, closely linked to ST1138 reported earlier from Hungary. All of these isolates retained susceptibility to eryrthromycin. One of the 5 serogroup 19A strains belonged to ST199 and another to ST226. Both STs are characteristic for Hungary. The ST226 strain proved our sole isolate showing high level resistance to penicillin.
Conclusion: *S. pneumoniae* strains causing invasive infections in children belong to both international and local clones in Hungary. The contribution of the individual clones (and serotypes) to the high rate and genetic mechanism of erythromycin resistance is diverse. Vaccination could substantially reduce both the incidence of infection and the rate of macrolide resistance.
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P527

Prevalence and typing of adenoviruses in stool of Iranian children with acute diarrhoea: a comparative study by molecular and serological assays

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Objective: Enteric adenovirus (Ead) is the second most frequently detected viral agent in childhood diarrhoea. However, due to the emergence of new variants and limitation of detection assays, the disease burden of this virus has not been well defined. Adenoviruses are classified to 51 serotypes within 6 subgenera (A–F). Types 40, 41 are associated with sever acute diarrhoea while types 31, 2 and 7 are supposed to have important contribution in infantile gastroenteritis. In the present study, by application of both serologic and PCR-based methods, we provide data of a comparative study for determination of the prevalence of the Ead types associated with diarrhoea in Iranian children.
Methods: Stool samples from 329 Iranian children less than 12 years old with acute diarrhoea were collected over a period of 1 year. All of the specimen were examined by Adenolex

(Orion Diagnostica, Finland) based on latex agglutination, for detection of Ead in Faeces. Genomic DNA was extracted from stools by NucleoSpinR (MN-france). A PCR-RFLP method based on amplification of conserved region of hexon gene by degenerate primers was exploited for both identification and typing of Ead in specimen (Allard et al, 2001).
Results: We could screen 12.4% (41/329) positive samples by PCR based method while this rate was 4.5% (15/329) for Adenolex assay. Moreover, all adenolex detected types were only Ead 41 but PCR-based assay could not only identify up to 21 samples of Ead41, but also by application of this PCR method, Ead 31, 7 and 40 were detected in 8, 2 and one stool samples respectively. We could also identify 2 specimens belonging to both genus C or D and one to genus E. Seven other positive samples were not processed for typing.
Conclusion: Prevalence rate of Ead in Iranian children with acute diarrhoea might be higher (>12.4%) than previously reported data (6.7%) which was based on application of commercial serologic assay. The reasons behind this difference may be new emerging types and mutants of Ead in different geographic regions and inability of most commercial assays to detects serotypes other than 40 and 41. Finally, despite the general anticipation that type 40 and 41 are considered for cause of most childhood gastroenteritis, our results indicated that types 41, 31 and 7 were the most frequently found Eads in Iranian children with acute diarrhoea.

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Molecular and physiological characterisation of fungal opportunists belonging to the genus *Trichoderma*

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Objectives: *Trichoderma* spp. are known as cosmopolitan soil inhabiting filamentous fungi. Certain members of the genus are emerging as causative agents of opportunistic infections in humans. Here we present the discriminatory power of different phenetic and phylogenetic approaches applied for the taxonomic characterization of clinical *Trichoderma* isolates.
Methods: Twelve clinical *Trichoderma* isolates were involved in the experiments. Molecular phylogenetic analysis was performed for the sequences of the internal transcribed spacer 1 and 2 (ITS1 and 2) regions of the rDNA cluster and for the 4th large intron of the gene encoding translation elongation factor 1-alpha (tef1). RFLP patterns of mtDNA were generated by *Bsu*RI and *Hin*6I. Phenotype profiles were examined by isoenzyme analysis of 7 enzyme systems with cellulose-acetate electrophoresis (CAE) and by carbon source utilization arrays performed on BIOLOG FF microplates.
Results: Based on morphological characters, the 12 clinical *Trichoderma* isolates were originally identified as members of 3 species from section *Longibrachiatum*: *T. longibrachiatum* (5), *T. pseudokoningii* (3), *T. citrinoviride* (1); and 2 species from section *Trichoderma*: *T. viride* (2) and *T. koningii* (1). However, the ITS barcode identification by TrichOKEY 1.0 (www.isth.info) revealed that all of them belong to the triplet of species *T. longibrachiatum/Hypocrea orientalis/ H. cerebriformis*. Phylogenetic analysis of tef1 sequences shows that 11 strains belong to the clade of *T. longibrachiatum*, while one is attributed to *H. orientalis*. The examination of further, non-clinical isolates indicated that the tef1 marker clearly separates these two species. RFLP of mtDNA revealed 7 and 10 different patterns with *Bsu*RI and *Hin*6I, respectively, resulting in 4 groups on the dendrogram, while CAE separated the strains into 4 distinct electrophoretic types. BIOLOG Phenotype Microarrays were performed for all clinical and a series of non-clinical isolates from several closely related species. Comparisons were done at 9 time points and at 3 temperatures in order to detect possible physiological shifts specific for clinical isolates.
Conclusions: Our results support that fungal opportunists belonging to the genus *Trichoderma* are restricted almost exclusively to section *Longibrachiatum*. Besides sequence analysis, the methods of CAE, mtDNA RFLP and BIOLOG Phenotype Microarrays proved also appropriate for the characterization of clinical *Trichoderma* strains.

P529

SeqNet.org: a European-wide certification trial for sequence-based typing of microbial pathogens

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Objectives: SeqNet.org is an initiative of currently 28 laboratories from 20 European countries in order to establish a European network of excellence for sequence based typing of microbial pathogens. The principle goal of SeqNet.org is to establish unambiguous, electronic portable, easily comparable typing data for local infection control and national and European surveillance of sentinel microorganisms. Here, we describe (i) the harmonization of sequencing methods for sequence based typing, (ii) the capacity building for DNA sequencing in diagnostic microbiology, and (iii) the certification trail for sequence-based typing of MRSA.
Methods: After the 'kick-off' meeting in Münster, Germany (November 2004), the participants received a protocol for typing of MRSA by *Staphylococcus aureus* protein A gene (spa) typing. Subsequent, five strains, 5 DNAs, and 5 forward and reverse chromatogram files of representative and well characterized MRSA strains were distributed to all participating laboratories to be typed until the end of 2005. The typing results were analysed and synchronized with the central server by using the Ridom StaphType software.
Results: All participating European laboratories built up capacities for sequence-based typing and established the spa typing method for typing of MRSA in the laboratories. Until today, the typing results for the certification trial were submitted by 24 of the participating laboratories. Each laboratory determined 2,783 bp (range, 206–422 bp per strain) and all participants reported exactly the same spa type for each of the analysed isolates and for the additional 5 chromatograms. Therefore, the intra- and inter-laboratory reproducibility of the sequencing results was 100% each. Online synchronization of the results proved the rapid exchange of high quality typing data based on nucleotide sequencing.
Conclusion: The SeqNet.org initiative enables laboratories European-wide to build up capacity for sequence-based methods. The spa typing results proved the unambiguous and highly reproducible nature and high portability of sequence data. The usage of a standardized nomenclature based on the software enabled an easy exchange of data.

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Genetic typing of enteroviruses for the investigation of two local outbreaks in France in 2005

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Enteroviruses are associated with a wide variety of diseases in humans and are responsible for summer outbreaks of acute meningitis in both children and adults. After the last and large meningitis outbreak in 2000, enteroviruses continued to be isolated until April 2005 and a virus alert from the French Health Ministry was declared in June because of the increasing number of occurring meningitis cases.

Objectives: Genetic typing of enteroviruses by sequencing of a specific PCR-based amplified portion of the genome (the VP1 encoding sequence) followed by phylogenetic analysis, for identifying viruses isolated in patients hospitalised during the year 2005.

Methods: Viruses were isolated in patients hospitalised in Clermont-Ferrand (n = 38) and Paris-Hôpital Trousseau (n = 16). They were recovered from 48 patients with meningitis (positive RT-PCR in cerebrospinal fluid specimens, n = 44) and 6 patients with cardiac manifestations (n = 2), sudden infant death syndrome (n = 1), neonatal infection (n = 1), hand-foot-and-mouth disease (n = 1) and pharyngitis (n = 1). A genetic typing method relying on the PCR amplification and sequencing of the complete VP1 sequence was designed with three sets of species specific primers. Virus identification was done by sequence comparisons (BLAST search) with enterovirus sequences in Genbank and was confirmed by phylogenetic analysis with the VP1 sequences of the enterovirus prototype strains.

Results: Virus isolates were identified in all patients with only one set of primers. All viruses were assigned to 11 different types within the Human Enterovirus B species by BLAST search and phylogenetic analysis confirmed the identification in all cases. In patients with meningitis (n = 48), echovirus 30 (E30) was involved in 25 cases (52 %). The other types were E18 (n = 5), E13 (n = 5), coxsackievirus B5 (CB5, n = 4), CB3 (n = 3), E6 (n = 2) and E11, E33, E7 and E4 (each n = 1). In patients with other symptoms, 3 types were observed CB5, CB3 and E3 (each n = 2). CB5 and CB3 were associated with cardiac manifestations and other symptoms. Among patients with an E30 infection, four different virus variants were evidenced by the phylogenetic analysis.

Conclusions: Genetic typing allowed the prospective identification of all isolates more effectively and rapidly than seroneutralization tests used during the 2000 outbreak in Clermont-Ferrand. E30 persists as the major type involved in meningitis despite it co-circulated with other enteroviruses in both outbreaks.

P531

Development of a novel SNP based assay to speciate *Brucella* isolates

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Objectives: Brucellosis is one of the most important and widespread zoonotic diseases. Therefore, rapid and unambiguous assays for the detection of *Brucella* species are essential. Here we describe a novel SNP based assay that can speciate *Brucella* isolates into their six classically recognised species. Previously, molecular approaches to speciation have been hampered by the homogeneity of the genus that has made identification of species-specific markers difficult. The SNP assay described here was developed to overcome the problems presented by this genetic homogeneity as well as mitigating the need for classical culture based biotyping which is both laborious and time consuming.

Methods: Several housekeeping genes were sequenced from a large number of *Brucella* isolates. This sequence data was analysed and a number of stable single nucleotide polymorphisms (SNPs) were identified that appeared to unambiguously define the six classically recognised members of the genus *Brucella*. Using the primer extension approach to SNP detection a single-tube multiplex assay was developed which incorporated six SNP interrogation primers.

Results: To date more than 400 culturally confirmed isolates have been correctly identified using this assay. These included 73 isolates from human blood cultures that encompassed the pathogenic species *B. melitensis*, *B. suis* and *B. abortus*. The SNP multiplex assay can be used to rapidly and clearly differentiate between the six *Brucella* species.

Conclusion: In comparison to current molecular based assays this approach is all encompassing and will identify members of all currently recognised biovars within *Brucella* species. Future objectives involve the inclusion of additional SNPs to facilitate higher resolution beyond the species level.

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Multiplex SCCmec typing of hospital, community and multi-resistant methicillin-resistant *Staphylococcus aureus*

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Objectives: To apply a multiplex SCCmec typing method for investigation of a range of strains to distinguish between Multi-resistant MRSA (MR-MRSA), Hospital-acquired MRSA (HA-MRSA) and Community-acquired MRSA (CA-MRSA). To compare the discriminatory capacity of multiplex SCCmec typing with that of Randomly Amplified Polymorphic DNA (RAPD) and Pulsed Field Gel Electrophoresis (PFGE) methodologies.

Methods: All typing methods were from previously published standardized protocols.

Results: Between 2001 and 2003, 47 HA-MRSA, 46 MR-MRSA and 34 CA-MRSA strains were obtained from the University Hospital Birmingham, NHS, UK. All 44 HA-MRMRSA and 2 CA-MRMRSA were SCCmec II. All 34 CA-MRSA were designated SCCmec IV. All of the community strains tested were SCCmec IV except in the case of multi-resistant phenotypes where isolates were SCCmec II. Of the 47 HA-MRSA, 43 were SCCmec IV and type I, Ia, III and IIIa were represented once by individual strains. Multiplex SCCmec typing was found to complement RAPD and PFGE groupings on the same strains by dendrogramatic representation.

Conclusion: Multiplex SCCmec typing identified all MR-MRSA as being SCCmec II and all CA-MRSA as being SCCmec IV. Multiplex SCCmec typing had a discrimination index (DI = 0.50) comparable to RAPD (DI = 0.55) which was less discriminatory to PFGE (DI = 0.88). Both HA-MRSA and CA-MRSA isolates can carry SCCmec IV indicating that it is not exclusive to community strains and SCCmec IV can disseminate within the hospital environment.

P533

Fast, accurate, and automated workflow for multi locus sequence typing of *Staphylococcus aureus*

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Objectives: Current Multi Locus Sequence Typing (MLST) methods do not offer an automated, fast, and accurate workflow for determining allelic profiles and sequence types of bacteria and other microorganisms. The current manual workflow can take a researcher about 4–5 hours of analysis time alone to determine the allelic profile for one bacterial sample. With our new workflow, we will show an example of *S. aureus* typing that significantly reduces this analysis time.
Methods: The following seven housekeeping genes were used in the MLST experiments: carbamate kinase (arcC), shikimate dehydrogenase (aroE), glycerol kinase (glpF), guanylate kinase (gmk), phosphate acetyltransferase (pta), triosephosphate isomerase (tpi), and acetyl coenzyme A acetyltransferase (yqiL) as specified by the MLST web site (www.mlst.net). The genes were amplified using two sets of primers. One set of primers were derived from the MLST website, while the second set are the same MLST primers tailed with −21 M13 Forward or M13 Reverse primers. The samples were sequenced using the Applied Biosystems 3130 Series Genetic Analyzers, BigDye® Terminator v1.1 Cycle Sequencing kit and automated data analysis and library matching with SeqScape® Software v2.5.
Results: For our experiments, we accurately determined the sequence types for eight unknown samples provided by M. Langvik. These samples were used in epidemiological studies. The use of M13-tailed MLST Primers, instead of the MLST primers for the sequencing reaction, gave more uniformed results and significantly streamlined the sequencing workflow. Furthermore, automated analysis between the 3130 Data Collection v3.0 with SeqScape® Software v2.5 significantly reduced the time to analyse and type the alleles for each sample of several *S. aureus* strains. With minimal sequence checking, the sequence type of each bacterial sample was obtained in 5 minutes or less.
Conclusion: The sequence types of eight unknown *S. aureus* samples were correctly determined using SeqScape Software. The amount of time to analyse the samples was fast and accurate, eliminating laborious manual sequence checking, trimming, alignment and allelic typing. This workflow will help enable researchers to accurately determine sequence types quickly for all pathogens, bacteria, and other organisms.

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Use of the isoschizomers *Sma*I and *Xma*I to generate a common PFGE pattern database for both *mef*-negative and *mef*-positive *S. pyogenes* isolates

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Objectives: PFGE is the choice method for *S. pyogenes* subtyping, following M-serotyping or its sequence-based successor, emm-typing. However, it has been observed that the routinely used restriction endonuclease (RE), *Sma*I, does not digest erythromycin-resistant strains of the M phenotype, harbouring a *mef* gene. Assuming that this could be due to inhibition of *Sma*I activity by DNA CpG methylation in its recognition site, we investigated the possibility of using a methylation-insensitive RE with the same recognition site. Our aim was double: to obtain PFGE patterns from *mef*-positive (*mef*+) isolates also, and to include all *S. pyogenes* PFGE patterns in a common database for molecular epidemiology.
Methods: Five *mef*+ isolates, belonging to emm-types 4, 12, and 75, and eight *mef*-negative (*mef*-) isolates, belonging to emm-types 1, 6, 12, 85 and 95 were typed by PFGE after *Sma*I (Fermentas) or *Xma*I (New England Biolabs) digestion of total DNA. Both isoschizomers recognise the sequence 5′-CCCGGG-3′: *Sma*I cuts after the third cytosine, which is a methylation target, while *Xma*I cuts after the first, which is not.
Results: Although *Sma*I digested only DNA from *mef*-isolates, *Xma*I digested all isolates' DNA. *Xma*I and *Sma*I patterns of *mef*– isolates were indistinguishable. Whilst, in general, isolates' profiles clustered according to serotype, the *mef*+ emm12 isolate did not cluster in the *mef*– emm12 group.
Conclusion: It has now been shown that a *mef*-encoding lysogenic phage also encodes a DNA CpG methylase (Euler C et al., XVIth Lancefield International Symposium on *Streptococci* and *Streptococcal* Diseases, abstract 14). To achieve PFGE typing of all *S. pyogenes* isolates and incorporation of all profiles in a single database for molecular surveillance, we therefore recommend digestion of *mef*+ isolates with *Xma*I, a more expensive isoschizomer of *Sma*I. *Sma*I should continue to be used for *mef*– isolates.
Acknowledgement: This work was conducted within the Strep-EURO EC project (contract number: QLK2-CT-2002-01398). In addition to the named authors, the Hellenic Strep-EURO Study Group includes: A. Avlamis, A. Bethimouti, M. Foustoukou, V. Gizaris, A. Halakatevaki, M. Iordanidou, G. Kouppari, S. Leveidiotou, E. Malamou-Ladas, E. Papafragas, V. Petroheilou.

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Genotypes of *Chlamydophila psittaci* causing zoonotic infection

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Objectives: Psittacosis is a disease caused by infection with *Chlamydophila psittaci*, an obligate intracellular bacterium. It is a zoonotic infection since birds are the main reservoir of *C. psittaci*. *C. psittaci* is divided in 8 serovars (A-F, M56, WC) and at least 9 genotypes. All genotypes are more or less associated with specific bird groups from which they are predominantly isolated. We genotyped all *C. psittaci* PCR positive human clinical samples available in our laboratory by ompA sequencing as the distribution of the genotypes of *C. psittaci* causing zoonotic infection is unknown.
Methods: The genotype of *C. psittaci* in ten human clinical samples was determined by ompA sequencing. The gene was amplified with primers located in the conserved regions of the ompA enclosing the four variable domains. After amplification, the PCR products were analyzed by agarose gel electrophoresis and the expected fragment and negative control samples were extracted from the gel and re-amplified for 20 cycles. Subsequently, overlapping sequences were obtained with six sequencing primers. The resulting sequences were aligned and a similarity index based on the translated 984 bp fragment was calculated. Similarity (1– distance) was calculated using the pairwise distance method generated by MEGA3. Reference

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ompA genotypes A–F and the *C. psittaci* 6BC type strain available in the GenBank database were included in this analysis.
Results: Five isolates were identical to the reference genotype A, three isolates were identical to genotype B and one isolate was identical to genotype C. We discovered one new genotype that was 99.4% similar to the genotype A reference, but even more similar to the *C. psittaci* 6BC type strain (99.7%).
Conclusions: In this study ompA sequencing of *C. psittaci* identified genotypes A, B, C and one new ompA genotype in human psittacosis cases. The new discovered *C. psittaci* ompA variant was most related to the *C. psittaci* 6BC type strain. Genotype A is mainly found in psittacine birds and is the most prevalent genotype (5 out of 10) in our clinical samples. Four isolates were genotype B and C. These genotypes B and C are predominantly isolated from European non-psittacine birds, among which pigeons (B) and ducks (C). The high prevalence of these genotypes (4/10) in our human clinical samples indicates that non-psittacine birds, in particular pigeons and ducks, should be considered a substantial part of the zoonotic reservoir for human psittacosis cases.

P536

A comparative study of *Escherichia coli* strains isolated from the intestinal mucosa of Crohn's disease patients and healthy subjects

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Objectives: To analyse and compare the *E. coli* strains present in CD patients with those from healthy controls.To identify and characterise the pathogenic strains.
Methods: We have used fresh biopsies which have been subjected to a mild sonication in order to discard the transient and loosely attached bacteria followed by a mild osmotic shock to release any intracellular bacteria from those outer epithelium cells. The isolation procedure consisted of the incubation of samples in TBX agar medium at 44.5°C o/n. Colonies were kept for a first confirmation screening of *E. coli* by the indole assay. Typing of isolates was performed by REP-PCR, using a primer set targeting the IS3 as described previously [1]. Clonality was further checked by PFGE.
Results: A total of 1600 presumptive *E. coli* from 8 CD patients, 10 healthy controls and 2 patients suffering from Ulcerative Colitis, were isolated. Several subtypes of *E. coli* were found in all specimens. Each screened person carried a unique set of *E. coli* subtypes that was different from the others. Some *E. coli* isolated from the transient microbiota were different from the attached counterparts. In addition, after application of osmotic shock, additional types were found in most of samples. Although the study of more samples is underway, preliminary results suggest that CD patients harbour a higher subtype numbers than healthy subjects.
Conclusion: The number and diversity of *E coli* attached to the intestinal mucosa of CD patients is congruent with some recent hypothesis on the implication of this bacterium in the pathogenesis of Crohn's disease [2–5].
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Molecular typing of *Shigella sonnei* isolates by RAPD-PCR and ribotyping

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Objective: Epidemiologic feature of shigellosis have not been well understood and the sensitive molecular typing methods have not been applied on the Iranian population for this organism. The aim of this study was to determine the drug resistance pattern of *S. sonnei* isolates as well as to characterize their genetic relationships by Random Amplified Polymorphic DNA (RAPD-PCR) and ribotyping.
Methods: This study was conducted on 1350 patients with acute diarrhoea visited in Tehran hospitals from Oct 2003 to Feb 2005. Eighty-six *S. sonnei* were isolated from 157 sporadic cases of shigellosis. Slide agglutination test was used to serotype the isolates by standard antisera (MAST Group Ltd). Isolates were screened for their susceptibilities to 14 different antibiotics by Kirby-Bauer method. All isolates were subtyped by ribotyping according to standard method (Coimbra et al. 2001). Isolates were subjected to RAPD-PCR using 1283, 1254-DAF, 1290 and 1247 AT primers. (Yumi Bando et al.1998).
Results: According to our results the percentages of multidrug resistance isolates were higher in comparison to previous report from Iran. The resistance rates to tetracycline, trimetoprim-sulphametaxazol and ampicilin were 100%, 97.7% and 90.7% were respectively. The figures of previous study for tetracycline and trimetoprim-sulphametaxazol were 73%, 70.4% in respect. All isolates were susceptible to Ciprofloxacin and Nalidixic acid. The endonuclease MulI was the best enzyme in differentiating the isolates in ribotyping and produced DNA patterns with high resolution. Isolates were grouped in 4 different patterns by ribotyping. RAPD analysis has the highest discriminatory power for typing of *S. sonnei* with 1283 primer. Analysis of isolates with this primer yielded 5 different patterns. Isolates within each genetic pattern belonged to specific drug resistant patterns.
Conclusion: RAPD analysis showed a higher discriminatory power for typing of *S. sonnei* isolates than ribotyping. It can be a very suitable tool in clinical practice and could be used as a complement to traditional methods. The ribotyping technique was a useful and reproducible method in subtyping of *S. sonnei* for epidemiological and phylogenetical studies of Shigellosis. Our results revealed that multi-resistant strains of *S. sonnei* are highly prevalent in Iran, and emphasize the importance of maintaining surveillance of these strains in order to asses local susceptibility patterns and empiric therapy.

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Evaluation of gyrB RFLP analysis for the typing of clinical and environmental strains of *Stenotrophomonas maltophilia*

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Objectives: *Stenotrophomonas maltophilia* is an opportunistic pathogen that is ubiquitous in nature and genetically diverse. Restriction fragment length polymorphism (RFLP) analysis of the polymerase chain reaction-amplified *gyr*B gene has previously been shown to identify broad genomic groups within this species. The aims of our study were to evaluate this method by using it to type a collection of clinical and environmental *S. maltophilia* isolates, and to determine whether particular *gyr*B RFLP-types dominate in the hospital setting or are more likely to cause infection.

Methods: We used *gyr*B RFLP analysis to type 102 clinical *S. maltophilia* isolates from two Western Australian hospitals, and 50 environmental *S. maltophilia* isolates from hospital wards and unrelated sites (non-hospital).
Results: All 152 isolates were typable by this method. Fifteen genomic groups were identified at a 100% similarity level, with 33.6% of isolates identified as a single *gyr*B RFLP-type. The second most common type included 23.7% of the isolates tested and the *S. maltophilia* type strain (ATCC 13637). Environmental isolates from non-hospital sites were more diverse than isolates from hospital sites which may indicate selection of certain *gyr*B RFLP-types in a hospital setting. Two *gyr*B RFLP-types were found exclusively in hospital environmental samples and clinical specimens; these types may be involved in nosocomial infections. The second most common *gyr*B RFLP-type from clinical isolates was also common in environmental samples from the intensive care unit but not in other environmental samples. Additionally, four *gyr*B RFLP-types were found only in clinical specimens. These five *gyr*B RFLP-types may be more likely to cause infection than the three types that were found only in environmental samples.
Conclusions: As a typing system for *S. maltophilia*, *gyr*B RFLP analysis showed excellent typability and reproducibility, and was rapid, easy to perform, and easy to interpret. Relatively low discriminatory power means broad genomic groups are identified and the characteristics of these groups can be investigated. In this study, *gyr*B RFLP analysis revealed distinct differences in *gyr*B RFLP-types present in hospital and non-hospital settings, and clinical specimens.

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Molecular analysis of *Mycobacterium kansasii* isolates from Taiwan

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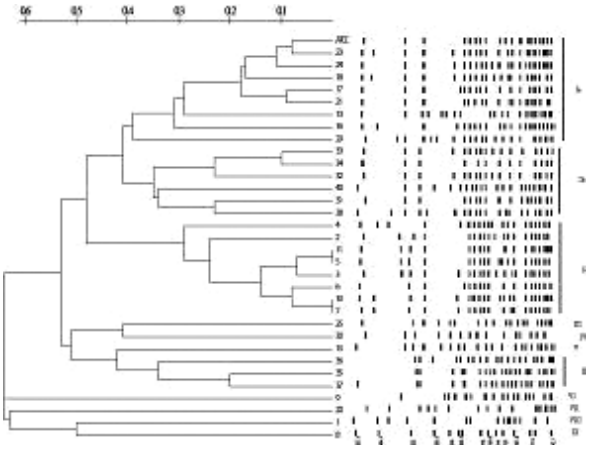
Objectives: *Mycobacterium kansasii* is an opportunistic pathogen of human disease. The aim of this study is to analyse clinical isolates of *M. kansasii* isolates from Taiwan by pulse-field gel electrophoresis (PFGE) with restriction enzyme *Ase*I and compare the results to those from Europe, Japan, and the United States.
Methods: From August 1999 through January 2003, a total of 40 clinical isolates of *M. kansasii* from 40 patients were collected from the Clinical Microbiology Laboratory of the Chang Gung Memorial Hospital, a tertiary referral medical centre in Taoyuan, Taiwan. Control strain was ATCC 12478 obtained from the American Type Culture Collection (ATCC). Clinical isolates were analysed by PCR restriction enzyme analysis (PRA) of the 441-bp Telenti fragment of the *hsp*-65 gene and PFGE of genomic DNA with restriction endonuclease *Ase*I. Isolates were considered clonal if they exhibited six or less band differences as defined by Tenover et al. for outbreak strains. The Gene Profiler program (Scanalytics, Inc., VA, USA) calculated the similarity index by setting the fragment length error tolerance at 2% and 0.4 DNA difference.
Results: With *Ase*I, only 32 clinical isolates were typable and separated to 9 clusters of genotypes. And 22 of 32 isolates (68.8%) generated one major cluster, including 3 similar genotype patterns (Figure). They were genetically related according to the definition described by Tenover et al. (1995). This major clone was indistinguishable from the major pattern seen in the studies of Europe, Japan, and the United States.



Conclusions: This study demonstrated one major clone of *M. kansasii* present in Taiwan, which has the indistinguishable pattern. Compared with the studies of Europe, Japan, and the United States, a major genotype is spreading worldwide and it should be potentially pathogenic.

Bacterial pathogenesis – I

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Resistance to colistin and carbapenems with detection of class 1 integrons on *Pseudomonas aeruginosa* clinical isolates

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Objective: Metallo-beta-lactamases (MBLs) are emerging resistance determinants in Gram-negative nosocomial pathogens, including *Pseudomonas aeruginosa*. It is known, that colimycin (colistin), the old polymyxin derivative is active in vitro against the pan-resistant *P. aeruginosa* strains. In this work, we present an outbreak due to colistin and carbapenem resistant *P. aeruginosa* in an Intensive Care Unit (ICU) of a Greek tertiary hospital.
Methods: Six multi-resistant *P. aeruginosa* isolates were recovered from four patients over a two-week period between September and October 2005, in our ICU. The source of the specimens was: 1 from blood culture, 2 from bronchial secretions, 1 from pleural fluid, 1 from pus and 1 from IV catheter. Commercial ID panels identified the strains and susceptibility to a broad panel of antimicrobial agents was determined by broth microdilution method according to CLSI guidelines. E-test also determined resistance to colistin and carbapenems. Isolates were investigated for the presence of the blaVIM-1 allele by PCR. Class 1 integrons were detected and molecularly characterized by sequencing.
Results: All isolates were found resistant to the following antimicrobial agents tested: aminoglycosides amikacin, gentamicin, tobramycin, netilmicin-, piperacillin, piperacillin/

tazobactam, ticarcillin, ticarcillin/clavoulanate, carbapenems imipenem, meropenem, quinolones ciprofloxacin, ofloxacin, pefloxacin, moxifloxacin-, trimethoprim/sulfa and colistin. Three isolates were found susceptible to aztreonam with MIC of aztreonam 2 mcg/ml. The blaVIM-1 gene was detected in all strains. The same class 1 integron was found in all strains. Its sequence analysis revealed the presence of other antibiotic resistance genes, apart from the blaVIM-1 allele.
Conclusion: This is a rare report of a nosocomial outbreak caused by colistin resistant *P. aeruginosa* producing MBL. Resistance to colistin was correlated with the increased use of colistin over the last year in ICU, due to prevalence of MBL mediated carbapenem resistance in *P. aeruginosa* in ICU patients. All isolates harboured class 1 integrons, which means a rapid spread of the resistant genes in nosocomial environment. Control measures (hand hygiene and restriction of colistin use) resulted to disappear the above colistin resistant *P. aeruginosa* isolates.

P541

Molecular epidemiology of *Pseudomonas aeruginosa* strains isolated from intensive care unit

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Introduction: *Pseudomonas aeruginosa* is ubiquitous bacteria prevalent in the nature. This microorganism is opportunistic pathogen. *P. aeruginosa* has often been reported as the cause of outbreaks of nosocomial infections in hospital units. *P. aeruginosa* strains exhibit high rates of resistance to antibiotics and are frequently multidrug-resistant.
Objective: The aim of this paper was the epidemiological investigation of the group of twenty *P. aeruginosa* strains isolated from patients treated in Intensive Care Unit (ICU).
Materials and methods: Twenty *P. aeruginosa* strains were isolated from respiratory track, wounds and urine. Eleven of them derived from three patients: six came from patient No. 1, two from No. 2 and three from No. 3. They were isolated in different time during hospitalisation. The isolates were identified in the ATB system (bioMerieux) using ID 32 GN strips. The antibiotic susceptibility was performed using disk-diffusion method. RAPD-PCR analysis of investigated strains were carried out with primers ERIC-2 and PAL-2.
Results: Examination of RAPD fingerprints using ERIC-2 primer in the group of six strains isolated from patient No. 1 revealed no differences in genotypes, despite the fact that they presented different phenotypes of antibiotic susceptibility. Whereas primer PAL-2 revealed two clusters of genotypes. RAPD analysis of two strains obtained from patient No. 2 using two primers enabled to characterize strains of nondistinguishable genotype. Genotyping of strains of patient No. 3 using ERIC-2 primer showed no differences between isolates, whereas PAL-2 primer confirmed the occurrence of two clusters of genotypes. In the group of nine *P. aeruginosa* strains isolated from different patients analysis of ERIC-2 fingerprints displayed seven clusters of genotype. One of them included three isolates from different patients. PAL-2 showed six unique patterns. One of them included two strains, second genotype included three *P. aeruginosa* strains. All isolates were acquired from different patients. Strains number 19 and 20 isolated from remaining patients were nondistinguishable using both primers.
Conclusions: Current study indicated a high degree of genetic diversity among *P. aeruginosa* strains isolated from patients of ICU. Our investigations confirmed among patients subjected to longer treatment during stay in ICU lack of changes in genotypes in comparison with phenotypes connected with their antibiotic susceptibility.

P542

Increase in incidence of serogroup C invasive meningococcal disease in Italy and rise of decreased susceptibility to penicillin

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Objectives: To monitor the rapid increase in proportion of serogroup C strains circulating in Italy and the rise of decreased susceptibility to penicillin (0.06 > MIC < 1 mcg/ml) to see whether they are linked to the circulation of specific phenotypes.
Methods: The strains were serotyped with specific antisera. The susceptibility to penicillin was performed by the E-test. A Real-Time PCR protocol was also applied to discriminate between penicillin susceptible and intermediate strains. Sequence analysis of the penA gene was performed to monitor mosaicism. MLST following the methodology described by Maiden et al (http://neisseria.org/nm/typing/mlst/), was performed to assess the lineages and complexes to which these isolates belong.
Results: Starting in the year 2002, an increase in proportion of serogroup C isolates was observed and in 2004 and first six months of 2005 they accounted for roughly 57% of all *meningococci* circulating in Italy compared to an average 23% during the 1990s. Before the year 2001 the main serogroup C phenotype had been C:2a:P1.5 (ST11/ET37). In 2002, C:2b:P1.5 (ST8/A4, ST1860/ET37), became the prevalent phenotype accounting for 40% of all group C strains and rising to almost 50% the following year. In the year 2004 a new shift occurred and C:2b:P1.5,2 (ST8/A4) became the most frequent (50%). Before 2002 an average 3% of group C isolates had shown intermediate susceptibility to penicillin but this proportion rose to 43% in 2002, 51% in 2003 83% in 2004 and 87% in the first 6 months of 2005. The same exogeneous DNA fragments in the penA sequences were detected in all C:2b:P1.5,2 *meningococci.*
Conclusions: Besides the increase in proportion of serogroup C isolates the last few years have also featured changes, with unexpected rapidity, in the major group C phenotype accompanied by a noteworthy rise in decreased susceptibility to penicillin.

P543

Detection and identification of bacterial DNA in cardiac valves from infective endocarditis cases

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Objectives: The study investigates the usefulness of culture-independent PCR and subsequent DNA sequencing for establishing the aetiology of infective endocarditis (IE) in surgically removed valves.
Methods: Cardiac valves from patients undergoing valvular replacement due to clinical necessity were examined by culture and by PCR/DNA sequencing. Valves from ten patients without suspicion of IE (controls) and from13 patients with IE (patients) were included. The valves were divided and one part was cultured using standard methods, and the other was subjected to a PCR targeting part of the bacterial 16S rRNA gene. The DNA sequence was determined on any product resulting from

PCR and bacterial identity was established using BLAST search in the NCBI database.

Results: Results from culture and PCR /DNA sequencing are shown in Table 1. Bacterial DNA was identified in valves from nine of the 13 patients. The species identified represented typical endocarditis bacteria in eight of these patients. Six of the DNA based identifications were in accordance with previous blood isolates from the same patients. DNA from *Propionibacterium* acnes was detected in two of the ten controls, and may represent contamination.

Table 1

Control (C) or patient (P)	Valve	Blood culture	Tissue culture	PCR and DNA sequencing
C 1	Mitral	Neg*	Neg	Neg
C 2	Mitral	Neg	Neg	Neg
C 3	Aortic	nd**	Neg	Neg
C 4	Mitral + Aortic	nd	Neg	Neg
C 5	Aortic	nd	Neg	*Propionibacterium acnes*
C 6	Aortic	nd	Neg	Neg
C 7	Mitral	nd	Neg	Neg
C 8	Aortic	nd	Neg	*Propionibacterium acnes*
C 9	Aortic	nd	Neg	Neg
C 10	Mitral	nd	Neg	Neg
P 1	Aortic	*Enterococcus faecalis*	Neg	*Enterococcus faecalis*
P 2	Aortic	*Staphylococcus aureus*	Neg	*Staphylococcus aureus*
P 3	Mitral	nd	Neg	*Streptococcus sanguinis/Streptococcus mitis****
P 4	Aortic	*Streptococcus gordonii*	Neg	Neg
P 5	Mitral	*S. aureus*	Neg	Clostridium species
P 6	Aortic	*Enterococcus faecalis*	Neg	*Enterococcus faecalis*
P 7	Mitral	*Streptococcus gordonii*	Neg	*Streptococcus gordonii*
P 8	Aortic	*Streptococcus gordonii*	Neg	*Streptococcus gordonii / Streptococcus mitis****
P 9	Aortic	Neg	Neg	Neg
P 10	Mitral	*Streptococcus oralis*	Neg	*Streptococcus oralis / Streptococcus mitis****
P 11	Aortic	*Actinobacillus actinomycetemcomitans*	*Actinobacillus actinomycetemcomitans*	*Actinobacillus actinomycetemcomitans*
P 12	Mitral	*P. acnes* / Neg when repeated	Neg	Neg
P 13	Mitral	*Streptococcus mitis*	Neg	Neg

*Neg = Negative, **nd = not done, ***Species differentiation not possible

Conclusion: Detection and identification of bacterial DNA seems a promising method for establishment of the aetiology of IE, but further studies are required for final validation.

P544

Spectrum of bacterial pathogens causing IE in our hospital

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Objective: The amplification of DNA from bacterial and fungal pathogens is being increasingly used in the microbiological diagnosis of infective endocarditis (IE). Molecular techniques, mostly based on PCR amplification, have been demonstrated useful on achieving a precise and rapid identification of the infectious aetiology of endocarditis episodes if applied on heart valves tissue samples. The aim of our study was to determine the most common bacterial pathogens causing IE endocarditis in our hospital.

Methods: DNA was isolated from homogenised heart valve tissue samples of fifty-nine IE patients suspected of IE (collected in years 2003–2005) undergoing valve replacement surgery. A broad-range 16S rRNA PCR technique followed by sequencing and sequence similarity analysis was used.

Results: PCR results demonstrated the presence of bacterial DNA in the heart valves obtained from 42 patients suspected of IE. The largest portion of IE cases is represented by two bacterial groups *Streptococci* (16 cases) and *Staphylococci* (16 cases). The most frequent pathogen was identified as *Staphylococcus aureus* (13 cases). Rest of cases was caused by less common bacterial pathogenes like *Corynebacterium diptheriae*, *Abiotrophia elegans*, Actinobacillus actinomycetemcomitans or *Bartonella* sp. The causative microorganism for one patient with definite culture negative endocarditis was identified as a rarely reported *Aerococcus urinae*.

bacterial pathogen	cases
Abiotrophia elegans	1
Actinobacillus actinomycetemcomitans	2
Aerococcus urinae	1
Bartonella sp.	1
Corynebacterium diptheriae	1
Enterococcus faecalis	1
Gemella sp.	1
Propionibacterium acnes	1
Pseudomonas aeruginosa	1
Staphylococcus sp.	
S. aureus	13
S. epidermidis	3
Streptococcus sp.	
α-hemolyticic group: *S. suis*	1
β-hemolytic group: *S. pyogenes, S. agalactiae*	3
viridans group: *S. anginosus, S. mitis*	8
D group: *S. bovis, S. macedonicus*	4

Tab.1 Bacterial spectrum in cases of IE

Conclusion: PCR-based molecular detection of pathogens in valve samples from surgically treated IE patients is fast, sensitive and reliable. The technology along with thorough validation and clinical interpretation is a promising tool for routine testing of IE.

P545

Molecular characterisation and prevalence of tick-borne diseases pathogens in Lithuania

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Background: The tick *Ixodes ricinus* is involved in the transmission and maintenance of a wide variety of pathogens of species *Borrelia*, *Ehrlichia/Anaplasma* and *Babesia*. The infection is initiated by inoculation of the bacterium into the skin during a tick bite. Lyme borreliosis is the prominent human infectious disease; *Ehrlichia/Anaplasma* and *Babesia* are also regarded as human pathogens.

Objectives: The purpose of the present study was to determine the prevalence of *Borrelia*, *Ehrlichia* and *Babesia* in *I. ricinus* ticks by molecular genetics methods.

Methods: More than 2000 ticks were collected in West, North, East and South regions of Lithuania (WL, NL, EL, SL). For detection of *B. burgdorferi* s.l. in infected ticks, the 1408 adults and 151 nymphs were analysed individually by the PCR with fla gene specific primers. For *Borrelia* genotyping were used multiplex PCRs with genospecies-specific primers for *B. burgdorferi* s.s., *B. garinii* and *B. afzelii*. The presence Ehrlichia/Anaplasma group was determined by using PCR with specific primers. The positive samples were reamplified and the biotinylated Ehrlichia/Anaplasma PCR products were hybridised with different oligonucleotide probes in the reverse line blot assay. The Babesia divergens was detected by RT-PCR with the ABI Prism system.

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Results: *B. burgdorferi* s.l. was detected in all Lithuanian regions: WL – 7% (8/113), NL – 19% (98/525), SL – 15% (41/278) and EL – 10% (62/643) and common in country – 13% (209/1559). In the NL ticks are infected distinctly more. It could be explained by the zone of sympatry of few Ixodes species and biodiversity of vectors could cause intensity of infection. Of the 243 individually processed ticks, 5 (2%) were positive for Babesia divergens, 12 (5%) were positive for Ehrlichia/Anaplasma (HGE – 3, HGE variant – 1, *E. schottii* – 2 and 6 were not identified), 38 (16%) for Borrelia genotypes: an absolute domination of *B. afzelii* – 25 (66%) is observed; *B. garinii* – 12 (32%); *B. burgdorferi* s.s – 1 (3%). It is accordance with data from Baltic region of Russia (Kurish Spit) and Norway.
Conclusions: The known pathogenic species (Borrelia, Ehrlichia/Anaplasma and Babesia) found in Europe are also present in the Lithuanian host-seeking tick population. It was detected that *B. afzelii* was the dominant genospecies in Lithuanian ticks (10%) and Ehrlichia/Anaplasma and Babesia were found in ticks too and might cause human diseases.
Acknowledgement: The study was supported by the Lithuanian Science and Study Foundation.

P546

Tick-borne Rickettsiae in Russia

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Objectives: Three spotted fever group (SFG) rickettsioses, transmitted by hard tick bites are known in Russia. These include Siberian tick typhus (STT) caused by *Rickettsia sibirica* sensu stricto (*R. sibirica* sensu stricto) in the Asian part of the country, Astrakhan spotted fever (ASF) caused by *R. conorii* subspecies caspia in Astrakhan, and far eastern rickettsiosis, caused by *R. heinlinjangensis* in Russian Far East. Recently, additional tick-borne rickettsiae were detected from ticks in Russia. Herein, we collected ticks in five regions of Russia, from the European part of the country to Far East, and detected and identified using PCR and sequencing, SFG rickettsiae.
Methods: We attempted amplification and sequencing of a 590-bp fragment from the *omp*A gene as well as the complete *glt*A gene from all ticks. The 5'-end of ompA was amplified using the 190-70 and 190–701 primers, whereas amplification of the *glt*A gene was performed using the two primer pairs CS1d-CS535r and CS409d-RP1258n. PCR products were sequenced using an ABI Prism 3100 automated Sequencer (Applied Biosystems, Foster City, CA, USA). All sequences were performed twice in both directions. Sequences were identified using the BLASTn software by comparison with sequences available in GenBank.
Results: We detected *R. sibirica* sensu stricto in six tick species collected in Eastern Siberia, Zauralye, and Russian Far East. *R. heilongjangensis* was identified in ticks from Siberia and Far East. We also detected three new species known to be pathogenic in other countries, i.e., *R. slovaca* in *D. marginatus* ticks in the European part of Russia and Zauralye, *R. helvetica* in Ixodes persulcatus ticks collected in Western Siberia, and *R. aeschlimannii* strain Stavropol in Hyalomma marginatum in the Stavropol region. In addition, rickettsiae of unknown pathogenicity were detected, including *R. sibirica* strain BJ-90 in *D. silvarum* collected in Far East, *Rickettsia* sp. strains RpA4, DnS14 and DnS28, and Candidatus "*Rickettsia tarasevichiae*".
Conclusions: A minimum of 11 rickettsiae are distributed in hard ticks in Russia. These include 5 human pathogens in addition to the ''classical'' *R. sibirica* sensu stricto. Therefore, clinicians should be aware that spotted fever rickettsioses in Russia may be caused by several species with different severities.

P547

Microbiological characterisation of invasive group A streptococci in the UK during 2003–2004

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Objectives: As part of the strep-EURO project (QLK2.CT. 2002.01398), an enhanced surveillance of severe group A streptococcal (GAS) infections was undertaken in the UK during 2003–2004, to obtain disease burden estimates and to characterise strains isolated. Microbiological characterisation of invasive GAS isolates collected are described alongside clinical and risk factor data to identify significant associations.
Methods: Invasive GAS isolates from laboratories in England, Wales and Northern Ireland were sent to the Streptococcus & Diphtheria Reference Unit for further characterisation. Cases were defined by the isolation of GAS from a normally sterile site and/or any clinical information indicative of a severe infection. Microbial data available include *emm*/M type, *spe*A, B and C genes and antibiotic resistance profiles. Analyses were performed using STATA software to test for any significant associations between laboratory and clinical data using chi-square and logistic regression analyses.
Results: A total of 3639 invasive GAS cases were reported during 2003–2004 and 2483 (68%) isolates were available for characterisation. In total, 74 different M/emm types were identified, with M1, 3, 87 and 89 comprising 48% of all invasive GAS isolates. Spe toxin results were available for 282 isolates; 112/282 (40%) were *spe*AB positive; 99 (35%) possessed speBC; 55 (20%) speB; 15 (5%) speABC positive. There was a significant association between *spe* gene and M type ($P = 0.001$), notably with *spe*AB and M1 and 3, and speBC with M12, 28 and 87. Susceptibility results against 4 antibiotics were available; none were resistant to penicillin; 8 (0.8%) were clindamycin resistant; 56 (3.6%) erythromycin resistant; 181 (14.0%) tetracycline resistant. The tetracycline resistant isolates correlated with M43 and 83 ($P = 0.001$). Toxic shock syndrome and necrotising fasciitis were significantly associated with M1 and 3 isolates, and those containing *spe*A ($P < 0.01$). Intravenous drug users comprised 363/2184 (17%) cases, with M83, 82 and 43 and tetracycline resistance all strongly linked with this major risk group ($P = 0.001$).
Conclusions: The UK is again observing changes in GAS type distributions with the emergence of higher M types, especially M87 and M89. Characterisation of these isolates using techniques such as *spe* gene toxin detection and antimicrobial susceptibilities has further enriched our capacity to understand the relationship between clinical manifestations and microbiological characteristics.

P548

Detection of *pca*A gene in *M. tuberculosis* strains isolated from clinical specimens

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Background: The aim of this study was to investigate the prevalence of *pca*A gene in *M. tuberculosis* strains isolated and typed by spoligotyping. The associated risk factors among patients with different nationalities residing in Iran were also determined.

Methods: The study population involved a total of 439 patients that referred to the NRITLD, the referral tuberculosis centre in Iran; during March 21st, 2003 to March 21st 2004. The isolated *Mycobacterium tuberculosis* strains have been characterized by performing susceptibility tests against four first-line antituberculosis drugs and were then subjected to spoligotyping characterization. PCR was used for detection of *pca*A gene and its nucleotide sequence was also determined.
Results: Spoligotyping of *M. tuberculosis* strains resulted in 140 different patterns that divided into three evolutionary groups (É, ÉÉ, ÉÉÉ). One hundred twenty-two (87.1%) of these spoligotype isolates were unique and reported for the first time. The remaining18 (12.8%) spoligotype patterns were previously reported from other geographical regions of the world. Interestingly, 6.3% of the strains belonged to the Beijing family. The MDR (multi drug resistance), double and triple resistance were seen in group É of evolutionary scenario. The *pca*A gene was detected in *M. tuberculosis* clinical isolates but not in saprophyte strains such as *M. kansasi*.
Conclusion: The results showed that multi drug-resistances were more prevalent in bacteria isolated from Afghani TB patients residing in Iran. In addition, spread of *M. tuberculosis* strains belonging to the Beijing family among Iranian patients has to be considered seriously. This study confirmed the widespread existence of *pca*A gene in almost all the clinical isolates. It is also important to undertake studies to identify which factors are the most significant to consider in tuberculosis control program.

P549

Isolation and identification of *Legionella* (legionnaires' disease agents) from fish ponds and environmental water sources by culture and PCR methods

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Objectives: *Legionella* are the causative agents of pneumonia in human and it is reported that up to 90% cases of legionnaires' disease are due to *Legionella pneumophila*. These organisms are ubiquitous distributed in natural and man made water sources. They are spread to human by inhalation of contaminated aerosols which are originated from these sources. We studied some of man-made water sources in view of presence of *Legionella*, by two methods of culture and PCR.
Methods: 150 water samples (each one 500 ml) collected from different sources, such as: Fish ponds, swimming pools and cooling towers were studied. These sources were located in Ahwaz city and some other cities of Khoozestan Province in Iran. After centrifugation of water samples, the pellets were treated by Hcl-Kcl buffer (pH 2.2) and resuspended pellet was inoculated into BCYE and BMPA (Oxoid) media. Isolated colonies were identified by morphological and biochemical tests. DNA was extracted from the bacteria in another portion of the same pellet and then it was used as template in PCR technique. DNA pattern of *Legionella* were identified after electerophoresis of DNA products.
Results: Survey of water samples from 117 fish ponds, 20 swimming pools and 13 cooling towers were resulted in 11 strains (7.3%) of *Legionella pneumophila* by culture, and identification of 23 strains (15.3%) of them by PCR. The highest rate of *Legionella pneumophila* isolation was 4.3% and 8.6% by culture and PCR respectively, from Bioz fish ponds (around Ahwaz city). Susceptibility and specificity of PCR in this survey were 100% and 92% respectively.
Conclusion: The results of this study showed that legionnaires' disease agents were widely spread in our examined water sources, so the treatment of these sources by chlorinization, heating or refreshing of water are necessary to eliminate of these agents.

P550

Three females with asymptomatic infection of *Mycoplasma pneumoniae*, carrying genes for MDS and lymphoma detected with FISH technique

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Objectives: It is established that chronic infections may cause hematological disturbances. The aim of this study is to show that persistent asymptomatic infection with Mycoplasma pneumonia can produce a clinical picture similar to chronic fatigue syndrome and even more can stimulate genes involved in hematological malignant disorders.
Patients and methods: This work reports the presence of a chronic fatigue syndrome in three female patients. Their age was 33,40 and 54 years old respectively. There whole blood check-up was normal except of a low value of IgG immunoglobulin; 698 mg/dl (normal range: 859–1515 mg/dl). Further more there was a 2-fold titre of Mycoplasma pneumonia-IgM type antibody in all, using ELISA technique. Bone marrow aspiration and trephine biopsy were not diagnostic of a special disease. CT of neck, thorax, and abdomen were normal with no signs of any disease. Peripheral blood lymphocyte cultures were prepared using standard techniques. We applied FISH technique on metaphase chromosomes using LSI EGR1 (5q31) Spectrum oarnge, LSI D7S486 (7q31) spectrum orange /CEP7 spectrum green probe, searching for a Myelodysplastic Syndrome (MDS), and LSI IGH/CCND1 Dual fusion translocation probe, LSI IGH/BCL2 Dual colour, Dual fusion translocation probe for Lymphomas (VYSIS).
Results: The karyotypes up to 30 cells using GTG banding technique were normal. FISH revealed that one pt had a deletion of 7q31 chromosome that is present in MDS and it is a bad prognosis factor, while the other two had cells carrying the translocation t (11; 14)(q13; q32), which is found in Non-Hodgkin's Lymphoma.
Conclusions: The three pts had serologically Mycoplasma pneumonia infection of IgM-type without any sign of active disease. Their fatigue syndrome existed for more than 3 years. The essential mechanism between the Mycoplasma pneumonia infection and low IgG is unclear since it is not included even in rare extra pulmonary complications. Studies with larger samples should be done for a better evaluation of its involvement in human malignant disease. The chronic asymptomatic infection probably acts as a stimulator to genes responsible for pre- or for malignant hematological diseases.

P551

Aseptic meningitis: aetiologic diagnosis

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Introduction: Although aseptic meningitis is in general a benign clinical situation, a rapid etiological diagnosis can have an important impact on patient care. With the implementation of polymerase chain reaction techniques (PCR) in cerebrospinal fluid (CSF) analysis, it is now possible to identify not only usual but also emerging pathogenic microorganisms.

Aim: To investigate the aetiology of aseptic meningitis.
Methods: Aseptic meningitis was defined when CSF cytosis exceeded 6 leukocytes/μl and a negative standard bacterial culture. Herpes simplex virus 1/2 (HSV), Epstein barr virus (EBV), Cytomegalovirus (CMV), West Nile virus (WNV), and Enterovirus (EV) were detected with commercial available PCR reagents and *Leptospira* spp. with an ''in house'' real time PCR test. An ''in house'' and commercial PCR test were used to detect Toscana virus (TV). Varicella zoster virus (VZV), mumps virus and *Coxiella burnetii* infections diagnosis were based on clinical signs and/or serology.
Results: During 3 years we studied 315 CSF samples. Two hundred and nineteen (70%) patients were younger than 15 years and 187 (59%) were males. CSF pleocytosis ranged from 6 to 2100 cells/μl. Bacteriological study was negative in all samples. Etiological diagnosis was achieved with molecular methods in 148 (47%) patients. PCR detected 110 (35%) EV, 20 (6.3%) HSV, 6 (1.9%) Leptospira, 4 (1.3%) EBV, 5 (1.6%) TV and 3 (0.9%) CMV infections. All samples tested negative for West Nile virus. Serology detected one EBV and one *Coxiella burnetii* infection and in eighteen patients a diagnosis of VZV (13) or mumps (5) infection was based on clinical grounds. Overall the diagnosis was clarified in 168 (53%).
Conclusions: The sensitivity, specificity and versatility of PCR make this methodology an ideal tool to investigate the aetiology of central nervous system infections. In this study EV is the more common aetiology of aseptic meningitis. The investigation of Toscana virus, although less frequent should go on in summer cases of aseptic meningitis in Portugal as in other Mediterranean regions. The vector of West Nile virus is present in our country but no cases of infection by this virus was detected. Other agents need to be considered in future studies to further clarify this clinical situation.

P552

Characterisation of the recombinant S-adenosylhomocysteine hydrolase from *Cryptosporidium parvum*

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Cryptosporidium parvum is a unicellular, obligatory intracellular, parasitic protist that infects mammalian gastrointestinal epithelium. It can produce a self-limited diarrhoea in healthy adults and children, but potentially life-threatening infection in immunocompromised persons for which no effective cure is known. S-adenosylhomocysteine hydrolase (SAHH), which catalyses hydrolysis of S-adenosylhomocysteine (SAH) to yield adenosine and homocysteine, regulates methionine cycle and synthesis of S-adenosylmethionine (SAM). SAM is a major donor of methyl groups for methylation reaction, and its decarboxylated form is a donor of aminopropyl group for polyamine synthesis. Polyamine metabolism of *C. parvum* differs substantially from its mammalian hosts. Drug development which would target both enzymes of the methionine cycle and polyamine synthesis can be effective against human cryptosporidiosis. *C. parvum* SAHH (CpSAHH) was cloned. CpSAHH is a single copy intronless gene of 1479 bp and encodes a protein of 493 amino acids that contains all conserved amino acid (aa) residues necessary for enzymatic activity. In contrast to mammalian hosts, CpSAHH contains plants-like 49 aa insertion. RT-PCR analysis proved that CpSAHH is expressed in both *C. parvum* sporozoites and intracellular stages. CpSAHH was cloned into the expression vector pMAL-c2X to yield pMAL-CpSAHH fusion. This maltose-binding protein (MBP) fusion was expressed in the competent *E. coli* TB1 strain and affinity purified over an amylose column. Recombinant protein of expected size of 98.3 kD was examined by SDS-PAGE. MBP-CpSAHH fusion was cleaved using 2.5% Factor Xa protease and CpSAHH was finally divided from MBP on hydroxyapatite column. The assay of CpSAHH activity in the hydrolytic direction was performed spectroscopically at 412 nm by measuring the rate of the product (homocysteine) formed by reaction with dinitrilo-dithiodibenzoic acid (DNTB). The reaction mixture contained 4 units of S-adenosyldeaminase, 200 μM DNTB and varying concentration of SAH (0.1–100 μM) in the 50 mM potassium phosphate buffer of pH 7.2 containing 1 mM EDTA. Michaelis-Menten kinetics estimated a Km of 2.49 (±0.53) μM for MBP-CpSAHH fusion protein and Km of 1.42 (±0.38) μM for the purified recombinant CpSAHH. The inhibition of recombinant CpSAHH by adenosine analogs (3-deazaadenosine, 7-deazaadenosine and 3-deoxyadenosine), specific SAHH inhibitors will be presented.

P553

VIM-1-producing *Klebsiella pneumoniae* strains with class 1 integrons isolated from bloodstream infections

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Objective: Metallo-beta-lactamases (MBLs) mediated resistance in *Klebsiella pneumoniae* has become an emerging problem. This resistance is often associated with low level carbapenem MICs and may be misidentified. This study was performed to investigate the prevalence of MBL in blood isolates of *K. pneumoniae*, collected at our tertiary care hospital in the year 2005.
Methods: All consecutive *K. pneumoniae* isolates from blood cultures of 61 inpatients (16 in medical wards, 7 in surgical wards, 30 in ICU and 8 in other departments) were tested. They were identified by standard methods and MICs were determined by the broth microdilution method, according to CLSI guidelines. MBL production was screened by E-test MBL. blaVIM-1 alleles were detected by PCR. The presence of class 1 integrons was verified by PCR with specific primers, designed on the basis of the 5 conserved segment (5-CS) and the 3-CS of class 1 integrons.
Results: Twenty-four of 61 isolates exhibited reduced susceptibility or resistance to carbapenems, imipenem (IMI) and meropenem (MER) with MICs ranged from 0.5 to ≥8 mcg/ml. In two isolates the MIC of IMI was 1 mcg/ml, in thirteen isolates was 2 mcg/ml, in five isolates was 4 mcg/ml and in four isolates was ≥8 mcg/ml. The MICs of MER were: 0.5 mcg/ml one isolate, 1 mcg/ml eleven isolates, 2 mcg/ml five isolates, 4 mcg/ml four isolates and ≥8 mcg/ml three isolates. They were shown to produce an MBL activity by the E-test. The same twenty-four isolates, 39.3%, were found positive for the presence of the blaVIM-1 gene. All of these isolates harboured class 1 integrons of different molecular weights, carrying a variety of genes that confer resistance to antibiotics.
Conclusion: The presence of the blaVIM-1 gene in 39.3% of our *K. pneumoniae* blood isolates is high. These results confirm that the spread of MBLs in *K. pneumoniae* is becoming a clinical concern, despite the fact that most isolates remained susceptible, according to CLSI breakpoints. Continuous surveillance and control measures are necessary in order to eliminate the MBLs.

P554

Molecular detection of the cefoxitin resistance gene, *cfx*A, and the 3′ regulatory sequence of its resistance element, MTn4555

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Objectives: Detection of the level of cefoxitin resistance, detection of the *cfx*A resistance gene and characterization the structure of the 3′ sequence of the resistance mobilizable tranposon, MTn4555, possibly involved in the regulation of *cfx*A.
Methods: 64 Bacteroides strains were examined for the level of cefoxitin resistance by the E-test method, and were screened for the *cfx*A and the upstream hypothetical insertion element (IS) transposase (TpI) genes by PCR. For a detailed analysis of the upstream regions of the *cfx*A genes, they were amplified by PCR using primers in conserved regions, and were subsequently sequenced.
Results: *cfx*A genes were found in 13 isolates (20%). All had cefoxitin MICs that fell in either the intermediate (=32 mg/l) or the resistant category (>32 mg/l). One additional strain was also resistant to cefoxitin but it displayed resistance to carbapenems too. Two IS element sequences (TpI) were found all in *cfx*A-positive strains and they were mapped to the upstream region of the *cfx*A genes; this structure corresponded to the reference sequence of the mTn4555 of B. vulgatus CLA341. In 8 strains, the regions upstream of the *cfx*A genes were devoid of the IS-like sequence of *B. vulgatus* CLA341 and an additional 360 bp region that had direct and inverted repeats at its ends. In 3 strains, the upstream regions could not be amplified or had a size of 500 bp or insertion of an IS614-like element.
Conclusion: We developed a method with which we could detect the cephamycin-specific resistance of Bacteroides strains, demonstrated the heterogeneity of the 3′ region of the resistance transposon, MTn4555, and described the prevalent IS-less structure of its 3′ region.

Emerging microbial infections

P555

Severe cutaneous anthrax and toxaemic shock

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Objective: With the September 11 attack, anthrax is a re-emerging disease. Majority of cases are cutaneous (95%) and a mild disease, in some cases cutaneous reaction may be more severe and threat patients' life.
Methods: The cases diagnosed as cutaneous anthrax in the last 2 years were evaluated and presented. The diagnose was carried out with an exposure, clinical presentation compatible with anthrax, gram stain, positive culture from lesion.
Results: Nine cases (6 male, 3 female, aged between 30–64 years) were evaluated. Of these, 3 cases were severe form of cutaneous anthrax, 1 severe form and toxemic shock, 1 toxemic shock and 4 mild form. Eight patients gave a history of an exposure to an animal dying. One patient was a raw leather worker. Incubation period was between 3–12 days. Clinical presentation of severe form cutaneous anthrax in 3 cases was characterized with fever, hemorrhagic bullae surrounded with an extensive erythema and edema and leukocytosis. Toxemic shock was observed in 2 cases; 1 had a severe cutaneous lesion on the right arm with an extensive, non-pitting edema from lesion to the shoulder including posterior and anterior chest wall. Another case had a small lesion on the anterior neck surrounded by an extensive erythema and non-pitting edema from lesion to the all anterior chest wall, neck and face and respiratory distress. Both cases had also a low systolic blood pressure (<9 mmHg), apathy and toxemic appearance, leukocytosis, hypoalbuminemia, hyponatremia, high level of CRP. Shock was resolved with intravenous fluid infusion in one case, other case was required dopamine infusion. Intravenous fresh plasma was given in both cases. Intravenous penicillin G was given 4 cases (10 days in 1 case, 7 days 1 case, 5 days in 2 cases), clindamycin in 1 case for 5 days because of penicillin allergy. Other 4 cases with mild form, 3 cases received amoxicillin for 3–5 days and 1 case received procain penicillin intramuscularly for 5 days. A deep tissue necrosis healing with a large black eschar (more than 10 cm diameter) developed in all 4 cases having severe cutaneous anthrax lesion. This eschar was leaved with surgically after 3 weeks of disease. After 4–6 weeks of disease, the wound was grafted.
Conclusion: Severe cutaneous reactions or toxemic shock threat the patient's life. Penicillin is still the first choice of drug in naturally occurring anthrax. The duration of therapy may be 3–5 days in cutaneous anthrax.

P556

Visceral leishmaniasis in immunocompromised patients: 2 cases and review of the literature

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Objective: Visceral leishmaniasis is rare in Western Europe. Morbidity and mortality is low in immunocompetent but may be fatal in immunosuppressed patients. Control of infection and immunity is achieved by the generation of leishmania-specific CD4-T-cells of TH1-type, the secretion of IFN-a and IL-2 and consecutively activation of macrophages to kill intracellular amastigotes. The infection can be reactivated years later in case of cellular immunosuppression.
Methods: We describe two patients with reactivation of latent infection: the first after induction chemotherapy for acute lymphatic leukemia (ALL) and the second after treatment with steroids.
Results: The first patient, a 41-year-old man from Turkey, was treated for newly diagnosed B-cell ALL with Cytarabine and Idarubicin. He developed fever in neutropenia, mucositis with diarrhoea and abdominal cramps. The patient deteriorated while on empirical treatment with broad spectrum antibiotics and Amphotericin B. Because of an acute abdomen, a laparotomy with resection of the duodenum was performed. The histology revealed an intestinal leishmaniasis. Despite adequate therapy with liposomal Amphotericin B the patient developed fatal septic shock with multiorgan-failure. The second patient was a 66-year-old swiss woman with a diagnosis of allopurinol-induced hepatitis. A liver biopsy showed fibrin ring granulomas. Prednison (75 mg/day) was started and 3 weeks later the patient was readmitted with pancytopenia. A bone marrow aspiration revealed leishmaniasis, identified as Leishmania infantum by PCR. A repeated liver biopsy showed infiltration of leishmania without evidence of fibrinous granulomas as shown on the first biopsy.

The history revealed a travel to Malta 2 years earlier. Liposomal amphotericin was started. The patient developed septic shock and died after 15 days on the intensive care unit.
Conclusion: Visceral leishmaniasis may become more frequent in non endemic regions because of international travel and increasing numbers of immunosuppressed patients. Reactivation of leishmaniasis should be considered particularly in patients with fever in neutropenia or with pancytopenia and fever under steroids.

P557

Nontyphoidal *Salmonella* invasive bloodstream infections resistant to quinolone and/or an extended spectrum cephalosporin in a country with extensive use of these antimicrobials in food animals

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Objectives: Nalidixic acid and extended spectrum cephalosporin resistant nontyphoidal *Salmonella* infection has been a growing global problem and more worrisome in certain countries. In our country, enrofloxacin, a veterinary fluoroquinolone, is extensively used in virtually all food animals. In addition, ceftiofur, a veterinary third-generation cephalosporin, is also widely used here in swine, not only for treatment but also for disease prevention. With complex plasmids capable of carrying both antimicrobial-resistance and virulence genes in a package, a general principle of microbes sacrificing virulence for resistance may not always apply. We have embarked on exploring clinical and molecular aspects of this growing problem, and wish to report our pilot survey here to alert the medical community.
Methods: Archival nontyphoidal *Salmonella* isolated from bacteremic patients in our university hospital from January 2003 to October 2005 and from bacteremic patients nationwide sent to The WHO National Salmonella and Shigella Center during the first half of 2005 entered the study. These collections are non-overlapping. E-test was used to evaluate MICs of nalidixic acid, ciprofloxacin, and ceftriaxone and susceptibility was defined using Clinical Laboratory Standards Institute (CLSI/NCCLS) 2005 criteria for *Salmonella*.
Results: The bacteria were resistant to the antimicrobials tested at very high rates (Table). All Choleraesuis isolates with ceftriaxone resistance also expressed high levels of nalidixic acid resistance (MIC > 256 μg/ml) and thus reduced susceptibility to ciprofloxacin (MIC = 0.125 μg/ml or more). Of 73 nalidixic acid resistant isolates, 55 (75%) had ciprofloxacin MIC at 0.125 or more, 14 (19%) at 0.094, and 4 (6%) at 0.064 μg/ml. A case of aortitis from a ceftriaxone resistant organism resulted in a fatal ruptured mycotic aneurysm.

Sources	serovars	NA-sensitive (%)	NA-resistant (%)	Ceftriaxone sensitive (%)	Ceftriaxone intermediate +resistant (%)	Resistant to both (%)
Our hospital	Choleraesuis	0/10 (0)	10/10 (100)	7/10 (70)	3/10 (30)	3/10 (30)
	Non-Choleraesuis	8/27 (30)	19/27 (70)	27/27 (100)	0/27 (0)	0/27 (0)
	Total	8/37 (22)	29/37 (78)	34/37 (92)	3/37 (8)	3/37 (8)
WHO *Salmonella* Center	Choleraesuis	20/44 (45)	24/44 (55)	39/44 (89)	2+3/44 (11)	2+3/44 (11)
	Non-Choleraesuis	21/41 (51)	20/41 (49)	41/41 (100)	0/41 (0)	0/41 (0)
	Total	41/85 (48)	44/85 (52)	80/85 (94)	2+3/85 (6)	2+3/85 (6)

Conclusion: Compared to susceptibility patterns in the past, current nontyphoidal *Salmonella* infections in humans in our country are obviously more resistant to quinolone and cephalosporin without sacrificing its virulence. Nalidixic acid susceptibility correlates well with reduced susceptibility to ciprofloxacin. Alarming ceftriaxone resistance in *Salmonella choleraesuis* may be associated with inappropriate ceftiofur usage in pig farming. A reconsideration and probable major revision in policy on antimicrobial use in food animals for various purposes in our country is warranted.

P558

Detection of *Coxiella burnetii* and anti-*Coxiella* antibodies by molecular and serological methods among risk groups

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Objectives: Q fever is due to *Coxiella burnetii* and usually found as a professional fever disease and can be presented in its acute or chronic forms. Isolation of *C. burnetii* should be done in a 3rd level security laboratory which increase the value of serologic diagnosis for the institutions which do not have it. The aim of this study was detection of *C. burnetii* and anti-Coxiella antibodies by molecular and serological methods among risk groups.
Methods: Of 92 people studied were 85 males and 7 females with ages ranging from 18 to 60 years. Among 92 people, 30 were veterinary doctors, 30 were farm workers and 32 were butchers. Sera were collected from all 92 people and presence of anti-*Coxiella burnetii* antibodies was studied using *C. burnetii* ELISA IgG and *C. burnetii* ELISA IgM kits (Vircell, Spain). The positive or equivocal samples with ELISA were studied further by IFA. IFA test were done using *Coxiella burnetii* Phase I+II kits (Vircell, Spain). Presence of *C. burnetii* was also studied in all cases by PCR using specific primers Trans1: 5′-TGGTA-TTCTTGCCGATGAC-3′, Trans 2: 5′-GATCGTAACTGCTTA-ATAAACCG-3′.
Results: A total of 12 (13.0%) and 8 (8.7%) people were positive and equivocal by ELISA IgM, respectively. Among 92 people studied 32 (34.8 %) and 9 (9.8%) people were positive and equivocal by ELISA IgG, respectively. The ELISA positive and equivocal sera were studied further by IFA and in 7 (7.6%) cases IgM and in 39 (42.4%) cases IgG presence were confirmed. All IgM positive cases were also positive for IgG but one, so 40 (43.5%) cases were *C. burnetii* seropositive. There was no significant difference for *C. burnetii* seropositivity among three professional groups (veterinary doctors, farm workers and butchers) ($p > 0.05$). Only 4 (4.3%) cases PCR was positive.
Conclusions: Coxiella seropositivity was found to be 43.5% among risk groups which is higher than the rates reported among general population in Turkey. For this reason especially among risk groups in case of atypical *pneumoniae*, granulomatous hepatitis, and fever with unknown aetiology, Q fever should be thought and searched for differential diagnosis.

P559

Dermatophytosis by *Trichophyton violaceum*: an emerging pathogen in European urban areas

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Objectives: The aim of this study is to present eleven cases of dermatophytoses caused by *Trichophyton violaceum*, a new

emerging pathogen in Baix Llobregat (metropolitan area of Barcelona). We summarize the epidemiological, microbiological and clinical data.
Methods: This report is based on eleven cases of mycotic infection due to *Trichophyton violaceum* in Baix Llobregat area from January 2001 to September 2005. Epidemiological, clinical and laboratory findings were analyzed.
Results: All patients were attended in units of dermatology and samples were submitted to laboratory for culture. Ten patients were children (four women, six men; age range, 2–12 years). The other patient was a 71-year-old female. Eight patients were immigrants from Africa, and the others patients were spanish. The only adult patient was not immunosupressed. Nine patients presented with tinea capitis, consisting of spotty alopecia and crust formation. The other two cases presented with tinea involving nose and cheek. Skin scrapings and hair samples were cultured in Sabouraud dextrose agar (Chloramphenicol-cycloheximide and gentamycine-chloramphenicol). The growth of flat violet-coloured colonies was detected in all the cultures. Microscopically, tangled, branched, and irregular hyphae were noted, with absence of microconidia and macroconidia. All patients were treated with oral antifungals drugs.
Conclusion: A progressive increase in the number of cutaneous infections caused by *T. violaceum* has been observed during last years. In our geographic area, *T. violaceum* has been isolated since 2001. Almost all our cases occurred in children and were related with immigration. Spreading between brothers was demonstrated in four cases. In contradistinction with previous reports, the only non-paediatric patient of our study was not immunosupressed. Incubation of cultures must be at least one month because colonies of *Trichophyton violaceum* have an extremely slow growth.

P560

Characterisation of *Listeria monocytogenes* strains isolated from human and ruminant clinical cases and from food products by serotyping and automated ribotyping

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Objectives: *Listeria monocytogenes* is a food-born pathogen capable of causing serious disease in susceptible individuals. Only recently a new non invasive form of listeriosis that causes febrile gastroenteritis in people with no predisposing conditions increased the public health significance of *L. monocytogenes*, suggesting the possibility of a wider range of infection vehicles. As the major infection factor of food contamination seems to occurs in the environment of processing plants, where it can be imported from slaughter facilities, we investigated the epidemiological role of ruminant as possible zoonosic source of listerial pathogenic strains.
Methods: 52 *L. monocytogenes* strains isolated in Northern Italy since 1998 (20 isolated from cattle and sheep and 11 from patients with sporadic clinical listeriosis, 21 from food products) were subjected to characterization of somatic (O) and flagellar (H) antigens (Kit Seiken, Denka Seiken Co., Ltd, Japan). The same strains were ribotyped using the automated RiboPrinter® system.
Results: 4b, 1/2a and 1/2b were found to be the most represented serotypes, while ribotyping, that offers a partially independent and more accurate subtyping scheme, identified 3 major ribogroups, classified with the Dupont Identification pattern Number (DUP-ID) 1038, 1039, 1042, shared by human, feed and animal isolates.
Conclusions: Our data indicate that a variety of *L. monocytogenes* ribotypes, which have been shown to be associated with human sporadic and epidemic listeriosis all over the world, where commonly present in ruminant cases in our study. The finding of three common clusters within humans, feed and animals supports the hypothesis that ruminant could play an important epidemiological role both as reservoir and source of listerial infection for human beings.

P561

Lethal *Ehrlichia ruminantium* infections in humans in South Africa

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Objectives: *Ehrlichia ruminantium*, is a tick-borne intracellular bacterium that parasite endothelial cells. It causes heartwater in ruminants in sub Saharan Africa and the French Indies.
Patients and Methods: A 56-year-old not immuno-compromised woman, became sick about 2 weeks after her pet dog died from 'biliary fever'. She died a week later and no further clinical details are available. A 6-year-old boy initially presented with a history of headache and fever. He presented with gait disturbance (ataxia) and progressive sleepiness and rapidly became comatose. A CT scan of the brain performed at this stage revealed edema and hypodense lesions in the cortex. Encephalitis was diagnosed and he was transferred to ICU, where he died 3 days later. Post mortem examination revealed extensive brain vasculitis, most prominent in the midbrain and the pons. A second child, who had evidence of tick bite, died in January 2005 with similar symptoms although he had been treated for tick bite fever. DNA was extracted from acute phase serum samples of the three individuals and subjected to polymerase chain reaction (PCR) amplification using primers specific for 16S, pCS20 and citrate synthase (*glt*A) gene of *E. ruminantium*. Amplicons of appropriate sizes were cloned into a plasmid vector for sequencing or were directly sequenced. Sequences were aligned with previously determined *E. ruminantium* sequences to check for genotype identity. Samples from two patients were similarly examined for the presence of *Rickettsia* spp.
Results: Both tested patients were negative for *Rickettsia* spp. In all three patients, 16 S r DNA and pCS20 sequences identical to known *E. ruminantium* genotypes were detected in the acute phase serum. A *glt*A sequence identical to that of the Welgevonden stock of *E. ruminantium* was also detected in one patient.
Conclusions: This is the first molecular evidence, that *Ehrlichia ruminantium* infection occurs in humans. In view of the severity of the infections, we aware physicians of this possible diagnosis in patients being bitten by tick in sub-Saharan Africa and all the *E. ruminantium* endemic areas. Treatment should be based upon doxycycline including in children.

P562

Value of serology in diagnosing *Lymphogranuloma venereum*

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Objective and background: An outbreak of *Lymphogranuloma venereum* (LGV) caused by Chlamydia trachomatis L2 was reported recently among MSM in Europe. Confirmed diagnosis of LGV requires identification of *C. trachomatis* L-genotypes in

symptomatic patients. However, due to the invasiveness of LGV-strains they may not always be detectable in lesional swabs. Since LGV is associated with induction of a strong antibody response, serology may be helpful in identifying affected patients, even retrospectively. To characterize the predictive value of serology for LGV, serum samples of patients with and without *C. trachomatis* infections were analyzed using different commercial C. trachomatis antibody tests

Methods: Serum specimens from patients with invasive *C. trachomatis* infection (LGV) (n = 15), epithelial *C. trachomatis* infection (urethritis, cervicitis) (n = 17), and without any evidence for infection with *C. trachomatis* (n = 20) were tested for antibodies against *C. trachomatis* by complement fixation (CF), LPS-based (genus-specific) EIA (LPS-EIA), MOMP-based (*C. trachomatis*-specific) EIA (CT-EIA) and a line assay with recombinant antigens (LA). Presence or absence of *C. trachomatis* was analyzed by SDA of anogenital swabs obtained from these patients.

Results: Specificity and sensitivity for LGV was 89.2% and 93.3% (CF), 92.9% and 73.3% (LPS-EIA, IgA), 94.6% and 26.7% (LPS-EIA, high IgG), 73.0% and 86.6% (CT-EIA, IgA), 91.9% and 73.3% (CT-EIA, high IgG), 86.2% and 85.7% (LA, IgA), and 75.9% and 85.7% (LA, high IgG). Assuming a prevalence of 20% (confirmed cases among patients suspicious of LGV) the PPV ranges between 44.5% (CT-EIA, IgA) and 72.1% (LPS-EIA, IgA). NPVs were higher, ranging between 81.0% (LPS-EIA, high IgG) and 98.2% (CF).

Conclusion: Positive serology (IgA-positive or high IgG titre) does not necessarily indicate LGV, but may also result from *C. trachomatis* infections caused by non-L strains, whereas in case of negative serology the presence of LGV is very unlikely.

P563

Investigation of *Francisella tularensis* using real time TaqMan PCR in water sources in Turkey where tularaemia cases seen mostly as pharyngeal form

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Objectives: Several epidemiologic reports indicate that typeB disease has a close connection with water. In Turkey, tularemia cases have been seen mostly as pharyngeal form. But, up to now, isolation of *F. tularensis* or its DNA from water samples have not been reported from Turkey. It is the first report from Turkey to evaluate the link between tularemia outbreaks and water sources.

Methods: Two litres of water samples from every water source were obtained and studied by TaqMan 5′ nuclease assay. Water samples were put through 0.22-μm-diameter cellulose acetate filters. Surfaces of filters were washed with sterile distilled water for 15 minutes in a shaker, and lysis buffer (Guanidine isothiocyanate [5M], Na acetate [1/10 (v/v)], Sarcosil % 0.5) was added to this pellet. DNA isolation depended on the binding of DNA to glass beads (Glassmilk, Bio 101, La Jolla, CA) was performed. Regions targeted for TaqMan assay were specific for *Francisella tularensis* and included the tul4 (91 bp) and fopA (87 bp) genes. tul4 (encoding 17 kDa lipoprotein) and fopA (encoding 43 kDa outer membrane protein) primers and 5′-FAM and 3′ TAMRA labelled probes for *F. tularensis* TaqMan 5′ nuclease tests were used. TaqMan 5′ nuclease assay was performed by a real-time PCR device (Quantica; Techne Inc., UK).

Results: Beside network water, natural stream water was available and widely used for drinking and cooking purposes in rural area of Turkey. To investigate water as a possible source of infection, natural springs and network water were sampled for testing with TaqMan PCR. In this study, water sources were sampled from five different provinces. They were named Suluova, Golcuk, Edirne, Duzce, Zonguldak as Site A-B-C-D-E, respectively. They are located at least 200 km from each other. Totally 44 l of water were filtered in related to 22 water sources in five outbreaks. *F. tularensis* DNA in three water samples were found positive in Site A, Site C and Site D. In Site B and Site E, all water samples were negative by TaqMan PCR assay.

Conclusions: This study shows that the contagion come from drinking water in some outbreaks in Turkey. Real time TaqMan PCR is a useful method to detect the DNA of *F. tularensis* from water sources.

P564

Outbreak of tularaemia: the first case-control study in Turkey

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Objective: The aim of the study is to identify the potential factors associated with infection sources and modes of transmission during a recent outbreak (October 2004), of tularemia at Suluova, Turkey.

Methods: Active surveillance was initiated for compatible cases at Suluova County to identify cases of tularemia,. A matched case–control study with analysis based on the first 43 cases and 49 matched controls. A standardized questionnaire was used to collect information about general demographics, exposure to all known sources of tularemia infection, as well as potential risk factors related to water and animals (i.e., fishing and farming, hunting and other activities) and environmental conditions of houses. Microagglutination test was used for serological diagnosis. Clinical and water samples were screened for evidence of the tularemia agent by PCR.

Results: The overall attack rate was 0.23% (86/38.000). The single most common presenting symptom was lymphadenopathy present in 95.3 %, followed by fever (83.7%) and sore throat (79.1%). 28 out of 43 were reported to have painful lymph nodes. *Francisella tularensis* was shown by PCR in samples obtained from ulcerated lesion of two patients. In multivariate logistic regression model, keeping domestic animal in the garden was associated with the risk of contracting the disease; OR = 12,32 (CI 95 %: 1.43–106.0) (p = 0.022). *F. tularensis* was detected by PCR in the water sample obtained from the rivulet which passes through Suluova.

Conclusion: The results of this study show that case–control studies might be useful for analysing the epidemics and for identifying the source of infection. In order to prevent water-related zoonotic infections, water and sewerage systems should be improved.

P565

Tularaemia: a misdiagnosed disease in France

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Objectives: Tularaemia is a zoonotic disease caused by the gram-negative coccobacillus *Francisella Tularensis*, which is endemic in the Northern Hemisphere notably in Europe. *F. tularensis* is a bioterrorism agent and declaration of any case of

tularaemia has been mandatory in France since 2002. As a reference centre for tick-borne disease and for Bartonella we routinely test for *F. tularensis* any suspected tick borne ''rickettioses'', "atypical" pneumonia or cat scratch disease. We report the epidemiological, clinical, biological features of tularaemia diagnosed through systematic testing in our reference laboratory.

Methods: Between June 2003 to October 2005, we enrolled all patients for whom a positive serology and/or a positive polymerase chain reaction assay and/or a positive culture for *F. tularensis* from blood, skin, lymph nodes and liver samples was obtained. We collected epidemiological, clinical, biological features and outcomes after treatment for each patient with a standardised questionnaire and used the Chi-square test for the statistical analysis.

Results: Eighty-eight cases fulfilled the above criteria. Their clinical presentations were glandular forms (37.7%), ulceroglandular forms (18.7%), pneumonic forms (28.1%), digestive forms (12.5%) and oculoglandular forms (3%). Pneumonic forms are significantly associated with risk of relapse ($p < 0.022$). Only patients who were more than 40-year-old developed pneumonic forms ($p < 0.019$). Farming, gardening and being bitten by a tick were statistically independent risk factors for developing tularaemia ($p < 0.003$ respectively). The cases reported in spring are associated with a great exposure to tick bite. Finally, tetracyclines are more often used for treatment of any forms of tularaemia in our study ($p < 0.042$).

Conclusion: Tularaemia is a common disease in France but often misdiagnosed as suspected CSD, "atypical" pneumonia or tick borne "rickettioses". Clinical features and risk factors are similar to those reported in endemic countries.

P566

Anaerobic bacteraemia in patients with leukaemia and lymphoma. Oropharyngeal mucositis and status of haematologic disease are the most important predisposing conditions

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Herein we report our experience about Anaerobic Bacteremia (AB) in oncohematologic pts. Over a 10-year period, 34 episodes of AB were identified in 34 different pts (16 males, 18 females, median age 35, range 17–69 yrs) for a rate of 0.5 AB per 100 patient admissions; it accounted for 4% of all bacteremic episodes that occurred in our Department during the study period. The majority of pts had a refractory/relapsed leukemia (20/26) or lymphoma (5/8) and 5/34 (15%) had received a BMT procedure before infection. 28/34 (82%) of pts were neutropenic at onset of AB with a WBC count less than 100 cells/mmc and 26/34 (76%) had a severe oral mucositis following intensive chemotherapy (grade III°–IV° WHO in 20/26 cases). Bacteremic episodes occurred after a median of 16 days of severe neutropenia (range 5–28) and of these 8/34 (24%) were polymicrobial. Pulmonary infiltrates were observed in 4/34 (12%) of pts. The most frequently isolated: *Fusobacterium nucleatum* (18 cases), *Bacteroides* sp. (8 cases), *Peptostreptococcus* sp. (5 cases) and *Clostridium* sp. (3 cases). The first line antibiotic therapy was piperacillin-tazobactam in 13 pts, carbapenems in 10 and glycopeptide plus ceftazidime in 4. The median duration of therapy was 9 days (range 3–18). The response rate of the first antibiotic treatment was 72% and the overall response was 83%. The mortality AB related was 15% (5/34). The causes of death were *Clostridium* sp. bacteremia (2 cases), *Bacteroides* sp. bacteremia (2 cases) and *Fusobacterium nucleatum* bacteremia (1 case). *In vitro* the most active antibiotics against anaerobes were piperacillin, imipenem, amoxacillin-clavulanate and clindamycin. To identify the risk factors for infections a retrospective case–control study was performed (for each case 2 control pts). Univariate analysis revealed that the severe mucositis (WHO III°–IV°) and the status of refractory/relapsed haematologic disease were significantly associated to development of AB ($P = 0.008$ and $P = 0.01$ respectively). (A) Our data confirm the rarity of AB in neutropenic pts (4% of all bacteremic episodes) but this kind of infection, if underestimated, can be a fatal complication. (B) *Fusobacterium* sp., *Bacteroides* sp., *Peptostreptococcus* sp., and *Clostridium* sp., were the most common isolates causing AB in onco-hematologic pts. (C) Predisposing conditions to AB are severe oropharyngeal mucositis and refractory or relapsed status of haematologic disease.

P567

Non-toxigenic *Corynebacterium diphtheriae* as a cause of bacterial endocarditis in children with congenital heart defects

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Introduction: Non-toxigenic strains of *C. diphtheriae* have been increasingly recognised as a cause of invasive disease. Bacteraemia and endocarditis caused by these strains have been reported with increasing frequency. Other invasive diseases such as septic arthritis, splenic abscesses and mycotic cerebral aneurisms have also been described. To date at least 67 cases of non-toxigenic *C. diphtheriae* causing endocarditis have been reported worldwide. Most of these cases were in either native or prosthetic heart valves and in IV drug abusers.

Objective: We report on 4 cases of infective endocarditis caused by non-toxigenic *C. diphtheriae* in patients with underlying congenital heart defects, occurring at the Pretoria Academic Hospital over the past 7 years.

Method: Endocarditis was confirmed by the presence of diphtheroid organisms in the blood cultures of all the patients. A final diagnosis was made on microscopy (Gram and Albert's stains), culture on blood agar and Hoyle's medium, in-house biochemical tests substantiated by API CORYNE (Biomerieux) An elek test for toxin production was performed on all isolates.

Results: The ages of the 4 patients were between 3 and 16 years. Positive blood cultures (Bactec 9240 System) were obtained from all patients on multiple occasions. Characteristic ''Chinese letter'' arrangements of the bacilli were seen on both Gram and Albert's stains, as were metachromatic granules. In-house biochemical tests validated by API CORYNE confirmed all organisms to be *C. diphtheriae* var gravis. Elek tests in all cases indicated no toxin production. Sensitivities to a number of antibiotics (ampicillin, penicillin, erythromycin, gentamicin, piperacillin and cefuroxime) were determined by the Kirby-Bauer disc diffusion method. With the exception of penicillin and ampicillin resistance in one patient, all antibiotics tested were sensitive. Patients were treated with penicillin and gentamicin parenterally and all survived without complications.

Conclusion: Non-toxigenic *C. diphtheriae* is an infectious pathogen, and detection of coryneform bacteria in the blood can no longer be dismissed as contamination and must be investigated. Failure to recognise this pathogen can delay final diagnosis and initiation of appropriate chemotherapy. Species identification is important as mortality differs with the different biotypes. The importance of this organism as emergent pathogen should not be underestimated.

P568

Shiga toxin producing *Escherichia coli* isolated from one-humped camels (*Camelus dromedarius*)

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Objectives: The aim of the study was to investigate the presence of Shiga toxin (Stx) producing *Escherichia coli* (STEC) in faecal samples of camels, i.e. animals widely farmed in the Middle East and Africa.

Methods: Two hundred and one stool and rectal samples collected from individual animals in the Eastern region of the Abu Dhabi Emirate (United Arab Emirates) were studied. DNA extracts of the mixed coliform cultures were tested for the presence of the Stx gene (*stx*) by PCR. From positive specimens STEC was identified by applying the same PCR to test a total of 200 colonies in pools of decreasing sizes. The *Stx* production of the strains was determined by ELISA, while enterohaemolysin production was investigated on agar plates containing washed erythrocytes. The isolates were genotyped by PCR using primers specific to *stx*1, *stx*2, *stx*2e, and with those recognizing genes of intimin, enterohaemolysin, enteroaggregative plasmid, enteroaggregative heat-stable enterotoxin (EAST), cytolethal distending toxin B, cytotoxic necrotising factor, genes located on the yersinia high pathogenicity island (fyuA and irp2), and with primers identifying sequences of putative STEC adherence factor genes *saa*, *iha*, *tox*B, *efa*, respectively. The *stx* genes of the isolates were sequenced. The O and H antigens of the isolates were determined and they were further typed by pulse-field gel electrophoresis following *Xba*I digestion of the genome and also by ERIC PCR.

Results: From the specimens of the 201 animals three *stx*-carrying strains were recovered (1.5% of the samples). One strain (O8:H9) isolated from an adult animal did not produce any detectable *Stx* while carrying the complete *stx*2e gene. It did not carry the genes of intimin, nor any of those of the putative virulence factors tested. Two other strains (O76:HNT) indistinguishable by the typing methods used and isolated at a different farm from rectal samples of healthy baby camels produced Stx and carried the gene *stx*1c. Both isolates secreted enterohaemolysin and gave positive PCR reaction with primers specific to putative adherence factor genes *saa* and *iha*.

Conclusion: These data show for the first time that one-humped camels, whose meat and the often un-pasteurised milk are frequently consumed in countries of the Middle East and Africa, can also be carriers of STEC posing a potential health risk to humans.

P569

Achievements of the first European project on severe group A streptococcal disease

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Objectives: The main objective was to improve understanding of severe group A streptococcal (GAS) disease in Europe.

Methods: Enhanced surveillance was launched in January 2003 in 11 countries (Table1). Questionnaires were sent to hospitals and laboratories for collection of patient data and isolates. The criteria for inclusion of patients were the isolation of GAS from a normally sterile body site, or from a non-sterile site in the presence of a clinical diagnosis of streptococcal toxic shock syndrome or necrotising fasciitis. Collected isolates were characterized serologically, and by molecular methods. Antibiotic susceptibility was determined by MICs and E-test. Two sets of EQA strains for antibiotic susceptibility and one set for typing were sent to all participating laboratories.

Results: More than 5000 cases were identified, considerably higher than anticipated. Around 3000 were from the UK, yielding an annual incidence (2003) of 3.8/100,000. A similar incidence was documented in Sweden, Denmark and Finland but lower in some other participating countries. The incidence in France reached 2.7 during 2004. The M/emm type distribution of GAS also varied markedly. In a few countries types 1, 3 and 28 were predominant; however, an overall increase of new invasive types (77, 81, 82, 89) was noticeable. High rates of MLS antibiotic resistance in some countries (France, Italy) and tetracycline resistance in almost all countries was found. In the UK, intravenous drug use was found to be a major risk factor, following a previously reported trend. In France, the spread of a clone of GAS, type emm28, resistant to MLS drugs and bacitracin, was reported as mostly associated with puerperal sepsis. Early results from the pathogenesis work package have identified that low antibody levels against some newly described cell wall-attached proteins of GAS may predispose to severe GAS disease.

Table1: Strep-EURO Study Group QLK2-CT-2001-01398

Lund, Sweden	B. Luca, L. Björck, H. Tapper, U. von Pawel-Rammingen, C. Schalen, A. Jasir
Germany	R. Lütticken, M. van der Linden, R. Reinert,
UK	T. Lamagni, S. Neal, A. Tanna, M. Emery, C. Keshishian, A. Efstratiou
Czech Republic	L. Strakova, P. Urbaskova, J. Motlova, P. Kriz
Greece	A. Stathi, L. Zachariadou, A. Pangalis, P. Tassios, J. Papaparaskevas, N.Legakis
Italy	R. Creti, L. Baldassarri, G. Orefici
Finland	J. Iivonen, J. Jalava, T. Siljander, P. Ruutu, J. Vuopio-Varkila.
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Cyprus	I. Ioannou, M. Koliou
France	J. Loubinoux, L. Mihaila-Amrouche, A. Bouvet

Conclusions: Comparable data across Europe were achieved. The apparent overall increase of invasive cases may partly depend on the role of Strep-EURO in enhancement or establishment of surveillance systems but careful analysis of data should be considered for each country depending on national system for collection of data, and treatment strategies of cases. With regards to broad distribution of emm types any future development of vaccine based on M protein has to be considered. There is a need of a working group for validation and definition of new emm-types and subtypes.

Vaccines and immunotherapy

P570

Evaluation of gamma interferon kinetic in vaccinated BALB/c mice by gD expression vector of HSV-1 and its recombinant protein

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Objectives: Herpes simplex virus type 1(HSV-1) is the causative agent of localized skin infections in the oral, ocular, and neural regions; the need for a vaccine is widely recognized. HSV glycoprotein D (gD) is the major target of vaccination studies. IFN-g enhances CD8 T-cell cytotoxicity, up regulates major histocompatibility complex (MHC) class II, and reverses down regulation of MHC class I by HSV-1 ICP47. Furthermore, high level of IFN-g indicates the Th1 response. Regarding the importance of IFN-g in restriction of infection the ability of DNA and subunit vaccine was evaluated.
Methods: Female BALB/c mice were immunized with 100 micro gram gD expression vector (as DNA vaccine) and 1 x 10^6 Sf9 cells infected by recombinant expressing gD-1 baculovirus (as subunit vaccine) on days 0 and 21. Immunized BALB/c mice were sacrified after 14 days. Spleen cells were cultured (in 24–well plates) and were stimulated with five MOI of the inactivated virus. At different hours after stimulation (8, 16 and 32 h) total RNA was extracted from 1x10^6 cells and cDNA was synthesized using random hexamer. The semi-quantitative PCR reaction was performed using IFN-g and B2microglobulin (as internal control) specific primers. The primers have been designed in different exon of gene to prevent DNA contamination. The PCR products were evaluated by band-densitometer software.
Results: Our results reveal that vaccinated and infected (positive control) mice 8, 16 and 32 hrs after *in-vitro* stimulation comparing to negative control showed a significant immunity. The IFN-g production kinetic at 8, 16 and 32 hrs after stimulation in all groups displays no significant differences.
Conclusion: This investigation show that although 8 hrs stimulation is sufficient for expression of mRNA and significant detection of IFN-g in memory T-cells but 32 hrs post stimulation can't be sufficient to comparison between different vaccines, as mice groups that immunized by DNA and subunit vaccines after 32 hrs didn't show significant difference in IFN-g level.

P571

Is MMRV vaccination cost-effective in HCWs in Turkey?

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Objectives: To investigate the immune status of healthcare-workers (HCWs) against measles, rubella, mumps and varicella zoster and to promote an adequate vaccination program among HCWs in Turkey.
Methods: Voluntary HCWs in two hospitals, one is a children' hospital, and the other is a general hospital were included to the study between March and May 2005. The specific Ig G antibodies against measles, rubella, mumps, and varicella zoster viruses were screened quantitatively with use of immunosorbent enzyme-linked assay (ELISA) kids (EUROIMMUN®).
Results: Three hundred and sixty three heathcare-workers(HCW) were participated to the study from two hospitals. Of these, 186 (51%) were physicians, 118 (33%) were nurses, 36 (10%) were housekeeping staff, 23 (6%) were medical technicians. The positive rates for the antibodies against measles were 98.6%, rubella 98.3%, mumps 92.2%, varicella 98%. No statistically significant differences were found between two hospital for measles, rubella, and varicella but the HCWs in childrens' hospital were found more susceptible to mumps. There was no relationship the immunity against measles, mumps, rubella, and varicella between two hospital, gender, age, duration of work, professions and department of work. The positive rates of the past history of, measles, rubella, mumps and varicella were 18 %, 23%, 19%, 20%, and that of previous MMR and varicella vaccination were 11%, 5%, respectively. The overall cost for prevaccination screening of the 363 HCWs for MMR and vaccination of susceptible person was approximately $3,204. Assuming all of the HCWs was susceptible and would receive vaccination with MMR cost of these procedure is $ 3,630. Cost for prevaccination screening of varicella and then vaccination of susceptible HCWs $2,564. Cost of vaccination of 363 HCWs is $35,211.
Conclusion: The majority of the HCWs are immune to measles, mumps, rubella, and varicella. However, HCWs in Turkey seem to have little knowledge about their history of these diseases or vaccinations, prevaccination serologic screening seems to be necessary to identify susceptible. Without screening test MMR vaccination is cost effective in HCWs in Turkey, whereas prevaccination serologic screening is cost effective for varicella.

P572

Parasitic diseases in children as one of the reasons for postvaccinal complications

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Objectives: On a background of high prevalence of the diseases essentially changing child organism's immunological reactance, decrease of postvaccinal immunity efficiency, on the one hand, and increase of postvaccinal complications frequency, on the other hand, have been observed. The presented research was aimed at investigating the reasons of occurrence of postvaccinal complications (PC) (hyperthermia, local tissues reactions, regional lymphadenitis, skin rashes) at children being suffered from bronchial asthma (BA) and from BA combined with parasitic diseases, and that is why having immunological deregulations.
Methods: PC frequency at 85 BA-patients and 115 BA-patients, infested by some kind of parasites (Enterobias verm, Ascarias lumbr, Giardia int) was compared. The BA and parasitic diseases were diagnosed and treated according to the standard requirements. The statistical importance of distinctions was estimated by T-criterion Wilcocson.
Results: PC were fined out at 8.2% non-infested BA-patients and at 13.1% infested BA-patients. Therewith the age-dependence of results was observed: among children 0–7 years the difference was more (7.4% against 23.1%), and in 8–16 years group are less (8.6% against 10.1%). Within 6 years of supervision all patients received antiasthmatic and antiparasitic treatment in accordance with BA gravity and parasitic species. During this period the repeated vaccinations

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were carried out at decretal age. As a whole, PC frequency has decreased at non-infested BA-patients up to 1–2%, and at infested BA-patients up to 3.5%. Even so, the former age dependence was kept (see tab). [Note: Tab's data in %; all distinctions are statistically significant (T-criterion for N = 2, $p < 0.05$).]

The main disease	BA		BA + parasitic disease	
Age	Before treatment	After treatment	Before treatment	After treatment
0-7 years	7,4	0,0	23,1	7,7
8-16 years	8,6	1,7	10,1	2,2

Conclusions: The widespread at childhood parasitic diseases (enterobiosis, ascariasis, giardiasis) significantly influence the postvaccinal answer. The reason of such reaction on vaccination material can be the presence of antiparasitic antibodies in child organism. The parasitic antigenic complex is heterogeneous owing to what the antiparasitic immune answer is characterized by low specificity. Contact with parasites results in production of the antibodies, capable to cooperate not only with parasite epitopes, but also with other cells expressing similar molecules. It is important for clinicians to provide successful scheduled child vaccination so parasitological inspection and if necessary antiparasitic treatment can promote this purpose.

P573

Vaccination with adeno-associated virus vector encoding severe acute respiratory syndrome coronavirus S protein induces potent and prolonged antibodies

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Objectives: Severe acute respiratory syndrome coronavirus (SARS-CoV) is the etiological agent of a novel infectious disease SARS. SARS-CoV S plays important roles in immune responses and mediates the virus binding to its receptor angiotensin-converting enzyme 2 (ACE2). Recombinant adeno-associated virus (rAAV) is a powerful delivery vector widely used in gene therapy. Therefore, the Objectives of this study are to detect functional fragments of SARS-CoV S using the AAV system and to pursue a good candidate for developing SARS vaccines.
Methods: In this study, a rAAV vector encoding SARS-CoV S was developed and used to induce SARS-CoV specific antibodies. Genes of RBD and other four SARS-CoV S fragments were inserted into an AAV plasmid to generate rAAV vectors, which were used to vaccinate mice intramuscularly to evaluate the immunogenicity *in vivo*. Mouse sera were detected by an ELISA for specific antibodies against SARS-CoV and measured by a neutralizing assay for antibody neutralizing activities. rAAV encoding RBD was further applied to immunize mice intradermically and intranasally to detect its ability in inducing antibodies via different vaccinations.
Results: Our results demonstrated that vaccination with five rAAV vectors encoding SARS-CoV S fragments elicited SARS-CoV specific IgG antibodies in vaccinated mouse sera, but the antibody titer and the time for maintaining antibodies varied in different rAAV vectors. Results Also showed that rAAV encoding the receptor-binding domain (RBD) provoked potent and prolonged antibodies with neutralizing activities, indicating that it can deliver a prolonged immune response. Furthermore, intradermic and intranasal vaccinations with rAAV encoding RBD induced SARS-CoV specific IgG antibody in mouse sera and IgA antibody in mouse lung flush, illustrating that this live vector is able to elicit specific antibody responses, including respiratory tract local immune response, through different vaccination pathways.
Conclusion: Our study provided a novel database that rAAV encoding SARS-CoV S fragments produced high SARS-CoV specific antibodies in immunized mice, giving the clue that it is worthwhile to further study the function of these fragments. In addition, results that rAAV encoding RBD elicited specific IgG and IgA antibodies with neutralizing activities via intramuscular, intradermic and intranasal vaccinations imply that this live vector might be a good candidate for development of SARS vaccines.

P574

Appropriateness of rabies post-exposure treatment received by patients presenting to an anti-rabies treatment unit

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Objective: With increased availability and accessibility of rabies post exposure treatment (PET), costs borne by the Government of Sri Lanka in the purchase of anti-rabies vaccines have increased dramatically. The country spends around 500 million Sri Lankan Rupees (1 Euro = 121 SLR) on rabies PET vaccines alone, per annum amounting to almost about one tenth of the country's annual expenditure on drugs and vaccines. This large proportion of the health budget spent on rabies treatment needs justification. Hence, the present study was carried out to determine the appropriateness of rabies PET received by patients presenting to a tertiary care unit.
Method: A hospital based descriptive study was carried out in the newly commenced anti-rabies treatment unit of the National Hospital of Sri Lanka (NHSL). An interviewer administered questionnaire assessed the socio-demographic characteristics of each patient, severity of exposure and the health seeking behaviour following exposure. Details of treatment were taken from patient registration cards.
Results: Of those who seek out patient treatment from the NHSL on a given day, 2.5% reported exposure to animals suspected of having rabies. Of the 367 patients studied, the majority were males, employed and belonged to 21–30 year age group. Exposure to domesticated dogs (70%), which were unvaccinated (82%), was the commonest presentation. Only 35% of the patients presented for treatment within the first 24 hours and the mean time duration taken was 2 days (SD 3.75). Around 15.5% of the patients did not wash the exposed area with soap and water immediately following the exposure. This practice showed a significant association with level of education ($p = 0.025$), exposure being to a dog ($p = 0.004$) and when the exposure was to a domesticated animal ($p = 0.016$).In assessing appropriateness of PET received by these patients, the study found that the use of vaccine was inappropriate in 9.4% (25) of new cases and 8% (8) of those who received day 30 and day 90 doses of anti-rabies vaccine.
Conclusion: Appropriateness of anti-rabies PET received was altogether around 91% in this sample. This was over and above the expert predicted appropriateness of anti-rabies treatment in the country. Setting up specialized units to treat patients who require anti-rabies treatment will help to optimize treatment and reduce treatment costs in the long run.

P575

Adverse effects of rabies postexposure prophylaxis

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Solid organs from a rabies infected donor were transplanted in six individuals on 1st January 2005 at five German hospitals. Rabies postexposure prophylaxis (PEP) was initiated for health care workers (HCWs) who had contact to rabies infected patients.

Objective: To investigate the incidence of adverse effects of rabies PEP in an homogeneous population.

Methods: PEP was administered according to the ''Essen schedule'' (Berirab® immunglobulin 20 IU per kg BW, Rabipur® purified chick embryo cell vaccine (PCECV) day 0, 3, 7, 14, 28). A standardised follow-up form was used to record adverse effects of rabies PEP. Only adverse effects that occurred up to 24 hours after a vaccination were considered.

Results: In four hospitals complete follow-up data from a total of 370 individuals that received PEP were collected. At least one reversible local adverse effect was recorded in 67.9 % of all cases [local pain (60.2%), erythema (10.7%), swelling (9.8%) and malfunction (7.5%)]. Systemic effects were recorded in 38% of all cases [tiredness (30.1%), malaise (25.8%), headache (26.8%), dizziness (16.7%), fever (7.2%), chills (13.4%), nausea (10.0%), vomiting (1.9%), myalgias (5.3%), arthralgias (5.3%), diarrhoea (2.9%), paraesthesias (7.7%) and lymph nodes swellings (3.8%)]. Persisting adverse effects were paraesthesia and artrial fibrillation in two HCWs. In two other HCWs, symptoms were suspicious for a mild form of meningitis, but symptoms were reversible. In 15 cases (4.0%) PEP was interrupted due to adverse effects (severe headache, dizziness, paraethesias and allergic reaction). Whereas incidences of tiredness, malaise, headache, dizziness and fever declined significantly (all p-values < 0.05) from vaccination to vaccination, the incidences of paraesthesia did not change ($p = 0.7$).

Conclusion: Rabies PEP was safe in 370 vaccinated HCWs. The incidence of adverse effects of PEP according to the ''Essen schedule'' included mild adverse effects more frequently than described before. It is not clear if the high incidence of adverse effects during the first week was due to the additional administration of immunoglobulin on day 0 or if an accelerated booster reaction led to these effects. The reoccurrence of paraesthesias after each vaccination may represent an adverse effect specific for the active immunization. However, rabies PEP remains the only effective measure to prevent rabies and should always be given if individuals were possibly exposed to rabies containing secretions.

P576

Design and construction of an expression vector containing immunogenic epitopes of HIV-1 P24 and gp 41 proteins as a vaccine candidate against HIV-1

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Introduction: An effective vaccine is urgently needed to stop the AIDS epidemic worldwide. DNA vaccines induce conformational-dependent antibodies and mimic live vaccines without their pathogenic potential. They can also easily be made in a polyvalent form based on identification of T and B cell epitopes in the virus. The P24 and gp41 play many of important roles in host-virus' interaction and pathogenesis. These proteins are considered as attractive vaccine candidate in which their immunogenicity and immunomodulatory effects have been confirmed.

Materials and methods: In this study, an expression vector (PCDNA3.1 hygro) containing P24-gp41 immunogenic sequences under the control of IE HCMV promoter was designed. The expression of the recombinant peptides was analysed in a eukaryotic system (COS-7 cells). Immunofluorescence and western blotting confirmed the presence of expressed proteins.

Conclusions: The above polyvalent P24-gp41 vector will be used in an animal model for evaluation and generation of effective immune responses.

P577

Clinical course study in post BCG vaccination adenitis

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Objectives: The most common complication of post B.C.G vaccination is lymphadenopathy or lymphadenitis, usually in neck or axilla. The aim of this study were evaluation various manifestations of post vaccination adenopathy and effectiveness of different treatment modalities on them.

Method: We studied 82 infants (range of age 2–26 months) within 2 years (May2000–2002). These patients who were affected by post B.C.G vaccination lymphadenitis referred to pediatric infectious clinic .The patients were follow up by physical examination monthly for 6–18 months and some time intervention treatment till adenopathy disappear or fistulized. We observed the duration of healing in affected lymph node.

Results: No specific treatment was performed on 50 patients who had only cervical and axillar lymph node. The lymph nodes were resolved spontaneously within 3–9 months. In 30 patients out of 50 mentioned above resolution occurred without fistulization. In the others resolution of lymph node occult with fistulization.6 patients with disseminated adenitis required needle aspiration. The lymph nodes in these patients were resolved in 2 months. Oral Erythromycin was administered in three patients. These treatments not have any significant affect on duration.

Conclusion: Generally in adenitis post B.C.G vaccination surgery or needle aspiration rarely needs. Cleaning area of lymph node and careful following up patients are highly recommended.

P578

A flow cytometric opsonophagocytic assay for measurement of functional antibodies elicited after immunisation with the *Neisseria meningitidis* serogroup A capsular polysaccharide-serogroup B outer membrane vesicle conjugate vaccine in animal model

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Introduction: Production of effective vaccine formulations is dependent on the availability of assays for the measurement of protective immune responses. Antibody- and complement-

mediated phagocytosis is the main defence mechanism against *Neisseria meningitidis*.
Methods: Therefore, a newly developed phagocytosis assay based on flow cytometry (flow assay) was using sera obtained from rabbit postvaccination with a bivalent conjugate of *Neisseria meningitidis* serogroup A capsular polysaccharide (CPSA) to serogroup B outer membrane vesicle containing PorA (OMV-PorA), an OMV-PorA of *Neisseria meningitidis* serogroup B and the CPSA (as control), was done in order to evaluation of the potential efficacy of (experimental) meningococcal vaccines. The conjugate and control were injected intramuscularly into groups of five rabbit with boosters on days 14, 28 and 42 after the primary immunization. The following groups were used as control: CPSA; OMV-PorA; normal saline. The serum on days 0, 14, 28, 42 and 56 were collected and stored at - 20°C for next analysis. Phagocytic function of and intracellular oxidative burst generation by rabbit PMN, against *Neisseria meningitidis* serogroup A and B, were measured with flow cytometer (Coulter Epics- XL-Profile USA), using dihydrorhodamine-123 as probes, respectively. In these experiments non-heat-inactivated standard strain *Neisseria meningitidis* serogroup A(CSBPI,G-243) and B(CSBPI,G-245) were used.
Results: The results of quantitative flow cytometric analysis of rabbit PMN function in hyperimmun sera with the glycoprotein conjugate revealed a highly significant increase in opsonophagocytic responses against serogroup A meningococci after 56 day in comparison with the CPSA and OMV-PorA control group ($P < 0.05$). opsonophagocytic responses against serogroup B meningococci of the conjugate showed no significant difference in comparison with the OMV-PorA containing control ($P > 0.05$).
Conclusion: Our results indicated that the CPSA--OMV-PorA conjugate could be as a candidate for bivalent vaccine toward serogroup A and B meningococci.

P579

Immunological evaluation of *Neisseria meningitidis* serogroup B outer membrane vesicle containing PorA conjugated with *Neisseria meningitidis* serogroup A capsular polysaccharide in rabbit

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Introduction: Bacterial meningitis caused by different groups of *Neisseria meningitidis* is still one of the serious health problems world wide.
Methods: In at present investigation, standard serogroup B strain of *N.meningitidis* (CSBPI,G-245) were grown under controlled-submerge cultural conditions in fermenter containing modified Frantz medium. The cells were harvested at late exponential growth phase. Outer membrane vesicle containing PorA(OMV-PorA) were extracted from cell wall according to the Deoxycholate Extraction Technique and further purified by sequential centrifugation and ultracentrifugation steps. Molecular evaluation of OMV-PorA was done by micrograph scanning in electron microscope and SDS-PAGE. Identity of the OMV-PorA was determined by Double Diffusion Gel Technique using hyperimmune rabbit antiOMV-PorA antisera against purified vesicles. Then *N.meningitidis* serogroup A capsular polysaccharide (CPSA) was conjugated to serogroup B OMV-PorA in order to test the possibility of obtaining a bivalent serogroup A and B meningococcus vaccine. The conjugate and control were injected intramuscularly into groups of five rabbit with boosters on days 14, 28and42 after the primary immunization. The following groups were used as control CPSA plus OMV-PorA; CPSA OMV-PorA;and IV normal saline. The serum collected on days 0,14,28,42 and 56 were tested by complement mediated bactericidal assay and ELISA titer for induction of protective immunoresponses in rabbit model.
Results: In micrograph scanning of electron microscope, more than 70–90% of the OMV-PorA retained their native configurational structure after extraction procedures. Purified vesicle showed a strong band of 40–45 KD molecular weight when run on 10%polyacrylamide gel electrophoresis with SDS. A strong precipitate line between hyperimmune rabbit anti OMV-PorA antisera and purified vesicles was shown by Double Diffusion Gel Technique. The results of immunological evaluation of the glycoprotein conjugate revealed a significant increase in serum bactericidal titre as well as ELISA titre against serogroup A meningococci after 56 day in comparison with the CPSA and OMV-PorA control group. Bactericidal and ELISA titre against serogroup B meningococci of the conjugate showed no significant difference in comparison with the OMV-PorA containing control.
Conclusion: The results indicate that the CPSA–OMV-PorA conjugate could be a candidate for bivalent vaccine toward serogroup A and B meningococci.

P580

Efficacious vaccination against serogroup C meningococcal disease by one shot at the age of 14 months in the Netherlands

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Objectives: To evaluate the efficacy of vaccination against serogroup C meningococcal disease in the Netherlands.
Materials and methods: Nation-wide vaccination of the population in the age group 14 months–19 years with the conjugate serogroup C capsule polysaccharide had been accomplished in the period June-November 2002 and one shot vaccination at the age of 14 months has been implemented in the national vaccination program since September 2002. Meningococcal isolates were characterised in the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) by serogrouping, serotyping, and sequencing of the variable regions of porA, encoding the PorA epitopes. The number of cases of meningococcal disease in the period January 2000–April 2002 (pre-vaccination period) was compared with that in the period January 2003–April 2005 (post-vaccination period).
Results: The number of cases of serogroup B meningococcal disease was 1004 and 638 in the pre-vaccination period and post-vaccination period, respectively. The number of cases of serogroup C meningococcal disease was 527 and 59 in the pre-vaccination period and post-vaccination period, respectively. Among persons with age between 14 months and 19 years the number of cases of serogroup C disease reduced from 332 cases in the pre-vaccination period to only three cases during the post-vaccination period. These three patients had not been vaccinated. The number of serogroup C isolates among persons younger than 14 months decreased from 35 to 18 cases and that among persons older than 18 years was reduced from 160 to 36 cases in the post-vaccination period. This reduction of cases of serogroup C disease among non-vaccines might be indicative for herd protection, although the bimonthly distribution of cases of serogroup C disease shows that the

decline started already before the introduction of the vaccine independent of the age group.
Conclusions: Since 2002, the incidence of meningococcal disease in the Netherlands is declining, which is partly caused by the natural fluctuation in the incidence of serogroup B as well as serogroup C meningococcal disease. Nevertheless, the vaccination against serogroup C meningococcal disease by one shot at the age of 14 month was very effective and contributed significantly to the decline in the incidence of meningococcal disease; after the introduction: of the vaccine, cases of serogroup C meningococcal disease were no longer observed among vaccines by the NRLBM.

P581

Streptococcus pneumoniae: proteomics of surface proteins for vaccine development

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Objectives: Currently, two formulations of pneumococcal vaccines are available, which prevents invasive disease in adults and children. However, these vaccines will not protect against the majority of *Streptococcus pneumoniae* serotypes. Highly conserved surface proteins as vaccines may circumvent this problem.
Methods: *S. pneumoniae* surface proteins were isolated after mutanolysin treatment and identified via the proteomics platform at ACE BioSciences. The technology applied includes one-dimensional and two-dimensional polyacrylamide gel-electrophoreses as well as an in-solution based strategy, in combination with mass spectrometry. Identified proteins were extensively screened in a process, including in silico, *in vitro* and *in vivo* validation criteria. As an example, target RNA was detected with RT-PCR in infected tissue and sequenced in different *S. pneumoniae* serotypes to assess the possible role during infection, the variability and the conservation amongst these serotypes. Finally five candidates were selected, expressed in *E. coli* and purified for immunisation experiments. Animal efficacy data was demonstrated in a mouse sepsis model, where mice were vaccinated with the candidate proteins and challenged with *S. pneumoniae* D39 strain. Immunogenicity was tested applying ELISA technology.
Results: We identified more than 280 *S. pneumoniae* surface proteins. Five proteins were selected as vaccine candidates. These proteins were detected in at least 40 different serotypes of *S. pneumoniae* and were expressed in *S. pneumoniae* during infection. Moreover at least two candidates showed protection in a sepsis animal model ($p < 0.05$ (*t*-test) for CFU/ml blood 6h after challenge with *S. pneumoniae* D39 compared with a group vaccinated with an unrelated protein)
Conclusion: We identified two promising *S. pneumoniae* vaccine candidates with the ACE BioSciences proteomics platform, which were validated *in vivo* in an animal sepsis model. These candidates will be excellent runners for novel protein based pneumococcal vaccines.

P582

Seropathotypes, adherence ability and inflammatory effect induced to cellular substratum by enteropathogenic *E. coli* strains isolated from aquatic environments

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Introduction: Enteric pathogens infecting the human gastrointestinal tract (e.g. *Salmonella*, *E. coli*, *Shigella*, *Yersinia*) rapidly upregulate the expression of a program of intestinal epithelial cell genes, the products of which chemoattract and activate populations of leucocytes that are important for the onset of host protective mucosal inflammatory responses. The type III secretion system of enteropathogenic *E. coli* (EPEC) strains has been associated with the ability to induce secretion of the proinflamatory cytokines, NO and antimicrobial peptides in cultured intestinal epithelial cells.
Purpose: Serotyping, screening for virulence markers, investigation of the *in vivo* adherence capacity to cellular substratum and evaluation of the *in vitro* inflammatory response induced to $CaCo_2$ epithelial cells infected with 12 aquatic *E. coli* strains.
Methods: Serotyping according to Kauffmann-White scheme; PCR-genotyping using eaeA, bfpA and eaf genes; *in vitro* study of adherence and invasion capacity to HeLa cells investigated by Cravioto's method; adherence to an inert substratum evaluated by the slime test; RT-PCR for the expression level of the genes encoding IL-8, GAPDH and hBD-2.
Results: All aquatic *E. coli* strains belonged to typical (eae + bfpA + eaf+ and eae + eaf + genotypes) and atypical (eae + bfp - eaf - genotype) EPEC of non-EPEC serogroups. The adhesion to the inert and cellular substratum proved to be a general feature of aquatic EPEC showing a localized/ diffuse adherence patterns that do not affect the cellular density of peritoneal liquid variable in composition in chronically infected mice. Bacterial production of CDF type exfoliante toxins and mitogenic induced effect to epithelial cells were observed. EPEC strains induced low expression levels for the IL-8, GAPDH and hBD-2 genes, according with the values obtained for IL-8/ GAPDH (0.161 minimal value) and hBD-2/GAPDH (0.325 minimal value) ratios.
Conclusion: Predominance in aquatic environments of EPEC strains with seropathovars different than the clinical EPEC strains and relatively distinct virulence profiles. Aquatic EPEC strains predominantly induce a low inflammatory effect on $Caco_2$ cells, which might be due to the involvement of different cytokines combinations. This study proved that aquatic media represents an important reservoir and source of dissemination or transfer for opportunistic pathogens and different virulence markers, including antibiotic resistance genes, with major implications in human pathology.

P583

Characterisation of intestinal lactobacilli as potential second-generation probiotics

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There is increasing interest in developing second generation probiotics (SGP), e.g. the application of *Lactobacillus* species as vaccine delivery vectors. The effect of probiotics is greatly influenced by their ability to persist in gut, a property that differs among various strains. Therefore, besides *L. casei* and *L. plantarum* that are most frequently used for transformation, more strains and species should be tested.
Objective: To characterize intestinal lactobacilli reflecting their potential use as SGP.
Methods: The study included 93 strains isolated from the faeces of healthy children. Strains belonged to the culture collection of the Department of Microbiology, University of Tartu, and included ten species: homofermentative *Lactobacillus acidophilus*, *L. crispatus*, *L. delbrueckii*, *L. salivarius*, and heterofermentative *L. paracasei*, *L. plantarum*, *L. brevis*, *L. buchneri*, *L. coprophilus* and *L. fermentum*. At first, all strains were tested for autoaggregation as adhesion marker, then 76

most promising strains were tested for resistance to low pH (pH 3.0; 2.5; 2.0) and bile content (2%). Thereafter, 21 strains with best characteristics were selected and tested for resistance to pancreatin (0.5%) and antibiotic susceptibility pattern (13 different antibiotics).
Results: 59% of strains were aggregating to some degree, with the strongest value among strains in *L. acidophilus* group. The lowest pH tolerated by lactobacilli was 2.5 (3 *L. acidophilus* strains), whereas 50% of strains (nine homofermentative and 29 heterofermentative strains) resisted pH 3.0. Nearly all strains were resistant to bile at a concentration that was six times of the physiological concentration and all strains were resistant to pancreatin at five times of the physiological concentration. Only two *L. plantarum* strains differed from the innate resistance pattern of lactobacilli, revealing resistance to tetracyclines due to a possible mobile genetic element.
Conclusions: Several human homo- and heterofermentative lactobacilli, in particular strains of *L. acidophilus*, *L. paracasei* and *L. fermentum*, possess properties required for their persistence in gut and could be used for the development of novel SGP.The study was supported by the Commission of the European Union (BIODEFENCE 508912).

P584

Immunological determinants of disease pathogenesis in Indian leishmaniasis

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Objective: Visceral leishmaniasis or Kala-azar (KA) is a severe systemic disease associated with suppression of cell mediated responses, fatal if not treated. The disease affects 12 million people and 350 million peoples are at risk. In India 5–15% of apparently cured KA patients develop an unusual dermal form of the disease termed Post Kala azar dermal leishmaniasis (PKDL). Characterization of the circulatory and localized immune responses was undertaken in Indian KA and PKDL patients to understand the disease pathogenesis.
Methods: We exploited Flowcytometry, reverse transcriptase PCR (RT-PCR) and microarray technology for characterization of immune response. Radiolabelled cDNA probe prepared from RNA isolated from tissue, was hybridized to nylon membrane gene array chip comprising of 268 cytokine/receptor genes. Clinical samples were collected from KA (serum, n = 35; tissue, n = 10) and PKDL (serum, n = 29; tissue, n = 25) and control (serum n = 18; tissue n = 6).
Results: Analysis of circulating cytokines using CBA kit showed significantly elevated levels of IFN-g, IL-6 and IL-10 during active KA and their restoration to control values at the end of treatment. In PKDL serum, TNF-a was found significantly elevated compared to KA or controls while other cytokine levels were comparable to controls. Estimation at tissue level by RT-PCR revealed significant elevation in message transcripts of above mentioned cytokines during active KA and PKDL as compared to control. Further to understand the broader picture of disease pathogenesis, we exploited the microarray technology for analysis of mRNA levels of 268 cytokine/receptor genes. The results showed altered expression of several cytokines, chemokines, receptors, CD markers and apoptotic genes. Up regulation of IFN-g, IL-6, IL-10 TNF-a and TGF-b was evident on the array. Interestingly, the expression of IFN-gR1 was found significantly lower in both KA and PKDL which was also validated with RT-PCR.
Conclusion: Data implicates the role of several cytokines in the pathogenesis of KA and PKDL. Elevated level of IFN-g coupled with low level of IFN-gR1 indicates how unresponsiveness to type1 stimuli prevails during active disease. High levels of serum protein and message level of TNF-a in PKDL patients suggest that investigation of the TNF locus in Indian Leishmaniasis patients is warranted since polymorphism at this locus has been associated with autoimmune and infectious diseases including Leishmaniasis.

P585

Association of polymorphism in genes encoding human innate immunity factors and persistent nasal *Staphylococcus aureus* colonisation

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Nasal carriage of *Staphylococcus aureus* provokes a neutrophil-mediated inflammatory host response resulting in elevated concentrations of anti-microbial peptides at the site of colonization. Polymorphism in, allele frequency of and genotype of genes encoding innate immune factors were determined in volunteers (n = 109) with a known *S. aureus* carriage status (37 persistent carriers, 22 intermittent carriers and 50 non-carriers). We determined polymorphism in the non-coding parts of the alpha- and beta-defensin genes and in the promoter- and coding regions of the mannose-binding lectin gene. An heterozygous genotype in HNP 1–3 was found to occur more frequently among persistent carriers (0.92) than among non-carriers (0.75) (P = 0.041). Carriers differed from non-carriers at the baseline level of HNP 1–3 peptide production as well (214 + 159 versus 108 + 82 μg/ml, P = 0.016). No significant association between HNP 1–3 production levels and polymorphism was documented. Allele frequency in HBD-1 and MBL genes does not affect *S. aureus* nasal colonization in humans. The MBL haplotype A was overrepresented in the persistent carrier group (P = 0.038). Our results: indicate that overall production of the HNP 1–3 peptides is associated with protection against *S. aureus* nasal carriage. Polymorphism in the other genes encoding innate immune factors or variation in allelic- or genotype frequency thereof did not affect the *S. aureus* nasal colonization status in humans.

P586

Interference with pathogenic bacteria in cystic fibrosis

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Objectives: To investigate the preventive effect of alpha-haemolytic *Streptococci* on recolonization of the airways with *Pseudomonas aeruginosa* in patients with cystic fibrosis (CF) and thereby reduce the number of treatments with antimicrobial agents.
Methods: Saliva samples from healthy children (n = 38), healthy adults (n = 24) and saliva (n = 10) and sputum (n = 20) samples from CF-children have been collected. The saliva samples were diluted and inoculated on selective agar plates for *Streptococci*. The sputum samples were inoculated undiluted on blood-agar plates. Streptococcal strains with different morphology were collected and isolated in pure cultures. In total 299 strains of alpha-haemolytic streptococci were identified and tested initially for inhibition of growth of clinical isolates of three strains of *P. aeruginosa*, one strain of *Stenotrophomonas maltophilia*, two strains of *Staphylococcus aureus*

and two strains of *Burkholderia cepacia*. Strains with inhibitory activity against at least three of the pathogens were kept for further analyses. Further tests were performed with an agar overlay technique to avoid interfering factors like the effects of pH. The pathogens used were 32 clinical isolates of *P. aeruginosa* from both non-CF and CF patients. To be able to identify the strains in clinical samples streptomycin resistance was induced.
Results: Strains of *Streptococci* with the strongest inhibitory effect on growth of pathogens common in CF-patients were isolated in healthy children and adults. Strains isolated in CF-patients had no or weak inhibitory effect. Of the eight strains with the strongest inhibitory capacity one was excluded because of an extreme production of extracellular dextran and two were further excluded because of difficulties in inducing streptomycin resistance. The remaining five strains were identified as *Streptococcus oralis*.
Conclusion: Strains of alpha-haemolytic streptococci have been identified with inhibiting effect on *P. aeruginosa* and other pathogens common in CF-patients. The *in vivo* interfering properties of the strains will initially be tested in a mouse model. The preventive effect of the strains will finally be evaluated in patients suffering from cystic fibrosis being intermittent colonized with *P. aeruginosa*.

P587

New insights into the role platelets in antifungal host defence

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Background: We observed that serotonin (5 HT) acts fungicidal against *Aspergillus* species and decreases fungal virulence *in vitro*. In humans, 5 HT is stored in platelets and the 5 HT concentration in granules is about 65 mM. These data and the coincidence of an increased infection rate and low 5 HT levels in certain diseases let us to examine the role of platelets in antifungal host defence.
Methods: We investigated the expression of platelets surface receptors (CD 62 P, CD 63 and CD 154) following *Aspergillus* (hyphae) exposition by fluorescent labelling. Platelets were mixed with fungi (ratio 100:1, 10:1) and incubated for 1 h at 37°C. Platelets activation and adherence on hyphae of *Aspergillus* sp. was investigated with a Zeiss DSM 950 scanning electron microscope (SEM). The XTT test was applied to assess platelets and platelets/neutrophils effects on viability. The influence on fungal growth was examined by assessing hyphal elongation. Clinical isolates of *Aspergillus fumigatus* (n = 2) and *Aspergillus terreus* (n = 2) were used for this study; each test was performed in triplicate and repeated three times.
Results: All *Aspergillus* spp. induced platelet activation as the CD 62P, CD 63 and CD 154 antigens were clearly induced and visualized by fluorescence microscopy; strain dependent expressions were observed. Spread of irregular shaped platelets over hyphal surfaces was shown by SEM. Platelets decreased the ability of hyphae to reduce XTT, co-incubation with polymorphonuclear neutrophils increased fungal damage significantly ($p < 0.05$). Hyphal elongation was significantly decreased (23 + 8 μm) by platelets in comparison to untreated hyphae (47 + 9 μm) after 12 h of incubation.
Conclusion: The impaired capacity of fungi to reduce XXT, the expression of membrane receptors and surface markers for platelet activation and the decreased hyphal elongation confirm that platelets have the potential to play an important role in host defence against *Aspergillus* species.

P588

The role of mannose-binding lectin in susceptibility to infection in premature neonates

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Background: Infection remains one of the most frequent clinical complications in premature neonates. This is in part due to suboptimal host defences. In the present study we have explored the impact of Mannose-Binding Lectin (MBL) genotype/phenotype on susceptibility to infection and outcome to sepsis.
Methods: MBL-exon 1 & promoter polymorphisms were studied by PCR followed by heteroduplexing.MBL serum protein levels were measured by ELISA. A variety of statistical tests were utilised as appropriate for each analysis. Wild type alleles were denoted as "A" and variant alleles as "O".
Results: 166 premature neonates (99 Polish, 67 British) were recruited. The genotype frequency for exon-1 mutations were as follows: -Polish: A/A 71%, A/O 25.5%, O/O 3.5%;-British: A/A 59%, A/O 38%, O/O 3%. Off all variables tested including sex, gestational age, birth weight, as expected genotype was the most important determinant of MBL levels. Gestational age and birth weight were found to influence MBL levels. MBL levels in neonates born <30 weeks & with birth weight <1500 g (VLBW) were lower than those of >30 weeks of gestation & birth weight >1500 g. MBL levels increased significantly during the first month of life ($p < 0.01$). Among the Polish neonates (n = 81) 68% were diagnosed with infection & 46% with sepsis. The proportion of cases with variant alleles (A/O, O/O) increased with severity of infection: 8% had localized infection, 18% had sepsis with 5 % having no infection. A trend for lower MBL levels was seen in neonates with infection (1680 ng/ml) vs no-infection (2228 ng/ml). In neonates with VLBW, the presence of a variate allele (13/51) significantly increased susceptibility to sepsis (12/13).
Conclusion: MBL polymorphism associated with low MBL levels appears to be associated with increased risk of infection & sepsis in preterm neonates.

P589

Immunoprophylaxis of wound and urinary tract infections in urological patients by application of immunostimulator Urostim

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Objective: To detect protective effect of oral polybacterial immunomodulator Urostim (U) for prophylaxis of wound and urinary tract infections (UTI) in urological patients
Method: An oral polybacterial immunomodulator composed of killed cells and their lysates from *Escherichia coli* expressing type 1 and R-pili, *E. coli* R mutant, *Proteus mirabillis*, *Klebsiella pneumoniae* and *Enterococcus faecalis* was used. Patients enrolled in study received orally 50 mg U daily for a period of three months.
Patients: Two groups of 65 urological patients with and free of UTI. Urostim was administered 15 days before and after the operation.
Results: The prophylactic effect of the U in terms of hospital acquired wound or UTI was recorded on the basis of comparative study with control groups with only antibiotic prophylactic. Obtained results showed Urostim immunoprophylaxis yelds positive results: in urological practice. In 75% of cases development of complications was prevented. Only in 14.7% of cases microbiological findings persisted. In the control group the number of complications and uncured cases was more than 3-fold higher and consisted 45% of the group.

Conclusion: The results provide evidence for positive immunomodulating effect of Urostim. Predominantly treat with antibiotics of UTI leads to the appearance of multiresistant strains, mainly gram-negative spp. Vaccine administration combined with antibiotic broadens the therapeutic opportunities in urologic patients and is alternative approach for the SSI prevention and control of UTIs such as mucosal vaccine and immunomodulator.

P590

Faropenem enhances the bactericidal activity of human neutrophils *in vitro*

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Objectives: Polymorphonuclear cells (PMNs) otherwise known as neutrophils are the body's first line of defence against bacterial infection. Some antibiotics appear to modulate PMN function *in vitro*. Post-antibiotic leukocyte enhancement (PALE) has been reported as a mechanism of increased bacterial clearance by PMNs *in vitro* due to sensitization of bacteria via pre-exposure to antibiotic. In a mouse thigh infection model faropenem (FAR) was previously shown to be 3- to 4-fold more active against *Streptococcus pneumoniae* in normal vs. neutropenic mice. The objective of this study was to test whether FAR initiates a PALE.
Methods: PALE was conducted using FAR at therapeutically achievable plasma concentrations (free drug) in *Haemophilus influenzae* as well as at sub-MICs for *Staphylococcus aureus*. FAR was removed via centrifugation and the bacteria were then introduced to isolated PMNs. Samples were taken at 0, 1, 2, and 4 hours during incubation and plated for bacterial viability. Comparator agents included amoxicillin, amoxicillin/clavulanate, and cefuroxime.
Results: FAR produced a positive PALE effect in all studies. Compared to the effect of PMNs alone, pre-treatment with FAR for 60 minutes followed by removal of the drug resulted in 6-fold greater reduction in viable CFUs by PMNs in *S. aureus*. In *H. influenzae* the viable colony count was reduced below the level of detection after 60 minutes in bacteria pre-treated with FAR and exposed to PMN; this is in comparison to untreated bacteria in the presence of PMN with only 18% reduction in CFUs. FAR treatment at 0.5X (sub-MIC) and 1X the MIC of *S. aureus* showed a reduction in CFUs of 1.5 to 2 log10 in comparison to the control.
Conclusions: A brief, non-lethal exposure of bacteria to FAR considerably enhanced the bactericidal activity of PMNs to both *S. aureus* and *H. influenzae*. Faropenem may enhance PMN mediated killing of selected organisms and may play some role in enhancing leucocyte mediated killing of bacteria. Since faropenem targets cell wall biosynthesis, sub-MIC concentrations of the drug may affect cell morphology that may contribute to enhanced killing by leucocytes. Together, these effects may explain, in part, the increased efficacy of FAR in normal vs. neutropenic hosts.

P591

Light microscopic appearances of resident macrophages in the adrenal glands of pregnant and non-pregnant guinea pigs, and their immuno-endocrinological significance

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Objectives: Macrophages are non-lymphoid immune cells with phagocytotic functions. Although some tissue-specific macrophages such as Kupffer's cells and microglia are well-known, any knowledge about the adrenal macrophages is not yet documented in the textbooks. For this aim, we conducted a light microscopic study to show resident macrophages of the adrenals.
Methods: Eight nonpregnant and eight pregnant guinea pigs were used. After sacrificing all animals, the adrenals were promptly removed. They were fixed in Bouin's solution, and dehydrated in ethanol, and embedded in paraffin. Sections were stained with haematoxylin-eosin (H-E) and periodic acid-Schiff (PAS), and examined by a light microscope.
Results: We found many macrophages primarily in the zona reticularis close to the medulla of the adrenals, especially adjacent to sinusoids. They have a stellate appearance due to cytoplasmic processes extending from the cell body. Their cytoplasm was stained yellowish with H-E, suggesting that they are rich in lipofussin pigment granules, and was PAS (+), indicating their excessive lysosomal content. We saw much more adrenal macrophages in the pregnant animals in comparison with the nonpregnants.
Conclusions: Macrophages have been recently thought as favourable cells for interactions of the neuro-endocrine organs with the immune system because they can also secrete cytokines. During the immune response, the whole organism is usually affected, as reflected by the neuro-endocrine and metabolic alterations. One of the effects of immune stimulation is elevation of glucocorticoid blood levels. As known, glucocorticoids are released from adrenocortical cells. Beta-adrenergic receptors have been characterized within the adrenal cortex, indicating its susceptibility to sympathetic stimulation. A regulatory function of medullar products on steroid secretion by the cortical cells may, hence, if present, be expected to be mediated in a paracrine manner. Cytokines released by macrophages, such as tumour necrosis factor (TNF)-alpha, interleukin (IL)-1 and IL-6, may influence adrenocortical steroidogenesis through medullary catecholamines. It was recently shown that IL-1 and TNF-alpha have a direct, ACTH-independent, stimulatory effect on corticosterone secretion in rats. So, macrophages may have a pivotal role in the bidirectional immune-adrenocortical communication within the adrenal. Finally, resident macrophages of the adrenals are waiting for being documented in the textbooks.

P592

Blood lymphocyte subsets in patients with tick-borne encephalitis

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Prediction of tick-borne encephalitis (TBE) clinical course is problematic usually. Therefore several immunological parameters are looked for severity prognosis in certain phases and clinical forms of TBE.
Aim: The aim is to study the changes of some immunological parameters in TBE patients and to provide additional prognostic information about course of pathological process.
Patients and methods: 119 patients with TBE treated at the Infectology Center of Latvia are included in this study. Diagnosis of TBE was confirmed by ELISA Enzygnost Anti-TBE IgM in blood and/or in cerebrospinal fluid. Detection of lymphocyte subpopulation in blood was performed by flow cytometry method. Traditional clinical criteria were used for detection of severity of TBE.
Results: The analysis of changes in the number of lymphocyte subsets in 70 patients with moderate TBE meningitis in

dynamics showed the following: a decreased level of T-lymphocytes (CD3): in acute stage 1122 ± 62 (contrary to reconvalescence 1750 ± 65; P = 100%); a decreased level of CD3/HLA-DR (activated T-lymphocytes) in acute stage 276 ± 27 (in reconvalescence 429 ± 32; P = 100%); a decreased level of HLA-DR (activated B-lymphocytes) in acute stage 313 ± 19 (in reconvalescence 376 ± 23; P = 99%); a decreased level of CD4 (helpers) in acute stage 683 ± 41 (in reconvalescence 1064 ± 48; P = 100%) and CD8 (suppressors) in acute stage 464 ± 30, contrary to reconvalescence 708 ± 35, P = 100%. The analysis of changes in the number of lymphocyte subpopulations in 34 patients with severe TBE meningoencephalitis in dynamics showed the following: a decreased level of T-lymphocytes (CD3) in acute stage 1081 ± 172, if compared with reconvalescence 1720 ± 128 (P = 99%); a decreased level of HLA-DR (activated B-lymphocytes) in acute stage 275 ± 31 (in reconvalescence 376 ± 39; P = 97%) and a decreased level of CD4 (helpers) in acute stage 585 ± 62 contrary to reconvalescence 1059 ± 93 (P = 100%).

Conclusion: Significant time-dependent changes of blood lymphocyte subsets during TBE course were observed, but no differences in lymphocyte subpopulations between moderate and severe TBE clinical forms were found.

P593

Evaluation of a new immunoassay to detect antibodies against *Treponema pallidum* in sera samples

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Syphilis can be transmitted sexually of through blood transfusion. The seriousness of this disease is the high risk of complications and congenital infections. Laboratory diagnosis is largely based on serological tests and the immunoassays are widely used as the syphilis screening because is highly sensitive and can easily automated. This study compares the performance of Architect Syphilis assay with a routine screening protocol. We have studied 420 sera samples with Syphilis antibody requested. The current protocol for to screening Syphilis antibodies in our laboratory includes an immunoassay for to detect total specific IgG + IgM antibodies (Enzygnost Syphilis, Dade Behring). Reactive samples were again tested by TPHA method (TPHA Syphilis, Biokit). The discrepant results are resolved by line-blot immunoassay (Innolia Syphilis, Innogenetics). The Architect Syphilis is an immunoassay for to detect anti-treponemal IgG + IgM antibodies integrated in the Architect i-2000 robot platform. One-hundred-ninety-nine samples were categorized as reactive following the screening protocol. All samples were reactive by Architect Syphilis. Seventeen samples were TPHA but confirmed by line-blot. These sera were reactive by Architect Syphilis but her median was 2.79, lower to 23.32, median in the TPHA reactive samples. Two-hundred-twenty-one samples were negative in the screening protocol. Only one sample was reactive by Architect and considered as false reactive, in contrast with Enzygnost Dade Behring found five samples false reactive. According to results Architect Syphilis has demonstrated a sensitivity, specificity, positive predictive value and negative value of 100, 99.5, 99.5 and 100. As conclusion is remarkable the excellent performance of the Architect Syphilis assay used as syphilis serological screening test. The speed and automation make it a recommended test in laboratories with high load in routine.

P594

Monocyte chemoattractant protein-1: role in the pathogenesis and progression of acute pyelonephritis in infants

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Objectives: There is an increasing body of evidence that Monocyte Chemoattractant Protein-1 (MCP-1) plays a major role in the pathogenesis of renal disease. MCP-1 is involved in the initiation and progression of tubulointerstitial damage and lately there is evidence in humans of correlation of the glomerular expression of MCP-1 with the degree of renal damage in inflammatory and non-inflammatory models of glomerular injury.

Patients and methods: We studied the role of MCP-1 in the pathogenesis of progression of acute pyelonephritis in 20 febrile infants (2–18 months of age). Initial DMSA scan of all patients revealed renal lesions in 12 (60%) patients. Follow-up scans, performed 6 months later in all of the 12 patients, showed reversible lesions in three of them and renal scars in nine. MCUG performed in all 20 patients, revealed significant vesicureteral reflux (Grade III) in 5 (25%) and mild reflux (Grade II) in two infants respectively. We measured plasma levels of MCP-1 with a LINCOplex Human Cytokine/chemokine kit using the Luminex xMAP technology, on admission of the patients and three days after initiation of treatment.

Results: MCP-1 was increased significantly in all patients at diagnosis of the urinary tract infection (312.0 ± 35.0 compared to 135.0 ± 28.0 pg/ml of controls), ($p < 0.001$). We compared the values of MCP-1 in patients with normal DMSA and in patients with renal lesions and we observed that MCP-1 either remained high or further increased in patients whose DMSA was abnormal. MCP-1 was not ''affected' by the initiation of treatment in this group of patients and especially in those patients who ended up with renal scarring in their follow-up scan. Similar results were found in the group of patients with the severe vesicureteral reflux. We also observed that MCP-1 decreased after treatment, although not in normal levels, in the group of patients whose DMSA scan was initially normal, as it also decreased in the small group of patients with the reversible lesions in the DMSA.

Conclusion: Our findings showed that MCP-1 levels were increased in all infants with febrile urinary tract infection and this increase was more pronounced in patients with true renal damage. Apparently, MCP-1 is more than just a chemoattractant. Apart from its experimentally proven role of MCP-1 as a direct elicitor of an inflammatory response induced by cytokines and adhesion molecules expressed in the kidney, may also play a clinical role in the prognosis of an acute renal damage.

P595

Vaccination delays maedi-visna lentivirus infection in naturally infected sheep flock

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Maedi-visna (MV), a slow lentivirus infection of sheep, is common in the Mediterranean region of Europe. A small field trial using inactivated whole virus vaccine with alun (Icelandic MV strain K796) was carried out in one naturally infected sheep flock in Cyprus. One female twin lamb in 30 female twin pairs was vaccinated, 1) at birth, 2) 3 weeks later, 3) at 3 months of age. The other twin served as unvaccinated control. After

vaccination all the twins were kept in the flock under natural conditions and bled regularly.
28 months later 11 of the 60 twins were seronegative, nine vaccines and two unvaccinated twins. Mothers of 13 twin pairs were seroconverting at the time of birth of the lambs. In eight of their twin pairs the unvaccinated twin seroconverted at 3–4 months of age but the vaccinated twin at 11–16 months. In three pairs both twins seroconverted at 3–4 months of age and in two pairs the vaccinated twin became infected earlier than its sibling. In 11 of the 17 twin pairs born by seronegative mothers the vaccinated twin was infected later than its unvaccinated sibling or not at all during 28 months. In four pairs the vaccinated twin was infected earlier than its sibling and in two pairs both twins seroconverted early in their 2 year of life.
The virulent Icelandic vaccine strain K796 is different from the low and slow MV strains isolated from this sheep flock. There is a slight cross-reaction between the local strains and the vaccine strain detectable in serological tests. Yet, in 19 of the 30 vaccines this vaccination apparently delayed and possibly in some cases prevented natural infection for 28 months in this heavily contaminated environment.

AIDS and HIV

P596

Free anonymous HIV testing sites are an opportunity to offer hepatitis B virus vaccination to high-risk non-immune patients

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Objectives: Prevention of hepatitis B virus (HBV) transmission relies partly on vaccination of high risk subjects which are numerous among patients attending Free Anonymous HIV Testing Sites (FAHTS). Our purpose was to assess the risk profile, vaccination history and serologic status of a representative sample of high risk patients attending a FAHTS in Paris.
Methods: A sample of 1016 anonymous patient files was randomly selected among 5169 files from patients having attended a FAHTS in Hôpital Bichat – Claude Bernard, Paris, in the year 2004. Sociodemographic profile, risk factors, vaccine history and serologic profile of these patients were depicted using descriptive statistics.
Results: Among 1016 patients, 450 (44.3%) had one or more risk factors for HBV infection and were hence tested for HBV: 171 females (38%) and 279 males (62%). Mean age (SD) was 29.2 (8.9) years. Their birth countries were France (58%), sub-Saharan Africa (17%), north Africa (11%), other European country (6%) and others (8%). HBV risk factors were: multiple sexual partners (62%), originating from high (20%) or medium (18%) endemic area, history of sexually transmitted disease (15%), professional exposure (8%), history of transfusion (3%) or intravenous drug use (2%). Nearly a third (31%) of these patients had a history of complete HBV vaccination, 7% reported an incomplete or ongoing vaccination, the remaining 62% had no known history of vaccination. HBV serology showed that 36% of these patients had natural or vaccine-induced immunity and 1.8% were HBs antigen carriers. Thus more than 62% of these high risk patients had no HBV immunity.
Conclusion: Patients with a high risk of HBV infection are numerous among attendants of FAHTS in French large cities. Nearly two thirds of these patients have no HBV immunity. Thus FAHTS consultations appear to be a good opportunity to identify these patients and offer HBV vaccination.

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Morphologic and molecular evidence of viral infection in human brainstem

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Objective: In the literature, majority of studies consider the diencephalic and telencephalic locations of viruses, with correlated histopathologic findings, while minor attention has been paid on brainstem, a structure involved in the regulation of autonomic functions. Aim of this study was to identify in the brainstem the genomic sequences of a series of neurotropic viruses (HIV, JCV, BKV, SV40, EBV, CMV, HHV-6, HHV-8, HSV-1, HSV-2) and to detect histopathologic findings ascribable to viral infection.
Methods: Brainstems of 25 subjects who died of acute opiate intoxication were studied through histologic and immunohistochemical (antibodies anti-CD68, -CD45, -CD3, -CD20, -CD8, -GFAP) stainings. In each case, Real-Time PCR search of viral sequences was performed. Histopathologic findings were compared between PCR-positive and -negative brainstems (Mann–Whitney Method) and among the different levels of section (one-way ANOVA and Newmann-Keuls test).
Results: In 11 out of 25 (44%) samples, sequences HIV proviral DNA were identified. In one of these HIV- positive samples, HHV-6 DNA was also found and in another sample BKV and SV-40 DNA were detected too. In 1 of HIV-negative samples a genomic sequence of HHV-6 was also found and in another sample a CMV sequence was detected too. Both HIV-positive and -negative brainstems showed the presence of oedema, perivascular or parenchymal inflammatory infiltrations and microglial proliferation, with sporadic microglial nodules. Perivascular infiltrations were more numerous and with major cellularity in HIV-positive brainstems (mean index of perivascular infiltration ± SD: 2,3 ± 0,9 vs 0,5 ± 0,6; $P < 0,01$) and were mainly composed of CD8+ T-cells. No differences were detected among the different levels of section.
Conclusions: Our results confirm brainstem tropism of HIV and HHV-6 and demonstrate, also for BKV, SV40 and CMV, the possibility of location in this district. Moreover, our study showed, as reported in the literature for diencephalic and telencephalic structures, that, also in brainstem of HIV-positive presymptomatic subjects, there are histopathologic findings due to location of the virus in the nervous tissue.

P598

Prevalence and risk factors of QTc interval prolongation in HIV-positive patients

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Objective: QTc interval prolongation is an electrocardiographic (ECG) abnormality that may cause severe arrhythmias including torsades de pointes and ventricular fibrillation. Many drugs administered to HIV-positive patients and, according to some Authors, the HIV infection itself, can induce QTc interval

prolongation. The aims of our study were to calculate the frequency of QTc prolongation in a cohort of 402 HIV-infected outpatients and to identify the risk factors associated with this ECG abnormality.

Methods: In 2004, at our Infectious Disease Unit, we performed ECG recording of 402 consecutive HIV-infected subjects followed up in our outpatient clinic. For each subject the following data were collected: demographic features, risk factors for HIV infection, ongoing therapy and prophylaxis, cardiologic risk factors, CD4+ cells count, HIV viraemia and the results of the main haematochemical tests. ECG was evaluated by two independent cardiologists. QTc was calculated as QT/RR1/2. A ''nested'' case-control study was performed using as cases those patient with QTc >0.44 sec (males) or >0.46 sec (females) and as controls (1:4) HIV infected subjects matched by gender and age (more or less 5 years). The association between QTc interval prolongation and potential risk factors was evaluated in terms of Adjusted Odds Ratio (AOR), with their 95% Confidence Intervals (CI), by using a multivariable logistic model.

Results: In our cohort, ECG abnormalities were recorded in 225 out of 402 (56%) subjects. Twenty-two subjects (5.4%), 17 males and 5 females, showed a QTc interval prolongation. At multivariate analysis, the following variables were associated with the presence of a long QTc: use of Cotrimoxazole as prophylaxis against *P. jiroveci pneumonia* (AOR 6.20, C.I. 95% 1.91–20.12), Efavirenz as part of the current antiretroviral regimen (AOR 3.28, C.I. 1.07–10.03), and the presence of a serum triglyceride level <220 mg/dL (AOR 5.91 C.I. 95% 1.46–24.00).

Conclusions: According to our data, QTc interval prolongation is associated with the use of drugs commonly administered to HIV-infected patients, such as Cotrimoxazole and Efavirenz. The role of low serum triglyceride level needs further investigation. Clinicians should perform a strict cardiologic follow-up of HIV infected subjects in those situations that may potentially result in QT prolongation and life-threatening arrhythmias.

P599

Prevalence of paediatric septicaemia in a tertiary hospital in Tanzania and the impact of aetiology, antimicrobial susceptibility and HIV co-infection on clinical outcome

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Objectives: Septicaemia is a common cause of hospitalization, morbidity and death in children. Septicaemia caused by drug-resistant organisms is difficult to treat. This study assesses the prevalence of septicaemia in children at Muhimbili National Hospital, Dares Salaam, Tanzania, and the impact of microbial aetiology, antimicrobial susceptibility and HIV co-infection on clinical outcome.

Methods: In a prospective cohort study from August 2001 to August 2002, we investigated 1828 consecutive admissions of children aged 0–7 years with signs of systemic infection. Blood cultures were obtained, bacterial isolates identified by standard methods and susceptibility tested by the disk diffusion method. Blood was taken for malaria and HIV testing. HIV positive antibody tests in patients younger than 18 months were verified by determination of HIV-1 RNA. Clinical data were obtained from standardized questionnaires, patient records and departmental records.

Results: Septicaemia with bacteria or fungi was confirmed by culture in 13.9% (255/1828) of children with symptoms of systemic infection. The most frequent isolates were *Klebsiella pneumoniae* (n = 48), *Escherichia coli* (n = 36), various *Salmonella enterica* serotypes (n = 37), *Staphylococcus aureus* (n = 20), *E. faecium* (n = 17), *E. fecalis* (n = 15) and *Candida* spp. (19). Malaria was found in 21.3% (257/1204) of those tested, including 32 patients with concomitant septicaemia. Malaria was associated with lower case-fatality rate (18.9%) than septicaemia (34.2%) and particularly gram-negative septicaemia (42.8%). Multidrug-resistance, including extended-spectrum beta-lactamase phenotype was common in gram-negative isolates and associated case-fatality rates in excess of 70%. HIV infection was present in 17.3% (157/909) of patients tested. In a multiple regression model, recovery of gram-negative bacteria (OR: 3.5 95%CI 2.1 to 5.9), ESBL phenotype (OR: 5.5, 95%CI: 1.4 to 21.9) and HIV co-infection (OR: 2.1, 95%CI: 1.4 to 3.2) were independently associated with fatal outcome.

Conclusion: While malaria appears to be a slightly more common cause of systemic infection in children in this Tanzanian hospital, septicaemia carries a significantly higher case-fatality rate, partly because of widespread multidrug-resistance, particularly in Gram-negative bacteria.

P600

Microarray analysis during adipogenesis identifies new genes altered by antiretroviral drugs

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Objectives: Aim of the study was to elucidate the pathogenesis of highly active antiretroviral therapy (HAART)-associated lipodystrophy, by investigating the effects of antiretroviral drugs on adipocyte differentiation and gene expression profile.

Methods: 3T3-L1 preadipocytes, a widely used *in vitro* model of adipogenesis, were induced to maturation into adipocytes and treated with nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs). Adipocyte differentiation was studied by Oil Red O staining, whereas gene expression profile was evaluated by DNA microarrays and quantitative RT-PCR analyses.

Results: Under standard adipogenic differentiation protocols, PIs significantly inhibited adipocyte differentiation, as demonstrated by cell viability assay and Oil Red O staining and quantification, whereas NRTIs had mild effects on adipogenesis. Gene expression profile analysis showed that treatment with NRTIs modulated the expression of transcription factors, such as Aebp1, Pou5f1 and Phf6, which could play a key role in the determination of the adipocyte phenotype. PIs also modulated gene expression toward inhibition of adipocyte differentiation, with up-regulation of the Wnt signalling gene Wnt10a and down-regulation of the expression of genes encoding master adipogenic transcription factors (e.g., C/EBP-alpha and PPAR-gamma), oestrogen receptor gamma, and adipocyte-specific markers (e.g. Adiponectin, Leptin, Mrap, Cd36, S100A8).

Conclusions: This study indentifies new genes modulated by PIs and NRTIs in differentiating adipocytes. Abnormal expression of these genes, which include master adipogenic transcription factors and genes involved in lipid metabolisms and cell cycle control, could contribute to the understanding of the pathogenesis of HAART-associated lipodystrophy.

P601

Indeterminate HIV Western blot profiles: how may we proceed?

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Objectives: Western Blot (WB) is the most widely accepted confirmatory assay for detecting HIV antibodies. Indeterminate WB reactivity occurs in certain individuals. Many diagnosis criteria's to interpret WB tests have already been proposed, among which three are mainly applied worldwide: World Health Organisation (WHO), Center for Disease Control and Prevention (CDC) and American Red Cross (ARC) criteria's. WHO criteria's are used at our institution. We conducted a review study to check if applying CDC and ARC criteria's to subjects with indeterminate HIV profiles would help us to better interpret their status and to try determining alternative means in cases of uncertainty.
Methods: We reviewed retrospectively the charts of all patients tested for HIV between February 2000 and May 2004 at our institution and having indeterminate WB results (Biorad) and re-interpreted their profiles according to ARC and CDC criteria's.
Results: 16 patients were identified, 6 women, 10 men, 15 originated from central Africa, mean age was 31 years. Two pregnant women were given an ARV treatment for prevention of potential mother to child transmission of HIV. All had incomplete patterns on WB according to WHO criteria's making HIV status indeterminate. Applying CDC and ARC diagnosis criteria's to re-interpret their WB results showed obvious discrepancies: eight patients showed positive profiles, six (37.5%) according to CDC and three (18.75%) according to ARC, (one patient was positive by ARC and CDC simultaneously). Eight patients had positive ELISA screening tests (Biorad/BioMerieux - Vidas) for HIV with non conclusive WB patterns by any of the three criteria's. Among bands screened-for on WB, gag and env gene's products reactivity was frequently encountered compared to pol gene's products reactivity. All patients had negative HIV RNA screening in blood when tested initially and none proved to be positive on further long term follow up testing.
Conclusions: Applying CDC and ARC criteria's to re-interpret indeterminate HIV WB profiles, according to WHO, showed tremendous discrepancies and didn't prove to be a convenient alternative to advance work up in our experience. We think it is advisable to at least screen for plasma HIV RNA in such cases, a negative result being a solid argument against any potential HIV infection and to insist on necessity of long term follow up.

P602

Best practices in model-based HIV drug development

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Over 20 HIV drugs are now approved by the FDA, and dozens more have reached at least early stage clinical development. In HIV, as in other therapeutic domains, the path to market for successful compounds is long, costly, and sometimes inefficient. Elias Zerhouni, MD, Director of NIH, has set Translational Research as a priority. Many NIH institutes have formed offices and programs to accelerate progress from discovery to market. FDA believes that ''… A new product development toolkit– containing powerful new scientific and technical methods such as animal or computer-based predictive models, biomarkers for safety and effectiveness, and new clinical evaluation techniques– is urgently needed to improve predictability and efficiency along the critical path from laboratory concept to commercial product.'' Pharsight has conducted over 20 HIV projects using model-based drug development techniques over the past seven years, in all development phases and covering critical issues in trial design, dose selection, trial sequencing, and development strategy. This experience covers most mechanisms of action including protease inhibitors, NNRTIs, NRTIs, and two novel mechanisms.
This presentation reviews best practices in the application of tools for providing quantified insight on HIV drug development decisions. Case examples will show how to integrate relevant sub-models, estimate key parameters from trial data, and simulate candidate trial designs. Key parameters critical in HIV include patient adherence or compliance to the prescribed regimen, compartmental pharmacokinetic parameters, viral inhibition (*in-vivo* IC50), virus and immune cell characteristics (depending on the patient population), and trial characteristics such as dropout rates. HIV disease models of varying complexity will be discussed, together with the presenter's views of the advantages and disadvantages of complex vs. simpler models. HIV models typically include at a minimum uninfected cells, actively infected cells, latently infected cells, and multiple viral strains. Differential equations describe the virus-cell interaction over time, including resistance development.
The goal of this presentation is to give the audience an improved ability to use model-based drug development techniques to accelerate HIV development decisions.

P603

Primary pneumocystis infection in children hospitalised with acute respiratory tract infection

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Objectives: A serologic response to *Pneumocystis jiroveci* (P.j.) develops in most persons during childhood. The purpose of this blinded, retrospective study was to determine the prevalence of P.j. harboured in the respiratory tracts of children with acute respiratory tract infection, and whether clinical and laboratory characteristics separate those with and without P.j.
Methods: Nasopharyngeal aspirates (NPA) collected from children hospitalized at Hvidovre or Amager Hospitals from 1999 to 2002 were included. 461 NPA from 423 patients with 432 episodes were available for analysis. One HIV-infected child with PCP was excluded. 64% of the episodes received a diagnosis of lower respiratory tract infection (LRTI), 28% of upper respiratory tract infection (URTI), and 8% of ''other''. The median age was 112 days (range 7–4430), and 52.7% were male. Samples were analysed using a closed-tube quantitative real-time PCR, using fluorescent probes for detection. Enzymatic carry-over prevention and external and internal controls were included. Positive samples were re-run for confirmation. Clinical data were collected by review of medical records.
Results: The patients tested positive for P.j. in 68 episodes (16%) No differences in sex distribution [(36/224) male vs. (31/201) female] or RSV infection [(30/195) RSV + vs. (38/234) RSV-] were observed. 96% of the P.j.-positive cases occurred in patients in the inter quartile age range (50–265 days). Infants aged 50–112 days (2nd age quartile) were 47 (OR, CI 11–203) times more likely than children younger than 50 days (1st age quartile) to

harbour P.j., and infants aged 112–265 days (3rd age quartile) 8.7 (CI 1.9–40) times more likely by multivariate analysis. Infants hospitalized with an episode of URTI were 2.0 (CI 1.05–3.82) times more likely to harbour P.j. than infants with a LRTI by multivariate analysis. The copy numbers detected in P.j.-positive samples are normally distributed after logarithmic transformation suggesting one biological phenomenon.
Conclusion: We found a prevalence of P.j. of 16% among children admitted with symptoms requiring a diagnostic NPA. This could represent either colonization or sub-clinical or overt infection. To our knowledge, there is no previous evidence of clinical illness or specific symptoms in connection with the primary infection in immunocompetent children. Our data suggest that P.j. may present itself as a self-limiting URTI in infants, and that the infection is acquired early in life.

P604

Increasing reports of gynecomastia among HIV-infected patients treated with highly active antiretroviral therapy. Epidemiological and clinical correlates, and suggestions for pathogenetic investigation

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Background: Gynecomastia (G) is an emerging untoward event in patients treated with HAART.
Methods: Through a cross-sectional study performed on around 1000 HIV-infected patients (p) treated with antiretrovirals at our reference centre, we identified all cases of G related to the administration of at least 12 consecutive months of HAART, to assess possible correlations of G with a spectrum of clinical, laboratory, and therapeutic variables (and including all adverse effects of HAART itself). All p with true G (as distinguished from lipomastia by an ultrasonography assay) were considered evaluable, while p with other predisposing conditions (endocrine disease, alcohol abuse, liver cirrhosis, and use of drug possibly predisposing to G), were carefully ruled out.
Results: Twenty-one out of 616 evaluable HIV-infected male p (3.4% of our population), developed a true G when aged 12–58 y. Seven p of 21 never received protease inhibitor (PI)-containing therapies, while efavirenz-based regimens apparently prompted G in 7 p who were naïve for PI, and worsened this disturbance in 3 further p who abandoned PI for efavirenz. Considering nucleoside analogues (NA), 2 p developed G during treatment conducted with dual isolated NA. Comparing the different adminstered NA, stavudine seemed to be the most commonly used compound, also taken for the longest time ($p < 0.01$). A complete hormonal workup did not detect significant abnormalities, save in one p, who had slight FSH, LH, and testosterone anomalies (with normal prolactin levels). When considering the eventual correlation with the most common HAART-induced disturbances, some forms of lipodystrophy was concurrent in all the 21 p with G, while hypertriglyceridemia, hypercholesterolemia, and hyperglycemia were found in 15, 9, and 3 p, respectively. During the subsequent 12–36-month follow-up, a spontaneous ameliorement of G was never observed, notwithstanding eventual HAART modifications. Due to local hypersthesia, two p resorted to surgery.
Conclusion: G is probably an underestimated problem in the setting of HAART. The frequent association of G with other HAART-related dysmetabolism suggests a possible common pathogenetic causes.

P605

Insulin resistance and glucose intolerance in HIV-infected subjects during their protease inhibitor treatment: three oral hypoglycemic drugs in comparison

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Introduction: HAART-related dysmetabolic alterations recently emerged in their frequency and clinical correlates. When considering protease inhibitor (PI)-treated patients (p) a high prevalence (30–80%) of insulin-resistance and hyperinsulinemia have been found versus a lower (<10%) incidence of altered glucose tolerance or frank diabetes.
Methods: Aim of our randomized, prospective study is to assess the frequency of hyperglycemia in p treated with HAART, and the efficacy-safety of gliclazide versus metformin and versus rosiglitazone in p with altered glucose metabolism. Two hundred and 89 p who started a PI-based HAART regimen from years 1998 to 2002 were prospectively followed for >12 months. All evaluable p had a fasting glycemia repeatedly normal before starting their novel HAART: 32.5% of p were antiretroviral-naïve. During the entire study period all p with hyperglycemia persisting for >6 months and resistant to a >3 month dietary-exercise program, were randomized to receive gliclazide (80 mg/ day), metformin (500 mg twice daily) or rosiglitazone (4 mg/day) and were followed-up for >12 months.
Results: After the first 12 months, elevated serum fasting glucose levels were found in 36 of 289 evaluable p (12.5%). During the follow-up period, glucose abnormalities were often mild in severity in 28 p (110–140 mg/dL), followed by moderate severity in 8 p (140–200 mg/dL) while a severe hyperglycemia (>200 mg/dL) was never observed. In 36 p hyperglycemia was associated with the finding of elevated C-peptide levels (mean value 7.4 ng/mL) glycosilated haemoglobin (mean value 9.7%), and hypertriglyceridemia (mean level 238 mg/dL). Other significant associations included a proportionally advanced age (over 55 years), a BMI above 28, a more prolonged overall exposure to antiretrovirals (and HAART regimens) and a more advanced HIV disease, while no significant correlations emerged between the abnormalities of glucose metabolism and the use of each single anti-HIV compound. After >12 months of gliclazide, metformin, or rosiglitazone therapy, a mean drop of mean glycemia of 27.2 ($p < 0.02$), 24.1 ($p < 0.02$), and 30.3 mg/dL ($p < 0.02$) respectively, was observed versus baseline levels, in absence of significant difference among the three tested drugs.
Conclusion: A PI-based HAART may be related to a moderate, but non-negligible risk of hyperglycemia-hyperinsulinemia. Oral hypoglycemizing drugs (either gliclazide, metformin, or rosiglitazone), proved equally effective in our preliminary experience.

P606

Advanced, lethal acute myelogenous leukaemia during HIV disease favourably managed with HAART

R. Manfredi, S. Sabbatani, F. Chiodo *(Bologna, IT)*

Introduction: Extremely infrequent episodes of HIV-associated acute myelogenous leukaemia (AML) were described.
Case report: A 49-year-old HIV-infected patient (p) suddenly developed a dyshomogeneous isoechogenic tender left thyroideal mass associated with dysphagia, fever and the

appearance of an AML-suggestive peripheral blood smear, concurrent with increased LDH levels. HIV infection was controlled by an HAART regimen (viral replication suppressed; CD4+ count 653 cells/μL). A thyroideal fine needle aspirate detected numerous blasts. Bone marrow studies disclosed a 46 XY, inv (16)(p13q22) AML (12 methaphases). The immunophenotypic analysis showed ~75% of cells expressing monocyte receptors CD14, CD86, and myeloid markers CD13-CD33, leading to a diagnosis of acute M5 AML (FAB staging). A CT scan disclosed multiple upper pulmonary metastases. A 3/7-day cytarabine-daunorubicin course was delivered together with growth factors, blood-platelet transfusion, antimicrobial prophylaxis and HAART. A neutropenia-related *S.haemolyticus*-Corynebacterium JK febrile bacteremia occurred 3 weeks later and was controlled by antibiotics-antifungals. A remarkable improvement of general-local signs-symptoms paralleled the complete normalization of blood cell count in 3 weeks. Ten weeks after chemotherapy a marrow aspirate confirmed disease remission so that a consolidation cytarabin-daunorubicin cycle was delivered. Again, chemotherapy-associated leukopenia-thrombocytopenia were corrected and empiric levofloxacin-fluconazole avoided superinfections. A remission was maintained during 10 months, when peripheral blood cell count recovered, HIV disease remained stable and a repeated marrow aspirate, karyotype study (30 metaphases) and immunophenotypic examination proved normal, although a chromosomal translocation remained.

Conclusions: Less than 40 cases of HIV-associated AML were described, the majority with a monocyte-myelomonocyte phenotype. Type M5 AML represented <10% of cases and the outcome was strongly related to CD4+ count. The advanced (FAB stage M5) and the initial, massive thyroid involvement of our p have no literature analogues to the best of our knowledge and should prompt differential diagnosis with intrinsic, opportunistic or neoplastic thyroideal disorders. Besides the lack of consensus on AML prognostic factors in the general population and the absence of controlled trials to guide decision-making in the HIV setting, however an induction chemotherapy associated with HAART appears indicated in HIV-infected p with AML.

P607

Isolated, dual nucleoside analogue antiretroviral therapy in the year 2005. Frequency, reasons, significance and outcome of this persisting phenomenon, nine years after the introduction of HAART

R. Manfredi, L. Calza, F. Chiodo *(Bologna, IT)*

Introduction: No controlled data are available about the long-term evolution of patients (p) on isolated dual nucleoside/nucleotide analogue (NA) antiretroviral therapy, although this phenomenon still exists in daily clinical practice, notwithstanding that antiretroviral guidelines did not include these regimens since 1997 due to their demonstrated suboptimal potency.

Methods: At our reference centre, p still on a dual NA treatment are nearly 15% of the 1041 treated ones.

Results: Of 152 p on dual NA therapy since at least 12 months, the majority receive zidovudine-lamivudine (30.3%) followed by lamivudine-stavudine (27%), zidovudine-didanosine (21.7%), didanosine-stavudine (13.8%), lamivudine-tenofovir (5.9%). The majority of these p (118 of 152:77.6%) never received an HAART regimen, while the remaining 34 p came from a triple therapy, usually refused after adverse events or intolerance and strongly denied the adjunct of another (''third'') anti-HIV drug. During the last 12 months of observation, their viremia ranged from undetectable to 8.9×104 HIV-RNA copies/mL (mean 8.6×103 HIV-RNA copies/mL), with a CD4+ lymphocyte count ranging from 212 to 978 cells/μL (mean 413.9 cells/μL). No clinical signs-symptoms of HIV disease progression became apparent during this prolonged, isolated dual NA therapy.

Discussion: A stable HIV disease course under long-term isolated dual NA therapy is consistently observed nine years after the introduction of HAART regimens, based on at least three different antiretroviral compounds. Should long-term non-progressor p received a ''not-proper'' anti-HIV therapy in early nineties, or the proportionally low efficacy of dual isolated NA was sufficient to contain disease evolution and also resistance development, however the great majority of p who still receive this ''obsolete'' antiretroviral therapy do not have the requirements for introducing an HAART based on three different drugs, based on virological and immunological markers of the 2005 updated guidelines of antiretroviral therapy, so that they are prone to continue their ''out-of-the-scheme'' regimens. In absence of controlled trials, most of raised questions remain unanswered, and the long-term balance among HIV infection, immunologic-genetic background and the even suboptimal effect of two NA, probably does not require treatment intensification.

P608

The two non-nucleoside HIV reverse transcriptase inhibitors: significantly different dysmetabolic profile between efavirenz and nevirapine

R. Manfredi, L. Calza, F. Chiodo *(Bologna, IT)*

Background: Dysmetabolism is an emerging feature of treated HIV infection, but poor information exists about non-nucleoside HIV reverse transcriptase inhibitors (NNRTI) use.

Methods: Among 1018 patients (p) treated with HAART for >12 months, the metabolic pattern of NNRTI was assessed in three different scenarios. The first one included p naïve to all drugs, starting a NNRTI-based regimen; the second one included a broad spectrum of p pre-treated with 2–10 therapeutic lines, but still NNRTI-naïve; the third group represented p who added for the first time a NNRTI while undergoing salvage regimens including >4 drugs, including PI.

Results: 324 p treated with efavirenz (E) were compared with 299 p taking nevirapine (N) in our prospective observational survey lasting 6–24 months, by a multivariate analysis of serum lipid-glucose levels, and other metabolic anomalies. Among the 183 p naïve to antiretrovirals, hypertriglyceridemia was more common ($p < 0.001$) in the E versus the N group. When considering the 295 experienced p who introduced a NNRTI for the first time, the frequency of hypertriglyceridemia appeared greater in the E group ($p < 0.0001$), with early development in p on E versus N ($p < 0.0001$). Also in the 145 p on a salvage HAART, the rate of hypertriglyceridemia-hypercholesterolemia-hyperglycemia tested greater among p treated with E versus N ($p < 0.02$–$p < 0.006$), and the time to peak alterations was earlier in the E group. Comparing the 324 p receiving E with the 299 p on N, the frequency of high triglyceride-cholesterol-glucose levels was greater in E-treated p ($p < 0.0001$–<0.0004). Some grade of lipodystrophy was present in 207 pre-treated p, but some improvement occurred after NNRTI introduction in seven p only of the E group, versus with 25 p on N ($p < 0.0006$).

Conclusions: A prolonged follow-up shows that E may not resolve (or might prompt) dysmetabolism. The two available NNRTI have a notably different toxicity profile. The pathways of the different NNRTI dysmetabolic patterns deserve in-depth pathogenetic studies.

P609

Opportunism related to a late, first AIDS diagnosis. Paradoxical increasing frequency at the time of HAART

R. Manfredi, L. Calza, F. Chiodo *(Bologna, IT)*

Background: Notwithstanding the availability of HAART, AIDS notifications continue to occur, with increasing prevalence for patients (p) who missed-neglected their underlying condition.
Methods: All AIDS cases notified since the year 2001 were compared with those found in the decade preceding HAART availability (1986–1995).
Results: Compared with the pre-HAART era, the drop of frequency of overall AIDS cases occurred: from a mean 58.3 ± 11.2 p-year in the decade 1986–1995, to 17.1 ± 7.2 p-year from 2001 ($p < 0.001$), together with an increased mean age ($p < 0.003$), female gender ($p < 0.02$), sexual versus i.v. transmission ($p < 0.001$), and proportion of immigrant p ($p < 0.03$). In the HAART era, the most evident drop of frequency interested opportunistic diseases linked to a CD4+ lymphocyte count <50–100 cells/μL, while a proportional rise of tuberculosis, pneumonia, lymphomas, and other neoplasms was observed. Both *Candida esophagitis* and *P.cariniii* pneumonia remained steadily the first two notified AIDS-related conditions. After HAART availability, the following diagnoses were neurotoxoplasmosis, wasting syndrome, and AIDS-dementia complex. P with multiple AIDS-defining diseases, and also AIDS diagnoses made at death, even showed a paradoxically increased frequency and absolute number during the HAART era versus the prior decade ($p < 0.001$ and <0.02), while no difference was found as to HIV-associated immunodeficiency. Surprisingly, an underlying anti-HIV therapy was a more common event until 1995, versus the HAART era ($p < 0.001$), since during recent years AIDS notification tends to be increasingly associated with the first HIV infection diagnosis.
Conclusions: When facing p with suspected opportunism, clinicians should maintain an elevated suspect for an advanced (but missed or untreated) HIV disease. A continued attention will help a more rapid recognition and an appropriate management of p who did not benefit from HAART, since they remained unaware of (or removed) their underlying disease.

P610

Immigration and HIV infection in North-Eastern Italy. Inpatient admissions, 2000–2004

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Background: Immigration is a recent phenomenon in Italy, caused by the sudden arrival of waves of foreign citizens, refugees, and individuals escaping from civil war. This phenomenon is of great concern due to its socio-economic, cultural, and health care impact.
Methods: A prospective survey of all charts of patients (p) hospitalized or followed on day-hospital (DH) basis at our Infectious Disease ward until end-2004, allowed us to assess the frequency of admission of immigrants from extra-Western Europe (eWE), and to analyze multiple variables related to their epidemiological and clinical features.
Results: The rate of p immigrated from eWE showed a significant increase among our inp, and at a lesser extent and later for DH admissions: 7.7% and 3.1% during year 2000, 10.1% and 4.6% in 2001, 13.2% and 6.2% in 2002, 17.9% and 7.9% in 2003, up to 21.3% and 8.9% in 2004 ($p < 0.0001$ for inp; $p < 0.008$ for the DH p). Around 60% of p came from Africa, followed by Eastern Europe, Asia, and America. When comparing the admission features of WE citizens with those of p coming from abroad, no differences were found as to duration-intensity of assistance, with HIV disease prevailing among regular admissions (37.2%), and DH access (39.1%), followed by acute-chronic hepatitis, CNS and respiratory tract infection, and STD. HIV-infected immigrants were frequently (>62%). AIDS presenters, and <5% of these p were already on anti-HIV therapy. While the frequency of HIV-associated admissions did not show differences in the considered six-year period, p from eWE had an increasing frequency of tuberculosis, skin-soft tissue infection, exanthems, gastroenteric-parasitic diseases, and malaria (from $p < 0.05$, to <0.0001).
Conclusions: An informed screening and a continued monitoring of this phenomenon are strongly warranted, to improve a sustainable social-cultural network, to plan health resource allocation, and to define adequate and targeted prevention measures.

P611

Management of hyperlipidaemia related to antiretroviral therapy with the novel statin rosuvastatin: a pilot study

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Background: A hypolipidemic drug therapy becomes recommended in HIV-infected patients (p) when HAART-associated hyperlipidemia is severe or tends to persist for a long time, although the selection of single compounds is often difficult, because of potential drug-drug interactions, including anti-HIV compounds.
Methods: Aim of our prospective open study is to assess efficacy and safety of a novel potent statin (rosuvastatin, at 10 mg/day) in the therapy of HIV-infected p treated with HAART, who had an elevated hypercholesterolemia lasting for six or more months, not responsive to a hypolipidemic diet and an anequate physical exercise program. An interim analysis was planned after 24 weeks.
Results: Seventeen p have been enrolled, and were followed for at least 24 weeks until now. At the end of this observation period, a mean reduction of serum triglyceride levels of 21.7% (range 14.6–30.4%), and 30.1% for cholesterol (range 18.5–35.4% was achieved, compared with baseline values; $p < 00.01$). A significant drop of mean LDL cholesterol levels was also obtained (- 22.4% versus baseline; range 15.8–34.9%), as well as an increase of HDL cholesterol (+28.5%; range 17.6–372%) ($p < 00.01$).
Conclusion: In comparative clinical studies, the novel potent statin rosuvastatin demonstrated a hylipidemic activity significantly greater compared with that of atorvastatin, simvastatin or pravastatin, associated with more contained risks of pharmacological interactions with all molecules metabolized by the liver cytochrome P450 (including HIV protease inhibitors). In our preliminary experience, rosuvastatin proved effective in the management of HAART-related mixed dyslipidemia, with a spectrum of favourable activity extended to LDL and HDL cholesterol levels, in absence of untoward clinical and/or laboratory adverse events.

P612

Increased frequency of syphilis among patients who remained unaware of their underlying HIV infection

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Introduction: After the early years of HIV pandemic, sexually-transmitted diseases (STD) recently increased their frequency also among patients (p) with HIV, who had their life expectancy and QoL significantly improved by the HAART availability.
Methods: Through a retrospective survey of all clinical-microbiological data, 72 p co-infected with HIV and syphilis were identified from 1999 to August 2005 (6 y, 8 mo). Some epidemiological, clinical and laboratory data were compared between p diagnosed in the 1999–2002 period versus the last 20 months (2003–2005).
Results: Among the 72 episodes of HIV-syphilis co-infection, 46 occurred in the period 1999–2002,and 26 from 2003 to August 2005. Assessing these last 25 p,7 (26.9%) had a primary lues (one p), a secondary syphilis (3), a latent disease (2), or a tertiary lues (one p), all recognized before HIV infection, which was diagnosed during their follow-up. In the same period (2003–2005), 19 further cases of syphilis were found in p who were already aware of their HIV seropositivity. Considering the preceding 1999–2002 period, all the 46 p had lues diagosed together with a known HIV disease ($p < 0.0001$). Comparing the 46 episodes diagnosed in 1999–2002 with the 25 cases occurred since 2005, a significantly increased frequency ($p < 0.04$), an increased HIV sexual transmission ($p < 0.0001$) and a decreased of mean p's age ($p < 0.0001$) were observed and this last figure is in countertendency compared with the known increase of mean age of HIV-infected p, therefore underlying that HIV-lues co-infection tends to occur in proportionally younger life ages. No significant differences among the two study groups emerged as to p's gender, immigration, use of HAART and specific combinations, adherence levels, HIV disease stage, further concurrent diseases (chronic hepatitis and opportunism) and the virological-immunological HIV disease course. A typical syphilis presentations (including meningoencephalitis and acute-subacute hepatitis,) characterized even 8 of 26 cases (30.8%) diagnosed in the 2003–2005 period, making its prompt recognition more difficult.
Conclusion: Health care workers engaged in the management of HIV and STD should maintain an elevated clinical suspicion since a missed/delayed diagnosis of syphilis and HIV may be responsible for disease progression and increased sexual spread of both infections. Educational campaigns need a careful re-appraisal including preventive messages targeted on the population who still remains at risk of acquiring STD and HIV.

P613

Loss of bone mass in chronically HIV-infected patients. Significant association with male gender amd protease inhibitor administration

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Objectives: The aim of our study was to evaluate the prevalence of osteopenia and osteoporosis in a cohort of patients with HIV infection, and to assess the possible correlation with demographic variables and the selected antiretroviral association.
Methods: Patients were enrolled among adult subjects with HIV infection referring to our tertiary care outpatient centre of Bologna, Italy. Bone mineral density was measured in lumbar spine and femoral proximal head by dual energy X-ray absorptiometry (DEXA) technique.
Results: A total of 95 HIV-infected patients (45 males and 50 females), were enrolled until now: 12 subjects were naïve to antiretroviral therapy, 18 received three nucleoside reverse transcriptase inhibitors (NRTIs), 28 were treated with two NRTIs plus one non-nucleoside reverse transcriptase inhibitor (NNRTI), and the remaining 37 patients received two NRTIs plus one protease inhibitor (PI). The overall prevalence of osteopenia and osteoporosis according to lumbar T-score was 37.9% and 9.5% respectively, and osteoporosis was significantly more frequent in males than in females (20% versus 0%; <0.001). The mean value of lumbar T-score was significantly lower in PI-treated patients (-1.32 ± 0.48) than in antiretroviral-naïve subjects (-0.62 ± 0.24), or in those receiving three NRTIs (-0.68 ± 0.34), or a NNRTI-based anti-HIV regimen (-0.86 ± 0.44) ($p < 0.05$).
Conclusions: The alterations of bone metabolism associated with HIV infection have probably a multifactorial pathogenesis, but according to our experience bone mass loss seems to be significantly prompted by the male gender and a PI-based therapy. Prospective studies conducted in more extensive patient series are certainly needed, to better understand the epidemiology, the clinical features, the ethiopathogenesis, and the outcome of bone metabolism abnormalities associated with HIV infection.

P614

Different HIV 1-subtypes and diseases observed in a group of immigrants hospitalised between 2001–2005

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Objectives: To analyze the origin countries, the diagnosed diseases, the immuno-virological characteristics and the HIV-subtypes in 78 seropositive individuals
Patients and methods: We developed a data base for analysing a group of 78 HIV + immigrants attended as in-patients during the last 5 years (2001–2005). HIV subtyping was performed by phylogenetic analysis of the proteasa and retrotranscriptase genes in 55 (70%) plasma specimens. Statistical study was done by SPSS 11.0.
Results: Nationality could be evaluated in 72 subjects: Forty one (57%) were from sub-Saharian Africa (30 Equatorial Guinea, 6 Nigeria, 3 Camerun), 19 (26.4%) from South America (7 Ecuador, 3 Brasil, 3 Peru, 3 Colombia), 8 (11.1%) from another European countries, 2 from Asia and 2 from Marocco. Forty (51.3%) had been infected by heterosexual contact and HIV was diagnosed at the hospitalisation time in 35 cases (45%). More frequent diagnosed diseases were tuberculosis in 16 subjects (20.5%), candidiasis in 19 (24.4%), pneumonia in 15 (24.4%) and malaria in 17 (21.8%). Positive antibodies to hepatitis C virus (HCV) was detected in 12 patients (15.4%), to hepatitis B virus (HBV) in 5 (6.4%) and 6 had active syphilis (7.7%). Mean CD4+ cells was 261 cells per mm3 (r: 3–1176) and mean viral load was 118000 copies/ml (r: 50–500000). Subtype B was recognized in 26 patients and none of them was African. HIV-1 subtypes and recombinants were recognized in 29 (53%) out of the 55 subtyped specimens: 2A, 2C, 2D, 16G, 5GA, 1JG and 1GK recombinants.Therefore, clade G and AG recombinants were the most frquent variants (55,2% and 17,2% respectively). Moreover, intersubtype recombinants at the pol gene appeared in 24% of cases carrying non-B

subtypes. All non-B strains infected Africans but in contrast, South Americans and Europeans carried uniformly subtype-B variants. There was not relationship between HIV-subtype and clinical manifestations.
Conclusions: A relatively high proportion of HIV-1 non-B variants, mostly carrying clade G sequences, caused HIV-1 infection in the studied population. Regardless the subtype, a great number of subjects had acquired HIV infection through heterosexual contacts, did not know the HIV infection, had an elevated viral load and developed seriously illness. Epidemiological implications, plasma viremia quantification, susceptibility to antiretroviral drugs and clinical implications of the presence of those variants in this collective need to be further studied.

P615

Immigrants with HIV infection: hospitalisations during 5 years

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Objective: Immigrants with HIV infection are an emergent health problem in our country and our objective was to analyse their epidemiological, clinical and immunovirological characteristics when they are hospitalised.
Patients and methods: We reviewed the clinical history of 78 non-Spanish HIV patients hospitalised during the last 5 years (2001–2005). A data base was developed and only the first episode was studied. HIV subtype was performed by pathogenetic analysis of the proteasa and retrotranscriptase genes in 55 (70%) plasma specimens. Statistical study was done by SPSS 11.0.
Results: The 78 patients represented 3,9% of admissions. After this first episode, 23 patients had another hospitalisations and the number was ranged from 1 to 16 (mean: 2 episodes/person). Nationality could be evaluated in 72 patients: Forty one (57%) were from sub-Saharian Africa (mainly Equatorial Guinea and Nigeria), 19 (26.4%) from South America (mainly Ecuador), 8 (11.1%) from another European countries, 2 were from Asia and 2 from Marocco. Mean age was 39.5 years (r: 20–66), 55 (70.5%) were men and 40 (51.3%) had been infected by heterosexual contact. HIV was diagnosed at the hospitalisation time in 35 cases (45%) and only 22 (28%) were receiving HAART. Forty patients (51.2%) were seriously ill, 38 (48.7%) had CD4+ cells less than 200 per mm^3 and 18 (23%) less than 50 per mm^3. Relationship between immunodepression and origin countries was not found. HIV-subtype B was isolated in 26 patients (47.2%), none Africans. Twenty-nine people (52.8%) were non-B subtype, mainly G (16 cases, all Africans). The more frequent diagnosed diseases were: tuberculosis (mainly disseminated) in 16 subjects (20.5%), oral and/or oro-esophagical candidiasis in 19 (24.4%), pneumonia in 15 (24.4%) and malaria in 17 (21,8%). There were not relationship between diagnosed diseases and origin countries apart from malaria only diagnosed in Africans. Sixty-eight patients (87.2%) were discharged without any problem and nearly 50% of them were diagnosed as CDC C3. Mean diseases diagnosed each patient was 2.6 (r: 1–9) and only 3 subjects passed away.
Conclusions: Most HIV- infected immigrants attended in our hospital were from Africa and South America and non-B subtypes were predominant. They acquired the infection through heterosexual contacts and nearly 50% did not know that they were infected. They were very immunodepressed and with AIDS defining illness at admission.

P616

Poor response to trimethoprim-sulfamethoxazole in HIV-infected patients with *Pneumocystis jiroveci* pneumonia and concurrent opportunistic infections

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Objectives: *Pneumocystis jiroveci* pneumonia (PCP) remains a common opportunistic infections in patients infected with human immunodeficiency virus (HIV). Trimethoprim–sulfamethoxazole (TMP-SMX) is the most effective drug for treating PCP. Nevertheless, some patients have poor responses from the treatment with this drug. We aim to study the contributing factors of poor response to TMP-SMX in HIV-infected patients with PCP.
Methods: We retrospectively reviewed medical and laboratory records of adult HIV-infected patients who were diagnosed PCP and received TMP-SMX as the first regimen during 1999 and 2004. Poor response defined as pulse rate >100 /min, blood pressure <90/60 mmHg, respiratory rate >20 /min, and needed oxygen therapy or oxygen saturation <90% on the 5th day of treatment.
Results: A total of 168 HIV-infected patients with PCP were reviewed. Eighty-five patients (51%) were men and mean age was 36 ± 11 years. Median CD4 cell count was 29 (range, 0–190) cells/mm^3. Of these, 14 patients (8.3%) and had received antiretroviral therapy and 14 patients (8.3%) had received PCP prophylaxis before they developed PCP. Steroids was given in approximately 90%. Of 168 patients, 77 patients (45.8%) had poor response. Among 91 patients with good response, there were 12 patients who had concurrent opportunistic infection(s) whereas 47 from 77 patients with poor response had concurrent opportunistic infection(s) (13.2% vs 61.0%, $p < 0.0001$). The most common concurrent opportunistic infection were pulmonary tuberculosis (27 patients), cryptococcal meningitis (8), bacterial sepsis (6), *Salmonella* infection (5), and cytomegaloviral pneumonia (5). Patients who had poor response had a longer median length of stay than those who had good response [8 (range, 1–68) days vs 5 (range, 2–22) days, $p < 0.0001$]. The overall mortality rate was 26.8% (45/168 patients). All patients who died were in the poor response group. Patients with concurrent opportunistic infection(s) also had higher mortality rate than patients with PCP alone (42% vs 18%, $p = 0.001$).
Conclusion: There is a high rate of concurrent opportunistic infection(s) among HIV-infected patients with PCP especially those who had poor response to TMP-SMX. It conveys high mortality and morbidity rate. Early recognition of concurrent infection(s) in patients presented with PCP and appropriate therapy are essential issues.

P617

Impact of occult HBV infection in HIV patients naïve for anti-retroviral therapy

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Objective: To study the impact of occult HBV infection in 115 consecutive anti HIV positive, HBsAg negative patients, naïve for antiretroviral treatment.
Methods: Of these 115, 86 patients were followed up at least 6 months (range 6–36 months) by serial determination of laboratory data including HIV-RNA and HBV-DNA by PCR.

Results: Of the 86 patients having a follow-up, plasma HBV DNA was detected in 17 cases (19.8%) by PCR: in 13 patients at the first observation and in 4 only during follow-up. HBV-DNA positivity was more frequently found in patients with antibody to HBV (14 of 45, 31.1%) than in those without (3 of 41, 8.8%, $p < 0.01$). Twenty-eight patients (32.5%) experienced a hepatic flare during the follow-up; this event occurred more frequently in the 17 patients showing HBV-DNA than in those 69 HBV-DNA negative (64.7% vs. 24.6%, $p < 0.005$). Of the 13 patients HBV-DNA positive at the first observation, 11 were treated with HAART containing lamivudine and became HBV-DNA negative during the follow-up: of these 11, four turned HBV-DNA positive and showed a hepatic flare during lamivudine treatment, and two after lamivudine was discontinuated. A hepatic flare under lamivudine treatment occurred also in 2 of the 4 patient in whom HBV become detectable during the follow-up. Of the 49 patients with no hepatitis viruses marker, a hepatic flare was observed in 26.3% of the 19 receiving HAART and showing signs of the immune reconstitution inflammatory syndrome (IRIS), in 11.8% of the 17 patients receiving HAART with no evidence of IRIS and in none of the 13 patients left untreated during the follow-up.
Conclusion: The study suggests that HBV occult infection, relatively frequent in anti-HIV positive patients, is frequently involved in the pathogenesis of hepatic flares.

P618

Studies of the mechanism of the inhibitory effect on HIV-1 infection by stimulation of the VPAC2 neuroendocrine receptor

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Objective: Previously, we have reported that stimulation of the VPAC2 neuroendocrine receptor by specific agonist peptides profoundly inhibits productive HIV-1 infection in cell lines and human primary cells (Can J Infect Dis 15 (Suppl A): 15A, 2004). We hypothesize that VPAC2-stimulation generates a signalling pathway that targets a specific stage or stages of the HIV-1 life cycle that Results: in the inhibition of infection.
Methods: HIV-1IIIB was used to infect Jurkat cells and helodermin was used to stimulate VPAC2. Total phosphotyrosine (pTyr)-containing proteins was assessed using Western immunoblotting. A Malachite green assay was used to measure protein tyrosine phosphatase (PTP) activity. Polymerase chain reaction (PCR) was used to detect viral cDNA. 2-LTR circles were examined using nested PCR of U3 and U5 regions of viral cDNA. Proviral integration analysis used nested PCR based on the HIV-1 LTR region and Alu repeats of the host DNA. PCR products were cut with HinfI and hybridized to a radioactive LTR probe to identify a single fragment of predicted size. A pseudoenvelope-typed virus where the nef gene is replaced by a luciferase (luc) gene was used to measure the level of HIV-1 transcription.
Results: Western blot indicated that VPAC2 agonists decrease the basal level of pTyr-containing proteins. This observation was supported by showing an increase in PTP activity. The PTP activation was transient and inversely proportional to the inhibitory effect on HIV-1 infection. To sustain the inhibitory effect, cells required daily treatments with VPAC2 agonists and this resulted in sustained PTP activation. VPAC2 agonists did not affect HIV-1 entry or reverse transcription of viral RNA; however, transcription of the integrated HIV-1 was significantly inhibited for VPAC2 agonist treated cells and 2-LTR circle formation and proviral integration were profoundly inhibited.
Conclusion: VPAC2 agonists are strong inhibitors of HIV-1 infection by interfering with proviral integration. This inhibitory effect correlates with the generation of a signal that results in activation of a PTP. Activation of this PTP has no effect on formation of viral cDNA. Instead, it may halt the integration of HIV-1 cDNA into the host DNA. Further studies to elucidate the exact signalling pathway and target responsible for the block in the ability of HIV-1 provirus to integrate may provide insight into the pathogenesis of the virus as well as lead to novel future treatments for HIV/AIDS.

P619

Dramatic changes in survival and death rates after AIDS through the evolution of antiretroviral therapy, Paris

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Objectives: We explored changes in the survival of persons with AIDS (PWA) according to the availability of antiretroviral drugs from 1994 to 2002. We tested whether changes in the hazard ratio (HR) of progression to death have been homogeneous among various groups of PWA.
Methods: This study included PWA diagnosed in Paris in 1994–2001, reported to the National Institute for Public Health Surveillance by 2002 and followed for vital status up to October 2002. No individual information on treatments was available through AIDS case reporting. Data on antiretroviral drugs prescribed in Paris among PWA were obtained from the French Hospital Database on HIV. According to these data, calendar period was divided into 4 periods: monotherapy (1994–1995), transition dual therapy-HAART (1996), early HAART (1997–1999), late HAART (2000–October 2002).
A Cox regression in which calendar period was modelled as a time-dependent covariate, was used. HR of progression to death during a given period was compared with the calendar period of reference (monotherapy) adjusting for confounding variables. Cox regressions stratified by age, transmission category, CD4 cell count, and initial AIDS defining illness (es) (ADIs) were used.
Results: 4158 PWA contributed 7690 years at risk. The mortality rate (per 100 patients years) declined from 57.9 (monotherapy), to 38.8 (transition dual therapy-HAART), 23.7 (early HAART) and 7.1 (late HAART period). Adjusted HR of progression to death reached a minimum in the late HAART period (HR 0.22, 95% CI: 0.19–0.26). No difference in the decrease of the HR of progression to death has been found by age. HR decreased and was marked during the late HAART period across all HIV transmission categories, including intravenous drug users (0.23, 0.15–0.35). Among PWA diagnosed with tuberculosis, the HR decreased significantly only in the late HAART period (0.34, 0.19–0.63), while it decreased earlier and stronger for all other ADIs, also for progressive multifocal leucoencephalopathy, HIV dementia and tumours. Since HAART introduction the decrease of HR was stronger for PWA with a CD4 cell count $\leq 200/mm^3$ compared with those with a CD4 cell count $>200/mm^3$.
Conclusions: Survival has continued to increase since the Introduction: of HAART but was however heterogeneous according CD4 cell count at AIDS diagnosis and ADIs. Our study suggests that cardiovascular diseases and the risk of emergence of HIV resistance has not affected mortality until 2002 in PWA.

P620

Determinants of persistent nasal carriage and population structure of *Staphylococcus aureus* in HIV patients

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Objectives: We investigated whether CD4 cell counts or antiretroviral therapy were determinants of persistent *Staphylococcus aureus* nasal carriage and whether methicillin-resistant (MRSA) and Panton-Valentine Leukocidin (PVL) positive strains were circulating in a population of HIV-patients in The Netherlands. Furthermore, we compared the genetic structure of *S. aureus* isolated from HIV patients with the previously determined population structure of healthy individuals with *S. aureus* nasal carriage.
Methods: Between February 2004 and June 2005 all HIV patients visiting the outpatient department of Erasmus University Medical Centre (Rotterdam, The Netherlands) were asked to participate in this study. Participants were interviewed and screened for *S. aureus* carriage using two quantitative nasal swab cultures. According to an earlier validated culture rule, two carriage patterns were distinguished: non-or-intermittent versus persistent carriage. Potential determinants of persistent carriage were evaluated using logistic regression. *S. aureus* strains were tested for the presence of mecA and PVL by PCR. The genetic structure of *S. aureus* was determined by AFLP-analysis.
Results: For 443 patients two cultures were available of which 131 (29.6%) were persistent carriers. Male sex [odds ratio (OR) 2.22; 95% confidence interval (CI), 1.32–3.73], current smoking (OR 0.58; 95% CI, 0.38–0.90), *Pneumocystis jiroveci* pneumonia (PCP) prophylaxis (OR 0.39; 95% CI, 0.16–0.97) and antiretroviral therapy (OR 0.61; 95% CI, 0.38–0.98) were independent determinants of persistent carriage. CD4 cell counts were not associated with persistent carriage (P = 0.629). Only two strains were mecA positive (1.2%) and no PVL positive strains were detected. The population structure of *S. aureus* strains isolated from HIV patients appeared to be strongly overlapping with that of *S. aureus* isolates from healthy individuals from the same geographic region.
Conclusions: HIV patients have an increased risk of persistent nasal carriage of *S. aureus* compared to healthy individuals. Male sex (+), smoking (−), PCP prophylaxis (−) and the use of antiretroviral therapy (−) are independent determinants of persistent *S. aureus* nasal carriage in this cohort of HIV-patients. No PVL positive strains were found to be circulating and the prevalence of MRSA was low. No unique *S. aureus* clones specific for this cohort were identified.

P621

Pulmonary hypertension among HIV positive subjects: findings from the Latium Regional Registry

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Objective: To describe the frequency and characteristics of HIV-associated pulmonary hypertension (PH) among a population of HIV-positive subjects followed up in the Infectious Diseases Units (IDU) of the Latium region, Italy, and to evaluate the factors associated with PH.
Methods: The Latium Regional Registry of PH in HIV infected patients was created in April, 2004 with the aim to collect all cases of PH (according to WHO classification) occurring among the population of adult HIV-positive patients cared for in the Latium IDU, i.e. about 5000 subjects. All patients with mean pulmonary artery pressure (PAP) of more than 25 mmHg at rest, or more than 30 mmHg with exercise, assessed by trans-thoracic echocardiography, were included in the registry. For each patient the following data were ascertained: demographics, CDC classification, HIV risk factors, HIV RNA, CD4, signs and symptoms, NYHA class, antiretroviral and cardiovascular treatment, ECG findings.
Results: Upto October, 2005, a total of 33 cases of PH in HIV positive patients were recorded. Of these, 26 presented with signs and symptoms suggestive for PH; the remaining 7 were found to have raised PAP value during an echocardiographic screening of 300 HIV-positive patients without cardiovascular sign or symptoms, performed in one centre. Males were 22 (66%); mean age was 43 years (range, 31–56). Seven of them (21%) had HIV-related PH (0.12 × 100 subjects). According to CDC classification, 4 patients (12 %) were in class A, 17 (51%) in class B and 12 (36%) in class C; mean CD4 cells count was 327/mm^3 (range, 43–783); median HIV RNA was 1714 copies/mL (range, <50 to >500.000). Nineteen (58%), 12 (36%), and 2 (6%) patients were in NYHA class A, B, and C, respectively. Mean PAP was 39 and 45 mmHg among asymptomatic and symptomatic patients, respectively. Two of the 33 patients died during one-year follow up.
Conclusions: New and effective antiretroviral drugs have decreased the mortality of HIV infection; however, emerging syndromes, including cardiopulmonary involvement, represent a concern for HIV infected patients. We found that the prevalence of PH among the population of HIV infected patients is not negligible; moreover, PH can occur in patients with CD4 cells count higher than 200 cells/mm^3. As early diagnosis of PH can lead to a beneficial treatment, clinicians should be aware of the risk of cardiopulmonary involvement at any phase of HIV infection.

P622

Proteinuria in tenofovir-exposed and non-exposed patients: audit of screening processes within a United Kingdom HIV cohort

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Background: Proteinuria occurs in 7% to 14% of HIV positive patients and has been associated with CD4 count, HIV viral load, tenofovir use, black race and chronic hepatitis C infection. Proteinuria has been shown to predict chronic kidney disease in HIV. Regular testing for proteinuria in HIV positive outpatients was introduced as a standard of care at North Manchester General Hospital in June 2003.
Objective: The Objective: was to audit the use of urinalysis in HIV positive outpatients.
Methods: A retrospective case-note review of 164 consecutive HIV positive patients attending an outpatient HIV service between June 2003 and August 2004 was performed. Demographic and clinical data were entered into an Access (for Windows) database and statistically analysed using CLINSTAT (St. George's Hospital Medical School). Associations between variables were statistically tested using non-parametric tests. Proteinuria was defined as ≥10 mg/dl of urinary protein detected by dipstick urine test on at least 1 occasion during the audit period.

Results: Of 164 case-notes reviewed, 100 (61%, 95% CI 53% to 68%) had at least 1 urinalysis recorded. Of these, 61% (95% CI 51% to 71%) had one, 29% (95% CI 20% to 39%) had two and 10% (95% CI 5% to 18%) had three recorded. Of the 100 patients with at least 1 urinalysis, 84% were male and 77% were Caucasian. The mean CD4 count was 414 cells/ml; 22% had a CD4 count <200 cells/ml. 73% had a HIV viral load <400 HIV copies/ml. Proteinuria was found in 20% of all patients. Proteinuria was detected more frequently in patients currently taking tenofovir (56% versus 17%; c2 = 5.33, P = 0.02). The frequency of urinalysis between currently tenofovir exposed and non-exposed individuals did not account for this finding (% having one urinalysis performed in tenofovir exposed and non exposed groups, respectively = 13 (50%) and 44 (67%), two urinalyses = 8 (31%) and 18 (27%) and three urinalyses = 5 (19%) and 4 (6%); c2 = 4.22, P = 0.1). Length of tenofovir use and the length of all HAART use in those on tenofovir was not significantly associated with proteinuria. No cases of renal impairment were identified.
Discussion: The audit shows that urinalysis was sub-optimally performed. Proteinuria was a frequent finding overall. As expected, tenofovir use was associated with proteinuria. This, and the recognised adverse effects of this commonly used antiretroviral, emphasises the importance of auditing this standard of care in HIV practice.

P623

A meta-analysis of the frequency of side effects for HAART drugs

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Objectives: The Highly Active Antiretroviral Therapy (HAART), comprising a combination of one to three drugs, causes severe side effects lowering the patient's life quality and threatening the continuation of the therapy. The determination of the frequency of side effects for each HAART drug enables us to predict the effects of a HAART combination as a whole and to trace a specific side effect back to the drug most likely to cause it.
Methods: A Meta analysis of side effects, reported in clinical trials of HAART, was conducted, including as sources the NIH web site, controlled clinical trials and branches and manufacturer's disclosures. It was assumed that the frequency of a given side effect for a given HAART combination is the sum of frequencies for each drug in the combination. This allows us to formulate the determination of the frequencies of side effects as a regression problem, where the regression coefficients are the frequencies of side effects per drug and the dependent variables are the observed frequencies. The linear regression coefficients and their confidence intervals were determined using SPSS.
Results: A total of 112 entries (24022 patients) were included, yielding information on 27 individual HAART drugs and 38 side effects. Controlled trials with several branches were represented by one entry per branch. On average, 48 entries report on a given side effect, ranging from 6 entries on kidney stone to 106 entries on increased alanine transaminase, although for some entries side effects with zero frequency may not have been mentioned. The reported frequency of side effects ranged from 0.08% (osteopenia) to 24.1% (diarrhoea), on average 5.5% for a given side effect. For example, 70 entries report on severe vomiting with an average frequency of 7.1%. Out of the 25 HAART drugs in these entries, 5 drugs had significant ($p < 0.05$) frequencies of severe vomiting (abacavir 26%, amprenavir 20.9%, lamivudine −3.8%, indinavir 5.3%, ritonavir 13.4%). The negative frequency for lamivudine may be due to a true reduction of vomiting, or it could be due to a breakdown of the assumption of additivity of the frequencies, i.e. due to interaction with other drugs. The model fit for severe vomiting is satisfactory (R square = 0.77).
Conclusion: Meta analysis of published clinical trials can give some indication of the frequencies by which HAART drugs cause side effects, but limitations in the data do not allow frequencies for all side effects to be determined for all drugs.

P624

Immune-virological status and psycho-social factors concerning vertically contamined HIV-infected children at the time of transfer to adult care

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Objective: To analyse a cohort of HIV-infected children contamined via mother-to -child transmission at the time of their transfer to adult care.
Method: Retrospective study of all children followed in Nice since the discovery of their HIV-infection. The following variables were analysed: demographic characteristics, immuno-virological data (CD4 T-cell count, viral load, resistance tests), clinical status (stage, lipodystrophy) and psycho-social aspects (treatment adherence, education, psychological and behavioural assessment, family context).
Results: Of the 96 children infected at birth followed on a regular basis in a paediatrics department between 1985 and 2004, 33 died before age 18. Eighteen became adults in 2004 and have since been followed in the infectious diseases department. Mean age is 19.4 ± 1.9(15–24) years with known duration of HIV infection of 19.1 ± 1.6 (14–22) years. Median value of most recent CD4 T-cell count was 454(4–1251) with 33,8% >500 and 27.7%<200/mm^3. Viral load was 3.1 log ± 1.3 (1.3–5.7) and above detection limit (200 copies/ml) in 79% of patients. Only one child had never received antiretroviral treatment, 66.6% had received at least 5 different treatment combinations. Regarding current treatment, 11.8% never took protease inhibitors, 11.8% received a combination including a fusion inhibitor and at least one PI and 2 nucleoside analogues. Six children were CDC stage A, 3 B, 9 C. Sixteen displayed severe lipodystrophie. Most patients had major treatment compliance difficulties which may explain the high prevalence rate of resistance mutations. Only one child passed the final school certificate, none lived with both parents, and 13 were mother orphans.
Conclusion: This cross-sectional retrospective study illustrates the difficulties in managing these young adults. The frequent virological failures appear multifactorial (treatment adherence difficulties, psychological and behavioural disorders related to adolescence and family disruption). Optimal use of antiretroviral combinations accompanied by psychological support for the children and their families could improve these patients' future which presently appears severely compromised.

P625

Variation in interpretation and counselling of blood exposure incidents

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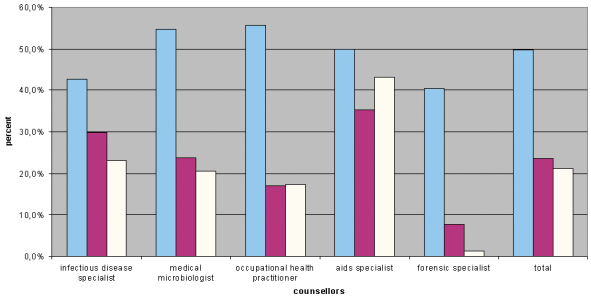
Background: Blood exposure incidents pose a risk both for healthcare workers and in public health. Despite several guidelines (national and international), counsellors often differ in opinion with regard to the risks caused by these incidents.

Little is known about the influence of the counsellor's background, profession and medical training on the proposed treatment and the related costs for the healthcare system.
Objective: To study the influence of counsellors profession, on assessment of blood exposure incidents.
Subjects: Subjects included infectious disease specialists (IDS) and forensic specialists (FS) from public health departments and medical microbiologists (MM), occupational health practitioners (OHP) and aids specialists (AS) from hospital settings.
Method: Surveys were sent to five different kinds of counsellors in the Netherlands asking questions about assessing different kind of blood exposure incidents as well as prophylaxis and treatment they would consider adequate to prevent transmission of these blood borne pathogens. Indications for administration of immunoglobulins and post exposition prophylaxis, the testing of sources and time limits for action were questioned. Questions were ranked for Hepatitis B virus (HBV), hepatitis C virus (HCV) and HIV risks.
Results: Of the 488 surveys sent, 184 were returned and 171 were taken into account. In the HBV risk counselling OHP and MM in general showed a more aggressive way for treatment. ($p = 0.026$) In HCV risk counselling in general IDS and AS were more aggressive ($p = 0.001$) while in HIV counselling AS were far more aggressive in their treatment than the other groups ($p < 0.001$). For seven of the total of twelve questions these differences were significant.

Figure 1: Agressive approach for treatment in blood exposure incidents



Conclusions: Different groups of counsellors assess similar blood exposure incidents differently. Differences are shown for all three blood born viruses. These differences can influence the risk of transmission of blood born viruses, the costs of the healthcare system as well as the care and information given to patients.Although the prevalence of blood born viruses in the Netherlands is relatively low, a national agreement on a protocol with regard to counselling and treatment of blood exposure incidents is essential.

Detection of viruses

P626

The measurement of 9-carboxymethoxymethylguanine in human body fluids by high-performance liquid chromatography

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Objectives: Aciclovir (ACV) is a safe and effective drug for managing infections with herpes simplex and Varicella-Zoster viruses. Approximately 85% of a dose is excreted in the urine unchanged and the remainder as the metabolite 9-carboxymethoxymethylguanine (CMMG). CMMG may be the cause of ACV neurotoxicity, and a recent study correlated serum CMMG concentrations with CNS symptoms. It is not known whether symptoms also correlate with CSF CMMG concentrations. We describe a simple HPLC assay that appears suitable for monitoring serum and CSF CMMG levels, and for pharmacokinetic studies. Monitoring may indicate action needed to prevent the development of toxicity.
Methods: Chromatography was performed on a 5 μC-18 100 × 4.6 mm HPLC column. The mobile phase was 1% octane sulphonic and 1% ortho-phosphoric acids pumped at 1 mL/min. Samples were mixed with equal volumes of 7% perchloric acid, left for 5 min then centrifuged at 12,000 g for 5 min. 20 mL of the supernatant was injected. CMMG was detected by UV absorbance at 255 nm and quantified by reference to an external standard. For identification and characterisation it was also measured at 214, 234 and 300 nm. Data were analysed by a chromatography management system.
Results: The retention time (RT) of CMMG was approximately 12 min (ACV 15 min). The assay was reproducible (at 2.5 mg/L intra-assay CV = 0.5%, inter-assay CV = 1.4%) and linear (range 0.25–20 mg/L), with an accuracy of between 4–13%. Recovery from serum was approximately 95% and from CSF > 99%. In both fluids the limits of detection and quantification were 0.1 mg/L and 0.25 mg/L respectively. RT, height to area ratio, and ratios of heights at different wavelengths identified peaks as CMMG. No interference was seen with clinical samples containing other antimicrobial agents. In serum from patients receiving ACV, CMMG was detected at levels from 0.23 to 3.4 mg/L, which were between 3–87% of the ACV level. In 7 sample pairs, the ratio of CMMG to ACV was lower post-dose than pre-dose in 3 cases, higher in 2 and unchanged in 2.
Conclusion: This assay is suitable for monitoring CMMG levels and for conducting pharmacokinetic studies in biological fluids. In some clinical samples CMMG levels approached those of ACV. The rate of conversion appears to be variable and in some cases CMMG levels rose rapidly. It may be possible to identify a subset of patients or clinical conditions where CMMG monitoring is indicated, particularly among patients with renal failure.

P627

Transient HBsAg reactivity after hepatitis B vaccination

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Objective: To investigate the occurrence of HBsAg reactivity on the ARCHITECT® i2000SR and on the AxSYM® instrument short-term after Hepatitis B vaccination.

Methods: Serum samples of 43 patients, neither HBV vaccinated nor with any personal HBV history, were collected on day 1 and day 8 after a first HBV (ENGERIXTM 20 μg/1.0 ml) vaccination. All 43 samples were tested for presence of HBsAg on the ARCHITECT® i2000SR with the ARCHITECT® HBsAg test kit, and on the AxSYM® instrument with the HBsAg (V2) test kit. Verification of HBsAg positive results was done with the ARCHITECT® HBsAg Confirmatory test kit. Finally a complete HBV status of all 43 patients was investigated 4 months after the first HBV vaccination.
Results: When clinical specimens of 43 patients were tested on day 1 after first HBV vaccination, 9.3% of all sera tested with the ARCHITECT® HBsAg test kit, and 2.32% of all sera tested with the HBsAg (V2) on the AxSYM® instrument were found to be positive for HBsAg. All 4 HBsAg positive results were confirmed with the ARCHITECT® HBsAg Confirmatory test kit. On day 8 after first vaccination all 43 sera were reinvestigated with both assays and 1 sample was still found to be positive for HBsAg on the ARCHITECT® i2000SR. The final HBV status, investigated 4 months after the first vaccination, showed negative HBsAg results in all specimens, and an increase in protective HBV surface antibodies.
Conclusion: Both, the ARCHITECT® HBsAg test kit and the HBsAg (V2) on the AxSYM® instrument are known to be sensitive methods to detect HBsAg in clinical samples. Follow up of 43 clinical serum samples showed a transient HBsAg reactivity in 4 samples on the ARCHITECT® i2000SR, and in 1 sample on the AxSYM® instrument short-term after HBV vaccination. The possible occurrence of HBsAg short-term after HBV vaccination is an important fact for the routine diagnostic laboratory.

P628

Development of human anti-rubella IgG and IgM assays for the Abbott ARCHITECT Instrument

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Objectives: Develop a panel of human anti-rubella immunoassays on a high throughput automated platform. The ARCHITECT Rubella IgG assay is intended to be used for the quantitative measurement of IgG antibodies to rubella virus in human sera or plasma to aid in the determination of immune status to rubella virus. The ARCHITECT Rubella IgM assay is intended to be used for the qualitative measurement of IgM antibodies to rubella virus in human serum or plasma to aid in the diagnosis of primary or acute infection.
Methods: The prototype rubella assays for the ARCHITECT instrument are two-step immunoassays utilizing rubella whole virus coated paramagnetic microparticles for the capture of anti-rubella antibodies. An acridinium labelled monoclonal antibody conjugate directed against human IgG or IgM is utilized for detection. Samples from pregnant women, blood donors, hospital patients, IgG/IgM screened negative samples, known rubella IgM positive samples, vaccine serial bleeds and seroconversion panels have been tested on the new ARCHITECT Rubella assays in comparison to the Abbott AxSYM Rubella IgG and IgM assays. Discrepant samples were tested on 1 additional Rubella IgG or IgM on-market assay and resolved with the consensus.
Results: The ARCHITECT Rubella IgG shows a resolved relative sensitivity of 99.89% and a resolved relative specificity of 100% compared to AxSYM on the population described above (N = 1198). The ARCHITECT Rubella IgM assay shows a resolved relative sensitivity of 98.08% and a resolved relative specificity of 100% compared to AxSYM on the population described above (N = 1059). The seroconversion sensitivity of the ARCHITECT Rubella IgG and IgM assays were equivalent and better than the AxSYM Rubella IgG and IgM assays, respectively. The ARCHITECT Rubella IgG and IgM assays exhibited equivalent or greater sensitivity than the AxSYM Rubella IgG and IgM assays on serial bleeds as well as before and after vaccination.
Conclusion: The performance of the 2 new ARCHITECT Rubella IgG and IgM assays is better than or equivalent to the AxSYM Rubella IgG and IgM assays.

P629

Preliminary evaluation of the Abbott ARCHITECT anti-cytomegalovirus IgG, IgM and IgG avidity assays

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Objectives: Preliminary evaluation of a panel of human anti-Cytomegalovirus (CMV) immunoassays on the automated ARCHITECT instrument.
Methods: The three CMV assays for the ARCHITECT instrument are two-step immunoassays utilizing CMV virus lysate coated paramagnetic microparticles for the capture of human anti-CMV antibodies. The CMV IgM assay contains both viral lysate and the recombinant protein CKS-pp150, pp 52 (UL32, UL44) coated onto paramagnetic particles. An acridinium-labelled monoclonal antibody directed against human IgG or IgM was utilized for human anti-CMV antibody detection. Samples from pregnant women, blood donors, hospital patients, transplant recipients and seroconversion panels were tested on the new ARCHITECT CMV assays in comparison to best in class on-market CMV assays. The performance of the ARCHITECT CMV IgG and IgM assays was compared to the Abbott AxSYM CMV IgG or IgM assays. Samples with discrepant results were tested on two additional CMV IgG or CMV IgM assays and resolved with the consensus. The performance of the ARCHITECT CMV IgG avidity assay was evaluated by comparison to the Radim CMV IgG Avidity assay and clinical information.
Results: The ARCHITECT CMV IgG assay has a resolved relative sensitivity of 100% and a resolved relative specificity of 99.5% when compared to AxSYM CMV IgG on a population as described above (n = 1154). The seroconversion sensitivity of the ARCHITECT CMV IgG and IgM assays was equivalent or better than all reference CMV IgG or IgM assays tested. The resolved specificity of the CMV IgM assay was 99.2% on blood donors and 98.7% on pregnant women. The ARCHITECT CMV IgG avidity assay using ''AVIcomp'' technology displayed 99.6% clinical specificity on CMV immune blood donors and pregnant women (n = 256). The clinical sensitivity of the avidity assay was 97% on 23 seroconversion panels with 72 bleeds drawn within 4 months post-seroconversion. The clinical sensitivity and specificity of the ARCHITECT CMV IgG avidity were superior to the reference avidity assay.
Conclusion: The performance of the three new ARCHITECT CMV immunoassays is better than or equivalent to the reference assays.

P630

A novel method for distinguishing between bacterial and viral infections that incorporates standard clinical laboratory data and quantitative analysis of neutrophil complement receptors, CR1 and CR3

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Objectives: Since severe sepsis with acute organ dysfunction is a major threat to life, it is customary to start empirical antimicrobial therapy in all patients hospitalised for a suspicion of systemic infection. However, treating viral illnesses or non-infective causes of inflammation with antibiotics is ineffective, and contributes to the development of antibiotic resistance, toxicity, and allergic reactions, leading to increasing medical costs. Therefore, to avoid the unnecessary use of antibiotics, early and accurate diagnosis of bacterial infections is of primary importance. Hence, a new method for distinguishing between bacterial and viral infections was developed.
Methods: Standard clinical laboratory data [neutrophil (PMNL) count, serum C reactive protein level (CRP), erythrocyte sedimentation rate (ESR)] and quantitative analysis of neutrophil complement receptors, CR1 and CR3, were obtained from 135 hospitalised febrile patients with microbiologically or clinically diagnosed bacterial (n = 89) or viral (n = 46) infection. The same variables excluding CRP and ESR were obtained from 60 healthy controls. A measurement of receptor expression was obtained by determining the mean fluorescence intensity (MFI) of isolated neutrophils by flow cytometry after incubation with FITC-labelled anti-CR1 (CD35) and PE-labelled anti-CR3 (CD11b) monoclonal antibodies.
Results: In bacterial infections, all measured variables were significantly increased compared to viral infections [average (SD); PMNL: 7.8 (3.8) vs. 3.3 (1.9) x 109/L; $p < 0.0001$, CRP: 224 (121) vs. 40 (42) mg/L; $p < 0.0001$, ESR: 67 (28) vs. 20 (18) mm/h; $p < 0.0001$, CR1: 20 (8.8) vs. 5.9 (3.0) MFI; $p < 0.0001$, and CR3: 101 (47) vs. 55 (26) MFI; $p < 0.0001$, for bacterial and virus infections, respectively]. We described a novel marker of local and systemic bacterial infections, designated 'clinical infection score (CIS) point', which varied between 0 and 10, and displayed 98% sensitivity and 97% specificity in distinguishing between bacterial infections [CIS points: 7.2 (2.3) vs. 0.7 (1.2); $p < 0.0001$, respectively]. CIS point incorporates standard clinical laboratory data and quantitative analysis of neutrophil complement receptors, CR1 and CR3.
Conclusion: The reliability of differential diagnoses made using CIS point is high over a wide range of prevalence of bacterial infections. Therefore, the proposed CIS-based diagnostic test could potentially assist physicians in deciding whether antibiotic treatment is necessary.

P631

Epitope specific IgG response to Epstein-Barr virus capsid protein p18 among different age groups

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Objective: Determination of IgG avidity against viral capsid antigen (VCA) frequently have been used for the diagnosis of acute Epstein–Barr virus (EBV) infection and for estimation of seroconversion age in epidemiological studies. Recombinant protein or synthetic peptides with different size often used as diagnostic target. The aim of this study was to compare IgG response to antigenic epitopes: AE1 – located on position 1–119 aa (expressed as GST fusion protein) and AE2 located on position 124–153 (modelled by synthetic peptide) of VCA p18 among different age group.
Materials and methods: A total of 93 serum samples from adult (normal blood donors) and 166 serum samples from children (age range 0–17-years old) were analysed by commercially available anti-EBV-VCA IgG enzyme immunoassay (Diagnostic Systems, Russia). All EBV-VCA IgG positive samples were additionally tested for presence of IgG specific to AE1 and AE2 regions individually. The determination of IgG avidity was performed with 8 M urea as a dissociative agent. Serum samples were divided for 7 groups: less 4 months old, n = 43(1), 4 months to 1 year old, n = 18(2), 1–2 years old, n = 12(3), 3–5 years old, n = 24(4), 5–9 years old, n = 26(5), 10–13 years old, n = 20(6) and 14–17 years old, n = 43(7).
Results: Epitope-specific distribution of anti-VCA-IgG activity was significant different in term of both IgG level (for groups 1, 4–7 and adults) and IgG avidity (for groups 1, 3, 4 and adults) (Table 1).

Table 1. Age distribution of IgG and low-avidity IgG to AE1 and AE2 antigenic domains of VCA p18

Age range	Anti VCA positive samples	Frequency of low-avidity anti VCA	Anti AE1 positive samples	Frequency of low-avidity anti AE1	Anti AE2 positive samples	Frequency of low-avidity anti AE2
less 4m	37(86%)	9 (24%)	17 (40%)	9 (53%)	35 (81%)	5 (14%)
4-12 m	8 (44%)	5 (63%)	5 (13%)	3 (60%)	8 (44%)	2 (25%)
1 – 2 yrs	14 (100%)	1 (7%)	10 (71%)	2 (20%)	11 (79%)	0
3 – 5 yrs	22 (92%)	2 (9%)	13 (59%)	3 (23%)	20 (83%)	0
5 – 9 yrs	28 (100%)	3 (11%)	14 (50%)	2 (14%)	25 (89%)	2 (8%)
10 –13 yrs	20 (87%)	4 (20%)	12 (53%)	0	19 (83%)	3 (16%)
14 - 17yrs	40 (93%)	1 (3%)	30 (70%)	3 (10%)	41 (95%)	1 (2.5%)
Adults	89 (96%)	3 (4%)	50 (54%)	13 (26%)	90 (97%)	3 (3.3%)

Conclusion: Specific IgG response to different antigenic epitopes located on VCA p18 of EBV differs in IgG level and IgG avidity among various age groups. Our results strongly indicated that selection of antigenic epitopes (or nature of diagnostic target) for the routine avidity determination of anti VCA IgG may be a critical point in the design of the EIA protocols for both clinical and epidemiological study.

P632

Antigenic properties of new recombinant polypeptide from TBEV IgE protein

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Objectives: Tick-borne encephalitis virus (TBEV) is a pathogenic human flavivirus endemic in some parts of Europe and Asia. Detection of specific antibody is a base for diagnosis of TBEV-infection. Recent studies have shown the necessity of further improvement of existing TBEV-tests regarding both sensitivity and specificity. Majority manufactures used cultural antigens (virion or purified protein) producing from Central European type of TBEV (Naidorff strain). The common problems of this approach are: (1) insufficient purity of antigen; (2) problem with recognition of other TBEV-types (Sibirean and Far-East); (3) possible cross reactivity with another flaviviruses. In this study we investigated antigenic properties of new recombinant protein corresponding to domain III of

TBEV IgE protein. Domain III is highly antigenic, consists primarily of linear epitopes and have high degree of homology among all types of TBEV.
Methods: Recombinant polypeptide comprising 296–414 aa region of TBEV IgE-protein was produced in *E. coli* as GST-fusion protein. Assay conditions for the detection of anti-TBEV-IgG were optimised to reduce the possibility of false positive and false negative results. Two groups of sera have been used to evaluate sensitivity and specificity of the test: serum samples from TBEV-infected individuals collected in European part of Russia (n = 78) and sera from normal blood donors (n = 109). All specimens were previously tested for IgG anti-TBEV activity by commercially available EIA.
Results: All sera from TBEV-infected patients were positive in EIA with new recombinant protein. The average of signal to cutoff ratio was 24, 5.108 out 109 samples from normal blood donors sera were negative. Assay sensitivity was calculated at 100%, assay specificity – 99.1%.
Conclusions: The recombinant protein derived from C-term of IgE TBEV used in this study demonstrated significant potential as diagnostic reagent in EIA for the detection of specific IgG to TBEV in serum specimens.

P633

Development a new enzyme immunoassay for detection anti-EBNA1 antibody to Epstein–Barr virus based on new p72 mosaic protein

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Background: The Epstein–Barr virus (EBV) is a human herpes virus 4 (HHV4) that infects and establishes latency in B-lymphocytes. Primary infection leads to a life-long past infection which is normally asymptomatic. EBV expresses a number of genes, however, Epstein–Barr nuclear antigen 1 (EBNA1) p72 is the only viral protein which characterizes EBV past-infection.
Objective: The aim of this study was to evaluate diagnostic relevance of new artificial protein composed of 2 antigenic epitopes of the EBV EBNA1 and to develop and evaluate a screening enzyme immunoassay (EIA) for the detection of anti-EBNA1 IgG activity to EBV in serum specimens.
Materials and methods: Two potential antigenic epitopes of EBNA1 protein have been predicted by bioinformatics analysis. Mosaic of two antigenic domains from the protein p72 (1–98 aa) and (408–498 aa) of HHV4 was expressed in *E. coli* as hybrid proteins with Glutathione S-transferase to develop an assay for the detection anti-EBNA1 antibodies. Assay conditions were optimised to reduce the possibility of false positive and false negative results. The new IgG-EIA was evaluated using serum specimens obtained from EBV PCR positive patients (n = 51), HIV-infected individuals (n = 72) and from normal blood donors (BD) (n = 504). All PCR positive specimens were additionally tested for IgG anti-EBNA1 activity by commercially available EIA based on full-length EBV nuclear antigen. The specificity was estimated on the EBV negative samples (n = 23).
Results: The EBV past-infection for PCR positive patients was confirmed by the detection of high avidity IgG to EBV Viral Capsid Antigen (VCA). All of the 51 EBV PCR positive patients had IgG antibodies to EBNA1 on the novel EIA. Concordance with commercially available EIA was 98.03%. The frequency of IgG antibodies to EBNA1 in all investigated groups were as follows: 92.21% for health blood donors, 95.39% for HIV-infected individuals and 90.23% for children. Specificity of the assay was around 95.63%.
Conclusion: Recombinant protein comprising theoretically predicted antigenic epitopes of EBNA1 protein demonstrated a significant potential as diagnostic reagent. The new EIA is highly specific diagnostic assay for the detection of anti-EBNA1 IgG HHV4 activity in serum specimens and in combination with VCA IgG and IgM may be useful tool for routine diagnosis of acute EBV infections or EBV immune status.

P634

Rapid detection of rotavirus in children: comparison of Vikia rota-adeno and Diarlex MB, two immunochromatographic tests

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Purpose: The purpose of this study was to compare two immunochromatographic tests: Vikia Rota Adeno (bioMérieux, France) and Diarlex MB, with centrifugation (Orion Diagnostica), used in rotavirus detection in the case of gastroenteritis episodes in paediatrics.
Methods: 189 stool samples in total were tested: 116 frozen stools (-20°C) studied retrospectively and 73 fresh stools studied prospectively (December 2004–August 2005). Of the frozen stools, 50 were selected as containing bacteria or yeasts responsible for diarrhoea and 45 were selected as containing rotavirus using the laboratory's routine method (Diarlex MB). For all the stools, the rapid tests were performed on the same day under the same conditions at Hôpital Debrousse and the reference method IDEIA rotavirus (Dako) was performed in blind mode by bioMérieux.
Results: On the frozen stools, Vikia produced the same results as the reference method and Diarlex MB produced two false negative results. On the fresh stools, Vikia produced two non-interpretable results and two false negatives, and Diarlex MB produced two non-interpretable results and two false negatives. For three frozen stools, Vikia posed methodological problems associated with the migration on the strip which were resolved in a second assay. On the 50 frozen stools containing bacteria or yeasts, no cross-reaction was detected with the two tests. For the 189 stools, the reference method, Vikia, and Diarlex MB detected 81, 79 and 76 stools positive for rotavirus. The sensitivity and specificity values, positive predictive value and negative predictive value are 97.5; 100; 100 and 98.2% for the Vikia test and 95; 100; 100; and 96.4% for the Diarlex MB test, respectively. No statistically significant difference was observed either between the two tests themselves or between the tests and the reference method.
Conclusion: Equal in terms of performance, the Vikia test offers a simpler and more rapid methodology.

P635

Evaluation of rubella IGG and IGM assays on the new vidia instrument

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Objective: The aim of the present study was to evaluate the performance of the new VIDIA Rubella IgG and IgM assays on the fully automated VIDIA instrument, easy to use with a high level of traceability.
Material: Sensitivity and specificity of the VIDIA RUB IgG (bioMérieux, France) were evaluated on 680 serum samples in comparison with VIDAS Rub IgG (bioMérieux, France), AXSYM Rubella IgG (Abbott, USA) and ACCESS Rubella IgG (Beckman Coulter, USA) assays. The performance of VIDIA Rub IgM (bioMérieux, France) was also evaluated in comparison to AXSYM Rubella IgM (Abbott, USA) and Platelia Rubella IgM

TMB (BioRad, USA) assays on 401 serum samples. Discrepancies were resolved considering patients' clinical data, when available, as well as with complementary tests: Western Blot for IgG discrepancies and Avidity test for IgM discrepancies.
Results: Sensitivity: For VIDIA Rub IgG, sensitivity was 100% for 2 of 3 methods. For VIDIA RUB IgM, the relative sensitivity was 90.17% compared to Axsym Rubella IgM, but after resolution of discrepancies, the absolute sensitivity was close to 100%. In comparison with Platelia Rubella IgM, the relative sensitivity was 89.39%. Taking into account clinical information and Avidity results, the absolute sensitivity was close to 100%. Specificity: For VIDIA Rub IgG, the specificity has been established at 99.17%. In fact, 2 samples positive with VIDIA and negative with the compared methods, were found positive by Western Blot. Taking this into account, the absolute sensitivity was found 100%. The relative specificity of VIDIA Rub IgM determined in comparison with Axsym Rubella IgM and Platelia Rubella IgM assays was respectively 96.98% and 97.83%. After the resolution of discrepancies, the absolute specificity was close to 100%.
Conclusion: The two evaluated assays, VIDIA Rub IgG and VIDIA Rub IgM, show an excellent sensitivity and specificity.

P636

RNA extraction from respiratory samples using the NucliSens easyMAG system

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Objective: An enhancement was made to the RNA extraction procedure for sputum and BAL samples using DNAse I activity. The objective of this study was to measure the efficacy of the new RNA extraction procedure for the recovery of RNA from respiratory samples in combination with the NucliSens easyMAG system (bioMérieux).
Methods: In total, 40 samples (30 sputum and 10 BAL) were included in the study. Samples were treated with proteinase K and DNAse I, in order to liquefy the samples. Next treated samples were extracted with the NucliSens easyMAG system (bioMérieux). The efficiency of the extraction procedure was measured by spiking internal control RNA. In addition, RSV was spiked at a concentration of 300 TCID/100 μl to 10 sputum samples. Extracted samples were analysed with real time NASBA using NucliSens EasyQ® RSV A + B assay (bioMérieux), NucliSens EasyQ® Mycoplasma pneumoniae assay (bioMérieux) for 10 and 30 samples, respectively.
Results: RSV was detected in 10/10 (100%) of the samples spiked with RSV. Inhibition (no detection of the internal control RNA) was measured in 1/40 (2.5%) of the samples tested.
Conclusions: In this study, RSV RNA and Internal control RNA were efficient detected from respiratory samples with the enhanced RNA extraction procedure that uses DNAse I activity. The use of this procedure might increase the sensitivity for the detection of viral and bacterial RNA in respiratory samples, however this remains to be tested in follow up studies.

P637

Evaluation of automated sample processing using the MagNA Pure LC instrument for use with the COBAS AMPLICOR HCV (ver 2) test

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Objectives: Determine the limit of detection, linearity, reproducibility and correlation between clinical results of the COBAS AMPLICOR HCV test v2.0 (CA HCV) using the MagNA Pure LC (MPLC) for automated specimen preparation.
Methods: A range of analytical standards and clinical specimens were used to determine each of the evaluation parameters as follows: Limit of detection and linearity 16 replicates of a 7 member dilution panel of the WHO 2nd International Standard HCV RNA covering the range 0–1000 IU/mL.
Reproducibility 80 replicates of HCV 100 IU/mL WHO 2nd International Standard HCV RNA.Concordance Split sample analyses of 100 clinical specimens tested using both methods of sample preparation. Sample preparation was performed using the Total Nucleic Acid Isolation Kit (Roche Diagnostics Australia) on the automated MPLC (Roche Diagnostics Australia) platform according to a modified in-house validated protocol. Nucleic acid from the 100 clinical samples was also extracted in duplicate using the COBAS AMPLICOR HCV test v2.0 (Roche Diagnostics Australia) manual sample preparation protocols. Testing for HCV was performed using the COBAS AMPLICOR HCV test v2.0 (Roche Diagnostics Australia) on the Roche COBAS Amplicor (Roche Diagnostics Australia) according to the manufacturer's instructions.
Results: The performance criteria for the CA HCV assay following sample preparation by MPLC were determined. Preliminary results to date demonstrate that the linearity across the range of HCV RNA levels tested (0–1000 IU/mL) is acceptable and that the lower limit of detection is comparable to the manufacturer's claimed sensitivity for manually processed samples (50 IU/mL). For the 100 clinical specimens tested using both methods of specimen preparation, results were concordant for 99/100 specimens. After resolution of the discordant results the sensitivity and specificity of CA HCV following MPLC specimen preparation were 99.0% and 100%, respectively, when compared to manual specimen preparation.
Conclusions: The MPLC instrument is a suitable front end platform for use with the COBAS AMPLICOR HCV test v2.0. The modified protocol using the Total Nucleic Acid Isolation Kit on the MPLC resulted in a reliable and labour-saving method for the extraction of nucleic acid. The performance of CA HCV PCR testing on samples processed on the MPLC was comparable to that on samples processed manually.

P638

Development of sensitive and specific real-time PCR assay for simultaneous diagnostics and genotyping of cytomegalovirus

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Background: Human cytomegalovirus (HCMV) is an important pathogen capable of establishing lifelong persistent infections, which normally remain asymptomatic. Previous studies indicated that sequence variation among CMV strains frequently occurs even in highly conserved genes' regions. Genetic variation of functionally important genes may complicate CMV diagnostics.
Objective: The purpose of this work was designing primers and probes for simultaneously Real-Time PCR diagnostics and genotyping of CMV.
Materials and methods: In this study 527 serum samples obtained from pregnant women and 3 samples from children with CMV congenital infection were used. Virus DNA was extracted by using the MagNA Pure DNA purification kit (Roche, Indianapolis, IN, USA). 4 sets of primers directed to IE2,

gN, gO and gB CMV genes and 10 sets of probes for Real-Time detection with LidhtCycler instrument (Roche, Indianapolis, IN, USA) have been designed and evaluated. Genotyping was being carried out by sequencing analysis on ABI 3100 Avant Genetic Analyzer instrument (ABI, Foster City, CA, USA). Phylogenetic analysis of the nucleotide sequences was conducted with Clastal X, the Tamura-Nei substitution model, Grow tree based on Neighbour-Joining tree building method, and Maximum Parsimony method implemented in MEGA 3.0 package.
Results: The Real-Time PCR analysis with IE2 gene detected CMV activity in 72 isolates among 527 analyzed samples The PCR tests with previously described primers to gN, gO and gB genes revealed 7, 1 and 2 positive samples accordingly. The PCR test sensitivity was defined with quantitative CMV control (ABI, Foster City, CA, USA). The sensitivity of PCR test on IE2 gene was 20 copies per 50 μl. The phylogenetic analysis of IE2 region sequences demonstrated that this region could be successfully used for virus genotyping. The Real-Time FRET test divided all analyzed samples into two groups, those that had a melting peak like laboratory strain Davies and those that had a melting peak like Towne and AD169 laboratory adapted strain. Two pairs of specific hybridisation probe covering two mutations inside IE2 gene's region were used to confirm it.
Conclusion: Nested PCR with primers to IE2 gene incorporated with Real-Time FRET analyse described here, provides a sensitive and specific assay for detecting CMV in clinical isolates. The IE2 gene can serve as a target for simultaneously detecting and genotyping of CMV using the Real-Time PCR opportunities.

P639

Detection of human papilloma virus (Type 16, 18) in pathological sample from patients with cervical cancer by PCR and RFLP methods

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Objectives: Infection with human papilloma virus (HPV is the most frequent sexually transmitted disease world wide. HPV types 16, 18, 31 and 33 are considered as important causes of cervical cancer. This study was carried out to detect HPV types 16 and 18 among 64 pathologic blocks from patients with cervical cancer in Tehran.
Methods: Primers HP6133 F 5′-TGG ATT ATA AAC AAA CAC A-3′, HP6133 R 5′-GTG GTA TCT ACC ACA GTA ACA-3′, HP168 F for types 6, 11, 31 and 33 and 5′-GAA TAT GAT TTR CAG TTT ATT TT-3′, HP168 R 5′-TCT YKA GAA AAC TTT TCC TTT-3′ for types 16, 18 and 35 were designed from the sequences of HPV (Genebank accession numbers E54157, U34171, M74117, AF067049) and used in PCR. These primers produced amplicons with sizes of 564 and 269 bp respectively. The PCR products were digested with BamHI and EcoRI and the fragments were separated by electrophoresis in agarose gel.
Results: Human papilloma virus DNA was detected in (59.4%) of the cases. HPV type 16 was the most common one (22/64, 34%) followed by HPV type 18 (16/64, 25%). Digestion of PCR products with BamHI and EcoRI differentiated type 16 and 18 respectively as the amplicons from each type had one restriction site for one of the enzymes used.
Conclusion: HPV type 16 was the predominant infection (34%) in our study. This rate in our country is still lower than in other countries such as Croatia (50%), Australia (53%), Spain (66%) and China (48.8%). However, in compare with the previous study in Iran (26.7%) infection with type 16 shows an increase. Of 16 cases infected with HPV18, 11 had squamous cell carcinoma. Statistically, there was significant correlations between infection with HPV type 18 and development of squamous cell carcinoma ($p = 0.019$). Such relation was not found for type 16. PCR and PCR-RFLP are sensitive and useful in identification and typing of papiloma virus and differentiation of their types.

P640

Detection of human papillomavirus by PCR genotyping and immunostaining in population of Bosnian women

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Objectives: To determine prevalence of human papilomavirus (HPVs) among Bosnian women.
Methods: Cervical swab samples and smears on glass slides were collected from 97 women from three disease defined groups: Group 1: patients who had an abnormal Papanicolaou (PAP) cytology report (N = 34); Group 2: patients who had a history of genitourinary infections (N = 22), Group 3: patients not in either group 1 or 2 (N = 41). Age groups were defined as Group A (20–24 yr), Group B (25–29 yr), Group C (30–34 yr). Group D (35–39 yr), Group E (40–44 yr), Group F (45–49 yr), Group G (>50 yr). Specimens were collected from December 2004 to January 2005 at two sites in Sarajevo: the Department of Gynaecology of the University Medical Center and the Institute for Health Protection of Women and Motherhood. Specimens were shipped to Johns Hopkins University, for testing. Swab samples were tested for high risk (HR) and low risk (LR) types HPV by research Polymerase Chain Reaction – PCR (Roche, primer set PGMY 09/11) and for HR HPV by the Hybrid capture-2 (HC2) test (Digene). Slide samples for stained for P-16 protein biomarker (Dako).
Results: The overall prevalence for high risk HPV by HC2 test was 22.68% (22/97). The highest HR-HPV DNA prevalence by HC2 was 31.81% (7/22) in Age Group B. The lowest prevalence was in 4.54% (1/22) in Age Group A. Total of both HR-HPV and LR-HPV prevalence by Roche PCR was 29.89% (29/97). The highest prevalence 34.48% (10/29) was in Age Group B. Positive cytoplasmic immunostaining by p16 biomarker showed prevalence 9.27% (9/31) of total HPV positive patients (HC2 and PCR). By Roche testing the most prevalent HPV genotypes were: HPV 16 (9/29), HPV 52 (4/29), HPV 31, 53, 67 (3/29).
Conclusion: High-risk HPV infection was high among Bosnian women. These results demonstrated that HPV DNA testing was a useful indicator for prevalent of HPV infections and could be adjunct test to PAP testing as a part of regular cervical screening programs among women in Bosnia and Herzegovina in the future.

P641

A novel system for the simultaneous detection of seven respiratory viruses

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Objectives: To develop a kit (Respiratory Viral Assay or RVA) for amplifying, detecting, and differentiating seven respiratory viruses: human parainfluenza (HPIV) 1, 2 and 3, influenza (INF) A and B, and respiratory syncytial virus (RSV) A and B.
Methods: Multiplex RT-PCR of extracted patient samples or RNA control transcripts was carried out using primers identical in sequence to those in the Prodesse Hexaplex® assay. Following amplification, automated detection was carried out on the

NanoChip® 400 system and its accompanying electronic microchip in four distinct steps: (1) biotinylated capture oligonucleotides complementary to the seven respiratory viruses as well as an optional internal control were electronically targeted to specific sites across the 400-site cartridge; (2) a dilution of the RT-PCR reaction was electronically biased to a set of pads comprising the full set of capture oligonucleotides, allowing any generated amplicon to hybridise to its complementary capture sequence; (3) each additional RT-PCR reaction was sequentially biased to a unique set of sites across the cartridge; (4) passive hybridisation using a set of bi-functional discriminator oligonucleotides (half of the molecule binds a specific amplicon and half binds to a reporter molecule) and 2 fluorescently-labelled reporter oligonucleotides allowed for detection of the amplicons.
Results: Analytical sensitivity of 500 copies/mL or 12 copies/reaction of viral specimen equivalence in a matrix of negative patient eluant was achieved with the RVA kit for each of the seven systems when individually infected. With simulated dual infections, an analytical sensitivity of 5000 copies/mL was achieved. No cross-reactivity with 20 organisms or viruses known to give flu-like symptoms was observed. A total of 205 patient samples were analyzed with both the Hexaplex assay and RVA kit. In comparison to the Hexaplex assay, the RVA kit yielded a clinical sensitivity of 94.8% (HPIV 1 = 18/19; HPIV 2 = 19/19; HPIV 3 = 23/23; INF A = 16/19; INF B = 24/25; RSV A = 22/25; RSV 25/25) and a clinical specificity of 99.6%. Precision testing was performed by three operators using a panel of RNA transcripts to obtain measures of repeatability and reproducibility. In this study, an overall accuracy of 99.5% was observed.
Conclusion: The RVA kit has been proven to be an accurate, user-friendly methodology for detecting the presence or absence of seven respiratory viruses.

P642

Evaluation of the multiplex reverse transcription real time PCR ProFlu-1 LC real-time assay for the detection of RSV, influenza A and influenza B in a single test

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Objectives: Nucleic acid-based assays are more sensitive than culture or antigen testing to detect respiratory viral infections. Specific antiviral treatments are now available or in development and they provide better efficiency when they are given early after the onset of symptoms highlighting the need to have a quick diagnosis. A multiplex molecular assay based on a reverse transcription combined with a real time PCR (ProFlu-1 LC Real Time Assay, Prodesse, Waukesha, Wis) was developed and used to rapidly detect RNA of respiratory syncytial viruses (RSV), influenza viruses A (IA) and B (IB) in nasal wash or bronchoalveolar lavage specimens in a single test.
Methods: We tested 48 samples from children and adults known to be infected by either RSV (n = 16) or influenza viruses (A, n = 18; B, n = 14) as determined by culture and single classical PCR and 10 samples positive for parainfluenza viruses or negative for any respiratory virus. The samples were spiked with an internal control (IC). We carried out reverse transcription and amplification in glass capillaries on the Light Cycler Instrument 2.0 (Roche Applied Science, Meylan, France). The Real-Time Supermix contains MuIV reverse transcriptase (Applied Biosystems, Foster City, CA) and Platinium Taq polymerase (Invitrogen, Carlsbad, CA), primers complementary to highly conserved regions of genetic sequences for these respiratory viruses and dual-labelled exonuclease probes: RSV (FAM, BHQ1), IA (Cal Orange, BHQ1), IB (Cal Red, BHQ2) and IC (Q670, BHQ2). The fluorescence signals were measured for RSV, IA, IB and IC on the channels 530 nm, 560 nm, 610 nm and 705 nm respectively.
Results: All samples positive for RSV, Influenza A and Influenza B were detected by the ProFlu-1 LC Real Time Assay with Ct values ranging from 13 to 31. The species determination according to the specific channel analysis was in agreement with those expected. No sample was found dually positive. No amplification was detected for the respiratory virus negative samples positive nor for parainfluenza virus positive samples. The assay turn around time was 80 min.
Conclusion: Our data suggest that ProFlu-1 LC Real Time Assay is a rapid, sensitive, and specific multpliex PCR test for the diagnosis of infections with RSV, Influenza A and B viruses.

P643

Detection of respiratory pathogens including coronaviruses using PCR

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Objectives: We routinely use nucleic acid amplification tests to identify parainfluenza virus, influenza virus A and B, respiratory syncytial virus, human metapneumovirus, *Chlamyophila pneumoniae*, *Mycoplasma pneumoniae* and *Bordetella pertussis* in patient respiratory samples. In a 1-year period we also included a broad range PCR for detection of coronaviruses in the same samples. The aim of this study was to estimate the impact of coronaviruses as a respiratory pathogen in a Norwegian population.
Methods: A multiplex RT-PCR with subsequent probe hybridisation was used to identify parainfluenza virus, influenza virus A and B, respiratory syncytial virus and human metapneumovirus (Hexaplex, Prodesse, USA) in patient respiratory samples. The cDNA generated in the Hexaplex procedure was also amplified with coronavirus specific primers. Coronavirus PCR products were sequenced to identify the type of coronavirus. Real time PCRs for detection of *Chlamyophila pneumoniae*, *Mycoplasma pneumoniae* and *Bordetella pertussis* were performed on all samples.
Results: In the 1-year period from March 2004 about 2000 samples were analysed. In about two thirds of the samples no pathogen was found. In 12% of the samples one of the three bacteria were found. Infection with human metapneumovirus had a peak incidence in December–January with positive rates of 24 and 15% respectively. Infection with the influenza viruses had a peak incidence in February–March 2005 with positive rates of 24 and 29% respectively. In total 75 of the samples were positive for coronavirus, most of them OC43-like, with a peak incidence in January–February 2005 with positive rates of 9 and 14% respectively. In addition to the OC43 coronaviruses, three of type 229E, two of type NL63 and four of type HKU1 were detected. Very few samples contained more than one pathogen, but in 15 of the coronavirus positive samples another virus was also detected. All HKU1-like coronavirus positive samples also contained another respiratory pathogen.
Conclusion: In the winter months infection with coronavirus OC43 had a high incidence in this population of patients with respiratory illnesses. This suggests that this virus is an important cause of respiratory tract infections. As all of the coronavirus HKU1 positive samples also contained another

respiratory pathogen, and the pathogenicity of coronavirus HKU1 is difficult to estimate from this study.

P644

An evaluation of the predictive value of HPV testing and genotyping in the mass-screening against cervical cancer in Norway

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Cervical cancer is a progressive disease with detectable pre-invasive stages. Since 1995 every woman between 25 and 69 years of age is invited to participate in the national mass-screen against cervical cancer. The recognition of a causal role of high-risk human papilloma virus types (hr HPV) in cervical cancer aetiology has opened new alternatives for prevention of cervical cancer and guidelines for screening are under revision. HPV type 16, 18, 31, 33 and 45 have proven to be the most relevant oncogenes for cervical cancer in Europe and North America. To investigate the prevalence of hr HPV genotypes in women attending the mass-screening program in our region of Norway, and to evaluate and compare the predictive value of two different HPV-tests, we have set up a study aimed to proceed for 5 years involving a total of 2500 women. The study groups consists of 1000 women over the age of 30 with no history of abnormal cytology (control population), 500 women with inadequate cytology samples, 500 women with atypical (ASCUS) or low-grade (LSIL) squamus cells and 500 are women that have been treated by laser conisation for high grade cytological changes. Following standard cytology (Pap-smear) all participants are tested by the PreTect HPV proofer (Norchip, Norway) and the Amplicor HPV test (Roche Molecular Systems, Switzerland). PreTect HPV Proofer detects the E6/E7 mRNA transcripts of the hr HPVs 16, 18, 31, 33 and 45 and discriminates between genotypes (16, 18/31 and 33/45). Amplicor HPV detects the L1 gene of 13 hr HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) without any discrimination between these genotypes. For samples that are DNA positive but RNA negative, the genotype is identified using the newly launched HPV Linear array kit (Roche Molecular systems, Switzerland). So far (October 2005), a total of 1435 samples have been tested and 37.5% of the samples are classified as HPV DNA positive and 14.4% as E6/E7 RNA positive. 23.8% of the DNA positive samples are negative in the RNA test, whereas 2.4% of the RNA positive samples are negative in the DNA test. Half of the analysed samples belong to the control group and reveal normal cytology, and for these samples 11% are DNA positive and 3.5% are RNA positive. Linear array genotyping has been implemented revealing a domination of the genotypes HPV 51, 16, 31, 33 and 18. So far it is to early to draw any strict conclusions on the predictive values.

P645

Comparison between two commercial assays for HBV monitoring

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HBV viral load monitoring is essential to keep the virus under surveillance in chronic infected patients, to avoid complications such as flares, resistance or non-compliance to treatment. In transplanted patients, instead, it is necessary to closely monitor and detect the virus to minimize the impact of a disease re-activation.

Objectives: To compare two different commercial assays based on different technologies for monitoring of HBV viral load in high and low HBV positive samples.
Method: Eighty-five clinical samples ranging from undetectable to high HBV load were analysed by two HBV assays. One assay was based on conventional PCR format measured by an enzyme mediated reaction, affigene® HBV VL (HBV VL; Sangtec Molecular Diagnostics, Sweden). The other assay was based on real-time PCR technology, affigene® HBV trender (HBV trender; Sangtec Molecular Diagnostics, Sweden). For HBV VL, viral DNA was extracted from 100 μl of plasma according to the manufacturer's protocol. For HBV trender, 400 μl of plasma was used for the extraction of viral DNA using the EZ1 extraction system (Qiagen GmbH, Germany).
Results: The two assays correlated well in samples from 100 to 10E+06 IU/ml, where the assays were equally good at quantifying HBV genomes. The correlation calculated was r = 0.93 for all 85 samples. One fifth (20%) of all positive samples, evaluated by HBV trender were found HBV negative using HBV VL. Moreover two samples, among these, sowing a viral load of 1000 IU/ml, were identified as positive only by HBV trender. Clearly, the real-time PCR assay, HBV trender, is more sensitive than the conventional PCR assay, HBV VL. Moreover, the manufacturer states that the primer design is different between the two assays. In particular, the newer assay, HBV trender, was designed to detect all known HBV genotypes (A–H) with the same efficiency. Two samples reached the upper limit of HBV VL and could therefore not be quantified accurately. On the opposite, HBV trender could easily measure the same two samples.
Conclusion: The real-time PCR assay, affigene® HBV trender is more sensitive and has a higher upper limit than the conventional PCR assay, affigene® HBV VL. Thus, affigene® HBV trender is better than affigene® HBV VL for monitoring of HBV patients since a sensitive assay with a broad quantitative range is important in order to give a more appropriate result to the clinician.

P646

Detection of herpes simplex viruses type I and II in dermal, genital, oral, cerebrospinal fluid, and lower respiratory specimens by a Roche HSV I/II analytic specific reagent kit on real-time polymerase chain reaction

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Objective: To contrast traditional cell culture and real-time polymerase chain reaction (PCR) methods for detecting herpes simplex virus type I and II (HSV I/II) in several types of clinical specimens.
Methods: A total of 112 specimens [dermal (n = 22), genital (n = 34), oral (n = 11), cerebrospinal fluid (n = 7), and lower respiratory (bronchial wash, n = 32 and bronchoalveolar lavage n = 6)] were tested blindly by using both traditional viral cultural and a HSV I/II analytic specific reagent (ASR) kit (Roche, Indianapolis, IN) at the same time. DNA was extracted by using a MagaZorb mini-prep kit (Cortex Biochem, San Leandro, CA) and the PCR was performed on Roche LightCycler with 45 cycles used. 20-μl of total volume (15-μl master mixture and 5-μl sample) was used in the PCR. Data analysis was carried out either through describing quantization issues (crossing point) or by charting the melting curves. PCR results were compared to culture findings to determine sensitivity and specificity.

Results: The positive control on PCR was established by using Roche HSV I/II template DNA. The positive diagnosis on PCR was made by using the cut off of 100 copies of the template DNA (which will be adjusted in the near future because of the ASR qualification test for the HSV). Among all of the 112 patients' samples, 68/112 showed negative results by both methods; 25/112 showed positive by both; 6/112 showed clearly positive by PCR, but not viral culture; 11/112 showed peaks on the PCR designed HSV I/II positive ranges, but were unable to be diagnosed as positive compared to the cut off of the100 copies; 1/112 viral culture showed positive, but not PCR. 1/112 showed positive on viral culture and low peak on PCR.
Conclusion: Real-time PCR is a rapid test for HSV I/II diagnosis in the clinical virology laboratory with high sensitivity (25/27) compared with the traditional viral culture method. For the group which showed positive on PCR but not viral culture, DNA sequencing would be performed. This experiment is in progress and the results will be shared in the future.

P647

Survey of Crimean-Congo haemorrhagic fever in Iranian suspected patients by Elisa and RT-PCR method

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Introduction: CCHF is a viral zoonotic disease. The virus is from *Nairovirus* genus and Bunyaviridae family. The disease is transmitted to humans by infected tick bite or contact with virus contaminated tissues, blood or discharges of mammals. The mortality rate could be up to 50%. Early treatment with ribavirin sharply decreases this rate.
Method: In the period from June 2000 to October 2005 serum samples from 774 suspected patients for CCHF have been sent to the Arboviruses Lab (National Center) of the Pasteur Institute of Iran and have been analyzed serologically by ELISA method for specific IgM and IgG and molecularly by RT-PCR method for detection of a fragment in the S segment of the virus genome.
Results: Between 774 suspected human cases, 297 cases were positive serologically and molecularly. Between the 297 cases, 264 were IgM positive and 33 cases RT-PCR positive. Between the 264 IgM positive, 49 persons were also RT-PCR positive. Between the 297 positive, 53 died, among them 31 were IgM positive and 22 were RT-PCR positive and IgM negative. 55% of the IgM positive cases were in the age range 21–40 years. The Sistan-Baluchestan province, by having 67% of IgM positive cases, was the most infected province and the Isfahan province (12%) and Fars province (5%) were the second and third most infected province. The most exposed professions were: Farmer (21%), worker (20%), housewife (18%) and butcher (13%).
Discussion: Considering the results, CCHF is the most important haemorrhagic fever in Iran. Concerning the prevalence of the disease, more precautions must be taken by professionals in relation with livestock during slaughtering of domestic animals and handling of their carcass. In the survey carried out, it has been shown that due to the short viremia period, the probability of funding the virus genome in serum samples is decreased. On the other hand in certain patients who died fulminantly and had not enough time to generate an immune response, virus genome can be detected by molecular method. So molecular method together with serological method in CCHF suspected patients is the best way to diagnose the disease.

P648

Comparative evaluation of the vidas and liaison toxoplasmosis, cytomegalovirus and rubella panels in a French university hospital

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Objective: The aim of the present study was to evaluate the performance of VIDAS and LIAISON toxoplasmosis, cytomegalovirus and rubella panels in routine conditions in our laboratory.
Material: A study was performed during 3 weeks on 269 sera either from the laboratory routine or from the site sample collection for toxoplasmosis, 126 sera cytomegalovirus and 125 for rubella IgG testing. We have evaluated the performance of the VIDAS TOXO IgG II, VIDAS TOXO IgM, VIDAS CMV IgG, VIDAS CMV IgM, RUB IgG, VIDAS assays (bioMérieux, Marcy l'Etoile, France) in comparison with the LIAISON Toxo IgG, LIAISON Toxo IgM, LIAISON CMV IgG, LIAISON CMV IgM, LIAISON Rub IgG assays (DiaSorin, Saluggia, Italy). The VIDAS TOXO IgG and CMV IgG avidity and LIAISON Toxo IgG and CMV IgG avidity assays were used in case of IgG and IgM positive results (14 sera for toxoplasmosis and 13 for CMV). Complementary testing was performed in case of discrepancies such as immunofluorescence, ISAGA, IF and other ELISA tests. In addition, the following parameters were assessed for both systems: instrument set up, maintenance, time to result, ergonomics, general practicability.
Results: The correlation obtained for rubella IgG, CMV IgG, Toxo IgG and avidity assay for CMV or toxoplasmosis was good for both systems. Regarding the CMV, we obtained 1 discrepant result for the IgG when 9 were found for the IgM; perfect correlation on the avidity tests between both system was found. Regarding the Toxoplasmosis we observed 3 discrepant results for the IgG and 10 for the IgM, for the avidity 2 samples were equivocal, 1 in each method. The VIDAS System is more specific for IgM detection, in fact the LIAISON results showed more frequently no specific or residual IgM detection. The ergonomics of each system shows advantages and disadvantages inconvenient. The decision to choose one of these two systems is related to organisation strategies according to the laboratory specificity and panels available.
Conclusion: The results obtained during this study demonstrate that VIDAS is more specific for the diagnosis of acute CMV or Toxoplasmosis infections even if the LIAISON is more adapted to high throughput routine testing. These two systems can be easily integrated and used in the same laboratory.

P649

Different sensitivity of chemiluminescent immunoassay for the detection of HBsAg

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Background and aims: Sensitivity is the fundamental analytical requisite of assays for the surface antigen (HBsAg) of hepatitis B virus (HBV), since the determination of HBsAg is carried out both for blood donation screening and for screening and diagnosis of the general population; assay specificity is also important in low-prevalence settings. We aimed to investigate the diagnostic accuracy of two automated chemiluminescent immunoassays for HBsAg (Abbott Architect and Ortho Vitros) on selected repository specimens.
Materials and methods: We selected 94 serum samples who gave a repeat reactivity by our routine screening assay for HBsAg (Abbott AxSYM HBsAg) and that were classified as true or false positives according to the result of a confirmatory

neutralization assay and/or by serological or clinical criteria. All specimens were assayed under code by Architect and Vitros HBsAg in a different lab and the results were reported to our Center.
Results: The samples were divided into two groups: (a) 30 false positives by AxSYM, all negative by Vitros and 28 (93.3%) negative by Architect; (b) 64 true positives (57 neutralized, 3 viremic, 4 positive for other markers and with a history of HBV infection). The Architect assay gave a positive result on 63 specimens, the only negative being a patient clearing HBsAg after an acute infection. In contrast, the Vitros assay was positive only in 34 cases, grayzone negative in 4 and negative in 26. Interestingly, 7 of the samples negative by Vitros showed a strong reactivity by Architect, [sample/cutoff (S/CO) ratios from 100 to >5000], and three showed also a strong reactivity for anti-HBs (>240 mUI/mL), suggesting the possible presence of surface antigens escape mutations.
Conclusions: The assay for HBsAg on the Architect analyser was accurate in the discrimination of true from false positives, whereas the Vitros assay gave a surprisingly high rate of false negatives. In 19 cases this was probably due to a lower analytical sensitivity, but for the remaining seven specimens, showing a strong positivity for HBsAg by both AxSYM and Architect HBsAg, the negativity by Vitros may be related to its limited capability to recognize HBsAg mutants, as reported in the literature. In our opinion, it is urgent to clarify the prevalence and clinical impact of surface antigen variants in the current epidemiological setting in Italy as well as in the other countries in which anti-hepatitis B vaccination policies are established.

P650

Evaluation of a new assay for simultaneous detection of HCV core antigen and anti-HCV antibodies

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Objective: The serological HCV diagnosis may present false negative results in the window period, which can be reduced by doing HCV RNA or HCV core Ag. A new ELISA technique for simultaneous detection of HCV core Ag and anti-HCV antibodies has been studied and evaluated for laboratory routine use.
Materials and methods: 1491 sera with anti-HCV antibodies request received in our laboratory and 3 seroconversion panels with 24 serum samples were analysed. All these samples were tested by a routine assay which detects anti-HCV antibodies (Assay 1, Ortho HCV 3.0 ELISA), and by the new one that detects both HCV core Ag and anti-HCV antibodies simultaneously (Assay 2, Monolisa HCV Ag/Ab Ultra, Bio-Rad). All positive sera were confirmed by recombinant immunoblot (INNO-LIA de Innogenetics).
Results: The number of positive (303) and negative (1188) sera was the same for both techniques; however, 6 sera had divergent results. They are shown in the following table: Considering routine technique 1 as reference, the Ag/Ab assay had a sensitivity of 99.01% and a specificity of 99.75%. 99.07% of the negative sera and 93.72% of the positive ones presented an index far from the cut off. Considering the seroconversion panels, in all cases the combination assay reduce the window period.

	Assay 1	Assay 2
1	+	-
2	-	+
3	-	+
4	-	+
5	+	-
6	+	-

Conclusions: The new assay for simultaneous detection of HCV core Ag and anti-HCV antibodies presents very good sensitivity and specificity values and it can differentiate very well positive and negative results. This makes it a valuable assay for routine diagnosis which allows early detection of HCV infection.

P651

Comparison of molecular and serological assays in the diagnosis of HCV infection

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Objectives: There are different assays available for HCV infection diagnosis and treatment follow-up (anti-HCV antibodies, HCV-RNA, viral load, genotyping and free HCV core antigen). A new ELISA technique for simultaneous detection of HCV core Ag and anti-HCV antibodies has been evaluated. Its sensitivity has been assessed considering the potential influence of RNA-VHC, free VHC core Ag, viral load or the infecting genotype on it.
Materials and methods: A total of 143 positive anti HCV-antibodies sera have been tested for simultaneous detection of HCV core Ag and anti-HCV antibodies using Monolisa HCV Ag/Ab Ultra, Bio-Rad. In all of them HCV core antigen was analysed by ELISA (TrackC Ortho), serum HCV RNA was measured by PCR (Cobas Amplicor, Roche) and HCV genotyping was carried out by INNO-LIPA.
Results: 140 of the 143 samples were positive for the new combination assay (sensitivity = 97.9%). The 3 divergent sera were tested for confirmation by recombinant immunoblot, being 1 of them positive and 2 negative, therefore 141 were true positive being the sensitivity of 99.20%. Among these 141 positive sera, 72 (51.06%) were HCV RNA and 73 (51.77%) HCV Ag positive, although both parameters were not detected at the same time in all of them as it is shown: ARN-/Ag-: 60, ARN-/Ag+: 9, ARN+/Ag-: 8, ARN+/Ag+: 64. The most prevalent genotype was 1b (18.57%) and the assay performance was not influenced by the infecting genotype.
Conclusions: The technique for simultaneous detection of HCV Ag and anti-HCV Ab has very good sensitivity which is not influenced by the HCV infecting genotype, the presence of free HCV Ag, RNA or viral load. Also it has a good discriminative capacity for positive results; therefore it could be use in routine microbiology laboratories.

P652

Evaluation of an automated multiplexed assay of heterophile antibody in comparison with a latex agglutination test for assessment of Epstein-Barr virus induced infectious mononucleosis

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Objectives: The detection of heterophile antibodies, and specific antibodies against Epstein-Barr virus (EBV) antigens, has proven useful to categorize patients with no history of infectious mononucleosis, patients who are acutely infected,

patients with evidence of a prior or remote infection, and patients with reactivation of latent infection. The objective of this study is to compare the results of a fluorescence-based automated multiplexed assay for heterophile determination to results acquired with a conventional latex agglutination spot test.

Methods: Using the Bio-Rad BioPlex 2200 system, a fully automated high throughput multiplex platform, we report the detection of IgM antibodies to heterophile antigens and compare them to the Wampole Mono-Latex® test, a qualitative subjective methodology. The BioPlex 2200 results are reported as a semi-quantitative index value relative to negative and positive calibrators. For each sample, three internal quality control beads are evaluated simultaneously to verify instrument and chemistry performance, supplementing traditional quality control samples and calibration curve checks. The multiplex assay uses 5 μl of specimen and the instrument processes one hundred patient samples per hour.

Results: Serum specimens (N = 523 non-randomly selected) were analyzed by both assay methods. About 12% of the sera were positive for heterophile antibody. Compared to the Mono-Latex assay, the BioPlex 2200 gave a sensitivity of 91.5%, specificity of 99.3% and overall agreement of 98.3%. Six samples were initially categorized as false-negatives and three as false-positives. Upon repeat testing of the discrepant specimens, the automated test system exhibited a sensitivity of 97.1%, specificity of 99.6%, and overall agreement of 99.2%. The Mono-Latex test changed status on five of the nine discrepant samples, highlighting the difficulties interpreting visual readouts. No status changes for the discrepant samples were observed with the automated system. Additionally, the BioPlex 2200 system has excellent precision with CVs of less than 5% for samples near the cut-off.

Conclusion: The two heterophile assay systems showed excellent concordance. The BioPlex 2200 system, however, offers practical advantages that allow for rapid and fully automated evaluation of heterophile and IgM EBV antibodies.

P653

Real-time polymerase chain reaction and HCV RNA quantification: comparison of Cobas Ampliprep-Cobas TaqMan and branched DNA technology

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Background: Management of therapy for hepatitis C virus (HCV) infection is based on quantitative measurement of HCV RNA and the decision of treatment discontinuation on the basis of a 2 log decline at week 12 is a widely accepted rule.

Aims and methods: In the present study, we evaluated the performance of the completely automated system Cobas Ampliprep-Cobas TaqMan (CAP/CTM, Roche Diagnostics, Branchburg, NJ) for HCV RNA quantification in clinical serum specimens. CAP/CTM is a real time Polymerase Chain assay that relies on an automated nucleic acid extraction from 1050 μl of serum followed by RNA capture using magnetic particles, purification and elution. The system has a reported lower detection limit of 15 IU/ml and a dynamic range from 43 to 6.9E7 IU/ml. CAP/CTM results were compared for quantification of HCV genotype 1–4 with those from a signal amplification assay based on the branched DNA (bDNA) technology, the Versant Quantitative 3.0 (Bayer Diagnostic, Tarrytown, NY, detection limit 615 IU/ml and dynamic range from 1185 to 1.5E7 IU/ml).

Results: Sixty-three clinical specimens from patients with HCV infection were studied. The two assays were concordant (r = 0.85) with a mean ± SD interassay difference of −0.06 ± 0.8 log IU/ml. However, when the CAP/CTM values were analyzed for genotype, the mean ± SD interassay difference with bDNA were as follows: genotype 1 (n = 26), 0.3 ± 0.4, genotype 2a/2c (n = 12), 0.1 ± 0.2; genotype 3a (n = 18), −0.6 ± 1.4; genotype 4 (n = 7), −0.4 ± 0.7 log IU/ml, thus appearing that values obtained from CAP/CTM were in general 0.5 log lower than those from the bDNA for genotype 3a and 4. CAP/CTM detected HCV RNA in 8 (62%) out of 13 samples below 615 IU/ml with the bDNA (CAP/CTM levels from 1.4 to 3.5 log). Six out of the 8 CAP/CTM+/bDNA- specimens were from genotype 1 infected patients.

Conclusion: CAP/CTM showed a good correlation with bDNA with a better sensitivity that is crucial for the management of anti-HCV therapy, particularly for genotype 1. However, it seem that CAP/CTM underestimates HCV RNA levels in genotype 3 and 4 and this is clinically relevant as decisions during the clinical management of patients are based on measurements of HCV RNA levels and HCV RNA decline in patients on anti-HCV therapy.

P654

Automated fractal microscope for virus-cell monitoring

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Objectives: Virus-cell interaction could be monitored in a standard way through direct infected cells counting in the optical luminescent microscope. This way is time- and effort-consuming and requires additional colouring of the cell culture and the sample preparation process takes from 24 to 48 hours. The reliability of this method is not sufficient because it monitor only a small part of the specimen surface. Taking into account the results of our previous experimental investigations in the field, we may suppose that practically every stage of virus-cell interaction could be described in objective quantitative manner using the fractal approach and corresponding original instrumentation.

Methods: We have studied the interaction of Herpes simplex virus strain US-1 (HSV-1) with the specific cell culture Hep-2 modified by addition of proteolysis inhibitor E-aminocaproic acid (E-ACA) and Acyclovir taken in various concentrations. The monolayer of the cell culture was placed between the glasses support. The described samples were washed out with Hanks solution and afterwards fixed with ethanol. The fractal microscope included the single-mode and intensity stabilized Spectra-Physics He-Ne laser with 5.0 mW output and wavelength of 0.6328 + 0.01 microns. The diffraction patterns (DP) of the samples were taken with the use of Olympus Z-8080 digital camera and introduced with the use of USB cable into the port of Pentium IV computer. The fractal dimension D of the DP was evaluated automatically in an express manner (2 minutes per sample).

Results: The main result of our experiments was, of course, the multi-fractal type of the DP clusters. The registered minimal sizes of the fractal cluster elements were practically about the cell size ~1 mkm and HSV-1 virion ~0.1 mkm. The DP picture on the target is Fourier transform of the object and the real picture of the virus-cell system is obtained as the reverse Fourier transform of the DP.

Conclusions: The proposed device and method are applicable and highly competitive in laboratory and clinical antiviral research as well as in the drug design and testing process due

to its high sensitivity, express character of data taking and precise quantitative way of data processing.

P655

Comparison of HCV genotype and subtype determination using Inno-LiPA HCV II and a laboratory-developed HCV 5′UTR sequencing procedure

S. Gabella, T. Allice, S. Varetto, F. Pittaluga, V. Ghisetti *(Turin, IT)*

Background: Genotyping and subtyping of hepatitis C virus (HCV) is clinically relevant to epidemiology, prognosis and therapeutical management of HCV infection.
Objectives: To compare HCV genotyping and subtyping for clinical samples using an established Line Probe assay (InnoLiPA HCV II, Innogenetics, Ghent, B) and a laboratory-developed HCV 5′UTR direct sequencing protocol (5′UTR Seq) with ABI Prism 310.
Methods: Twenty-five clinical samples cross-representing HCV genotypes 1–5 were analysed with InnoLiPA and a direct 5′UTR sequencing method. A library of 5′UTR HCV prototypes (n = 64) was constructed; BioEdit 7.0 and ClustalW were used for alignments and phylogenetic analysis of samples. Concordance for genotype and subtype determination was compared between the commercial test and HCV sequencing. HCV viral load was quantified in each sample using the bDNA technology (VERSANT HCV RNA 3.0 Assay).
Results: 5′UTR provided genotype determination in all the samples. Concordance with InnoLiPA for genotype determination was 92% (22/25). Discrepant results occurred in 3 samples misclassified as genotype 2, 4 and 5 by InnoLiPA versus genotype 3 and 4 (2 samples) by 5′UTR Seq. Subtype concordance was much lower (44%, 11/25). 5′UTR Seq provided subtype determination in 96% of the clinical samples (24/25; one sample subtype 5a by InnoLiPA was classified as type 4r/4m without further subtyping), while InnoLiPA accounted for subtyping 13 of 25 samples (52% of all samples). 5′UTR Seq classified the 24 samples in 8 subtypes: 1a (n = 2), 1b (n = 5), 1c (n = 1), 2a (n = 2), 2c (n = 1), 2k (n = 1), 3a (n = 6) and 4a (n = 6), one sample misclassified as 4r/4m. InnoLiPA allowed the classification of the samples in only 5 subtypes: 1a (n = 2), 1b (n = 4), 3a (n = 4), 4e (n = 1) and 5a (n = 1) (13 not subtyped samples). Average (+SD) viral load for all genotypes was 5.9 + 0.5 log IU/ml and no significant differences were observed according to the genotype distribution.
Conclusion: 5′UTR Sequencing protocol provided HCV genotype determination for all samples and therefore it is suitable for determining HCV genotypes from clade 1–5 in clinical samples from patients with HCV infection. 5′UTR Sequencing allowed a much wider subtype determination from all the clades but particularly for genotype 1 and 2 compared with the commercial established InnoLiPA HCV II assay. HCV subtype sequencing provides a relevant tool for epidemiological purposes and the surveillance of nosocomial transmission of HCV in high risk units.

P656

Comparison of IMMULITE® 2000 anti-HAV IgM and anti-HAV total to Abbott AxSYM® assays

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Monitoring for the presence of IgM antibodies to hepatitis A virus (HAV) has proven beneficial in identifying acute or early events in infected individuals. Similarly, measuring the total antibody response to HAV has been shown to be advantageous in identifying previous exposure or ongoing viral infection. Chemiluminescent enzyme immunoassays were developed on the DPC IMMULITE® 2000 automated analyser for the detection of IgM and total antibodies to HAV in serum or plasma. The purpose of this study was to evaluate the performance of the IMMULITE 2000 assays compared to the Abbott AxSYM® HAVAB-M and HAVAB. In an initial study, the IMMULITE 2000 Anti-HAV Total assay was evaluated on 94 samples using the Abbott AxSYM HAVAB assay as a reference. The comparison demonstrated a relative sensitivity of 94%, specificity of 100% and overall agreement of 96.8%. In a separate study, the IMMULITE 2000 Anti-HAV IgM assay was compared to the Abbott AxSYMâ HAVAB-M assay on 116 samples. Results indicated relative sensitivity, specificity and agreement of 93.8%, 100% and 99.1%, respectively, compared to the reference. The assay exhibited intraassay precision of 18.2%, 10%, 11.4%, 6.8% and 5.5% for ratios of 0.35, 0.72, 0.96, 1.52 and 4.2, respectively. The interassay precision for these same samples was 18.4%, 11.7%, 10.1%, 7.3% and 6%, respectively. These data indicate that the IMMULITE 2000 Anti-HAV IgM and Anti-HAV Total assays perform comparably to the Abbott AxSYM assays and suggest the suitability of the DPC assays for the serological detection of IgM and total antibodies to hepatitis A virus.

Nosocomial infections

P657

National Resource for Infection Control (NRIC)

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The introduction of the 'Health Bill' in England later next year and subsequent 'Code of Practice' will give a firm statutory footing to what is accepted as best practice and evidence based policy and guidance on infection prevention and control in all healthcare settings. It will require a rolling programme of evidence based infection prevention and control policies to ensure that all policies and procedures are continually reviewed and updated. To assist in this process, the National Resource for Infection Control (NRIC – www.nric.org.uk) is an ongoing project developed by and for infection professionals, a single access point to the existing evidence base and resources for infection prevention and control. This project is funded by the UK Department of Health and endorsed by the UK National electronic Library of Infection (NeLI – www.neli.org.uk), a digital library bringing together the best available quality appraised online evidence-based resources on the investigation, treatment, prevention and control of infectious diseases. National policy and guidance documents are available on NRIC as well as templates to aid in writing local policies. Evidence based information is available and organised by settings, clinical practice tasks, modes of transmission and diseases/organisms. The level of evidence for each resource is clearly noted, as well as its regional coverage. Each infection

control resource is quality appraised before being added to the portal, and many of the resources have a full review, written by infection professionals. In addition, there are algorithms for easy access and templates to be adapted to meet local needs. The implementation of online discussion of the resources allows further debate of reviews so as to ensure all issues are addressed. The resource is not intended to replace local infection prevention and control policies/guidance but to assist in their writing and/or updating and to achieve a more standardised approach to the evidence used. Progress with the development of NRIC has been excellent and feedback from infection control professionals has been positive with roughly 1000 unique users per month as of October 2005. Although the site contains primarily UK policy, guidance and research, the concept will be of interest in this era of global challenges which includes the need for joint working and collaboration with colleagues in infection prevention and control internationally.

P658

An independent review of computerised systems for alert organism and alert condition surveillance

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There is a demand and requirement for information systems to support the activities of infection control and infection control teams in acute hospital trusts in England. ''A Systems Evaluation Project for Infection Control (ASEPTIC)'' defined the user requirements for infection control functions in acute hospitals, assessed current systems and recommended those to be piloted against the requirements [1]. The Infection Control IT Implementation and Evaluation (ICIT/IAE) project was undertaken to implement and further evaluate the three systems recommended by ASEPTIC, namely EpiQuest, ICE, ICNet.

Objective: The principal aim of the ICIT/IAE project was to provide recommendations with regard to the use of infection control systems to support local infection control teams and infection control practices in the National Health Service (NHS) acute hospital trusts. The scope of the project was to implement and then evaluate three infection control systems for local use. The systems were tested in nine NHS trusts in England.

Method: The evaluation was based upon a questionnaire that assessed the: Installation, configuration and interfacing with the Laboratory Information Management System (LIMS) alone; communication, professionalism and approaches of each of the suppliers; ability of the systems to support local trust internal alert organism reporting, outbreak detection and case management.

Results: Eight of the nine trusts experienced delays during implementation. By 31st March 2005, seven of the nine systems were installed and being used locally. As of 31st May 2005, seven of the nine trusts had decided to keep the IC software that they piloted and have completed business cases for funding. This includes two EpiQuest sites, all three ICE sites and two ICNet sites.

Conclusion: The key outcomes from ASEPTIC, endorsed by the ICIT/IAE Project Board were that three systems should be piloted. These were EpiQuest, ICEnterprise, and ICNet. These systems were installed and independently reviewed by nine UK NHS trusts. The recommendations of the ICIT/IAE project board will be presented and include details of the companies results and recommendations on implementation of infection control IT systems. (1) ASEPTIC final report. August 2003. http://www.hpa.org.uk/infections/publications/pdf/aseptic_Report.pdf.

P659

Knowledge, attitudes and practices of health care workers in Kosovo hospitals regarding nosocomial infections

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Objectives: To assess the level of knowledge, attitude and practices amongst doctors and nurses towards nosocomial infections in Kosova hospitals.

Methods: A confidential, self-administrated questionnaires were distributed randomly to 550 health care workers at four Kosova hospitals during the march 2005. The questions in the survey assessed general knowledge about nosocomial infections, attitudes and infection control practices.

Results: The questionnaire response rate was 63.6%. Of the 350 respondents, 39% were doctors and 61% were nurses. The median age of respondents was 38.1 years. Only 16.8% of respondents knew the complete definition of nosocomial infection. Sixty-nine per cent of health care workers knew that contact is the most common mode of transmission. Regarding the transmission pattern of hepatitis B and C, 62% of respondents gave the correct answer. Ninety-four per cent knew that instruments should be cleaned before sterilization and disinfection. The majority of health care workers reported hand washing after using gloves (84%). Forty-seven percent of them thought disinfection is the process of complete destruction of all forms of microbial life. During collection of blood samples, one-fourth of respondents withdraw blood immediately after skin disinfection and 47% 5 sec later. Sixty percent of HCW were vaccinated against hepatitis B. Knowledge of risks of HIV transmission from an infected patients after needlestick injury was very low(8.5%). Fifty-seven percent of HCW reported that they had suffered a needlestick injury and 26% of them did not report them to authorities. There was no significant difference between doctors and nurses concerning needlestick injuries ($p > 0.05$).

Conclusion: The study demonstrated that knowledge of medical staff in Kosova hospitals about nosocomial infections is insufficient and some infection control practices are poor. Therefore, further education and training are necessary to improve the level of knowledge and infection control measures in our hospitals.

P660

Worldwide implementation strategy of the World Health Organization ''Guidelines on Hand Hygiene in Health Care''

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Objective: The aim of the 2005–06 Global Patient Safety Challenge (GPSC) of the World Alliance for Patient Safety is to reduce health care-associated infections (HAI) worldwide, with hand hygiene (HH) as the cornerstone. The advanced draft of the World Health Organization (WHO) ''Guidelines on HH in Health Care'' (HC) was recently issued and presents an unprecedented approach targeted at the promotion of HH in a global perspective.

Methods: The essential tools and methods to implement the WHO Guidelines were identified through evaluation of lessons learned from HH campaigns existing worldwide, extensive literature review and expert advice. Several consultations and meetings with renowned international experts, field practitioners, as well as patient and industry representatives, were organized to achieve a consensus strategy.
Results: To meet the GPSC, the WHO Guidelines on HH in HC are integrated with other interventions in the field of blood, surgical procedure, injection and water and waste management safety. Different components of the Challenge are being implemented in several countries in a pilot testing phase with the expectation of a mutual reinforcement among the different actions. For optimal HH promotion, the essential elements for implementation are: healthcare worker education, appropriate technique and indications for HH, system change (alcohol-based handrubs available at point of care, and water access and quality), appropriate use of gloves, monitoring system for impact evaluation and staff feedback, and administrative support. The tools made available from WHO are: educational model to be tailored to the cultural background; validated WHO formulations for local production of alcohol-based handrubs; posters; glove use guide; monitoring model; and patient information leaflet.
Conclusions: For the first time, HH is being globally promoted as the cornerstone of a series of infection control interventions. The goal is to reduce HAI worldwide, regardless of the type of HC setting and the level of country development. Key elements must be considered to achieve a standardized implementation and meet the minimum requirements for a successful evidence-based strategy. This pilot testing phase represents a remarkable added value to the WHO ''Guidelines on HH in HC'' for their validation and, after revision, to be issued in a final version based on the lessons learned.

P661

Considerations on optimal glove use within the World Health Organization ''Guidelines on Hand Hygiene in Health Care''

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Objective: Glove use and re-use are crucial issues which can significantly affect health care workers' compliance with hand hygiene (HH) practices. Within the advanced draft of the World Health Organization ''Guidelines on HH in Health Care'' recently issued, a specific section is dedicated to this topic in the perspective of promoting HH worldwide.
Methods: Indications for glove use and advantages and disadvantages of glove use during patient care were analyzed through extensive literature review and consultation with international experts. Glove reuse should be avoided, but stock disruption is still a reality in several settings worldwide, and methods for glove reprocessing were also discussed.
Results: The experts acknowledged that wearing gloves can be a potential barrier to perform HH when indicated during patient care. Situations when gloves should be never/always used were carefully identified and the need for non-sterile rather than sterile gloves was evaluated for each opportunity. Continuous glove-wearing during patient care should be considered as a missed opportunity for HH when indicated. Glove reuse should be avoided, but if it occurs in situations with limited resources, the following steps should be performed: (1) evaluation process to verify the reasons and need for reuse; (2) safe handling of contaminated gloves by healthcare workers during reprocessing should be guaranteed; (3) selection process: only surgical latex gloves can be reused for examination purposes and/or for surgical double-gloving; (4) reprocessing: decontamination with 0.5% chlorine solution, cleansing with soap and water, autoclave sterilization or steam high-level disinfection, and integrity test with air or water filling. Solutions for sticky gloves and excess tearing and rupturing were provided.
Conclusions: The issue of glove use and reuse should be carefully considered when planning a HH promotion campaign in healthcare. Indications and contraindications for wearing gloves should be carefully detailed during education activities. In the case of glove reuse, a careful evaluation process, together with an adequate reprocessing method should always be performed. The possibility of decontaminating gloves with alcohol-based hand rubs during health care when contact isolation precautions are in place should be investigated as a research issue.

P662

Alcoholic hand disinfectant use in a general hospital: impact of an intervention

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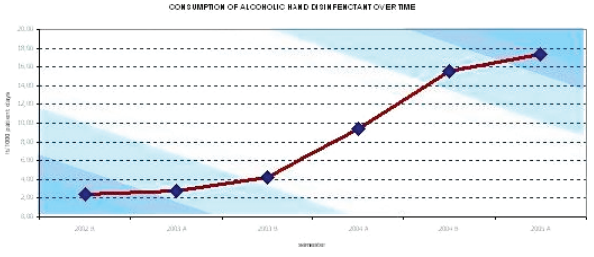
Introduction: The volume of alcohol-based hand disinfectant (ABHD) used, is currently considered as a performance indicator for hospital infection control. Infection control is not a first priority issue for many Hellenic hospitals. The aim of our study was to locate areas problematic in terms of infection control in our hospital (''A. Fleming'' General Hospital – 300 beds), by using the data for ABHD consumption. Besides, we wanted to assess the results of a relevant intervention (mounting of ABHD dispensers in every patient room).
Methods: We used data from the pharmacy computer for the ABHD distribution to various departments of the Hospital. Data were collected for 6-month periods beginning from the second half of 2002 to the first half of 2005. Consumption was expressed as litres of disinfectant/1000 patient days.
Results: The consumption was 2.5, 2.8, 4.2, 9.4, 15.6, 17.3 liters/1000 patient days for 2002B, 2003A, 2003B, 2004A, 2004B and 2005A semesters (Figure). Furthermore, more ABHD was used in medical than surgical departments and the difference was significant for all study periods ($p = 0.007$, paired t test). It is worth noting that during 2004A semester, dispensers for ABHD were mounted in every patient room.



Conclusions: (A) Mounting of ABHD disinfectant dispensers in every patient room resulted in a significant increase in ABHD consumption for the hospital as a whole. (B) The impact of this increase on nosocomial infection rate is currently under evaluation. (C) It seems that surgical departments' personnel are more reluctant to use hand disinfectant and should be the first target group for education on infection control.

P663

Does sufficient hand hygiene influence hospital-acquired infections?

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Objective: To improve hand hygiene practice as a part of a strategy to prevent hospital acquired infections (HAI).
Method: In April 2004 Aarhus University Hospital, Skejby Sygehus started to promote hand rub with alcohol in a hospital wide agenda containing campaigns, training of hygiene link nurses, introducing of an E-learning programme and by improved hand hygiene facilities. Sufficient hand disinfection is described as a part of an evidence based clinical guideline for hand hygiene. We performed four surveys based on observations of health care workers (HCW) hand hygiene practice. Three performed in 2004 and one in 2005. Observations in 2004 were done by seven special trained key-link nurses. This included a baseline survey before the efforts to improve hand disinfection practice in April 2004. In 2005 the observations were carried out by 50 hygiene link nurses from most clinical wards. In all surveys we recorded potential opportunities for hand disinfection as described in the clinical guideline. Inter observer variation was not estimated but minimized through audit discussions. In parallel we monitored the hand-alcohol consumption and the monthly incidence of hospital acquired septicaemias as expressed by the number of blood stream infections per 1000 bed-days.
Results: The average of HCW observed in all four studies was 27%. At baseline, compliance for hand disinfection was 58% (567 of 917). The two following studies in 2004 did not show any improvement in compliance with hand disinfections. In 2005 we succeeded to improve compliance for hand disinfection to 73% (1985 of 2735). As showed in other studies compliance for observed physicians was in 2005 lower (51%; 166 of 324) than for other groups of HCW. The consumption of hand-alcohol increased with 100% from 1252 l in the first quarter of 2004 to 2503 l in the second quarter of 2005. The incidence of hospital acquired septicaemias did not decrease in the period between the first quarter of 2004 (1.8 per 1000 bed-days) and the second quarter of 2005 (1.7 per 1000 bed-days).
Conclusion: The increase in observed compliance for hand disinfection and consumptions for hand-alcohol in this study seems not to have influenced the rate of hospital acquired septicaemias. The compliance for hand disinfection practiced by physicians is still unacceptable low.

P664

Adherence to hand hygiene in an Italian hospital

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Objective: To evaluate the adherence to hand hygiene (HH) in an Italian hospital.
Methods: The study, of the observational type, was performed at the Istituti Ospitalieri di Cremona, in Lombardy, Italy, an 800 bed non-teaching facility. In the hospital work 1478 health care professionals (HCP): 270 medical doctors (MD), 796 nurses, 67 physiotherapists, and 192 ancillary staff. The analysis was performed by 6 trained researchers. HCP performance on all potential opportunities that required HH was recorded on charts, following the Centres for Disease Control and Prevention guidelines. The observation was performed using Pittet's methodology, i.e. each observation lasted about 20 min. Analysis was stratified by HCP category, department, and contamination risk, divided into low, intermediate and high risk. High risk procedure were defined as: before intravenous care, before respiratory care, and passing from a dirty to a clean body site. Univariate and multivariate analysis were performed to identify risk factors associated with adherence to HH.
Results: During 257 observation periods performed in 28 wards and services, we recorded 3241 HH opportunities performed by 1639 HCPs (1113 nurses, 257 physicians, 165 ward maits, and 99 other HCPs). The overall adherence to HH was 25.5%. Analysis performed by working class, showed that nurses had the highest adherence to HH (29.3%). MD cleaned their hands in 19.5 of occasions. Adherence to HH was highest in medical wards (41.6%) and lower both in Intensive care units as well as in paediatric wards – 13.6% and 10.5% respectively. Multivariate analysis showed that the conditions more strictly associated with a non compliance with hand hygiene were working as a physiotherapist and as ward mait (OR = 2.86; and 2.33 respectively), working in a paediatric ward (OR = 2.73), and performing high risk procedures (OR 2.21)
Conclusions: Adherence to HH by HCP is happens in about 1 in 4 occasions, as reported in other countries. Adherence is higher among nurses than other HCPs. Adherence is very low (18.8%) during high risk procedures. This adherence to HH, the simplest and probably most effective strategy to prevent nosocomial transmission of multiresistant germs is unacceptably low. HH campaigns need to be implemented to improve adherence to this procedure.

P665

Handwashing and glove use frequencies of intensive care nurses

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Objectives: The aim of this study was to asses the frequency of hand washing and glove use among intensive care nurses.
Methods: The study was conducted between December 2004–February 2005 in General Surgery, Neurosurgery, Cardiovascular Surgery and Anaesthesia and Reanimation intensive acre units (ICUs) at Ege University Medical Hospital. A ''direct observation'' method was used. Nurses were observed according to procedures requiring hand washing with the recommendations of The Centers for Disease Control and Prevention (CDC) and The Association for Professionals in Infection Control and Epidemiology (APIC). Totally, 1257 procedures performing by the nurses were observed. Data were analyzed using SPSS (version 10.0, SPSS, Inc, Chicago, IL, USA) and represented as frequency and percentage.
Results: The hand washing rate of nurses was found very low both before (0.2%) and after (23.7%) procedures. However, the rate of glove use (72%) was relatively high. We also found that nurses did not wash their hands in 96.9% before entering the ICUs, whereas they washed in 93.4% after leaving ICUs, thinking us they washed hands to protect themselves mostly.
Conclusion: The hand washing frequencies of ICUs were below the literature. We recommended that researches should be conducted to determine the causes of not washing hands among nurses, if there is a need, education should be given to the nurses for enhancing their knowledge and compliance to hand washing. The rate of infections based on regular surveillance also should be reported to the departments.

P666

Evaluation of parameters influencing compliance of HCWs with hand hygiene in two university departments of internal medicine in a Greek tertiary hospital

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Objectives: The aim of this study is to evaluate factors and situations at risk which may influence compliance of HCWs with hand hygiene in two University Departments of Internal Medicine of a tertiary Greek Hospital.
Methods: Behaviour of HCWs regarding the appropriate use of alcohol-based hand-rubs before and after contact with patients was assessed by two independent observers via a standardized registry form. The study was conducted during two periods, from April to May and May to June 2005, before and after the application of hand hygiene policies, in two Departments of Internal Medicine named ''A'' and ''B''. Hand hygiene policies included the installation of a bed-rail hand-rub dispenser system in ''A'' (this system had already existed in ''B''). Besides, during the same time-frame, a campaign by posters related to hand hygiene was launched both in ''A'' and ''B''. Assessment of HCWs' compliance with appropriate use of antiseptic on hands was performed regarding also variables as time of observation (morning afternoon, day of hospital emergencies), patients at risk (hepatitis, HIV, AIDS, neutropenia and multi-drug resistant bacterial infections).
Results: They are summarized as follows: in "B", overall compliance reached 36.5%, ranging from 96/215 (44.6%) in the morning to 7/53 (13.2%) in the afternoon ($p < 0.001$). No difference was observed regarding behaviour between emergency and non-emergency days (31% vs 37%) and between high and low risk patients (31% vs 38%). Compliance in "A" was improved after the initiation of the bed-rail system from 102/281 (36.3%) to 70/136 (51.4%) ($p = 0.004$) but it is worse in the afternoon than in the morning (21.8% vs 56.7%, $p = 0.01$).
Conclusions: Although compliance of HCWs with the appropriate use of antiseptic on hands in ''B'' was quite high, it was also significantly worse in the afternoon. No difference on compliance between patients with and without high risk of transmissible infection was observed. Similar observations were assessed in ''A'' where a newly applied bed rail system of alcohol-based dispersers was recently established. In ''A'' as in ''B'', compliance with hand hygiene in the afternoon is significantly worse than in the morning despite the infection control measures. We conclude that other factors, like the lack of staff and the absence of surveillance in the afternoon may influence behaviour of HCWs regarding hand hygiene. A new strategy of infection control is required in order to stabilize HCWs' hand hygiene behaviour during 24 hours a day.

P667

Nosocomial meningitis after spinal anaesthesia

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Objectives: Nosocomial bacterial meningitis is a rare but serious complication of spinal anaesthesia. The aims of this study were to investigate an outbreak of nosocomial meningitis caused by *Serratia marcescens* in University Clinical Centre of Kosova among patients after spinal anaesthesia and to implement the appropriate control measures.
Methods: During September 8–11, 2003, three patients were referred from orthopaedic and abdominal surgery to the Infectious Disease department with clinical signs of meningitis after interventions proceeded by spinal anaesthesia. An epidemic investigation was therefore started to identify the source and to control the outbreak. A retrospective case control analysis of the clinical charts in the previous year, extensive microbiological sampling and review of the unit practices were performed. Aetiological diagnosis was based on culture of cerebrospinal fluid.
Results: Three cases during September 2003 had undergone spinal anaesthesia in the departments of orthopaedic and abdominal surgery. Patients developed meningeal syndrome 24–49 hours after spinal anaesthesia. The patients were from 16–50 years old. *S. marcescens* was isolated from cerebrospinal fluid culture of two patients. Cultures were obtained from potential environmental resources including anaesthesia equipment, medication solution, needles and syringes. Samples from vials containing fentanyle were positive for *S. marcescens*. In both orthopaedic and abdominal surgery departments, fentanyle containers were used as multi-dose vials covered by sticking-plaster. This practice was justified with limited resources. Antimicrobial susceptibility testing of isolates from patients and vials yielded the same pattern suggesting common source of infection. The outcome of disease was favourable in all patients. Retrospective review of patient's charts in previous year detected five cases of nosocomial meningitis from patients after spinal anaesthesia. In one case culture was positive for *Serratia marcescens*.
Conclusions: Our findings are consistent with break in aseptic techniques that could result in introduction of *Serratia* into the cerebrospinal fluid through multiple-use of anaesthetic solution. To prevent nosocomial iatrogenic infections caused by *Serratia* and other multiresistant bacteria, compliance with strict aseptic rules and comprehensive infection control procedures are recommended.

P668

Infections after open heart surgery

C. Ezpeleta, E. Gomez, C. Busto, I. Atutxa, J.A. Alava, J. Unzaga, R. Cisterna *(Bilbao, ES)*

Objective: To know the rates of NI (Nosocomial Infection) and Surgical site infections (SSI) in patients operated on open heart surgery in our hospital and to compare with those of HELICS (Hospital in Europe Link for Infection Control trough surveillance).
Patients and Methods: The 1416 patients (pt) operated on open-heart surgery in our hospital from January 2000 to September 2005 were included. Preoperative protocol regarding infection control includes: shower with 4% clorhexidine soap the night before surgery and repeated the day of surgery. Hair clipping just before surgery. Antibiotic prophylaxis: Cefuroxime 15 g. IV/8 h during 24 h. All the pt are prospectively studied since the day they are operated until the end of the episode by the infection control team. Variables under surveillance are age, sex, underlying illnesses, predisposing conditions, ASA physical status classification, NNIS risk index, antibiotic prophylaxis, nosocomial infections, microorganisms, length of hospital stay, treatment and outcome. A computer based surveillance system (INOZ) is used during admission and continued 1 year after discharge. CDC definitions of nosocomial infection are used.
Results: Age and sex: 967 (68.3%) men, mean age 66.1 y (SD 11.1). Mean preoperative stay in the hospital 5.3 days (SD: 9.1). Nosocomial infections: 282 pt acquired 388 NI, 66 of them were

surgical site infections (SSI), Respiratory 132, Urinary tract (UTI) 95, Catheter related 37, Bacteremia 29, and other locations 29. Cumulated incidence of patients with NI 19.92%. Surgical site infections: 22 incisional superficial, 17 deep incisional and 27 organ space. NNIS score 0: 103 pt, 1% SSI; Score 1: 926 pt, 3.3% SSI; Score 2: 372 pt 7, 5% SSI, Score 3: 15 pt, 26.7% SSI. Antibiotic prophylaxis was administered in 99.2% of the cases. The dosage, time, drug and duration of the prophylaxis were appropriated (100%, 99.4%, 100% and 93.5% respectively). Microorganisms in SSI: Coagulase Negative *Staphylococci* (CNS) 20, *P. aeruginosa* 8, *S. aureus* 7, *Enterococcus* spp. 5, *S. marcescens* 2, Polymicrobial 10. Cumulated incidence of SSI 4.66%.
Conclusions: Comparing our data of SSI (4.66%) with the HELICS report our results are higher than the total results (2.7%), near of those of our country (6% in Spanish hospitals) and below the rates of percentile 90. Gram-negative bacilli are the most common isolated microorganisms in SSIs in our hospital, in the HELICS report gram positive cocci are recovered in 81.7% of cases.

P669

Surgical site infections after colon surgery

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Objective: The aim of this study is to know the rate of Surgical Site Infection (SSI) and other nosocomial acquired infections after colon surgery in our hospital and to compare our results with the HELICS (Hospital in Europe Link for Infection Control trough surveillance) report.
Methods: The Infection control team prospectively studied all patients operated on colon surgery from January 2002 to September 2004. Variables under surveillance are age, sex, underlying illnesses, predisposing conditions, ASA physical status classification, NNIS risk index, antibiotic prophylaxis, nosocomial infections, microorganisms, length of hospital stay, treatment and outcome. Antibiotic prophylaxis schedule in our hospital is Neomycin + Erythromycin po and Amoxicillin + Clavulanate IV. Case definitions: CDC definitions for Nosocomial infections. Surveillance after discharge: 1 month.
Results: 484 patients were studied, 63.42% of them were males, mean age 67.5 y (SD 12.9). 141 patients (29.13%) had 209 NI: 112 surgical site, 48 UTI, 20 catheter related, 8 respiratory, 12 bacteremia, and 9 other locations. Cumulated incidence of infected patients 29, 13/100. Antibiotic prophylaxis was administered in 99.5% of the cases. The dosage, time, drug and duration of the prophylaxis were appropriated (100%, 99.6%, 94.2% and 90.0% respectively). Surgical site infections (SSIs) 112 cases: superficial incisional SSIs (54), deep incisional SSIs (26) and organ/space SSIs (32). Cumulated incidence patients with SSIs 21.28%. NNIS Score 0: 110 patients, 11.8% SSIs; Score 1: 186 patients, 18.8% SSIs; Score 2: 132 patients, 32.6% SSIs; Score 3: 36 patients, 22.2% SSIs; score M 20 patients, 20% SSIs. Microorganisms in SSI: *E. coli* 55, *Morganella* 11, *Enterococcus* spp. 8, *P. mirabilis* 6, *S. aureus* 6, Anaerobes 14. Mean length of hospital stay 31.8 days (SD 29.9) in patients with NI and 13 days (SD 9.8) in patients without NI. Crude mortality 4.3%: 8.5% patients with NI and 2.6% without NI.
Conclusions: Cumulative incidence of SSIs in our hospital is similar to that of Spanish hospitals included in the HELICS report (21.28% vs 21.6%), but very different from other European countries. There are some differences in case definitions between countries and again major differences between countries were observed in the type of reported SSI (Superficial/deep/organ space). The use of common surveillance systems and definitions should be encouraged.

P670

Hospital-acquired infection: a hospital-wide prevalence study at a London hospital

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Objectives: To conduct a hospital-wide prevalence of infection study to determine relative value of alert organism verses alert condition surveillance in the detection of hospital acquired infection (HAI).
Methods: In November 2002 in addition to existing alert organism surveillance (as recommended by the UK Department of Health), the Infection Control Team performed a pan-hospital prevalence study, over 5 days, to capture all patients being treated for bacterial infection. Drug charts were reviewed for all in-patients. The primary inclusion criterion was treatment with antibiotics. Patients receiving antiviral and antifungal agents or antibiotics for prophylaxis were excluded. Cases were followed-up by review of medical notes, interview with the patient's clinical staff members and review of culture results from the Medical Microbiology database, where available.
Results: 91.3% of the 945 beds surveyed were occupied. 52% of the patients receiving antibiotics were male and 48% female. 40% of these in-patients were over 65 years old. A total of 396 antibiotics were prescribed for the 261 patients, with 42 (16%) receiving three or more agents. The Critical Care group had the highest percentage of patients on antibiotics (63%) followed by Specialist medicine (51%) and Renal (48%). 185 patients were followed-up. Among these the highest rates of infection were wound followed by respiratory tract infections and bacteraemias. Ten patients had multiple infections. The overall prevalence of infection was 25% and 40% of these were hospital acquired. For the study period, the ratio of patients with 'alert organism' HAI versus non-alert organisms was approximately 1:4. As there were approximately 393 patients with an 'alert organism' HAI in 2002, using the above ratio we estimate there were 1965 patients who had a hospital-acquired infection at Kings during 2002.
Conclusion: Current surveillance methodology recommended by the UK Department of Health only detects one quarter of patients being treated for HAI. This highlights the need for increased alert condition surveillance to inform strategy and target resources more appropriately in the management of HAI.

P671

Surveillance of nosocomial bloodstream infections

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Objectives: To establish an electronic system for surveillance of nosocomial blood-stream infections (BSI) providing feed-back to the clinicians with the purpose of reducing the frequency of BSI. In retrospect, to describe the rate of BSI in our hospital over a 5-year period.
Methods: All patients admitted to Aarhus University Hospital, Skejby in the period 2001–2005 are included. The hospital comprises about 500 beds and includes departments of paediatrics, obstetrics and gynaecology, infectious diseases, cardiology, nephrology, urology and thoracic surgery, and an intensive care unit. A BSI is defined as the growth of a pathogen in the blood and the concomitant treatment of the patient with antibiotics. To be registered as a nosocomial BSI, the infection must appear later than 48 hours after admission unless the patient is readmitted within 7 days or has gone through a

surgical procedure within 30 days. A new BSI episode is defined as the growth of a new pathogen more than 2 days after a previous pathogen or by the growth of the same pathogen more than 7 days after its first detection. We combine data from the patient-administrative system, the laboratory information system, and the drug database in the electronic patient medical records. The number of new episodes of nosocomial BSI per 1000 bed-days is calculated for each month. A gliding average of BSI episodes within the last 6 months was also calculated monthly.
Results: The overall hospital rate of BSI did not change between 2001 and 2005. The monthly gliding average of BSI ranged from 1.5 to 2.3 episodes per 1000 bed-days. The rate of new episodes was higher in July compared to the other months during the observation period. The BSI rates for the individual department did not change over time. For one department, a seasonal distribution of BSI was observed. A pronounced difference in BSI with specific microorganisms was observed within some of the departments.
Conclusion: The surveillance of nosocomial BSI based on combined data from various electronic hospital registries is cost-effective and provides documentation of trends and sudden changes in the rates of the BSI for the hospital overall and the individual clinical departments. Importantly, the system provides a mean for monitoring the consequences of changes in procedures to reduce the rate of nosocomial BSI.

P672

A morbidity and mortality conference to evaluate nosocomial infections and hospital mortality

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Objectives: The authors had for aim to determine the relationship between the hospital deaths and nosocomial infections (NI) in a morbidity and mortality conference.
Methods: The study was made in St Philibert Hospital (Lomme, France) on 306 patients' deaths from December 2004 to May 2005 (mortality rate: 3.23%). Medical records of the 271 consecutive patients who died at least 48 h after admission were reviewed for cause of death, NI and disease severity, before admission and before NI onset. The contribution of NI to death was assessed by an expert comity including hospital physicians having dealt with the patient.
Results: The median age was 76 years (33–101), with 54% male patients. The disease was cancer in 30% of the cases, shock and heart failure in 11%, respiratory disease in 7%, liver disease and cirrhosis in 6%, infectious disease in 0.3% and violent death in 0.3%. Seventy two patients presented with at least one nosocomial infection (23.5%): 30 with a bacteriemia (41.7%), 20 with a pneumonia (28%), 16 with a urinary tract infection (22%), 3 with an infection of a surgical-site infection (4%) and 3 with other infection (4%). Mortality was attributable to NI in 4.24% of the cases and death was considered as preventable in 8 cases (2.6%), of which 3 were attributable to NI (1%).
Conclusion: Based on a consensual and thorough review of each patient's clinical story, this study confirms that a NI increases the risk of death. If the same scale were used on a national level, the number of deaths attributable to NI in France would reach 14,000, of which 3300 considered as preventable. To improve healthcare quality, morbidity and mortality conferences are needed in hospitals to identify those circumstances that might be associated with the onset of severe NI contributing to death, and preventive measures should be targeted at these.

P673

Point prevalence of nosocomial infections in Western Greece

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Objectives: The purpose of this study was to estimate the frequency of nosocomial infections (NI) in the region of Western Greece and to determine the impact of different risk factors. A further aim was to prioritise infection control measures and to develop more detailed incidence surveillance of NI.
Methods: All 7 acute care hospitals in W-Greece were invited to participate in two one-day prevalence studies, on 16.12.2004 and on 10.2.2005. The Laboratory of Public Health at the University of Patras supplied the hospitals with questionnaires including demographical and clinical data and written instructions. In each clinic, a physician and a nurse were assigned to collect data on the day of survey. They were to confirm the presence of NI by on-site observation. The total number of inpatients, the number and the site of NI as well as data on individual risk factors were recorded. Statistical analyses were performed using SPSS v.12.0.
Results: A total of 2305 hospitalised patients were registered during the two studies. There were 34 males and 32 females identified with NI (mean age 64 years). The regional prevalence of all recorded NI was 2.9% (2.1% in 2004 and 3.7% in 2005) and ranged from 0–6.8% between the facilities and from 0–22.7% between the different specialities. The overall highest prevalence was found on Neurosurgery and Intensive Medicine. The mean interval between admission and the onset of NI was 12 days. In both surveys, NI were located most frequently in the urinary tract (33.4%), followed by pneumonia (13%), surgical sites (10.1%) and septicaemia (10.1%). Overall, 90% of patients with NI had predisposing factors, such as cardiovascular disease, diabetes or cancer. The total number of NI confirmed by the microbiological laboratory was 59%. The most frequently isolated microorganisms were *E. coli* (25.6%), *Enterococci* (15.4%) and *S. aureus* (10.3%).
Conclusion: The study reveals a comparably low overall prevalence of NI, but remarkable differences between the hospitals and clinics. This fact may be due to comprehensible medical reasons but also due to underreporting of NI in certain clinics. However, the study highlights the need for repeated point-prevalence surveys, which seem to be an acceptable, cheap and easy way of assessing NI rates. With adequate education of the hospital staff and enhanced surveillance, they can contribute to improving infection control programmes and enabling effective interventions to reduce the risk factors of NI.

P674

Levofloxacin plus rifampicin treatment of staphylococcal prosthetic-joint infections with prosthesis retention

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Objectives: To describe a series of *Staphylococci*-infected prosthetic joints after hip and knee total replacement, treated with debridement and prosthesis retention plus long-term levofloxacin and rifampicin.
Methods: Prosthesis-joint infections due to staphylococcal species were defined by isolation of *Staphylococci* from at least two cultures of consecutive samples of joint aspirate, surgical intraoperative specimens in the debridement procedure, or at least three cultures on three different days from discharge of the sinus tract, in the presence of clinical criteria. Patients received

long-term oral levofloxacin 500 mg o.d. and rifampicin 600 mg o.d.

Results: Sixty patients (age 74.6 ± 8.4 years) were included: 28 knee infections and 32 hip infections. In knee infections, coagulase-negative *Staphylococci* were significantly more frequently isolated (78.6% vs. 21.4%; p = 0.00001), with no differences in hip staphylococcal aetiology (46.9% for *S. aureus* vs. 53.1% for *S. epidermidis*). One third (33.3%) of *S. aureus* isolates were methicillin-resistant. Time from arthroplasty to symptoms onset was significantly (p = 0.03) higher in coagulase-negative vs. positive *Staphylococci* (mean time free of symptoms: 1.7 vs. 0.9 months, respectively). Global failure was 35% (42.8% for knee infections and 28.1% for hip infections), and ranged from 16.6% to 69.2% (p = 0.0045) in patients with <1 to >6 month symptoms duration. When analysing cure versus failure cases, significantly shorter time of symptoms duration (2.5 vs. 6.7 months; p = 0.001) and time to diagnosis (3.5 vs. 7.3 months; p = 0.01) were found in cured patients. Among *S. aureus*, higher (p = 0.08) failure rates were obtained for methicillin-resistant (5 out of 7; 71.4%) than for methicillin-susceptible (3 out of 14; 21.4%) isolates.

Conclusions: Efficacy of treatment with levofloxacin plus rifampicin and debridement with prosthesis retention was higher in patients with shorter time of duration of symptoms, earlier diagnosis, hip infections, and methicillin-susceptible isolates.

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Prosthetic joint infections: results of a collaborative protocol between orthopaedist surgeons and infectious diseases physicians

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Background: Successful treatment of an arthroplasty infection requires a combination of meticulous surgical approach and effective antimicrobial therapy. Antibiotic treatment must be individualized and the therapeutic decisions can be very complex.

Methods: Prospective open observational study, 2 years of follow-up. A cooperative protocol for management of prosthetic joint infections was started and an infectious diseases specialist established the proper antibiotic treatment for every PJI. This therapy was controlled by him, who decided the optimal moment of drug suspension. The individualized surgical procedure and the time of realization was consensuated with the orthopaedist.

Results: 40 patients were analysed. Sex: Men 23 (57.5%), Women 17 (42.5%). Mean age: 70.03 ± 9.84 years (R:43–87). Prosthesis location: Knee (K): 13 (32.5%), Hip (H): 26 (65%), Shoulder (S):1 (2.5%). Time presentation: Early: 21 (52.5%), Delayed: 6 (15%), Late: 13 (32.5%). Positive cultures were obtained in 37 patients: Coagulase-negative *Staphylococci*: 15, *Staphylococcus aureus*: methicillin-susceptible: 5, methicillin-resistant: 2, *Streptococcus* species: 3, *Enterococcus* species: 3, *Enterobacteriaceae*: 8, Nonfermenters: 3. Surgical procedure: Debridement (D): 13 (32.5%), One-stage exchange (1s): 7 (17.5%), Two-stage exchange (2s): 18 (45%), permanent removal of prosthesis: 2 (5%). Medical therapy: Initial: Rifampin (R) + Quinolone (Q): 4, R + Glycopeptides (G): 11, G alone: 4, Cephalosporins + Aminoglycoside (A) 3, synthetic Penicillin (Pn) + betalactamase-inhibitor: 3, PnG: 2, Others:13. Maintenance: R + Q: 11, R + G: 1, G alone: 1, Linezolid: 2, R + trimethoprim-sulfamethoxazole: 7, Others:18. Long-term therapy (ST): 3. Outcome: See table 1. Lost of follow-up: 11. Side-effects: Acute Renal Failure 1, Skin rash: 1. The degree of acceptance of therapeutic recommendations by orthopaedists surgeons was 100%. The index of satisfaction (perceived quality) on the part of the patients was very high.

	Location	Cure	Failure	Lost	Recidive	Days of treatment
D	K	5	1	3	0	157,5±45
	H	2(+2 supressive Therapy)	1	3	0	112,5±95,5
1 s	H	6	1	0	0	125,1±57
2 s	K	6	1 (not related death)	2	2	90±48
	H	5	0	3	0	90±54
	S	1	0	0	0	60
A	H	2	0	0	0	70±21
ST						3 patients still on treatment

Conclusions: The infectious diseases specialist must have a main role in management of patients with prosthetic joint infections. A narrow collaboration between the different specialists involved in the care of the patient, is essential to obtain the best possible results.

P676

Predictive value of oral colonisation by *Candida* yeasts on the onset of a nosocomial infection in elderly hospitalised patients

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The incidence of nosocomial yeast infections has increased markedly over the past decades, especially among the elderly. The present study was therefore initiated in order to determine the predictive value of oral colonisation by yeasts in the onset of a nosocomial *Candida* infection in elderly hospitalized patients (>65 years), but also to clarify the promoting factors of infection and to establish a relationship between the intensity of oral carriage and the onset of yeast infection. During this prospective cohort study, 256 patients (156 women and 100 men with a mean age of 83 ± 8 years) were surveyed for yeast colonisation or infection. Samples were collected every four days from D0 to D16 from four sites in the mouth, and intrinsic and extrinsic factors that might promote infection were recorded for each patient. Pulsed field gel electrophoresis was performed on *C. albicans* isolates from all infected patients. Poor nutritional status was observed in 81% of the patients, and hyposalivation in 41%. The colonisation rate was 67% on D0 (59% *C. albicans*) and a heavy carriage of yeasts (>50 c.f.u.) was observed for 51% of the patients. The incidence of nosocomial colonisation reached 6.9% on D4 (6.1% on D8 and 2.7% on D12), and that of nosocomial infection was 3.7% on D4 (6.8% on D8, 11.3% on D12 and 19.2% on D16). Of the 35 patients infected, 57% were suffering from oral candidiasis. The principal risk factors for colonisation were a dental prosthesis, poor oral hygiene and antibiotherapy. The risk factors for infection, in addition to those already mentioned for colonisation, were endocrine disease, poor nutritional status, prolonged hospitalisation and high colony counts. Genotyping revealed person-to-person transmission in two patients. Thus this study demonstrated a significant association between oral colonisation and the onset of yeast infections in elderly hospitalized patients. Therefore, oral samples should be collected at admission and antifungal

treatment should be administered in case of colonisation, especially in patients presenting a heavy carriage of yeasts. Genotyping of the strains confirmed the possibility of person-to-person transmission. UPRES-EA 3142

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Impact of antifungal treatment on *Candida-Pseudomonas* interaction

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Objective: A pathogenic interaction between *Candida* and *Pseudomonas* has recently been demonstrated (1). In addition, Candida bronchopulmonary colonization (BPC) is significantly associated with *Pseudomonas* ventilator-associated pneumonia (VAP) (2). However, no cause-to-effect relationship has been established. The aim of this study was to determine the impact of antifungal treatment on bronchopulmonary infection (BPI) and/or BPC due to *Pseudomonas*.
Methods: Retrospective observational cohort study conducted in a 30-bed ICU during a 1-yr period (2004–2005). All patients intubated and ventilated >48 h with *Candida* BPC were eligible. Patients with *Pseudomonas* BPC and/or BPI diagnosed before or at the same time than *Candida* BPC were excluded. Routine screening for *Candida* and *Pseudomonas* was performed at ICU admission and weekly. Antifungal treatment was at physicians' discretion. Risk factors for *Pseudomonas* BPC and/or BPI were determined using univariate and multivariate analyses.
Results: 102 patients were included, *C. albicans* was the most frequently isolated *Candida* (66%). 36 patients received an antifungal treatment. Mortality rate (53% vs 31%, OR [95% CI] = 2.3[1–5.5], $p = 0.032$), mechanical ventilation duration (31 ± 22 vs 14 ± 10 d, $p < 0.001$), and length of ICU stay (36 ± 25 vs 16 ± 14, $p < 0.001$) were higher in patients who received antifungal treatment than in those who were not treated. 19 patients (18%) developed a *Pseudomonas* BPC and/or BPI. Rates of *Pseudomonas* BPC and/or BPI (20% vs 17%, $p = 0.4$), and of *Pseudomonas* VAP (13% vs 10%, $p = 0.4$) were similar in patients who received antifungal treatment compared with those who did not receive antifungal. However, mean duration of mechanical ventilation between intubation and first *Pseudomonas* BPC and/or BPI (20 ± 14 vs 13 ± 10 d, $p = 0.003$) was significantly longer in patients who received antifungal treatment than in those who did not receive antifungal. Hospital acquired pneumonia at ICU admission [OR (95% CI) = 5.5(1.4–21.7), $p = 0.014$] was the only factor independently associated with *Pseudomonas* BPC and/or BPI.
Conclusion: Antifungal treatment is not associated with *Pseudomonas* BPC and/or BPI. However, *Pseudomonas* BPC and/or BPI occurred significantly later in patients treated with antifungal than in patients who were not treated.
References 1. Hogan DA et al. Science 2002; 296:2229–32.2. Azoulay E et al. Réanimation 2005; S1: SO 71 (abstract).

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Nosocomial urinary tract infections caused by extended-spectrum beta-lactamases producing *Enterobacteriaceae* in Latvia

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Objectives: To discover tests for screening and confirmation of extended-spectrum β-lactamases (ESBLs) production in different genera of the family *Enterobacteriaceae*. To evaluate ESBL producing strains isolated from hospital patients with urinary tract infections (UTI) in Latvia.
Methods: 316 non-duplicate *Enterobacteriaceae* strains collected from urine UTI patients from Infectology Center of Latvia and children hospital ''Gailezers'' during January 2004 to October 2005 and two quality control strains were tested. Identification was performed in mini API system, for susceptibility testing – disk diffusion, E-test and ATB mini API were used. Screening of ESBL positive strains was obtained in mini API ATB Expert system. ESBL production was confirmed by disk diffusion (NCCLS, 2002) and E-test ESBL: both cefotaxime/cefotaxime + clavulanic acid and ceftazidime/ceftazidime + clavulanic acid (AB Biodisk, 2000).
Results: The screening of ESBL producing strains revealed 12.0% positive *Enterobacteriaceae* (38 of 316): among isolated *Klebsiella* spp. 27.5% (11 from 40 strains), *Escherichia coli* 6.9% (15 of 218), *Enterobacter* spp. 31.5% (5 of 16), *Proteus* spp. 37.5% (6 of 16), *Morganella morganii* 11.1% (1 of 9). There were not ESBL producing strains in two *Serratia* spp. and five *Citrobacter* spp. strains in screening. The confirmatory tests occurred to be positive in all *E. coli* and *Klebsiella* strains, identified as resistant phenotype ESBL, five of six *Proteus mirabilis* strains, marked as resistant phenotype ESBL. In *Enterobacter* species only two of five, marked in screening as ESBL possible by Expert, were ESBL positive by confirmatory tests. As concerns one *M. morganii* ESBL suspicious strain was not confirmed.
Conclusion: Mini API ATB Expert system should bee recommended: as screening for ESBL production in *Enterobacteriaceae* strains, especially *Klebsiella* species, *E. coli* and *P. mirabilis*. ESBL screening marked as ESBL possible must be confirmed with confirmatory tests by discs and E-test ESBL.