Immunology and immunotherapy

P1315 Kinetics of the release of nitric oxide and tumour necrosis factor-alpha after activation of microglia via Toll-like receptors-2, -4, and -9

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Objectives: Microglial cells, the major constituents of innate immunity within the brain, express Toll-like receptors (TLRs) recognising exogenous and endogenous ligands. The present study aimed at quantifying the activation of microglial cells in response to stimulation with one or two simultaneously administered specific agonists of TLR-2, -4, and -9 at concentrations causing submaximum and maximum effects.
Methods: Primary mouse microglial cell cultures were stimulated with the TLR agonists Pam3Cys and heat-killed Acholeplasma laidlawii (TLR-2), endotoxin and pneumolysin (TLR-4) and oligonucleotides containing unmethylated cytosin-guanosin motifs (CpG) (TLR-9). Nitric oxide (NO) release was quantified using the Griess reaction. TNF-α release was measured using the Quantikine M Mouse TNF-α Immunoassay (R&D Systems GmbH). Cell viability of microglial cells was determined using the WST-1-Cell Proliferation Reagent (Roche Applied Science).
Results: Maximum stimulation of TLR-2, -4, and -9 resulted in approximately equal amounts of NO release. LPS was most potent in stimulating microglia (concentration causing the half-maximum effect [EC50]-NO: 0.00037 μg/mL; EC50-TNF-α: 0.024 μg/mL), followed by pneumolysin (0.024 μg/mL; 0.136 μg/mL), CpG (0.119 μg/mL; 0.685 μg/mL) and Pam3Cys (52.3 μg/mL) (EC50-values represent means of six experiments with at least triplicate measurement, respectively). Pneumolysin was a potent activator of microglial cells at concentrations below 3 μg/mL; at higher concentrations it reduced cell viability. No cytotoxicity was noted with the other TLR agonists. Co-stimulation with maximum concentrations of two TLR agonists did not further increase NO release. Co-stimulation with sub-maximum concentrations had an additive effect.
Conclusions: Stimulation of microglial cells via different TLRs resulted in a relative uniform release of NO and TNF-α. Simultaneous stimulation with low concentrations of two agonists led to an additive effect. Not only microbial products, but also endogenous compounds can act as agonists in the TLR system. The additive effect of stimulating more than one TLR does not only increase the sensitivity of microglia during infections, yet may also entail interactions among exogenous and endogenous agonists of TLRs. This may be one reason for the exacerbation or induction of autoimmune diseases by infections.

P1316 Drug modulation of Toll-like receptors expression by human macrophages stimulated with lipopolysaccharide and bacteria

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Objectives: Human Toll-like receptors (TRL) TLR2 and TLR4 recognise the specific patterns of a variety of bacterial cell-wall components and transduce signals in cells during an early phase of the innate immune response. Glucocorticoids (GC) are potent immunomodulatory drugs. Although GC can supress many functions of macrophages (Mph), a role of GC in enhancing host-immune responses, particularly through an effect on TLR expression in Mph, remains unknown. The aim of the study was to evaluate the effect of dexamethasone (DXM) on TLR2 and TLR4 expression and cytokine production by Mph upon stimulation with LPS or *Salmonella typhimurium* (STM). We also determined modulation of these parameters by ceftriaxone (CFX), a cefalosporin antibiotic used in the treatment of bacterial infections.
Methods: PBMCs were isolated from human buffycoats by Histopaque (Sigma,USA) density gradient centrifugation. Monocytes were isolated by adherence to plastic (3 h, 37°C). Adherent monocytes were stimulated with LPS (100 ng/mL) or STM (300 CFU/mL) for 24 and 48 h. DXM and/or CFX (Sigma, USA) were used at concentrations 1 μM a 5 μg/mL, respectively. CFX was added into some wells after 24 h. The supernatants were collected and frozen at −70°C for quantification of IL-6, IL-10 and IL-12 by ELISA (Pharmingen, USA). Mph were harvested and stained for flow-cytometric analysis using mAb against CD14 (Becton-Dickinson, USA), TLR2 and TLR4 (eBioscience, USA).
Results: TLR2 expression by Mph was enhanced by LPS or STM ± DXM. Unlike TLR2, DXM inhibited IL-6, IL-10, and IL-12 by LPS-stimulated Mph. However, DXM increased IL-10 production by STM-stimulated Mph. Interestingly, Mph stimulated by LPS or STM and treated with CFX showed lower expression of TLR2 compared with those without CFX. This was associated with a decrease in IL-6 and IL-10. We did not observed any effect of LPS or STM on TLR4 expression by Mph.
Conclusion: Our results demonstrate the ability of GC to increase the expression of TLR2 on human Mph. This indicates a possible immunostimulatory effect of GC. However, involvement of TLR2 induction by GC in subsequent innate and specific immune responses requires further study. Decreased TLR2 expression associated with lower production of IL-6 and IL-10 in Mph treated by CFX indicates a role of TLR2 in the immunomodulatory effect of this antibiotic.
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P1317 Exopolysaccharides from marine thermophilic bacilli induce a Th1 cytokine profile in human PBMC

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Objectives: The exopolysaccharides (EPS) produced by a strain of Geobacillus thermodenitrificans and a strain of *Bacillus licheniformis* were used to evaluate their immunoregulatory and antiviral effects. The two strains were isolated from a shallow marine hot spring of Vulcano Island (Italy). In view of the important regulatory role in antiviral defence played by cytokines modulator factors, we analysed both the effects of EPS of two marine bacteria on cytokine production and the antiviral activity on peripheral blood mononuclear cells (PBMC).
Methods: PBMC were obtained from healthy donors, after centrifugation of heparinised venous blood over a Ficoll-Hipaque gradient. Supernatants were harvested and analysed for the presence of IFNγ, IFNα, TNFα, IL-12, IL-18, IL-4. In order to establish if the EPS antiviral activity was related either to the marked production of Th1 cytokines or to a direct interaction with virus replication, we used WISH cell line. To evaluate the effects of the on HSV-2 replication in PBMC and WISH cell line, the amounts of virus production were titrated and expressed as PFU/mL.
Results: The EPS trigger mononuclear phagocytes in releasing cytokines involved in Th1 profile, such as IL-12, IFNγ, IFNα, TNFα, IL-18, whereas did not induce the production of Th2 cytokine (IL-4). Furthermore, treatment of HSV-2 infected PBMC with EPS down-regulated virus replication. This effect on HSV-2 replication seems to be related to the pattern of cytokines induced in PBMC by EPS. The addition of EPS to WISH cells, infected with HSV-2, did not show any antiviral effect.
Conclusion: We have shown that the two EPS are powerful stimulators of Th1 cell-mediated immunity. They are immunomodulatory agents which could be potentially used as a therapy in the immunocompromised host.

P1318 Differential induction of IL-8 expression by LPS and LTA

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Objectives: Lipopolysaccharide (LPS) from Gram-negative bacteria and lipoteichoic acid (LTA) from Gram-positive bacteria are key inflammatory principles which differ regarding the cytokine pattern they induce: LTA is a stronger inducer of chemokines, i.e. IL-8 and G-CSF, than LPS, which is a stronger inducer of pro-inflammatory cytokines, such as TNFα. We have shown previously for LPS stimulation that IL-8 and G-CSF production is increased by db-cAMP, while TNFα production is decreased, and described a novel activating CRE in the G-CSF promotor. Thus we now investigated whether the IL-8 promotor also has an activating CRE element, which might explain the parallel strong induction of IL-8 and G-CSF by LTA.
Methods: HL-60 cells were transfected with mutated or wild-type promotor constructs with a luciferase reporter. β-galactosamine was co-transfected and values were normalized to its activity. mRNA expression in whole blood was measured by real-time PCR. Protein release in whole blood supernatants was quantified by ELISA.
Results: Screening of the IL-8 promoter identified a putative CRE (TTTCgTCA). We transfected HL60 cells with a minimal IL-8 promoter containing different disrupted transcription factor binding sites, namely NFkB, AP-1 and the putative CRE with a luciferase reporter. However, LPS and LTA induced the same signal height both in cells with normal and mutated CRE, indicating this is a nonfunctional element, while NFkB and AP-1 were confirmed as essential elements. Moreover, cAMP elevation in human whole blood led to an increase in LPS-induced IL-8 release but a decrease in LTA-induced IL-8 release. This indicated that the strong LTA-induced IL-8 release was dependent on a different parameter, so we characterised this induction more closely. IL-8 mRNA expression peaked at 4 h in LPS-stimulated whole blood but continued to rise until 24 h in LTA-stimulated blood. In line, IL-8 protein levels induced by LTA became significantly higher than those induced by LPS only at time points after 8 h following stimulation and peaked at 28 h (stimuli 10 μg/mL: LPS, 64 ± 17 ng/mL IL-8 vs. LTA, 205 ± 47 ng/mL IL-8, $P < 0.01$, $n = 16$). This divergence was retained in plasma-free blood but was not observed in PBMC. However, addition of erythrocytes to PBMC reconstituted this effect.
Conclusion: The strong induction of IL-8 by LTA in whole blood appears to depend on an amplification or presentation factor supplied by erythrocytes and not on increased cAMP levels.

P1319 Mannose-binding lectin (MBL) and susceptibility to infection in preterm neonates

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Rationale: Premature infants are particularly susceptible to infections. It is estimated that up to 50% of neonates born at <32 weeks gestation may suffer from clinical sepsis. The reasons for this are complex but immaturity of the immune system with sub-optimal levels of circulating immunoglobulins is at least partly responsible for high mortality and morbidity associated with infection. Another protein, mannose-binding lectin (MBL) has also been shown to be low in premature infants and may be important in defending premature infants from infection. Genetic polymorphisms resulting in deficiencies of MBL have been shown to increase susceptibility to infection especially in children. The aim of the current study is decide the role of MBL in determining the susceptibility and outcome of premature neonates to severe sepsis.
Methods: A total of 50 premature neonates have been recruited to the study. Serum levels of MBL were measured by enzyme immunoassay and polymorphisms at codons 52, 54 and 57 within exon 1 (A, wild type, 0, variant) and a promoter polymorphisms (X/Y) of the MBL gene were determined by PCR and heteroduplex analysis.
Results: Birth weight and gestational age were both found to influence MBL levels. Neonates born at <30 weeks gestation and <1500 g were significantly lower than neonates of >30 weeks and >1500 g. This difference persisted ($P < 0.0005$). 40% of preterm neonates had exon 1 mutations (A/0 or 0/0). 50% of these neonates were diagnosed with sepsis and/or multiple organ system failure. In comparison, only 26% of neonates with wild-type alleles (A/A) were diagnosed with sepsis. When MBL levels were analysed in all 50 neonates, levels of MBL were significantly less in those with sepsis when compared with those without sepsis (mean 1 492 ng/mL (SD 1 451 ng/mL) vs. 2 342 ng/mL (SD 2 070 ng/mL), $P = 0.037$).
Conclusion: In this preliminary study, MBL deficiency appears to be associated with increased susceptibility to severe sepsis in premature neonates. Further studies are required to elucidate the true impact of this finding on neonatal susceptibility to infection.

P1320 Immunotherapy with RUTI, a new useful vaccine for treatment of *Mycobacterium tuberculosis* infection

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Objectives: To study the usefulness of RUTI in the treatment of *M. tuberculosis* infection in a experimental murine model. RUTI is a vaccine (patent pending) composed by *M. tuberculosis* fragmented cell wall, detoxified (MtbFCW) and liposomed.
Methods: Female mice DBA/2 and C57BL/6, 6 weeks old, were infected by aerosol using a Middlebrook device. On week nine postinfection treatments were started: (I) Control, inoculated with 50 mL of empty liposomes on weeks 9, 11 and 15 postinfection, s.c; (II) chemotherapy from week 9–17; (III) chemotherapy plus RUTI on weeks 17, 19 and 21 i.n. and (IV) chemotherapy plus RUTI on weeks 17, 19 and 21 s.c. Chemotherapy with 25 mg/kg isoniazid the first 4 weeks and rifampicin 10 mg/kg the following last 4 weeks was administered orally through gavage 5 days/week. 50 uL of RUTI containing 185 μg of MtbFCW were injected in every inoculation. On week 22 postinfection, we determined colony forming units (CFUs) in lungs and spleens; quantified cytokine mRNA in lungs using by Real-Time PCR and levels of antibodies against MtbFCW antigens through western blot. Finally we determined the area occupied by granulomas with digital microphotography and processed with appropriate software.
Results: A significant and synergistic reduction of CFUs was revealed in groups III and IV in DBA/2 mice compared with group II. No differences were detected in C57BL/6 mice as treatment with only chemotherapy revealed a high efficiency. Relative increase of IFN-γ was detected in both mouse strains treated with RUTI, specially in i.n. Inoculated groups. levels of TNF were kept low. After i.n. C57BL/6 mice elicited a strong IgG2b response against a broad spectrum of cell wall antigens. DBA/2 increased the IgG2a and IgG2b ones. After s.c. inoculation, C57BL/6 enhanced also the IgG1 and IgG3 levels, while DBA/2 increased just the IgG1. High IgA levels were detected in the BAL of DBA/2 mice after i.n. and s.c. immunisations. In C57BL/6 only after i.n. Histometrical analysis did not reveal any significant increase regarding on the area occupied by granulomas in RUTI-treated mice, but in intranasal inoculated C57BL/6 mice.
Conclusions: The use of RUTI as a coadjuvant of chemotherapy has demonstrated its usefulness in DBA/2 mice with a combined Th1/Th2 response against *M. tuberculosis* infection without provoking an increase of pulmonary parenchymal lesions.
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P1321 Coadministration of intranasal RUTI with chemotherapy improves the treatment of *Mycobacterium tuberculosis* infection

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Objectives: To improve the use of RUTI as a coadjuvant of chemotherapy in the treatment of *M. tuberculosis* infection in the mouse strains that triggers the strongest resistance against this infection.
Methods: Female mice BALB/c and C57BL/6, 6 weeks old, were infected using a Middlebrook device, allowing the inoculation of 10 to 20 bacilli in the lungs. On week nine postinfection we defined four different groups according intranasally inoculation of RUTI. BALB/c mice treated with chemotherapy from week 9–15 postinfection, and inoculated on week 13 with 50 μL of empty liposomes (group I) or RUTI (group II). C57BL/6 mice were treated with chemotherapy from week 9 to 17 postinfection, and inoculated on weeks 13, 15 and 17 with three doses of empty liposomes (group III) or RUTI (group IV). Chemotherapy was done orally through gavage 5 days a week with 25 mg/kg isoniazid. 50 μL of RUTI containing 185 μg of *M. tuberculosis* fragmented cell wall (MtbFCW) were injected in every inoculation. We determined colony forming units (CFUs) in lungs and spleens on week 15 (groups I–II) and 15 and 28 postinfection (groups III–IV). Expression of cytokine was quantified in lungs using Real-Time PCR. We also determined the ratio between the area occupied by granulomas and the total area analysing four consecutive 5 μm thick sections of two lobes from the right lung of each mouse stained with haematoxilin–eosin and photographed at 10x in a Nikon stereoscopic microscope. Images were processed with appropriate software.
Results: A significant and synergistic reduction of CFUs was revealed in groups II and IV in both mice strains. No significant differences on IFN-γ and TNF expression were found in both groups of BALB/c mice. Besides, these cytokines were significantly low in RUTI treated C57BL/6 mice on week 15 and 28, reflecting a decrease on inflammatory response. Histometrical analysis did not reveal any significant difference in both mice strains on week 15.However, control mice doubled the area when compared with RUTI treated group on week 28.
Conclusions: The use of RUTI as coadministrated with chemotherapy inoculated intranasally has demonstrated its benefit in chemotherapy treatment even in those mice strains able to trigger the strongest immunological response against *M. tuberculosis* infection.
Research support: Fondo de Investigaciones Sanitarias. Grant no. 01/0341.

P1322 Early prognostic markers of outcome in severe sepsis

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Objective: Since mortality in severe sepsis remains high, the aim of this study was to estimate the prognostic value of certain early immunological markers for the clinical outcome.
Methods: Thirty patients (15 male, 15 female, mean age 68.5 $\pm$ 9.7 years) with severe sepsis (sepsis plus at least one organ dysfunction) were studied. The aetiology of sepsis was: pneumonia ($n = 11$), pyelonephritis ($n = 5$), intra-abdominal infection ($n = 9$), skin or joint infection ($n = 5$). The control group included 14 healthy subjects (seven male, seven female, mean age 67.9 $\pm$ 8.6 years). The percentage of monocytes expressing human leucocyte antigen-DR (CD14-HLADR) was determined by flow cytometry on admission, days (d) 3, 10, 13 and on discharge. Serum cytokine levels were determined by using ELISA (Quantikine, R & D Systems, Minneapolis) on the same days. Stepwise multiple regression and logistic regression analysis were used for statistical analysis.
Results: Seventeen patients died 1–14 days after admission. A significant contribution to positive outcome was detected for CD14-HLADR on admission [odds ratio (OR), 1.04; 95% confidence intervals (CI), 1.00–1.09, $P = 0.03$], while serum interleukin-10 (IL-10) levels on day 3 (OR, 0.92; 95% CI, 0.86–0.99, $P = 0.03$) and IL-10 on day 10 (OR, 0.80; 95% CI, 0.65–0.98, $P = 0.04$) were found to be predictors of poor outcome. The strongest effect on IL-10 levels on day 10 was attributed to CD14-HLADR on day 3 ($r^2 = 0.4$; $P = 0.008$) without further contribution of other cytokines (tumour necrosis factor-α, IL-4, IL-6, IL-8, transforming growth factor-β).
Conclusions: (i) Immunosupression associated with low levels of monocyte HLA-DR expression precedes immunosupression correlated with high levels of IL-10. (ii) Monocyte HLA-DR expression is an early prognostic marker of outcome in severe sepsis and a diagnostic tool for identifying high risk patients.

P1323 Evaluation of cardiac troponin I in elderly patients with sepsis

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Sepsis is a common cause of organ dysfunction and finally multiple organ failure. Cardiac dysfunction in septic patients is due to myocardial ischaemia and endothelial-leucocyte interactions that result to microcirculatory tissue damage. Measurement of cardiac troponin I (TnI) provides a sensitive and specific indicator of cardiac injury over a wide diagnostic window. Aim of this study was to investigate if serum troponin I correlates with severity of sepsis in elderly patients and to evaluate whether TnI might be a prognostic marker in these patients
Subjects and methods: A total of 46 patients with sepsis of various origin, 26 females and 20 males, aged 76 $\pm$ 11 years old, were included in this study. Blood samples were collected and TnI levels was measured within 6 h after hospital admission. Patients with possible myocardial infarction or unstable angina were excluded from this study. Serum TnI was determined by colorimetric immunoassay in DADE-BEHRING biochemical analyser.
Results: A total of 24 patients had TnI values >0.2 ng/mL and 22 had levels <0.2 ng/mL. The CPK–MB values were not elevated and ECG did not differ between groups. In patients with higher TnI levels APACHE II score and DIC score (criteria of International Society on Thrombosis and Haemostasis), were calculated and they were found higher in comparison with those of patients with low TnI (24.2 $\pm$ 7.8 vs. 19.4 $\pm$ 5.3, $P = 0.025$ and 3.45 $\pm$ 0.3 vs. 2.26 $\pm$ 0.3, $P = 0.009$, respectively Student's *t*-test). A positive correlation between TnI and APACHE II score as well TnI and D-Dimers values were also observed ($P = 0.0037$ and $P = 0.015$, respectively, stepwise regression analysis)
Conclusion: Serum troponin I may be of value in the recognition of clinically unrecognised myocardial injury in septic elderly patients and a cut-off prognostic value could be determined and used as a means for risk stratification of these patients.

Host defenses

P1324 Influenza and Sendai viruses induce a differential IFN, chemokine and IKKe gene expression in A549 lung epithelial cells

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Lung epithelial cells are the primary targets for respiratory viruses such as influenza A, influenza B and parainfluenza viruses. To study the ability of these viruses to induce host cytokine and chemokine gene expression we infected human lung epithelial A549 cells with influenza A (H1N1 and H3N2 strains), influenza B and Sendai viruses, isolated total cellular RNA and analysed host cell cytokine and chemokine mRNA expression by Northern blotting and RT-PCR. Influenza A and influenza B virus infection lead to a relative weak induction of interferon (IFN-α/β) and IFN-like genes, IL-28 (IFN-λ2/3) and IL-29 (IFN-λ1). The expression of IRF-3 and IRF-7 activating kinase, IKKe was also weakly induced by influenza viruses. In similar experimental conditions Sendai virus infection activated the expression of IFN-α, IFN-β, IL-28, IL-29 and IKKe genes very well. Similarly, while Sendai virus readily induced TNF-α, CCL2 (MCP-1), CCL5 (RANTES), CXCL8 (IL-8) and CXCL10 (IP-10) gene expression, influenza A and influenza B virus-induced expression of these genes was either lacking (TNF-α) or occurred at a relatively low level. Pretreatment of A549 cells with IFN-α lead to a dramatic increase in influenza A virus-induced IFN, IL-28, IL-29 and chemokine gene expression. This induction correlated with enhanced expression of virus-activated signalling molecules, TLR-3, IKKe and IRF-7. The results indicate that in human lung epithelial cells influenza A and B viruses are relative poor inducers of type I IFN, IL-28, IL-29 and chemokine genes, but their ability to induce these genes can be fully restored by pretreatment of cells with type I IFNs.

P1325 Monoclonal antibodies against recombinant spike protein neutralise the infection of severe acute respiratory syndrome coronavirus (SARS-CoV) through inhibition of virus binding to vero E6 cells

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Objectives: To generate monoclonal antibodies against spike protein that can neutralise the infection of severe acute respiratory syndrome coronavirus (SARS-CoV) SARS-CoV infection.
Methods: In this study, the coding sequence (amino acid 268–1255) of spike glycoprotein of SARS-CoV was cloned into prokaryotic expression vector, pET101/D-TOPO, and recombinant protein (rS268) was purified to near homogeneity. After immunisation with recombinant proteins, specific monoclonal antibodies were obtained and confirmed both by ELISA, Western blot and immunofluorescent assay (IFA). The neutralization effect of monoclonal antibodies against recombinant spike protein of SARS-CoV was evaluated in Vero cells by Western blot.
Results: Results showed that some of these monoclonal antibodies could effectively neutralise the infection of SARS-CoV in a dose-dependent manner. Furthermore, binding inhibition assay revealed that these neutralising monoclonal antibodies, but not SARS patient's serum, could inhibit SARS-CoV binding to Vero E6 cells.
Conclusion: To our knowledge, this was the first report revealing neutralising monoclonal antibodies, generated by immunising mice with prokaryotic cell-expressed recombinant spike protein, that inhibit infection of SARS-CoV in Vero E6 cells through inhibiting virus binding to cells. Result from this study should facilitate description of the receptor-binding domain and target epitope of the spike protein of SARS-CoV, and peptide-based subunit vaccine as well.

P1326 Interaction of respiratory tract pathogens and epithelial cells

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Objectives: Epithelial cells play an important role in the regulation of local inflammatory and immune response in reply to bacterial infection. In the present study we analysed the epithelial cell cytokine release of interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-1α (IL-1a) after stimulation by different strains of *Streptococcus pyogenes* and nontypeable *Haemophilus influenzae* (NTHi).
Methods: All investigated strains were clinical isolates from patients with community acquired respiratory tract infections in 2003. The human bronchial epithelial cell line NHBE was seeded into 24-well tissue culture plates and was infected by group A beta-haemolytic streptococci (GAS) and NTHi at a ratio of 100 bacterial cells to one bronchial cell. After different incubation times within 24 h, the invasion medium was aspirated and centrifugated. IL-6, IL-8 and especially IL-1a were quantified in the cell culture supernatant of infected NHBE by enzyme-linked immunosorbent assay (ELISA).
Results: In response to infection by *S. pyogenes*, NHBE released elevated levels of IL-6, IL-8, and especially IL-1a. Different cytokine kinetics' were detected after infection by clarithromycin-susceptible and resistant GAS. Apart from only one NTHi strain, isolated from a patient with cystic fibrosis, ampicillin-sensitive and β-lactamase-positive nontypeable *H. influenzae* induced similiar cytokine kinetics with high concentrations of IL-6, IL-8, and IL-1a.
Conclusion: After stimulation by both respiratory tract pathogens, NHBE released increased cytokine levels of IL-6, IL-8, and IL-1a. *H. influenzae* induced the epithelial cells to secrete high concentrations of all tested cytokines, while *S. pyogenes* caused a high release of only IL-1a. Additionally, we demonstrated a difference in the time-dependent NHBE cytokine induction provoked by clarithromycin-susceptible and resistant GAS.

P1327 Role of dendritic cells in the immune regulation of primary Epstein-Barr virus infection

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Epstein-Barr virus (EBV) is a human gamma herpesvirus that infects 95% of human populations and usually persists without harm for the lifetime of the host. Yet, it is a highly transforming virus as shown by its association with a number of malignancies of which nasopharyngeal carcinoma and nasal lymphoma are prevalent in the Chinese. The virus-specific CD8+ cytotoxic T-cells are important in effecting the intricate balance between the host and the virus. It is not known, however, how the primary immune response is regulated.
Objectives: As dendritic cells (DCs) are the most potent antigen presenting cells, we investigate whether exposure to apoptotic or necrotic EBV-transformed lymphoblastoid B cell lines (LCLs) could mediate neonatal DCs maturation and subsequent T cell priming.
Methods: To study primary EBV infection, samples of fetal cord blood are collected, from which LCLs are established by infection of cord blood mononuclear cells with B95-8 EBV producer cell lines. Autologous monocyte-derived DCs and T cells are generated after positive selection by anti-CD14 and anti-CD3 immunomagnetic microbeads.
Results: We found that neonatal DCs phagocytosed both apoptotic and necrotic LCLs efficiently after 5 h coculture. Phagocytosis of both necrotic and apoptotic LCLs led to a lightly up-regulation of CD40, HLA-DR, CD80 and CD86 when compared with LPS activation. T-cell proliferation and cytotoxicity assays are in progress

to determine the capacity of neonatal DCs in activating the cord blood derived naïve T cells.
Conclusions: In conclusion we suggest that neonatal DCs are efficient in phagocytosing dying cells. The relative immaturities of DCs phenotype may due to the compromised antigen presentation in neonatal DCs.

P1328 Role of endogenous tumour necrosis factor alpha and beta on cytomegalovirus replication in human placental cells

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Objectives: The proinflammatory cytokine tumour necrosis factor (TNF) has been identified as mediator of human cytomegalovirus (HCMV) stimulation and reactivation in human progenitor cells. We postulated that TNF may have an antiviral activity and play an important role in limiting of HCMV replication in placental tissues. The aims of our study were: to characterise the antiviral activity of placental TNF in an organ culture (OC) and isolated placental cells system, and to compare TNF production in normal placentas and amniotic membranes (AM), and in placentas infected with HCMV *in utero*.
Methods: The studies were performed in organ cultures and isolated placental cells (cytotrophoblasts, macrophages) obtained from normal placentas infected with HCMV strain AD169 *in vitro*. The neutralising antibodies to TNFα or/and β were used as a means of blocking cytokine activities. Treatment of cells were started immediately after HCMV infection of cultures or 24 h before HCMV infection and continued for another 14 days.
Results: In villous culture infected with HCMV *in utero*, an increase in TNF production was found compared with that in uninfected cell cultures. Infection of decidual tissue with HCMV resulted in production of massive amounts of TNF. Treatment of organ cultures with anti-TNF-α or anti-TNF-β anibodies resulted in increase in peak levels of HCMV release, intensely in cultures treated with anti-TNF-α. In individual experiments, treatment with anti-TNF antibody resulted in a 100-fold increase in virus levels. Increases of 10-fold to 100-fold of HCMV replication in the cytotrophoblasts and macrophages treated with anti-TNF-α or/and anti-TNF-β were observed. We found that the enhancement of HCMV replication by TNF was present mainly in fetal part of organ.
Conclusion: Obtained results indicate the importance of the endogenous TNFs in placental immunity. This work was supported by the State Committee for Scientific Research, grant no. 3 P05E 001 25.

P1329 Assessment of the correlation between anti-inflammatory therapy and concentration of pro-inflammatory cytokines in cerebrospinal fluid (CSF) and its effect on the outcome of bacterial meningitis (BM)

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Objectives: To determine the influence of antiinfalmmatory therapy with dexamethasone (DX) and DX with pentoxifilline (PX) on concentration of TNF-α, IL-1β, IL-8 in CSF and the course of BM.
Methods: Forty-two patients, 13 (31%) female and 29 (69%) male with BM were investigated. They were divided into three groups: A – treated only with antibiotics, A + DX – treated with antibiotics and DX, A + DX + PX – treated with antibiotics, DX and PX. Cytokines concentration was measured in CSF by ELISA at the day of admission (before initiation of therapy) and 72 h later.
Results: Antiinflammatory therapy did not have impact on the resolution of inflammation (pleocytosis, protein, glucose level) in CSF. However it was established that adjuvant therapy with DX and PX has beneficial effect on the course of BM. In this group 61.5% patients recovered, in comparison with 28.6% in the group A + DX and 26.7% in the group A. Mortality rate was: in the group A – 33%, A + DX – 21.4%, A + DX + PX – 7.7%. Correlation between outcome of the BM in the investigated groups and cytokines concentrations in CSF was observed. In the group A + DX + PX all patients respond to the therapy with decrease of cytokines concentration, and coefficients of variation were low (TNF-α, 1%; IL-1β, 23.6%; IL-8, 18.9%). Also in the group A + DX decrease of cytokines concentration in CSF was observed, however not such significant (coefficients of variation were: TNF-α, 28.7%; IL-1β, 31.4%; IL-8, 53.8%) In the group A concentration of cytokines in CSF varied and coefficients of variation were high (TNF-α, 91.4%; IL-1β, 33% and IL-8, 21.4%).
Conclusion: (i) Anti-inflammatory therapy with DX + PX inhibits the release of cytokines in CNS and has beneficial effect on the course of BM. (ii) Anti-inflammatory therapy with DX alone also inhibits cytokines production in CNS but its effect is not such well defined.

P1330 Complement activation during meningococcal disease: engagement of alternative and lectin pathway and association with disease severity

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Objectives: Invasive infection with *Neisseria meningitidis* leading to meningococcal meningitis or fulminant meningococcal sepsis (FMS), is an important cause of childhood mortality and morbidity. Activation of the complement cascade has been implicated in the pathogenesis of FMS, but the initial pathways that activate complement, the relation between complement activation and activation of the cytokine- and coagulatory- system, or disease severity still remains subject to investigation.
Methods: In the present study, 11 patients with meningococcal disease admitted to the paediatric intensive care unit (PICU) of the UMC St Radboud were included, informed consent was obtained from both parents. Complement activation products, coagulation parameters and cytokines were measured at fixed time intervals after admission.
Results: C3bc and terminal complement complexes C5-9 (TCC), both reflecting total complement activation, were significantly increased at admission. Unsurpassed by any other inflammatory parameter, C3bc and TCC were highly correlated with disease severity (PRISM-score): Spearman rank correlation with PRISM for TCC, $R = 0.95$ ($P < 0.001$), for C3bc $R = 0.89$ ($P < 0.001$). TCC was also significantly correlated with platelet and fibrinogen consumption ($R =$ -0.93 for platelet count and $R =$ -0.71 for fibrinogen) and fibrinolytic activity ($R = 0.93$ for D-dimer). In addition, TCC at admission was significantly correlated with cytokine concentrations ($R = 0.88$ for IL-1b, $R = 0.64$ for IL-10, $R = 0.61$ for IL-12 and $R = 0.76$ for IFN-γ). During the first 8–24 h after admission TCC and C3bc slowly declined. The early complement activation was caused by alternative ánd lectin pathway engagement, but not by classical pathway activation as shown by repeated measurements of C3bBbP (alternative), C4b (classical or lectin), and C1rs-C1inh-complexes (classical). However, after 24 h C1rs-C1inh complexes slowly increased, reflecting classical pathway activation from this time onwards.
Conclusions: Our data indicate that initial activation of the complement system during meningococcal infection is highly correlated with disease severity. Complement activation occurs via the alternative and lectin pathway, whereas classical pathway engagement develops only after 24 h.

P1331 The importance of cytokine and cortisol levels in cerebrospinal fluid as clinical and biological markers of bacterial meningitis

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Objectives: Despite the advent of modern antibiotical treatments, bacterial meningitis (BM) continues to be associated with signifi-

cant morbidity and mortality. It was suggested that an exaggerated intrathecal immune response plays a pivotal role in disease pathogesis. This response is characterised by an increased intrathecal production of proinflammatory cytokines – interleukin (IL)-1β, IL-6, IL-8, IL-12 and tumour necrosis factor (TNF)-α. Production of these cytokines is controlled by antiinflammatory mediators such as IL-10 and cortisol. The aim of this study was to assess the clinical relevance of cytokine and cortisol levels in the cerebrospinal fluid (CSF) from patients with BM.

Methods: We examined 20 samples of CSF from BM patients obtained by diagnostic lumbar puncture performed after admission. The samples were analysed with three colour flow cytometry using Cytokine Bead Array (BD Biosciences, San José, USA). CSF cortisol levels were measured by radioimmunoassay purchased from the DSL (Webster, USA). Severity of the illness was determined using APACHE II and Sepsis Organ Failure Assessment (SOFA) scores; level of unconsciousness was scored with Glasgow Coma Scale (GCS).

Results: CSF levels of cortisol correlated positively with APACHE II ($r = 0.596$, $P = 0.041$) and negatively with GCS ($r = -0.743$, $P = 0.01$). CSF levels of IL-10 correlated negatively with GCS ($r = -0.631$, $P = 0.012$). CSF levels of IL-12 correlated negatively with SOFA ($r = -0.636$, $P = 0.018$). Moreover, the levels of IL-10 correlated positively with CSF protein concentration and number of neutrophils ($r = 0.604$, $P = 0.01$; $r = 0.498$, $P = 0.049$) and with IL-1β ($r = 0.50$, $P = 0.033$), IL-6 ($r = 0.693$, $P = 0.01$) and TNF-α ($r = 0.611$, $P = 0.01$) in the CSF.

Conclusion: Our results indicate that severe course of BM is associated with a high concentration of antiinflammatory mediators in the CSF. Furthermore, increased levels of IL-10 are closely connected with elevated concentrations of proinflammatory cytokines in the CSF during BM. These findings allude to IL-10's attempt to suppress the intrathecal proinflammatory response. Thus, IL-10 and cortisol levels in the CSF could be used as markers of the disease severity and progression.

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P1332 Two-year follow-up analysis of the class-specific immune response after infection with *Yersinia pseudotuberculosis* followed by erythema nodosum and reactive arthritis – case report

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Objectives: *Yersinia enterocolitica* and *Y. pseudotuberculosis* are important causative agents of enteric infections in humans. Postinfectious complications, such as reactive arthritis and erythema nodosum, may also follow the infection. Isolation of bacteria from faeces in such cases is not achieved, and consequently the diagnosis depends on serology. In this study the results of 2 years serological investigation after infection with *Y. pseudotuberculosis* followed by erythema nodosum and reactive arthritis in a 27-year-old women are described.

Methods: The IgM, IgG and IgA class antibodies were measured in nine serum samples, collected at 2–12 weeks intervals, by two ELISA, using *Yersinia* outer membrane proteins (YOPs) and lipopolysaccharides (LPS) extracted from *Y. enterocolitica* serotypes O:3, O:5,27, O:8, O:9 and *Y. pseudotuberculosis* serotype I and III. The antibody responses were also studied by immunoblotting (Western Blot, Mikrogen).

Results: In the first serum sample, obtained 3 days after onset of the clinical symptoms of erythema nodosum and arthritis, were diagnosed a very high levels of IgA, IgG and IgM antibodies to YOPs and to LPS extracted from *Y. pseudotuberculosis* serotype I. In the immunoblot analysis we found antibodies, in all of the immunoglobulin classes, only against YopD protein (33–36 kDa). In the second serum sample, obtained after 14 days, we observed a decrease of antibody titres to LPS and increase of antibody titres to YOPs. Obtained results with the next seven serum samples showed that antibody titres measured against LPS antigens in comparison to YOPs antigens decreased more rapidly after reconvalescence. Antibodies to YOPs, in all immunoglobulin classes, were still demonstrable at high level at the end of the follow-up period of 2 years. At this late period the antibodies to LPS were not found in a diagnostically significant titres.

Conclusion: Results of our study indicate that after infection caused by *Y. pseudotuberculosis*, complicated by erythema nodosum and reactive arthritis, a maximum levels of specific IgA, IgG and IgM antibodies to YOPs are reached later than antibodies to LPS. However, antibodies against YOPs in comparison to antibodies against LPS persist longer after reconvalescence. Demonstration of IgM-class *Yersinia* antibodies 2 years after onset of the disease showed that this class of antibodies cannot be considered in all cases as good marker of recently acquired infection.

P1333 Pro-inflammatory cytokine production by human monocytes in response to *P. aeruginosa* infection mainly involves TLR2 and mannose receptor

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Pattern recognition receptors play an important role in protection against pathogens linking innate and adaptive immunity. *P. aeruginosa*, an oppurtunistic pathogen, is capable of establishing both chronic and acute infections in immunocompromised hosts. We have recently shown that Slime-GLP, an extracellular product of *P. aeruginosa* containing mannose, is the most important stimulant for TNF-α production by human monocytes and a potent inducer of NFêβ-mediated transcriptional activation. In this study we sought to investigate the role of different pattern recognition receptors to inflammatory pathway triggered by *P. aeruginosa*. To this end, we have treated human monocytes with *P. aeruginosa* purified LPS (5 ng–50 μg/mL), Slime (5–5 μg/mL), or viable bacteria (10 bacteria/monocyte) in the presence of blocking antibodies specific for TLR4 (5–2 μg/mL), TLR2 (5–25 μg/mL), and Mannose receptor (5–25 μg/mL) and measured TNF-α, IL-1β and IL-6 production for 2–24 h. An isotype matching, nonspecific antibody was used as negative control. TNF-α, IL-1β and IL-6 induction was inhibited by 80–90% in the presence of anti-Mannose receptor and anti-TLR2 blocking antibodies, whereas blocking of TLR4 caused a less prominent inhibition (40%). Comparable levels of inhibition were obtained when monocytes were stimulated by *P. aeruginosa* Slime-GLP or whole viable bacteria. *P. aeruginosa* LPS caused a weak proinflammatory cytokine induction not amenable to blocking of TLR2, TLR4 or mannose receptor. Our results suggest that TLR2 or mannose receptor-mediated pathway could be a specific target for inhibition of inflammatory responses triggered by *P. aeruginosa*.

P1334 *Pseudomonas aeruginosa* LPS and slime glycolipoprotein (GLP) differentially activate the same mitogen-activated protein kinase signalling pathways for tumour necrosis factor alpha in fresh human monocytes

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TNF-α production has a central role in the development and progression of *P. aeruginosa* septic shock. We have previously shown that *P. aeruginosa* GLP is the most potent stimulant compared with homologous LPS, for TNF-α production and NF-kβ activation in human monocytes. In this study, using fresh human monocytes stimulated for 15 min–6 h with *P. aeruginosa* LPS (5 ng/mL–50 μg/mL) or GLP (10–50 μg/mL), we show that secretion of TNF-α induced by *P. aeruginosa* GLP and LPS was paralleled by phosphorylation of mitogen-activated protein kinases (MAPK's) ERK1/2, p38 and the stress-activated protein kinases c-jun N-terminal kinases 1 and 2 (JNK). Phosphorylation of p38 and ERK1/2 correlated with an increase of activity. TNF-α levels were significantly reduced by (60–80%) and by (80–95%) when

inhibitors of ERK1/2, PD98059 (50uM), or p38, SB203580 (10 μM) respectively, were added in the culture 1 h before stimulation. Combination of both inhibitors almost abolished TNF-α induction. *P. aeruginosa* GLP differed from the homologous LPS only regarding the strength of p38 and ERK1/2 activation, with GLP leading to a stronger activation of p38 and ERK1/2. No differences were observed at the level of JNK activation between LPS and GLP. Involvement of TLR-2 and TLR-4 for phosphorylation of p38 was shown by employing specific blocking anti-human TLR-2 (10 μg/mL) and TLR-4 (10 μg/mL) antibodies. Activation of p38 induced by *P. aeruginosa* GLP was dramatically reduced in the presence of anti-TLR2 antibody and to a lesser degree in the presence of anti-TLR4, whereas the LPS induced stimulation was inhibited only in the presence of anti-TLR4. Our results suggest that *P. aeruginosa* GLP stimulates the MAP kinase pathway more effectively than the homologous LPS, probably by using different combination of TLRs.

P1335 *In vitro* stimulation of innate immunity by susceptible and resistant *Escherichia coli*

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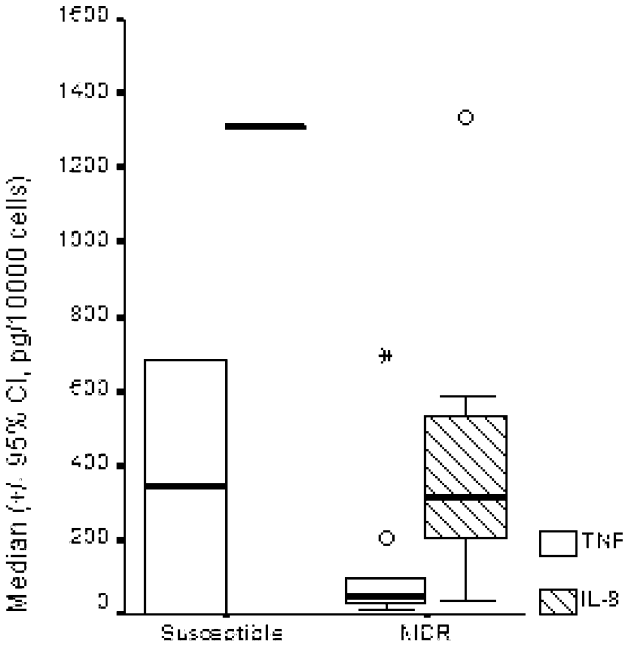
Objective: It has already been shown that susceptible and multidrug-resistant (MDR) *Pseudomonas aeruginosa* differ on their stimulatory effect on innate immunity (Giamarellos-Bourboulis *et al. Clin. Exp. Immunol.* 2004). A similar study has been performed on susceptible and MDR *E. coli*.
Method: Seventeen isolates of *E. coli* were studied, eight susceptible and nine MDR all pathogens of different cases of pyelonephritis. Mononuclear cells separated from whole blood of healthy volunteers were suspended in flasks and monocytes isolated after removal of nonadherent cells were resuspended in RPMI with 10% FBS and 2 mM glutamine. After incubation of 1 h the monocytes were triggered by a 4.7 log 10 and a 6.7 log 10 inoculum of each isolate. Tumour necrosis factor-alpha (TNF-α) and interleukin-8 (IL-8) were measured in culture supernatants after 2 h of incubation, with an enzyme immunoassay.
Results: They are given in the figure.



Conclusions: Triggering by susceptible and MDR isolates of *E. coli* lead to different production of cytokines, results that might reflect difference in virulence.

P1336 The role of human beta-defensins during *Campylobacter jejuni* infection

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Objectives: *Campylobacter jejuni* (*C. jejuni*) is one of the commonest causes of infective diarrhoea worldwide effecting mainly young children (<5 years) both in developing countries and in the western world. The clinical spectrum varies from mild watery to bloody, inflammatory diarrhoea and is frequently associated with postinfectious extraintestinal complications such as Guillain-Barré Syndrome. Despite the serious health problem caused by the bacterium, disease pathogenesis remains poorly understood. Bacterial adhesion and invasion of the intestinal epithelium is known to be a critical feature of infection. Human beta defensins (hBDs), a family of epithelial antimicrobial peptides are a major component of innate host defence. We and others have highlighted the role of these peptides during gastrointestinal infection and inflammation. In the present study we have investigated the role of hBDs during *C. jejuni* infection.
Methods: Human colonic intestinal cell line (Caco-2) was infected with virulent strain (NCTC 11168) of *C. jejuni*. Both time and dose dependent studies of hBD gene expression were assessed by RT-PCR. The bactericidal activity of recombinant hBD-1, -2 and -3 against wild type and isogenic mutants of *C. jejuni* was evaluated by broth-dilution assay.
Results: A marked time-dependent induction of hBD3 was observed with maximal expression at 8 h postinfection. In contrast hBD2 gene expression was modest. Importantly the bacterium was found to be highly susceptible to the antimicrobial action of hBD-3 amongst the peptides tested.
Conclusions: The present study provides evidence for dynamic cross talk between the bacterium and host epithelial innate defence. The potent bactericidal activity of hBD3 may contribute to the self-limiting nature of the infection via enhanced bacterial clearance in a healthy host.

P1337 Association between *Helicobacter pylori* infection and chronic idiopathic urticaria

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Objectives: The present study intends to understand the association between *Helicobacter pylori* infection and chronic idiopathic urticaria.
Methods: We studied 30 patients with chronic idiopathic urticaria diagnosed for *H. pylori* infection with the urea breath test using carbon 14, 22 patients were infected and received triple eradication therapy (amoxicilin, claritromicin and omeprazol) and antihistamine (loratadine); and eight noninfected individuals received only antihistamine treatment. Serum concentrations of total IgE and specific IgG against *H. pylori* was determined. In addition, we standardised an ELISA to detect specific IgE against *H. pylori*, at time 0 and 6 weeks after concluding treatment.
Results: Bacteria was eradicated in all the patients infected, with variable clinical improvement (14 without clinical signs, eight with partial clinical signs); patients without *H. pylori* infection fail to improve after treatment.
Conclusions: The present study established a statistical significant difference ($P < 0.0001$) between the eradication of *H. pylori* infection and the improvement of clinical signs for chronic idiopathic urticaria, when compared with the noninfected group. In patients with moderate symptoms, we observed the best treatment response with clinical improvement ($P = 0.0083$). There were not statistically significances between the concentrations of total IgE, IgG anti *H. pylori* and the values for *H. pylori*-specific IgE, except in those patients with moderate partial improvement, where the *H. pylori*-specific IgE significantly diminished ($P = 0.0286$) after eradication. The eradication of *H. pylori* clearly improves chronic idiopathic urticaria, by an unknown mechanism independent of IgE.

P1338 Some biochemical and serological aspects of Q fever diagnosis

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Coxiella burnetii, an obligate intracellular parasite of eucaryotic cells, is the aetiological agent of Q fever. The disease is a widespread zoonosis and is endemic throughout the world. In humans, the acute form of Q fever is characterised as a flue-like illness or atypical pneumonia, or less frequently as granulomatous hepatitits with a significant incidence of neurologic complications. Persistent infections may lead to chronic form of the disease, which may be associated with endocarditis. Human infection is aquired most often by inhalation of contaminated aerosols, or less frequently, by drinking infected milk, infection through skin trauma, sexual contact or mother to fetus transmission. Upon serial laboratory passages in embryonated hen eggs, *C. burnetii* undergoes a virulent (phase I) to low-virulent (phase II) variation, which is accompanied by noticeable modifications in both composition and structure of the cell outer-membrane components. During the acute Q fever, antibodies against the phase II antigen are detected earlier and at higher titres than those against the phase I antigen. In contrast, titres to phase I antigen are higher during the chronic form of the disease. We have found that the phase I antibodies are mostly directed against the O-specific chain of the *C. burnetii* smooth-form lipopolysaccharide (LPS). A remarkable decrease in the serological activity of the LPS was observed when two unusual sugars virenose (Vir) and dihydrohydroxystreptose (Strep) were selectively removed from its O-specific chain. This indicates that most phase I antibodies are directed against the epitopes containing terminal Vir and Strep. Thus, our results may suggest that Vir and Strep are involved in the immunobiology of the disease. Mild acid treatment of the *C. burnetii* phase I cells resulted in a considerable degradation of the LPS and exposure of the surface proteins exhibiting a high-immune response to the phase II antibodies. 1D SDS-PAGE revealed the most intense silver-stained proteins at about 29 and 60 kDa. The subsequent, initial proteomic studies have identified the latter protein as the Chaperonin 60 kDa (GroEL protein, heat shock protein B). This protein is localised in cytoplasm of several bacteria but a significant fraction of it is also associated with their cell envelopes. Most likely, the protein has a dual association also in *C. burnetii* as it is immunogenic. Further peptide mass fingerprinting is in progress.

P1339 Evaluation of inflammatory capacity of fungal spores in human whole blood

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Objectives: Fungal spores are clinically relevant contaminants as they are ubiquitous, inhalable and release mycotoxins. Cell wall structures such as glucans and mannans seem to play an important role in inflammatory processes but no defined structure could be described up to now as responsible for the inflammatory potential of fungi. In addition to well-known human-pathogenic fungi, like *Candida albicans* and facultative pathogens, e.g. *Aspergillus fumigatus*, nonpathogens – particularly moulds – endanger human health in immunosuppressed patients.
Methods: Over 50 different moulds and yeasts were cultured and their spores were prepared in order to measure their inflammatory capacity in comparison to endotoxin from *E. coli* O-113/. We used a standardised whole blood assay to detect pyrogenic activity of the fungal spores on the basis of release of cytokines, e.g. IL-1β, IL-6 and TNF-α as pro-inflammatory cytokines, IL-8 as a chemoattractant and IL-10 as an anti-inflammatory cytokine.
Results: We detected considerable differences in the immunological response to spores between different fungal species. We found these differences in cytokine induction between representatives of different fungal genera and between members of the same genus. However, there was a strong correlation between cytokine inductive capacity and estimated total spore surface. The variance between the response of individuals to fungal spores was greater than that towards endotoxin. We investigated whether structures on intact spore surfaces are recognized by toll-like receptors (TLRs) by incubating fungal spores with bone marrow cells from TLR-4 and TLR-2 deficient mice, and found that the TNF α-inducing activity of none of the tested species was fully dependent on TLR recognition, only some species were partially dependent on these TLRs.
Conclusion: Cytokine induction capacity seems to be dependent on the exposed total spore surface rather than on the spore count. Immune recognition does not appear to be centrally dependent on TLR activation. Further studies will attempt to define the role of different surface structures of selected fungal spores in cytokine induction.

P1340 The differentiation of human monocytes into dendritic cells is tunable and exploitable by micro-organisms to modulate the host's adaptive immune response: the *Candida albicans* model

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Objectives: To investigate whether the ability of *Candida albicans* to convert from yeast (Y) to mycelial forms, through germ-tube (GT) formation, that is considered a key feature of its transition from commensalism to virulence, could be related to an abnormal human monocyte differentiation into dendritic cells (DCs).
Methods: Noninfected and Y or GT infected monocytes were allowed to differentiate into DCs by culture with GMCSF and IL-4. After a 6 days culture, cells were analysed for their ultrastructure, phenotype and function.
Results: Human monocytes cultured with GM-CSF and IL-4 after phagocytosis of Y forms did not differentiate into DCs: they retained CD14, did not acquire molecules of the CD1 family, and were unable to express the maturation markers CD83 and CCR7. Moreover, they produced TNFα, IL-6 and IL-10 rather than IL-12, and were able to induce proliferation of alloreactive memory, but not naïve, T lymphocytes. Conversely, monocytes that had phagocytosed GT forms differentiated into mature CD83+ and CCR7+ DCs with, however, a failure in the up-regulation of MHC class II and CD80, irrespective of LPS treatment. In addition, they synthesised TNF-α, but not IL-6, IL-10 or IL-12. These cells were able to prime naïve T cells, but not to induce their functional polarisation into effector cells. In details, expanded T lymphocytes were unable to secrete cytokines, in particular IL-4 and IFN-γ suggesting that they could be ineffective in helping the macrophage killing of microorganisms. These data indicate that phagocytosis of Y and GT forms have profound and distinct effects on the differentiation pathway of monocytes. These data imply that differentiation of human monocytes is tunable by microorganisms, which may exploit this process to elude immune-surveillance.

P1341 Monocyte chemotactic protein-1 production by human astrocytes and astrocytoma cell lines during toxoplasmic infection: differential regulation by interferon-gamma and D-609

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Objective: We have used human astrocyte cultures to investigate the cellular responses of central nervous system resident cells to intracellular *Toxoplasma gondii* infection. The objectives of this study were to evaluate the secretion and the expression of monocyte chemotactic protein-1 (MCP-1) and to measure the effect of IFN-γ and D-609, a specific inhibitor of phosphatidylcholine-specific phospholipase C (PC-PLC), on the regulation of the expression and the secretion of this CC chemokine during infection by *T. gondii*.

Methods: Human astrocytes *ex vivo* and human astrocytoma cell lines (U-373, CCF) were infected by the virulent RH strain and parasite multiplication and penetration were measured. Using a real-time PCR technique and a ribonuclease protection assay, the copies of mRNA encoding MCP-1 and seven other chemokines were analysed 24 h postinfection (p.i.). MCP-1 levels were determined by ELISA in culture supernatant at 24 h p.i. D-609 was added 45 min before the infection in culture medium. Furthermore, the effect of preincubation with IFN-γ (1000 IU/mL) was studied.
Results: Penetration and multiplication rates of *T. gondii* were higher in human astrocytoma cell lines than in human fibroblasts ($P = 0.01$ and $P = 0.009$, respectively). Parasitic infection increased MCP-1 production by astrocytes. Preincubation of these cells with IFN-γ did not modify significantly the secretion of MCP-1 by astrocytoma cell lines in our experimental conditions. Nevertheless, MCP-1 production by astrocytoma cells lines treated by D-609 were decreased compared with those not treated by this inhibitor.
Conclusion: Astrocytes and astrocytoma cell lines are significantly more infected by *T. gondii* than fibroblasts and the parasite induces production of MCP-1 in these cells. Moreover, in our protocol, both secretion and expression of this CC chemokine are regulated by D-609, a PC-PLC inhibitor, but not by IFN-γ. These results suggest that the upregulation of MCP-1 may be involved in the pathogenesis of toxoplasmic encephalitis and regulated by intracellular messengers.

P1342 Yersinia toxin YopE Targets granulocytes in the intestinal tract and associated lymphatic tissues

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Objective: To determine the immune cell targets of *Yersinia Yops* during infection.

Background: *Yersinia pseudotuberculosis* colonises the gastrointestinal tract (GI) and associated lymphatic tissues, such as mesenteric lymph nodes (MLN) and Peyers' patches (PP) after orogastric infection of humans or mice. Colonisation requires *Yersinia* outer proteins (YOPS), which are virulence factors that are translocated into mammalian cells by a type III secretion system where they inhibit cellular functions. Competition studies in mice coinfected with wild type (WT) *Yersinia* and single yop deletion mutants (yopE, yopH, yopO) demonstrated that these Yops are important in colonization of the GI, MLN and PP. Previous work demonstrated that YopE, YopH and YopO have antiphagocytic activity in cultured cells. As phagocytes are an early defence used by the host to fight bacterial infection, we tested whether YopE, YopH and/or YopO target phagocytes in mice.
Methods: To study a possible role of these Yops in inhibiting the bactericidal effects of phagocycytes during an infection, CD18 $-/-$, granulocyte-depleted and CD18 $-/-$ granulocyte-depleted mice were orogastrically infected with an equal mixture of WT and yopE, yopH or yopO mutant strains. The ratio of WT to mutant bacteria recovered from GI, MLN and PP tissues was quantified and compared with the ratio from WT mice. If a higher number of a yop mutant is recovered in the deficient mice compared with the nondeficient mice, then the mutant yop strain is more susceptible to phagocytes in the tissue studied. Hence, the missing Yop likely functions to neutralise the bactericidal effects of phagocytes.
Results: Our results show that there is a high recovery of yopE mutant strain in the MLN, PP and caecum of the granulocyte-depleted mice. In contrast, yopO mutant strains are recovered in the small intestinal lumen and mesenteric lymph nodes in the CD18 $-/-$ mice. CD18 $-/-$ granulocyte-depleted mice showed that yopE and yopO were rescued in most of the tissues studied.
Conclusion: Our data documents the significant role of yopE and yopO in neutralising phagocytes during infection, specifically YopE is crucial to inhibit granulocytes function while YopO targets other CD18-expressing cells.

Nosocomial infection: Gram-positive micro-organisms

P1343 Surveillance of glycopeptide-resistant enterococcal bacteraemias in England

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Introduction: Glycopeptide-resistant enterococci (GRE) have emerged as important nosocomial pathogens in recent years. As part of the Department of Health's programme of mandatory surveillance of Healthcare Associated Infection in England, a surveillance scheme for GRE bacteraemias is being developed. A questionnaire was devised to obtain background data to inform the development of the proposed surveillance scheme.
Objectives: (i) To establish current approaches to detection of GRE (ii) To review technical problems associated with detection and reporting of GRE (iii) To review issues related to the significance of reports of GRE bacteraemia and the comparability of data from different hospitals
Methods: A questionnaire reviewing methods, approaches to testing and risk factors for GRE bacteraemia was designed by the national group, piloted and administered to all laboratories in England ($n = 205$) in June 2003.
Results: A response rate of 82% ($n = 169$) was achieved; several questionnaires were not fully completed. Thirty-four per cent ($n = 55$) of respondents identified GRE Bacteraemias in 2002 and of those laboratories, 29% ($n = 16$) saw more than five in that year; 33% ($n = 18$) of identified bacteraemias were not reported via the routine communicable disease reporting system; 87% ($n = 142$) of respondents reported using three or more routine methods of GRE identification and 22% ($n = 37$) did not identify isolates to species level; 80% ($n = 13$) of respondents did not test vancomycin on all enterococci from bacteraemias. Glycopeptide susceptibility testing was primarily carried out using the disc-diffusion method (91%, $n = 148$). Specialist units such as bone marrow and liver transplantation units were the predominant sites for routine screening of patients' faeces for GRE. The majority of respondents (69–99%, $n = 108$–156) could provide data for indicators of clinical significance of GRE bacteraemias.
Conclusions: The majority of laboratories in England did not see GRE bacteraemias in 2002. Routine reporting of these infections in 2002 was incomplete. A varied approach to the identification of enterococci from blood cultures and glycopeptide-susceptibility testing was observed. Most laboratories could provide data on the clinical significance of GRE bacteraemias. A standardised procedural guideline is required if robust data are to be produced as part of a mandatory surveillance scheme in England.

P1344 Epidemiology of vancomycin-resistant enterococci in a Greek tertiary hospital: comparison of two point prevalence studies on intestinal colonisation

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Objectives: Two point prevalence studies were undertaken in order to investigate the intestinal colonisation with vancomycin-

resistant enterococci (VRE) among the patients of our hospital. The first study took place on February 1, 2001 and the second 22 months later.
Methods: The prevalence studies were performed by the same methodology. All patients cared in high-risk departments including: the intensive care unit (ICU), the cardiology intensive care unit (CICU), the renal unit, and the neonatal unit were tested. In addition, through a systematic random sampling process, 25% of all patients cared in all other wards (non high-risk departments) were also tested. Faecal samples or rectal swabs were inoculated into enterococci broth with vancomycin 6 mg/L and subcultured on bile-esculin azide agar with vancomycin 6 mg/L. Enterococci were identified by conventional methods. Glycopeptides susceptibility testing was performed by disc diffusion, Etest, and MIC (agar dilution) methods. Characterization of van genotypes was performed by a multiplex PCR assay using specific primers for vanA, vanB, van C1, vanC2/C3, ddlE. faecalis, ddlE. faecium, and rrs genes.
Results: The prevalence rate of VRE faecal carriers increased from 18.8% (first study) to 30% (second study). VanA, vanB, and vanC prevalence rates in the first study were 1.8, 3.7, and 13.8%, respectively, while in the second study were 10.3, 5.9, and 14.8%, respectively. In the first study, vanA-harbouring isolates were detected in ICU and renal unit only, but the second study revealed a further dissemination within the hospital. VRE prevalence in high-risk departments did not present any significant difference, but in surgical wards increased from 10.4–45.5%, and in medical wards from 15.9 to 33.9%. The haematology, the nephrology, and the orthopaedic wards contributed significant number of VRE faecal carriers.
Species distribution in the first study was: *E. faecium*, 11 (27%); *E. faecalis*, one (2%); *E. gallinarum*, 24 (57%); and *E. casseliflavus/flavescens*, six (14%), but in the second study changed to: *E. faecium*, 24 (38.1%); *E. faecalis*, four (6.3%); *E. avium*, four (6.3%); *E. hirae*, one (1.6%); *E. gallinarum*, 28 (44.4%) and *E. casseliflavus/flavescens*, two (3.2%).
Conclusion: Twenty-two months after the appearance of VRE in our hospital the prevalence of VRE faecal carriers increased, especially in surgical and medical wards. VanA isolates were detected in more wards and distributed in more species.

P1345 Clonal dissemination of van A-type glycopeptide-resistant *Enterococcus faecalis* between hospitals located in two cities in the State of Sao Paulo, Brazil

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Nosocomial dissemination of glycopeptide-resistant enterococci represents a major problem in hospitals worldwide. In Brazil, the dissemination among hospitals in the city of Sao Paulo of polyclonal DNA profiles was previously described for vancomycin-resistant *E. faecium*.
Objectives: To describe the dissemination of VanA phenotype *E. faecalis* between two hospitals located in different cities in the state of Sao Paulo.
Methods: The index outbreak occurred in a university hospital in the city of Sao Paulo (HCUSP) and 3 years later a cluster caused by the same strain was recognised in a tertiary care hospital located in the country side (CMC). From May to July 1999, 10 strains of *E. faecalis* vancomycin-resistant were isolated from 10 patients hospitalised in the HCUSP. From May to July 2002, three strains of vancomycin-resistant *E. faecalis* were isolated from two patients hospitalised in CMC and both patients were colonised by the VRE in skin lesions. All the isolates were tested for susceptibility to vancomycin and teicoplanin using Etest®. The detection of Tn1546 was performed by PCR method and the strains were typed using PFGE.
Results: All strains had the presence of the transposon Tn1546 and were closely related when typed by pulsed-field gel electrophoresis.
Conclusions: The dissemination of VanA phenotype *E. faecalis* to hospitals located in different cities is of great concern and monitoring measures are necessary to reduce the risk of dissemination.

P1346 Virulence factors and antimicrobial resistance in *Enterococcus faecalis* strains of clinical origin

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Objectives: *Enterococcus faecalis* has long been identified as a cause of endocarditis, bacteraemia, urinary tract and neonatal infections. Knowledge on the pathogenic factors of *E. faecalis* is still limited, although several virulence factors have been described, such as cytolysin, aggregation substance, surface proteins. The aims of this work were to investigate the presence of known virulence determinants in 135 *E. faecalis* strains isolated from clinical specimens in Sardinia, and to determine their antimicrobial resistance patterns.
Methods: *Enterococcus faecalis* strains were mainly isolated from patients with urinary tract infections. The following virulence genes, gelE (gelatinase), esp (enterococcal surface protein), agg (aggregation substance) and cylM (post-translational modification of cytolysin) were amplified by PCR using published specific primers. Production of gelatinase and haemolysin was also phenotypically determined. *In vitro* susceptibilities of the clinical isolates towards 13 antibiotics were determined by the Vitek method.
Results: Of the 135 strains examined, only seven did not harbour any of the virulence determinants tested; the majority possessed two or three factors (51 and 41%, respectively) and 16 carried all the genes investigated. Most strains presented the gelE and agg determinants (65 and 64% positivity, respectively), while esp and cylM were detected in about half of the isolates. Several strains contained apparently silent gelE and cylM determinants, when comparing genotypic and phenotypic results. Multiple antimicrobial resistance was uncommon, the majority of strains being resistant to two antibiotics (86%) only. A large proportion of isolates was resistant to phosphomycin (90%) and tetracycline (79%) and only one strain was resistant to vancomycin. All the isolates were susceptible to ampicillin and teicoplanin.
Conclusion: Our results confirm the presence of various virulence genes in clinical isolates of *E. faecalis*, providing further supporting evidence for their role in pathogenesis. It is worth noting the generally low rate of antibiotic resistance in the clinical strains examined, especially when vancomycin-resistance is considered, if compared with data reported from other countries.

P1347 Five-year molecular epidemiology of high-level gentamicin-resistant *Enterococcus faecalis* in a Belgian academic hospital

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Objective: To monitor the molecular epidemiology of high-level gentamicin-resistant *E. faecalis* (HLGRE) from 1995 to 1999 and to evaluate the impact of control measures on their nosocomial transmission
Patients and methods: During the period 1995–1999, a total of 130. A total of 114 patients were admitted at Erasme Hospital, a 850-bed tertiary-care University Hospital. HLGRE strains (gentamicin MIC > 2000 μg/mL) were isolated from 370 patients. The infection control team reviewed the patient charts to define if strains were imported/nosocomial and responsible of infection/colonisation. Since May 1996, all colonised/infected patients were placed in contact isolation. HLGRE incidence rate was monitored in the pre-intervention period (1 January 1995 to 30 April 1996) and the postintervention period (01 May 1996 to 31 December 1999). PCR analysis of aacA-aphD gene and macrorestriction analysis (SmaI) resolved by pulsed-field gel electrophoresis (PFGE) were performed.
Results: The rate of HLGRE nosocomial acquisition was 0.24 and 0.21% for the pre- and postintervention period, respectively. Among nosocomial HLGRE strains, 31% were isolated from intensive care units, 17% from gastroenterology ward and 13% from

the surgical vascular ward. All HLGRE strains harboured the aacA-aphD gene and a total of 49 PFGE types, defined as patterns differing by ≥7 DNA fragments, were found among the 286/370 HLGRE strains typed. Major epidemic PFGE types 1, 3 and 4 were recovered from 18, 37 and 12% of patients with nosocomial and from 8, 29 and 31% of patients with community-acquired strains, respectively. Type 1 was recovered predominantly during the preintervention period whereas types 3 and 4 were isolated during both periods.
Conclusions: Typing data showed a major shift in transmission of a few predominant HLGRE epidemic types in our hospital. There was continuous introduction of patients colonised with types that appear to be regionally endemic. Contact precautions appeared to have no significant impact on the long-term incidence of nosocomial acquisition of HLGRE.

P1348 Epidemiology and antimicrobial patterns of multiresistant nosocomial strains of *Enterococcus faecium* isolated over 3 years in a Bulgarian university hospital

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Objectives: *Enterococcus* spp. are among the common organisms associated with hospital-acquired infections. The emergence of enterococci as significant pathogens is a matter of concern because these organisms are inherently resistant to a multiple antimicrobials, especially *Enterococcus faecium*. The aim in this study was to examined *in vitro* activities of different antimicrobial agents to 54. *E. faecium* isolates collected in Queen Joanna University Hospital, Sofia, Bulgaria during 3-year period and to determined theirs prevalence among different wards in our hospital.
Materials and methods: Between November 2000 and December 2003 a total 54 isolates of *E. faecium* were collected that causes the nosocomial infection. The identification and susceptible to antibiotics has been performed in automated system Sceptor *Streptococcus* MIC/ID (Becton Dickinson) MIC/ID panels as described by the NCCLS.
Results: There was an increase in the proportion of *E. faecium* isolated in 2003 (25%) compared with 2002 (15%) and 2001 (3.6%). About of 60 % of all *E. faecium* strains were multidrug resistant and exhibited ampicillin resistance, high-level gentamicin (HLGR) and quinolones resistance. The strains were susceptible only to linezolid and glycopeptides. Nine (27%) of them were isolated from ICU's, 14 (42%) from abdominal surgery, four (12%) from neurosurgery and 19% from medical units. Isolates were recovered from surgical-site infections (48%), urinary-tract infections (33%), primary blood stream infections (9%) and other infections (10%).
Conclusion: The emergence of multidrug resistant *E. faecium* and theirs dissemination in our hospital has highlighted the need for continual surveillance. The most important problems determined were the high frequencies of resistance to ampicillin, aminoglycosides and quinolones. The most active antimicrobials to which no resistance has been found were linezolid and glycopeptides. It is concluded that glycopeptide resistance is not yet an important problem for enterococcus isolates.

P1349 Hospital and community-acquired *Staphylococus aureus* bacteraemia: risk factors, location of inflammation and outcome

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Objectives: To access the incidence, to recognise risk factors and describe location of specific tissue inflammation along with the outcome of patients with community acquired and nosocomial *Staphylococcus aureus* bacteraemia (SAB), treated in a tertiary medical department.
Methods: This is retrospective study of 7250 patients who were hospitalised in a medical department, during 7-year period (January 1995 to January 2002). Community acquired and nosocomial SAB were determined according to positive blood cultures, taken less or >72 h from admission, respectively. Categorical variables were compared with Chi-square techniques.
Results: During the 7-year period, 55 (28 male, 33 female) and 182 (85 male, 97 female) patients developed community acquired and nosocomial SAB corresponding to an incidence of 1 per 1000 person years and 3.5 per 1000 person years, respectively. Risk factors for the developed of SAB bacteraemia in group A vs. group B, was: injection drug use: 12 (21.8%) vs. 17 (9.3%) ($P = 0.02$), haemodialysis-dependent patients: seven (12.7%) vs. 18 (9.9%) (P = NS), diabetes mellitus: 20 (36.4%) vs. 16 (8.8%) ($P = 0.01$), neoplasmatic diseases: five (9.1%) vs 18 (9.9%) (P = NS), corticosteroid use: eight (14.5%) vs. 62 (34.1%) ($P = 0.01$), haematologic disorders: three (5.5%) vs. 28 (15.4%) ($P = 0.05$), surgery within the previous 30 days: 0 vs. 10 (5.5%) (P = NS) Determination of location of septic inflammation was feasible in 29 (52.7%, group A) vs. 94 (51.6%, group B) (P = NS), including: deep tissue abscess: five (9.1%) vs. 23 (12.6%) (P = NS), psoas abscess: two (3.6%) vs. 18 (9.9%) (P = NS), vertebral osteomyelitis: four (7.3%) vs. 12 (6.6%) (P = NS), soft-tissue infection: 10 (18.2%) vs. eight (4.4%) ($P = 0.01$), septic arthritis: two (3.6%) vs. nine (4.9%) (P = NS), septic thrombophlebitis: four (7.3%) vs. eight (4.4%) (P = NS), endocarditis: two (3.6%) vs. eight (4.4%) (P = NS), meningitis: zero vs. eight (4.4%) (P = NS), infection of orthopaedic prosthetic devises: zero vs. 13 (7.1%) (P = NS). Mortality was three (5.5%, group A) and 28 (15.4%, group B), respectively ($P = 0.05$).
Conclusions: Among risk factors for community acquired SAB are injection drug use and diabetes mellitus, while for nosocomial SAB is use of corticosteroids and haematologic disorders. Soft-tissue infection was more frequent on group of community-acquired SAB. Higher mortality rate was noticed in the group of nosocomial SAB.

P1350 Endocarditis is not a common complication of catheter-related *Staphylococcus aureus* bacteraemia

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Background: *Staphylococcus aureus* is probably the most important pathogen isolated from intravascular (IV) catheter-related (CR) bloodstream infections (BSI). Recently, it has been suggested that 25% of cases of *S. aureus* CRBSI are complicated by infective endocarditis (IE).
Objective: To characterise the clinical and epidemiological features of CRBSI due to *S. aureus* at our institution
Methods: Prospective cohort registry started in August 2002. All consecutive adult patients with ≥1 positive blood cultures (BC) were evaluated for the following data: demographics, microbiological and epidemiological characteristics of positive-blood cultures, including number and duration of positive BC, source of bacteraemia, presence, type and duration of catheterisation, underlying conditions, type and duration of treatment and outcome. Transoesophageal echocardiography (TEE) was recommended for all patients and performed if an informed consent was obtained.
Results: During a period of 15 months, there were 187 episodes of nosocomial *S. aureus* BSI, of which 115 (61%) were primary and occurred among patients with IV catheters. Of these 115 episodes, 48 (42%) were IV-related, 67 (58%) were IV-associated, 24 (21%) were haemodialysis catheter-related and 74 (64%) were due to MRSA. There were nine cases of definite hospital-acquired IE, of which four (6%) were diagnosed among 64 episodes of CRBSI in which TEE (59 cases) or TTE (five cases) were performed. These four cases of IE presented with at least one finding (predisposing heart condition, clinical signs or persistent bacteraemia after removing the catheter) that would have required TEE. There were 10 episodes of relapse, all but one occurring among patients treated for ≥14 days. Of the 94 episodes treated with antibiotics for

≥10 days the mean duration of bacteraemia was significantly longer for vancomycin than for β-lactam drugs (6.5 days vs. 1.0 day, respectively, $P < 0.001$). The overall mortality rate of patients with CRBSI was 26%.
Conclusion: *Staphylococcus. aureus* CRBSI is an important cause of morbidity and mortality in our institution. Episodes of bacteraemia treated with vancomycin persisted for significantly longer period of time than that observed in episodes treated with β-lactam antibiotics. This preliminary data show that IE, as a complication of *S. aureus* CRBSI, appears to be less common than previously reported.

P1351 Characteristics of staphylococci from hip prostheses infections

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Objectives: Study of virulence factors of staphylococci isolated from patients with infected total hip prosthesis.
Methods: Swabs sampled from inflamed places of total hip prosthesis (THP) of patients from 1st Department of Orthopaedics were examined by standard microbiological methods in the period from January 2000 to June 2003. Antimicrobial susceptibility was tested according to NCCLS. Detection of β-lactamase was performed by nitrocefin method, and production of PBP2a was measured by latex agglutination test. Mec A gen detection was performed by PCR. Production of slime was detected by use of congo red agar plates. IcaA gen in 11 selected strains was detected by PCR. The surface hydrophobicity in all staphylococcal strains was evaluated by method based on bacterial adhesion to hydrocarbon-xylene. Haemolysins, coagulase, and DNase were tested by standard microbiological methods.
Results: Staphylococci represented 51% of all bacterial isolates; their occurrence showed increasing tendencies during the study period. Coagulase-negative staphylococci (CoNS) prevailed (62 of 91 strains; 68%). All tested staphylococcal strains were vancomycin susceptible. 84 strains (92%) produced β-lactamase and 37 CoNS strains (60%) produced PBP2a and were mec A gen positive. Slime (a significant virulence factor in the development of endoplastitids) was produced in 10 strains (34%) of *S. aureus* and in 23 strains (37%) of CoNS. In 11 CoNS (a mixture of positive and negative for slime production in congo red method), icaA gen presence was tested. Only three strains were icaA positive. 12 strains (41%) of *S. aureus* produced α-haemolysin and two strains (7%) δ-haemolysin. Delta-like toxin was produced by 24 strains (39%) of CoNS. 26 of 29 *S. aureus* strains produced DNase.
Conclusions: Staphylococci were the most frequently isolated bacteria in patients with THP infection. The occurrence of methicillin-resistant CoNS is still rather high. This fact should be taken in account in the regular updating of antimicrobial pre-, and perioperative prophylaxis of THP patients.

P1352 Five-year review of *Staphylococcus aureus* bloodstream infections in renal patients, 1998–2002

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Objectives: *Staphylococcus aureus* bloodstream infection (BSI) is common amongst patients on renal dialysis and serious complications may follow. Beaumont Hospital houses the national renal transplant unit and all patients with *S. aureus* BSI are clinically reviewed by the microbiology team.
Methods: A retrospective analysis of the incidence, source and outcome of *S. aureus* BSI was carried out on renal dialysis patients from January 1998 to December 2003 using clinical records and the laboratory database. Antibiotic susceptibilities, source and outcome were recorded for each isolate.
Results: There were 185 patient episodes between January 1998 and December 2002. A total of 400 blood cultures yielded *S. aureus*; of which 41% ($n = 236$) were resistant to methicillin. By comparison 38% of all BSI isolates of *S. aureus* in our hospital were methicillin resistant in 2002. The majority (85%) of renal patient episodes were secondary to catheter-related sepsis. Complications were documented in 19% of episodes, infective endocarditis (40%) being the most common. 4% ($n = 7$) of patients died. The data for 2003 is currently being updated.
Conclusions: *Staphylococcus aureus* BSI is common amongst renal dialysis patients; infective endocarditis is a significant complication but the mortality is low. Interventions to reduce the incidence are a priority in this compromised patient group.

P1353 Antimicrobial resistance of nosocomial strains of *S. aureus* isolated from patients with skin and soft tissue infections in Russia

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Objective: To determine the rates of antimicrobial resistance in nosocomial strains of *S. aureus* isolated from patients with skin and soft tissue infections in different parts of Russia.
Methods: A total of 624 *S. aureus* strains isolated from patients with nosocomial skin and soft tissue infections were studied. Patients were hospitalised in 17 hospitals in different parts of Russia – four in Central region, two in north-west region, three in south region, two in Volga region, three in Ural region, three in Siberia. Antimicrobials tested included chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, levofloxacin, linezolid, lincomycin, moxifloxacin, mupirocin, oxacillin, quinupristin/dalfopristin, rifampicin, tetracycline, trimethoprim/sulphamethoxazole, vancomycin. Susceptibility testing and its interpretation were performed by agar dilution according to NCCLS guidelines where applicable.
Results: Majority of strains (45%) were isolated from patients hospitalised in general surgical units, 25% of patients were hospitalised in burn units, 14.9% to traumatology/orthopedic units, 8% in ICUs, 4.5% in neonatal units, 2.6% in general medical units. Results of susceptibility testing are presented in the Table.

	$I+R_1$, %	MIC_{50}/MIC_{90}, mg/L	MIC ranges, mg/L
Vancomycin	0	1/1	0.5–4
Linezolid	0	2/2	1–4
Fusidic acid	0	0.125/0.125	0.03–2
Mupirocin	0.3	0.25/0.25	0.125–16
Trim./Sulfa.	1.1	0.125/0.5	0.06–64
Quinu./Dalfo.	1.9	0.5/1	0.125–16
Rifampicin	7.5	0.03/0.03	<0.03–128
Levofloxacin	8.3	0.25/0.5	0.06–16
Ciprofloxacin	12.5	0.5/4	0.125–64
Clindamycin	29	0.125/256	0.06–>256
Gentamicin	32.9	0.5/256	0.125–>256
Oxacillin	35.9	0.5/128	0.125–>256
Tetracycline	37.7	0.5/64	0.125–256
Erythromycin	41.2	0.5/256	0.125–>256
Chloramphenicol	47	8/128	0.5–256
Lincomycin	NA	2/256	0.25–>256
Moxifloxacin	NA	0.06/0.125	<0.015–4

Conclusions: (i) The overall rate of methicillin-resistance was 35.9%; (ii) the most potent antimicrobials were linezolid, vancomycin and fusidic acid, to which no resistant strains were found, followed by quinupristin/dalfopristin, mupirocin and co-trimoxazole with rates of resistance lower than 5%; (iii) macrolides,

lincosamydes, tetracyclines and chloramphenicol should not be used for the empiric therapy of nosocomial skin and soft tissue infections caused by *S. aureus*.

P1354 *Staphylococcus hominis* subsp. *novobiosepticus* – new pathogen in nosocomial infections

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Objectives: To evaluate phenotype properties of 67 strains of *Staphylococcus hominis* subsp. *novobiosepticus*, cultured in the Department of Bacteriology, Ceske Budejovice Hospital in 1999–2003.
Methods: Isolates of coagulase-negative staphylococci from clinically important material have been analysed using biochemistry identification system STAPHYtest 16. Resistance to novobiocine was determined by a disc-diffusion test. Novobiocine-resistant strains were evaluated by ORIDES system. Susceptibility to antimicrobials was tested by quantitative dilution test. The results were confirmed in the National Reference Laboratory. This facility also tested the capability to produce slime using the method of growth in Congo red, and the presence of staphylococcal delta lysine by exposing its synergy with beta β-haemolysine.
Results: The majority of evaluated strains of *Staphylococcus hominis* subsp. *novobiosepticus* tested in Staphytest 16 (Pliva-Lachema Brno) with their typical biochemical profile. The glucose-novobiocine test was, however, in most cases false-positive. The strains were easy to be identified in the ORIDES system. One 100% of strains were resistant to oxacillin. Resistance rate to erythromycine, clindamycine, chloramphenikol and ciprofloxacine exceeded 90%. All strains were susceptible to teicoplanin and vancomycine. Slime was produced by 98.5% of strains, and staphylococcal delta haemolysine was produced by 79% of strains.
Conclusions: In 1999–2003, we have isolated from clinically important materials total of 67 strains of *Staphylococcus hominis* subsp. *novobiosepticus*. Blood cultures accounted for 48% of these strains, vascular catheters 42, and 10% were found in other clinically significant materials. This recently recognised *Staphylococcus* subspecies mostly evades to be diagnosed, since it is missing in the database of majority of marketed diagnostic sets. Strains of this subspecies manifest unusually high resistance to antimicrobials and significant production of virulence factors – namely slime and staphylococccal delta lysine. These properties can make it a dangerous nosocomial pathogen.

P1355 Pathogenic *Staphylococcus epidermidis* bloodstream isolates: PFGE analysis and biofilm formation

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Background: In this analysis, 60 bloodstream isolates of patients with clinically defined infections and 60 nonpathogenic *Staphylococcus epidermidis* were investigated for particular genotypes responsible for infection.
Methods: During the years 1998–2000 blood stream isolates from 60 patients with *S. epidermidis* foreign body infections, and 60 isolates from case-matched controls without foreign body infection were collected at the University Hospital of Vienna. Cases were matched with regard to hospital placement and time of bacteraemia. All isolates were typed by pulsed-field electrophoresis, and essayed for formation of biofilms in static biofilm model. Isolates were clustered by the unweighted pair group method of arithmetic averages. Similarity (SAB) between paired isolates A and B was calculated from the constructed dendrogram. Isolates were considered to be of the same strain if SAB was 100%; to be related or closely related variants if SAB was $\geq$80 or $\geq$90%, respectively; or to be distinct strains if SAB was <80%.
Results: Thirty-one clusters of related or closely related strains were identified. Twelve small clusters with identical strains were identified occurring in 12 patients and in 11 controls. Strains of three of the clusters only were isolated at the same ward at the same time strains of the other nine clusters were distributed over the whole hospital. Patients had infections of implanted pacemakers, central venous catheters and prosthetic valves. They had significantly higher temperatures and a lower platelet count than controls (38.8 + 1.0°C vs. 37.9 + 1.1°C, and 158 + 91 vs. 233 + 122 g/L, respectively, $P < 0.05$). Biofilms were formed by 87% of the pathogenic isolates.
Conclusion: Small clusters of *S. epidermidis* strains were isolated from patients and controls as well, thus distinct strains causing bacteraemia and infection were not identified.

P1356 Resistance profiles in biofilm-positive strains of *Staphylococcus epidermidis* isolated from blood cultures

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Objectives: Biofilm formation on the surface of medical devices represents a serious problem that is magnified with the increasing use of prosthetic devices. The biofilm-forming bacteria difficult to eradicate with antibiotics often cause chronic infections. Determination of minimum inhibitory concentration (MIC), based on activities of antimicrobial agents against planctonic bacteria, is conventionally used for antibiotic susceptibility testing. Biofilm phenotypes have different susceptibility profiles. The Minimum Biofilm Eradication Concentration (MBEC) was measured on biofilm grown on pegs of the polystyrene plate. The aim of this study was to determine the resistance profiles of 45 blood culture-isolates, which were biofilm-positive by genotypic and phenotypic methods.
Methods: The 45 *Staphylococcus epidermidis* strains repeatedly isolated from blood cultures were used in this study. All isolates were ica-positive and showed the phenotypic ability to form biofilm. As the control, five ica-negative, biofilm-negative strains were used. For the antibiotic testing, the plates of polystyrene with 96 pegs that fit into standard microtitre plates were used. The pegs of the plates were coated with poly-L-lysine and transferred into microtitre plate with a fresh overnight culture of staphylococcal strains to enable primary adhesion. For the quantification of attached cells sonification was used. After 18 h of biofilm formation the plates were transferred into microtitre plates containing logarithmic dilutions of antibiotics (penicillin, oxacillin, chloramphenicol, tetracycline, co-trimoxazole, erythromycin, clindamycin, ciprofloxacin, gentamicin, teicoplanin and vancomycin). After overnight cultivation the plates were transferred into colorimetric medium, which indicates metabolic activity of surviving cells. The changes of the medium were detected spectrophotometrically. The MBEC was determined as the minimal concentration of antibiotics that eradicates the bacteria and so inhibits their metabolic activity.
Results and conclusions: All the tested strains showed drastically higher resistance to all the above-mentioned antibiotics when tested in biofilm form. All antibiotics exceeded maximal concentrations achievable in human plasma. That explains why the treatment of these infections with normal dosage of antibiotics often fails. The study was supported by the grant IGA 6818-3 of the Ministry of Health and grant 0463-2003 FRVS.

P1357 *Staphylococcus lugdunensis*: an emerging pathogen in bone and joint infections? Report of 17 cases

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Objectives: To describe epidemiological and clinical features, and outcome of 17 cases of *S. lugdunensis* bone and joint infections in traumatologic and orhtopaedic units in two university hospitals in Toulouse (Southern France)

Patients and methods: We conducted a retrospective study over a 7-year period (1996–2002) in two orthopaedic-traumatological units. Inclusion criteria were: patients with articular signs or fistula, positive pure cultures for *S. lugdunensis* from articular fluid or surgical samples. First identtification criteria for *S. lugdunensis* were: ornithine décarboxylase, L-pyrrolidonyl arylamidase and catalase-positive tests, negative-coagulase test. Full identification was assessed by API ID32 Staph Gallery (Bio Métieux°). Criteria for a favourable outcome were: lack of pain, disability or local inflammatory signs.
Results: Our study included 17 patients (10 male and seven female). Mean age was 56 years (30–87). Seven patients had comorbidity factors (history of cancer: three, rheumatoid arthritis: one, rheumatoid spondylitis: one, diabetes mellitus: two). Fifteen had a bone or joint foreign body (joint prosthesis: nine (hip: five, knee: four), osteosynthesis: six). Mean time between surgical operation and *S. lugdunensis* identification was 52 weeks (3–26), mean time between clinical signs and *S. lugdunensis* identification was 12 weeks (2–104). Twelve patients had local inflammatory signs, only four had fever. In all cases pus and/or macroscopic inflammatory signs were noted by surgeons during operation. All strains of *S. lugdunensis* were susceptible to oxacillin and only six (35.3%) and one (6%) were, respectively, resistant to penicillin and fluoroquinolones. However 13 (76.5%) were resistant to phosphomycin. All patients received antibiotics (combination: 11 cases), for a mean period of 12 weeks (3–63): quinolones: nine, oxacillin : 8, rifampin : 8, fusidic acid : 2, clindamycin : 2, pristinamycin : 3, miscellaneous: three. All patients but two had also a surgical treatment. Mean follow-up time was 15 months (2–48). A favourable clinical course of the *S. lugdunensis* infection was observed in all cases except for the two patients who had no surgical treatment.
Conclusions: *S. lugdunensis* is an emerging pathogen, associated with infections of orthopaedic devices. Inflammatory signs and macroscopic aspects are similar to those described in *S. aureus* infections. As for other pathogens, treatment of such infections must associate surgery and general antibiotherapy.

Nosocomial infections: multiresistant Gram-negative bacteria

P1358 Clinical presentation and outcome of multi-resistant *Enterobacter aerogenes* infections: a review of 116 episodes

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Objectives: Multi-resistant *Enterobacter aerogenes* (MREA) is a major nosocomial pathogen associated with epidemics in several countries, including Belgium. The objective of this study was to assess the clinical and therapeutic aspects and outcome of MREA infections in a 858-bed academic hospital.
Methods: We retrospectively reviewed the medical files of patients (pts) with a positive culture for MREA over a 5-year period and selected those with infection according to CDC criteria. Antimicrobial susceptibility was determined by standard disc diffusion and extended-spectrum β-lactamase (ESBL) screening was performed by double-disc potentiation test. Isolates were considered multiresistant if resistant to third generation cephalosporins and to a fluoroquinolone and/or an aminoglycoside.
Results: MREA was isolated in a total of 347 hospitalisations and was responsible for infection in 122 (35%). Among the 116 studied episodes, foci of infection were: urinary (43), respiratory (39), abdominal (20), soft tissue (eight), blood (three), central venous catheter (two), and bone (one). Infection was polymicrobial in 36 cases and associated with bacteraemia in 17. At total of 63 episodes were complicated by sepsis (24), severe sepsis (18) or septic shock (21). Most pts with urinary infection (UI) had no signs of sepsis by contrast to pts with pneumonia and peritonitis (27/39 and four of six, respectively). ESBL production was identified in 79% of the strains. Appropriate treatment consisted of meropenem (71%), temocillin (22%, given only for UI) and cefepime (6%) combined with aminoglycosides in 43 episodes (37%). Clinical outcome was favourable in 58% of the cases; 38 patients died (34%), 32 due to infection. The highest mortality rate was found in patients with peritonitis (67%), catheter-related infection (50%) and pneumonia (41%) while the lowest rate was found in patients with urinary infection (9%). Multivariate analysis outlined sepsis as the only factor statistically associated with increased risk of mortality. Microbiological eradication was obtained in 46.5%. Temocillin in uncomplicated urinary infection had a favourable clinical outcome in 97% of the cases and microbiological eradication in 50%.
Conclusion: Nonurinary MREA infections can be associated with severe initial clinical presentation and non-negligible mortality. Given the favourable clinical outcome, temocillin could be used for selected patients with uncomplicated urinary tract infection.

P1359 ESBL-producing *Enterobacter aerogenes* outbreak in neonates hospitalised in a neonatal unit

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Objectives: Analysis of a nosocomial ESBL-producing *Enterobacter aerogenes* outbreak in neonates.
Methods: We analysed medical reports and results of bacteriological and epidemiological data from October 2002 to April 2003 in our Department of Neonatology (63 beds with eight on NICU). Identification and susceptibility was performed by routine methods and VITEK (bioMerieux). Production of ESBL was detected by double disc-diffusion method. Colonisation was determined by a positive rectal/throat culture without any sign of infection.
Results: Outbreak strain which was susceptible only to imipenem, meropenem and ciprofloxacin was isolated from 71 patients: 41 with respiratory distress syndrome, 19 congenital infection, five dysadaptation, two persistent fetal circulation, two asphyxia, two NEC. Only four of the neonates were born at term, other were premature (48, born in 30–38 Hbd and 18 before 30 Hbd). 41 were colonised and 30 had infections: nine with sepsis, 12 pneumonia, 11 UTI, two osteomyelitis, one soft-tissue infections (several patients had more than one infection). *E. aerogenes* ESBL+ was isolated in October–April from 1.8, 8.6, 14, 17, 13, 7.4 and 2.9% of all hospitalised neonates, respectively.
Conclusion: We considered that 42% patients developed infection and were therefore treated with carbapenems. The outbreak ended after the implementation of the strict infection control.

P1360 Study of antibiotic resistance, production of ESBL and other virulence factors in enterobacterial strains isolated from nosocomial infections in cardiovascular devices inserted patients

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Introduction: Since 1980s, ESBL-producing microorganisms, mostly Enterobacteriaceae emerged in the whole world. The increasing use of broader-spectrum cephalosporins is one of the major factors responsible for the high rate of selection of ESBL-producing microorganisms in Romanian hospitals.

Purpose: To establish the incidence of ESBL-producing enterobacterial strains isolated from cardiovascular devices (CDs) inserted patients in the intensive care unit of the Institute of Heart Disease 'C.C. Iliescu' and to correlate the ESBL production with other virulence factors and with the plasmidial profile of these strains.
Methods: Antibiotic susceptibility determined by the disc diffusion; double disc-diffusion for ESBL phenotype detection; *in vitro* study of adherence and invasion capacity to HeLa cells investigated by gentamycin-protection assay; adherence to an inert substrate evaluated by the slime test; other virulence factors: Kanagawa and sheep erythrocytes haemolysins, DNase, lipase, lecitinase, amylase, gelatinase, mucinase and caseinase, Congo red test were tested on specific media; plasmid DNA isolation by alkaline lysis method.
Results: In our study enterobacterial strains are placed on the second place in the aetiology of infections in CDs inserted patients, after staphylococci. The incidence of the main antibiotic markers was: 65% for aminopenicillins, 60% for third generation cephalosporins, 20% for aminoglycosides and 25% for fluoroquinolones. The ESBL screening test was positive in 60% of these strains. The multi-resistance feature was not associated with the production of other enzymatic virulence factors, which proved to be very poor for the tested strains. In exchange, the adhesion to the inert and to the cellular substrate proved to be a general feature of ESBL-producing enterobacterial strains. The plasmid DNA analysis revealed the presence of variable number of plasmids (ranging from 2.5 kbp to 30 kbp) in 20% of strains.
Conclusion: All isolated enterobacterial strains proved to be multiple drug resistant and of great concern is the fact that the third generation cephalosporins resistance and ESBL-production were highly prevalent in these strains suggesting the emergence of nosocomial infections with β-lactamases producer strains. The genetic determinism was in the great majority of cases nonplasmidial. The resistance to β-lactams is probably accompanied by changes in the bacterial wall structure promoting the adhesins expression.

P1361 Urinary pathogens in a general hospital in Greece: antimicrobial resistance and incidence of ESBL producing strains during 2001–2003

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Objectives: The aim of this study was to determine the incidence of urinary pathogens, their antimicrobial resistance and the detection of ESBL-producing strains, which is necessary to guide antibacterial therapy selection.
Methods: A total of 12640 urine samples were examined in our laboratory in northern Greece during 2001–2003. The Auto Scan-4 system of Dade Behring was used to identify the bacteria isolated and evaluate their susceptibility to common antibiotics. Discs of ceftazidime 30 μg, ceftazidime/clavulanic 30/10 μg, cefotaxime 30 μg, and cefotaxime/clavulanic 30/10 μg were used to determine the presence of ESBL production among *E. coli* and *Klebsiella* spp. and the results were interpreted according to NCCLS guidelines.
Results: A total of 2852 urine cultures were positive, (22.5%). The most frequently isolated pathogens were: *E. coli* 1912 (67%), Proteus spp. 253 (8.9%), *Klebsiella* spp. 189 (6.6%), Pseudomonas spp. 113 (3.9%), *Enterococcus* spp. 102 (3.6%), *Enterobacter* spp. 78 (2.7%), *Acinetobacter* spp. 38 (1.3%), Citrobacter spp. 28 (1%), *Serratia* 16 (0.6%), *Staph aureus* 21 (0.7%), *Staph epidermidis* 20 (0.7%), *Staph saprophyticus* 11 (0.4%), other CoNS 19(0.7), and fungus 34 (1.2%). The incidence of ESBL producing strains among the isolated *E. coli* and *Klebsiella* spp. was 3.24% (62 of 1912) and 8.47% (16 of 189) respectively. The resistance rates (%) for *E. coli*, *Proteus* spp. and *Klebsiella* spp. to antimicrobial agents were as follows: amoxicillin/clavulanic 12/21/12, cefuroxime 10/17/17, ceftazidime 5/8/7, ciprofloxacin 11/20/12, trimethorpim/sulphamethoxazole 20/37/14, amikasin 6/6/5. The resistance of *Pseudomonas* spp. to amikasin was 26%, ceftazidime 19%, cefotaxime 75%, cefipime 26%, ciprofloxacin 40%, imipenem 16%, piperacillin/tazobactam 5%. *Enterococcus* spp. were resistant to ciprofloxacin 35% while strains resistant to *Vancomycin* and *Teicoplanin* were not observed.
Conclusions: As expected the prevalent urine pathogen was *E. coli*. It was observed that acinetobacter and enterobacter were more sensitive to ampicillin/sulbactam (60.67%) than amoxicillin/clavulanic (25.20%). Ceftazidime is more efficient against *Pseudomonas* spp. than cefotaxime and cefipime. The rates of ESBL producing strains of *E. coli* and *Klebsiella* spp were similar to those of other studies reported from our country and these strains showed decreased susceptibility to nonlactamic antibiotics compared with non-ESBL producing strains.

P1362 Clinical and epidemiological analysis of *Klebsiella pneumoniae* bacteraemia in a neonatal department

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Objective: The aim of this study was to analyze the epidemiological and clinical characteristics of patients with extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* (KP ESBL) bacteraemia.
Methods: Study period covered ten months from April 2002 to January 2003. We retrospectively studied the records of patients with KP ESBL detected in blood. Specimens were incubated in automated system BacT/Alert (Bio Merieux). Identification and susceptibility was performed by VITEK system (Bio Merieux) and disc-diffusion method according to the NCCLS standards. Molecular study of isolates was done using ADSRRS-fingerprinting.
Results: KP ESBL was recovered from blood of 10 patients. All of them were earlier colonised by KP ESBL. Neonates (60%) were female, mean gestational age was 32.7 weeks, mean birth weight was 1638 g, 70% of deliveries were by cesarean section, mean 1 min Apgare score was 5.7, in 50% of neonates mechanical ventilation were necessary and 40% were ventilated longer than 5 days, all of neonates had parenteral nutrition in the beginning of life, mean hospital stay was 52 days. Bacteraemia occurred between 4 and 45 days of life. Comparing infected and colonised neonates ($n = 86$) we found: higher gestational age (34.2 weeks), higher birth weight (2089 g), higher 1 min Apgar score (6.6), shorter hospital stay (30.1 days) in colonised patients. 44.2% of colonised neonates were mechanically ventilated however only 10.5% of analysed population were ventilated more than 5 days. Molecular study with ASDSRRS-fingerprinting confirmed that only one genotype was responsible for that outbreak.
Conclusions: We detected an outbreak of KP ESBL bacteraemia affecting immature neonates with some risk factors. ADSRRS-fingerprinting which is a new typing method proved useful in determining the strain responsible for infections.

P1363 Molecular epidemiology of ESBL-producing *Klebsiella* from different perinatal intensive care units (PICUs) in Hungary

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Objectives: Molecular and phenotyping of 132 ESBL-producing *K. pneumoniae* and *K. oxytoca* strains from seven PICU outbreaks in five Hungarian county hospitals.
Methods: Confirmation of ESBL production by E-test, double-disc diffusion and agar dilution methods according to the NCCLS; PCR analysis for β-lactamase detection using specific primers; phage typing, resistance transfer, plasmid profile analysis, genomic fingerprinting by ERIC-PCR and PFGE.
Results: The isolates were multidrug-resistant but were still susceptible to ciprofloxacin. Of 132 isolates, 103 belonged into six different phage types, but 29 were not typable. ERIC-PCR was performed on all strains and we found five major ERIC-types and nine subtypes. PFGE analysis revealed seven different genetic clones at 85% homology level. All isolates harboured plasmids

ranging from 2 to 140 MDa in 12 plasmid profiles. The sequence analysis of the SHV PCR products showed that SHV-2a was presented in two outbreak clones, SHV-5 in five outbreak clones, and two isolates carried both SHV-5 and SHV-26. Of these, SHV-2a and SHV-5 were on transferable plasmids.
Conclusions: These results show that SHV-2a and SHV-5 are the most prevalent ESBLs producing by klebsiellae in hungarian PICUs. Our work also confirms that ERIC-PCR, when it is combining with phage typing, plasmid profile analysis and PFGE is an effective tool for investigating the epidemiologies of ESBL-producing *K. pneumoniae* and *K. oxytoca* strains.

P1364 Adhesion to and invasion of human epithelial cells by ESBL and non-ESBL-producing *Klebsiella pneumoniae* strains

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Objective: Extended spectrum β-lactamse (ESBL)-producing *Klebsiella pneumoniae* strains are suggested to possess higher pathogenic potential than non-ESBL-producing strains, mainly by virtue of their higher ability to adhere to human epithelial cells, and to resist the bactericidal activity of serum and the phagocytosis by polymorph nuclear leucocytes. Because the adhesion to and invasion of epithelial cells are considered to be major virulence factors of bacteria that are prerequisites for the infectious process we sought to compare the ability of ESBL and non-ESBL-producing *Klebsiella pneumoniae* isolates to adhere to and invade human epithelial cells.
Methods: The invasion of human ileocecal (HCT8), bladder (T24) and lung epithelial cells (A549) by ESBL-producing and non-ESBL-producing *Klebsiella pneumoniae* strains was tested using an imipenem killing assay. In total, 28 ESBL-producing and 48 non-ESBL-producing *Klebsiella pneumoniae* strains were tested. Six ESBL-producing strains bearing the ESBL-coding plasmid were retested for their adhesion and invasion ability after eliminating of the R-plasmid.
Results: No significant differences could be shown between the ability of ESBL-producing strains and non-ESBL-producer to invade the HCT8 ileocaecal ($2.7 \pm 3.4\%$ of inoculum vs. $2.7 \pm 3.7\%$), the T24 bladder ($0.94 \pm 1.8\%$ vs. $0.75 \pm 0.8\%$) or A549 lung epithelial cells ($0.06 \pm 0.27\%$ vs. $0.12 \pm 0.54\%$) ($P > 0.05$). Likewise, the adhesion of the ESBL and non-ESBL-producing isolates to the HCT8 cells did not differ significantly ($51.6 \pm 26.3\%$; 63 ± 17.6) ($P > 0.05$). Plasmid curing did not influence the ability of the strains to adhere to or invade the HCT8 cells, all six plasmid-cured derivatives retained the same adhesion and invasion potential to the HCT8 epithelial cells ($54.6 \pm 43\%$ and $2.9 \pm 3.1\%$) ($P > 0.05$).
Conclusion: The results indicate that the ESBL-coding plasmids do not contribute to the sadhesion or invasion potential of *Klebsiella pneumoniae*.

P1365 Isolation of multi-resistant strains of *Klebsiella oxytoca* from neonatal intensive care unit – colonization or infection?

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Introduction: Existence of MDR strains of Gram(−) rods creates serious epidemiological danger and therapeutic problem in newborns' infections and it is important to differentiate between colonization and infection.
Objective: The aims of the study were: analysis of the occurence of ESBL and antibiotic susceptibility of *Klebsiella oxytoca* (*K. ox.*)isolated from newborns, determine the genetic relatedness between strains and differentiation between colonisation and infection in the cases of newborns from whom *K. ox.* was isolated.
Material and methods: A total of 26 *K. ox.* isolated from newborns hospitalised in NICU between b October 2002 were tested for antibiotic susceptibility (by NCCLS) and the DD test for detection of ESBL. To molecular typing of *K. ox.* isolates was used PFGE (Xba). 13 cases of the newborns with MDR *K. ox.* were analysed. Attention was paid: Apg. scale, HBD, birth weight, clinical state during the hospitalisation and duration, the day of hospitalisation in which *K. ox.* was isolated, treatment and potential coexisting diseases.
Results: All strains of *K. ox.*: produced ESBL, were sensitive to: amox.-clav. acid, aminoglicosides and carbapenems. Analysis of the banding pattern for the isolates can be divided into two types: A (three newborns) isolated between 8 September 2002 and B (13) 9 October 2002.Two of them were isolated from one newborn in different time. In analysed 13 newborns' group: four of colonisation (without any symptoms) and nine cases of infection was found. In all of colonised newborns *K. ox.* was isolated up to fifth day of hospitalisation. Ampicillin and meropenem were administrated in prophylaxis in two cases with low weight. In all of infected newborns *K. ox.* was isolated after 10th day of hospitalisation. Ampicillin, amikacin, meropenem were administrated. Characteristics of infected newborns: one born in good condition with proper weight, diarrhoea was the only symptom; eight premature birth: one born in good condition with weight 1750 g developed NEC; seven born in medium or severe manifestations appeared (pneumonia, meningitis and sepsis). One of them died. The duration of hospitalisation 13–57 days.
Conclusions: All ESBL + *K. oxz.* belong to two genetic types. Isolation of the same type from different newborns during 3 months spreading of the bacterium clone on the department. Colonisation developed in cases of healthy newborns. Infection developed in cases of newborns with low weight and severe or medium condition, more often during long hospitalisation.

P1366 Antimicrobial resistance of Gram-negative bacilli isolated from patients with bloodstream infections in ICUs

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Objectives: To investigate antimicrobial resistance of Gram-negative rods causing bloodstream infections (BSI) in the ICU of a university hospital in Turkey.
Methods: The study included 27 *Pseudomonas aeruginosa*, 44 *Acinetobacter* spp, 16 *Klebsiella pneumoniae*, and 12 *Escherichia coli* isolated from the blood samples of patients having bloodstream infections in the period of 2000–2003. The antibiotic susceptibilities and extended-spectrum β-lactamase (ESBL) production were determined by Etest (AB BIODISC) using Mueller–Hinton agar (OXOID) according to NCCLS recommendations.
Results: The antibiotic resistances and rates of ESBL production of the strains are presented in the table.

Table. The antibiotic resistance percentage of the micro organisms

Pathogens ($n = 99$)	MEM (%)	IPM (%)	CAZ (%)	CIP (%)	P+T (%)	TOB (%)	PM (%)	CTX (%)	ESBL *n* (%)
Acinetobacter spp ($n = 44$)	84	98	100	100	100	98	100	98	
P. aeruginosa ($n = 27$)	59	85	85	70	85	81	77	92	
K. pnemoniae ($n = 16$)	0	43	81	94	87	94	69	62	10 (62%)
E. coli ($n = 12$)	0	0	33	50	66	66	25	25	3 (25%)

MEM: Meropenem, IPM: Imipenem, CAZ: Ceftazidime, CIP: Ciprofloxacin, P+T: Piperacillin-tazobactam, Tom: Tobramylin, PM: Cefepime, CTX: Cetotaxime

Conclusions: Among the microorganisms causing BSI multi-drug resistant *Acinetobacter* spp was the leading pathogen. Regarding all the strains, carbapenems and cefepime are identified as the most effective antimicrobial agents.

P1367 Risk factors for ICU-acquired imipenem-resistant Gram-negative bacterial infections

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Objective: Intensive care units are high-risk areas for imipenem-resistant finfections. The main objective of this study was to investigate risk factors for ICU-acquired imipenem resistant Gram-negative bacterial infections and to develop strategies to prevent imipenem resistance in our hospital.
Patients and methods: This study was conducted prospectively in three surgical and one medical ICUs from April to December 2002 at a tertiary care hospital in Ankara. The patients who had ICU-acquired Gram-negative infections were included in the study. The patients were assigned as imipenem-resistant (IR) cases if they had IR bacterial infections and as imipenem-sensitive (IS) cases if they had IS bacterial infections. ICU-acquired Gram-negative infections were detected in 128 patients during the study period. Overall 163 Gram-negative infections were diagnosed. Of the 128 patients, 42 had IR Gram-negative infections and 86 had IS Gram-negative infections. Overall 173 Gram-negative bacteria were isolated from the clinical specimens of the infected patients. Of these bacteria, 52 were IR and 121 were IS. The highest imipenem resistance rate was detected in *Acinetobacter* spp (67.4%), and followed by *P. aeruginosa* (48.3%). IR Gram-negative bacteria were most frequently recovered from surgical site cultures (30.8%). However, IS Gram-negative bacteria were most frequently recovered from urine cultures (49.7%). According to the univariate analysis, hospital stay before ICU admission, hospitalisation period before ICU, length of ICU stay, surgical ICU stay, surgical operation, previous antibiotic use and previous administration of imipenem, meropenem, piperacillin-tazobactam, fourth generation cephalosporin, aminoglycoside and glycopeptide were significant risk factors for IR infections ($P < 0.05$). In multivariate analysis, length of ICU stay (OR, 1.02; 95% CI, 1.00–1.04), surgical operation (OR, 4.31; 95% CI, 1.79–10.35) and previous carbapenem use (OR, 3.31; 95% CI, 1.22–8.98) were independently associated with imipenem resistance.
Conclusions: This study suggests that long ICU stay, surgical operation and administration of carbapenem were major risk factors for IR Gram-negative infections.

P1368 Epidemiology of integron-associated resistance in two ICUs during a nonoutbreak period

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Objective: Integrons (ints), present on plasmids and transposons in Gram-negatives play a role in horizontal transfer (HT) of antibiotic resistance. Little is known about the prevalence, incidence, and HT of int-associated resistance in Enterobacteriaceae in a non-outbreak period in an intensive care unit (ICU).
Methods: During an 8-month period all patients (pts) admitted to two ICUs were screened for rectal colonisation with Enterobacteriaceae with reduced susceptibility to cephalosporins (ERSC) by means of rectal swabs taken on admission and twice weekly thereafter and cultured on agar with cefpodoxime. Isolates (iso) were identified using the VITEK. Two iso of each species/pt were selected for susceptibility testing, int-specific PCR, and AFLP genotyping. Ints were characterised by CS-PCR, RFLP and DNA sequencing. Demographics and data on antibiotic use were collected.
Results: In total, 458 pts were admitted to these ICUs of which 121 were colonised with ERSC. A total of 61 pts were colonised on admission and 56 acquired colonisation in the ICU. A total of 174 iso were selected and 52 iso of 29 pts (24%) carried at least one int. The daily endemic prevalence of ints was 7% (range: 0–33%). Multivariate analysis did not reveal risk factors for acquisition of int-positive isolates. Characterisation revealed three groups containing the majority of int-positive iso. Two of these groups were only found in *E. coli* iso collected from ICU-1. The first group comprised 11 iso carrying two ints with the aadA2 and the aadB/catB3 genes. The second group comprised four iso carrying an int with the dfrIa and aadA1a genes. The third group comprised eight *E. cloacae* iso, one *K. oxytoca* iso, and one *K. pneumoniae* iso from ICU-2, which carried an int-containing the aadB gene. Iso carrying identical ints were, with a few exceptions, part of the same AFLP clusters. HT of a complete int-carrying organism (i.e. cross-transmission) was identified four times (two times in each ICU). HT of an int was suspected in two patients colonised with genetically different iso of *E. coli*. Tranfer of an int from *E. cloacae* to *K.oxytoca* isolated within a pt was identified once.
Conclusion: No risk factors for acquisition of int-positive isolate could be identified. HT of a complete int-carrying organism is more likely than HT of individual ints.

P1369 Intravenous (IV) colistin for the treatment of multiresistant Gram-negative bacilli: review of experience in 30 patients

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Objectives: Colistin (IV) could be a therapeutic option for the treatment of infections due to multiresistant Gram-negative bacilli. As data on efficacy and tolerance with this drug are limited, we report our experience with colistin.
Methods: We reviewed the medical charts of all patients (pts) treated with IV colistin for systemic infection due to multiresistant Gram-negative bacilli.
Results: From January 2001 to October 2003, 32 episodes of infection (two with different multiresistant strains) due to *Pseudomonas aeruginosa* (22), *Enterobacter* spp (three pts), *Acinetobacter baumannii* (three) or others (six) were treated with IV colistin in 30 pts (12 F, 18 M, median age 61 years). At initiation of treatment, the median APACHE II score was 16 (range: 8–27). Nineteen pts were treated in the ICU. Infections were pneumonia (16, including five ventilator-associated pneumonia), intra-abdominal (nine), urinary (five), catheter-related infections (five) and others (five). Ten pts were bacteraemic. Twenty-seven of these 32 infections had been previously treated with other antibiotics. Colistin was used at a median dosage of 3.5 (range: 1–8) million unit daily for a median of 10 days (1–70 days). In all cases, another antibiotic was added despite *in vitro* resistance: meropenem (13; eight standard dose, six double dose), ceftazidime (15; 11 by continuous infusion), cefepime (three) or aztreonam (one). Fourteen episodes benefited from concomitant drainage or catheter removal. A good clinical outcome was achieved in 13 patients (43%). Seventeen patients died after a median of 11 days of colistin treatment (range 1–26 days), 11 probably related to infection. Microbiological eradication was obtained in eight of the 23 pts (35%) who were treated for at least 7 days. Significant side effects were reported in eight patients: alteration in renal function (doubling of serum creatinine) in seven pts (renal function returned to near baseline in all survivors) and myoclonia due to excessive dose in one pt.
Conclusions: These retrospective data suggest that IV colistin could represent a valuable therapeutic option for the treatment of infections caused by multiresistant. Gram-negative bacilli. since tolerance of the drug appears to be good, higher doses may be considered to improve outcome.

P1370 The frequency of multidrug-resistant bacteria from clinical samples

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Objectives: To analyse the frequency of multiresistant bacteria isolated from urine, blood and wound swabs culture.
Methods: During 3-month study we analysed isolates from urine, blood and wound swabs, which were resistant to three and more

antibiotics. Susceptibility testing was performed by Kirby-Bauer disc-diffusion method following NCCLS guidelines.
Results: In urine culture 470 (24.5%) of total 1918 isolates were multidrug resistant. The most frequently isolated microorganism was *Klebsiella pneumoniae* with total of 165 (34.2%), followed by *Escherichia coli* with 123 (26.2%) and *Proteus mirabilis* 42 (9%). Urine samples from neurology and nephrology departments had the highest rate of multidrug-resistant microorganism. In blood culture 115 (58%) of bacteria were multidrug-resistant. The most frequently isolated microorganisms among a total of 115 isolates were: coagulase-negative staphylococci (CNS) 60 isolates (52%), *Acinetobacter calcoaceticus* 11 (9.5%), *Pseudomonas aeruginosa* nine (7.8%), *Klebsiella pneumoniae* eight (7%), *Serratia marcescens* seven (6%), *Enterococcus faecalis* six (5.2%) ans *Staphylococcus aureus* five (4.3%). Blood samples from paediatric, haematology and Department for Infectious diseases had the highest rate of multidrug-resistant microorganisms. In wound swab culture 109 (15.8%) of isolates were multidrug resistant. *Staphylococcus aureus* was the most frequent with total of 54 (50%), followed by *A. calcoaceticus* 17 (15.5%), *E. faecalis* 12 (11%) and *S. marcescens* 9 (8.3%). Orthopaedic and plastic surgery departments had the highest rate of multidrug-resistant bacteria in wound samples. All Gram-negative bacilli expressed high frequency of resistance to Ampicillin, cefalosporins I and II generation, aminoglycosides and quinolones, moderate resistance to cephalosporin IV generation -Cefepime, and high sensitivity to Imipenem and Amicacin. Gram-positive cocci *Staphylococcus aureus* and coagulase-negative staphylococci were in high per cent resistant to oxacillin. *E. faecalis* expressed resistance to almost all antibiotics, except to amoxicillin + clavulanic acid.
Conclusions: Resistance to antibiotics has been permanently developing as well. *Klebsiella pneumoniae*, coagulasa-negative staphylococci and *Staphylococcus aureus* are the most frequently isolated multiresistant bacteria which reflected ecosystem in our hospital. The obtained data point to the need of changing the empirical antimicrobic therapy and better (antibiotic policy).

P1371 Occurrence of extended-spectrum β-lactamase-producing *Escherichia coli* strains in a university hospital

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Objectives: To determine the occurrence and the antimicrobial resistance of the extended spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC) strains in an University Hospital, Pleven, Bulgaria.
Methods: Non-replicated isolates (845) of *E. coli*, collected from hospitalised patients during a 1-year period were studied. ESBL production was determined by the double disc synergy method. Susceptibility to antimicrobials was tested by a disc diffusion method according to the NCCLS recommendations.
Results: ESBL-EC strains accounted for 9.2% of total *E. coli* isolated from clinical specimens during 2002. According to the date of isolation the prevalence of *E. coli* ESBL producers was relatively lower (4.6%) in January–June but it was increased to 13.3% in July–December. Isolates were most often recovered from urine (48.7%), soft tissues (33.3%), respiratory tract (7.6%), and blood cultures (5.1%). Colonisation/infection with ESBL-EC isolate was detected in 77 patients. The majority of ESBL-EC originated from ICU and urology, where 25.4–28.8% of *E. coli* isolates were producers of ESBL. The ESBL producers were more resistant than nonproducers to gentamicin, ciprofloxacin, and trimethoprim-sulphamethoxazole (89, 85, 62% and 22, 21, 28%, respectively). Both groups showed similar low resistance rates to amikacin (12 and 8%, respectively), and were fully susceptible to imipenem.
Conclusions: The data suggest that in our hospital there is an ongoing outbreak of ESBL-producing multiresistant *E. coli*. Further investigations will be necessary to characterise the type of ESBL and the epidemiology of strains.

P1372 *Serratia marcescens* in clinical specimens over 3 years

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Objectives: The aim of the study was to determine the role of *S. marcescens* in tertiary care hospital.
Methods: The analysis of microbiological records from 2001 to 2003 was done. We used Vitek GNI+ cards (Biomerieux) to identify isolates and disc-diffusion method to determine their susceptibility. ESBL production was detected by double disc method.
Results: About 140 000 clinical specimens were send for culture over 3 years. 501 specimens were positive for *S. marcescens*. It was isolated from: wounds/drainage (35%) respiratory tract (22%), urine (16%), blood (12%) and catheter tips (3%). Most of 225 affected patients were from surgery (43%) and internal medicine (32%) wards. Twenty-three patients suffered from bacteraemia. Considering one isolate per patient the incidence of ESBL-producing isolates was 29%. The number of ESBL isolates decreased from 33 in 2001 to nine in 2003. Most isolates displayed cefotaximase phenotype which was in agreement with our earlier study (1996–2000) showing dominance of CTX-M-3 enzyme in *S. marcescens* population.
Conclusions: *Serratia marcescens* seems to be an endemic pathogen in our setting responsible for numerous infections. The incidence of ESBL-producing isolates has decreased over time, which is probably due to strict implementation of infection control measures. CTX-M phenotype predominates among ESBL.

P1373 Genotyping of *S. marcescens* and *K. pneumoniae* nosocomial isolates from newborns

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Objectives: *Serratia marcescens* and *Klebsiella pneumoniae* are well-known causative agents of nosocomial infections in newborns. The aim of the present work is to investigate, by the use of molecular typing methods, two putative epidemic clusters of newborn cross-infections due to *S. marcescens* and *K. pneumoniae*, respectively, in order to establish clonal relationships among the isolates and to identify the possible source and mode of transmission.
Methods : Over a 6-month period, 11 newborns, from an intensive care unit, where either infected or colonised by *S. marcescens*, 10 by *K. pneumoniae*, three by both of them. Four premature neonates among these patients yielded invasive infections. Repetitive positive blood cultures were associated in two cases with *K. pneumoniae*, in one case with *S. marcescens*, in another fatal case with both microorganisms. The nurses and the environment were checked by microbiological studies. The antibiograms and the results from two molecular typing methods (automated Riboprinting and ERIC-PCR) of all bacteraemic or colonising isolates from newborns and from patients elsewhere in the hospital during the same time period, were compared in order to investigate the epidemiology of the clusters.
Results: Totally 20 *S. marcescens* isolates, including 12 from newborns and eight from other patients of the same hospital, and 10 *K. pneumoniae* strains from newborns were examined. An initial analysis performed after the identification of the first nine newborns contaminated by *S. marcescens* established clonal relationships among all the isolates, including the two bacteraemic strains. A second analysis revealed one different pattern in three *S. marcescens* strains successively isolated. Six genotypes unrelated to newborns isolates were found in the eight strains from the other examined patients. The three *K. pneumoniae* bacteraemic strains resulted genetically related among them but unrelated to the colonising isolates. *S. marcescens* isolates among different molecular patterns showed identical antibiograms. A focal source for both microorganisms was not identified.

Conclusion: Typing results revealed that two different microorganisms (*S. marcescens* and *K. pneumoniae*) were involved at the same time in invasive nosocomial infections in premature newborns and documented two contemporaneous clonal clusters of cases. Heterogeneous genotypes among both species were also demonstrated to be simultaneously present in the neonatal intensive care unit.

Surveillance studies

P1374 Prevalence and risk factors for nosocomial infections in 19 hospitals of Veneto Region (Italy)

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Objective: To estimate the prevalence and risk factors for nosocomial infections (NIs) in 19 hospitals of Veneto Region (northern Italy).
Methods: A 7-day period-prevalence survey was conducted in May 2003 in 19 selected acute care hospitals, for a total of 10 598 beds (63% of the total regional hospital beds). An investigation team for each hospital was trained for the purpose. Standard definitions for NI according to CDC were used. Eligible patients (pts) were older than 1 year and present in the 24 h preceding the survey day on surgical, medical (excluding dermatology, psychiatry, long-term care) and intensive-care wards (except for neonatal intensive care units). A standardised questionnaire was completed for all eligible pts and an additional one for pts with NI, identified by reviewing of nursing and medical records. All data were checked and validated by the local co-ordinator and by the co-ordinating centre. A descriptive, univariate and multivariate (step-wise regression model) analysis were performed.
Results: A total of 6352 pts were suitable for analysis (55%, medical area; 45%, surgical area; 5% ICU). The average age was 62.5 years; the mean length of stay pre-survey was 10.2 days. Overall, 1931 pts had surgery prior to the study point. The total number of NIs was 483 (rate 7.6%), occurring in 441 pts (rate 6.9%); prevalence of NIs in ICU, medical and surgical wards was 25.8, 6.5 and 5.1%, respectively. Urinary-tract infections accounted for 28.4%; surgical site infections 20.3%, bloodstream infections 19.3%, pneumonia 17.5%, other infections 14.5%. Rates of NIs in the participating centres ranged from 2.6 to 17.7%. The NI was microbiologically proven in 65.2%;Gram-positive bacteria were 47.9%, Gram-negative 42.8%, fungi 8.6% and anaerobes 0.7%. In the multivariate analysis, the following variables were independently associated with an increased risk for NI: presence of central venous catheter (CVC) (OR 2.2; 95% CI 1.6–3.0), urinary catheterisation (UC) (OR 2.5; 95% CI 1.9–3.2), intubation (OR 3.1; 95 CI 2.0–5.0), and length of stay >15 days (OR 12.9; 95 CI 9.3–18.1).
Conclusions: This is the first prevalence study performed at regional level in Veneto. It displayed a great interhospital difference in NI rates, with higher rates observed in larger hospitals; it confirmed the importance of length of stay, intubation, UC, CVC, as major risk factors for NIs; it also showed a frequency of bloodstream infections higher than expected.

P1375 Tn21-like elements disseminating carbapenemase genes in Italy: report from the SENTRY antimicrobial surveillance programme

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Objective: To analyse the genetic context of metallo-β-lactamase (MBL) containing integrons in *Pseudomonas aeruginosa* strains isolated from geographically distinct regions of Italy.
Methods: MBL expressing strains were collected from three geographically distinct hospitals in Italy. These were SENTRY sites no. 75 (Genoa, northern Italy), no. 86 (Rome, central Italy) and no. 85 (Catania, Sicily). These strains were previously characterised with respect to their PFGE profile, the integrons they contained and the order of the gene cassettes within these integrons. The genetic context of these integrons was further analysed by a combination of PCR based approaches. These included (i) amplification using primers anchored to 5′ and 3′ conserved sequences (CS) together with random primers designed to amplify upstream and downstream sequences, (ii) PCR amplification using primers designed against Tn21 tnpR and tnpA sequences and (iii) primers designed to amplify the insertion site of the Tn402/Tn5090 integron.
Results: All SENTRY site no. 75 isolates contained blaVIM-1 containing integrons harboured on a Tn21-like transposon with a complete tnpR gene and a partially deleted tnpA gene. SENTRY site no. 86 isolates were of two different types. Type (A) consisted of an blaIMP-13 containing integron harboured by a complete Tn5051-type transposon and type (B) a blaVIM-1 containing integron harboured by a Tn21 like transposon with a complete tnpR gene but the tnpA gene could not be amplified. This integron was also characterised by having the insertion sequence IsPa7 inserted downstream of the integrase gene. SENTRY site no. 85 isolates also contained two different types. Type (C) contained a blaVIM-1 integron on a Tn21 type transposon with intact tnpA and tnpR genes and like type (B) had an identical IsPa7 insertion sequence in an identical place. Type (D) also contained a blaVIM-1 integron harboured on an intact Tn21-like transposon and a defective IsPa7 insertion sequence.
Conclusions: All MBL containing integrons in this study were harboured on either an intact or defective Tn21-like transposon. This may give an additional level of mobility to the MBL gene cassettes harboured on the intact transposons. Interestingly integrons from one strain isolated in Rome and two strains isolated in Catania harboured the identical insertion element IsPa7 in the exact site just downstream of the class 1 integrase gene. This suggests that this integron has spread across a large geographical area.

P1376 Clinical efficacy of cefepime in hospitalised patients with severe nosocomial infections. Results of a German surveillance study

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Objectives: Cefepime (CEP) is a fourth generation cephalosporin (group 4) with a broader spectrum of activity against Gram-negative pathogens than ceftazidime and other extended spectrum cephalosporins and a higher activity against Gram-positive cocci such as streptococcal and staphylococcal species. The broad spectrum including *Pseudomonas aeruginosa* and *Enterobacter* spp. together with low rates of resistance and favourable pharmacokinetic properties make cefepime a drug of choice for initial empiric treatment of severe nosocomial infections. Excellent efficacy and tolerability has been proven in prospective, randomised clinical trials and was also investigated under daily routine conditions in hospital.
Methods: This study was done as multicentre, retrospective surveillance study in German hospitals. Data were collected and documented for a period of 11 months.
Results: In total 639 patients have been evaluated. About 75% were ICU patients. Main indications were pneumonia ($n = 322$) and septicaemia ($n = 188$), followed by UTI infections and bile/gall-bladder infection. A total of 188 pneumonia patients were mechanically ventilated. In total 252 patients received antibiotic pretreatment before cefepime therapy was initiated. The median

duration of cefepime treatment was 8 days, most patients received 2 × 2 g cefepime daily as monotherapy. In 197 patients an additional antibiotic (combination therapy) was given. Clinical success rates were 83.2% in pneumonia patients, 79.2% in septicaemia patients, 86.9 in UTI and 93.8% in gall-bladder/bile infections. Overall the tolerability was very good.
Conclusion: This surveillance study confirms high clinical success rates for cefepime in hospitalised patients with severe nosocomial infections.

P1377 Evolution of nosocomial infections rates during 5 years in a university hospital

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Objectives: To investigate the epidemiology of nosocomial infections at Dicle University Hospital in Turkey.
Methods: The study was carried out at the Dicle University Hospital (1050 beds). A prevalence study was performed prospectively, at the hospital from January 1997 to October 2001. Nosocomial infections searched for in 89 270 in-patients.
Results: During 5-year follow-up period, 1558 nosocomial infection episodes were detected in 1441 patients out of 89 270 inpatients. The overall prevalence rate of patients with nosocomial infections were 1.7% and for each year as follows: 1997, 4.3%; 1998, 1.3%; 1999, 1.2%; and 2000, 1.4%; and 2001,1.6%. The most common nosocomial infections by primary site were urinary-tract infection (31.9%) and surgical-site infections (27.1%), followed by bacteraemia (21.1%) burn infections (6.7%), skin infections (4.6%), pneumonia (3.5%) and sepsis (2.6%). Nosocomial infections were seen frequently in the department of burn unit (23.5%), urology (7.1%), general surgery (3.7%), neurology (3.7%), orthopaedics (3.2%), cardiovascular surgery (2.9%), internal medicine (2.2%), neurosurgery (2.2%), infectious disease (1.2%). and paediatric surgery (1.1%). The most prevalent microorganisms were *Escherichia coli* (26%), *Pseudomanas* spp (15%), *Staphylococcus aureus* (12%), *Klebsiella* spp (13%), coagulase-negative staphylococci (10%) and *Enterobacter* spp (9%). The most frequent associated procedure was vascular access (86.3%), followed by indwelling urethral catheter (64.6%). and draining catheter (20.1%), The most common predisposing risk factors associated with infection were usage of histamine-2 blockers (36.3%) followed by transfusion (17.6%) and renal failure (17.1%).
Conclusions: The surveys indicate that the prevalence of nosocomial infections has been reduced over the last 4 years in Dicle University Hospital. The routine application of standard infection control practices may reduce the incidence of nosocomial infections.

P1378 Nosocomial infection in Italy: results from the first point prevalence for the INF-NOS 2002–2004 surveillance

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Objective: The aim of the present study was to assess the frequency of NI in Italian hospitals and wards, as wide national available data date back to 1983.
Methods: The survey of NI was carried out through the point prevalence studies method, repeated every 12 months for the 1st year (2002–2003) and every 6 months for the 2nd (2004) year. Participating Hospitals all round Italy were given a standardised protocol, a software designed *ad hoc*, a local training on the study procedures and, as feedback to sites, a statistical data report available on the web at http://www.6-gsk.it between one prevalence and the other. A descriptive analysis was conducted to evaluate the prevalence of NI, of risk factors and of their association.
Results: A total of 186 wards participated to the first prevalence, performed in 1 day during October–November 2002: 44.1% were medical, 38.2% surgical and 17.7% critical services belonging to 31 Italian hospitals. A sample of 3210 subjects was surveyed with a NI global prevalence of 6.8% that, stratified by type of ward was: 4.2% (95% CI: 3.2–5.5) in the surgical; 4.5% (95% CI: 3.6–5.6) in the medical; 42.5% (95% CI: 36–50) in the critical area. The microbiological diagnosis was performed in 80.8% of NI: *P. aeriginosa* was the most frequent pathogen isolated (20.6%), followed by MRSA (7.8%) and *E. faecalis* (7.8%). In the Table below the sites of NI are reported.

Site of Infection	N (v/n)
Lower Respiratory Tract	90 (36.7)
Urinary Tract	58 (23.7)
Surgical Site	35 (14.3)
Blood Stream	31 (12.7)
Gastrointestinal	7 (2.9)
Skin/Soft Tissue	7 (2.9)
Higher Respiratory Tract	7 (2.9)
Bone and Joint	2 (0.8)
Central Nervous System	2 (0.8)
Reproductive	1 (0.4)
Not Localized/Other	5 (2.0)
Total	245 (100)

Conclusions: This study represent an organised and economic effort aimed to highlight the relevance of the problem, the importance of continuous surveillance on an issue with huge implications on quality of care.

P1379 Surveillance of bloodstream infections in Czech hospitals: epidemiological background of CZ-EARSS

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Objective: Bloodstream infections (BSI) are associated with high mortality, prolonged hospital stay and extra costs. Globally growing resistance of invasive pathogens represents serious threat. Detailed knowledge of changing epidemiology of BSI at the local, national and international level is critically important for their successful control. Priorities of BSI surveillance have to be identified continuously.
Methods: Prospective study on the incidence of BSI in five hospitals of different structure and size was performed during 3-month period (April–June 2003). The internet based protocol of National Register of Nosocomial Infections was applied. Clinical cases were characterised by the date of infection, diagnosis, classification (primary, secondary, nosocomial or community-acquired), pathogens, invasive procedures and devices, risk factors, sources of secondary BSI, patient and hospital stay characteristics. BSI rates, their origin and aetiology were analysed and compared with CZ-EARSS results to obtain more detailed characteristics of their importance.
Results: Total number of 313 cases of BSI were found during study period. Incidence of BSI per 1000 admissions in particular hospitals (A, B, C, D, E) was 18.9, 9.5, 9.5, 10.5, 5.6; percentage of nosocomial cases 97.6, 60.9, 51.0, 70.5, 73.5%; percentage of catheter-related cases 41.2, 18.3, 17.6, 9.1, 10,5%; percentage of ICU-acquired cases 70.2, 37.4, 9.8, 27.8, 10.5%, while percentage of ICU beds was 38.9, 10.8, 8.8, 5.6, 8.1%. Source of secondary BSI was the most frequent in the urinary, biliary, gastrointestinal and lower respiratory tract. Frequency of pathogens (total percentage and variations in incidence per 1000 admissions) was: *S. aureus* 14.0% (0.5–2.1), *E. faecalis* 6.7% (0–2.0), *E. faecium* 1.9% (0–0.9), *S. pneumoniae* 2.6% (0–0.4), *E. coli* 19.5% (1.2–3.4), *K. pneumoniae* 13.4% (0.5–4.3), *E. cloacae* 6.1% (0–1.8), *P. aeruginosa* 3.5% (0–1.8), *Acinetobacter* spp. 3.5% (0–1.1) and *Candida* spp. 4.5% (0–1.4). Polymicrobial BSI were found with following percentage: 11.9, 7.0, 5.9, 27.3, 10.5%.

Conclusion: Important differences in the incidence, origin and aetiology of BSI were observed in the particular hospitals. Pathogens included to the EARSS project were found in high rates, although other nosocomial pathogens, frequently associated with multidrug resistance and high mortality, were also of great importance, including *Candida* spp.

P1380 Bloodstream infection: comparing the epidemiology of the late 1980s to that of the late 1990s at a community hospital in Brazil

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Objectives: Verify temporal trends in incidence, aetiology, antimicrobial (ATM) resistance, therapeutic adequacy and mortality of hospital acquired (HA-BSI) and community-acquired bloodstream infections (CA-BSI) in a Brazilian community nonuniversity hospital.
Methods: Data were collected prospectively for 15 years by the infection control team. Outcome was measured by 14 days and in-hospital mortality. Two periods were chosen for comparison: 1987–1989 (P1) and 1997–1999 (P2). Only in P2 the automated BactAlert and Vitek systems were used.
Results: BSI rates were stable at 9.6 and 9.5/1000 admissions. HA-BSI rates decreased from 5.8 to 4.1/1000 adm ($P < 0.01$) while rates for CA-BSI increased from 3.8 to 5.4/1000 adm ($P < 0.01$). Most patients were immunocompromised. Gram-negative bacilli were involved in most BSI in both periods; neither the incidence of Gram-positive cocci nor fungi augmented. Unusual bacteria, mainly *S. maltophilia*, emerged in both CA and HA-BSI, and the presence of central venous catheters in CA-BSI increased (16–33%). CA oxacillin-R *S. aureus* was detected in P2, but only in patients with links to the healthcare system. Inadequate treatment of HA-BSI was more frequent in P1. BSI had a better outcome in women, in P2 and if CA. There was no difference in BSI mortality if an appropriate antimicrobial agent (ATM) was introduced until the second day or between the third or fifth day after BSI. Initiating an appropriate ATM until the fifth day had a better outcome (12.9% mortality) than after the fifth day or not at all (30.6% mortality). The 14-day mortality was lower in the P2 (23% in P1 vs. 11% in P2), both for CA-BSI (17% vs. 6%) and for HA-BSI (28% vs. 18%). In-hospital mortality also decreased from 34% in P1 to 17% in P2, both for CA-BSI (24% vs. 9%) and HA-BSI (41% vs. 28%). Among adequately treated BSI, the outcome improved in the P2; among those inadequately treated however, the outcome was similar in both decades.
Conclusions: (i). Incidence of BSI was stable; (ii). many so-called CA-BSI were healthcare-related infections acquired outside the hospital; (iii). the decrease in HA-BSI probably reflects transference of patients under care to the outpatient and home care settings; (iv). Gram-negative bacilli remain the most frequently isolated BSI agents in this hospital; (v). *S. maltophilia* emergence probably reflects ATM usage patterns; (vi). Oxa-R *S. aureus* did not occur in 'pure' CA-BSI; 7. Treatment and mortality associated with BSI improved with time.

P1381 Nosocomial infections: two prevalence studies (2000–2003)

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Objectives: As it is well known surveillance of hospital-acquired infections (HAIs) is an important component of an effective nosocomial infection control programme. Prevalence surveillance is a rapid and inexpensive way to estimate the problem of HAIs. To study the problem of nosocomial infections in our hospital, two prevalence studies were made from our team during the years 2000–2003.
Methods: The first study included 273 patients and the second 330 (the total number of hospitalised patients at the time of the study).
Results: In the first study a nosocomial infection was found in 21 patients and in the second in seven patients. Regarding age the highest incidence of HAIs occurred in the third age group. The overall prevalence of HAIs was 7.7 and 2.1% for the two studies, respectively. In the first study, urinary-tract infections were seven (33.3%), lower respiratory-tract infections were five (23.8%), upper respiratory-tract infections were four (19.04%), surgical site infections were three (14.3%), and other were (9.5%). In the second study, urinary-tract infections were four (57.1%) and lower respiratory-tract infections were three (42.9%), The incidence of multiresistant bacteria was primarily *Enterococcus* spp and secondary, *Pseudomonas aeruginosa*, *Escherirchia coli*, *Staphylococcus* spp., *Klebsiella pneumoniae*. Unjustified prescription of prophylactic chemotherapy was found despite the suggestions of the infection control committee.
Conclusion: Repeated prevalence surveillance is a valid way to estimate the problem of HAIs.

P1382 Five prevalence surveys of hospital-acquired infections in a Greek hospital

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Objectives: As it is well known surveillance of hospital-acquired infections (HAIs) is an important component of an effective nosocomial infection control programme. Prevalence surveillance is a rapid and inexpensive way to estimate the problem of HAIs. To study the problem of nosocomial infections in our hospital, four prevalence studies were made from our team during the years 1994–2003.
Methods: The first study included 288 patients, the second 288 too, the third 265, the fourth 273 and the fifth 330 (the total number of hospitalised patients at the time of the study).
Results: In the first study a nosocomial infection was found in 20 patients, in the second in 15 patients, in the third study in 13 patients, in the fourth in 21 patients and in the fifth in seven patients. The overall prevalence of HAIs was 6.9, 5.2, 4.9, 7.7 and 2.1% for the five studies, respectively. In the first study, among HAIs, urinary-tract infections were 12 (60.0%), lower respiratory-tract infections were six (30.0%) and surgical site infections were two (10.0%). In the second study, urinary-tract infections were four (26.7%), lower respiratory-tract infections were 2 (13.3%), surgical site infections were five (33.3%) and bloodstream infections were four (26.7%). In the third study, urinary-tract infections were six (46.1%), lower respiratory-tract infections were three (23.0%), surgical site infections were three (23.0%) and bloodstream infections was one (7.7%). In the fourth study, urinary-tract infections were seven (33.33%), lower respiratory-tract infections were five (23.80%), upper respiratory-tract infections were four (19.04%), surgical site infections were three (14.28%) and other were two (9.52%). In the fifth study urinary-tract infections were four (57.10%) and lower respiratory-tract infections were three (42.90%). The incidence of multiresistant bacteria was primarily *Enterococcus* spp and secondary *Pseudomonas aeruginosa*, *Enterobacter* spp, *Klebsiella pneumoniae*, *Escherirchia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*. Regarding age the highest incidence of HAIs occurred in the third age group. Unjustified prescription of prophylactic chemotherapy was found despite the suggestions of the infection control committee.
Conclusions: Repeated prevalence surveillance is a valid way to estimate the problem of HAIs.

P1383 Multi point prevalence investigation of nosocomial infections in a Greek tertiary hospital

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Objective: To describe the epidemiology of nosocomial infections (NI) in a Greek tertiary general hospital and the distribution of isolated microorganisms.
Method: Analysis of surveillance data on 2757 inpatients from medical, surgical and intensive care units. A number of 11 1-day surveys were performed between 1995 and 2003 by the infection-control practitioners of the hospital. The criteria by the CDC (Atlanta, USA) were used for the definition of NI.
Results: A total of 122 patients with at least one NI were found. The overall prevalence was 4.43% (range 2.4–6.8%). The most frequent of all NI were urinary-tract infections (UTI) 34.6%, followed by lower respiratory tract infections (LRTI) 27.6%, surgical site infections (SSI) 16.5% and primary bloodstream infections (BSI) 8.7%. Various other infections were reported at a rate of 12.6%. The isolated microorganisms were for UTI: *E. coli* (38,5%), *Acinetobacter* spp. (12.8%), *Klebsiella* spp. (12.8%), *Enterobacter* spp. (7.7%), *S. aureus* (5,1%) and *Candida albicans* (2,6%); for LRTI: *Acinetobacter* spp. (45,2%), *Pseudomonas aeruginosa* (6,5%), *Staphylococcus epidermidis*, *S. aureus* and S. haemolyticus (6,5% each one); for SSI: *Klebsiella* spp. *S. haemolyticus* and *Enterobacter* spp. (11,1% each one), followed by *E. coli*, *Acinetobacter* spp., *S. aureus*, *P. aeruginosa* and *S. epidermidis* (5.6% each one); for BSI: *Acinetobacter* spp. and *S. simulans* (22.2% each one), followed by *Micrococcus*, *C. albicans*, *S. hominis*, *S. haemolyticus* and *S. epidermidis* (11.1% each one). No microorganism could be isolated in 7.7% of UTI, in 6.5% of LRTI and in 38.9% of SSI.
Conclusions: These data show that the rate of NI in our hospital is in relative low level. The distribution of isolated microorganisms differs by site and hospital unit. We assume that multi point prevalence studies are easy to perform and can be useful for monitoring and surveillance of NI.

P1384 Prevalance of nosocomial infections in Ilam's hospitals, Iran

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Objectives: Nosocomial infections are one of the most important health problems in this century. according to researches in different countries, the rate of nosocomial infections differs from 2.5 to 21%.
Methods: In this study, prevalence of nosocomial infections has been investigated in three hospital of Ilam during January 1999 to September 2001. For this purpose, 1786 patients who were hospitalised >72 h, were monitored. Clinical findings helped us for diagnosis of infections during hospitalisation. Blood agar, chocolate agar, EMB agar and manitol salt agar madia were used to isolation of bacteria.
Results: According to clinical findings, 216 patients (7.75%) were suffering from nosocomial infections. In Mostafa Khomini hospital, 122 cases (9.5%) of nosocomial infections were observed, so that its rate was more than two other hospitals. the highest rate of infections has been observed in burn ward 41 patients (35%) and ICU 25 patients (8.2%).The most common infections were urinary-tract infection 64 cases (29.7%) and surgery and burned wound infection 59 cases (27.3%). The causative agent of infections have been isolated by culture method just in 194 cases (89.8%) . The most common isolated orgnisms were coagulase-negative staphylococci 43 (22.2%), *E. coli* 37(19.1%), *Pseudomonas aeruginosa* 30 cases (15.5%) and *Staphylococcus aureus* 22 cases (11.3%).
Discussion: Results of this study showed that, the rate of nosocomial infections varis among different hospitals in Ilam, and this difference was significant ($P = 0.0048$). The rate of nosocomial infection also showed a significant differences between different age groups ($P = 0.044$).

P1385 Surveillance for nosocomial infections using special monitoring protocol in Austrian ICU

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Objective: Nosocomial infections (NI) in the intensive care unit (ICU) significantly influence mortality rates and also cause substantial additional costs. The objective of the present surveillance study was to evaluate the current frequency of NI, the predominant infecting organisms, the risk factors for developing NI and its impact on outcome of critically ill patients in order to better stratify for future management of critically ill patients.
Methods: Data were prospectively collected by the Austrian Center for Documentation and Quality Assurance in intensive care medicine in accordance with the protocol of the study surveillance for NI on the Austrian ICU's. All patients consecutively admitted to a combined medical-surgical ICU within teaching hospital between June 1 2003, and November 3 2003 were included in this study. All patients were monitored for NI at six body sites. The data included the presence or time course after admission; the most common cause and antibiotics administration; level of provided care (TISS-28); length of ICU stay and outcome data.
Results: Of 125 evaluated patients, 31 (24.8 %) acquired NI. It occurred after 4.2 days in patients without antimicrobials, after 3.2 days in patients receiving prophylaxis and after 7.3 days in patients receiving antimicrobials for treatment already at admission. Regarding bacterial agents the most common pathogens were Gram-positive cocci, Gram-negative no Enterobacteriaceae and fungi or parasite, which were respectively 15.2, 9.7, and 7.3% of the isolated agents. Enterobacteriaceae accounted for 5.7%.The most commonly observed NI was pneumonia. In infected patients length of stay was significantly longer than in noninfected patients (21 days vs.7 days, *t*-test, $P < 0.05$). With regard to provided level of care, infected patients showed significantly higher level of care than patients without NI (782 vs.266 TISS-28 score/patients ICU stay, $P < 0.05$) The mortality rate was comparable in the two groups.
Conclusion: The mean overall patient infection rate was 25 NI per 100 patients. The documented data confirmed clinical impression, that patients with NI have a prolonged length of stay and a increased need of care. Despite enormous progress in the intensive care, the rate of NI remains a problem. The knowledge about the ICU population have an important impact on risk estimates and may provide better strategies in the patients management.

P1386 Calculation of incidence and repartition of nosocomial infections

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Objectives: Calculation of incidence and repartition of nosocomial infections (NI).
Methods: A total of 431 neonates hospitalised 48 h or more, in 2000 and in 2002, were studied: gestational age, birth weight, artificial ventilation and central vascular catheter utilisation were noted.
Results: We have counted 61 NI and 51 infected neonates. Patients ($n = 431$); total number of hospitalisation days (6180) Sixty-one NI (14.1% of entries) for 51 infected neonates (11.8% of entries) : 9.9 NI/1000 hospitalisation days and 8.2 infected neonates/1000 hospitalisation days. Forty bacteriaemias: 67.2% of NI; 6.6 bacteriaemias/1000 hospitalisation days; 15.7 bacteriaemias/1000 device days (vascular catheter utilisation). A total of 14 pulmonary infections: 23% of NI; 2.3 IP/1000 hospitalisation days; 9.8 IP/1000 days artificial ventilation. Infection risk for a weight $\leq$1500 g: RR** = 7.45 – infection risk for gestational age $\leq$32 weeks: RR** = 3.48 – CRIB* >4: RR** = 4.80.
Conclusions: The infection risk is far higher when the birth weight is $\leq$1500 g. When gestational age is $\leq$32 weeks and when the CRIB is >4, relative risk is multiplied by 4.80. The first micro-organism responsible for bacteriaemias is *Staphylococcus epidermidis*. When a

device, such as a central vascular catheter is used, the RR of NI is multiplied by 80. In 2000, one possible death was noted. *CRIB: clinical risk index for babies; **RR: relative risk.

P1387 A computerised system of administrative data for nosocomial infection monitoring

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Objective: To assess the accuracy of a computerised system (CSy) linking data from hospital discharge forms (HDF) and microbiological reports for monitoring nosocomial infections (NI) in the Veneto Region, northern Italy.
Methods: Within the frame of the regional project for NI surveillance and control, we have set up a data base applying a linkage between all the HDF and the microbiological reports of patients (pts) discharged from regional hospitals. We selected a list of ICD-9 CM diagnosis proxy of NI [urinary tract (UTI), and wound infections (SSI), pneumonia (PNEU) and bloodstream infections (BSI)] and a list of microorganisms (isolated from urine, blood, wound swab and respiratory samples) commonly recognised as nosocomial pathogens. Then, three groups of discharged pts with events proxy of NI could be identified: one group with a diagnosis, a second with a nosocomial isolate, and a third with both diagnosis and nosocomial isolate. A sample (control group) of pts from each group was randomly selected and their medical records reviewd for NI according to the CDC definitions.The positive predictive value (PPV) of the CSy in identifying NI events, when compared with the NI detected in the control group (true NI), was calculated.
Results: Three regional hospitals (Vicenza, Verona and Treviso) have been analysed for the 2001. Discharged pts were 93 740 (pts days 81 8737). Overall, our CSy identified 5906 events proxy of NI in 4518 pts (rate 6.3%). In the control group (1800 of 4518) the CDC criteria of NI were met in 1244 cases, and the PPV of the CSy was 69.1%.The PPV for PNEU, BSI, UTI, and SSI were 77, 73, 72 and 72%, respectively. Microbiological records had the highest PPV (81%), in comparison to ICD-9 CM (42%). By applying the PPV estimates obtained from the control group to all HDF, the adjusted rate of NI proxi detected by our CSy was 3.5% (7.2/1000 patient days).
Conclusions: Our CSy based on linkage of currently available informations from administrative records is able to produce an adequate evaluation of NI epidemiology in comparison with data obtained by a retrospective review of medical records. This method could be convenient in terms of cost sparing compared with active surveillance of NIs. It can be easily applied on a large scale, allowing a continuous monitoring of NI at regional level, and a useful comparison of control programmes among different hospitals.

P1388 Cumulative microbiological laboratory data: an important tool for management of health care quality in the hospital

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Objectives: To describe the collection, analysis and use of the cumulative microbial identification and antimicrobial susceptibility data in our hospital.
Methods: A locally developed data management system is used to analyse cumulative microbiological test data. Patient demographic information, specimen information and test results are exported from the laboratory information system to the analysis software. Detection of duplicate isolates is based on patients' names and identification numbers, organism identification and susceptibility patterns. Isolates from screening specimens are excluded.
Results: Listing of identification and antibiotic susceptibility test results are generated each month. All species are presented, regardless of the number isolated. Specific subsets are tabulated such as different patient groups (e.g. inpatient, outpatient, specific wards, intensive care units), specimen types (blood, urine) and special patient groups (cystic fibrosis). Semiautomatic detection of deviations (± 2 and SD = 3) is used for surveillance of isolation and resistance frequencies. Other applications are monthly listings of all patients with bacteraemia, daily listings of patients with resistant or highly transmissible micro-organismes and an expert system for detection of patients with possible nosocomial infection. Cumulative antimicrobial susceptible data of relevant species are presented in tabular form. Separate tables are made for specific subsets if needed. graphs are made to follow accumulated data over several years. These data are used to update empiric therapy schemes.
Conclusions: Cumulative identification and antimicrobial susceptibility reports can give useful information if obtained in a consistent matter. They are not only very helpful in surveillance of nosocomial pathogens and their resistance patterns but also in the quality assesment of the laboratory and in the selection of empiric therapy. Accessible and clear presentation of cumulative microbiological data can convince healthcare professionals to comply with prudent use of antibiotics and hygienic precautions.

P1389 AMBU-KISS: quality control in ambulatory surgery

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Introduction: Surgical site infections are associated with considerable morbiditiy and additional healthcare costs arising from lengthy hospitalisation in these patients. According to the German Infection Protection Act implemented on 1 January 2001, hospitals and ambulatory surgery institutions are required to assess and document nosocomial infections. The data must be available to health organisations on demand.
Method: We report on a new Surveillance Module (AMBU-KISS) designed to assess and document surgical site infections in ambulatory surgery. The objective is to create a reference database for these institutions.
Results: Preliminary results have been obtained and for two indicator procedures show no significant differences in surgical site infection rates between ambulatory surgery institutions and the hospital setting (OP-KISS). The arithmetic mean values of surgical site infection rates in arthroscopic surgery of the knee are 0.09% in AMBU-KISS and 0.11% in OP-KISS (http://www.nrz-hygiene.de). For inguinal hernias the respective rates are 0.65 and 0.78%. A significant difference was observed for vein-stripping procedures with surgical site infection rates of 0.38% in AMBU-KISS and 0.64% in OP-KISS.
Conclusions: The results indicate that the surveillance module (AMBU-KISS) is reliable in assessing surgical site infections in ambulatory surgery.

P1390 Investigation of the antibiotic susceptibility patterns of pathogens causing nosocomial infections using the Etest

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Objectives: We investigated the antibiotic resistance patterns of nosocomial bacteria according to the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Programme. The outcome of this resistance was followed for 3 years.
Methods: During 2000–2002, the resistance patterns a total of 570 bacteria (390 Gram-negative, 180 Gram-positive) against meropenem, imipenem, ceftazidime, cefotaxime, cefepime, piperacillin/tazobactam, ciprofloxacin and tobramycin were investigated using the Etest. Extended-spectrum β-lactamase (ESBL) production was determined using ceftazidime and ceftazidime/clavulanic acid Etest strips.
Results: Meropenem was the most effective antibiotic against Gram-negative organisms (89.0%); this was followed by imipenem (87.2%) and piperacillin/tazobactam (66.4%). The most active

antibiotic against Gram-positive bacteria was imipenem (87.2%) and this was followed by, piperacillin/tazobactam (81.7%) and meropenem (77.8%). The rates of production of ESBL by *Escherichia coli*, *Klebsiella pneumoniae* and *Serratia marcescens* were 20.9, 50.0 and 46.7%, respectively. ESBL production increased each year (21.7, 22.1 and 45.5% in 2000, 2001 and 2002, respectively). All of the ESBL producing isolates were sensitive to meropenem and 98.5%, sensitive to imipenem. AmpC β-lactamase was produced by 20.9% of the *Enterobacter* spp., *Citrobacter* spp. and *S. marcescens*. All of these were sensitive to meropenem and 77.8%, to imipenem and ciprofloxacin. Multi-drug resistance (MDR) rates were 45.4% in *Acinetobacter* spp and 37.7% in *Pseudomonas aeruginosa* isolates.
Conclusion: As in the entire world, resistance to antibiotics is a serious problem in our country. Solving of this problem depends primarily on prevention of the development of resistance.

P1391 A 6-year study of risk factors, aetiology and outcome of late-onset septicaemia in premature neonates treated in a NICU

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Objectives: To analyse perinatal and postnatal risk factors, clinical symptoms, biochemical and haematological disorders and results of treatment of late-onset septicaemia (LOS) in premature neonates admitted to NICU between 1997 and 2002
Methods: A retrospective chart review of first incidence of LOS in premature infants. Diagnosis of LOS was made based on positive blood culture and inflammatory signs appearing after 72 h of life.
Material: A total of 151 (86 boys and 65 girls) premature neonates (52% of all septic prematures) treated in the NICU within 6 years. Birth asphyxia in 60%, ELBW in 25%, gestational age below or equal to 28 weeks in 30%, other perinatal risk factors in 91% of cases were noted. Eighty-two per cent of infants were mechanically ventilated and 75% received parenteral nutrition before they developed septicaemia. All of them underwent vessel catheterization.
Results: The most common bacteria isolated from blood were meticillin-resistant *Staphylococcus epidermidis* (33%) and multidrug-resistant *Klebsiella pneumoniae* (21%). Other staphylococcal strains in 14%, *Pseudomonas aeruginosa* in 15%, others Gram-negative bacteria in 11%, *Enterococcus* in 4% and *Candida* (*C. sake*, *C. albicans*) in 1.3% were found. Typical clinical symptoms comprised of pneumonia (79%), shock (59%), gastrointestinal disorders (50% including NEC in 21%), purulent meningitis (30%) and DIC syndrome (28%). Metabolic acidosis (84%), increased CRP level (89%) and hyper or hypoglycaemia (16%) were the most frequent biochemical disorders. Thrombocytopenia in 53% and stab cells over 5% in 42% were noted. Despite of intensive antibiotic therapy based on antibiograms 29 (19.2%) premature infants died, their mean birthweight was 1111.5 $\pm$ 357 g. The aetiology of septicaemia of infants who died was Gram-positive in 14 cases, Gram-negative in 13 and *Candida* in two cases. In 48% severe brain injury and 31% bronchopulmonary dysplasia were main cause of death.
Conclusion: Multidrug-resistant *Klebsiella pneumoniae* and methycillin-resistant *Staphylococcus epidermidis* are potentially important pathogens in aetiology of first incidence of late-onset septicaemia in premature infants. ELBW, birth asphyxia and severe chronic lung disease are the factors of bad prognosis in LOS in premature infants.

P1392 Antimicrobial resistance patterns of bacteria isolated from intensive care unit infections

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Objectives: Hospital-acquired infections, especially among intensive care unit (ICU) patients not only increase the rates of mortality and morbidity, they also lead to higher treatment costs with growing antimicrobial resistance. In this study performed from January 1999 through January 2001, aetiological agents of ICU infections and their resistancy patterns to various antimicrobials were evaluated.
Methods: Clinical specimens from suspected sites of infection of ICU patients were cultured onto 5% sheep blood agar, eosin-methylene-blue agar and Sabouraud's dextrose agar. The identification and antimicrobial sensitivity of bacterial pathogens were performed with Sceptor system (Becton Dickinson, USA).
Results: Of 871 specimens belonging to 612 patients, 771 pathogens were isolated and identified. Four major infection sites represented 91.83% of all reported infections; lower respiratory-tract infections were most frequent (31.52%), followed by urinary-tract infections (27.88%), bloodstream infections (23.09%) and surgical-site infections (9.34%). Most commonly identified microorganisms were as follows: *Pseudomonas aeruginosa* (20.36%), *Candida* species (15.04%) and *Staphylococcus aureus* (12.97%). Among the Gram-negative microorganisms *P. aeruginosa* were mostly resistant to third generation cephalosporins (71–98%), while *A. baumannii* were resistant in all cases to piperacillin, ceftazidime and ceftriaxone. Coagulase-positive staphylococci were mostly resistant penicillin and ampicillin (95%), whereas coagulase-negative staphylococci were 98% resistant to methicillin and in all cases resistant to ampicillin and tetracyclin.
Conclusion: To reduce the emergence and spread of antimicrobial-resistant pathogens in ICUs, monitoring and optimisation of antimicrobial use should be considered carefully.

P1393 Isolation frequency and antibiotic resistance of bacteria recovered in a respiratory ICU in the years 1997 and 2003

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Objectives: We studied and compared the isolation frequency and resistance phenotype of clinical isolates in intensive care respiratory unit of 'Sotiria' Chest Diseases Hospital of Athens for the years 1997 and 2003.
Methods: The hospitalised patients were 140 and 131, respectively, while the total of isolates consisted of 106 (65.4%) Gram(−) and 56 (34.6%) Gram(+) in 1997 and 207 (70.2%) Gram(−) and 88 (29.8%) Gram(+) in 2003.
Results: In 1997, among Gram(−) strains, Enterobacteriaceae were 27/106 (25.5%), *Ps. aeruginosa* 40/106 (37.7%) and *A. baumanii* 39/106 (36.8%). In 2003, Enterobacteriaceae were 70/207 (33.8%), *Ps. aeruginosa* 67/207 (32.4%), *A. baumanii* 59/207 (28.5%) and *S. maltophilia* 11/207 (5.3%). Regarding the prevalence of Gram(+) strains in 1997, 26/56 (46.4%) were *S. aureus*, 19/56 (33.9%) *S. epidermidis*,8/56 (14.4%) *Enterococcus* spp and 3/56 (5.4%) *Corynobacterium* spp, while in 2003, 34/88 (36.8%), 28/88 (31.8%), 11/88 (12.5%), 3/88 (3.4%), respectively, plus 12/88 (13.6%) *S. haemolyticus*. In relation to the clinical source, in 1997 and 2003, lower respiratory-tract isolates (LRT) were 105/162 (64.8%) and 213/295 (72.2%), i.v. catheter isolates 22/162 (13.5%) and 31/295 (10.2%) and blood isolates 19/162 (11.7) and 24/295 (8.1%) respectively.
Conclutions: Concerning LRT isolates, the incidence of *A. baumanii* has decreased from 34.3 to 21.1%, while Enterobacteriaceae show an increasing incidence from 15.2% to 27.2% with *Klebsiella pneumoniae* being the most frequently isolated strain (58.6%). As far as antibiotic resistance patterns, we noted a significantly increased isolation of imipenem-resistant *Kl. pneumoniae* (from 11% in 1997 to 28.5% in 2003) and *A. baumanii* (0 and 66%, respectively).

P1394 Incidence of surgical site infections (SSI) in Italian surgical settings

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Objectives: To assess the incidence of SSI in Italian surgical units during in-hospital stay and after discharge, and to identify associated risk factors.

Methods: One-month, prospective national multicenter surveillance study of patients undergoing selected interventions. Thirty-day postdischarge surveillance by telephone call and by surgical-outpatient cards was conducted. Data on 4665 surgical interventions in 48 surgical units were collected. Interventions included gastric surgery, colon surgery, cholecystectomy, hernia repair, vascular surgery, hysterectomy, mastectomy, caesarean section. SSI were defined according to criteria developed by the Centers for Disease Control and Prevention, Atlanta. A multivariate analysis of risk factors for SSI was performed.
Results: The global SSIs attack rate was 5.4% (252/4665; 95% confidence intervals: 4.7–6.1); 43/1443 (3.4%) occurred in clean surgery, 137/2629 (5.2%) in clean contaminated surgery, 51/518 (9.8%) in contaminated surgery, and 21/75 (28%) in dirty surgery. The rate of SSIs was 6.3% (195/3067) in general surgery, and 3.6 (57/1598) in obstetrics and gynaecology. SSI rate was 3.6, 7.7, 15.0, 18.2 according to Infection Risk Index (RI) 0, 1, 2, and 3, respectively. 98% of patients were actively followed-up after discharge. Of the 252 SSIs, 148 (58.7%) were identified prior to discharge, and 104 (41.3%) postdischarge. In a multivariate analysis, the variables independently associated with the risk of SSI were class of intervention, IRI, presence of surgical drainage, and sepsis. In half of the cases, an open surgical drainage was used. Finally, in one third of patients the length of surgical prophylaxis was longer than 24 h.
Conclusions: Our study showed that 40% of infections were detected after discharge; thus, an effective postdischarge follow-up programme is essential for a reliable assessment of SSIs. In our study the SSIs incidence rate is higher than that reported by other European studies, but some of these did not include postdischarge surveillance. Finally, our study showed that a better policy regarding the use of surgical drainages and antibiotic prophylaxis is needed. Work supported by Ministry of Health funding Ricerca Finalizzata IRCCS.

P1395 Incidence of nosocomial infection in Italian surgical wards

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Objectives: In Italy the last multicentre postoperative study in surgical patients date back to 1989 and show a NI rate of 10.5%, being surgical site infection (SSI) overall rate of 6.2%. Principal aim of this study was to update NI rates in surgical wards.
Methods: The study was carried out from the 1st of April to the 31st of May 2002 in 32 general surgeries belonging to wide hospitals all round Italy. All operated patients were surveyed by the surgeon during and after hospital stay up to 30 days from operation. The diagnosis of NI was defined according to CDC criteria. At discharge each patient received a questionnaire to register onset of any NI symptoms in the follow up time frame. The statistical analysis was descriptive: NI incidence rates were calculated both as number of patients with NI and number of NI every 100 operated patients; SSI rates were calculated through the NNIS method.
Results: In 2972 patients (male = 48.6%) were performed 3066 operations, 75.4% of whom with NNIS index = 0–1. NI onset in 226 patients (7.6%) for a total of 236 NI (7.9%); SSI (5.3%) and urinary (1.1%) were the most common infections. Among SSI, 98 (62%) and 23 (14.6%), were respectively at superficial and at deep incisional, 35 (23%) at organ-space site; 48.6% of patients with organ-space SSI (OS-SSI) required a new operation and 31.4% died. The average postoperative hospital stay and the average onset of SSI was respectively 9 and 9.2 days from operation: 6.6 and 13.4 days in patients with pre and postdischarge SSI. Fifty-five SSI (34.8%) were diagnosed after discharge, 74.5% of whom at superficial incision. The postdischarge questionnaire was given to 2256 (86.0%) patients, among them only 55.6% replied: 110 SSI symptoms were reported. The agreement between the diagnosis of SSI made by surgeon and the presence of SSI symptoms reported by patients was only 36%.
Conclusions: This study allowed to update incidence SSI rates in Italian general surgeries, stratify the rates through the NNIS method and confirm the risk for mortality in SSI patients, especially with OS-SSI. These NI should be targeted by special control programs in the surgeries. Although postdischarge surveillance is fundamental to estimate correctly SSI frequency, the method of postdischarge questionnaire seem misleading in our experience.

P1396 Surgical site infection after cholecystectomy (laparoscopic vs. open cholecystectomy)

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Objective: To know the rate of surgical site infection (SSI) after cholecystectomy in a university hospital and to compare laparoscopic cholecystectomy with open cholecystectomy.
Methods: Infection control team prospectively studied patients admitted to our hospital and operated on cholecystectomy from May to December 2001 and August 2002 to February 2003. Patients under surveillance were followed during their length of stay in hospital and evaluated about surgical site infection. Postdischarge surveillance was done until 30 days after operation. Infection rates were stratified according to NNIS methods and surgical site infection was defined using the criteria established by CDC.
Results: In this study, 207 patients underwent cholecystectomy (88 laparoscopic and 119 open), however, laparoscopic cholecystectomy increased from 34% in 2001 to 52% in 2002. There were 86 men and 121 women. Median age in laparoscopic and open cholecystectomy were 51.22 and 61.69 years, respectively. Laparoscopic cholecystectomy was associated with a shorter postoperative stay (3.52 days vs. 10.93 days). There were six SSIs detected, yielding an infection rate of 2.89%. The SSI rate was 4.67% in 2001 and 1% in 2002. All SSIs were in open cholecystectomy. The overall rate of SSI was lower for laparoscopic cholecystectomy (0%) than for open cholecystectomy (5.04%). The number of cholecystectomy with NNIS risk categories −1, 0, 1, 2 and 3 were 56, 78, 55, 11 and 7, respectively. Five SSIs belong to NNIS risk category 1 and one to NNIS risk category 3. According to the CDC definition, there were two superficial incisional, two deep-incisional and two organ/space infections. There were polymicrobial infection in two cases. The microorganisms isolated were similar for laparoscopic and open approach. Enterobacteriaceae (three) and *P. aeruginosa* (three) were the most commonly microorganisms isolated.
Conclusions: Although the use of a laparoscope in cholecystectomy has been incorporated into the NNIS risk-index, SSI rates following cholecystectomy should be also stratified by the type of technique to evaluate the results.

P1397 Surveillance of the infection in valvular replacement in cardiac surgery: an evaluation over 4 years

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Objectives: To determine the incidence and aetiology of surgical site infections in cardiac surgery with valvular replacement.
Methods: Wound infections after cardiac surgery with valvular replacement have been monitored at the Hospital Universitario de Canarias for 4 years (2 months in 2000, three in 2001 and six in 2002 and 2003) . A prospective study of 207 patients operated, all were followed-up for 1 year except corresponding to 2003, and stratified

according to intrinsic risk factors of infection NNIS System (Nacional Nosocomial Infection Surveillance, CDC de Atlanta). All infections are categorised using standard Centers for Disease Control definitions.

Results: During this period, five infections were documented, three organ-space infections and two superficial incisional infection. The wound infection rate [wound infection 100/ n° surgical patients (1)] were 0 in 2000, 10 in 2001, 2.3 in 2002 and 0 in 2003. The aetiology of the five infections was: *S. epidermidis*, *Corynebacterium* sp., *S. conii* and *P. aeruginosa* in the three organ-space infections (one case were polymicrobial infection), *S. aureus*, *S. epidermidis* and in two superficial incisional infections.

Table. SSI rates by risk index catogery 2000–2003, and NNIS* data (pooled mean rate).

	NNIS 0			NNIS 1			NNIS 2			NNIS 3		
	N	SSI	I-ISQ	N	SSI	I-SSI	N	SSI	I-SSI	N	SSI	I-SSI
2000	1	0	0	7	0	0	4	0	0	1	0	0
2001	8	0	0	19	3	15,7	1	0	0	2	0	0
2002	25	1	4	53	1	1,8	7	0	0	1	0	0
2003	0	0	0	69	0	0	9	0	0	0	0	0
2000-03	34	0	2–9	1–48	4	3–7	21	0	0	4	0	0
NNIS*			**			1–54			2–25			2–25

N: operative procedures
SSI: surgical site infections
I-SSI = N/SSI*100
*National Nosocomial Infections Surveillance (NNIS) (2002) System Report, data summary from January 1992 to June 2002. Issued August 2002. *Am J Infect Control*, **30**, 458–475.
**no data available.

Conclusion: Comparing our data with those of the NNIS report, our hospital rate in NNIS 2 and NNIS 3 is lower than the percentile 10, and NNIS 1 is higher than the percentile 90. Staphylococci is the most common pathogen recovered in both major and minor infections. It is important to monitor the surgical care of the patients and extensive use of antistaphylococcal antimicrobials in clinical practice.

P1398 Evaluation of postoperative nosocomial infections in a university faculty of medicine department of general surgery, Izmir

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Objectives: To evaluate the nosocomial infection rate and the risk factors associated with nosocomial infections in the Ege University Faculty of Medicine Department of General Surgery.

Methods: A total of 1125 patients who were operated in the Ege University Faculty of Medicine Department of General Surgery between September 17, 2001 and March 17, 2002 have been followed-up with the patient based active surveillance method for nosocomial infections.

Results: A total of 51 of 1125 patients operated in this period were found to have nosocomial infection. 52.9% of these patients had surgical wound infection, 23.6% pneumonia, 21.6% bacteraemia/septicaemia, 1.9% urinary-tract infection. The patients, who had nosocomial infection had a mean of 12.5 days of (range 3–101) extra hospitalisation and mortality rate was 27.5%.

Number of patients (51)	Clean-Contaminated (6)	Contaminated (45)
Open surgery	5	45
Laparoscopic surgery	1	..
Morning shift operations	6	33
Night shift operations	..	12
Emergency	2	17
Elective	4	28
Duration of surgery		
<1 hour	..	..
1–2 hour	3	4
2–3 hour	2	11
>4 hours	1	30
Pre disposing factors		
Age > 65	2	13
Obesity	2	1
Diabetes mellitus	..	5
Malignity	..	12
Additional disease[1]	..	5
Invasive application[2]		
Yes	4	4
No	2	41
Prophylactic antibiotic use		
Yes	6	51
No	..	..

[1]Chronic obstructive pulmonary disease, tuberculosis, renal failure, infective endocarditis, hepatitis B and C infection
[2]Urinary catheters, nosogastric sound, tracheostomy central venous catheters.

Discusion: The length of operation time, emergency and night shift operation, dirty- contaminated or contaminated wounds, diabetes mellitus, malignancy and additional diseases were the risk factors associated with nosocomial infection.

P1399 Evaluation of surgical site infection rates by surveillance after hip arthroplasty

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Background: Artificial joint replacement of the hip (HPRO) is one of about 20 operative procedures of the surveillance of surgical site infections (SSI) according to the United States National Nosocomial Infections Surveillance System (NNIS) and the national hospital infections surveillance system (KISS) in Germany. Periprosthetic infection is predominantly due to bacterial contamination of foreign material during the time of implantation. The period from implantation to clinically manifestation of periprosthetic infection may last for longer than the hospitalisation period of the patient (according to CDC SSI definition up to 1 year). We report on an assessment of infection rates by extended postdischarge follow-up in addition to predischarge surveillance.

Material and methods: A total of 508 orthopaedic patients were evaluated after they underwent HRPO ($n = 425$ primary; $n = 83$ revision). SSI were recorded according to KISS-criteria during their hospitalisation period. Patients without predischarge SSI were contacted by postal questionnaire 12 months following operation; nonresponders were reminded after 3–4 months. Questionnaires were analysed and in case of reported SSI further information was gained from clinicians and GPs. From these data standardised and stratified infection rates were calculated.

Results: The response rate of the postal questionnaire survey was 76% after first contact and 85.4% after reminder. A total of 16 (3,15%) SSI were noticed, 12 (75%) were recorded by predischarge surveillance, distributing among type A1($n = 4$), A2 ($n = 5$) and A3 ($n = 3$) of SSI, defined by the CDC criteria. Four cases (25%) of SSI were found by postdischarge questionnaire, all belonging to type A3 of SSI with the need of reoperation. Time

between discharge and diagnosis of these cases ranged from about 8 days up to 8 months, but all cases were noticed and reported according to KISS already. Standardised wound infection rate was with 1.25 over the pooled arithmetical mean surgical site infection rate of KISS (0.87) but below 75%-quantil (1.5).
Conclusions: Our results underline the importance of the surveillance of nosocomial infections beyond the time of discharge, but present methods seem to be reliable. The pursuit of shorter hospitalisation periods in surgical medicine may challenge the methods of surveillance systems.

P1400 Bacteraemia in patients with haematological malignancies

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Objectives: To investigate the incidence, pathogens and antibiotic resistance of isolates from bacteraemic patients of the Haematology unit over a 3-year period (2001–2003).
Methods: A total of 193 patients presented one ore more episodes of bacteraemia during a 3-year period. The identity and MIC determination of isolates were performed by the Vitek II system (bioMerieux, France), conventional methods and the Etest method (Solna, Sweden).
Results: Bacteraemia was microbiologically documented in 193/321 (60.1%) cases. Polymicrobial bacteraemia was observed in 22/193 cases (11.4%). A total of 265 strains had been isolated, among which 163/265 (61.5%) were Gram-positive bacteria, 88/265 (33.2%) Gram-negative bacteria, 10/265 (3.8%) fungi and four of 265 (1.5%) anaerobes. Of our Gram-positive isolates 23/163 (14.1%) were 'infrequent' in earlier years: *Listeria monocytogenes* six of 163 (2.3%), *Corynebacterium* spp 14/163 (8.6%), *Streptococcus viridans* two of 163 (1.3%) and *Leuconostoc* spp one of 163 (0.6%). Staphylococci coagulase negative were the most common pathogens, 69/265 (36.2%). Of them, 66/96 (68.7%) were methicillin-resistant and six of 96 (6.25%) were identified as glycopeptide (teicoplanin)-intermediate *Staphylococcus* species (GISS). *Enterococcus faecium* was more common than *Enterococcus faecalis* (25 strains vs. six strains) and 18/25 (72%) of them were resistant to glycopeptides (vanA). *Klebsiella pneumoniae* and *E. coli* strains produced ESBLs in 15/17 (88.3%) and five of 20 (25%) cases, respectively. One strain *Ps. aeruginosa* was resistant to all tested agents.
Conclusion: Gram-positive organisms were the predominant pathogens in our patients, especially the coagulase-negative staphylococci, followed by Gram-negative bacteria and fungi. Methicillin resistant Staphylococcus species (MRSS) accounted for approximately 68.7% and teicoplanin resistant Staphylococcus species (GISS) for 6.25% of coagulase negative Staphylococcal infections. Enterococcus faecium, the most common Enterococcus species, was 72% resistant to glucopeptides (vanA). Ongoing cooperation between haematologists and microbiologists is important to detect the distribution of pathogens, which can be used to design policies for empirical antibiotic therapy and infection control measures.

Infectious diseases and public health issues

P1401 Surveillance study of incidence of HIV-positive patients in a low-risk group of community-acquired pulmonary tuberculosis

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Introduction: Jaipur in India has been considered a low prevalence state for the HIV epidemic and HIV/AIDS was therefore not a thrust area in government-implemented public health initiatives. However HIV positivity is seen to be spreading into the hitherto low risk groups in the community at an alarming rate warranting immediate energetic efforts to arrest the epidemic.
Objective: To establish the incidence of HIV positivity in patients suffering from pulmonary tuberculosis hospitalised in a civil hospital in Jaipur, India and thereby highlight the alarming rate of spread of the HIV epidemic in low risk groups. Using this as baseline data upscale government policy initiatives towards public awareness campaigns, preventive measures and care and support of HIV-positive population.
Method: A total of 1000 hospitalised patients in the Civil TB hospital, through random sampling were identified as target group. They were screened over a period of 6 months for HIV using ELISA method on serum.
Results: A total of 1000 serum samples were screened. 44 samples were found positive (4.4%) and retesed with a different kit to confirm positivity.
Conclusion: The marked increase in HIV positivity (from <1% as per figures in 2001–2002) in a low risk group of population suffering from community-acquired TB infections is cause for concern. Data from this study discussed with Department of Health Government of Rajasthan, and has lead to upscaling preventive initiatives, which is discussed.

P1402 Epidemiological investigation of tuberculosis in Western Greece and the examination of the completeness of notifications

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Objectives: The objectives of this study were (i) to describe the epidemiology of TB in western Greece (W-GR) in the last 4 years by using all reported cases to the three Public Health Departments (PHD) of this region, (ii) to evaluate the completeness of notifications to PHDs and to the Hellenic Centre for Infectious Disease Control (KEEL) and (iii) to examine the sufficiency of the surveillance and monitoring system for notifiable diseases in Greece.
Methods: TB notifications for the region of W-GR for the period 2000–2003 were obtained from the three relevant PHDs and KEEL. Statistical analyses were performed using the SPSS. incidence rates were calculated on the basis of the 2001 census for the population in W-GR. For the purpose of evaluation of the completeness of TB notifications, records from two representative tertiary care hospitals – the Specialised Hospital for Pulmonary Diseases (Thorax-H) and the University Hospital of Rio Patras (Uni H) were compared with the statutory notifications to the PHDs in W-GR.
Results: During the 4-year study period a total of 161 cases of TB were reported to the PHDs in W-GR, 50 in Achaia, 55 in Aitoloakarnania and 56 in Ilia. The average annual incidence rate per 100 000 persons per year was found to be 5.4 (four for Achaia, six for Aitoloakarnania and 7.2 for Ilia), while the official data presented by KEEL indicate an incidence of 3.8 for W-GR. A clear male preponderance was found (m/f ratio 2:1). In contrast to the reported data, the hospital-documented cases were significantly higher: from the 155 recorded TB cases in the Thorax-H only 5% were reported to the PHD of Achaia, 45% to Aitoloakarnania and

67% to Ilia, whereas the notifications rates for the 46 documented cases in the Uni-H were 75, 70 and 50%, respectively.
Conclusion: This study demonstrates a significant inaccuracy and insufficiency of the obligatory TB monitoring system in Greece. In order to improve the accuracy of the notification system the reasons for underreporting and a proper and sincere co-operation with the physicians, the health centres and the hospitals are required. An effective disease control and prevention in Greece, a country with a high proportion of immigrants and refugees coming from regions with high TB incidence can be achieved only with a well-organised surveillance of tuberculosis at the local, regional and national level, in order to evaluate and plan programmes, to target resources and to develop appropriate policies.

P1403 Survey of brucellosis seroprevalence in risk groups in Erzurum, Turkey

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Objective: Brucellosis is a systemic and a serious infection that may affect many systems in the body. Most cases of human brucellosis occur in people directly exposed to infected animals, their excreta, products of abortion, infected carcasses, and unpasteurised milk and processed dairy foods from infected animals. So the disease is closely related to certain occupational groups, such as abattoir workers, farm workers, veterinarians, and meatpacking employees. Erzurum, where the present study was conducted, has been known as an endemic area for brucellosis, since the economy is mainly depended on agriculture and livestock. In this regard we aimed to investigate the seroprevalence of *Brucella* antibodies in risk groups working in this district.
Methods: Blood samples were collected from 280 persons belonging to the four different risk groups. They were consisted of 58 veterinarians, 112 butchers, 60 abattoir workers and 50 farm workers. Additionally 100 serum samples from normal population were included as controls. During sampling, a questionnaire concerning the age, gender, length of employment, educational level and protective precautions during work if available, was administered to each individual in the risk groups. Sera obtained from both risk and control groups were analysed by the standard tube agglutination test (STAT) using *B. abortus* antigen provided from Pendik Veterinarian Research Institute, Istanbul/Turkey. Sera with titres equal or more than 1l40 were accepted as positive. The data and the results of the laboratory tests were analysed by using software SPSS.
Results: Screening of 280 serum specimens of risk groups and 100 of control group by STAT gave positive result in 50 (17.9%) and four (4.0%), respectively. The differences between risk and control group was found to be significant ($P < 0.001$). The seropositivity rates were 24.1% in the veterinarians, 19.6% in the butchers, 16.7% in the abattoir workers and 8.0% in the farm workers. The differences in the seropositivity rates obtained from the occupational groups were not significant. However, seropositivity was not associated with the demographic characteristics of the individuals.
Conclusion: The results of the present study indicate that seroprevalence of brucellosis is higher in certain occupational groups concerned with the aninmal husbandry when compared with the normal population, mainly due to greater exposure to infected livestock and their excreta.

P1404 Epidemiological and economic effects of the proposed recommendation for universal varicella vaccination in Germany

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Objectives: In the past recommendations for varicella vaccination in Germany focussed on risk groups only. However, universal vaccination of children aged 1 year has recently been recommended by the German STIKO and shall become effective in 2004. The objective of this study is to analyse the potential epidemiological and economic effects of this recommendation over the next 30 years compared with the risk group strategy.
Methods: The analysis is based on the validated, dynamic model EVITA (Economic Varicella VaccInation Tool for Analysis). This model allows to analyse the number of varicella cases and their complications, hospitalisations and varicella-related death in a population. Different vaccination strategies defined by age-group and coverage rate can be analysed. For the risk group strategy, a coverage rate of 10% among adolescents aged 12–15 years has been assumed based on the number of vaccination doses sold. The development of the coverage rate in children aged 1 year was calculated from the development in those US states, which do not require a certification of immunity before entering day care centres, comparable with the situation in Germany. For the base case scenario which is based on the mean coverage rate in these US states coverage was assumed to increase from 11 to 85% over 8 years and remain constant afterwards. Pessimistic and optimistic scenarios based on the slowest and the fastest development have also been analysed. Future costs have been discounted with a discount rate of 5%.
Results: Under the risk group vaccination strategy, 721.400 varicella cases, 38.700 complications, 5.500 hospitalisation and 21 varicella-related death occur each year in Germany. Average, annual varicella cost for the society amount to 86.5 million . About 43% of the costs are paid by sickness funds. Universal varicella vaccination reduces the average, annual number of cases and related events by about 77% in the base case and about 75 and 80% in the pessimistic and optimistic scenarios. Costs for society are reduced by 44% in the base case scenario, the ratio of savings (in treatment and work loss costs) and of the vaccination costs (benefit-cost-ratio = BCR) equals 3.94 reflecting the net savings. Even for sickness funds, net savings occur with a BCR of 1.58.
Conclusions: Universal varicella vaccination of children is a very effective and cost-saving strategy to reduce the considerable burden of varicella in Germany.

P1405 Infectious diseases imported to the Czech Republic between 1998 and 2002

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Objective: In agreement with the national law on public health, the Public Health Service (PHS) of the Czech Republic and the National Institute of Public Health also monitor imported infectious diseases by means of EPIDAT. The study objectives were to assess the relevance of this monitoring to public health and to analyse the trend in imported infections.
Methods: Geographical, seasonal and age distribution of imported infections were analysed separately for foreigners and the Czech population. Case histories were taken from the records of diagnosing physicians and PHS epidemiologists.
Results: Between 1998 and 2002, 6758 imported infectious diseases were reported. They were imported from 139 countries of the world and 10 regions or continents, with the country not being specified. Imported morbidity was detected in 3735 (55.3%) foreigners, 2663 (39.4%) Czech tourist travellers and 352 (5.2%) Czech business-travellers. Among the five countries from which infections were imported most frequently are Vietnam (22.0%), Afghanistan (13.6%), India (12.9%), Sri Lanka (10.5%) and Slovakia (6.9%). Among the five countries from, which infections were imported by Czech tourist travellers are Slovakia (14.8%), Croatia (10.7%), Tunisia (8.5%), Spain (7.0%) and Italy (6.3%). Among the most frequently imported diseases are salmonellosis (17.7%), campylobacterosis (7.1%), shigellosis (4.7%), viral hepatitis A (2.3%) and malaria (1.7%). Frequent are also clinically less important parasitic diseases (ancylostomiasis 11.9%, trichuriasis 10.9%, ascariasis 10.8%, scabies 8.5%, giardiasis 7.5%). In contrast to infections imported by foreigners, morbidity imported by Czech tourist travellers showed a clear seasonal trend peaking in

August and peaks not only for children and parents but also for the third age (retired people).
Conclusion: In the light of the vast range of the infections imported and worldwide distribution of their possible source countries, the EPIDAT monitoring is of crucial relevance to protection of public health in the meaning of an early warning system. Although secondary cases of imported infections are not regularly reported so far, the imported pathogens with different biological characteristics as identified by the respective national reference laboratories must have circulated among the Czech population not sufficiently protected by specific immune mechanisms.

P1406 Harmonising the acute respiratory infection Reporting System in the Czech Republic with the European Community Networks

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Acute respiratory infections (ARI) are the most frequent human diseases with considerable health and economic impact. In 1951, the influenza morbidity monitoring programme started in the Czech Republic. The age-specific incidence of ARI and incidence of complications have been monitored weekly since 1968 and nowadays the system covers more than 5 million population (half of the Czech population). In an attempt to improve the healthcare information systems, substantial changes were made to the ARI reporting system in 2000–2002. Starting from season 2001/2002, each District Public Health Service enters the data from collaborating general practitioners and paediatricians in the central SQL database using a secured protocol https. The basic data processing is automated and uses a statistical model for early detection of unusually increased rates of the indicators monitored, based on a general linear model for left censored data. Direct standardization and weighting for the size of the monitored population are also used. In accordance with the European Commission decision on case definitions for reporting communicable diseases to the Community network one more change was made to the system. Starting from January 2004 the clinical data on incidences of influenza-like illness (ILI) within the same population and the same age groups as in ARI (0–5, 6–14, 15–24, 25–59, 60+ years) are also collected. Virological surveillance is performed by the National reference laboratory (NRL) for influenza and the NRL for noninfluenza respiratory viruses. The data on morbidity from epidemiological surveillance are integrated with those from virological surveillance. After approval and advanced assessment, the results are presented on a weekly basis. Comprehensive outputs to international centres such as the EISS or FluNet are provided by the NRL for influenza.
Conclusions: The ARI/ILI reporting system of the Czech Republic is a modern and efficient tool of surveillance based on collection of high quality data. Since using an internet-based platform, it is easily accessible and provides timely information. The project was partly supported by the Ministry of Health of the Czech Republic.

P1407 A prospective search for pertussis in Korean adults

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Objectives: Although pertussis is increasingly recognised as a cause of persistent cough in adults in Europe and North America, there are few reports, in East Asia, about the prevalence of pertussis in adults with prolonged cough. In South Korea, infection of *Bordetella pertussis* in adults who had a persistent cough was prospectively searched. Also, in community the prevalence of adult patients who were suspected to have pertussis was surveyed.
Methods: From September 2002 through May 2003, 102 adult patients visiting to a university health service centre and a general outpatient clinic at a municipal hospital (Seoul, Korea) due to a cough of 1–12 weeks' duration without underlying pulmonary disease were evaluated. The polymerase chain reaction (PCR) and culture were used for evidence of *B. pertussis* infection. The follow-up clinical data of laboratory-confirmed patients were obtained from the weekly telephone visits. In 1412 persons who participated in the routine health check-up for the faculty in a university (Seoul, Korea) at September 2002, the morbidity of cough illness and the presence of the classic symptoms of pertussis (paroxysmal cough, whoop and post-tussive vomiting) within past 6 months were surveyed by the self-administered questionnaire.
Results: Three patients (2.9%) were PCR-positive for *B. pertussis*, but there was no patient with positive culture. All PCR-positive patients had one or more of classic symptoms of pertussis and their cough was confirmed to persist for 3–7 weeks. In the community survey, we identified 34 cases (2.4%) who had a cough of >1 week's duration and one or more of classic symptoms of pertussis within past 6 months.
Conclusion: We confirmed that pertussis was a cause of persistent cough in Korean adults. Therefore, pertussis should be considered in the differential diagnosis of chronic cough illness in adults.

P1408 Seroprevalence survey of *Rickettsia typhi*, *Rickettsia conorii*, and Bar29 strain in the South of Spain

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Objective: Mediterranean spotted fever is endemic in the South of Spain. Recently murine typhus has been demonstrated as a cause of fever of intermediate duration in this area. Bar29 strain has been detected in Barcelona (north of Spain), but its presence in the south of Spain has not been determined. The objective was to evaluate the seroprevalences of *Rickettsia conorii*, *Rickettsia typhi*, and Bar29 in the population of Sevilla (south of Spain).
Methods: People living in the province of Sevilla were eligible. Those not able to answer the epidemiological survey or with a febrile disease in the previous 30 days were excluded. Sample size was established for an estimated seroprevalence of *R. typhi* of $5 \pm 2.5\%$ (confidence level = 99%) in 504 subjects. The population was prestratified according to age (0–14, 15–29, 30–44, 45–64, and >64 years), and living place [>50.000 inhabitants = urban (U); 5.000–50.000 = suburban (SU); <5.000 = rural (R)]. Epidemiological variables surveyed were gender, profession, living place, outdoors activities, travels in last 12 months, contact with animals, and insect bites in last month. IgG was measured by indirect immunofluorescence assays (*R. conorii*: Ref no. 75901, bioMérieux, France; *R. typhi*: Ref no. IF0100, Focus Technologies, USA; and Bar29: local strain, Barcelona, Spain). Past infection was considered when titres values ^{3}1:64. Group comparisons were performed using Chi square and Fisher tests, and correlations using Spearman's Rho. A $P < 0.05$ was considered significant.
Results: Seroprevalence of *R. conorii*, *R. typhi*, and Bar29 strain was 8.73, 3.77, and 3.37%, respectively. Past infection with *R. conorii* was present in 9.7% of subjects in U, 6.4% in SU, and 10.8% in R (P = ns), while *R. typhi* was present in 4.5% in U, 3.2% in SU and 0% in R (P = ns). Bar29 strain past infection was more common in R (10.8%) than in U [3.2%, $P = 0.05$; RR=3.6 (1.1-12.3)] and SU [1.9%, $P = 0.02$; RR = 6.2 (1.3-28.9)]. Older age was associated with past infecion with *R. conorii* ($P = 0.02$), *R. typhi* ($P < 0.0001$), and Bar29 ($P = 0.002$). Gender, contact with animals, travels to rural areas, or outdoors activities were not associated to past infection with any of the Rickettsia. The relative risk of past infection with *R. typhi* in those with an insect bite in the month previous to blood extraction was 3.1 (0.99–10.02).
Conclusion: These results confirm the presence and widespread of past infections by *R. typhi* and Bar29 strain in people from rural and urban areas in the south of Spain.

P1409 Dynamics of nasopharyngeal colonisation by *Haemophilus influenzae* in infants and young children

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Objective: The aim of the study was to analyse the dynamics of nasopharyngeal carriage of *Haemophilus influenzae* in asymptomatic children sampled twice a year using phenotypic and molecular methods.
Methods: A total of 77 children (43 from an orphanage OR; 34 from a crèche DCC) were examined twice a year, first in winter and again in spring. Clinical data and information about antibiotic treatment within the 1-month period before sampling, were collected by questionnaire. Nasopharyngeal swabs were processed and *H. influenzae* was identified by conventional microbiological methods. The MICs of antimicrobials were determined by the agar-dilution method. All *H. influenzae* isolates were tested for the presence of capsule gene and serological type by PCR. Pulsed-field gel electrophoresis (PFGE) of *Sma*I restriction fragments of the bacterial chromosome was used to determine the relatedness among *H. influenzae* isolates.
Results: Altogether 77 *H. influenzae* isolates were identified; 24 from DCC and 53 from OR. In a single case three different strains were obtained from the same child. Over 40% of investigated children were colonised by *H. influenzae* in winter and 60% in spring. Twenty-four children were colonised by *H. influenzae* both in winter and spring. Of them only two were colonized by the same strain during the second sampling as during the first one. In most cases, *H. influenzae* isolated at two different points of a year from the same child were unrelated but in particular groups the same strain was often isolated from several children. From all the 77 isolates of *H. influenzae*, three (3.9%) produced the capsule and all the capsular strains belonged to the serotype b (Hib). Almost all isolates of *H. influenzae* were fully susceptible to the antimicrobials tested.
Conclusions: Colonisation of nasopharynx by *H. influenzae* represents a dynamic process – bacteria are acquired, eliminated and reacquired over a period of 3–4 months. Long-term colonisation (over 4 months) involved only two of the investigated children. Small closed communities, such as OR and DCC, are places with a high percentage of nasopharyngeal carriers of *H. influenzae* and create a high risk of the clonal spread of potential pathogens.

P1410 Cell-detaching *Escherichia coli* activity in urinary-tract infection isolates

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As many as 90% of all community-acquired urinary-tract infections (UTIs) are caused by *E. coli*, this bacteria is one of the most common bacterial infections in humans. Symptomatic UTI is manifest in two syndromes: One is acute pyelonephritis generally perceived as being a kidney infection, the second is cystitis, which is generally perceived to be a bladder infection. A recently defined category of potentially diarrheagenic *E. coli* is cell-detaching strains, which were originally defined by their capacity to detach tissue culture cells from solid supports in adherence assays. However, epidemiological studies have found that these strains are not really associated with diarrhoea, and their significance remains unknown. In this report, we describe the identification of urinary *E. coli* strains (isolated from patients with UTIs) capable of detaching HEp-2 cells monolayers and characterise these strains in detail. Serotyping revealed that most of the strains belonged to serotypes associated with extraintestinal disease, O6 was the most prevalent serogroup. Fifteen of 40 *E. coli* isolates from UTIs had the ability to detach HEp-2 cells in the adherence assay. The culture supernatant of these cell-detaching bacteria (concentrated 10-folds) caused the same detaching effect and contain several secreted proteins. Two of them were identified as Sat (Secreted autotransporter toxin), and haemolysin. Some other protein bands appears on the gel after SDS-PAGE electrophoresis of the supernatant, the most prominent and consistent of these secreted proteins had apparent molecular masses of 176, 120–122, 110, 36–38 and 25 kDa. Western blot analysis shows that most of these proteins were recognised by secretory immunoglobulin A (sIgA) antibodies isolated from milk from Mexican women. The strong sIgA response to these secreted proteins obtained in this study indicates that such antigens stimulate intestinal or urinary-tract responses and may elicit protective immunity against uropathogenic disease.

P1411 Long-term surveillance of invasive group A streptococcal disease in the Netherlands, 1994–2003

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Objectives: After more than half a century of decreasing morbidity and mortality a resurgence and persistence of serious group A streptococcal (GAS) infections has been noted since the mid-1980s. There are however limited population based and prospective epidemiological studies.
Methods: A prospective, nation-wide, laboratory-based surveillance of invasive GAS infections was formally organised with all regional public health laboratories (national coverage: 45%) in the Netherlands from May 1994.
Results: Between May 1994 through May 2003,1431 patients with invasive GAS disease were identified. Isolates were predominantly obtained from blood (70%). There was a clear seasonal pattern with the preponderance of cases occurring in late winter and spring. Incidence varied between 4.0/100.000 person years (1995/1996) and 2.0 per 100.000 person years in 1999. The most frequently identified M-types were: M1 (23%), M3 (12%), M89 (11%), M28 (10%), M12 (7%) and M6 (4%). The annual relative contribution of M1 and M3 types showed a high degree of variation, which was not explained by local outbreaks. Over this decade of surveillance, the relative proportion of the >65 years age-group increased gradually from 39% to over 50%.
Conclusion: Population-based surveillance provides data for monitoring incidence trends and identifying the importance of virulent strains.

P1412 Early and late onset group B *Streptococcus* (GBS) disease at a central hospital, Harare, Zimbabwe

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Objective: The main focus of this study was to determine the prevalence, common presenting symptoms, risk factors and mortality rate associated with early and late onset neonatal GBS disease.
Methods: This was a prospective study comprising of 115 neonates suspected to have early and or late-GBS infection, presenting with sepsis and or meningitis during the period 1st February and 30th June 2003. On 69 of the neonates, a lumbar puncture to obtain cerebro spinal fluid (CSF) was performed for microbial analysis for pathogens. On 46 of the cases, a blood culture was performed for isolation and identification of pathogens. For both CSF and blood culture standard bacterial methods were used to isolate and identify pathogens. A questionnaire was also used to collect data concerning details of birth records, presenting symptoms and treatment history.
Results: Of the 115 samples 69 (60%) were lumbar punctures while 46 (40%) were blood cultures. Neonates (57%) were male and 43% were female. Apart from GBS, especially capsular type III, there were other causes of neonatal disease of which the predominant cause was CNS and the others included, *Klebsiella* spp, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, nonlactose fermenting coliforms (NLFC) and lactose-fermenting coliforms (LFC). Most babies were 0–7 days old (early onset),

however GBS disease was more frequent in babies 8–90 days of age (late onset disease).
Conclusion: Late onset disease is more common than early onset disease. GBS capsular type III is a cause of late onset neonatal disease, however there are other organisms also causing early and late onset neonatal disease at Harare Hospital. There are no clear guidelines dealing with neonatal GBS disease at Harare Hospital.

P1413 Emergence of MRSA strains containing the Panton-Valentine Leucocidin gene in the Netherlands

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Objectives: Recently, MRSA strains possessing the Panton-Valentine Leucocidin (PVL) gene were detected in the Netherlands. The PVL gene encodes a highly potent toxin associated with severe skin infections and lethal necrotizing pneumonia. PVL-positive MRSA strains have also been noticed in other European countries, in the USA and Oceania. Our objective was to study the presence of MRSA harbouring the PVL gene in the Netherlands, and to compare this with other studies on PVL-MRSA conducted in Europe and the USA.
Methods: Molecular subtyping (PFGE, MLST and SCCmec typing) was performed on PVL-MRSA isolates obtained from the national MRSA surveillance programme in the Netherlands, from 2000 till 2003. When available, epidemiological information was used to compare the Dutch PVL-MRSA isolates with those found abroad.
Results: In the Netherlands, approximately 10% of all MRSA isolates send to the RIVM (national surveillance programme) in the period 2000–2003 possess the PVL gene. Molecular subtyping (PFGE) on the Dutch isolates showed a predominant clone: cluster 28. The PFGE pattern of cluster 28 is identical to the pattern of predominant PVL-MRSA clones from several other European countries. By using multi-locus sequence typing (MLST), all cluster 28 isolates were sequence type (ST) 80. Thus far, MRSA with ST80 has not been observed in the USA, indicating that this PVL-MRSA genotype might be an European clone. PVL-MRSA ST80 has so far shown to be 100% resistant to fusidic acid, neomycin and tetracycline, used for first line topical treatment of staphylococcal infections. Since 2000, 24 patients were observed in the Netherlands with PFGE cluster 218. The PFGE pattern of this cluster is identical to the PFGE pattern of a predominant USA PVL-MRSA strain (USA300; ST8), suggesting transmission from the American continent. The majority of the Dutch PVL-MRSA isolates harbour a type IV SCCmec region, and therefore might be community-acquired.
Conclusions: A significant proportion of MRSA strains detected in the Netherlands contain the PVL gene. Relationships with other countries or continents seem obvious. The combination of the PVL gene (virulence) and mec cassette (resistance) and proven epidemicity (e.g. ST80) makes this a well-adapted pathogen, which can have severe implications, especially when more resistance markers are acquired.

P1414 Prevalence of group B streptococcal colonisation in Greek pregnant women

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Group B streptococcus (GBS) is the leading cause of severe infections in neonates with high mortality rate. Neonates born to mothers colonised at delivery with GBS are at risk of developing the infection.
Objective: Aim of the study was to determine the prevalence of GBS carriage among pregnant Greek women.
Patients and methods: A study of colonisation by GBS was conducted in 332 consecutive pregnant women at 35–37 weeks of gestation. Swabs were placed into 5 mL of selective broth with antibiotics and after 18 h of incubation were subcultured onto colistin/nalidixic acid (CNA) agar. Identification was made by conventional microbiological methods and positive agglutination test for GBS. The isolates underwent antibiotic susceptibility testing by the disc-diffusion method following the recommendations of the NCCLS.
Results: A total of 332 women aged 16–40 years were recruited. Thirty-six (10.8%) were colonised by GBS. All isolates were penicillin-susceptible while resistance to erythromycin and clindamycin was detected in 12 and 6.6%, of them respectively.
Conclusions: Antepartum screening for GBS colonisation and maternal-intrapartum chemoprophylaxis with antibiotics in the positive cases appear to be the most effective approach, in order to prevent the transmission of GBS to the newborn and to reduce the risk of early-onset disease.

P1415 Seroprevalence of rubella, cytomegalovirus and *Toxoplasma gondii* among women of reproductive age in the region of Thrace, Greece

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Objectives: The purpose of this study was to evaluate serological analysis of immunity to Rubella, cytomegalovirus (CMV) and *T. gondii* in a population of pregnant and nonpregnant women of reproductive age in the region of Thrace, Greece within 1-year period.
Methods: From February 2002 to February 2003, sera from 318 women were tested for detection of specific Rubella, CMV and *T. gondii* antibodies. There were three groups of women, group A: 182 (57%). Greek orthodox women, group B: 108 (34%) Greek Moslem women and group C: 28 (9%) Immigrants of Greek origin from Former Soviet Union. All tests to detect the levels of IgG and IgM antibodies were performed by MEIA msethology (AXSYM –Abbott).
Results: In group A 140 (77%) women were found to be positive for Rubella IgG, 130 (71%) for CMV IgG and 28 (15%) for *T. gondii* IgG. In group B 52 (48%) for Rubella IgG, 78 (72%) for CMV IgG and 34 (31%) for *T. gondii* IgG. In group C 20 (71%) women were found to be positive for Rubella IgG, 24 (86%) for CMV IgG and eight (29%) for *T. gondii* IgG. It was also measured the seropositive rate for IgM antibodies (marker of acute infection) for Rubella, CMV and *T. gondii*. The seropositive rate for Rubella IgM was for group A 0.5%, for group B 0% and for group C 0%. CMV IgM was for group A 2.2%, group B 4.6% and group C 25%. *T. gondii* was for group A 2.7%, for group B 2.7% and for group C 53% .
Conclusions: (i) This study showed that there is a significant prevalence of Rubella IgG in group A and C. A very low rate was found in group B, despite the fact that there is a routine Rubella vaccination programme. A total of 106 women (33%) were not immune to Rubella. (ii) A total of 248 women (78%) were at higher risk of acquiring *T. gondii* infection in pregnancy. This is indeed a large number of unprotected women. (iii) A total of 232 women (78%) were found to be positive for CMV IgG and 86 women (22%) were negative. (iv) The overall seropositive rate for Rubella was 0.3%, for CMV 2.5% and for *T. gondii* 1.6%. Further studies with larger population size may be needed in order to determine the seroprevalence of viruses and *T. gondii* in immigrants. It is important that all women should be checked prior to planned pregnancy, especially those from certain minorities that might have difficulties attending the existing vaccination programmes.

P1416 Bacterial evaluation of supermarket bought chickens in the Mafikeng area (RSA)

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Background: In developing countries, chickens are marketed live but in more industrially developed countries (South Africa),

chickens are dressed in a commercial processing plant before being marketed. The study was aimed at evaluating the level of bacteria contamination of chicken carcasses from the supermarkets.
Methods: A total of 48 refrigerated samples were collected from the some supermarkets (24 in winter and 24 in summer). The bacterial analysis was achieved both qualitatively and quantitatively. Qualitatively, a 10-fold serial dilution was performed from nutrient broth on which 10 g of chicken skin had been placed using saline. Dilutions were plated using plate count agar. Plates were incubated at 37°C for 24 h and later determined for the level of contamination in terms of the number of colony forming units (CFU)/mL of the sample. Quantitatively, the interest was on isolating *Salmonella* spp. and *Staphylococuss aureus*. Subcultures were performed from selenite broth and nutrient broth to XLD agar and mannitol salt agar (MSA), respectively. Plates were incubated at 37°C overnight. Gram-staining was done [Cruickshank (1975)]. Presumptive Gram-negative *Salmonella* isolates were further confirmed by the oxidase and the API 20E tests and Gram-positive *Staphylococuss aureus* isolates were further tested by the catalase test, DNase test and confirmed by the coagulase test. Both isolates were tested for resistance to various antibioticsm (Bauer 1966).
Results: Qualitatively, during winter, 4.2, 87.5 and 8.3% of the samples had bacterial loads that were insignificant, acceptable and were approaching or at a state of spoilage, respectively. Contrarily, the summer collection revealed 12.5, 70.8, 16.6% of the samples had bacterial loads that were insignificant, acceptable and were approaching or at spoilage, respectively. The level of spoilage was higher in summer (16.6%) as opposed to winter (8.3%). Quantitatively, in 48 and 76% of *Salmonella arizonae* were isolated in winter and summer respectively while 35 and 87% of *Staphylococuss aureus* were isolated from winter and summer respectively. Multiple antibiotic resistances (MAR) were observed for all *Salmonella* and *Staphylococcus* isolates.
Conclusion: With the preceding as background it was concluded that chickens are not free from bacteria contamination, rendering them unhealthy for human consumption if proper handling and preservation measures are not implemented.

P1417 Prognostic factors in adult tetanus

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Objectives: The objective of this study was to determine prognostic factors related to death from adult tetanus.
Methods: A total of 53 tetanus patients, 25 female and 28 male admitted to Infectious Diseases Department of Cukurova University Hospital between January 1994 and July 2000 were investigated and were followed for 60 days if they survived. As for the analytical techniques, the Chi-squared tests or when needed Fisher's exact test were used for the association between mortality and such categorical variables as gender, incubation period, tetanus type, etc. In evaluating the prognostic value of the scoring scheme, a trend test of Cochran and Armitage was employed. In addition to the fatality rate among tetanus patients was examined by multivariate logistic regression models, which include variables of incubation period and symptoms simultaneously.
Results: The mean age was 46.6 years. 41 patients (77.7%) came from rural areas. Most of the cases had minor trauma (64.1%), but 19 (35.8%) had deep injuries. The mean incubation period was 11.5 days. Mortality was high (52.8%), due to cardiac or respiratory failure or complications. Mortality was related to the length of the incubation period. In cases with an incubation period of 7 days or less the mortality rate was 75% ($P = 0.07$). Mortality was significantly associated with generalized tetanus ($P < 0.05$), fever 40°C, tachycardia >120 beats/min ($P < 0.05$), postoperative tetanus ($P = 0.03$) and the absence of post-traumatic tetanus vaccination ($P = 0.068$). Patients who were given tetanus human immune globulin or tetanus antiserum ($P > 0.05$) showed similar outcomes. Patients who were given penicillin had a similar mortality rate to patients who were given metronidazole ($P = 0.15$).
Conclusion: The fatality rate was higher in patients with severe tetanus (92%) than in patients with moderate diseases (53%). The binary logistic model revealed that the existence of tachycardia and being >60 years of age are simultaneously the most significant prognostic risk factors in relation to mortality for tetanus patients.

P1418 Quality of life after tick-borne encephalitis

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Objectives: South Bohemia is a large-size natural focus of tick-borne encephalitis (hereinafter TBE) in the Czech Republic. In the Infectious Department in Ceske Budejovice Hospital, TBE virus is the most frequently proven pathogen of neuroinfection. In recent years we have hospitalised annualy from 90 to 170 patients with clinically, by CSF, and serologically proven TBE. Convalescence period in these patients is very long and unpleasant. The objective of this study is to measure quality of life during convalescence period.
Methods: In 2003, we have diagnosed TBE in total 93 patients, 16 of them were children. The 74 adults were offered to fill an SF-36 questionnaire (1) in mean 3.4 months (range 0.5–5 months) after discharge. 50 of them have replied, one of them sent not fully completed questionnaire. Total 49 patients (31 males, 18 females) aged 48.7 ± 14.7 years (18–72) were included in our analysis. Patients with encephalitic course of disease responded more often compared with patients with meningitis: 31 (63%) responders have had encephalitic course of TBE. The responders were stratified by sex and encephalitic vs. meningitic course of disease. The results were compared with the control of general-population-based Oxford sample.
Results: TBE patients manifested worse results ($P < 0.05$) in the next segments of the SF-36 test (two-sided testing): physical function, role limitation-physical, role limitation-emotional, social functioning, pain, mental health, and general health perception. Vitality was not significantly affected. Women compared with men had significantly worse results ($P < 0.05$) in physical function, role limitation-physical, role limitation-emotional, social functioning, pain, mental health, and vitality. Differences in general health perception were not significant. Postencephalitic patients compared with those after meningitis had not significantly worse results.
Conclusion: Tick-borne encephalitis affects the patient's quality of life for months. The quality of life of women compared with men is significantly more affected. 1. Medical Outcomes Trust (1996) Boston MA, USA, Health Services Research Unit, 1996 Oxford, Great Britain. Czech version: Zdravotne socialni fakulta, Jihoceska Univerzita v C.Budejovicich.

P1419 The effect of short-time microwave exposures on *Escherichia coli* O157:H7 inoculated into hamburgers

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The bactericidal effect of short time exposures on *Escherichia coli* O157:H7 inoculated into beef hamburgers were studied *in vitro*. Hamburgers made from minced bovine meat, weighing 40 g each were contaminated with *E. coli* O157:H7 inoculums of concentration 105–106 CFU/g and subsequently cooked in a domestic microwave oven (SHARP R-7280, 2450 MHz, 650W). The hamburgers were cooked for 10, 20, 25, 30, 35, 40 and 50 s. Viable counts on selective media were performed following standard microbiological procedures. Time, temperature and reciprocal viable counts were recorded and the survival rate was assessed. After 30 s of exposure to microwave radiation the hamburgers looked well cooked; however, *E. coli* O157:H7 viable cells were recovered at concentration level 0.58×10^6 CFU/g. After 35 s of

microwave cooking and at a mean final temperature 68.1°C, the mean final concentration of *E. coli* O157:H7 viable cells was reduced to 500 CFU/g. Complete destruction of *E. coli* O157:H7 cells was accomplished after 50 s of exposure to microwave radiation, when the mean final temperature was 75.6°C. The results indicate that short time exposures to microwave heating are insufficient for complete decontamination of beef burgers potentially contaminated with *E. coli* O157:H7 and the safety of food cooked very fast are questioned.

P1420 A retrospective evaluation of *Listeria* infections in Bari, south Italy, from March 2002 to April 2003

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Objective: *Listeria monocytogenes* is an intracellular ubiquitous organism that affected patients with decreased cell-mediated immunity such as the elderly, transplant recipients, cancer patients, dialysis patients, patients with diabetes mellitus and those with HIV infection. Women are at particular risk during pregnancy because cell-mediated immunity is slightly decreased during pregnancy. We described 10 cases of listeriosis that occurred in elderly and adults in Bari, south of Italy.
Methods: Strains of *L. monocytogenes* were isolated according to standard protocols from various clinical samples. Data regarding serotyping, antibiotic resistance and clinical information were analysed by software Epi-Info 6.4.
Results: From March 2002 to April 2003, 10 cases of human listeriosis were reported to the Regional Reference Centre for pathogenic enterobacteria. Seven strains of *L. monocytogenes* were isolated from blood culture and three from cerebrospinal fluid culture. Feto–maternal infections (two cases of still-birth and one case of spontaneous abortion) and septicaemia in adults (two cases) and elderly (one case) were most frequent followed by central nervous system infections in adults (four cases). Seventy per cent of the patients presented a current conditions which favoured the triggering of the disease. The predominant serovars were 4ab and 1/2 b both frequent (40%), followed by serovar 1/2 a (20%). The prevalence of antibiotic resistance was lower.
Conclusions: The number of cases of listeriosis in Italy grew up dramatically in the period of observation. In the same period also in France was observed an epidemic cluster. No more cases were notified to the Regional Reference Centre for pathogenic enterobacteria from April 2004 after active surveillance of invasive listeriosis has been carried out. This may do a combination of increased regulatory activity, implementation of HACCP programmes and specific reccomendations to immunocompromised host and pregnant women on how to avoid foodborne listeriosis.

P1421 Microbiologic survey of long-term-care facilities in Greece

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Objectives: A microbiologic survey of LTCF was performed aiming to determine the prevalence of pathogens colonising the elderly residents.
Methods: A total of 21 LTCF were randomly selected from the public sanitation list of Attic province. Urine (U), nasopharyngeal (N) and wound (W) samples were collected from 662 residents; from each LTCF, we chose randomly 30% of the existing elderly population (minimum = 25 residents). Cultures and susceptibility testing were carried out and information was collected on facility and resident demographic data.
Results: The residents had a mean age of 86.6 years. The prevalence of residents with indwelling bladder catheters ranged from 1.8 to 32%, with a mean of 17.1%. A total of 358 patients (54.1%) had been receiving a systemic antibiotic during the preceding month; leading prescribed antibiotic classes were quinolones (39%) and cephalosporins (21%). The mean prevalence rates of recent (previous 120 days) hospitalisation, poor functional status and usage of feeding tube were 2.6, 42.2, and 9.5%, respectively. A total of 1369 specimens (662 U, 662 N, 45 W) were collected and 495 bacteria were isolated. The prevalence rate was 36.16%; the leading isolates were *Escherichia coli* (34.5% of all found), *Staphylococcus aureus* (22%-ÌRSA 3.2%), *Proteus mirabilis* (14%), *Klebsiella pneumoniae* (12.5%), *Providencia stuartii* (4.25%), *Morganella morganii* (4.25%), *Pseudomonas aeruginosa* (1.75%) and *Enterococci* species (1%). Of all bacteria found, Gram-negative isolates (387) accounted for 78.2% and Gram-positive (108) for 21.8%. Among U isolates (302/495, 61%), Enterobacteriaceae (EB) (83.5%) predominated. In N pathogens (142/495, 28.7%) and W isolates (51/495, 10.3%), the most frequent were *S. aureus* (19.5% and 4.9%, respectively) and *Proteus mirabilis* (6.6 and 2.9%, respectively).
Conclusions: (i) Gram-negative bacteria predominate in the bacterial flora of Greek LTCF. (ii) The high rate of NS colonisation with GNB and *S. aureus* is important and must be considered, especially in case of respiratory infections. (iii). Antibiotic usage is extremely high; further emphasis must be laid on antimicrobial use management in Greek LTCF.

P1422 Pseudo-contamination of blood components with *Burkholderia cepacia* during quality controls

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Objective: To investigate the microbiological detection of *Burkholderia cepacia* in blood components during quality controls.
Methods: Any blood components contaminated with *B. cepacia* found during microbiological quality controls at Freiburg University Hospital blood bank in July 2003 were recorded. The procedures employed in producing blood components were analysed and the individual steps in quality control were investigated. One of these entails inoculating the blood culture bottles with samples from the blood components. All the disinfectants were investigated, including those used for decontamination of the rubber stoppers belonging to the blood culture bottles.
Results: *Burkholderia cepacia* was found in three samples (thrombocyte and erythrocyte concentrate and apheresis plasma) investigated during microbiological quality control. No septic reactions associated with transfusions had been reported in patients over the last 6 months. A second microbiological control of the affected blood components was negative. No mistakes could be found regarding the processing and storage of blood components. Analysis of quality control procedures revealed that a disinfectant based on a quaternary ammonium compound (QAC) had been used to disinfect the rubber stopper of the blood culture bottle. The disinfectant was prepared with concentrate and tap water according to the manufacturer's instructions. *B. cepacia* was found in a sample taken from this disinfectant. The four isolates (disinfectant and blood components) were found to be identical in their biochemical reactions and resistance patterns. Genotyping was not done.
Conclusion: In Germany, leucocyte-depletion of blood components to reduce the risk of CJK transmission has been required by law since October 1st, 2001. However, this increases the risk of bacterial contamination of blood products. Our case proved to be a pseudo-contamination due to the use of a contaminated disinfectant during quality controls. This case report demonstrates that to ensure the qualitative validity of the results obtained, adherence to high standards of hygiene during quality control procedures is important. An alcohol-based disinfectant should be used to disinfect the rubber stopper of a blood culture bottle. QAC-based disinfectants are not efficacious against part of the spectrum of Gram-negatives and are therefore inadequate. After introduction of an alcohol-based preparation no more cases of *B. cepacia* contamination were found.

P1423 The proteolitic and lipolitic activity of *Pseudomonas* strains isolated from raw milk

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Our research is aimed to determine the *Pseudomonas* types which are isolated from the raw milk that indicating proteolytic and lipolytic activities accordingly. For such a purpose, the naming works of the bacteria, which are isolated from the 80 pieces of raw milk collected from 80 places and afterwards, by using calcium cazeinat agar (CCA) feeding place the proteolytic and Tribütyrin Agar (TBA) feeding place, the lipolytic activities have been worked. Of the 104 isolation made from 80 isolated samples are named as 19 (18.2%) *Aeromonas hydrophila*, 73 (70.2%) *Pseudomonas*, six (5.75%) *Yersinia*, one (0.69%) *Alcaligenes*, two (1.92%) *Proteus*, two (1.92%) *Providencia*, one (0.96%) *Serratia*. species name and per cents of *Pseudomonas* were indicated *Pseudomonas aeruginosa* 38 (36.5%), *Pseudomonas aurefaciens* three (2.8%), *Pseudomonas cepacia* four (3.8%), *Pseudomonas mallei* 1 (0.96%), *Pseudomonas pseudoalcaligenes* five (4.8%), *Pseudomonas pseudomallei* one (0.96%), *Pseudomonas fluorescens* biovar I, II, III, IV, V 8 (7.6%), *Pseudomonas stutzeri* one (0.96%), *Pseudomonas picketii* two (1.92%) vs. *Pseudomonas viridiflava* one (0.96%). The number of species which indicate proteolytic and lipolytic activitiy is 38 from the *Pseudomonas aeroginosa*', the number of species proteolytic and lipolytic activitiy is five from the *Pseudomonas alcaligenes*, the number of species proteolytic and lipolytic activitiy is three from the *Pseudomonas aurefaciens*, the number of species proteolytic activitiy is two, the number of species lipolytic activitiy is three from the *Pseudomonas cepacia*, the number of species proteolytic and lipolytic activitiy is one from the *Pseudomonas mallei*, the number of species proteolytic and lipolytic activitiy is five from the *Pseudomonas pseudoalcaligenes*, the number of species proteolytic activitiy is one from the *Pseudomonas putida*, the number of species proteolytic and lipolytic activitiy is three from the *Pseudomonas mendocina*, the number of species proteolytic and lipolytic activitiy is one from the *Pseudomonas pseudomallei*, the number of species proteolytic activitiy is seven, the number of species lipolytic activitiy is eight from the *Pseudomonas fluorescens*' in (biovar I, II, III, IV, V), the number of species lipolytic activitiy one is from the *Pseudomonas stutzeri*, the number of species proteolytic activitiy is one, the number of species lipolytic activitiy is two from the *Pseudomonas picketii*, the number of species proteolytic and lipolytic activitiy is one from the *Pseudomonas viridiflava*.

P1424 Endocarditis due to *Enterococcus faecalis*: risk factors and outcome in 21 cases from a 5-year national survey

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Objectives: Investigate the risk factors, therapy and outcome of native valve endocarditis due to *Enterococcus faecalis* (*E. faecalis*) in Slovak Republic.
Methods: Between 1992 and 1996 all cases of native valve-infective endocarditis of *E. faecalis* origin were prospectively evaluated using a protocol, which was submitted to all medical departments in Slovakia. No cases of prosthetic endocarditis were included. Major and minor criteria of the Duke endocarditis service were recorded for definition of endocarditis as definitive or possible. In a univariate analysis risk factors and outcome were compared for the 21 cases of enterococcal endocarditis and all 180 cases of endocarditis from the national survey.
Results: Among 180 cases 21 (11.7%) were caused by *E. faecalis*, which was the third most common pathogen after *Staphylococcus aureus* (33.3%). The most commonly observed risk factors were rheumatic fever (eight cases), gastrointestinal neoplasia (GIN) (four), surgery (five) and diabetes (three cases). Dialysis, i.v. drug use was recorded in one case. The mitral valve was infected in 10, the aortic valve in 10 and the pulmonal valve in one patient. two cases were probable and 19 were definitive endocarditis, 15 cases has various predisposing heart conditions, all presented fever, 10 emboli, 10 immunologic phenomena, 19 major and two minor echocardiographical findings. Only 10 patients were treated appropriately. Mortality attributable to *E. faecalis* endocarditis was 19%. All 55 isolates of *E. faecalis* from 21 cases were susceptible to vancomycin and 50 of them were also susceptible to ampicillin and gentamicin. Nine patients underwent valve replacement surgery (one died). From four deaths due to infection (heart failure, CNS emboli) three were treated inappropriately. GIN ($P < 0.04$) and age more than 60 years ($P < 0.05$) were related to enterococcal endocarditis.
Conclusion: (i) Only two risk factors GIN and age more than 60 years were related to *E. faecalis* endocarditis. (ii) Mortality attributable to *E. faecalis* endocarditis was 19%, which is lower than in some other studies. (iii). Good susceptibility to ampicillin and gentamicin is also different from other reports. (iv) High susceptibility to antienterococcal antibiotic and early surgical therapy (47.5% pts) were possible explanation for good outcome of our cases of *E. faecalis* endocarditis.

P1425 Knowledge, attitudes and practices of sexually transmitted infections in immigrant female sex workers

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Objective: To describe the KAPs on STIs in immigrant female sex workers in northern Italy.
Methods: Prospective study using a questionnaire administered by a trained health operator to FSWs working on the street. The questionnaire had seven components: demographical and social, knowledge on STIs and HIV, gynaecologic and obstetric history, sexual history, history of prostitution, sexual behaviours, access to public health services.
Results: From August to October 2003 50 FSWs answered to the questionnaire. Thirty (60%) were from Africa, 45 (90%) were illegal immigrants, 37 (74%) were <23 years old, and 76% were in Europe since 1 year or less. Thirty-three (66%) declared to have >4 clients/night and 40 (80%) reported rape or rubbery. While 43 (96%) were aware of HIV infection, almost all were unaware of *Chlamydia* and *Trichomonas* infections. Thirty-one (62%) had never done an HIV test; a pap-test had never been done by 47 (94%). The proportion of sexual acts with clients, which were protected with condom was 100% for vaginal, 82% for oral and 80% for anal intercourse. Clients reportedly requested sexual intercourse without condom to 84% of the women. Lubricants were used by 52% of the women, of whom 34% used inappropriate substances. Over the previous month 33 women (66%) reported at least one condom rupture (broken, slipped off, etc.), 20% >5 times. After condom rupture 50% performed vaginal douching, 20% urinated; none adopted measures to prevent pregnancy or STIs. Thirty females (60%) had a steady boyfriend with whom 50% did not use condom. Twenty-eight (56%) reported at least one abortion; 11 (22%) more than one. Almost 50% had never attend any public health service while in prostitution; 78% would attend STI services specifically devoted to immigrant FSWs.
Conclusions: This survey suggests that immigrant FSWs have limited access to preventive and curative health services, rarely do the HIV test, and are exposed to a significant infective risk due to condom misuse and rupture. Targeted interventions should aim at changing high-risk behaviours.

P1426 *Bacillus cereus* in orthopaedic and traumatology wards. An unrecognised pathogen? A 63-case study

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Objectives: To describe epidemiological circumstances and clinical signs associated with *B. cereus* isolation, to state its pathogenic role

in post-traumatic infections and to propose guidelines for treatment and prevention.
Methods: We conducted a retrospective 6-year study (1997–2002) based on all *B. cereus* isolations from patients in the orthopaedic traumatologic units in Toulouse University Hospitals (Southern France). The patients selected in this study were those presenting clinical signs compatible with joint, bone or soft-tissue infection including fever, local inflammation, purulent discharges, wound dehiscence, necrosis. We collected data on the types of accident. *B. cereus* was isolated from local samples, taken in the operative room, from wounds, open fractures or operative sites.
Results: Sixty-three patients were included in the study (47 male and 16 female). The average age was 42 years (16–82). The distribution of cases over the study period was homogenous. All patients were victims of accidents (road accidents: 63%, industrial or agricultural accidents: 30%, sport accidents: 7%). Patients (89%) had an open fracture. Almost two-thirds of the patients presented fever at the time of *B. cereus* isolation but none presented severe sepsis. Culture were polymicrobial in 44% of the cases. *E. cloacae* was the most frequently associated bacterium. Before isolation of *B. cereus* all the patients but three had received antibiotics [amoxiclav: 78% (with aminoglycoside: 22%)]. All the strains were resistant to penicillin, amoxiclav and cephalosporins, but susceptible to quinolones, aminoglycosides, macrolides, clindamycin, pristinamycin and imipenem. Treatment included a fluoroquinolone in 89% of the cases and miscellaneous *in vitro* active agents in the other cases. The healing of the wounds was obtained in 86% of the cases. Seven patients (two of them not treated by antibiotics) had to be amputated despite aggressive surgical treatment.
Conclusion: The role of *B. cereus* should not be underestimated in patients with clinical signs of infection after injuries soiled by tellurian environment. Quinolones could be proposed as the first choice for antibioprophylaxis or curative treatment of those infections.

P1427 Determination of the quantity of aflatoxin M1 in pasteurised milk in Shiraz

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Objectives: Aflatoxins are toxic mold metabolites produced by toxigenic strains of *Aspergillus* species. They have an important role in the occurrence of a number of human diseases such as liver cancer, chronic hepatitis and cirrhosis. When animals eat food stuffs containing aflatoxin B1, these toxins are metabolites and excreted as aflatoxin M1 in milk. These aflatoxins are resistant to thermal inactivation and are not destroyed completely by pasteurisation, autoclaving or a variety of food processing procedures. The aim of this study was to determine the quantity of aflatoxin M1 in pasteurised milk samples in Shiraz.
Methods: A total of 624 pasteurised milk samples from different supermarkets in Shiraz were collected during 6 months (April–September 2003). After centrifugation of milk samples upper cream layers were completely removed and the lower phases were analysed by enzyme immunoassay for the quantitative analysis of aflatoxin M1.
Results: Aflatoxin M1 was found in 100% of the milk samples examined. 17.8% of the samples were higher than the maximum tolerance limit (0.05 ppb) accepted by European Union. As compared with other studies, Turkish milk had higher contamination ($P < 0.00006$), Brazil was the same as our findings and Albania's contamination was less than our results ($P < 0.0004$).
Conclusion: Consequently this subject is a serious problem for the public health, especially in infants and children consuming these products. Therefore milk and dairy products have to be inspected routinely for aflatoxin M1 contamination. To achieve a low level of aflatoxin M1 in milk, the dairy cows' feeds should be kept away from fungal contamination as much as possible.

P1428 Fungal contamination in a cheese factory in Iran

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Objectives: Hygienic production and prevention of any kind of contamination is one of the most important principles of producing cheese. This study was carried out in the UF cheese factory with the 21 tones daily production, for the following goals: (i) Rate of fungal contamination. (ii) The type of fungal contamination. (iii) The source of contamination. (iv) To suggest effective methods of preventing fungal contamination.
Methods: To estimate the prevalence of cheese contamination (CI 95%), 180 samples were selected and cultured after 53 days. The sampling was repeated three times in both warm and cold seasons. The fungus was then identified by studying the characteristics of the colonies and staining the microscopical structures. To determine the sources of contamination, the production line was also examined at the following points. (i) Raw milk. (ii) Milk passing through the second bactofuge. (iii) Milk passing through the pasteurisator. (iv) Condensed and pasteurised milk. (v) Cultured reservoirs.
Results: The plates, waxy papers, aluminum foils and additives used in cheese production examined for possible contamination. Air contamination of the different parts of the factory was investigated by precipitation and filtration. The overall rate of cheese fungal contamination in three periods were 71, 55 and 48%, respectively with and average of 60%. The most prevalent type of contaminant was the *Penicillium* spp. with the rate of 31% followed by *A. niger* (3.3%), *Cladosporium* (2.77%), *Fusarium* (2.6%), *Alternaria* (2.2%) and *Paecilomyces* (1.3%). The other fungi were observed in <1% of the cases. The raw milk to the factory was 100% contaminated. This rate reached zero after pasteurisation. The rates of contamination of the additives were as follows: rennet 16.6%, antifoam (5.5%), antiseptic (5.5%), thermophile (11.68), mesophile (0%) and salt (0%). The plates, papers, aluminum foils and water showed fungal contamination rates of 4.3, 8.27, 7.6 and 5.5%, respectively. The air was 100% contaminated with *Penicillium* spp.
Conclusion: In spite of multi-factorial sources of contamination the factory air is the most important factor because the air was 100% contaminated with *Penicillium* spp. We concluded *Penicillium* spp. were the most prevalent type of cheese fungal contamination. Since the use of chemicals in dairy products is prohibited, it is suggested to utilise physical methods.

P1429 An investigation of infection risks of patients newly fitted with orthokeratology contact lenses

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Objectives: Wearing contact lenses overnight has been reported to significantly increase risk of ocular infection. This study aimed to determine the normal ocular flora of overnight orthokeratology (ortho-K) patients, and levels of contamination of their lenses, lens case and accessories, and to correlate compliance with contamination.
Methods: The lower conjunctiva of 23 new patients was swabbed on two occasions for culture of normal eye flora before commencing ortho-K lens wear. On six follow-up visits, further specimens were collected, and cultures performed on swabs from the lens, the case and suction holder. All isolates were fully identified. Patients were interviewed on lens care after the fourth follow-up visit. Any patient with contamination with *Pseudomonas aeruginosa* or considerable numbers of non-normal flora organisms, indicating breaks in compliance, was warned about increased risks of infection and importance of good lens care reinforced.
Results: Cultures from 21 patients before lens use yielded normal eye flora only; 16 of these yielding only normal flora after lens wear. *Staphylococcus aureus* was isolated from two patients before lens wear and from one of these after lens wear. Potential pathogens isolated in low numbers from five patients on only one

occasion after lens use, but not present in subsequent samples, were considered transient. Investigation of lens organisms yielded only normal flora organisms for 11 subjects on all occasions. Tap water organisms were occasionally isolated from lenses of two patients, and 10 patients had one isolate of potential pathogen. Four patients with lens contamination had the same organism contaminating their lens case. Suction holders of seven subjects were contaminated, three with organisms found in either the lens or the case. Following interview, 13 subjects were judged to have good compliance. eight patients reporting poor compliance had contamination of lens or accessories on one occasion. All were re-educated and improvement was observed.

Conclusions: Use of ortho-K lenses resulted in no change in levels or content of normal flora. Failure to regularly replace lens case, and disinfect lens case weekly were common errors correlating with presence of potential ocular pathogens. With one exception, patients reporting good compliance had minimal contamination of eyes, lenses or accessories. Intervention improved compliance and eliminated contamination, confirming the need for reinforcement of lens care procedures.

P1430 Evaluation of Bartels ELISA *Legionella* urinary antigen in water samples

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The aim of this study was to evaluate the utility of Bartels ELISA *Legionella* urinary antigen (Trinity Biotech) for the detection of *Legionella* in water samples in comparison with conventional methods.

Materials and methods: Samples A – ATCC strains *L. pneumophila* serogroup 1 to *L. pneumophila* serogroup 14, *L. bozemanii*, *L. longbeachae* and nine wild type strains were used to spike water samples to a final concentration of 10^4–10^5 *Legionella* per millilitre. The lower detection limit of the test for all reference strains was assessed by serial dilutions of water samples from 10^5–100 CFU/mL. Samples B – in addition to the spiked water samples, 34 different tap water samples will be run.

Sample treatment: One litre of all water samples were filtered over 47 mm, 0.2 mcm Millipore membrane filters. Each filter was placed into a test tube containing 3 mL of distilled water. Afterwards, all samples were sonicated for 2 min.

Culture and identification: All tap water samples will be cultured after heating treatment and acid treatment in selective BCYE-agar. The identification of the isolated will be done by biochemical tests and immunofluorescence methods.

Results: *Legionella* antigen was detected in all the filtered spiked samples except for *L. bozemanii* and *L. longbeachae*. In the water samples spiked with wild strains of *L.pneumophila*, soluble antigen was detected in all cases. The lower detection limit to detect soluble *L. pneumophila* serogroup 1 antigen was 780 CFU/mL. The sonication of spiked water samples did not increase the sensitivity of the technique. In tap water samples, *L. pneumophila* was not isolated by culture from any of the 34 water samples tested, while the Bartels ELISA was positive in three cases. However, we could not rule out the possibility that these three samples were actually true positives.

Conclusions: (i) Bartels ELISA is a rapid and useful method for the detection of *L. pneumophila* in water samples. (ii) In addition, the Bartels ELISA has the capability of detecting all *L. pneumophila* serogroups. (iii) The test should be used as a rapid screening method for the detection of *Legionella* in combination with culture.

Infection in the immunocompromised HIV-negative host

P1431 *Fusobacterium nucleatum* bacteraemia in paediatric haematology patients 1997–2003

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Anaerobic bacteraemias are still a rare entity, but in the last decade they have been described increasingly in adult haemato-oncological neutropenic patients. *Fusobacterium nucleatum* is an anaerobic, Gram-negative rod that is part of the normal flora of the mouth and intestinal tract. Over a 6-year period, we identified 10 episodes of bacteraemia due to *F. nucleatum* in eight patients (age 1.9–11.5 years) with a diagnosis of AML (5), ALL (2) and MDS (1). All children were febrile and neutropenic (granulocytes <100 cells/ml) by the time bloodcultures got positive. Two patients had undergone bonemarrow transplantation shortly before onset of bacteraemia (3 and 4 days). Six of the eight children had mucositis as possible source of bacteraemia. In all but one case, *F. nucleatum* readily grew on anaerobic cultures. In one case, growth failed, and *F. nucleatum* was identified by 16S rRNA PCR followed by sequence analysis directly from the bloodculture bottle. The patients were treated either with ceftazidime plus metronidazol or with meropenem for 5–14 days. All patients recovered, but one child had three recurrent bacteraemias due to *F. nucleatum*. Endocarditis was ruled out by a transthoracic echocardiography, but the port-a-cath was not removed. Another persistent focus such as an abscess or an infected thrombus was not found. Susceptibility data are available from seven of the 10 isolates. All strains were susceptible to metronidazol, amoxiclav, and clindamycin. *F. nucleatum* rarely causes bacteraemia in neutropenic paediatric patients. Molecular diagnostic methods can help to identify strains that do not grow on conventional media.

P1432 Bacteriological findings in children with haematological disorders

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Objectives: Infections in neutropenic children in the haematology ward is a significant therapeutical problem. The aim of this study was the analysis of frequency of isolation and antibiotic susceptibility of bacterial strains from children hospitalised in the Haematology Clinic in Wroclaw in the period 1999–2000 vs. 2001–2002.

Methods: Various clinical samples (blood, urine, faeces, material from respiratory tract and other materials) were examined. Isolated bacterial strains were identified by bioMerieux tests. Antibiotic susceptibility was examined by ATB tests (bioMerieux) and the disc diffusion method (NCCLS).

Results: During the periods compared the total number of isolated strains was 785 and 1923 (blood 194 and 283). Significant changes in percentage of Gram-negative and Gram-positive isolates from blood (from 30.4% to 55.8% and from 69.6% to 44.0%, respectively) were noticed. Among the 1923 bacterial strains isolated from all the materials in 2001–2002, 53% were Gram-positive and 47% Gram-negative. In the group of Gram-positive strains ($n = 1017$), coagulase-negative staphylococci (CNS), streptococci orale and enterococci were dominant (32%, 31% and 23% respectively); in the group of Gram-negative strains ($n = 906$) it was mainly *Escherichia coli*, nonfermentative rods (*P. aeruginosa* excluded), Klebsiella and Enterobacter (31%, 12%, 11% and 10%). The results of antibiotic susceptibility were as follows:

– in the group of Gram-positive bacteria glycopeptide-resistant strains were not found,

– there were 75% MRS (methicillin resistant Staphylococcus) strains among CNS and 24% among *S. aureus*,

– Increase in antibiotic resistance of Gram-negative rods especially among *E. coli* (to amoxicillin/clavulanic acid), *P. aeruginosa* (to netilmicin, ceftazidime) and other nonfermentative rods (to ceftazidime, piperacillin) was observed,

– Gram-negative rods were highly susceptible to imipenem (96%), meropenem (94%), piperacillin/tazobactam (84%), ciprofloxacin (80%) and ceftazidime (79%); among carbapenem-resistant strains there were mainly nonfermentative rods (*P. aeruginosa* inclusive).

Conclusions: 1. Rising trends were observed in the number of Gram-negative isolates from blood and in antibiotic resistance of all Gram-negative rods. 2. The empiric therapy with piperacillin/tazobactam plus amikacin in children with cancer treated in the Haematology Clinic seems to be the optimal option, but the monitoring of antibiotic susceptibility of bacterial strains is still required.

P1433 Surveillance of nosocomial sepsis and pneumonia in patients with bone marrow transplantation or peripheral blood stem-cell transplantation. A Multicentre project

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Objectives: To evaluate the incidence of nosocomial infections (NI) in patients with haematologic malignancies suffering from severe immunosuppression, and to generate reference data.

Methods: For surveillance of nosocomial bloodstream infections (BSI) and pneumonia in patients undergoing bone marrow transplantation (BMT) or peripheral blood stem-cell transplantation (PBSCT), a multicentre study was started in 2001 (within the framework of KISS – German Hospital Infection Surveillance System). To allow participation with limited resources, the ONKO-KISS project focuses only on BSI and pneumonia during neutropenia using CDC definitions with modified criteria for neutropenic patients (neutropenia: WBC $<1.0 \times 10$ to the power of 9 per liter).

Results: Over the first 26-month period, 1071 patients with 16 184 neutropenic days were investigated (12 participating hospitals). Of these, 698 (65%) had undergone allogeneic and 380 (35%) autologous BMT or PBSCT. The mean length of neutropenia was 15 days. In total, 231 bloodstream infections and 114 cases of pneumonia were identified. Site-specific incidence densities (pooled mean) were: 14.3 BSIs and 7.0 cases of pneumonia per 1000 neutropenic days, respectively. The main pathogens associated with BSI were coagulase-negative staphylococci (55%), followed by streptococci (10.9%).

Conclusions: Surveillance of NI is a key element of infection control, especially in critically ill patients such as those undergoing BMT or PBSCT. For the first time the ONKO-KISS project provides reference data (http://www.nrz-hygiene.de) and thus serves to improve the quality of care provided to BMT and PBSCT patients.

P1434 Nocardiosis trends in a teaching hospital: a 15-year study

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Background: Nocardiosis is an infrequent, but severe infection, especially in profoundly immunocompromised patients. However, clinical experience remains rather limited.

Objectives: The objectives were to establish the trends of nocardia infections, the type of antibiotic treatment and the outcome in patients.

Methods: A retrospective chart review was undertaken on hospitalised patients at the Geneva University Hospitals from 1989 to 2003. All patients in whom *Nocardia* sp. was isolated were included in the study.

Results: *Nocardia* sp. was obtained from 20 patients (70% male, median age 58.5 years). Sixteen patients (80%) had one or more underlying conditions: solid organ malignancy (4 patients), chronic lung disease (4), HIV (3), diabetes mellitus (3), organ transplantation (3), immunosuppressive therapy (6), lymphopenia (15), others (6). The median time between the symptom's onset and nocardiosis diagnosis was 30 days (range, 3–50). Pulmonary tuberculosis (TBC) was the most common initial diagnosis of nocardiosis (4). The sites of infection were the lung (16 cases), central nervous system (CNS) (2), skin (2) and disseminated (1). *N. asteroides* complex spp. were isolated in 19/20 cases. *In vitro* testing was performed for 14 strains. No resistance was detected for imipenem or amikacin. Treatment was given to 17 patients and consisted of trimethoprim-sulfamethoxazole (TMP-SMX) (14 cases), imipenem (7), sulfadiazine (3) and ciprofloxacin (4). Improvement was observed in 15/20 patients, one patient relapsed, one patient had a pulmonary nocardiosis complication with CNS involvement and three patients died (one had not been treated since the diagnosis had been attributed to TBC; for the other two patients the treatment was started at day 23 and 50 after onset of clinical symptoms with oral TMP-SMX and sulfadiazine).

Conclusions: Nocardiosis is a severe infection and mainly affects profoundly immunocompromised patients. Differential diagnosis, especially with TBC, often delays the time of diagnosis that worsens the outcome. Efforts for more rapid diagnostic techniques application, such as PCR, should be made. TMT-SMX was the most common prescribed treatment, however its oral form was not sufficiently efficient in two of our cases with severe infection.

P1435 Reactivation salmonellosis in cancer patients after antineoplasic chemotherapy

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Objectives: It is well documented that nonthyphoidal salmonella (NTS) infections occur more frequently in cancer patients. However, it is unclear whether this is due to an increased susceptibility to *de novo*, exogenous salmonella infection, or due to cancer chemotherapy induced reactivation of asymptomatic salmonella stool carriage.

Methods: Retrospective analysis at a Swiss tertiary care centre of all medical records (1996–2002) from patients with positive clinical samples for nonthyphoidal salmonella. Non-oncological patients and cases not meeting our criteria for reactivation NTS (see below) were excluded. A Medline search (1966–2003, all languages) was also performed to identify potential cases. Case-definition of reactivation NTS

1. Patient asymptomatic at admission to the hospital
2. Symptoms consistent with salmonellosis >72 h after hospital admission
3. Symptoms arising after antineoplastic chemotherapy
4. No evidence of nosocomial acquisition

Results: From 1996–2002, 235 positive specimens for NTS were identified in 214 patients. Five specimens were from cancer patients who had an illness compatible with reactivation disease following the administration of chemotherapy. Medline search yielded four more cases that met our criteria. Of all the nine cases, five were women, and the median age was 28 years (range 3 months–72 years). Five patients had diarrhoea; one had salmonelluria, while three patients in the literature had bacteraemia. In two patients asymptomatic stool colonisation was documented prior to reactivation disease. All patients were successfully treated with antibiotics, even while chemotherapy was continued in eight patients. There were no salmonella relapses despite additional chemotherapy cycles in five patients.

Conclusions: Asymptomatic salmonella carriers with malignant neoplasms, unaware of their condition, may develop symptomatic salmonellosis, triggered by gastrointestinal mucosal damage and immunosuppression associated with chemotherapy. Current guidelines recommend bacterial stool analysis after three days of

hospitalisation only for patients >65 years of age with significant co-morbidity, HIV infection, or neutropenia. However, reactivation NTS can occur in patients who are <65 years old and in the absence of neutropenia. Stool cultures should be obtained in cancer patients with significant chemotherapy associated diarrhoea even if hospitalised >3 days.

P1436 Pulmonary infiltrates in solid organ transplant recipients. Mortality risk factors

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Background: Pulmonary infections are a major cause of morbidity and mortality in transplant organ recipients. Our purpose is to establish the risk factors for mortality in solid organ transplant recipients with a new onset of pulmonary infiltrates (PI).
Methods: We prospectively evaluated the solid organ transplant recipients with PI from February 1998 to December 2002. All patients underwent a diagnostic protocol that included the obtaining of two samples of blood cultures, spontaneous or induced sputus specimen or bronchoaspirate (in patients with mechanical ventilation), and samples of blood and urine for antigen testing. In the case of diffuse and bilateral PI's and those without clinical or radiologic improvement after 3 days of treatment, all of them underwent invasive evaluation, that included fiberoptic bronchoscopy with protected-specimen brush (PSB), bronchial aspirate and bronchoalveolar lavage (BAL).
Results: We included 75 patients, 56 men (75%) and 19 women. Mean age was 55 ± 13 years (Range: 16–76). The type of transplant was: kidney 33 (44%), kidney and pancreas 4 (5%), liver 30 (40%), and cardiac 8 (11%). The most frequent diagnoses were: Bacterial pneumonia 22 (MRSA pneumonia 4 cases; and *P. aeruginosa* pneumonia 4 cases), Pulmonary aspergillosis 19 cases (3 associated with MRSA, 1 with CMV, 1 with Pneumocystis carinii, 1 with *Candida albicans* and Scedosporium prolificans, and 1 with Stenotrophomonas maltophila); and Pulmonary oedema 6 cases. In 22 cases, we found no etiologic diagnosis. Mortality was 33% (25 patients), and only four of them without diagnosis. The variables associated with great risk factor for mortality were: mechanical ventilator (RR: 3.5; CI [95%]: 2.1–5.9; $P < 0.001$); fungal infection (RR: 2.75; CI [95%]: 1.3–6.0; $P < 0.01$), ICU admission (RR: 2.2; CI [95%]: 1.6–3.0; $P < 0.001$).
Conclusions: The presence of PI in solid organ transplant patients is associated with high mortality. The most important risk factor for mortality is the need for mechanical ventilation. In our experience, bacterial pneumonia is the most frequent diagnosis, followed by pulmonary aspergillosis.

P1437 Prevalence of BK virus infection in solid organ transplant recipients

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Background: BK virus (BKV) is a polyomavirus that causes an emergent infectious disease particularly troublesome in kidney transplant recipients. BKV remains latent in the urinary tract of the general population and may reactivate during immunosuppression. BKV viruria is prevalent in renal allograft recipients and BK viremia has been related to nephropathy and graft loss in renal transplant recipients. Nevertheless, the prevalence of this viral agent in other solid organ transplant groups (SOT) (cardiac and liver) has not been well established.
Objectives: to determine the prevalence of BKV infection and disease in all types of SOT recipients in our centre.
Methods: Seventy-seven consecutive SOT recipients were studied: 19 renal, 23 cardiac and 35 liver transplant recipients. Samples were obtained, a mean of 1620 days after transplantation (range 3–9.481 days). The presence of BKV-DNA was initially screened by a nested-PCR in urine. Plasma PCR was performed only in patients with a positive urine PCR. A pre-established clinical protocol was fulfilled the day the sample was obtained. Transplantation procedure and immunosuppression was standard.
Results: Polyomavirus BK was found in 10.4% of urine samples. The prevalence of viruria was 15.8% after renal transplant, 17.4% after cardiac transplants and 3% after liver transplants. Only one patient had BKV viremia. He was a 65-year-old renal transplant recipient who had lost his first kidney transplant due to BKV-associated nephropathy and had again poor renal function 3 months after the second transplantation.
Conclusions: This study confirms that urinary shedding of BK virus is common in all types of solid organ transplant recipients. For the time being, we have only been able to find BKV disease in a kidney transplant recipient, in whom it relapsed early after transplantation after causing the loss of the first graft. Further studies are needed to understand the natural history of primary infection and reactivation of BK virus in SOT and its relation to graft function.

P1438 A retrospective study on *Pneumocystis carinii* pneumonia in critically ill patients

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Introduction: Despite major advances in diagnosis and management, *Pneumocystis carinii* pneumonia (PCP) is a severe complication of immunocompromised patients. We analysed the outcome of patients with PCP with a positive bronchoalveolar lavage (BAL).
Patients and methods: We retrospectively analysed the medical records of all patients with a positive BAL culture admitted to a tertiary intensive care department from January 1999 to September 2003. Demographic, clinical and microbiological data were collected.
Results: In the study period, 22 patients had a PCP, confirmed by clinical, radiological and microbiological data. Five of these patients had an acquired immunodeficiency syndrome and 17 had a severe immunocompromised state. Twelve patients had severe respiratory failure with a paO_2/FiO_2 ratio <300 mmHg, and among them nine received invasive mechanical ventilation. The rest of the 13 patients were managed by non-invasive mechanical ventilation. The degree of severity as calculated by APACHE II score was 17.2 ± 5.4. The length of stay in the ICU was 8 (5–18) days. Three of the patients received prophylactic antibiotic therapy at the time the BAL was performed. Overall mortality rate was 36%, for intubated patients mortality was 55%, and for non-intubated patients 23% ($P = 0.01$). There was no significant difference in the ICU and in-hospital mortality between patients with a PCP associated with a HIV infection compared with other immunocompromised states (40% vs. 35% and 50% vs. 45%, respectively). Risk factors for death were the need of mechanical ventilation ($P = 0.03$) and prophylactic antibiotic therapy ($P = 0.04$).
Conclusions: PCP is associated with high mortality rates in immunocompromised patients. Critically ill patients requiring invasive mechanical ventilation and prophylactic antibiotherapy have a worse outcome.

P1439 Detection of *Pneumocystis carinii* by polymerase chain reaction in bronchoalveolar lavage specimens: clinical significance and correlation with other diagnostic methods

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Objectives: To evaluate the clinical significance of positive *Pneumocystis carinii* (PCC) PCR-test in bronchoalveolar lavage (BAL) specimens from adult patients with pneumonia.

Methods: We evaluated retrospectively all patients treated in Turku University Central Hospital in 1992–2001 with a positive result in PCC-PCR-assay in BAL-fluid specimen. The patients were identified through microbiology registration system. Clinical symptoms and radiological signs of pulmonary infection, results from other diagnostic tests for PCC, antimicrobial chemotherapy and patient outcome were analysed. Patients were stratified into four groups according to the confirmation of diagnosis of *Pneumocystis carinii* pneumonia (PCP) (definite, probable, possible and non-probable). Clinical and radiological findings and response to specific PCP therapy were used in stratification.
Results: Altogether 441 patients (480 BAL samples) were tested during a 10-year period. 66 patients (15%) had at least one positive result in PCR-assay. The majority (97%) of the patients were immunocompromised. Immunofluorescence test (IF) was concomitantly positive with PCR in 35% of patients tested (19/54) and methenamine silver staining (MSS) in 29% (19/66), respectively. Eighty-three per cent of the patients with positive IF test and 95% of the patients with positive MSS had concomitantly positive PCR-assay. Only 18% of the patients with positive PCR-assay had a triad of typical symptoms for PCP (dry cough, shortness of breath and fever) and 65% had bilateral interstitial pulmonary infiltrates. The diagnosis of PCP was considered definite or probable in 48% (32 patients) of patients with positive PCR assay. In 24% (16 patients) the diagnosis of PCP was considered non-probable. Seventy-nine per cent of all patients with positive PCR assay and 94% of patients with definite or probable diagnosis of PCP had received appropriate antimicrobial treatment against PCC. Mortality in patients with definite or probable diagnosis of PCP was 25%. Seventy-five per cent of the patients whose diagnosis was considered non-probable had not received any treatment against PCP. Only one of these 12 patients died (invasive aspergillosis).
Conclusions: Positive PCC-PCR-assay in BAL specimen correlates poorly with clinical disease and with other diagnostic methods. PCR-assay should be used only as a supplementary test for immununofluorescence test and methenamine silver staining.

P1440 Bacterial infection in cirrhotic patients with variceal bleeding

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Objectives: Bacterial infection seems to be very important in cirrhotic patients with variceal bleeding. The aim of our study was to find out the prevalence of bacterial infection in cirrhotics admitted to hospital with variceal bleeding in comparison with patients with liver cirrhosis admitted because of other reasons.
Material and methods: Bacteriological investigation of urine, stool, throat smear, haemoculture and ascites were investigated in 40 cirrhotic patients admitted to hospital with variceal bleeding and 65 cirrhotics admitted because of other reasons.
Results: There were differences in bacteriological findings between the patients with and without variceal bleeding. Bacterial infection was significantly more frequent in the cirrhotic patients with bleeding – positive in 19 patients (47.5%), in comparison with patients without bleeding – positive in16 patients (24%). Bacterial sepsis was more frequent in groups of patients with variceal bleeding – 5 patients (12.5%) vs. 3 (4%) in a group of patients without bleeding. There was also a difference in the bacterial colonisation of proximal parts of the gastrointestinal tract. The patients with bleeding were colonised with Gram-negative bacteria in 32.5%, the patients without bleeding were colonised in 10%.
Conclusions: Bacterial infection is more frequent in cirrhotic patients admitted with variceal bleeding in comparison with patients with liver cirrhosis admitted because of other reasons. Also the colonisation of proximal parts of the gastrointestinal tract with Gram-negative bacteria is more frequent in the group of patients with bleeding than in the sample of patients with liver cirrhosis without bleeding. The study was supported by the Grant Agency of the Ministry of Health of the Czech Republic (NK6661-3/2001).

P1441 Diabetic foot infections: risk factors and microbiological spectrum

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Objectives: To determine risk factors and evaluate the microbiological spectrum of diabetic foot infections.
Methods: We studied 44 consecutive diabetic patients with foot ulcers, 26 men (59%) and 18 women (41%) (group A). Each patient was matched for sex, age (±3 years) and diabetes duration (±5 years) with two diabetic case-controls without foot ulcers (group B). All patients had been regularly followed-up during a 10-month period in our department. Mean age was 62.0 years (±13.8) in group A and 62.7 years (±10.3) in group B. Mean duration of diabetes was 14.2 years and 13.2 years in group A and B respectively. The two groups were compared for body mass index (BMI), mean glycosylated haemoglobin concentration, serum lipids (total cholesterol, HDL, triglycerides), hypertension, smoking (pack × years), angiopathy, neuropathy. Samples for culture were taken from all ulcers, excluding necrotic material.
Results: Mean BMI was 29.1 (±4.4) in group A and 29.3 (±4.8) in group B, P = NS. In group A, 22 patients (53%) were on insulin, and 19 (47.5%) on antidiabetic oral agents, while in group B 32 patients (39%) were on insulin and 50 (61%) on antidiabetic agents. Mean glycosylated haemoglobin concentration was 8.6 g/dl in group A and 7.5 g/dl, $P = 0.0008$. Mean serum cholesterol levels were 236 mg/dl in group A (mean HDL levels 39 mg/dl) and 225 mg/dl in group B (mean HDL levels 43 mg/ dl), P = NS. Mean serum triglycerides were 184 mg/dl in group A and 196 mg/dl in group B, P = NS. Eighty-seven microorganisms were isolated, 83 aerobes (35 Gram-positive and 48 Gram-negative) and four anaerobes. The most common pathogens were *Staph. aureus* (13.8%: methicillin-susceptible 11.4% and methicillin-resistant 2.3%), *Proteus* sp. (12.6%), coagulase-negative staphylococci (12.6%), *Pseudomonas* sp. (13.8%). The mean number of microorganisms per patient was 1.97. No statistically significant differences in the aforementioned risk factors were identified among patients with monomicrobial or polymicrobial infections. Rates of antimicrobial resistance were low, because the study group included only outpatients, not previously treated with antibiotics.
Conclusions: Poor diabetic control is the main risk factor for the development of diabetic foot infection. Most diabetic foot infections are polymicrobial. Gram-negative isolates, particularly Enterobacteriacae, predominated in our study group.

P1442 *Actinomyces neuii* isolation from foot necrotic ulcer in an immunocompromised patient

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Objectives: An unusual case of *Actinomyces neuii* isolation from a foot necrotic ulcer in a diabetic end stage renal disease (ESRD) patient on continuous ambulatory peritoneal dialysis (CAPD) is presented.
Methods: Specimens from ulcer after surgical debridement were cultured at 1st and 4th day of admission onto appropriate media. Also direct smears by gram stain were examined. The identification of the microorganism was performed by standard methods and API Coryne (bioMerieux). Susceptibility testing was performed by disc diffusion method and Etest.
Case report: A 68-year-old woman on ESRD, came to the hospital with fever (38 °C) and painful, oedematous, necrotic ulcer in the small finger of the foot. She was suffering also from diabetic complications (retinopathy, nephropathy, coronary artery disease, hypertension and generalised peripheral arteriopathy). She started CAPD 2 years ago and 18 months later she presented fungus peritonis. On admission she was treated with metronidazole 500 mg 1 × 3 I.V. and ciprofloxacin 100 mg 1 × 2 I.V. together with surgical debridement. WBCs count of blood was 14 700/il

(80% neutrophils). The ESR was 100 mm/h. Abundant leucocytes and gram(+) small robs were seen on direct smears by gram stain. *A. neuii* ssp *neuii* was isolated from the cultures of both samples. The growth was better under anaerobic conditions. The strain was resistant to cinolones, aminoglycosides, and susceptible to penicillin G, cefaclor, cefotaxime, erythromycin, clindamycin, vancomycin and teicoplanin. On the 3rd of hospitalisation, the antimicrobial treatment changed and clyndamicin 600 mg 1 × 1 IV was administered. On the 5th day because of worsening of local inflammation findings, an amputation of the small finger was decided with concomitant teicoplanin 400 mg 1 × 1 IV for 15 days and then for the next 10 days 400 mg/48 h IM administration. Follow-up cultures did not isolate *A. neuii*.

Conclusions: *A. neuii* is an unusual cause of severe ulcers and abscesses in immunocompromised and diabetic patients. Antibiotic treatment is seldom successful, without surgical cleaning or amputation. All the 'coryneform' isolates should be identified either then or grown in pure culture or coexist with other microorganisms.

P1443 Serum and peripheral blood mononuclear cells infectious burden: correlation to inflammation and atherosclerosis in haemodialysis patients

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Infectious agents may be implicated in the inflammatory atherosclerotic process. Not only specific microorganisms but also the infectious burden, defined as the number of pathogens to which a patient is exposed, has been associated with atherosclerosis. In this study, the infectious burden, determined directly – by identification of viable pathogens in peripheral blood mononuclear cells, PBMCs - and indirectly - by serum antibodies detection – is correlated to the inflammatory and atherosclerotic status in haemodialysis (HD) patients, a population at high risk for cardiovascular disease. The viable forms of four microorganisms (Chlamydia pneumoniae, Herpes Virus I and II and Cytomegalovirus) were identified in patients' PBMCs by cell cultures and subsequent polymerase chain reaction. Serum IgG against the above pathogens and *Helicobacter pylori* were also determined. Inflammation was assessed by C-reactive protein (CRP), serum amyloid A (SAA), three pro-, one anti-inflammatory cytokines and four adhesion molecules measurement. Atherosclerosis was defined by a scoring system using medical history data. The number of viable pathogens identified in PBMCs, in the 122 HD patients included in the study, were 0 in 22.1% of them, one in 33.6%, two in 43.4% and three in one patient. The number of IgG antibodies determined was one in 6.6% of the patients, two in 32%, three in 48.4% and four in 13.1% of them. Seropositivity wasn't significantly different between patients with or without the respective viable pathogen identified in PBMCs. Atherosclerosis was present in 40.2% of the patients and CRP, SAA as well as Interleukin-6 were increased in these patients. Neither inflammatory indexes nor atherosclerosis were significantly different in patients with a higher number of viable pathogens detected in PBMCs or in those with higher number of antibodies. The direct infectious burden determination (the number of viable pathogens in PBMCs) doesn't coincide with the serum (by IgG detection) infectious burden. Although inflammation correlates to atherosclerosis, neither PBMCs nor serum infectious burden is associated to these two entities in the inflamed and atherosclerotic HD patients.

P1444 Human herpesvirus 8 infection in haematopoietic stem cells transplant recipients

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Background: HHV8 is the cause of Kaposi's sarcoma or lymphoproliferative disorders in immunodeficient individuals. In western and central European countries, the seroprevalence of HHV8 infection is low. Increased HHV8 seroprevalence and occasional occurrence of Kaposi's sarcoma was described in solid organ transplant recipients. The aim of this study was to evaluate the risk of HHV8 infection in haematopoietic stem cells transplant (HSCT) recipients.

Methods: HHV8 seroprevalence was retrospectively studied in 80 adult allogeneic HSCT recipients. Antibodies to the lytic HHV8 antigen were detected in pre-transplant and late post-transplant sera using indirect immunofluorescence test. Nested PCR was used to detect the presence of viral DNA in consecutive peripheral blood samples from patients who seroconversed after transplantation.

Results: The HHV8 seroprevalence rates before and after transplantation were 2.6 and 3.8%, respectively. Two patients seropositive before transplantation tested negative for anti-HHV8 antibodies in the post-transplantation period. Two patients showed HHV8 seroconversion after HSC transplantation. Both exhibited intermittent presence of viral DNA in peripheral blood.

Conclusions: HSCT recipients were not at significant risk for HHV8 infection. Allogeneic HSCT recipients may lose HHV8 seropositivity after transplantation. Active infection with HHV8 during post-transplantation period in two HSCT recipients was associated neither with high viral load in peripheral blood nor with significant signs of clinical illness. In one patient, reactivation of HHV8 infection was associated with active CMV infection and GVHD.

P1445 Acquired Shapiros syndrome (recurrent hypothermia) due to human herpesvirus 6 after stem cell transplantation

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Introduction: Human herpesvirus-6 (HHV-6) is a human pathogen of emerging clinical significance. Although overt clinical disease is infrequent in adults, HHV-6 reactivates with immunosuppression. We describe a patient who developed an HHV-6 encephalitis characterised by recurrent episodes of hypothermia in association with extreme hyperhidrosis after a cord blood stem cell transplant. These clinical features resembled those described by Shapiro and Plum in patients with agenesia of the corpus callosum, that usually respond to treatment with clonidine (an alfa 2 agonist). After antiviral treatment with foscarnet associated with clonidine was started, a resolution of the symptoms was achieved.

Case report: The patient was a 34-year-old man who received a cord blood stem cell transplant for the treatment of a Philadelphia chromosome-positive acute lymphoblastic leukaemia. Conditioning was performed with cyclophosphamide, total body irradiation and anti-thymocite globulin. Prophylaxis against graft-versus-host disease consisted of cyclosporine and prednisone. Prophylaxis of infections was carried out with aerosolised pentamidine, oral acyclovir and with unspecific gamma globulin. On day 20, the patient developed episodes of hypothermia, profuse sweating and shivering as well as confusion and abdominal pain. Routine blood analysis revealed no abnormalities except for a severe hyponatremia (Na 111 mEq/l), hypoosmolarity and a high cyclosporine concentration. A magnetic resonance of the brain showed bilateral lesions involving amygdala and hippocampus while the corpus callosum was present. An electroencephalogram showed diffuse slow waves. The lumbar puncture demonstrated: mononuclear pleocytosis (44 cells/ul), a high protein level (103 mg/dl) with normal glucose level (71 mg/dl). Viral studies were negative except for HHV-6 DNA, which was isolated from cerebrospinal fluid by PCR. With the diagnosis of HHV-6 encephalitis, foscarnet was started. As the symptoms of our patients were similar to those of Shapiros Syndrome, clonidine was added to foscarnet. After combined therapy was initiated control cerebrospinal fluid HHV-6 DNA PCRs were negative and a remission of the symptoms was observed. Five months after transplant the patient died. The autopsy demonstrated the presence of HHV-6B infected cells in the hippocampus.

P1446 Ganciclovir-resistant Cytomegalovirus infection in a heart transplant recipient

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Objectives: Cytomegalovirus (CMV) infection is a serious complication for solid-organ recipients. UL97 phosphotransferase and DNA polymerase gene mutations can confer ganciclovir (GCV) resistance. We studied a documented case of disseminated GCV-resistant (CMV) infection in a heart transplant recipient, the associated risk factors and the viral monitoring of the process.
Methods: Viral load was retrospectively monitored by a real-time quantitative amplification of a 250 bp viral glycoprotein B (*gpB*) gene fragment from 200 μL peripheral blood extracted DNA using a LightCycler instrument (Roche Molecular Biochemicals); DNA quantification was performed through 10-fold serial dilutions of a plasmid standard (pDrive, Qiagen PCR Cloning) containing the primer-spanning region of the *gpB* gene; plasmid standard DNA concentration was calibrated by spectrophotometry at 260 nm. CMV pp65 antigenemia was prospectively performed at least once a week on a 200 000 peripheral mononuclear cell extension and detected with fluorescent monoclonal antibodies (BioRad). Different clinical samples were inoculated on diploid human fibroblasts, incubated for 4 weeks on CO_2 enriched atmosphere at 37 °C and examined twice a week for the specific cytopathic effect. Phenotypic and genotypic antiviral susceptibility studies were performed by plaque reduction assays, inoculating 24-well plaques with increasing concentrations of GCV (0, 6, 12, 24, 48 and 96 μM, respectively), and sequencing of the UL97 gene on an ABI Prism 377 DNA Sequencer (Applied Biosystems), respectively.
Results: Higher plasmatic viral load and *pp65* antigenemia values correlated with viral syndromes, in the setting of and at the end of GCV treatment. Viral load and *pp65* antigenemia did not become negative until the foscarnet treatment was implemented. CMV strains were isolated on cell cultures from blood and gastric biopsy samples. All viral strains presented an A594V mutation on the UL97 gene, with a GCV IC50>96 μM.
Conclusions: GCV-resistant CMV infection is exceptional in heart transplant recipients. D + /R- CMV serostatus, GCV treatment at subtherapeutic levels, high number of viral syndromes and persistent subclinical reactivation in the setting of antiviral prophylaxis are the main factors that have contributed to the infection with a GCV-resistant CMV strain.

P1447 Prevention of iatrogenic Creutzfeldt–Jakob disease in recipients of corneal transplants: genetic testing of donors

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Creutzfeldt–Jakob disease (CJD) is the most common, fatal and transmissible neurodegenerative disease, most important in a group of human prion diseases. Effective therapy is not available. CJD occurs as sporadic (the cause unknown), genetic (with CJD-specific mutation of the prion gene) and iatrogenic, caused by transmission of the infectious agent by contaminated tissue or instruments. The first iatrogenic CJD reported in 1974 was caused by corneal transplantation. Subsequent experimental studies demonstrated infectivity in corneas of animals inoculated with CJD agent. At present there is no specific method for definite diagnosis of CJD intra vitam, therefore asymptomatic carriers of CJD-specific mutations are excluded from a tissue donation. Slovakia is characterised by exceptionally high percentage (75%) of genetic CJD patients with CJD-specific mutation E200K, and by a 'genetic CJD risk group', represented by asymptomatic carriers of this mutation. About 59% of these long-term observed carriers have been found to develop the disease. This experience as well as an increasing number of corneal transplantations initiated introduction of preventive genetic testing of all corneal donors. The testing started in April 2001. Since then 608 donors have been tested. DNA was isolated from the peripheral blood. Mutation E200K and polymorphism at codon 129 of the prion gene, indicating susceptibility to iatrogenic CJD, were tested in all donors. Since donors are a randomly arisen group representing the general population, results on 129 polymorphisms correlate with data for normal controls. Obtained distribution of codon 129 polymorphism demonstrated that majority of donors are homozygots, either methionine (48.4%) or valine (8.6%), indicating high susceptibility to CJD. These data are not in agreement with preponderance of heterozygots, reported previously by other authors or observed by us, but obtained in significantly smaller tested groups. Presented results suggest an urgent need to reevaluate the generally accepted indicators of genetic susceptibility to prion diseases. Concerning the tested genetic CJD risk, unexpectedly, two out of the 608 DNA examined were positive for mutation E200K. Carriers of CJD-specific mutation, present in a relatively small group of donors, justify the preventive measure introduced exclusively in Slovakia.

P1448 Putative role of SV40 in BMT children

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Objectives: Polyomavirus SV40 has been found in human tumours and normal tissues. In bone marrow transplant patients, polyomaviruses, particularly BKV, were associated with post-engraftment haemorrhagic cystitis. Recently, a co-infection of BKV and SV40 has been observed in kidney transplanted adults with nephropathy. In paediatric patients, distinct SV40 strains were occasionally detected in transplanted kidneys suggesting that the human kidney could be a reservoir for polyomaviruses and that the host immunosuppression may favour viral replication. This study aimed to investigate the presence of SV40 in children who underwent allogenic BMT and whether SV40 infection can be correlated to clinical events.
Methods: During the period 2000–2002, 28 patients underwent allogeneic BMT in the paediatric hospital 'Burlo-Garofolo' of Trieste. DNA from PBMC and urine (sediment cells and supernatant) was analysed by PCR for HCMV, AV, BKV, JCV, and SV40. DNA filter hybridisation was carried out in all SV40-positive samples while direct sequencing was done for two patients.
Results: SV40 footprints were detected in the blood of seven out 28 patients (25%) by PCR. In five patients, SV40 infection was transient and unrelated to any clinical relevant condition. On the contrary, in two patients who developed a severe form of haemorrhagic cystitis, SV40 was revealed in blood and in urine. The positivity was found starting from the onset of HC and frequently during the follow-up (mean time +100 days). The direct sequencing of the SV40 Tag-N-C terminal and regulatory regions indicated that the two viruses were related to the SV40 archetypal strain 776.
Conclusions: In this study, SV40 infection was found in seven children who had undergone BMT; two of these later developed a severe post-engraftment HC. As we excluded a vertical transmission or the contamination of transfused stem cells, blood transfusions, received by one patient in the past, or other invasive procedures may have been a possible route for the SV40 infection. However, this explanation remains merely hypothetical, since the epidemiology of the SV40 in humans is largely unknown. In conclusion, this study points out that SV40 may infect children and suggests a possible role of SV40, in addition to BKV and AV, in the aetiology of severe HC after BMT deserving further evaluation.

P1449 Contribution to the studies of the role of Chlamydiae and human herpesvirus 6 (HHV-6) in pathogenesis of multiple sclerosis

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Objective: Sera and cerebrospinal fluids (CSF) of patients with proven multiple sclerosis (MS) and adequate controls were examined

for the presence of specific antibodies to chlamydiae ($n = 349$) and HHV-6 ($n = 250$).
Methods: IgM, IgG and IgA antibodies to common LPS antigen of chlamydiae were detected by ELISA. The species-specific antibodies to major outer membrane protein (MOMP) of elementary bodies and IgM and IgG antibodies to HHV-6 were detected by MIF. The findings of anti-chlamydial antibodies in sera were classified into the following categories: IgG only – anamnestic response; IgG and IgA – suspected chronic infection; IgM, IgG and IgA or IgM and IgA – suspected reactivation.
Results: No substantial difference between MS patients and healthy controls was found in antibody response detected by ELISA method. Antibodies to MOMP antigen were mainly Chlamydophila pneumoniae-specific. The findings of antibodies to Chlamydia trachomatis and Chlamydia psittaci were negligible both in MS patients and controls. The proportion of anamnestic response to MOMP was nearly identical in MS patients and controls. On the other hand, the signs of chronic infection or activation were found in 9.4% of MS patients, but in none of the control subjects. Anamnestic antibodies to HHV-6 (IgG only) were detected in 32.6% of MS patients and in 26.8% of controls. The signs of activation of infection were found in 27.5% of ill subjects but in only 15.5% of healthy controls. In CSF, specific antibodies to chlamydiae detected by ELISA or MIF as well as anti-HHV-6 antibodies were found only rarely, in no case exceeding 3% in both MS and control subjects.
Conclusions: In all the serological methods used, a similar proportion of anamnestic antibodies to both Chlamydophila pneumoniae and HHV-6 was found in MS patients and healthy controls, but signs of activation of chlamydial and HHV-6 infections were significantly more frequent in patients. Analysis of our results leads to the suggestion that chlamydia and HHV-6 infections probably do not play an important role in triggering the autoimmune process of MS and that the more frequent activations may be the result of immunosuppressive therapy.

P1450 Retrospective analysis of cancer patients with neutropenic fever and predictors of treatment success

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Objective: Infections remain the most frequent cause of morbidity and mortality in neutropenic cancer patients. We examined the characteristics of febrile neutropenic cancer patients and predictors of treatments success in a tertiary care university hospital, in Turkey, between January through December 1999.
Methods: Medical charts of 167 patients were analysed retrospectively. The febrile attacks were categorised as: 'microbiologically-' and 'clinically documented' infections and 'fever of unknown origin' (FUO). The results of the treatment were grouped as 'success' or 'success only after modification of treatment/failure'. If a particular patient experienced more than one febrile neutropenic attack during hospitalisation, only the first attack was included in the analysis.
Results: Of the patients 61.1% were male; the mean age was 48 ± 18 years; 55.1% of the underlying diseases were haematological malignancies. The infection was clinically documented (CD) in 52 patients, microbiologically documented (MD) in, and the aetiology of fever was undetected in 75 patients (44.9%). In multivariate logistic regression analysis, vancomycin use was a significant predictor of treatment success and patients with FUO were more likely to have a successful outcome compared with those with MD infection.

Variable	p-value	Odds ratio	95% confidence interval
Underlying disease (ref. = solid tumours)	0.693	0.818	0.302–2.214
FUO (ref. = MD infection)	<0.001	16.937	4.670–61.429
MD infection (ref. = CD infection)	0.682	1.285	0.388–4.255
Vancomycin use (ref. = no use)	0.016	7.787	1.470–41.251
Amphoericin (ref. = no use)	0.258	3.838	0.356–41.397
Resolution of fever n 3 days (ref. = no resolution)	<0.001	0.039	0.008–0.180

Conclusion: Our results indicate that therapy success is more common in case of FUO compared with both CD and MD infections, indicating a need for extensive care in the latter groups, particularly if fever is not resolved in 3 days. Success rate was higher in patients who received vancomycin during the course of the treatment compared with no use, yet, this finding does not justify the empirical use of this drug since all of our patients received vancomycin upon documentation of a Gram-positive infection.

P1451 High-dose levofloxacin for the treatment of febrile neutropenia

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Background/Objective: The variety of organisms isolated from febrile neutropenic patients (FNP) warrants empiric antimicrobial therapy broad enough to cover most clinically significant Gram positive and negative bacteria. In an adult, low-risk, subset of such patients, current IDSA guidelines include the option of giving ciprofloxacin (C) and amoxicillin-clavulanate (AC) jointly. Several features of the fluoroquinolone levofloxacin (L) make it an attractive potential monotherapy alternative to that of combination regimen. Its spectrum encompasses the same implicated pathogens, the serum and tissue levels achieved with the 750 mg dose exceed PD targets for those bacteria. It is administered orally once daily, and it has an extensive and reassuring tolerability and safety profile. We report here on two pilot studies using L in the management of FNP.
Methods: Talcott IV out-patients were randomised to receive C 750 mg q12 h plus AC 875/125 mg po q12 h (following at least one IV dose each of ceftriaxone (CTX) 2 gms and amikacin (AK) 15 mg/kg) or L 750 mg po qd (poster presentation, ICID, 2002). More severely ill in-patients were randomised to cefepime (CP) 2 g iv q8 h or L 750 mg iv qd. Fever defervescence was the primary endpoint in both trials; secondary endpoints included safety and tolerability and microbiologic eradication.
Results: In the outpatient study, the total defervescence in the L-treated patients ($n = 27$) was 74% compared with 63% of the comparator-treated patients ($n = 19$). The clinical cure rate at post therapy in the inpatient study was 72.7% for L-treated patients ($n = 11$) and 20.0% for the comparator-treated patients ($n = 15$). Adverse events were comparable between arms in both studies and none were considered to be drug-related.
Conclusion: These limited data suggest that L 750 mg iv/po qd may offer a safe, effective, and convenient new option in the antimicrobial management of febrile neutropenia. Since fluoroquinolone agents are cidal in a concentration-dependent manner, higher doses have been thought possibly capable of retarding the emergence of resistance. Recent PD studies *in vitro* are lending experimental support to this hypothesis and give one more reason for considering this higher dose of L in the setting of febrile neutropenia. Based on these results, we propose additional full-scale studies of L 750 mg qd in FNP.

P1452 Empiric antibiotic treatment of adults with neutropenic fever in a general internal medicine ward

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Objectives: To compare the efficacy of piperacillin/tazobactam (PTZ) 4.5 g q 8 h plus amikacin 15 mg/kg/day (AMK) to cefepime (CFP) 2 g q 2 h plus AMK in patients with neutropenic fever and underlying haematologic malignancies; and PTZ versus CFP in febrile neutropenic patients with solid tumours who were treated in a general medical setting.
Methods: A prospective randomised open label study of adult patients (>18 years old) with neutropenic fever enrolled from January 2000 to December 2002.
Results: A total of 151 patients were enrolled and randomised to treatment. One hundred and two (102) individuals had haematologic malignancies of which 56 were randomised to PTZ + AMK and 46 received CFP + AMK. There were 43 patients with solid tumour malignancies of which 19 received PTZ and 25 were assigned to CFP treatment. Treatment groups were found to be equal in terms of mean age, duration of neutropenia and number of individuals receiving antibiotic prophyllaxis. For patients with both haematologic malignancies and solid tumour malignancies there were no significant differences in the number of clinically documented infections, positive blood culture isolates (Gram positive or Gram negative) or the outcome of patients (cured/discharged or died from sepsis) regardless of which antibiotic regimen received.
Conclusions: PTZ + AMK and CFP + AMK are equally efficacious in neutropenic patients with haematologic malignancies, and monotherapy with CFP or PTZ is equally efficacious in neutropenic patients with solid tumours. Low dose CFP is safe and allows a reduction of cost and less antibiotic exposure.

P1453 Aspergillus: air quality monitoring in areas reserved for the treatment of immunodepressed patients

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Aims: To ensure that the hyphomycete contamination levels of areas reserved for the treatment of immuno-depressed patients meet the specifications of the IAQ-1998 guidelines, as reported in the Microbiology Reference Guide: total Hyphomycetes at 20 °C <15 cfu/m3; airborne level of Aspergillus <1 cfu/m^3.
Methods: Monitoring air quality in areas reserved for the treatment of immuno-depressed patients, making technical adjustments to the air-conditioning system, and establishing a clear code of conduct for hospital personnel. The presence of airborne hyphomycete spores was detected using both active and passive sampling techniques.
Results: Analyses on hyphomycete spores were carried out by Regional Office Hazard Prevention Environment. The results obtained from 60 active samples taken during 2002 showed that the mean level of airborne hyphomycete spores in the areas under investigation was within the norms established for IAQ-1998 in the Microbiology Reference Guide (total hyphomycetes at 20 °C <15 cfu/m^3). However, Hyphomycetes belonging to the Aspergillus family (though not those of fluvus fumigatus niger) were found in some areas, including the intensive care ward. The maximum airborne level of Aspergillus, as established by the IAQ guidelines, should be <1 cfu/m^3. Following these results, a series of maintenance operations were carried out on the air filters, air-conditioning and ventilation systems, with the aim of reducing and measuring the quantities of hyphomycete spores present. However, the results obtained in 2003, from both passive and active samples, were similar to those obtained in 2002.
Conclusions: Comparison of the results obtained in 2002 and 2003 suggests that the carrying out of regular maintenance checks on air filters, ventilation units and air-conditioning systems, together with the introduction of codes of conduct for hospital personnel, designed to reduce the production and dissemination of airborne particles within areas reserved for the treatment of immunodepressed patients, are not sufficient to ensure that the level of airborne hyphomycetes falls within the limits proposed by the IAQ-98. A more fundamental overhaul of the ventilation system would seem, therefore, to be required, along with a wider diffusion of the established codes of conduct among hospital personnel. To the latter end, a monitoring programme should also be set up to assess to what extent hospital staff are following the recommended procedures.

P1454 Control but not eradication of *Legionella pneumophila* sg1 in a teaching hospital

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Objectives: To examine the sources and methods for control of *Legionella pneumophila* serogroup 1
Methods: A renal transplant patient developed severe pneumonia in July 2003. Bronchial lavage (BAL) was examined by immunofluorescence against *L. pneumophila* and by culture for Legionella and other organisms. Cultures were taken from showers and other sources of water in the transplant unit and adjacent buildings. Earlier outbreaks of Legionella pneumonia had occurred in the same and other buildings of the hospital in 1993 and 1997. Control measures performed had included hot water flushing, heat disinfection of shower tubings and reconstruction of water pipes. Legionella isolates from 1993, 1997 and 2003 were compared, using monoclonal antibodies and amplified fragment length polymorphism (AFLP) as recommended by the European Working Group for Legionella Infections (EWGLI).
Results: *L. pneumophila* serogroup 1 (sg1) was isolated from the patient's BAL. The patient was cured from his legionella infection by treatment with macrolides in high doses. Patient and water isolates from 1997 and 2003 in the transplant unit were identified as *L. pneumophila* sg1 Knoxville or Oxford. AFLP patterns were identical. Other *L. pneumophila* sg 1 isolates from buildings in the hospital in the same years were closely related. Between 1997 and 2003, cultures from the water system of the building housing the transplant unit were stably negative, and no clinical Legionella cases were detected despite close surveillance with legionella cultures of all sputum samples.
Conclusions: Control of *L. pneumophila* sg 1 can be achieved by careful management and surveillance of hot water systems in hospital buildings. Negative cultures from water systems do not guarantee the complete eradication of Legionella. Legionella strains may still persist in the biofilm of water tubings. In severely immunosuppressed patients with pneumonia, Legionella infection must always be suspected.

Antimicrobial resistance mechanisms - II

P1455 Clindamycin resistance among *Streptococcus agalactiae* isolates and genotypes

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Background: Group B streptococci (GBS) are an important cause of serious infections among adults with underlying or chronic diseases. Penicillin is the antibiotic of election for this infections, and for penicillin allergic patients, the alternative is erythromycin (ERY) or clindamycin (CLN). The resistance rate for CLN in ERY-resistant strains of GBS is 58% in our area. The primary mechanism of CLN resistance in *S. agalactiae* is a ribosomal methylase [erm B, erm A (subclass erm TR)]. We studied the erm (B) and erm (A) (subclass erm TR) codes for a rRNA methylase which produces resistance to macrolide-lincosamide-streptogramin B (MLSB) antibiotics. We investigated the relative frequency of MLSB resistance determinants among CLN-resistant GBS isolates.
Methods: A total of 37 GBS isolates, with MICs to CLN higher than MICs to ERY were collected during nine years (1994 to 2002). These strains were characterised by *in vitro* susceptibility testing that was carried out by disc diffusion and E-test. The strains found to be CLN resistant according to the NCCLS guidelines have been phenotypically and genotypically characterised. We studied the isolates looking for the resistance phenotype by disc diffusion using 2 mg CLN and 15 mg ERY discs. The presence of macrolide resistance genes [erm (B) and erm (A) subclass erm (TR)] was detected by PCR. The following antibiotics were tested by the NCCLS agar dilution method: ampicillin (AMP), ERY, CLN, telithromycin (TEL), tetracycline (TET), vancomycin (VAN) and ciprofloxacin (CIP).
Results: The PCR amplification showed that 12 (32%) isolates harboured the erm B gene, 10 (27%) were positive with primers specific for erm A (subclass erm TR) gene and 15 (40%) had both genes erm B + erm A (subclass erm TR). The resistance rates of GBS isolates were 0% for AMP, 89% for ERY, 100% for CLN, 0% for TEL, 86% for TET, 0% for VAN and 3% for CIP.
Conclusions: The majority of our GBS strains had the presence of erm B + erm A (subclass erm TR) genes. All GBS were susceptible to AMP, VAN and TEL. The novel drug, TEL, retained good activity against CLN-resistant strains.

P1456 Spread of the new sul3 gene in human and animal *Escherichia coli* in Sweden

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Objectives: To investigate the molecular basis for sulfonamide resistance in clinical isolates of Enterobacteriaceae and to assess the spread of sul3 in Sweden. This study was part of a follow-up of an observed increasing resistance to trimethoprim and sulfonamides in clinical isolates of *Escherichia coli* despite a simultaneous decline in co-trimoxazole consumption.
Methods: Antibiotic susceptibility tests were performed on 105 urinary tract infection isolates of Enterobacteriaceae. Sulfonamide resistant strains were tested with PCR specific for the sulfonamide resistance genes sul1, sul2 and the recently discovered sul3. Prevalence of sul3 in sulfonamide resistant *E. coli* isolates from healthy Swedish chickens and pigs was also investigated with PCR. Sequencing and plasmid analysis has been performed to investigate the context of the gene.
Results: Of 64 sulfonamide resistant human isolates, 39 were positive for sul1 and 48 were positive for sul2, 25 isolates carried both genes. Two of the isolates were negative for both sul1 and sul2, but were in further tests shown to harbour sul3. Among 49 sulfonamide resistant isolates from chickens, none was positive for sul3, while five of the 40 isolates from pigs carried the new gene.
Conclusions: The only observations of sul3 so far are in *E. coli* from pigs in Switzerland and *E. coli* from pigs, cattle and poultry in Germany. The present study is the first to identify sul3 in human isolates. Additionally, it shows that the gene is spread both among humans and animals also in Sweden. These results imply a possible spread of resistance elements between pigs and humans. The origin and prevalence of this new gene is important to investigate and the genetic context of sul3 might provide clues to the emergence of a new resistance gene to such old antibiotics as sulfonamides.

P1457 Characterisation of a novel AAC (3) I enzyme found in a class 1 integron from a *Salmonella enterica* isolate causing traveller's diarrhoea

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Objective: Detection of class 1 integrons present in *Salmonella enterica* causing traveller's diarrhoea and characterisation of a novel AAC (3) I encoding gene.
Methods: Sixteen antibiotic-resistant Salmonella strains were recovered from faeces of travellers to developing areas. The presence of class 1 integrons was determined by PCR with specific primers. The amplified products were recovered and sequenced in order to establish the genes carried. The sequences were compared with those present in GeneBank. Analysis of plasmids, as well as conjugation was performed in all integron-borne isolates. Additionally, susceptibility to gentamicin C1a, gentamicin C1, sisomicin, neomycin, dibekacin, kanamycin, tobramycin, amikacin, netilmicin, apramycin, dactimicin, spectinomycin, streptomycin, lividomycin and butyrosin was established in the isolate carrying an AAC(3)I.
Results: Four out of 16 (25%) isolates presented at least one class 1 integron, all containing antibiotic-resistance genes. Three of the strains presented a single integron that contained aadB plus catB3 genes, dfrA17 plus aadA5, and aac(3)I-like plus aadA7 genes, while the remaining strain carried two integrons containing a carb2 and aadA2 gene, respectively. Only one strain carried plasmids but no positive conjugation was obtained with either this or the remaining isolates. The homology between the DNA and amino acid sequences of the AAC(3)I-like enzyme and the AAC(3)I enzyme were of 59% and 62%, respectively. The antibiotic-susceptibility to different aminoglycosides in this strain showed resistance or decreased susceptibility to gentamicin C1a, gentamicin C1, dactimicin, and sisomicin, in accordance with the presence of an AAC(3)I. Resistance to streptomycin and spectinomycin was also shown, probably due to the presence of the aadA7 gene.
Conclusions: A high frequency of class 1 integrons has been detected in antibiotic-resistant Salmonella isolates causing traveller's diarrhoea. These integrons present a high variability of resistance genes, mainly associated with aminoglycoside antibiotics. In this line, a novel AAC (3) I encoding gene carried into an integron has been identified.

P1458 ParE mutations in *Pseudomonas aeruginosa* clinical isolates with a high level of ciprofloxacin resistance

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Objectives: Our objective was to unravel the topoisomerase mutations in clinical isolates of *Pseudomonas aeruginosa* resistant to ciprofloxacin. We previously studied 30 *P. aeruginosa* strains for mutations in the quinolone resistance-determining regions of

gyrA, *gyrB* and *parC* (Mouneime *et al*. 1999). Gyrase mutation was observed for all of the isolates (90% in *gyrA* and 10% in *gyrB*), and an additional QRDR *parC* mutation in two-third of the isolates. In the present study, we searched for additional *parE* mutation in all the isolates and for mutation outside the QRDR in the isolates lacking QRDR mutation and harbouring a high level of resistance to ciprofloxacin.
Methods: PCR amplification and sequencing of QRDR *parE* was applied to 30 strains isolated in Pitie-Salpetriere Paris and three strains isolated in Tunis, not susceptible to ciprofloxacin (MICs 2 to 128 mg/L). For strains without mutation in *parE* QRDR, the 5′ end region of *parE* and of *gyrB* was also amplified and sequenced.
Results: *parE* mutation was observed in 10 out of 33 isolates, either in the QRDR (Asp420Asn) for eight strains or outside the QRDR (Val200Met, Ala474Val) for two strains. These 10 isolates shared a *gyrA* Thr83Ile mutation, no mutation in QRDR *gyrB* or in QRDR *parC*, and ciprofloxacin MIC > 4 mg/L. For three strains with a high level of resistance to ciprofloxacin but only one *gyrA* mutation, no mutation was observed in *parE*, or in the entire *gyrB*, leading to the hypothesis of another additional mechanism of resistance such as multiple efflux pumps enhancement.
Conclusions: *parE* mutations were observed in *P. aeruginosa* isolates with a high level of ciprofloxacin resistance (MIC > 4 mg/L) in addition to gyrase mutation (*gyrA* or *gyrB*), and in the absence of *parC* mutation. All *P. aeruginosa* isolates with at least two topoisomerase mutations harboured ciprofloxacin MIC > 4 mg/L whereas all isolates with ciprofloxacin MIC of 2 or 4 mg/L harboured only one *gyrA* mutation.

P1459 Contribution of outer membrane protein alterations and beta-lactamases in carbapenem-resistant *Acinetobacter* spp.

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Objectives: As part of the SENTRY Program, *Acinetobacter* spp. isolates have been screened for resistance to carbapenems (imipenem [IMP] and meropenem [MER]) and ceftazidime (CTZ). Thirty-five isolates exhibiting this profile recovered in 2002 from Buenos Aires, Argentina were evaluated.
Methods: All isolates were tested against CTZ, IMP, MER with and without the serine-b-lactamase inhibitor BRL 42715 or the pump inhibitor reserpine by agar dilution. Beta-lactamase activity against imipenem and meropenem was evaluated using standard spectroscopic techniques. The number of beta-lactamase was first evaluated using Isoelectric Focusing (IEF) experiments and PCR reactions were undertaken to identify b-lactamase genes present in the strains. The OMP profile for each strain was determined and the proteins with decreased expression were submitted to N-terminal sequencing. Four susceptible isolates, recovered from the same medical sites at the same period, were used as negative controls.
Results: The isolates showed MICs of >64 mg/L against CTZ, 16–32 mg/L against IMP, 8–16 mg/L against MER. No significant differences in the MICs with the inhibitors were observed. IEF experiments showed that the isolates possess from two to four different b-lactamases. These results clustered the isolates in three different groups according to the beta-lactamases profile:(I) isolates with pIs of 9.0, 6.7, 5.8 and 5.4, (II) pIs of 9.0, 6.7, 5.8 and (III) pIs of 9.0, 6.7. One strain of each group was submitted for PCR with customer primers to blaTEM, blaCMY, blaCTX-M, blaGES, blaKCP, blaIMIA, blaOXA, blaSHV b-lactamase genes. Sequencing analyses of the amplicons confirmed the presence of blaTEM-1-like gene.
Conclusions: The decreased susceptibility against carbapenems in Argentinean *Acinetobacter* spp. isolates can be due to decreased expression of porins associated to hyper-expression of beta-lactamases that normally have low affinity to carbapenems. The low levels of *in vitro* resistance against carbapenems may jeopardise the treatment of infections caused by this pathogen.

P1460 Post-genomic identification of a new subclasss B3 metallo-beta-lactamase (NOV-1) from *Novosphingobium aromaticivorans*

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Objectives: Due to the recent advance of genome sequencing projects, metallo-beta-lactamase (MBL) homologues have been found to be widespread in microbial genomes, being also present in those of higher organisms. These proteins share significant structural similarity with MBLs, defining the MBL superfamily, and can exhibit a variety of other functions, such as aryl- and alkyl-sulfatase, cyclase, glyoxalase, etc. In the genome of *Novosphingobium aromaticivorans*, a bacterium that presents notable biotechnological interest because of its ability to produce glycosphingolipids and to degrade aromatic hydrocarbons, an open reading frame (ORF) was detected (Saro0160) which encodes a protein sharing 17–28% identity with subclass B3 MBLs. In this work we demonstrated that Saro0160 actually encodes an MBL, and investigated the functional properties of this enzyme, named NOV-1.
Methods: *N. aromaticivorans* SMCC F199 was grown in mineral medium at 20 °C. Beta-lactamase activity was assayed spectrophotometrically. The blaNOV-1 ORF was cloned under the transcriptional control of the T7 promoter in the expression vector pET-9a to obtain plasmid pET-NOV-1. Recombinant plasmids were transformed in *Escherichia coli* BL21(DE3) to evaluate beta-lactamase production.
Results: A crude extract of *N. aromaticivorans*, prepared from cells grown in liquid medium, exhibited hydrolytic activity against imipenem (sp. act., 38 nmol/min.mg of protein), that was inhibited >90% after incubation in the presence of 5 mM EDTA. The Saro0160 ORF, which encodes a putative protein of 372 residues, was cloned in an expression vector. Expression of this ORF in *E. coli* led to the production of an EDTA-inhibitable imipenemase activity (sp. act., up to 1130 nmol/min.mg of protein). If compared with other subclass B3 enzymes, NOV-1 exhibits the highest identities with THIN-B and L1 (28% and 25%, respectively) and a notably longer N-terminal domain (85 additional residues in comparison with L1). A truncated gene, starting from an alternative ATG codon located 249 bp downstream and cloned in the same vector/host system, did not yield any beta-lactamase activity. The NOV-1 enzyme was subjected to functional characterisation.
Conclusions: The metallo-beta-lactamase homologue found in the *N. aromaticivorans* genome encodes a functional MBL. The NOV-1 enzyme is a new subclass B3 MBL that shows peculiar functional and structural features.

P1461 New aminoglycoside and chloramphenicol acetyltransferase-encoding gene cassettes in class 1 integrons from multiresistant *Pseudomonas aeruginosa* clinical isolates

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Objectives: The integron system represents a powerful site-specific recombination mechanism for the dissemination and expression of small mobile monogenic units, the gene cassettes. A large repertoire of gene cassettes carry resistance determinants, such as genes encoding drug modifying enzymes, low-affinity targets, and efflux pumps. Characterisation of integron cassette arrays from clinical isolates has led to the discovery of several new resistance genes. In this work, we report on the molecular characterisation of integrons from clinical isolates of *Pseudomonas aeruginosa*, and on the discovery of two original gene cassettes carrying new aminoglycoside- and chloramphenicol-acetyltransferases determinants.

Methods: *P. aeruginosa* 2306/01 and 208/02 were two epidemiologically unrelated clinical isolates from the University Hospital of Varese (northern Italy), that exhibited a multidrug resistant phenotype and produced the acquired metallo-beta-lactamases (MBLs) VIM-2 and IMP-2, respectively. Characterisation of the variable region of class 1 integrons was carried out by a PCR mapping and sequencing approach. The function of the new resistance determinants carried on gene cassettes was investigated by cloning and expression experiments in *Escherichia coli*.
Results: Molecular characterisation of isolates 2306/01 and 208/02 revealed the presence of two integrons in each of them. In addition to the MBL-encoding integrons, that were different in each isolates and contained known gene cassettes, both isolates carried an identical shorter integron with an array of two new gene cassettes. The first cassette encodes an AacA aminoglycoside acetyltransferase similar to AacA4 (97% identity with the AacA4 encoded by integron In110). The second cassette encodes a new CatB variant, named CatB10, that shares maximum identity with CatB3 and CatB2 (84% and 83%, respectively). The attC site of this cassette is also original, showing the strongest similarity (93% identity) with the attC site of the CatB5-encoding cassette found in Tn840. The function of the new resistance determinants was investigated by expression studies in *E. coli*.
Conclusions: Two new resistance gene cassettes have been identified. Systematic characterisation of gene cassettes present in integrons from clinical isolates is a powerful approach for discovery of new resistance genes and gene cassettes.

P1462 Hyperproduction of AmpC beta-lactamase in a clinical isolate of *Escherichia coli* associated with a 30-base pair deletion in the attenuator region of ampC

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Objectives: To describe a novel mutation in the attenuator of the ampC of an AmpC-hyperproducing clinical isolate of *Escherichia coli* (Ec 47/94).
Methods: Ec 47/94 was isolated from a urine sample submitted at the Clinical Microbiology Department, University Hospital Virgen Macarena, Seville, Spain (1994). A microdilution assay (NCCLS guidelines) was used to determine the MICs of cefoxitin (FOX), cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP) and cefpirome (CPM), alone or in combination with fixed concentrations (4 mg/L) of two inhibitors of serine beta-lactamases: BRL42715 (BRL) or clavulanic acid (CLV). The isoelectric point (pI) and the inhibition profile of beta-lactamases with CLV or cloxacillin (CLX) were determined by isoelectric focusing. Hydrolysis of cephaloridine (CFL) determined by spectrophotometry was used as an indicator of AmpC production. Mutations in the promoter and attenuator of ampC were determined by direct nucleotide sequencing of the PCR products generated using primers specific for this region of ampC.
Results: The MIC (mg/L) of CPM was not affected (0.5) in the presence of BRL. The MICs of FOX, CTX and FEP decreased from >256 to 16 (FOX), from 32 to 0.5 (CTX) and from 0.5 to 0.25 (FEP) by BRL. In contrast, the MICs of these cephalosporins were not reduced by CLV. A band of beta-lactamase with a pI≥9 (inhibited by cloxacillin but not by CLV) was observed. Hydrolysis of CFL was 435 nmoles/mg of protein. The promoter of ampC showed five point mutations at positions −88 (C to T), −82 (A to G), −42 (C to T), −18 (G to A) and −1 (C to T). A deletion of 30 nucleotides between positions +16 to +45 containing the dyad symmetry region of the ampC attenuator was also observed.
Conclusions: The hyperproduction of AmpC beta-lactamase in Ec 47/94 is related with a 30-bp deletion of the ampC attenuator.

P1463 *In vivo* selection of *Enterobacter aerogenes* with reduced susceptibility to cefepime associated with either decreased expression of an outer membrane protein of 40 kDa or hyperproduction of AmpC beta-lactamase

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Objectives: To evaluate the mechanism(s) of decreased susceptibility to cefepime (FEP) in clinical isolates of *Enterobacter aerogenes* (*Ea*).
Methods: Three consecutive isolates (*Ea1*, *Ea2*, and *Ea3*) cultured from bronchial aspirates from the same patient were evaluated. Identification was performed with the VITEK 2 system. The clonal relationship among isolates was determined by REP-PCR. MICs of FEP, cefotaxime (CTX), cefoxitin (FOX) and ceftazidime (CAZ) were determined by microdilution (NCCLS), alone and/or combined with 4 mg/L of clavulanate (CV) or BRL 42715 (BRL), or 250 g/L of cloxacillin (CX). Production of ESBLs was detected by disc diffusion (NCCLS) and Etest (CT/CT-CV and CZ/CZ-CV). The isoelectric point (pI) and the inhibition profile of beta-lactamases (BLs) with CV or CX were determined by isoelectric focusing. Hydrolysis of cephaloridine (CF) and FEP was determined by spectrophotometry. The presence of TEM- and SHV-type ESBL genes was assessed by PCR. The ampR-ampC genes were sequenced. The outer membrane proteins (OMPs) were studied by SDS-PAGE.
Results: The three isolates showed identical REP-PCR pattern. All three isolates were resistant (MICs in mg/L) to FOX (>256), CTX (>32) and CAZ (64). MICs of FEP were 0.5 (*Ea1*), 2 (*Ea2*) and 32 (*Ea3*). The MICs of CTX and FEP were reduced up to ≤1 and ≤0.25 by BRL, and to 0.5 and 0.03 (*Ea1*), 0.125 and 0.03 (*Ea2*) and 1 and 0.5 (*Ea3*) by CX. CV did not affect the MICs of CTX and FEP. ESBLs were not detected. Amplification of TEM- or SHV-type genes was not observed. The three isolates showed the same pattern of BLs (pIs 7.9-8.3, inhibited by CX, but not by CV). Hydrolysis (nmoles/mg) of CF and FP was 3741.0 and 1.3 (*Ea1*), 4000.6 and 2.1 (*Ea2*) and 3797.4 and 17.3 (*Ea3*). The sequences of ampR-ampC genes of *Ea1* and *Ea2* were identical to that of the *E. aerogenes* strain deposited in the GeneBank (accession AF211348). For *Ea3*, however, a point mutation in position 311 of the ampC caused a change of Val to Glu. Three OMPs of 51, 40 and 38 kDa were observed in the three isolates, but the expression of the 40 kDa-OMP in *Ea2* was reduced.
Conclusions: Decreased susceptibility to FEP in *Ea2* is related with reduced expression of an OMP of 40 kDa, whereas that resistance to FEP in *Ea3* is associated with the hyperproduction of AmpC.

P1464 Analysis of the mutations in the pbp genes of penicillin-nonsusceptible pneumococci from Turkey

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Objective: To investigate the alterations in pbp1a, 2b and 2x genes that cause penicillin resistance in *Streptococcus pneumoniae* isolated in Turkey.
Methods: pbp sequence analysis of a total of 21 *S. pneumoniae* (8 high level (PenR), 9 low level (PenI) penicillin resistant and 4 penicillin susceptible isolates) was performed by using ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit. The sequences were compared with PBP1a, 2b, 2x nucleotide and amino acid sequences of PenS isolate R6 and PenI/ PenR strains provided from GenBank by using Clustal X program. KA, KS and KA/KS values, and transition /transversion ratios were determined by Mega version 2.1.
Results: When compared with the sequence of R6, the nucleotide and peptide sequences of PenI/R isolates contained up to 14.9% and 8.5% divergence, respectively. Phylogenetic trees were constructed according to the pbp sequences and it was seen that most Turkish isolates clustered together with high bootstrap values at a separate branch, apart from the GenBank sequences. The most common alteration in the PBP1a sequences of our isolates, was a T371A mutation in the active site STMK motif. A 574NTGY577 block was

also detected in all isolates. A T451A and a E481G mutation was found in the PBP2b sequences in 65% and 86% of the PenI/R isolates, respectively. All isolates also possessed a SVES/TK block between the 570 and 574th aminoacids, instead of the QLQPT sequence of R6. The analysis of the pbp2x genes revealed a T338A alteration in the STMK motif in 71% of the isolates. Three isolates also had a Q552E change located near the KSG box. Moreover, a segment of 406 nucleotides that is 32.7% divergent from the R6 homologue, showed 97% similarity to the pbp2b sequence of *S. mitis*.
Conclusions: This is the first study that analysed the alterations in the pbp sequences of pneumococci isolated in Turkey. As reported previously, the emergence of penicillin resistant *S. pneumonaie* is a consequence of alterations in the three major PBPs.

P1465 Mutations in erm(B) associated with rare, low-level telithromycin resistance in *Streptococcus pneumoniae*: 3-year data from PROTEKT

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Objectives: Since 1999, the susceptibility of *S. pneumoniae* to telithromycin (TEL) has been tracked in the PROTEKT global surveillance programme, and strains showing phenotypic resistance to TEL have undergone molecular analysis to determine underlying resistance mechanisms.
Methods: *S. pneumoniae* isolates, 13 684 in number, were collected over three consecutive respiratory seasons (1999–2002) from patients with community-acquired respiratory tract infections in 32 countries. TEL MICs were determined centrally using the NCCLS microbroth dilution method and interpreted using NCCLS breakpoints (resistant $\geq$4 mg/L) as approved by the NCCLS SAST, January 2003. Multilocus sequence typing, pulsed-field gel electrophoresis and serotyping were performed on TEL-resistant (TEL-R) isolates. The full erm(B) gene (including promoter and control peptide regions) and 4 copies of the 23S rRNA L4 and L22 genes were amplified and sequenced.
Results: TEL showed potent antipneumococcal activity, with no evidence of an increase in TEL MIC over time (mode MIC and MIC90 of 0.008 mg/L and 0.12 mg/L, respectively, in each year). Among erm(B)-positive strains ($n = 2736$), the TEL mode and MIC90 were 0.03 mg/L and 0.5 mg/L, respectively. Only 10 TEL-R isolates (0.07%) were identified, two in Year 1, two in Year 2 and six in Year 3 (7 had MICs of 4 mg/L, 3 had MICs of 8 mg/L): all tested positive for the erm(B) gene and negative for the 23S rRNA L4 and L22 mutations associated with MLSB resistance. Five TEL-R isolates had a 2 base-pair change (TA to AG) in the SD2 site at the start of erm(B). Three TEL-R isolates had a 136 base-pair deletion in the erm(B) promoter region, resulting in removal of the SD2 site: fusion of the remnants of the control peptide and the erm(B) gene resulting in the formation of a new protein, 24 amino acids longer, with SD1 now controlling expression. No erm(B) mutations were identified in the remaining two TEL-R isolates. There was no evidence of reproducible clonal spread of TEL-R isolates between centres.
Conclusions: TEL demonstrates potent *in vitro* activity against *S. pneumoniae*, with no shift in susceptibility to this antibacterial noted over the 3-year study period. Resistance to TEL was rare (<0.1%) and of low level when it did occur, with no evidence of clonal spread. The mutations described here are thought to result in increased or constitutive expression of the erm(B) gene, however, these and other possibilities are yet to be determined.

P1466 Fitness costs of apramycin resistance plasmids conferring cross-resistance to gentamicin and tobramycin

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Objectives: The aminoglycoside apramycin (Apr) has been used extensively in animal husbandry in the United Kingdom since 1978. Apr is not used in humans, but plasmid-mediated apramycin resistance (AprR) has been detected in human isolates of Salmonella, Klebsiella and *Escherichia coli*. The aims of this study were to assess the cost of carriage of AprR plasmids and to determine their transfer frequencies.
Methods: Weekly faecal samples were collected for 3 months from birth, from 11 calves that had not been treated with aminoglycosides. AprR *E. coli* were selected with TBX agar containing 8 mg/L Apr. MICs were determined following BSAC guidelines. Plasmid transfer frequencies were measured between *E. coli* K12 strains by both the end-point method and as a ratio of transconjugants (T) to donors (D). Growth rates of plasmid-carrying and plasmid-free strains were measured in both minimal media (MM) and Luria Broth (LB) by serial dilution and OD600.
Results: AprR *E. coli* was found in six of 11 calves. All AprR *E. coli* (45) were cross-resistant to tobramycin, gentamicin, and netilmicin. The presence of AprR *E. coli* was unrelated to calf age ($F1139 = 1.26$, $P = 0.26$) or sampling date ($F1139 = 1.22$, $P = 0.27$). AprR was conferred by three conjugative plasmids (pUK2001, pUK2002 and pUK2003). pUK2001 and pUK2002 transferred at high frequencies (approximately 10e-2 T/D h-1, 1.16 $\times$ 10e-11 ml per cell h-1). pUK2002 and pUK2003 also carried tetracycline and streptomycin resistance. Analysis of variance of growth rates of plasmid-carrying and plasmid-free *E. coli* J53 demonstrated no significant differences in growth rates in either MM or LB, when measured by serial dilution or OD600 ($P = 0.707$). T-tests revealed that growth rates did not differ significantly when plasmid-carrying and plasmid-free *E. coli* K12 strains were grown in direct competition ($P = 0.722$). Plasmid segregation was discounted, as mixed-effect models of the changes in cell numbers over time revealed no significant difference in the recovery of plasmid-carrying and plasmid-free cells ($P = 0.166$).
Conclusions: AprR plasmids with high transfer frequencies were detected in commensal *E. coli* despite no aminoglycoside usage. The presence of AprR plasmids conferring cross-resistance to medically important drugs, and the apparent lack of a fitness cost associated with carriage, poses severe implications for the transmission of these resistance determinants into clinical bacteria.

P1467 High-level aminoglycoside resistance of *Streptococcus bovis* isolates in a teaching hospital in Hong Kong – studies on prevalence and resistance mechanisms

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Objectives: Optimal therapy for *S. bovis* endocarditis requires a combination of penicillin and aminoglycoside for synergistic effect (1,2). High-level gentamicin resistance (HLGR) is well recognised in enterococci due to the presence of the bifunctional enzyme Aac(6′)-Ie-Aph(2″)-Ia.(3) On the other hand, high-level gentamicin resistance in *S. bovis* has not been reported (4–6). The objectives of this study were to determine the prevalence of HLGR in blood culture isolates of *S. bovis* from year 1990 to 2002 and to investigate the mechanisms of resistance in these isolates.
Methods: A total of 59 unduplicated isolates of *S. bovis* were collected from the Prince of Wales Hospital, Hong Kong. *The S. bovis* isolates were identified to species level by the API 32 Strept system. Antimicrobial susceptibility testing was performed by the standard agar dilution method according to the NCCLS guidelines (7). The standard disc diffusion test using gentamicin 120 mg disc was also performed (7). To detect HLGR genes, PCR was performed according to the method of Vliegenthart *et al*. (8).
Results: Susceptibilities profiles of *S. bovis* were shown in Table 1. All except one HLGR isolates were from Year 2001 and 2002. In Year 2001, 4/9 (44%) of isolates were HLGR while in Year 2002, 2/7 (28%) were HLGR. The antibiotic susceptibility patterns of the seven isolates with HLGR were shown in Table 2. When standard disc diffusion test using gentamicin 120 mg disc was performed, all seven isolates had a zone diameter of 0 mm. Among the 59 isolates, aac(6′)-Ie-aph(2″)-Ia gene was detected in all seven isolates exhibiting HLGR. All other 52 isolates did not harbour this gene.

Table 1. Susceptibilities of 59 isolates of *S. bovis* to various antimicrobial agents

Antimicrobial agents	MIC range (mg/l)	MIC_{50} (mg/l)	MIC_{90} (mg/l)	% Resistant
Penicillin	0.06–0.12	0.12	0.12	0
Imipenem	≤0.015–0.06	0.03	0.12	0
Cefotaxime	0.03–0.25	0.12	0.12	0
Vancomycin	0.25–0.5	0.5	0.5	0
Erythromycin	0.03–>512	0.06	>512	52.5
Clindamycin	≤0.03–>512	0.06	512	52.5
Gentamycin	4–>4096	4	1024	11.8*
Kanamycin	32–>4096	64	> 4096	NA†
Amikacin	8–512	32	64	NA†
Netilmicin	1–512	4	16	NA†
Streptomycin	8–2048	32	64	1.6
Tetracycline	0.25–256	128	256	86.4
Chloramphenicol	2–16	4	8	1.6
Levofloxacin	1–64	2	4	1.6
Linezoid	0.5–2	2	2	NA†
Quinupristindalfopristin	0.25–8	1	4	NA†

*Breakpoint for high-level aminoglycoside resistance for enterococci (i.e. >512 mg/l for gentamicin and >200 mg/l for streptomycin) is used
†NA, no interpretive NCCLS breakpoints are available

Table 2. Antibiotic susceptibility profiles of the HLGR strains

Strain no.	Penicillin MIC (mg/l)	Erythromycin MIC (mg/l)	Clindamycin MIC (mg/l)	Tetracycline MIC (mg/l)	Gentamycin MIC (mg/l)	Kanamycin MIC (mg/l)	Streptomycin MIC (mg/l)	Netilmicin MIC (mg/l)	Amikacin MIC (mg/l)
SB10	0.06	>512	256	128	>4096	>4096	32	64	64
SB28	0.12	>512	16	128	>4096	>4096	64	512	512
SB30	0.12	>512	0.5	128	>4096	>4096	64	128	128
SB33	0.12	>512	512	256	2048	>4096	32	16	32
SB34	0.12	512	128	0.25	2048	>4096	32	32	32
SB40	0.12	>512	512	256	1024	>4096	32	16	32
SB41	0.12	>512	512	256	512	>4096	32	8	32

Conclusions: This is the first report of high-level gentamicin resistance in *S. bovis*. The rapid emergence of HLGR since year 2001 with up to 44% of isolates with HLGR is alarming. The most likely mechanism is due to bifunctional enzyme Aac(6′)-Ie-Aph(2′′)-Ia. Routine screening of HLGR is recommended for all clinically significant blood culture isolates in order to avoid inadvertent use of short course combination therapy of penicillin and gentamicin which may lead to treatment failure for endocarditis and unnecessary usage of gentamicin, a drug with potential toxicity.

P1468 Molecular characterisation of a new variant of IND-type metallo β-lactamase expressed in a clinical isolate of CDC group Gram-negative asaccharolityc rod

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Objectives: The aim of this work was that to characterise the metallo β-lactamase (MBL) determinant present in a Gram-negative rod belonging to the CDC group II B, isolated from sputum of a patient from the Haematology Unit of the Hospital Ferrarotto in Catania (Italy). The strain, did not grow in MacConkey agar and at 42 °C was urease-, indole-, and oxidase-positive, and was able to hydrolyse gelatine. It was resistant to imipenem, meropenem and ceftazidime but susceptible to piperacillin/tazobactam by both diffusion and dilution susceptibility testing.
Methods: MBL production was tested by spectrophotometric method. Genomic DNA was analysed by Southern blot using several metallo-β-lactamase probes including: blaIND-3, blaIND-2, blaVIM-1, blaIMP-1, blaGOB-1. PCR experiments were performed with 20 ng of genomic DNA and using the following primers: IND-for/ 5′ATGAAAAAAAGAATTCAGTTCTTTA and IND-rev/ 5′ TTATTTTTTGTTAAGAAGTTCAAGA. Amplicon generated by PCR was directly sequenced on both strands using an ABI PRISM 377 DNA sequencer. The deduced amino acid sequence of protein was compared with those of natural IND β-lactamases reported in GenBank. The amplicon was cloned in pGEM-T easy vector and the β-lactamase was expressed in *Escherichia coli* JM109.
Results: MBL activity was detected in a crude extract of the isolate. The Southern blot assay, performed with several metallo-β-lactamase probes, revealed the strongest hybridisation signal with the blaIND-3 probe. The PCR analysis using the IND primers yielded an amplicon of 720 bp, encoding an IND variant that was different from IND-3 by 18 amino acid residues.
Conclusions: A new IND MBL variant was identified in a CDC group II B clinical isolate. The present report points to the presence of MBL genes in clinical isolates of this group of bacteria. MBL production can be a mechanism of carbapenem resistance in similar isolates.

P1469 Characterisation of beta-lactam resistance among clinical *Escherichia coli* strains in Portugal

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Objectives: As part of an antimicrobial resistance surveillance program we evaluated the prevalence and mechanisms of beta-lactam resistance in *E. coli* in Portugal which is mainly attributed to beta-lactamase production.
Methods: Unduplicated strains, 2447 in number, (83% from urine) were collected consecutively from 15 hospitals and three public health laboratories, during 6 months in 1999. Disc diffusion selected amoxicillin resistant (AmxR) strains and MICs of AmxR *E. coli* (1176 strains) were determined against 20 antibiotics by agar dilution. Isoelectric focusing was used to characterise beta-lactamases. Extended spectrum beta-lactamases (ESBL) were selected by synergy with clavulanate and by a multiplex-PCR method and were identified by sequencing.
Results: Among 2447 *E. coli* 48% were AmxR. All AmxR strains were susceptible to imipenem, but 88%, 8%, 19%, 12%, 0.8% and 0.9% were resistant to Amx-clavulanate, piperacillin-tazobactam, mecillinam, cefuroxime, cefotaxime and ceftriaxone, respectively; 2.6% were resistant to both ceftazidime and aztreoname; 1113/2248 (49.5%) and 63/199 (31.7%) AmxR strains accounted for hospital and community acquired infections, respectively. We detected higher prevalence to AmxR in urinary tract infections from patients in ICU 15/24 (63%) and surgery 24/42 (57%) than in outpatients 60/191 (31%). Forty-six AmxR plus cefoxitin susceptible strains (3.8%) showed synergy between C3G and CL, and AmxR plus cefoxitin resistant strains with that synergy accounted to 23/1176 (2%). Molecular plus phenotype methods showed that beta-lactam resistance in *E. coli* was mainly due to the production of TEM-1, AmpC, OXA, SHV-1, ESBL (TEM-derived) and IRT beta-lactamases.
Conclusions: This study shows the high prevalence of beta-lactam resistance in *E. coli* strains from nosocomial origin, in Portugal. It also highlights the importance of rational use of this family of antibiotics, and the need to continue surveillance and infection control programmes.

P1470 Molecular basis of fusidic acid resistance in *Staphylococcus aureus* from patients with atopic dermatitis undergoing therapy with fusidic acid

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Objectives: We examined the basis of fusidic acid (fus) resistance in a collection of *S. aureus* strains recovered from 18 patients being treated for atopic dermatitis with topical fusidic acid.

Methods: PFGE-typing was conducted according to the HARMONY protocol, and susceptibility testing performed using Etest. Strains were examined for fus resistance polymorphisms in the drug target by PCR amplification and DNA sequencing of fusA. For detection of the acquired staphylococcal fus-resistance determinant, fusB, southern hybridisation was employed to probe both total DNA and purified plasmid DNA preparations. Strains were defined as resistant or susceptible to fus according to breakpoints recommended by the British Society for Antimicrobial Chemotherapy (MIC $\leq$1 μg/ml: susceptible, MIC $\geq$ 2 μg/ml: resistant).
Results: Strains from 18 patients were examined, nine of whom carried fus-sensitive strains at the start of treatment. In four of the latter group of patients, cross-infection with a fus-resistant strain occurred during the period of treatment as judged by the isolation of strains exhibiting $\leq$70% PFGE homology with the original, sensitive isolate. In the other five patients in this group, resistant strains developed during treatment. The resistant isolates from all nine patients carried mutations in fusA that involved analogous substitutions to those found in laboratory-derived fusR mutants. The remaining 9/18 patients carried strains that were fully- or intermediately resistant (MIC range of 1.5 to >256 μg fus/ml) from the outset, and no increase in resistance or cross-infection with more highly resistant strains was observed during treatment. Resistance in strains from this second group of patients resulted from the presence of the fusB determinant (2/9), resistance polymorphisms in fusA (3/9) and additional, unidentified mechanisms (4/9).
Conclusions: *S. aureus* strains recovered from 18 patients with atopic dermatitis being treated with fus exhibited various mechanisms of resistance to the antibiotic, including mutations in fusA (12/18), carriage of fusB (2/18) and the presence of other, uncharacterised determinants (4/18).

P1471 High frequency of association between CTX-M-beta-lactamase-coding genes and the ISEcp1 insertion element

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Objectives: CTX-M beta-lactamases are becoming an increasingly alarming problem throughout the world due to their fast spread. This is often addressed to the possible transfer of CTX-M-coding genes with mobile genetic elements. Indeed, several studies have shown an association of blaCTX-M genes with the ISEcp1 insertion sequence or the putative recombinase gene orf513. However, such studies have been confined to relatively small numbers of strains. We have studied the association of blaCTX-M genes with ISEcp1 using a large collection of CTX-M-producing enterobacterial strains obtained from 21 Russian hospitals.
Methods: The strains studied were 28 *Escherichia coli* (EC), 87 *Klebsiella pneumoniae* (KP) and 36 *Proteus mirabilis* (PM). They comprised 17, 48 and 22 distinct genetic types, respectively, as determined by ERIC-PCR and RAPD typing and produced CTX-M-1-cluster enzymes (94.7%), i.e. CTX-M-3 and CTX-M-15, and a CTX-M-2-cluster enzyme (5.3%) – CTX-M-5. Two PCRs with ISEcp1-specific primers were used to identify the linkage of blaCTX-M genes with ISEcp1. One forward primer (F1) matched the 3′-end sequence of tnpA and another (F2) matched the right terminal repeat (RTR) sequence of ISEcp1. A common reverse primer (R) was located internally to blaCTX-M. Previously characterised EC and *Citrobacter freundii* strains harbouring blaCTX-M genes associated with either ISEcp1 or orf513 were included as positive and negative controls, respectively.
Results: Positive PCR results with primers F2-R were observed for 28 (100%) EC, 86 (98.9%) KP and 35 (97.2%) PM isolates. Amplification with primers F1-R additionally confirmed the association of blaCTX-M genes with ISEcp1 in 25 (89.3%) EC, 85 (97.7%) KP and 34 (94.4%) PM isolates. According to the length of PCR products, ISEcp1 was located approximately 50 bp upstream of the CTX-M ORFs in all EC, KP and PM isolates expressing CTX-M-1-cluster enzymes and approximately 20 bp upstream of blaCTX-M-5 in eight EC isolates. An insertion of ~700 bp sequence between tnpA and RTR was detected in a single KP isolate of a unique genetic type. Two isolates (PM and KP) which failed to produce PCR products with either of the primer pairs also represented unique genetic types.
Conclusions: We conclude that CTX-M-coding genes of different genetic subtypes have a strong association with the ISEcp1 insertion element in nosocomial Enterobacteriaceae from Russia. This in part may explain their notoriously rapid spread.

P1472 Fluconazole resistance mechanisms in *C. krusei* clinical isolates. The contribution of efflux pumps

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Objective: Infections caused by *C. krusei* have purportedly increased during recent years and the inherent resistance of this agent to fluconazole is well known. The main resistance mechanism for fluconazole is the diminished sensitivity of the target enzyme cytochrome P450 sterol 14 μ-demethylase (CYP51) to inhibition by azole agents. According to studies using a limited number of strains, an alternative mechanism of resistance could be based on the activity of efflux pumps. The aim of our study was to know the possible contribution of efflux pumps in conferring resistance to fluconazole in a large study of *C. krusei* isolates.
Methods: We obtained 22 *C. krusei* strains from different sources: urine (4), mucous membranes (4), genital (3), respiratory (2), catheters (3), sterile fluids (4), wound (1) and 1 ATCC 6258 strain. The activity of efflux pumps was checked using the inhibitor CCCP (carbonyl cyanide 3-chloro-phenylhydrazone) which could decrease the minimum inhibitory concentration (MIC) if resistance was correlated to this mechanism. We established a concentration of 0.5 mg/ml of CCCP, and verified that it did not kill the yeast. The susceptibility patterns of our isolates for six antifungal drugs (amphotericin B, fluconazole, itraconazole, ketoconazole, flucytosine and voriconazole) were determined according to an NCCLS M27-A protocol modification (Sensititre Yeast One). We tested all the strains before and after adding the CCCP to the RPMI medium.
Results: The MIC90s and ranges of the drugs were identical before and after CCCP treatment: amphotericin B 0.5 mg/ml [1–0.25]; fluconazole 32 mg/ml [64–8]; itraconazole 0.125 mg/ml [0.25–0.03]; ketoconazol 0.25 mg/ml [0.25–0.03]; flucytosine 4 mg/ml [8–1] and voriconazole 0.125 mg/ml [0.25–0.06]. The MIC for fluconazole was higher than for the other antifungals. The new triazoles appeared to be quite active and the MICs were lower. Only one isolate showed a twofold decrease in MIC to fluconazole when CCCP was added. We did not find any multi-resistant strains.
Conclusions: According to our study with *C. krusei*, the role of efflux pumps inhibited by CCCP in the resistance to fluconazole is scarce.

P1473 Characterisation of the Ambler class B chromosome-encoded β-lactamases SLB-1 and SFB-1 from *Shewanella livingstonensis* and *Shewanella frigidimarina*

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Shewanella livingstonensis and *Shewanella frigidimarina* are psychrophilic Gram-negative rods belonging to the Alteromonadaceae family. Two strains isolated from Antarctic coastal areas were studied for their β-lactamase content since carbapenem-hydrolysing oxacillinases have been recently characterised from other Shewanella species, with demonstration of their role in acquired resistance in Enterobacteriaceae. Shotgun cloning experiments gave recombinant clones expressing Ambler class B enzymes. SLB-1 and SFB-1, identified from *S. livingstonensis* and *S. frigidimarina*, respectively, that hydrolyse penicillins and ceftazidime and at a lower level imipenem. These metallo-enzymes are inhibited

by EDTA but not by clavulanic acid, as for class B β-lactamases. SLB-1 and SFB-1 shared 67% amino acid identity, and are weakly related to the IMP-type (40%), VIM-type (30%) enzymes, and to SPM-1 (30%). This study underlines the fact that the Shewanella species are environmental bacteria that may constitute a reservoir for carbapenem-hydrolysing enzymes, as observed with Flavobacteriaceae. Interestingly, this study showed that *Shewanella* spp. may be the source of carbapenemases belonging to Ambler class B and Ambler class D groups.

P1474 Evidence for an efflux pump affecting carbapenem susceptibility in *Bacteroides fragilis*

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Objectives: We sought evidence for an efflux pump mechanism affecting carbapenems among clinical isolates of *B. fragilis*.
Methods: Recent isolates were obtained from a multicentre network of microbiology laboratories serving tertiary care hospitals in the USA. MICs were done by an NCCLS-approved broth microdilution method in the presence or absence of these pump inhibitors: reserpine (0.125–0.40 mcg/ml), verapamil (1.56–100 mcg/ml) and B-naphthylamide (3.13–100 mcg/ml). We tested ertapenem, meropenem and imipenem, as well as cefoxitin, cefotaxime, piperacillin-tazobactam, moxifloxacin and chloramphenicol. Two strains with discordant susceptibility for ertapenem (8–16 mcg/ml) and imipenem (2–4 mcg/ml) were studied in more detail: outer membrane proteins (OMPs) were separated by SDS-PAGE and stained with Coomassie blue. ATCC25285 was included in all MIC and OMP experiments.
Results: Imipenem susceptibility was affected by reserpine, but not the other inhibitors, in all strains (see Table):

			MIC various carbapenems in the presence of efflux pump inhibitor							
			Efflux pump inhibtor (EPI) concentration (ug/ml)-Reserpine							
Strain	Drug	Conc. range	0	1.25	2.5	5	0	20	40	fold decrease
ATCC25285	Imipenem	2–0.002	0.25	0.25	0.25	0.25	0.25	0.25	0.0625	4
	Meropenem	2–0.002	0.125	0.125	0.125	0.125	0.125	0.125	0.0625	2
	Ertapenem	2–0.002	0.125	0.125	0.125	0.125	0.125	0.0625	0.0313	4
LSU5	Imipenem	2–0.002	>2	>2	>2	>2	>2	2	0.125	>=32
	Meropenem	32–0.0313	16	16	16	16	16	16	8	2
	Ertapenem	16–0.015	8	8	8	8	3	16	4	2
LSU11	Imipenem	2–0.002	2	2	2	2		1	0.0625	64
	Meropenem	32–0.0313	8	8	8	8	4	4	"	8
	Ertapenem	16–0.015	2	4	4	4	4	4	"	4

In two clinical isolates, imipenem MICs declined 32 and 64 fold respectively; the ATCC strain showed a 4-fold decline. Other beta-lactams were affected to a lesser degree, 2 to 8 fold. OMP analysis revealed overexpression of a band at 48 kD in the two clinical isolates, which had higher MICs for ertapenem than imipenem. Sequencing of this protein is in progress.
Conclusions: 1. We found phenotypic evidence for an efflux mechanism affecting carbapenem susceptibility in these strains. Imipenem seemed to be the most affected. 2. The overexpressed 48 kD OMP in organisms with higher ertapenem MICs may be an efflux pump.

P1475 Macrolide resistance and its genetic mechanism in *Streptococcus pneumoniae* in Finland 2002, with special reference to telithromycin resistance

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Objectives: The aims of this study were to follow the development of macrolide resistance, especially telithromycin resistance, in *Streptococcus pneumoniae* in Finland and to investigate genetic mechanisms of macrolide resistance.
Materials and methods: *S. pneumoniae* strains, 1007 in number, were collected from 24 FiRe laboratories, which represent the whole country. Strains were isolated both from non-invasive (n = 878) and invasive (n = 129) infections. The mininum inhibitory concentration (MIC) to macrolides (erythromycin, azithromycin, spiramycin, telithromycin and clindamycin) was tested by using agar plate dilution technique. The presence of resistance genes: mef(A/E), erm(B), erm(TR), was tested with a multiplex-PCR -method from the strains with erythromycin MIC equal or higher than 0.25 mcg/ml.
Results: Of 1007 pneumococcal strains, 21% ($n = 215$) were resistant to erythromycin (I + R, MIC equal or higher than 0,5 mcg/ml). Five of these strains had intermediate susceptibility. Resistance to azithromycin was 33%, and to clindamycin 11%. Seven per cent of pneumococci ($n = 71$) had reduced susceptibility to telithromycin (MIC equal or higher than 1 mcg/ml). The genotype was tested from 224 strains (22%). Mef(A/E) was the most common genotype; 48% of strains were positive ($n = 107$), 40% of strains harboured erm(B) gene ($n = 90$), and erm(TR) was found from one strain only. Double mechanism, mef(A/E) + erm(B), was detected from 5 strains (1%). PCR results were negative in 9% of tested strains ($n = 21$). All those strains, which had reduced susceptibility to telithromycin, also had resistance mechanism in their genome (mef(A/E) $n = 35$, erm(B) $n = 33$, and double-mechanism $n = 3$).
Conclusions: Resistance to macrolide-lincosamides is widespread and is increasing among pneumococci in Finland. In macrolide resistant pneumococci, mef(A/E) seems to be still the most common mechanism in Finland, although erm(B) is also frequently present. The interesting finding was that unexpectedly high proportion of pneumococci (7%) had reduced susceptibility to telithromycin.

P1476 Antibiotic and biocide resistance mechanisms in *Escherichia coli* O157

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Background: Bacterial resistance to antibiotics and biocides is a prevalent problem, which may be exacerbated by the commonplace and often unnecessary inclusion of biocides into domestic products.
Objectives: This study investigated potential adaptive resistance in *Escherichia coli* O157 to commonly employed antibacterial agents in order to identify mechanisms underlying any resistance obtained.
Methods: *E. coli* O157 strains were serially exposed to sub-inhibitory concentrations of erythromycin (ERY), benzalkonium chloride (BKC), chlorohexidine (CHX) and triclosan (TLN). Following each passage the MIC of the antibacterial and any adaptive resistance was recorded. Adaptive resistance was readily promoted following only two sub-inhibitory exposures. Permeability changes in the outer membrane, including LPS, cell surface charge and hydrophobicity and the presence of an active efflux were investigated as possible resistance strategies. The outer membrane and LPS profiles were analysed by SDS-PAGE and visualised by Coomassie blue and silver staining, respectively. The cell surface charge and hydrophobicity were investigated employing microelectrophoresis and microbial adhesion to hydrocarbons (MATH assay). Efflux activity was examined by comparing the level of resistance in pre- and post-adapted strains in the presence of the efflux inhibitor, reserpine. In addition, the FabI gene of pre, 1st and post- adapted O157 was sequenced and compared, in order to investigate the presence of any possible mutations conferring increased resistance.
Results: Examination of the outer membrane LPS did not reveal any significant changes between pre- and post-adapted strains. There was no correlation between cell surface charge and hydrophobicity. However, the hydrophobicity of the cells increased as the cells were passaged and became adaptively resistant. An

efflux system was the most likely mechanism of BKC and CHX resistance, whereas a FabI mutation was associated with TLN-adapted strains. The adaptive resistance to erythromycin, BKC, CHX and TLN in cells of *E. coli* O157 was stable for over 30 passages in biocide-free media.
Conclusions: In summary, increased cell surface hydrophobicity, the presence of the FabI mutation in addition to that of an active efflux pump could facilitate the acquisition of antibacterial resistance in *E. coli* O157 providing cross-resistance to nine of 17 antimicrobials investigated.

P1477 A novel SHV-derived extended-spectrum beta-lactamase (SHV-52) that hydrolyses ceftazidime through a single-amino-acid substitution (L169R)

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ESBL producing bacteria comprise one of the most serious resistance problems in hospitals especially in nosocomial infections. New ESBLs have emerged rapidly because of the overuse of antibiotics. A new SHV-derived extended-spectrum beta-lactamase (temporarily designated as SHV-52) conferring high-level resistance to ceftazidime but not cefotaxime was identified from an island-wide surveillance in 1998. *Escherichia coli* TSARI 981223, which is resistant to ampicillin, cephalothin, cephaloridine, cefpodoxime and ceftazidime while being sensitive to cefoxitin, ceftriaxone, cefotaxime, imipenem and the first-generation cephem cefazolin was isolated from the urine of a patient treated with beta-lactam antibiotics. Resistance to beta-lactams was transferred by conjugation from *E. coli* TSARI 981223 to *E. coli* JP995, and the transferred plasmid was about 50 kbp. The pI of this enzyme was 8.2. The ceftazidime resistant gene was cloned from the transferred plasmid. The sequence of the gene was determined, and open reading frame of the gene was found to consist of 861 bases (The GenBank accession number is AY223863). Comparison of SHV-52 with other SHV beta-lactamases suggests that the substitution of arginine for leucine-169 is important for the substrate specificity. Characterisation of the enzyme by molecular biological and enzymological methods will provide us information of the extension of substrate specificity. The three-dimensional model of the enzyme will provide critical information in modelling and developing new antibiotics in the future.

Mycobacterial diagnosis

P1478 Evaluation of a RAPID™ bioactive amplification with probing *Mycobacterium tuberculosis* (RAPID™ BAP-MTB) assay for identification of *M. tuberculosis* in respiratory specimens

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Objectives: To evaluate a novel molecular diagnostic assay for identification of *M. tuberculosis* in respiratory specimens.
Methods: Six hundred respiratory specimens obtained from patients treated at National Taiwan University Hospital from July 2003 to October 2003. RAPID™ Bioactive Amplification with Probing (BAP) *M. tuberculosis* (RAPID™ BAP-MTB) assay was used and the results were compared with those obtained from acid-fast microscopy, conventional cultures using BACTEC MGIT 960 Systems, and clinical history.
Results: The RAPID™ BAP-MTB assay could detect as low as 10 fentogram of MTB genomic DNA. The overall time for processing the RAPID™ BAP-MTB assay took about 5 h. Among the 600 samples, 56 (9.3%) were culture positive for MTB. One specimen was culture positive for MTB but negative using RAPID™ BAP-MTB assay. Twelve samples were positive by the novel method but had negative cultures for MTB. The sensitivity of the novel method was 97.8% and the specificity was 97.3%.
Conclusions: RAPID™ BAP-MTB assay is a promising method for rapid identification of MTB in respiratory specimens.

P1479 Early laboratory diagnosis of pulmonary tuberculosis. How to evaluate the results?

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Objective: The culture examination in Lowenstein–Jensen media, although inexpensive and specific, requires several weeks since growth of Mycobacterium tuberculosis species relatively to the MB/BacT system. The aim of this study was to evaluate the efficacy of the MB/BacT culture system in pulmonary tuberculosis patients.
Methods: The authors realised a retrospective study of 22 clinical cases with pulmonary tuberculosis which diagnosis was confirmed by the mycobacteriologic test of sputum, bronchial aspirate and/or bronchoalveolar lavage. To evaluate the efficacy, we used the chi-square test relatively to the recovery rate, and the *t* student test to the detection time.
Results: The mean age was 52.5 ± 20.6 years and 15 were male. The major symptoms were productive cough (95.4%) and vespertine fever (72.3%). By the radiological image, 36.4% had pulmonary tuberculosis that was very extensive. Relative to the examination of the culture, we verified that in 18 patients the Lowenstein–Jensen media was positive with 81.8% of sensibility, while in the MB/BacT system the sensibility was more elevated, 95.5% ($P < 0.0001$). The detection time in MB/BacT system was 14.0 ± 9.2 days and in LJ media 23.0 ± 5.0 days ($P < 0.0001$).
Conclusions: The MB/BacT system had a higher recovery rate and a lower detection time than Lowenstein–Jensen media and could be applied as a routine method to detection of *M. tuberculosis*.

P1480 Rapid cultivation and improved detection of *Mycobacterium tuberculosis* and other mycobacterial species by use of culture supplements

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Objectives: Rapid detection of the growth of mycobacterial species and susceptibility testing in liquid media has greatly reduced the time by which the overall processing of culture-positive samples is completed. This procedure has also reduced efforts invested in culture negative samples. The detection of minimal growth at an early stage combined with the use of nucleic acid-based tests for mycobacterial species identification and their drug susceptibility markers provide a unique opportunity for more rapid diagnostics. However, the time required for growth and less than optimal media are still limiting factors for the performance of culture systems. Our aim is to improve the sensitivity and reduce the time for the detection of mycobacteria in clinical specimens.
Methods: Clinical samples investigated for the presence of mycobacteria were cultivated in the culture system BACTEC MGIT 960 system which employs the Middlebrook 7H9 medium and a fluorescent indicator for growth detection. The samples were cultivated in the regular BBLTM Middlebrook 7H9 medium with and without the addition of culture supplements. Among the culture supplements added were bovine serum albumin and hemin.

Results: Among the 800 clinical samples investigated for the presence of mycobacteria, 54 were positive for *Mycobacterium tuberculosis*. The culture supplements improved the recovery of *M. tuberculosis* from patients and increased the sensitivity of *M. tuberculosis* cultivation by 20%. Furthermore, the mycobacterial culture supplements reduced the time required for the detection of growth of *M. tuberculosis* 5.4 days from an average of 16.2 days (range 8–24 days) without supplement addition to an average of 10.8 days (range 2–22 days) with supplement. The sensitivity and rapidity of culture were also enhanced for the detection of other mycobacterial agents, but to various extents in the different species investigated.
Conclusions: Significantly shorter cultivation time and 20% higher sensitivity in *M. tuberculosis* cultivation was achieved by adding culture supplements to liquid Middlebrook 7H9 medium. Also, a number of non-tuberculous mycobacterial species grew faster with culture supplements addition than without. These results hold promise for significant improvements in culture-based mycobacterial diagnostics.

P1481 Identification of clinical nontuberculous mycobacteria isolates by using polymerase chain reaction–restriction enzyme analysis in a teaching hospital

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Objectives: Because of the increasing incidence of non-tuberculous mycobacteria (NTM) infections and their clinical importance, in terms of strategies for treatment and the epidemiological implications, the rapid differentiation of causative mycobacteria is important. Identification of clinical isolates of mycobacteria to the species level is primarily based on cultural characteristics and biochemical tests. These conventional tests take several weeks, and sometimes fail to provide precise identification. The use of liquid culture systems like BACTEC 460-TB system in the clinical laboratory improves the ability to detect the growth of mycobacteria. Recently developed molecular methods, such as DNA sequence analysis, Polymerase Chain Reaction–Restriction Enzyme Analysis (PRA), Single-Stranded Conformation Polymorphism Analysis (SSCP), and DNA probe tests, offer identification of this NTM from a positive liquid culture medium prior to detection of growth on solid media.
Methods: Seventeen NTM isolates identified using BACTEC 460 TB during the study period (March 2001–April 2003) were analysed by the PRA method for identification of NTM subspecies. Due to the low NTM prevalence in our laboratory, the identification of all mycobacterium by PRA method would increase cost and labour. So, we also added the evaluation of cord formation to our study as a simple, cheap and reliable method for the preliminary diagnosis of *Mycobacterium tuberculosis* complex (MTK). All MTK and NTM isolates were identified definitely by cord formation in this study.
Results: We detected 11 of 17 isolates as *M. gordonae*, one as *M. abscessus* type 1, one as *M. intracellulare* type 1, one as *M. nonchromogenicum* type 2, two as *M.genavense* and one isolate as *M. thermoresistible*.
Conclusions: PRA method is a rapid, easy and reliable method that provides detection of multiple subspecies in a single step for identification of NTM in developed microbiology laboratories. On the other hand, the evaluation of cord formation is cost-effective in the laboratories with low NTM prevalence and helpful for the selection of molecular study method.

P1482 Tuberculosis of the parotid gland. Diagnosis by polymerase chain reaction

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Introduction: Tuberculous (TB) parotitis is a rare condition in developed countries and most cases have been documented in Africa and India. Even where disease is prevalent, TB infection of the PG is rare as the sole disease focus. It presents as a swelling or abscess involving the parenchyma of the gland either through haematogenous spread or from infection of lymph nodes within or around it.
Case report: A 35-year-old Greek woman presented with a gradually increasing swelling of the left PG of 9 months' duration, but was otherwise well. She was given several courses of different antibiotics and underwent an evacuating puncture with no improvement. She had two discharging sinuses and the overlying skin was reddish, with a normal temperature. Left cervical lymphadenopathy was present. Laboratory investigations showed a mild lymphocytopenia. Tuberculin test had a weal of 9 mm. Staining for acid-fast bacilli (AFB) and culture of the discharge were both negative. Chest radiograph was normal. Contrast enhanced CT showed an enlarged left PG with filling defects and a mass extending to the parapharyngal region. Histopathological examination revealed a florid granulomatous process with areas of necrosis. Staining for AFB was negative but tissue was not cultured for Mycobacteria. Paraffin sections' PCR was positive for Mycobacterium tuberculosis complex and TB parotitis was diagnosed. Afterwards a three-drug antituberculous regimen was started and being now in the third month of therapy, she shows progressive improvement.
Discussion: TB parotitis is usually not suspected as a possible cause of parotid tumour, because there may be no clinical clues for this. It is very important for the clinician to have the opportunity to review the case when the suspicion of tuberculosis arises. Such an opportunity is provided by PCR, which is a rapid – within 48 hours – and sensitive technique for the detection of mycobacterial DNA in formalin-fixed and paraffin-embedded tissue stored even for more than 5 years.

P1483 Polymerase chain reaction based detection of *Mycobacterium tuberculosis* complex in lupus vulgaris: a case report

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In recent years, cutaneous tuberculosis (CTB) with an atypical clinical appearance has become more common in developing countries and is often confused with other granulomatous dermatosis. The diagnosis of CTB remains troublesome, because of the difficulty of detecting mycobacteria in skin lesions by conventional laboratory methods. Lupus vulgaris (LV), the commonest of all forms of CTB can affect earlobes as well as the other parts of the body. In this article, we present a 20-year-old male patient with LV of left earlobe. In the initial period, pyodermia was the misdiagnosis and the patient was treated superfluously with antibiotics for four years, elsewhere. The definitive diagnosis of the disease was confirmed by polymerase chain reaction (PCR). Mycobacteria could not be seen or isolated by stained smears and conventional and radiometric culture methods from the skin biopsy specimens. The lesion was treated successfully with antituberculous chemotherapy (ATBC). PCR is a reliable and a very rapid method for establishing or confirming the diagnosis of CTB as seen in our patient.

P1484 Failure to detect the IS6110 repetitive DNA element of *Mycobacterium tuberculosis* in exhaled breath condensate of patients with active pulmonary tuberculosis

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Objective: To detect the presence of IS6110 repetitive DNA element of *Mycobacterium tuberculosis* (MTB) in exhaled breath condensate (EBC), a simple, non-invasive method to sample pulmonary lining fluid of patients with active pulmonary tuberculosis.

Methods: Ten hospitalised patients with positive sputum smears for MTB were identified by routine microscopy. Sputum samples were sent for routine culture and susceptibilities. EBC was collected from these patients within 6 days of initiating anti-tuberculous therapy (median, 36 h). EBC samples were concentrated by high-speed centrifugation and then analysed by RT-PCR using primers designed to amplify a 245 base-pair IS6110 DNA fragment of MTB. Amplified DNA was detected by ethidium bromide staining, agarose gel electrophoresis and confirmed by SaI digestion. Exogenous MTB DNA was added to EBC samples to detect PCR inhibitors. EBC samples were also cultured on Lowenstein/Jensen agar plates for 6 weeks.
Results: MTB was identified in nine out of 10 routine sputum cultures. One isolate was identified as *M. kansasii*. The IS6110 repetitive DNA element of MTB was not detected in any of the 10 EBC samples. By contrast, exogenous MTB DNA added to EBC samples evoked the characteristic band pattern of MTB on agarose gel electrophoresis. Cultures of EBC showed no growth of MTB.
Conclusions: The IS6110 repetitive DNA element of MTB was not detected in EBC in patients with active pulmonary tuberculosis. This could be related, in part, to anti-tuberculous therapy initiated before collection of EBC. We suggest that EBC should be collected from these patients before initiating anti-tuberculous therapy.

P1485 Is it necessary to incubate for eight weeks specimens submitted to the mycobacteriology laboratory?

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Objectives: Traditionally, the recommended incubation time for specimens submitted to the mycobacteriology laboratory is eight weeks. Today, with the introduction of liquid media in laboratory routine, the time for detection of mycobacteria could be reduced. The aim of this study is to analyse the reduction of the incubation time from 8 to 6 weeks and to evaluate its impact on the number of mycobacteria recovered.
Methods: We studied all mycobacteria strains isolated during the period 2000–2002. The specimens were decontaminated by standard procedures and inoculated onto liquid media (MGIT), and solid media (Lowenstein-Jensen Pyruvate (LJP)). The incubation times for MGIT and LJP were 6 weeks and 8 weeks, respectively. Growth rates were: one-fold each hour for MGIT and one-fold each week for LJP.
Results: During the study period, we recovered 1769 mycobacteria, (907 *Mycobacterium tuberculosis* complex (MTB), 699 *Mycobacterium avium* complex (MAC), and 163 other non-tuberculous mycobacteria (NTM)). Of these, only 93 (5.3%) were recovered in LJP after more than 42 days although 72 out of 93 also grew in MGIT (incubation time <42 days). Therefore, 21 strains grew only in LJP after more than 42 days. These mycobacteria were grouped as follows: 15 MAC, 2 MTB and 4 NTM. We reviewed the total specimens of patients with these 21 mycobacteria and in all cases the mycobacteria were isolated in other specimens, with the result that a diagnosis could be made in all patients.
Conclusions: The reduction of incubation time from eight to six weeks does not involve a significant reduction in the number of mycobacteria recovered. It can lighten the workload of the mycobacteria laboratory and provide physicians with prompt results.

P1486 A new molecular method for the identification of mycobacteria in clinical samples

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Objectives: Nowadays, molecular methods for the identification of significant mycobacteria are tedious and cumbersome, and they are not available in all laboratories. This study evaluates two new assays for the identification of mycobacteria: Genotype® MTBC for the species included in *Mycobacterium tuberculosis* complex and Genotype® Mycobacterium for the most frequent mycobacteria.
Methods: We selected strains isolated in our laboratory and used ATCC strains as a quality control; MTBC isolates were previously identified by two different methods: AccuProbe® and biochemical tests (Bch T), and non-tuberculous mycobacteria (NTM) were identified by PRA, Bch T and GL-chromatography. Genotype®assay is based on the amplification of the 16S-23S rDNA spacer region followed by reverse hybridisation. The assay takes approximately 6 hours to complete.
Results: We analysed 10 MTBC + 22 NTM strains. Genotype®MTBC correctly identified five *M. tuberculosis*, four *M. bovis* (one of them BCG) and one *M. africanum*. Genotype® Mycobacterium accurately identified some strains of *M. chelonae*, *M. celatum*, *M. fortuitum*, *M. gordonae*, *M. kansasii*, *M. malmoense*, *M. peregrinum*, *M. intracellulare*, *M. avium*, and *M. tuberculosis* complex. Discrepant results were observed only in two strains.
Conclusions: Genotype® is a rapid, easy and reliable tool for the identification of significant mycobacteria. Genotype® MTBC is the only commercially available assay that permits the identification of *M. tuberculosis* complex at species level. Using Genotype® Mycobacterium it is possible to identify 13 species including the most relevant mycobacteria.

P1487 Fast and sensitive detection of the *Mycobacterium tuberculosis* complex by real-time PCR

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Objectives: Tuberculosis is the most frequent fatal bacterial disease worldwide and causes approximately three million deaths every year. Apart from *Mycobacterium bovis* BCG in humans tuberculosis can be caused by all members of the *Mycobacterium tuberculosis* complex. The number of mycobacteria in clinical samples can be very low and their detection by culture usually takes between one week and two months. For this reason we developed a real-time PCR assay (RealArt M. tuberculosis TM PCR Reagents) for the use with the ABI PRISM 7000 SDS instrument (Applied Biosystems, USA) which allows the fast and reliable diagnosis of tuberculosis even in patients releasing low numbers of mycobacteria.
Methods: The real-time assay amplifies a region of the mycobacterial 16S rDNA using primer binding sites that are conserved in all members of the *M. tuberculosis* complex. The generation of the specific amplicon is detected by a dual-labelled fluorogenic probe. In addition, the real-time assay contains a second heterologous PCR system for the detection of a so-called 'internal control'. This can be used to control the DNA purification procedure as well as the existence of possible PCR inhibitors. To assess the usability of the real-time assay for clinical diagnostics 48 *M. tuberculosis* culture positive and 48 culture negative sputum samples were examined.
Results: The real-time PCR assay detects the members of the *M. tuberculosis* complex with an analytical sensitivity of 10 bacterial genomes per PCR. Its specificity is very high since neither atypical mycobacteria nor several other bacteria tested generated a signal in the ABI PRISM 7000 SDS instrument. The examination of the 96 sputum samples showed that the real-time PCR assay exhibits a specificity of 100%. The sensitivity was 100% for smear positive and 95.8% for smear negative samples compared with the results from culture. One per cent of the samples showed an inhibition.
Conclusions: The described real-time assay is a sensitive and highly specific tool for the detection of the members of the *M. tuberculosis* complex. Its integrated internal control allows the exclusion of false negative results due to purification loss or inhibition of the PCR.

P1488 Five-year experience with Roche Amplicor PCR assay for *Mycobacterium tuberculosis*

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Objectives: The clinical indications and economic consequences of *Mycobacterium tuberculosis* (MTB) PCR test could be controversial. Before analysing the cost-effectiveness of this method, we evaluated the performances on the Amplicor(R) MTB test (Roche) in our hospital in Seine Saint Denis (northern suburbs of Paris) where there is a high incidence of tuberculosis. In 2001, the incidence was 34/100 000 compared with 11/100 000 nationally.
Methods: We investigated 732 decontaminated samples (NALC-NaOH) collected from 588 patients during five years. The routine prescription included 527 extra-pulmonary (EP) specimens and 205 respiratory specimens. The internal control was incorporated into the amplification reaction. The MTB PCR test was compared with Mycobacterial culture (Lowenstein–Jansen and coletsos media). Results were interpreted with an adjusted 'gold standard' incorporating clinical diagnosis.
Results: Of 732 specimens, 40 were inhibitory, 83 were culture +/PCR+, 13 were culture +/PCR− and 36 were culture−/PCR+. Among cultures + samples, 18 were smear positive. After interpretation of results, three samples culture−/PCR+ were interpreted as false positive results. The overall sensibility was 89% and the specificity 99%. For EP specimens the sensibility and specificity were 85% and 99%, respectively. One third of EP tuberculosis was diagnosed only with PCR and clinical context. Of 82 abscess aspirates and samples of spondylitis and spondylodiscitis, 28 were culture+/PCR+, 13 were culture-/PCR+, none was culture+/PCR-. After interpretation of discrepancies the sensibility was 100% and the specificity 95%.
Conclusions: Our study confirms that Amplicor(R) MTB test is a sensitive and specific method for detecting MTB complex. The results are particularly interesting in EP tuberculosis where we need a rapid and accurate laboratory diagnosis.

P1489 Comparison of the MB/BacT culture system and the Lowenstein–Jensen medium for detection of Mycobacteria from respiratory samples

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Objective: For the isolation of Mycobacteria from clinical specimens, the use of liquid media as well as solid media is recommended. The aim of this study was to compare the MB/BacT system to solid culture media, in terms of reliability and rapidity of mycobacterial growth from respiratory samples.
Methods: Clinical respiratory samples, 679 in number, were inoculated in the MB/BacT system and on two LJ slants; N-acetyl-L-cysteine-NaOH method (final concentration 2%) was used for decontamination and smear from the sediment were prepared from all specimens and examined for acid-fast bacteria by the Tan–Thiam–Hok method. The mycobacteria isolated were identified by the Accuprobe culture identification test. All tests were conducted according to the manufacturer's instructions. Recovery rate, detection time and contamination rate were calculated.
Results: In both methods, we isolated 124 strains (18.3%) identified as *Mycobacterium tuberculosis* complex (Mt), 92 (74.2%) from smear positive and 32 (25.8%) from smear negative samples. The recovery rate with the MB/BacT was higher than that with LJ [116 (93.5%) versus 100 (80.6%)], $P < 0.0001$. Contamination rate were 5.5% in MB/BacT and 5.0% in LJ. The overall correlation between the two culture media for detection of Mt was 74.8%, although it was higher in smear-positive samples (82.1%). The mean time to detection was 11.4 days for the MB/BacT and 18.7 days for the LJ ($P < 0.0001$).
Conclusions: These results indicate that the MB/BacT system is more efficient and faster than LJ, for the recovery of Mt in respiratory samples.

P1490 Comparison of the performance of different assays for the immune-diagnosis of tuberculosis based on ESAT-6 and CFP10 selected peptides

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Mycobacterium tuberculosis ESAT-6 and CFP-10 purified protein and its peptides are currently being evaluated as antigens for the immune diagnosis of TB. We set up an ELISPOT assay for IFN-gamma whose novelty consists of two multiepitopic peptides from ESAT-6 protein, selected by computational analysis, that allows the discrimination between latent and active TB and the monitoring of the efficacy of anti-TB therapy (Vincenti *et al*., Mol Med 2003; Carrara *et al*., Clin Infec Dis, in press). It is important to find new strategies to increase the sensitivity of the diagnostic assays. Thus, the objective of this study was to assess the performance of this assay based on ELISPOT against other technical assays used in immunology laboratories, such as ELISA on cell culture supernatants, ELISA on plasma from whole blood cultures, proliferation assay evaluated by thymidine incorporation, intracellular cytokine staining evaluated by FACS. PBMC or whole blood from patients with active TB microbiologically confirmed (positive culture from biological fluids and/or PCR from biopsies specimens) were seeded in the presence or absence of ESAT-6 and CFP-10 proteins, their selected peptides, PPD, recall antigens, mitogens and their own controls. The results were obtained within one day by the ELISA on plasma or by FACS analysis, within 2 days by ELISPOT, and 5 days by proliferation assay or ELISA on cell supernatants. We found that the sensitivity of the ELISPOT assay was significantly higher compared with the proliferation assay ($P < 0.05$, to ELISA on cell culture supernatants and to FACS analysis although not statistically significant. However, a similar sensitivity was found between the results obtained by ELISPOT assay and ELISA on plasma from whole blood cultures. On the other hand, FACS analysis allowed the identification of the single cells involved in *Mycobacterium tuberculosis*-specific responses. In conclusion, immune-diagnostic assays based on the response to ESAT-6 and CFP10 selected peptides obtained by ELISPOT or ELISA performed on plasma from whole blood are comparable, whereas a sub-optimal sensitivity is found with proliferation assay, FACS analysis and ELISA on cell culture supernatants.

P1491 Evaluation of the BDProbeTec ET system for direct detection of *Mycobacterium tuberculosis* complex in respiratory and extra-respiratory specimens

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Objective: To evaluate the usefulness of BDProbeTec ET system for direct detection of *Mycobacterium tuberculosis* complex (MTBC) by comparing results to those of conventional mycobacterial culture performed with BACTEC MGIT 960 and Lowestein–Jensen medium.
Method: Four hundred and ninety two samples (382 respiratory and 110 extra-pulmonary) from patients with negative smear microscopy and a high probability of tuberculosis were assessed by BDProbeTec and by culture for mycobacteria. In all samples the internal amplification control (IAC) was successfully performed.
Results: Of 492 specimens, 29 grew MTBC (and in five cultures grew nontuberculous mycobacteria). BDProbeTec ET detected 25 of the 29 MTBC culture-positive specimens and gave a positive result in 10 of the 463 negative cultures, resulting in initial overall sensitivity, specificity and positive and negative predictive values of 86.2%, 97.8%, 71.4% and 99.1%, respectively. BDProbeTec ET was positive in none of the nontuberculous mycobacteria. After analysing respiratory and extra-pulmonary samples separately,

the sensitivity and specificity values of the BDProbeTec ET were 90%, 97.5% and 77.7%, 99% respectively.
Conclusions: These data suggest that the performance is good for negative smear samples and that sensitivity increases when analysing respiratory specimens. The BDProbetec ET system offers the advantage of including an IAC in the specimen well.

P1492 Evaluation of timing, number and yield of blood cultures in the detection of *Mycobacterium tuberculosis* bacteraemia in HIV-infected patients in Portugal

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Objective: To investigate the number, timing and yield of mycobacterial blood cultures (BC) for the detection of *Mycobacterium tuberculosis complex* (MTC) in HIV-infected patients in Portugal.
Methods: BC (Myco/F-Lytic) collected from 1998 to 2001 from HIV-infected individuals with culture proven MTC were retrospectively studied, including time and number of BC, as well as time, number, specimen type and result of all concurrent mycobacterial cultures. Conventional, non-mycobacterial BC (Bact/Alert FA) was inoculated with serial dilutions of MTB to determine the ability of MTB to grow in the medium. Subsequently, all non-mycobacterial BC (Bact/Alert FA) collected from HIV-positive patients that remained negative after 7 days of incubation in the Bact/Alert instrument were prospectively investigated for the presence of MTB by auramine staining (and culture were indicated).
Results: In the retrospective part of the study, a total of 1181 BC from 858 patients were collected. One hundred and fifty-seven HIV-positive patients had culture proven TB (any kind of specimen). Of these, 44 patients (28%) had 48 positive BC for MTC. In 29 cases, BC were the quickest or only way to diagnosis with a mean of 22 days to positivity (range 11–46). In six of ten patients who had two or more BC collected, discordant results between the multiple BC were observed. Positive BC were collected significantly earlier during admission than negative BC from patients with extrapulmonary/disseminated TB (61% versus 19% within 48 hours, $P < 0.01$). The serial dilution experiments showed that the new Bact/Alert FA medium (supplement: charcoal) supports the growth of MTB down to an inoculum of less than 10 bacteria/ml and that auramine staining detected all positive BC. In the ongoing prospective part, 220 BC from 85 patients have been checked so far for the presence of mycobacteria. Five conventional BC from two patients yielded mycobacteria.
Conclusions: BC are useful to provide rapid cultural confirmation of TB in HIV-infected patients in a setting such as Portugal. They should be collected as early as possible after admission. Interestingly, the new Bact/Alert FA medium supplemented with charcoal seems to support the growth of MTB. The ongoing investigation of non-mycobacterial BC will try to establish if a single BC is enough to detect the mycobacteraemic episode or if a collection of multiple BC, perhaps at longer intervals (>12–14 h apart), may increase yield further, as very rarely is $^{3}1$ mycobacterial BC collected.

P1493 16S rRNA PCR for retrospective detection of *Mycobacterium* spp in formalin-fixed paraffin-embedded archival specimens

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After formalin-fixation of clinical specimens culture of *Mycobacterium* spp is impossible. PCR provides a rapid and sensitive alternative diagnostic method for the detection of *Mycobacterium tuberculosis* or non-tuberculous mycobacteria in these clinical samples. We evaluated the usefulness of the 16S rRNA PCR combined with reversed line blot for the retrospective detection of (non)tuberculous mycobacteria in lymph node biopsies in which granuloma were found microscopically according to the Nationwide Pathological Anatomic Automated Archive (PALGA). PCR results were compared with previous clinical cultures if available. Mycobacterial DNA was shown in 12 of 51 materials (24%). *M. tuberculosis* was found seven times (14%). The other samples contained *M. fortuitum* (4 times) and *M. avium*, respectively. Clinical culture of two more patients showed *M. tuberculosis*. Three samples containing mycobacterial DNA, which showed reactivity with genus probe pMyc5a but not with one of the specific species probes, were determined by sequence analysis. We conclude that, although sensitivity is lower than 100%, the 16S rRNA PCR is also applicable retrospectively for the detection of *Mycobacterium* spp. in formalin-fixed paraffin-embedded archival specimens of lymph node biopsies.

P1494 Identification of mycobacteria with 16S ribosomal RNA gene sequence analysis

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Biochemical identification of mycobacteria is a time-consuming process due to their slow growth. Molecular techniques can speed up this process. The currently used 16S rRNA PCR combined with reversed line blot can only determine to species level if specific probes, which show a variable amount of disturbing cross reactivity, are added. Sequential analysis of hypervariable regions of the 16S ribosomal RNA gene can determine more mycobacteria to the species level without time-consuming subculturing. The aim of the study is to develop an identification method of mycobacteria by sequence analysis of hypervariable regions of PCR-amplified 16S ribosomal RNA gene. The amplified region has a length of about 590 base pairs. Sixty positive cultures of mycobacteria (e.g. *Mycobacterium tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. chelonae*, *M. malmoense*, *M. scrofulaceum*, *M. marinum* and *M. gordonnae*) collected during the last 10 years were analysed, including various strains which could not be determined previously. The 16S rRNA genes sequence was compared with the public RIDOM database of mycobacteria available on the Internet. Results of the sequence analysis excellently matched with reversed line blot and biochemical methods. Cross reactivity in reversed line blot could be explained by probe and strain sequence homology and one strain previously considered as *M. tuberculosis* by mistake was now correctly identified as *Mycobacterium holsaticum*. We conclude that sequence analysis of mycobacteria is a reliable technique that is applicable for routine use in our laboratory.

P1495 The role of tuberculosis skin test conversion in detection of tuberculosis

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Objective: To determine the risk factors of tuberculin skin test (TST) conversion among health care workers (HCW).
Methods: Two-step TST was performed, at the Ankara Numune Education and Research Hospital and conversion test was performed one year later. In 2001, 1006 physicians, 584 nurses, 408 laboratory technicians, 20 pharmacists, 197 management and service officers, 133 housekeeping personnel were actively working. All HCWs working at the hospital were invited to participate in the study. Logistic regression was performed for multivariate analysis.
Results: Of the 491 participants 408 (83%) had two-step TST positivity. Fifty HCWs out of 83 TST negatives had the third TST. TST conversion rate was 20%. The mean age was 35. Conversion was detected in 12 HCWs, 92% were female. In univariate analysis, the number of BCG scars, working in internal medicine, follow-up TB patient within last year, age <30 years were found to be

significantly associated with the outcome ($P < 0.05$). TST conversion rate was higher among HCWs, with working duration of <1 year ($P = 0.146$), and an annual income <$2500 ($P = 0.278$), although statistically not significant. However the conversion rate was not associated with public transportation to the hospital ($P = 0.180$), entertained at crowded settings out of the hospital ($P = 0.705$). None of the subjects had a history of TB in their families. None of the TST converted subjects had a history of contact with a TB patient outside of the hospital. None had had BCG vaccination within 10 years. In multivariate analysis, the HCWs who followed up TB patients in the last year were found to have higher risk of TST conversion (OR;8, CI;1.2–55.6, $P = 0.035$), although all the HCWs declared that they had complied with the protective measures. For every extra BCG vaccination detected on physical examination, a five time increase in TST conversion rate was detected (OR;5, CI;1.4–18.4, $P = 0.014$). TST converted HCWs were screened by their chest X-rays. In the chest X-ray of one HCW, cavitation was detected. The computerised tomography supported the diagnosis. Acid-fast bacteria in her sputum were investigated three times, and the bacteria were detected in all the samples. She was treated for 6 months.
Conclusions: Younger female HCWs, who are following up the tuberculosis patients, are under risk of TB infection. A systematic surveillance of HCWs is helpful in Turkey, although the prevalence of TST positivity is rather high.

Mycobacterial infection: epidemiology

P1496 *Mycobacterium kansasii* infection in Spain: results from an epidemiologic national survey (2000–2003)

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Objective: *Mycobacterium kansasii* (MK) has been identified as an agent of disease worldwide. However, its incidence varies significantly from area to area. Nowadays, there are no available data on MK infection in Spain. The objectives of this study were (1) to establish the frequency and geographical distribution of MK infection in Spain; (2) to study the relationship with several demographic characteristics; (3) to know the distribution of the different subtypes of MK isolates in Spain.
Methods: A retrospective (3 years) and prospective (1 year) surveillance (2000–2003) of all patients infected with MK in the 17 Autonomous Communities of Spain was carried out. Cases of MK infection were identified from 92 microbiology laboratories of reference hospitals (GEMKA). Demographic, clinical and microbiologic data were recorded in a standardised questionnaire. Clinical significance of the isolates was ascertained according to the American Thoracic Society' criteria. Genetic characterisation to subspecies level of each MK isolated (one per patient) was performed by PCR-RFLP analysis of *hsp65* gene.
Results: A total of 598 cases were identified during the study period from 15 Autonomous Communities: Euskadi ($n = 215$), Catalunya ($n = 192$), Madrid ($n = 51$), Aragón ($n = 38$), Valencia ($n = 20$), Asturias ($n = 19$), Andalucía ($n = 16$), Navarra ($n = 13$), Castilla-León ($n = 12$), Canarias ($n = 7$), Cantabria ($n = 5$), Galicia ($n = 4$), Extremadura ($n = 3$), Murcia ($n = 2$), and Castilla-La Mancha ($n = 1$). The mean age was 53 years and 80.4% were male. The HIV-infected patients (12.1%) were younger than non-infected ones (87.9%) (mean age, 37 vs. 55 years, $P < 0.001$). Clinical MK disease was detected in 75% and 72.1% of HIV-infected and non-infected patients, respectively. Pulmonary involvement was observed in 94.5% of cases. Seventy-four of 80 (92.5%) MK isolates analysed from different geographical areas were subtype I.
Conclusions: *MK* is a pathogenic and frequent mycobacteria isolated from several geographical regions of Spain, especially in densely populated areas. Subtype I seems to be the most frequent subtype isolated.

P1497 A case of disseminated tuberculosis presenting as Crohn's disease

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A 29-year-old man presented with abdominal pain, diarrhoea, dysphonia, disphagia and weight loss. With these symptoms and colonoscopic finding of inflammatory bowel disease he was referred to our clinic as infected with Crohn's disease. Furthermore, he was given medical treatment according to this diagnosis consisting of corticosteroids. We planned to investigate tuberculosis in this patient whose complaints increased despite the steroid therapy. For the diagnosis, colonoscopy was performed at first and multiple ulcers were seen in the cecum, transverse colon and ascending colon; ileocecal valve was infiltrated. Regarding diffuse involvement of Crohn's disease, upper GIS endoscopy was performed in order to see the oral cavity, oesophagus, stomach and duodenum. As a result, the oesophagus was found to be normal; edema in the epiglottic area and pangastritis were observed. Colonic biopsies indicated granulomas and were positive for acid-fast bacilli. Acid-fast bacilli were also positive in the sputum. In addition to that, chest x-ray and thorax CT showed bilateral infiltrates in both pulmonary apices. PCR test for *Mycobacterium tuberculosis* was positive all for biopsy, sputum and stool. According to the diagnosis of disseminated tuberculosis, anti-tuberculous therapy (INH + Rifampin + Pyrazinamide + Streptomycine) was started and a good response was observed at the end of two weeks. Later on, the diagnosis was absolutely confirmed by the cultures. Tuberculosis should be added in differential diagnosis in the patients suspected of Crohn's disease particularly before starting immunosuppressive therapy.

P1498 Mycobacterial contamination of a transfusion unit: lessons for the routine screening of blood products

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Objectives: Current guidelines recommend screening of blood units for a large number of pathogens at the exclusion of non-treponemal bacterial and mycobacterial organisms, based on the assumption that bacterial infection in healthy donors are unlikely to be asymptomatic. In the present study, we describe the identification of *M. mucogenicum* contamination of a blood unit in an immunocompromised patient.
Methods: Samples from blood units were incubated in a BACTEC9000. Upon detection of bacterial growth, positive samples were inoculated into blood agar. DNA was extracted from bacterial colonies. A 439-bp fragment encompassing the hsp-65 gene was PCR-amplified and subsequently digested with BstEII and HaeIII endonucleases. Bacterial DNA was also PCR-amplified using a primer pair specific for conserved regions of 16S rRNA. The nucleotide sequence of the resulting amplicons was established and analysed using the BLAST software.
Results: A 76-year-old female patient with acute myelogenous leukaemia, developed severe rigors following transfusion of a blood unit. Cultures obtained from the blood unit grew Gram-positive weakly acid fast rods that were identified, based on morphological criteria, as *Nocardia* sp. However, PCR-RFLP analysis

revealed a restriction pattern compatible with that of atypical mycobacteria. The identity of the contaminant was ultimately established as *M. Mucogenicum*, based on BLAST analysis of the bacterial 16S rRNA sequence.
Conclusions: Mycobacteria have the capacity to grow on regular blood agar instead of traditional egg-based agar. This fact, which was recently demonstrated experimentally (JCM 41:1710), may lead to misidentification of the growing pathogen. Accordingly, the mycobacterium species isolated in the present study was initially categorised as *Nocardia* sp., and only recognised as *M. mucogenicum* following molecular typing. The present case suggests the need to assess the value of large-scale routine screening of blood products for bacterial and mycobacterial contaminants, especially before transfusion to immunocompromised patients.

P1499 Tuberculous meningitis in Northern Greece

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Objective: The aim of this study is the presentation of tuberculous meningitis cases of the adult department of the Thessaloniki Infectious Diseases Hospital, where patients of all Northern Greece are attended to.
Patients and Methods: The hospital archives were surveyed retrospectively and cases of meningitis, classified as tuberculous during the years 1990–2003, were studied. Data was collected regarding history, clinical, laboratory and radiological findings, therapy and outcome.
Results: Fifty-eight cases were identified, 30 males and 28 females, from 15 to 80 years old, mean age (x+/-SD) 52+/−6 years. Twenty-four (41%) patients were admitted in stage I, 27 (47%) in stage II and 7 (12%) in stage III. Symptoms had appeared for a median of 19 (2–120) days before admission. The diagnosis of 23 (40%) patients was definite, because *Mycobacterium tuberculosis* was cultured from CSF. The rest of the patients fulfilled some of the following criteria for the diagnosis of tuberculous meningitis: long periods of symptoms before the admission, high CSF protein level, low CSF glucose level, favourable response to antituberculous therapy. The duration of hospitalisation was 30 (3–90) days. CSF findings at the admission were (x+/-SD): cells 216+/−291 mm^3, protein 263+/-295 mg/dl, CSF glucose/blood glucose 0.29+/−0.07 mg/dl, lactate 47+/−18 mg/dl. The mortality rate was 10.5% (6/58), while 19% (11/58) of the patients had permanent sequelae. CTs or MRIs from 51 patients showed that: 17 patients had hydrocephalus, five had meningeal enhancement, three had infarction, two TB spondylitis, one intradural tuberculoma of spinal cord and two brain tuberculomas, while 21 patients had normal images. Morbidity and mortality rates were higher in patients >60 years old and in patients admitted in stage II or III. Patients >60 years old were admitted more frequently in late stage than younger ones.
Conclusion: Tuberculous meningitis still remains a very serious disease with a high incidence of mortality and morbidity, especially among elder people and in late stage.

P1500 Isolation frequency of *Mycobacterium* sp. in extrapulmonary clinical sources

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Objective: We investigated the isolation frequency of *Mycobacterium* sp. strains in extrapulmonary clinical sources, in the period 1998–2002.
Methods: From the total of 87 530 clinical specimens received in the National Mycobacterium Reference Center of Athens for the purpose of tuberculosis laboratory diagnosis, 12 362 (14%) concerned extrapulmonary body sites. In particular, 6197 (50.1%) were pleural fluid, 2486 (20.1%) urine and 1655 (13.4%) blood and bone marrow, while there were smaller numbers of ascetic fluid, cerebrospinal fluid, pericardial and peritoneal fluids, pus and biopsies from lymph nodes, skin and tissues. All specimens were processed by the NALC- NaOH method as recommended by CDC, stained and cultured in parallel on Löwenstein–Jensen medium, on the automated Bactec MGIT 960 system or on the radiometric Bactec 460TB system. All isolates were identified by Accuprobe tests (Gene probe, Inc.) and by classical biochemical criteria.
Results: Laboratory testing for mycobacterial infection was positive in 437/12362 (3.5%) extrapulmonary specimens. Specifically, 394/437 (90.2%) grew *M. tuberculosis* and 43/437 (9.8%) *M. avium*. Regarding *M. tuberculosis* isolates, 152 were derived from pleural fluids, 70 from urine, 55 from lymph nodes, 42 from pus, 22 from tissue biopsies, 14 from cerebrospinal fluids, 14 from ascetic fluids, 8 from blood and bone marrows, 6 from upper respiratory tract, 4 from bone biopsies, 2 from pericardial fluids, 4 from genital tract, and one from a skin biopsy. Concerning *M. avium* isolates, 16 were recovered from blood, 2 from bone marrows, 18 from lymph nodes, 4 from pleural fluids, 2 from pus specimens and one from a stool specimen.
Conclusions: The incidence of extrapulmonary tuberculosis was detected to be 3.5%. *M. tuberculosis* was recovered from a vast variety of clinical sources, while *M. avium* was recovered mainly from blood and bone marrow specimens of HIV-infected patients or from lymph nodes of young children.

P1501 Simultaneous occurrence of tuberculosis and non-Hodgkin's lymphoma within cervical lymph nodes

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Introduction: Tuberculosis of cervical lymph nodes is not unusual, even in developed countries. This area is also a common site of presentation of non-Hodgkin's lymphoma. Nevertheless, concurrence of both diseases in the same lymph nodes is extremely rare.
Case report: A seventy-year-old woman of Italian origin was admitted to our Institute due to unfavourable evolution of cervical lymphadenopathy thought to be of tuberculous origin. No other serious medical problems were referred. A familial history of pulmonary tuberculosis was present. Six months before the admission bilateral cervical masses were first developed and three months later a biopsy of the left side revealed tissue pathology compatible with TB. Acid-fast bacilli were visible with the Ziehl–Neelsen stain. PCR was positive for *M. tuberculosis* complex. A standard three-drug antituberculous regimen was initiated. Drug susceptibility testing followed identification and no resistance was proved. There was no evidence of pulmonary disease. Haematological and biochemical profile was normal. Despite strict adherence to the treatment, node enlargement and appearance of new masses occurred. As smears and cultures of fine needle aspirations turned negative and obstructive phenomena due to the compression of the trachea ensued, complete excision of the involved nodes was performed. The diagnosis of a diffuse large B-cell NHL was established on the basis of morphologic and immunophenotypic features. Chemotherapy with CHOP was followed by massive relapse of the cervical masses and a few weeks later the patient died.
Discussion: Although exceptional, we believe that the co-existence of NHL and TB is not random. Several studies propose that TB could be a predisposing factor for NHL. There is also enough evidence that NHL may cause TB reactivation. Increase in interleukin-10 levels, produced by the neoplastic B-cells and the inability of the CD4 T-cells of the malignant lymph node to express their helper function may be the responsible mechanisms.

P1502 TB and HIV rates in Odessa, Ukraine: a dramatic rise in the last decade

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Infection with HIV has continued to escalate across countries of the former Soviet Union including Ukraine. Less than 50 HIV cases were diagnosed annually before 1994 but by June 2002, 47 988 cases had been officially recorded with 11 388 cases (or 24% of the total) occurring in the previous 18 months. Unfortunately, the rates of tuberculosis have also increased in parallel. National rates of HIV, TB and HIV and TB co-infection underestimate the real situation. Odessa and S. Ukraine reported higher rates of HIV diseases than in the rest of the country.
Objective: To examine current and long-term trends of HIV, TB and HIV with TB dual infection in Odessa region.
Materials and methods: Analysis of TB and HIV incidence data in Odessa oblast from 1962 to 2002.
Results and discussion: In 1962, the incidence of TB in the Odessa region was 178 cases per 100 000 cases, but this declined to 73.0, 42.0 and 41.6 cases per 100 000 in 1972, 1982 and 1992 respectively rising to 80.4 in 2002. TB mortality in Odessa has nearly doubled in the same time from 10.2/100 000 to 21.6/100 000. Overall, TB incidence, TB prevalence, HIV incidence and HIV prevalence were 80.4, 330.1, 46.4 and 241.0 cases per 100 000 population respectively in 2002. Of those TB cases officially registered within the Odessa TB dispensary system, in 2001, 281 persons or 7.5% of the total were HIV positive. There are estimated to be 650 000 IVDU in the Ukraine. The proportion of HIV infection acquired through IVDU in the Ukraine has fallen (72.7% in 1997 to 54.2% in 2000), and this may represent an increasing role for heterosexual transmission. As part of an effective strategy to prevent the coalescing of the two outbreaks there is a need for (1) more detailed epidemiology on both HIV and TB regionally as well as nationally, including anonymous linked and unlinked HIV seroprevalence studies in the general population and risk-groups and drug resistance surveys in TB patients; (2) behaviour modification campaigns with appropriate education delivered by professionals and peer-groups; (3) harm intervention strategies including condom provision, interruption of mother-to-child transmission and needle exchange; (4) improved TB diagnosis and case management and institute-effective mechanisms to limit TB transmission within institutions such as hospitals and prisons, particularly to HIV-positive patients.

P1503 IS6110 RFLP analysis of *Mycobacterium tuberculosis* strains in the Free State province, South Africa

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Objectives: Genotypes of *Mycobacterium tuberculosis* (TB) strains vary in geographical areas, e.g. the W-Beijing strain has been reported as the cause of disease and outbreaks in Asia, across Africa and in the United States. The Haarlem strain is well reported while the recently reported DRF150 strain has so far been found only in South Africa. In South Africa epidemiological studies have been limited to the Western Cape and Mpumalanga provinces. The aim of this study was to determine the tuberculosis disease dynamics in semi-rural populations in the central Free State province of South Africa.
Methods: IS6110-RFLP typing was done according to internationally approved methods using TB strains isolated from smear positive patients attending nine clinics in three regions of the Free State province over a period of three years.
Results: Fingerprinting was performed on 218 strains. Almost 63% of the patients were men, 48% in the age group 20–39 and 43% in the age group 40–59 years. Seventy two percent of males were represented in clusters. Small clusters of 2–6 strains were found. One cluster of six consisted of strains with only four bands and two of these strains were subdivided using spoligotyping. All clusters had strains from newly diagnosed and previously treated TB patients. Contact could be established between members of two clusters while another cluster contained strains from patients from the same clinic suggesting interpersonal transmission. The Recent Transmission Rate was calculated as 20.18%. No strains similar to the W-Beijing family were found, a strain that is common in the Western Cape. One strain was found to belong to the newly described Cape Town DRF150 resistant cluster.
Conclusions: It is evident that the molecular epidemiology of tuberculosis in the Free State differs from that of the rest of the country. Bigger family groups were evident at a 65% similarity index and further studies could confirm the opinion that transmission rates might be higher and should be calculated differently. The fact that no W-Beijing strains were found could be due to high unemployment and therefore low migration of patients between study areas. The findings of this study indicate the need for continuous resistance monitoring, molecular epidemiological surveillance and active contact tracing as part of the Free State Tuberculosis Control Program.

P1504 Infections due to non-pigmented rapidly growing mycobacteria (NPRGM) in a university hospital

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Objectives: To determine the frequency, clinical significance, treatment and outcome of the isolates of NPRGM in a university hospital during the period 1979–2002.
Methods: Patients with isolates of NPRGM were selected for a retrospective study from the records of the mycobacteriology laboratory between June 1979 and December 2002. Clinical charts were reviewed according to a predefined protocol, and clinical significance was evaluated according to accepted criteria.
Results: NPRGM were identified in 71 patients (5.4% of all patients with mycobacterial isolates). Seven patients were from other hospitals, and 64 patients were selected for the study. After reviewing, in 19 cases the isolates were considered clinically significant, and two more cases were considered as doubtful. No respiratory isolate was considered significant. Eleven isolates were identified as *Mycobacterium chelonae*, two as *M. fortuitum*, two as *M. abscessus* and two as *M. peregrinum*. Seven patients had skin and soft tissue infections, three had disseminated infection, two cases each of osteomyeltis, peritonitis in CAPD, and diabetic foot infection, and one case each of urinary tract infection, arthritis, diarrhoea, conjunctivitis and surgical site infection. Antimicrobials were selected for therapy performed according to individualised susceptibility tests. Surgery for removal of foreign bodies or sequestra was performed when these bodies were present. All but one patient were cured. One case was treated with antituberculous drugs despite the identification and susceptibility study of the isolate, and persisted for several months.
Conclusions: NPRGM can be the cause of clinical syndromes in one third of the cases, mainly when the isolate is from a non-respiratory source in our media. Therapy with selected antibiotics and surgery, when needed, lead to cure of all patients.

P1505 Osteoarticular infections due to mycobacteria in a university hospital

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Objectives: To study clinical and microbiological features of osteoarticular infections due to mycobacteria diagnosed in our laboratory over the last 20 years.
Methods: We carried out a retrospective study of clinical charts of patients with isolates of mycobacteria from osteoarticular samples between 1982 and 2003.
Results: Thirty-four cases (11 men, 23 women, mean age: 60.06, range 5–88) of skeletal tuberculosis (33 *M. tuberculosis*, 1 *M. bovis*)

were found. Locations were vertebral in 13, knee in seven, hip in five, wrist in five, elbow in three and six from other locations. In five cases, the disease affected two or more joints. Previous pulmonary tuberculosis and contact transmission were the most important predisposing factors. No patient was immigrant, and only two patients were HIV+. Pain was the main clinical presentation (25) followed by fever (11). In one case, a hip prosthesis was present, and the strain was isolated also from it. The tuberculin test reaction was positive in 20 patients out of 23. In 14 cases, the acid-fast strain was positive at least in one sample. All strains were susceptible to all first-line drugs, except for *M. bovis* and pyrazinamide. Three patients died because of diseases other than tuberculosis. Nine patients (26.5 %) had residual abnormalities. All patients were treated with antituberculous drugs, 21 of them also required surgery. Four cases (all men) had isolates of rapidly growing mycobacteria (RGM). In three cases (two *M. chelonae*, one *M. abscessus*), the isolate was considered as significant (one joint, two from bone), representing an 8.1% of all osteoarticular infections due to mycobacteria. All three patients were cured with proper chemotherapy and surgery.
Conclusions: Osteoarticular tuberculosis is still an important form of extrapulmonary tuberculosis, but has different risk factors than other forms of the disease. The outcome of tuberculous disease is good in all cases, but residual abnormalities appeared in almost one quarter of the patients. Osteoarticular mycobacteriosis in our hospital have been caused by RGM. In these cases, proper chemotherapy and removal of foreign bodies or necrotic tissue was necessary for a good evolution of the patients.

P1506 *Mycobacterium malmoense* lymphadenitis in Spain: first two cases in immunocompetent patients

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Objectives: *Mycobacterium malmoense* is a slow-growing, non-photochromogenic mycobacterium that has been recognised as nontuberculous pathogen in northern and northwestern Europe. Recently, it has been isolated from clinical samples in other areas. It can cause pulmonary and extrapulmonary disease and also disseminated infection. The objective is to present two cases of *M. malmoense* lymphadenitis in two immunocompetent children in Spain. These are the first documented cases of extrapulmonary infection by *M. malmoense* in our country.
Case report and methods: Two patients, an eight-year-old boy and a two-year-old girl were referred for evaluation of an enlarged painless superficial lymph node on the cervical area. One fine needle aspiration biopsy was performed in both cases to establish the diagnosis. Specimens were decontaminated and seeded onto Löwenstein–Jensen slants and into a liquid system, the BacT/Alert(R) MP (BioMérieux, inc. Durham).
Results: Microscopic examination of specimens yielded few acid-fast bacilli in both cases. Cultures in liquid medium showed growth of mycobacteria after 72 days in the first case, and 36 days in the second. Primary cultures on Löwenstein–Jensen remained negative after 75 days. Cultures were confirmed as slow-growing mycobacteria, non-photochromogenic; *M. malmoense* was identified by polymerase chain reaction and restriction enzyme pattern analysis (PRA) and on analysis of the 16S-rRNA gene
Conclusions: Unilateral cervical lymphadenitis is the most frequent extrapulmonary infection due to *M. malmoense*, and predominantly affects children. To ensure a sensitive primary isolation of *M. malmoense*, it is crucial to carefully choose culture media and conditions. New clinical isolates from different countries and the isolation from the environment question the fact that *M. malmoense* is exclusively limited to specific zones. Probably, *M. malmoense* is present in many geographic areas and may colonise or cause infections in humans and animals. We want to draw the attention of microbiologists and clinicians to this emergent pathogen that should be added to the list of nontuberculous mycobacteria responsible for disease in immunocompetent patients.

P1507 Central nervous system tuberculosis: epidemiological, clinical features and outcome in 133 patients

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Objectives: To describe epidemiological, clinical features and outcome in adults with central nervous system tuberculosis.
Patients and methods: We performed a retrospective study of all cases of central nervous tuberculosis hospitalised in our department during a 20-year period.
Results: A total of 133 patients (81 females and 52 males) were included. The mean age was 37 years (ages ranged 17–88 years). 20 patients (15%) had a history of tuberculosis. The clinical symptoms and signs on the admission were fever in 83.5%, headache in 85.7% and neck stiffness in 87.2%, compatible with tuberculous meningitis; alteration in consciousness and focal neurologic signs are present in 63 patients (47.4%). Paraplegia or hemiplegia was present in 33 patients (24.8%) and extraneurologic tuberculosis was associated in 51.8%. The spinal fluid at admission was clear in 63% of patients, and in most cases, the cell count results were under 300 cells/mm^3, with predominantly lymphocytes in 85% of cases. Low level of glucose was seen in 101 patients (80%), whereas elevated proteins up to 1 g/L was observed in 70% of patients. In five cases without meningitis, the spinal fluid was normal. *Mycobacterium tuberculosis* was isolated in the cerebrospinal fluid of 31 patients (25%). Abnormal chest X-ray was found in 49.6% of the patients. Cranial CT scan and MRI showed hydrocephalus (36 cases), tuberculomas (30 cases), leptomeningitis (28 cases), infarction (16 cases) and abcesses (2 cases). In five cases, tuberculomas or abcesses were associated to hydrocephalus without meningitis. All the patients were treated with antituberculous drugs and steroids. The overall mortality was 19.5%. Permanent neurological sequelae were seen in 10 patients (7.5%).
Conclusions: Tuberculosis of the central nervous system continues to be an important problem in developing countries including Tunisia. The disease is still severe, and mortality and morbidity remains high. Early diagnosis should be considered to improve prognosis.

P1508 Information system of bacillary tuberculosis – 5 years transition

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Background: Tuberculosis has a long surveillance history in the Czech Republic. However, only in the past few years have we experienced important changes in epidemiology of tuberculosis as well as in implementation of improved notification approaches.
Objectives: The aim of this work was to present new features in the notification of tuberculosis in the Czech republic and summarise changes in epidemiology of tuberculosis evident in routine surveillance.
Methods: Notification of tuberculosis is done in two independent systems, the Register of tuberculosis and the Information System of Bacillary Tuberculosis. The first one, the Register of tuberculosis is based on obligatory reporting by physicians. The second one, the ISBT is based on laboratory reporting and it collects positive laboratory findings that are notified on compulsory basis also. The ISBT is controlled by the National Reference Laboratory for mycobacteria of the NIPH. This system serves as a source of microbiological information and validating device for the register of tuberculosis in laboratory-proven tuberculosis cases. We studied data from the last five years (1997–2002).
Results: The average yearly burden of requested investigations is about 200 000 and they are positive in approximately 3.5%. The majority of these requests is for therapy checking and contact investigations. Since 1998 we have observed a steady decrease in TB prevalence (from 10.3 to 8/100 000). The age structure of patients remained almost unchanged with an increase in men in late productive age (40–54). It is five times higher than that of

women in the same group. The trend of open tuberculosis prevalence is decreasing slower than the total figure. There are substantial geographical differences (from >12/100 000 in Prague and west Bohemia to <6/100 000 in some eastern regions). Prevalence of cases with MDR tuberculosis is fluctuating around 3% and do not show any trend yet. New cases of tuberculosis are diagnosed among legal migrants and immigrants and there is an increasing tendency. They are mainly young people and some of them are infected with MDR strain.
Conclusions: Surveillance of tuberculosis is gaining importance in the changing pattern of cases with increasing importance of foreign-born persons and threat of MDR TB being partly imported from abroad. In the Czech Republic, the low incidence country technical means have increasing importance in improving surveillance methods.

P1509 Evaluation of PPD conversions in prisoners in Bandarabbas, Iran

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Objectives: Prisoners are in the high-risk groups for TB infection and disease. Prevalence of TB in prison is higher than in the community (more than 100 times). PPD conversion in prisoners in Bandarabbas is studied in this research.
Methods: We carried out this descriptive research in the Bandarabbas prison for six months. We selected prisoners with over one year conviction from quarantine. In the first step, we did PPD test on 400 prisoners out of which were selected 120 prisoners that had PPD test less than 5 mm. After 6 months we repeated the PPD test in 87 prisoners who had negative PPD test. We could not carry out PPD tests in 33 prisoners for various reasons.
Results: We observed 53 cases (60.9%) that had PPD conversion with over 10-mm duration. So 34 cases (39.1%) that remained had negative PPD. The minimum age of the prisoners was 18 and the maximum was 74 years. The minimum of PPD conversion was 2 mm and the maximum was 36 mm. The maximum PPD conversion was observed in Iv drug abusers among the 25–34 age group.
Conclusions: The high prevalence of PPD conversion in this research indicates that there is a high contamination rate of TB in prison. So for prevention of TB we propose to start INH prophylaxy in those prisoners that have over 6 months conviction and have had PPD conversion.

P1510 Clinical manifestation of leprosy in a pregnant woman

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Objectives: More than five million people are infected with *Mycobacterium leprae* globally. Due to high socioeconomic standards, however, leprosy has become rare in the Western world. The objective is to describe the clinical manifestation of leprosy during pregnancy in a 29-year-old, otherwise healthy, southeast-Asian woman that presented with a rash, itching and arthralgia to the University hospital in Frankfurt/Main, Germany. Initially, the patient received prednisone for reactive arthritis, but was subsequently treated with dapsone, rifampin and clofazimine.
Methods: The clinical isolate was detected in a skin biopsy by stains for acid-fast bacilli and was amplified by PCR for *Mycobacteria* spp. Genetic identification was achieved by specific PCR for *M. tuberculosis* complex and *M. leprae*. The specimen was inoculated on liquid and solid mycobacterial culture media.
Results: Microscopy of skin smears revealed a multibacillary disease variant with microorganisms being arranged like cigars. PCR for *Mycobacteria* spp. revealed a weak signal that could only be further identified by a positive result in a PCR specific for *M. leprae*. PCR reaction specific for *M. tuberculosis* complex remained negative. The isolate was noncultivatable after 8 weeks of incubation.
Conclusions: In the present case, the pregnancy-induced immunosuppression has led to the manifestation of a previously subclinical *M. leprae* infection. Stains for acid-fast bacilli should be performed in patients with skin lesions and a travel history to tropical countries. The laboratory diagnosis can be largely made through the application of molecular techniques to infected tissues.

P1511 Tuberculous spondylitis in Cukurova, Turkey

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Objective: To evaluate features of tuberculous(tb) spondylitis cases in the Cukurova region of Turkey.
Methods: Twenty patients (pts) with tb spondylitis followed between January 1998 and August 2003 were evaluated. Tuberculin and brucella agglution tests were made. Diagnosis of spondylitis was made on the following criteria: (a) isolation of *Mycobacterium tuberculosis* showed asido-resistant basil with Ehrlich–Ziehl–Neelsen from specimens obtained from the site of spinal involvement and sputum; (b) clinical evidence of the disease; (c) histopathological examination; (d) radiological findings by plain radiography and computerised tomography or magnetic resonance imaging (MRI). All patients were followed up for at least 6 months.
Results: Of the 20 patients 9 were male. Mean age was 42. Mean time between the onset of the symptoms and the time of diagnosis was 30 months (min. 2 months–max. 9 years). Tuberculosis was disseminated in eight patients. Frequent constitutional complaints were; back pain (*n*: 19), weight loss (*n*: 8), paresis (*n*: 7), night sweating (*n*: 5). Physical findings were determined as follows: tenderness at the vertebrae region (*n*: 11), fever (*n*: 6) and tenderness of the sacriliac joint (*n*: 5).Tuberculin test were positive in five patients. Elevated erythrocyte sedimentation rates (>20 mm/hr) in 14 patients and increased CRP levels (>10 mg/L) in 11 patients were detected. Chest graphy in six patients was abnormal. In only five patients was involvement limited to one vertebra. Thoracolumbar vertebraes were the most affected (*n*: 9) followed by lumbar spine (*n*: 5) and thoracal spine (*n*: 4). *M. tuberculosis* was isolated from the site of infection in seven patients. Two of the strains were resistant to ýsoniasid. All of the histopathological examinations revealed caseation of granulomatous necrosis. The most frequent MRI findings were compression (*n*: 7), psoas abscess (*n*: 7), paravertebral tissue inflammation (*n*: 4), epidural abscess (*n*: 2). All the patients were given four drugs for antituberculosis treatment for at least nine months. One of the patients died because of sepsis under the therapy. At the end of the therapy 10 patients remained with sequelaes. While seven of them were mild to moderate, three of them were severe. During the follow-up period there wasn't any relapse.
Conclusions: Half the patients remained with sequelaes and 40% of the patients had disseminated disease. For early diagnosis and to prevent complications tuberculous spondylitis should be considered in patients with back pain, in our region.

P1512 Evaluation of mono- and multiresistance to primer antituberculous agents of *Mycobacterium tuberculosis* strains recovered from Greek and immigrant patients with newly diagnosed pulmonary tuberculosis

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Objective: We evaluated the Isoniazid (INH) and Rifampicin (RIF) monoresistance (resistance to only one of these drugs), as well as the multidrug resistance (resistance to INH and RIF) of *Mycobacterium tuberculosis* strains recovered from Greek and immigrant patients with newly diagnosed pulmonary TB, at the National Mycobacterium Reference Center of Athens, during the 10-year period from 1993–2002.

Methods: Clinical *M. tuberculosis* strains were obtained from 3368 Greek patients and 730 immigrant patients with newly diagnosed pulmonary TB. Among the immigrants, 189 were repatriated Greeks from Eastern European countries and 551 were foreigners from other countries. All *M. tuberculosis* strains were identified by Accuprobe tests (Gene probe, Inc.) and by classical biochemical criteria. The susceptibility testing of strains was performed by the method of proportion on Löwenstein–Jensen medium, or by using the radiometric Bactec 460TB system or the automated Bactec MGIT 960 system (Bactec SIRE; Becton Dickinson).
Results: For INH, the monoresistance rate was 4.5% for Greeks, 6.5% for foreigners and 17.9% for the repatriated Greek patients. For RIF, the monoresistance rate was 0.4%, for Greeks, 0.5% for foreigners and 0.5%, for the repatriated Greek patients. Multidrug resistance rate was 2.5% for Greeks, 5.4% for foreigners and 10% for the repatriated Greek patients. The comparison of the monoresistance rates, at the beginning (1993) to the end (2002) of the study period, showed a twofold increase for INH in both Greek and repatriated Greek patients, while in the foreigners it showed a slight decrease. For the multidrug resistance rates, there was an eightfold increase in Greek patients, a twelvefold increase in repatriated Greeks and a twofold increase in foreigners.
Conclusions: During the last 10-year period, in all the three groups of patients studied, a quite low incidence of RIF mono resistance (<1%) was detected, in contrast to the high incidence of INH monoresistance and multidrug resistance. This particular data should be an alert in using reliable and time-consuming methods of evaluating the susceptibility rates of antituberculous agents.

Nosocomial infections: II

P1513 A European consensus on infection control professionals' training

B. Cookson on behalf of the HELICS Education Group

Objectives: To prepare and disseminate an inventory of European training in nosocomial infection control and establish a European core standard curriculum validated by a DG SANCO funded HELICS (Hospital Infection Linked to Infection Control through Surveillance) education working party comprising people in charge of training programmes.
Methods: Eighteen experts were nominated by their country representatives for the HELICS Advisory Board. Questionnaires were designed and piloted in two or three volunteer countries and then sent to the rest of the group with indicated deadlines. Email was used to exchange questionnaires, data and views. Lichart qualitative scorings were used throughout the project. Excel^{TM} was used for data entry in some instances or to analyse responses. Infection control nursing (ICN) and doctor/epidemiologist (ICD) training requirements and courses were explored. Four syllabus templates were used to develop audit tools to assess the content of current courses in a 'proof of principle'.
Results: The group was happy to use an UK syllabus developed by a multidisciplinary group as a basis for the core curriculum questionnaire design. The various requirements for ICNs and ICDs and the current courses run in each country were agreed and an inventory established. Countries had very different training schemes, accreditation requirements and amounts of time available or expected for lectures and distance, or 'own-time', learning. Accreditation for infection control nurses and or doctors was implemented in many countries or was being considered in the rest. Most (nine) were in favour of problem-solving tests and written examinations and 11 liked projects. However, other methods were less popular. The templates and audit tools were well received. A core competency initiative was to be considered in the future and could help rationalise courses. The major stumbling block we had was identifying the sources of funding for possible international activities where 'fellows' could travel to other centres for training and reflection.
Conclusions: Considerable progress has been made in agreeing the syllabus and content of Infection Control courses. There is a real will to progress in EU qualification but central funding for this has not been identified thus far.

P1514 The Academy for Infection Management: AIMing to improve outcomes in nosocomial infections

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Objectives: The early use of appropriate empiric antibiotics in nosocomial infections reduces mortality, morbidity and length of hospital stay, with obvious benefits to patients, physicians and payors. The intention of the Academy for Infection Management (AIM) is to communicate this new treatment paradigm to healthcare professionals worldwide.
Methods: A faculty of multidisciplinary experts in infection management has defined a set of core principles and devised educational materials, which were introduced to a multidisciplinary audience of almost 3000 delegates at three international and 12 national AIM educational meetings during 2003 and 2004. The educational materials are available via a dedicated, free-access website, http://www.infectionacademy.org.
Results: The AIM website provides education for those involved in the management of serious nosocomial infections. More than 3000 healthcare professionals are registered users of the website. Materials can be downloaded and edited for use at national, regional or institutional meetings, or for self-guided learning. Patient case studies, in the form of slide presentations and interactive key question workmats, are a major feature of the website. Almost 80% of attendees at the first two meetings ($n = 101$) thought the educational standard of materials was very good/excellent. Summaries of key publications supporting the AIM principles are also available. Topics include clinical management, antibiotic resistance, pharmacokinetic/pharmacodynamic principles and pharmacoeconomic considerations for infection management. The website also provides a forum to ask members of the faculty specific questions on the management of nosocomial infections.
Conclusions: The AIM website provides a continuous educational resource for healthcare professionals with access to the latest materials supporting the concept of using appropriate antibiotics early in nosocomial infections.

P1515 Diversity of infection control policies and practices in European hospitals: report from the ARPAC project

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Objective: To assess the organisation and human resources allocated in 2001 to Infection Control Programmes (ICP) in European hospitals.
Methods: Questionnaire surveys on policies and practices for surveillance and control of nosocomial infection and antibiotic resistance in 2001 in 167 acute care hospitals in 34 European countries.
Results: In 96 hospitals (90%), an infection control (IC) committee was present, including mainly: microbiologists (87%), clinicians (80.2%), infection control nurses (ICN) (74%), infection control doctors (ICD) (64%), pharmacists (62%), chief executives (58%). One or more ICNs with specific training in infection

control were present in 79% centres whereas only 38% had one or more specially trained ICDs. A link nurse system was present hospital-wide in 29% or in high-risk units in 16% centres. An ICP with annual objectives/progress reports was developed in 72% and was reviewed by senior management 70%. Hand hygiene (HH) for health workers (HCWs) was promoted by education in 84% centres and by written guidelines in 88%. These had been updated 4—5 yrs in 40% centres. The guidelines recommended: wearing gloves for all contacts with body fluids (93%); washing/disinfecting hands after removing gloves (90%) and use of alcohol-based solutions (70%) and/or medicated/antiseptic soap (42%) for decontamination of non-soiled hands. Education sessions for HCWs in IC practices was reported by 77% hospitals and mainly targeted qualified nurses (71%), junior medical staff (51%) and cleaning staff (51%). Observation/feedback on HH practices was performed by 46% centres but only 1% audited any of their IC protocols. The IC Team provided regular reports to the IC Committee in 66% centres. Epidemiological reports on the prevalence of patients infected with multi-resistant 'alert' organisms were published by 38% of participants.
Conclusions: These results indicate a wide variation in ICP amongst European hospitals. The promotion of HH techniques is well developed but audit of other IC policies and feedback of surveillance data is limited. There is a need to define the minimum core element of effective ICPs and to strengthen resources to harmonise their implementation.

P1516 Infection control policies for multiresistant 'alert' organisms in European hospitals: report from the ARPAC project

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Objective: To assess the specific measures implemented to control transmissible antibiotic resistant bacteria or 'Alert Organisms' in European hospitals in 2001.
Methods: Questionnaire surveys on policies and practices for surveillance and control of Alert Organisms (AO) in 2001 in 167 acute care hospitals in 34 European countries.
Results: In 87% of hospitals, the laboratory provided AO detection reports to the infection control team, for the following organisms: methicillin resistant *S. aureus* (MRSA: 89%), glycopeptide resistant *Enterococci* (GRE: 60%), third generation cephalosporins resistant *K. pneumoniae* (C3RKP: 60%), carbapenem resistant *A. baumannii* (CRAB: 48%), *C. difficile* (CDIFF: 42%), gentamicin resistant *P. aeruginosa* (37%). For MRSA, active screening of carrier was performed in patients in 55% of hospitals and in health care workers (HCW) in 46%. Screening for other AO was performed by 6% to 17% of hospitals depending on the organism. Contact precautions were used for the care of MRSA patients in the majority of centres (gloves: 65%; gown: 56%). These precautions were used for other AO by fewer centres: GRE (gloves: 44%; gown: 40%), C3RKP (gloves: 45%; gown: 36%), CRAB (gloves: 41%; gown: 32%) and CDIFF (gloves: 48%; gown: 41%). In addition, placement of colonised patient in single rooms varied by organism, from 59% (MRSA) to 29% (CRAB). For MRSA patients some centres completed these measures with use of mask (40%), mupirocin decolonisation of patients (77%) and cohorting care (40%).
Conclusions: A majority of European hospitals participating to the ARPAC survey have implemented laboratory-based surveillance of antimicrobial resistant AO. Local AO control policies include a variety of special barrier precautions for the care of colonised patients. MRSA is the most frequently targeted AO. The determinants of this broad diversity of control programmes require further study.

P1517 Influence of prophylactic antibiotic regimen on resistance rates of *Staphylococcus epidermidis* in orthopaedic surgery

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Objective: Prospective analysis of the impact of prophylactic antibiotic regimen on resistance rates of *Staphylococcus epidermidis* in total hip and knee replacement.
Methods: Oxacillin (two doses of 2 g) was used for prophylaxis between 2000 and 2003. In 2002, the regimen was changed to a first generation cephalosporin and adapted to current resistance status on a yearly basis. Starting in October 2002, an intraoperative bacteriological sample was taken routinely in all total hip and knee replacements. Between October 2000 and October 2003, samples were obtained from 178 endoprosthesis and a bacterium could be grown in 18 (10.11%); 14(7.68%) with bacterial growth had no evidence of infection on clinical grounds and were classified as aseptic whereas the remaining seven (3.9%) procedures (early and late infection as well as referred cases) showed also clinical signs of infections and were classified as septic.
Results: In the area of prophylaxis with oxacillin septic cases showed a methicillin resistance of *S. epidermidis* (MRSE) in 48.7% and aseptic cases in 27%. After the switch to a first generation cephalosporin and yearly adaptation of the drug regimen according to the resistance status the MRSE rate gradually declined from 15.3% in 2001 to 3.1% in 2002 for aseptic cases and 54.7% in 2001 to 9.3% in 2002 for septic cases. This decline in resistance towards methicillin of *Staphylococcus epidermidis* was paralleled by a decline of infection rate from 1.8% in 2001 to 0.3 in 2002.
Conclusions: Prophylactic use of a first generation cephalosporin with yearly adaptation according to the resistance status proved beneficial both in reducing the methicillin resistance of *S. epidermidis* (27%–3.1%) and the overall infection rate (1.8%–0.3%) in endoprosthetic surgery.

P1518 New views on large bowel surgery chemoprophylaxis. Compared administration of one dose of ticarcilline/clavoulanic acid against two

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Objectives: According to recent studies, data concerning chemoprophylaxis in colorectal surgery have changed. In this prospective study, we present our experience from issuing one against two doses of Ticarcilline/Clavoulanic acid as chemoprophylaxis in colectomies. The necessity of administering postoperative chemoprophylactic agents is analysed as far as it concerns avoidance of surgical site infection.
Methods: From December 2002 to December 2003, 32 patients (19 men and 13 women with the mean age of 69.12 and 64.36 years respectively) underwent colectomy due to malignant (26 patients) or benign disease (six patients). Nineteen were deemed high-risk patients due to coexisting diseases (cardiac disease, diabetes mellitus, respiratory disease, renal insufficiency, hepatic insufficiency, obesity, cortisone therapy). All patients were divided into two groups at random. In group A, one dose of Ticarcilline/Clavoulanic acid was administered intra-operatively, while in group B, two doses of the same antibiotic were administered (the first one intra-operatively and the second postoperatively). Thereafter, all patients were under clinical and laboratory monitoring for surgical site infection.
Results: Surgical site infection was developed in six out of 32 patients under study. Four patients (two from each group) suffered from wound suppuration, while two had intra-abdominal abscess formation (equally presented in each group). Culture isolates were *Escherichia coli* in five patients and *Enterococci* in four. Treatment consisted of wound drainage and systemic antibacterial

agents in cases of wound suppuration while in cases of intra-abdominal infection, administration of two antimicrobials or Imipenem was performed. All patients were cured. The mean hospitalisation was 15.7 days.
Conclusions: Reviewing our material, it appears that benefits arising from administering additional postoperative antimicrobial prophylaxis in colorectal surgery were statistically insignificant. Nevertheless the number of patients under study is still small, so it would be unsafe to extract a conclusion.

P1519 New aspects concerning chemoprophylaxis in elective cholecystectomy

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Objectives: Intra-abdominal abscess can result from elective cholecystectomy due to spilled stones or bile, infested with bacteria. Cefuroxime has been considered as the most appropriate agent to be given for chemoprophylaxis in elective cholecystectomies. Nevertheless, *Enterococci* infest bile and are sensitive to Ampicillin/Sulbactam rather than Cefuroxime. Even though *Enterococci* are lethal if implicated in intra-abdominal sepsis, the need for anti-enterococcal prophylaxis has not been studied yet.
Methods: During the period extending from July 2002 to December 2003, 188 patients (71 male and 117 female, mean aged 60.86 and 56.85 years respectively) underwent elective cholecystectomy. Eighty-nine (47.34%) were deemed high-risk patients. Cultures from gall-bladder bile and mucosa were taken from all patients. Cefuroxime 1.5 g, or Ampicillin/Sulbactam 3 g were administered intra-operatively at random. The patients were under clinical and laboratory monitoring for postoperative infection of the surgical site.
Results: Forty-two (22.34%) patients revealed positive cultures. More specifically the cultures' results were the following: *Enterococci* isolated in 21 patients, *Escherichia coli* in 12, *Klebsiella* in four, *Staphylococcus epidermidis* in three, *Citrobacter* in three *Enterobacter cloacae* in three, *Streptococci* in two, *Citrobacter freundi* in two, *Bacteroides fragilis* in two, *Pseudomonas* in one, *Enterobacter aerogenus* in one and *Acinetobacter* in one. Among the patients that revealed negative cultures, two suffered from sterile collection of the wound and one presented on the 7th postoperative day with wound suppuration and CNS isolation in pus culture. From the patients that had a microbe isolated in the gall bladder and bile cultures, one developed wound infection with *E. coli* and *Klebsiella* (that patient revealed the same bacteria in bile and mucosal cultures), one suffered from postoperative suppuration of the wound and CNS isolation in the pus, while two patients were hospitalised in ICU due to respiratory insufficiency. Mortality rate was nil. The mean hospitalisation and the mean postoperative hospitalisation were 7.43 and 4.57 days respectively.
Conclusions: Chemoprophylaxis had the same results in both groups. Nevertheless anti-enterococcal prophylaxis should be considered as far as *Enterococcus* is isolated in a great number of patients with positive cultures.

P1520 Ceftriaxone vs. other antibiotics for surgical prophylaxis

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Objectives: To investigate possible differences in prophylaxis with ceftriaxone compared with other antimicrobial agents for surgical–site infections (SSI) and remote infections such as respiratory (RTI) and urinary tract infections (UTI).
Methods: The efficacy of ceftriaxone (CRO) was compared with that of other antibiotics in the perioperative prophylaxis of local (SSIs) and remote (RTIs and UTIs) infections in a meta-analysis of randomised controlled trials published between 1984 and 2003. The analysis was based on a 2×2 contingency table with classification by treatment and number of infections obtained from individual studies. The global estimate of the effective treatment was obtained with the weighted mean of the log OR (Odd Ratio) according to Mantel–Haenszel and associated confidence intervals (CI) at 95%. All the calculations have been performed using SAS vs.8. Chi-square test was performed.
Results: Evaluations were performed on 48 studies, for a total of 17 565 patients. Four hundred and six patients (4.8%) in the CRO group and 525 (6.3%) in the comparator group developed a SSI, log Odd Ratio -0.30 (CI -0.50 to -0.13) $P < 0.0001$. RTIs were observed in 292 (6.01%) patients in the CRO group and in 369 patients in the comparator group (7.6%), log Odd Ratio -0.30 (CI -0.55 to -0.09), $P = 0.0013$. UTIs were reported for 2.2% of CRO prophylaxed patients compared with 3.74% of patients prophylaxed with other drugs [log Odd Ratio -0.54 (CI -1.18 to -0.16), $P < 0.0001$]. Overall, in clean surgery 195 (5.1%) and 234 (6.2%) patients developed an SSI in the CRO and comparator group, respectively [log Odd Ratio -0.22 (CI -0.51 to 0.01), $P = 0.0476$]. RTIs were prevented for all but 1.57% of patients (CRO group) and 2.62% (comparators group), $P = 0.01$ in clean surgery and for 9.54% (CRO group) vs. 11.6% (comparators group), $P = 0.01$. While results observed in clean surgery did not evidence a statistically significant superiority of CRO in preventing UTI's insurgence [Log OR -0.21 (CI. /0.65), $P = 0.7702$), this was shown in the clean–contaminated surgery. In fact, 4.47% of patients in the CRO group vs. 7.52% of patients in the comparator group developed an UTI [log OR -0.56 (CI -1.25 to -0.16), $P < 0.0001$]. Adverse events were observed in a similar proportion in the CRO prophylaxis and the comparator group (0.35% and 0.23%). Duration of prophylaxis did not influence the outcome of infections.
Conclusions: The meta-analysis showed that CRO is statistically superior to other antibiotics in preventing both local and remote post-operative infections.

P1521 Monitoring of antibacterial prophylaxis in general surgery: results from a multicentre Italian incidence study

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Objective: the CDC recommendations represent the standard for antimicrobial prophylaxis of clean/clean contaminated surgery in which first or second generation cephalosporins are indicated. The aim of this study was to monitor antimicrobial prophylaxis in Italian general surgery wards where penicillin was routinely used.
Methods: This study was carried out from the 1st of April to the 31st of May '02 in 32 general surgeries performed in large hospitals all round Italy. All operated patients were surveyed by the surgeon during and after hospital stay up to 30 days from operation for nosocomial infection (NI) diagnosis; patterns of antimicrobial prophylaxis (timing, duration and type) were collected in an electronic case report form.
Results: Surgical procedures, 3066 in number, were performed, 75.4% of them with NNIS risk index 0–1 and 86.8% clean (with prosthesis: 42.7%) or clean/contaminated. More than 50% of the operations were represented by colcestectomia (20.0%), herniorrhaphy (17.1%), colon surgery (12.0%) and appendectomy (10.5%). Among operated patients with clean and elective clean/contaminated or contaminated surgery 86.4% received antimicrobial prophylaxis; 59.0% for clean interventions. Amoxocillin/clavulanate (28.3%), followed by ceftizoxima (11.4%) and ampicillin/sulbactam (9.6%) were the most prescribed antibiotics. Only one antibiotic was used in 88.8% of patient with prophylaxis. Treatment was given on the same day of surgery to 89.6% of treated patients, in 47.1% and 43.5% of cases before and at induction of intervention respectively. Patients treated with cephalosporins were at higher risk for developing NI [RR: 1.6; 95% CI: 1.05–2.44] with respect to patient treated with penicillin: the two groups were comparable for age, sex and NNIS risk index; however, a majority of herniorrhaphy and mastectomy were represented in the penicillin groups with respect to cefalosporin.

A separated analysis in herniorrhaphy patients confirmed an incremented RR of 3.18 in patients treated with cephalosporin.
Conclusion: A high use of antibiotics was observed in clean surgery, particularly a high use of penicillin/protected was documented in all class of surgery. Rationale for antibiotic treatment in clean non-prosthetic surgery is controversial.

P1522 Prognosis of unknown origin bacteraemias in critically ill patients

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Objective: To determine whether unknown origin bacteraemia (UOB) is an independent risk factor of mortality in critically ill patients with bloodstream infections.
Material and methods: All clinically significant bacteraemias in a 12-bed intensive care unit of a teaching hospital, from 1996 to 2003, were evaluated for clinical and microbiological characteristics. We assessed risk factors for hospital mortality by multivariate logistic regression with the SPSS package (9.0).
Results: Eighty-five (36%) of 235 episodes of bacteraemia in critically ill patients were of unknown origin. Eighty-six percent of episodes of UOB were hospital acquired. The mean age of patients with UOB was 64.4 ± 14.4 years. Factors associated with UOB were: nosocomial origin ($P = 0.034$) and non-severe sepsis ($P < 0.001$). The aetiology of UOB was CNS (32%), *Acinetobacter baumannii* (12%), *Staphylococcus aureus* (12%), *Enterococcus* spp. (11%), *Pseudomonas aeruginosa* (6%), *Escherichia coli* (5%) and others (22%). The prevalence of CNS was higher in episodes of UOB ($P < 0.001$), and the prevalence of *A. baumannii* and *E. coli* was lower in UOB than in known origin bacteraemias ($P = 0.007$ and $P = 0.001$). Global and related death rates were 51% and 21% in UOB, and 54% and 21% in episodes of known origin, respectively ($P = 0.616$ and $P = 0.208$). The factors associated with global mortality in all episodes of bacteraemia were severe sepsis, septic shock and nosocomial origin. Septic shock was associated with related mortality, but not with UOB.
Conclusions: Contrary to previous reports, an unknown source of bacteraemia was not independently associated with a fatal outcome in critically ill patients.

P1523 Attributable mortality of nosocomial candidemia caused by *Candida albicans* and *Candida non-albicans* species in critically ill patients

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Background: We previously reported a nonsignificant attributable mortality rate in nosocomial candidemia in intensive care unit (ICU) patients [1,2]. It remains uncertain whether candidemia involving *Candida non-albicans* spp are associated with worse outcome in comparison with *Candida albicans* candidemia in this particular patient population.
Methods: In a retrospective study (1992–2000) attributable mortality for *C. albicans* and *C. non-albicans* candidemia was investigated and compared in ICU patients. Two independent matched cohort studies were performed. Matching was (1:2-ratio) based on severity of underlying disease and acute illness (APACHE II score and admission diagnosis) and length of ICU stay prior to the onset of the candidemia. As expected, mortality can be derived from APACHE II. This matching procedure results in an equal prognosis for cases and control subjects. Attributable mortality is determined by subtracting the hospital mortality rate of the controls from that of the candidemic cases.
Results: During the study period 73 of the 29727 ICU admissions were complicated with microbiologically documented candidemia (2.5/1000 admissions). Fifty-one episodes were caused by *C. albicans* and 22 by *C. non-albicans* spp (17 *C. glabrata*, 3 *C. parapsilosis*, 1 *C. krusei*, 1 *C. tropicalis*). In the *C. albicans* matched cohort study an attributable mortality of 3.9% was found (95% CI: −13–21%) as mortality rates for cases and controls were respectively 43.1% and 39.2% ($P = 0.771$). In *the C. non-albicans* matched cohort study an attributable mortality of 9.1% was found (95% CI: −16–34%) as mortality rates in cases and controls were 59.1% and 50.0% ($P = 0.663$). Both attributable mortality rates were not statistically significant. Consequently, the difference between them was also not significant.
Conclusions: No difference in attributable mortality in candidemia involving *C. albicans* vs. *C. non-albicans* spp was observed in this cohort of ICU patients.
References
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P1524 Predictors of nosocomial *Acinetobacter baumannii* bacteraemia in an intensive care unit

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Objectives: Acinetobacter baumannii has been named as the methicillin-resistant *Staphylococcus aureus* of Gram-negative pathogens, specially after the steady rise in the number of isolates and the reports of many Intensive Care Unit outbreaks. The aim of this study was to identify independent risk factors for developing Acinetobacter baumannii hospital-acquired bacteraemia (ABB).
Material and methods: Study of nosocomial bacteraemias in a medical-surgical intensive care unit in a teaching hospital, during a seven-year period (from 1996 to 2003). To analyse the predisposing factors for developing ABB appropriate clinical and epidemiological variables were recorded from clinical charts. We used stepwise logistic regression analysis to determine independent predictors of ABB. SPSS (9.0) software was used for data analysis.
Results: Fifty-nine (29%) of 202 nosocomial bacteraemias were due to *Acinetobacter baumannii*. The mean age of patients with ABB was 60.5 ± 17.7 years and the relation between men/women was 2.4. The principal origins of ABB were respiratory (57%), catheter (17%) unknown (17%). Polymycrobial bacteraemia was identified in 55% of episodes of ABB. Septic shock was present in 31.6%. The mean length of stay in the hospital was 44.0 days and the mean of days of hospitalisation before the onset of bacteraemia was 19.7 ± 20. Among the strains identified, 74% were multidrug-resistant and 28% were imipenem-resistant. The global and related mortality rate for ABB was 45.3% and 25.4% respectively. Risk factors for ABB were mechanical ventilation (OR 4.35; IC 95% 1.06–17.86: $P = 0.04$), respiratory source of infection (OR 9.41; IC 95% 3.51–25.16: $P < 0.0001$), and previous use of cephalosporins (OR 3.61; IC 95% 1.40-9.40; $P = 0.007$) whereas less than ten days of hospitalisation before the onset of bacteraemia (OR 0.35; IC 95% 0.14–0.87: $P = 0.02$) and the presence of any cardiac comorbidity (OR 0.29; IC 95% 0.09–0.90: $P = 0.03$) were preventive factors in the multivariate analysis.
Conclusions: More than 10 days of hospitalisation, the use of mechanical ventilation, pneumonia as source of bacteraemia and the previous use of cephalosporins must be considered as risk factors for developing ABB.

P1525 Antibiotics cycling in ICU: results of 1.5-year experience

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Objectives: The aim of the investigation was to study changes of antibiotic resistance of VAP pathogens after the use of the strategy of 'antibiotics cycling'.

Methods: The material of the present study is the results of the investigation carried out during the period from June 2002 up to October 2003 in the medical ICU department of Moscow's municipal clinical hospital. From January 2000 to June 2002 (I period) the combination of ceftazidime (6 g/day) with amikacin (1 g/day) was used as empirical antibacterial therapy of VAP, and from June 2002 to October 2003 (II period) – cefepime (4 g/day) as a single-drug therapy. Nine hundred and thirty-seven strains of different pathogens of VAP were studied. Antibiotic resistance of *P. aeruginosa* and *Enterobacteriacae* as most widely spread causative agents of VAP (33.4% and 18.6% correspondingly) was studied. The material was obtained by BAL. Sensitivity of pathogens to the tested antibiotics was determined by the disc-diffusion method.
Results: The total consumption of amikacin, ceftazidime and cefepime in the ICU department during the first period was 2043, 1632 and 158 g and during the second period, consumption of cefepime was 2836 g while amikacin and ceftazidime were excluded.

Resistance of Enterobacteriaceae and *P. aeruginosa*, %

	Enterobacteriaceae		*P. aeruginosa*	
Antibiotics	I period	II period	I period	II period
Amikacin	66	43	86	71
Ceftazidime	63	78	26	19
Cefepime	7	21	11	5

Conclusions: Exclusion of ceftazidime and amikacin for 17 months resulted in 7% decrease of resistance of *P. aeruginosa* to ceftazidime. It was not possible to overcome the high level of resistance to amikacin during that period. It was not revealed to increase in resistance to cefepime despite its wide use during the 17 months.

P1526 Evaluation of the risk factors for mortality and nosocomial infection in intensive care units

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Objective: Evaluation of risk factors for mortality and nosocomial infection in ICU patients
Patients and Methods: We conducted a prospective study between May 2002 and November 2002 in surgical and medical ICUs of Ankara Numune Education and Research Hospital. The analysis involved 334 patients who were followed in the ICUs for at least 48 hours.
Results: Among the 334 patients, 104 (31.1%) had ICU acquired infections. The overall mortality rate in the ICU was 46.7%. The mortality rate was significantly higher in the patients with nosocomial infections (66.3%) than in noninfected patients (37.8%) ($P < 0.001$). In the multivariate analysis, the mean length of stay in ICU (OR 1.11, 95% –1.06–1.17), the mean length of intubation (1.15 95% CI 1.05–1.25), total parenteral nutrition (TPN) (OR 8.52, 95% CI 2.93–24.83) and enteral nutrition (OR 3.22, 95% CI 1.65–6.3) were independently associated with infection. The mean age (OR 1.03, 95% CI 1.01–1.05), mean APACHE II score (OR 1.11, 95% CI 1.04–1.17), mechanical ventilation (OR 6.28, 95% CI 2.50–15.79), stay in medical/surgical ICU (OR 0.31, 95% CI 0.14–0.69), central venous catheter (OR 4.89, 95% CI 1.79–13.23), use of antibiotic (OR 0.43, 95% CI 0.21–0.86) and coma (OR 2.29, 95% CI 1.20–3.26) were independently associated with mortality.
Conclusions: Nosocomial infection risk increased 8.5 times in the use of TPN and 3.2 times in enteral nutrition. The most important risk factors for mortality were older age, high APACHE II score and coma and among procedures, central venous catheters and mechanical ventilation. The mortality rate was 6 times higher in the mechanically ventilated patients.

P1527 Knowledge, attitude and behaviour of health care workers in two Indonesian teaching hospitals on the island of Java

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Objectives: Knowledge, attitude and behaviour of health care workers concerning infection control are important determinants of the level of hospital hygiene. They also reflect the success of the infection control organisation in an institution. Not much is known about infection control in Indonesia. Therefore our study investigated knowledge, attitude and (self-reported) behaviour of health care workers in two Indonesian teaching hospitals by means of an inquiry.
Methods: A questionnaire was carried out amongst healthcare workers of the departments of internal medicine, surgery, paediatrics, obstetrics & gynaecology and ICU. The goal of the questionnaire was explained and anonymous analysis of the results was guaranteed to the participants. Participants completed the questionnaire during sessions in which one of the researchers or infection control nurses were present to check whether they worked individually, and to answer questions.
Results: A representative sample of more than 50% of all doctors, nurses and assistant-nurses of the involved departments of both hospitals ($n = 1045$) completed the questionnaire. Their answers showed that, in both hospitals, only a minority of personnel is vaccinated against hepatitis B (41.6 and 30%), although the majority has experienced needle stick accidents (64.1 and 86%), but did not report them. The knowledge of blood-borne diseases is small and there are no clear guidelines about handling of needle stick accidents and the place of the infection control organisation in these matters. There are not enough hand washing points and there is an inconsistency in knowledge, attitude and behaviour concerning the role of hands in infection control. The supply of personal protective equipment such as gloves, masks and gowns is insufficient and the distinction between sterile and non-sterile gloves is not clear. There is limited knowledge about wound care and care for patients with a urinary catheter.
Conclusions: The questionnaire showed a deficit of knowledge regarding infection control in general and specifically regarding hand hygiene, safe blood handling and personal protective equipment.

P1528 Assessment of the impact of the microbiological reports on the antibiotic treatment of bacteraemia

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Objectives: Blood cultures rank among the most important investigations in febrile patients. This study aims to assess the microbiological reports' impact on the choice of antimicrobial agents for the treatment of bacteraemia.
Methods: A prospective study of 165 episodes of bacteraemia in a tertiary care hospital. Data collected included demographic characteristics, underlying diseases, focus of infection, culture results, antibiotic treatment and outcome.
Results: The 165 bacteraemia cases are divided into group A (92/165) and group B (73/165). Group A consists of 92 cases ($92/165 = 55.7\%$) that were treated with appropriate empirical therapy (microorganism sensitive to the antibiotic). Group B consists of 14 bacteraemias ($14/165 = 8.4\%$) that didn't receive empirical therapy and 59 cases ($59/165 = 35.7\%$) that were treated with inappropriate primary therapy (microorganism resistant to the antibiotic). After consultation with the laboratory microbiologist and reporting of the blood culture results, there was immediate change in therapy for 61/73 of group B bacteraemias and thus the proportion of appropriate treatment for the total cases reached $(92 + 61)/165 = 92.7\%$. For the remaining cases of group B, 3/73 were attributed to a multiresistant microorganism and for 9/73 the blood culture report occurred

after death of the patient. Change of therapy after the blood culture result was also observed for 29/92 group A episodes (it was appropriate, i.e. in compliance to the susceptibility reports). Among group A bacteraemias the outcome was cure from the infection for $82/92 = 89.1\%$ while for group B the respective rate of cure was $53/73 = 72.6\%$. This statistically significant difference (chi-square test, $P = 0.006$) in cure rates cannot be attributed solely to the difference in appropriateness of empirical therapy though.
Conclusions: Our study confirms that blood culture results are essential in optimising antimicrobial therapy. Furthermore, the consultation of the microbiologist with the physician for every case of bacteraemia is invaluable and increases the compliance of therapy to the antibiotic susceptibility reports (the rate of appropriate therapy increased from 55.7% to 92.7%)

P1529 Nosocomial infection prevention and control programme during hospital reconstruction

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Introduction: Nosocomial infection (NI) outbreaks have been associated with construction activities in hospitals. As our hospital, a Tertiary University Hospital with 600 beds and an average of 34 000 admissions/year, is constructing a new building with land excavation and building demolition, a NI prevention and control programme has been established. The design and results of this surveillance method are presented.
Methods: An NI weekly prospective incidence study, from February 2001 to November 2003, of 30175 patients (4689 with major surgical procedures) admitted in risk areas near the works was carried out. Data was collected and processed by the Infectious Diseases (ID) team. Basic data was obtained from the income census. Microbiological results, antimicrobial prescription and seriated visits of ID consultants were used as sources for case detection. CDC criteria and definitions of NI were used. Microbiological air quality was checked in HEPA protected areas every 3 months and monthly in non-protected areas (366 samples). When a problem was detected adequate correction measures were taken.
Results: The global incidence rate was 3.2%. Weekly incidence of NI oscillated from 1.1% to 4.9% with urinary tract infection being the most frequent (0.8–3.6%). No increase of respiratory (0–2.5%) or surgical infections (4–8%) was observed and no epidemic outbreaks of aspergillosis, filamentous fungi or legionellosis were detected. In patients with bone marrow transplant or heart transplant admitted in the haematology or cardiology services there was not an increase of the rate of invasive aspergillosis compared with previous years.
Conclusions: (1) Longitudinal and prospective incidence studies, air sampling and prevention measures are useful methods for the follow-up of a new hospital construction-related nosocomial infections. (2) No increase of nosocomial infection rates or epidemic outbreaks have been observed in our institution during hospital construction.

P1530 Environmental organisms from different wards in a teaching hospital

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Objectives: An Intensive Care Unit (ITU), Stroke Unit (SU) and Medical Day Bed Unit (MU) were subjected to a standardised screening programme over a four-month period. The aim was to examine environmental organisms from these wards and compare their bacterial resistances in association with antimicrobial usage on each ward.
Methods: Hand-touch sites such as door handles and computer keyboards, and other sites such as floors and sinks were screened using commercial dipslides. Organisms were quantitatively and qualitatively assessed and subjected to antimicrobial susceptibility testing. Antibiotic consumption data was obtained for the previous year for each unit and expressed in Defined Daily Doses (DDD)/1000 patient-days.
Results: The amount and identity of organisms from each ward were similar but there were differences between wards regarding microbial density from certain sites. Nearly 300 staphylococci were recovered as opposed to 64 Gram-negative bacilli. Antibiotic resistance was significantly associated with individual wards, particularly for staphylococci ($P < 0.0001$) but also for coliforms ($P = 0.04$) and other Gram-negative organisms ($P = 0.07$) despite fewer numbers. ITU consumed 3110 DDD/1000 patient-days, six times more than SU and MU. There were further associations made between beta-lactam consumption and methicillin resistance, and aminoglycoside consumption and gentamicin resistance.
Conclusions: Antibacterial resistance is the only significant difference between environmental bacteria from different wards, and appears to reflect prescribing pressures placed on those wards. The dirtiest ward visually (SU) had the least heavy microbial growth from hand-touch sites and the least number of resistant bacteria. ITU appeared much cleaner but demonstrated a higher proportion of heavily contaminated hand-touch sites with multiple resistant bacteria. Thus, visual inspection of a ward may not provide a reliable guide to the potential risk of infection from its environment. The findings have implications for local antibiotic policies, infection control and cleaning schedules.

P1531 New way of microbe decontamination from air – MFI method

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Objectives: In many branches of medicine, the microbiologically clean air is a significant condition either of microbial pollution or infection reduction, i.e. in operating place, or medical staff infections.
Methods: The MFI method (Multifunction Ion Air Cleaning) of Genano Oy, Finland was examined, using Nanobio E310 device, from the point of its air decontamination efficiency. Tested device work parameters are: cleaning capacity 250 m^3/h, at air flow velocity 0.5 m/s. The microbiological purity of exhausted air was measured. Two methods were applied for qualitative and quantitative tests; De Ville Biotechnology (MicroBio device), and contact plates method (Oxoid). There were 10 test bacterial and fungal types from ATCC collection (Rockville, USA). In quantitative tests, a microbial suspension (aerosol) was used, density 10 6–10 8 cfu/ml, aseptic conditions.
Results: Microbiological purity of air, after passing through the air cleaning MFI device, determinated the removing and destroying of both bacteria and fungi from air. It is to be highly effective towards a wide spectrum of microbes. The extent of microbiological air contamination does not influence effectiveness of air cleaning with the MFI method. Long-term (7 days) work of Nanobio E310 device does not result in any changes in efficiency of air cleaning with the MFI method, even in conditions of high microbiological pollution of internal air. Research done in experimental conditions, points out to significant microbes reduction in cleaned air. It appears to be at least 10% bacteria and fungi less than in the beginning of the test, what equals effectiveness 99.999% at particles range equal or more than 0.003 μm.
Conclusions: The application of MFI technology, regarding appropriate assembling of air cleaning devices and preserving the air exchange frequency, adequate to room cubature, equipment and number of employees, enable reaching at least B air class of microbial purity (<10 cfu/m^3 of air) and/or 100, M3.5 air class, ISO 5 according to the United States Federal Standard 09 E and ISO standards as well.

P1532 Disinfection of surfaces in hospital isolation rooms with ultraviolet C (UVC) light, compared with chloramine

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The germicidal effect of ceiling- and wall-mounted ultraviolet C (UVC) light at 254 nm in isolation units with negative pressure (−45 Pascal) was examined and compared with disinfection with chloramine during end-disinfection after patient stay. Microbial samples were taken from surfaces before and after disinfection with UVC (33–47 min) and chloramine (5%, 1 h exposure) using standard contact plates (20 cm^2). The UVC-distribution in the isolation units was monitored at 165 positions. The output on the floor varied between 0.08 and 3.2 W/m^2, with an average (±SD) of 2.2 ± 0.5 W/m^2 in the patient room, 2.0 ± 0.7 W/m^2 in the sluice and 1.4 ± 0.5 W/m^2 in the bath/ decontamination room. On other places, the values varied from 0.08 to 6.82 W/m^2. The units were UVC-disinfected for 33–47 min, corresponding to doses ranging from 160 J/m^2 in shadowed area to 19 230 J/m^2 at the highest exposed site. According to published UVC-dosimetry, the survival of bacteria and bacterial spores are reduced by 90% with doses ranging from 4–120 J/m^2 and 100–365 J/m^2, respectively. Thus, UVC doses used in this study should be high enough to inactivate most bacterial organisms, including spores, even on surfaces not directly exposed to UVC. UVC-disinfection significantly reduced the bacteria on surfaces directly or indirectly exposed to UVC to a very low number (from *c.* 30 to 1–2 cfu/plate), as did 5% chloramine disinfection (from *c.* 30 to 1–2 cfu/plate) alone; $P < 0.001$, and $P < 0.001$, respectively. Since cleaning before disinfection may be a risk for the staff in isolation units, disinfection with UVC- or chemicals should always be performed first. The presence of completely shadowed areas in the isolation unit (the bed rail, lockers, mattresses etc.) still needs disinfection by chemicals before cleaning. Therefore, UVC may not be used alone, but is a good additive to chemical disinfection, to lower the biological burden of infectious agents in isolation units for high-risk infectious patients.

P1533 Controlling procedures for reprocessing flexible gastrointestinal endoscopes – the value of g-control charts

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Objectives: In our quality control (QC) program and in the international literature, infection has been related to high-risk flexible gastrointestinal endoscopes (FE). Especially after over night proliferation of micro-organisms in FE channels, patients can be at infection risk. We used a Number-Between g-Type statistical Control Chart and associated statistical methods to identify unstable FE reprocessing procedures.
Methods: We used microbial counts, CFUs, from flush water from the water channel before an endoscopy in our QC program. Clean, critical and high-risk FEs were defined. The g-control card is a figure where the *X*-axis represents the consecutive number for a critical or high-risk FE, and the *Y*-axis the number of clean FE between every critical or high-risk FE. A Center Line (CLwd) illustrates the expected quality in reprocessing FE defined for a concrete type of washer-disinfector (WD). A CLmc line shows the expected quality in reprocessing FE using only manual cleaning (mc) of FE with no proliferation of micro-organisms during storage. Thirteen endoscopy units from eight hospitals in Copenhagen Hospital Corporation (H:S) and Copenhagen County (KAS) participated in the program. CLwd was determined for three different WDs and CLmc from previous experiments. g-control charts were produced for each endoscopy unit allowing out of control evaluation in relation to occurrence of critical and high-risk FEs.
Results: 7351 QC samples from FE were evaluated. We present data on a g-control chart over a 5-year period from a department using three 10-year-old Olympus ETD WDs showing the development of an out of control situation. The FE reprocessing went from in control to out of control with consecutive control points below the CLwd and CLmc and with the occasional observation of high-risk FE during the last two years. Out of control situations can be detected if eight consecutive control points or 12 of 14 consecutive control points fall below the CLwd or CLmc.
Conclusions: The g-control chart was found to be a valuable statistical tool allowing identification of out of control procedures for reprocessing FE. These data can show out of control manual cleaning and give early signs of WDs in need of repair or are in such bad condition they should be replaced. g-control charts are a great improvement over the previously used p-control charts.

P1534 Preventing the spread of Legionella in hospital wards: measures adopted in a polyclinic, Bologna

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Aims: Legionella represents a serious problem for old hospitals that over the years have expanded in size and, as a result, have added to the plumbing systems of the original buildings. In the Policlinico S.Orsola-Malpighi which has highly specialised wards for the treatment of patients who are particularly vulnerable to infection such as transplant and cancer patients some parts of the plumbing network are over 50 years old. A comprehensive and radical overhaul of the plumbing system in order to eradicate Legionella completely was not a realistic goal and so the target set by the hospital was to prevent high-risk patients from being exposed to infection.
Methods: Filters were fitted to the taps and shower heads in the rooms of high-risk patients in the following sectors: haematology, oncology, paediatric onco-haematology, nephrology, surgery (transplant patients). A monitoring programme was set up to assess the efficiency of these measures. Until February 2003, single samples were taken at intervals during the year. However, with this method, low-level or intermittent contamination at outlets where filters had been installed could not be identified and so any evaluation of the plumbing system risked being distorted by false negative results. For this reason, a new monitoring programme was set up in March 2003 which involved taking regular samples at every outlet under inspection.
Results: Over the years, about 50% of outlets not protected by filters have shown Legionella contamination (32/year). With one exception, all the levels of contamination found were within the range where vigilance is recommended by existing regulations. In contrast inspections of shower heads and taps fitted with filters have given only negative results (490 samples). Recently outlets have also been monitored by repeated sampling to avoid false negative results because of low-level or intermittent contamination. Outlets fitted with filters were found to be uncontaminated even when subjected to this type of inspection. The only exception was one outlet, present in a room kept shut for several days, which was found to be contaminated in two instances.
Conclusions: The data from the monitoring carried out indicate that fitting water outlets with filters is an effective way of avoiding Legionella contamination so long as certain basic requirements are met: filters must be installed in the correct position in the plumbing system and must undergo periodic maintenance and replacement.

P1535 Nosocomial Legionella infections confirmed by molecular typing

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Objectives: Nosocomial origin of Legionellosis must be confirmed by molecular typing of Legionella strains isolated from patients and from hospital water supply. In this study we used pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA PCR (RAPD-PCR) to type Legionella strains isolated

from four patients, that acquired legionellosis in four different hospitals, and related hospital environmental strains.
Methods: *L. pneumophila* serogroup 1 was isolated from the first patient (male, 68 years, immunosuppressed) and *L. pneumophila* 1, 2, 3, 6 in the related hospital water. *L. pneumophila* serogroup 1 was isolated from the second patient (male, 81 years, cardiopatic) and *L. pneumophila* 1, 3, 4, 6 in the respective hospital water. *L. bozemanii* was isolated from the third patient (female, 44 years, immunosuppressed with autoimmune disease), *L. pneumophila* 1 and *L. bozemanii* in the related hospital water. In the last case, *L. pneumophila* sero group 3 was isolated from patient (female, 77 years, immunosuppressed) and *L. pneumophila* serogroup 3 and *Legionella* spp. from hospital environment. Strains from patients and related hospital water supply were typed. PFGE of DNAs cleaved with SfiI and NotI was performed on 1% agarose gel (CHEF DR III). Amplification for RAPD-PCR was performed with two primers using Ready-to-Go RAPD analysis beads and visualized by staining with ethidium bromide and by electrophoresis on polyacrylamide gel with GenePhor System.
Results: In all four cases, strains from patients and related hospital environmental isolates showed the same genomic profiles by PFGE and RAPD-PCR, while different patterns were found for unrelated strains.
Conclusions: Molecular typing demonstrated that strains from patients and from hospital hot water were genetically indistinguishable, showing that hospital water was the source of infection. The nosocomial origin would be probably unrecognized without molecular techniques, that are very useful epidemiological markers.

P1536 Experience with a chlorine dioxyde water treatment system to control Legionella in a hospital hot water supply

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Objectives: We evaluated the efficacity of a chlorine dioxyde (ClO2) system to reduce the significant amount of Legionella present in our hot water supply.
Methods: After an evaluation of the amount of Legionella in our hot water supply, a chlorine dioxyde system marketed by Gulldager® was installed in October 2000. This system includes three components: one pump injecting in the reaction chamber an aquous stabilised solution of chlorine dioxyde [Activ OX20®]; one pump injecting in the reaction chamber an activator [Activ 8®] and the reaction chamber. The Activ product is injected at the entrance of the hot water supply. We controlled the amount of Legionella at different places of the supply between December 2000 and March 2002. We also investigated the risk of corrosion of the water pipes by placing small pieces of steel or steel and zinc at a point before the injection of ClO_2 and at another point after the injection.
Results: Pretreatment measures revealed in July 2000 the presence of Legionella in 19 of 22 (86.3%) water samples. In nine of 22 (40.9%) water samples the number of Legionella was above 10^3 ufc/L. The mean number of Legionella in the samples was 1541 ufc/L. Seven controls were made between October 2000 (installation of the ClO_2 system) and march 2002. A 14-month period was necessary to see the efficacity. In December 2001, two of nine samples (22.2%) were positive but with <500 cfu/L and in March 2002, three of 10 samples (30%) were positive but only one sample had 10^3 cfu/L (mean number 468 cfu/L). Concerning the corrosion of the steel pieces, the loss of weight after 96 days was 149.8 mg (initial weight 14.648 g) without ClO2 and 150.4 mg (initial weight 14 704 g) with ClO_2. For the steel plus zinc, the loss of weight was 1550.8 mg (initial weight 12.456 g) without ClO_2 and 1415,1 with ClO_2 (initial weight 12 953 g)
Conclusions: As a result of the structure of our water pipes network and the presence of biofilms it appears that ClO_2 was only effective in decreasing the Legionella level after 1 year. Moreover the adjunction of ClO_2 does not increase the risk of corrosion.

P1537 Sustained antimicrobial activity of a novel triclosan hand preparation

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Objectives: (1) To assess the antimicrobial efficacy of a novel 2% triclosan hand preparation (THP) in the clinical setting; (2) To determine the sustained antimicrobial activity of THP when adopted as a part of routine hand hygiene; (3) To determine the effect of repeated hand washing with soap and water on the sustained antimicrobial activity of THP.
Methods: A controlled, randomised, prospective open trial was undertaken during 2003. One hundred and two health care workers (HCW) were recruited from acute surgical wards, critical care units and a radiology department based at the University Hospital Birmingham NHS Trust, UK. Baseline levels of micro-organisms on the hands of HCW were determined by standard microbiological techniques. Fifty-two HCW applied THP as recommended by the manufacturer; fifty HCW did not apply THP and served as controls. To assess the immediate antimicrobial efficacy of THP, hands were sampled for micro-organisms at one minute following application. HCW continued with their normal clinical practice and hands were further sampled for micro-organisms at 1 and 3 h to determine any sustained antimicrobial activity of THP. Data regarding the frequency of hand washing and duration gloves were worn during the investigation was also obtained. Alcohol hand rubs and medicated soap washes were excluded during the assessment of THP.
Results: There was a significant reduction in mean bacterial counts in the study group compared with controls after 1 min, 1 h and 3 h following application of THP ($P < 0.0001$, <0.0001 and 0.0359 respectively). There were no significant differences between the groups in the number of times hands were washed with soap and water [$P = 0.092$ (1 h); $P = 0.43$ (3 h)] and glove wearing [$P = 0.47$ (1 h); $P = 0.13$ (3 h)].
Conclusions: THP has immediate and sustained antimicrobial activity on the hands of HCW in the clinical setting for at least 3 h. The efficacy of THP is unaffected by repeated hand washing with soap and water. THP may be a beneficial adjunct to normal hand hygiene practice and may facilitate in reducing cross-infection.

P1538 Evaluation of bacteriological agents isolated from operating room staff after hand scrubbing in Yazd medical university hospitals

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Objective: Nosocomial infection is one the most important problem that causes mortality in all hospitals around the world. This problem is more emphasised in operation room's staff dealing with. The aim of this study was to determine the rate infection among operation room staff in the Yazd University hospitals of Yazd city, Iran.
Method: One hundred and thirty-four personals of operation team staff (81 men + 53 women) including surgeons; nurses and student were involved in this cross-sectional study. Following swapping from their hands after betadine scrubbing from four parts of their hands, all samples were cultured using standard method for detection of any possible bacteria. All detected bacteria were diagnosed using related biochemical tests. Simultaneously the demographic status such age, sex and period scrubbing time for all cases was recorded separately in a special form.
Results: Species detected were staphylococci coagulase positive (5.03%) and coagulase negative (40.7%). 70.9% of person's nail were infected with c+staph and 12.7% with c-staph 38.1% of staff's palm were infected with c-staph and 5.2% with c+staph, 23.9% of over hand of staph were infected with c-and 2.2% with c+staph. 29.9% of the staff's wrist were infected staph c- was detected on 29.9% of the staff's wrist. Infection among men was found to be significantly more (16%) than women (1.9%) for those

staff who scrubbed their hands between 5 and 10 min. Only 3% of studied population carried more than 50 colonies of bacteria.
Conclusions: The present study shows that men are more careless against infection in scrubbing and nosocomial infection has been found to be dominating among operation room's staff, and their nails are significantly carried more infections agents. It may be suggested that at least, 5 min scrubbing are recognised to minimise the infection.

P1539 Evaluation of preventative methods in blood-borne pathogens among health care workers

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Objectives: To estimate the risk and to evaluate the conditions of exposure to blood-borne pathogens among health care workers (HCWs). To conduct a comparison between present and past data of our hospital.
Methods: The data was collected using a questionnaire and included: characteristics of the workers, type of occupational exposure, immunity status of the exposed worker, infectivity of the source patient and follow up serologic testing of the worker. Eight hundred and eight incidents were enrolled between January 1998 and December 2002. The data was compared with data collected of the similar cohort during previous period (1990–97).
Results: We noticed an increase in the absolute number of reported incidents (808 vs. 284). Nurses appeared to have the highest injury rate per year among all HCWs (4.1% vs. 3%). Lack of attention remained the main cause of incidents (68% 1998–2002 vs. 51% 1990–97). Workers with short work experience (0–5 years) were primarily affected ($P < 0.001$). Our results indicated a serious increase in the rate of incidents during the resheating of used needles (46.7% vs. 18.3%), which was taken into consideration for appropriate adjustment of the relevant hospital policy. Interestingly the study demonstrated a decrease in the rate of incidents involving HbsAg (+) and anti-HCV (+) patients (8.7 and 6.9% during 1998–2002 vs. 20 and 17% during 1990–97 respectively). During this period none of the reported incidents progressed to seroconversion of the HCWs, where immune status to blood borne pathogens was estimated. This finding is in line with our previous survey.
Conclusions: An awareness of the HCWs as regards early reporting of such incidents was observed. Efforts by the infection control committee need to be more intense, in order to identify unsafe practices and provide more adequate preventive measures.

Ventilator-associated pneumonia

P1540 Evaluation of patients with ventilator-associated pneumonia

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Objective: To determine the epidemiology of ventilator associated pneumonia (VAP) and to examine the characteristics and outcomes of the patients with VAP.
Methods: Patients with the diagnosis of VAP, in surgical and medical intensive care units (ICUs), were enrolled to the study and followed prospectively. The diagnosis of VAP was based on positive quantitative culture of deep endotracheal aspirate ($\geq 10^5$ CFU/mL) with new or increased purulent sputum and chest radiograph showing new or progressive infiltrate. The demographic details, APACHE II score, length of ICU stay, length of mechanical ventilation, surgery, comorbidities, nutritional status, isolated micro-organisms and susceptibility results were recorded on individual forms.
Results: During study period VAP was diagnosed in 26 patients, 18 (69.2%) of them were male. Mean age was 50.9 ± 21.4. The reasons for ICU admission were respiratory failure (46.2%), trauma (23.1%), postoperative care (11.5%), cardio-pulmonary arrest (3.9%), and other reasons (15.4%) respectively. The mean duration of ICU stay was 26.8 ± 22.3 days and the mean duration of mechanical ventilation was 17.7 ± 19.1 days. Comorbidity was obtained in seven (26.9%) patients. APACHE II scores were between 10 and 19 in 16 (61.5%) patients and above 20 in six (23.1%) patients. VAP occurred at the 10.9 ± 9.9th day of the mechanical ventilation. *Acinetobacter* spp. was the most frequently identified pathogen, found in nine (34.6%) of the episodes, followed by *Pseudomonas aeruginosa* (19.2%), *Klebsiella pneumoniae* (19.2%), methicilline resistant *Staphylococcus aureus* (15.4%). Overall mortality rate was 76.9%, but in seven of 20 died patients, pneumonia was the direct reason of death. Risk factors leading to death in VAP patients were longer ICU stay (42.4 ± 31.5 and 21.1 ± 15.2 days, respectively, $P = 0.028$) and longer ventilation time (30.9 ± 33.3 and 12.8 ± 6.7 days, respectively, $P = 0.03$). No statistically significant difference was found between APACHE II scores of the patients died because of VAP and survived. Three of seven patients who died because of VAP, were infected by *Acinetobacter* spp. which were resistant to all antibiotics.
Conclusions: Ventilator-associated pneumonia is the most important complication of mechanical ventilation among ICU patients. Preventive measures for nosocomial infections and appropriate antibiotic treatment may be the most important step to reduce the mortality because of VAP.

P1541 Usefulness of serial quantitative cultures of bronchoalveolar lavage to control the development of antibiotic resistance in *Pseudomonas aeruginosa* causing ventilator-associated pneumonia

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Background: Ventilator-associated pneumonia (VAP) caused by *Pseudomonas aeruginosa* (PA) is a severe complication with high mortality. *In vitro* data have shown that the majority of PA isolates develop stable resistance after exposure to serial passages containing antibiotics.
Objectives: To investigate whether antibiotic therapy for VAP determines selection of antibiotic resistant PA *in vivo*; and to determine whether there is a change in PA strains during antibiotic therapy.
Methods: A prospective cohort study started in September 2002. Diagnosis of VAP required a positive quantitative culture of BAL; new or persistent infiltrate on chest X-ray; >2 of the following: hyper/hypopirexia, leucocytosis; purulent bronchial secretions; and onset >48 h after starting mechanical ventilation (MV). Chest X rays, haemochrome, SAPS II, and mini-BAL were obtained before starting therapy and every 72 h. Genotyping was performed by automated laser fluorescence analysis of digital fingerprinting data.
Results: Twenty-six patients fulfilled the diagnostic criteria for VAP within the first year of study. Eleven patients (42%) with PA VAP were enrolled with 49 serial BAL. The majority of patients were males (72%) with a mean (SD) age 53 (18) years. Ninety per cent of patients received antibiotics within 30 days. VAP had been diagnosed after 17 (12) days from the MV. At enrolment, resist-

ance to >2 antibiotics has been detected in 55% of the strains. All patients presented leucocytosis [mean, 17099 (8636/mmc)]. Six patients (54%) had a negative culture of a mini-BAL obtained after 72 h of therapy, the remaining 5 had PA still isolated. Compared with patients with negative BAL at 72 h those with positive BAL had excess of mortality (80% vs. 20%, OR 0.8, $P = 0.03$), and higher leucocytosis (26.566 vs. 17.566/mmc, $P = 0.02$). Radiological pictures and mean SAPS II did not change significantly after 72 h. Two patients developed resistance to piperacillin-tazobactam and imipenem after 8 and 11 days of therapy, respectively. All patient-unique isolates of PA were genetically different, while serial isolates from the same patients were identical.
Conclusions: Stepwise selection of resistance has been observed *in vivo* during antibiotic therapy for VAP. Mortality rate is significantly higher in patients with PA still isolated after 72 h. These preliminary data suggest that a mini-BAL after 72 h from therapy may be useful to control antimicrobial susceptibility of the isolate and to reduce mortality.

P1542 Preliminary bronchoalveolar lavage culture results in ventilator-associated pneumonia

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Objectives: To determine the value of preliminary bacterial colony counts for predicting final Bronchoalveolar Lavage (BAL) results in Ventilator Associated Pneumonia (VAP).
Methods: A total of 434 isolates from 208 quantitative BAL cultures were analysed, in an observational cohort from September 2001 to March 2003, in one Intensive Care Unit, in a University teaching hospital. Preliminary and final colony counts for each isolate were categorised as either no growth, insignificant (1-99,999 cfu/mL),or significant (≥100 000 cfu/mL).VAP was diagnosed on the basis of positive quantitative cultures. Sensitivity, specificity, positive predictive value, negative predictive value of preliminary results were estimated .Concurrent antibiotic therapy was recorded if no growth or insignificant growth was detected on preliminary results.
Results: On preliminary colony counts reports there were 100 isolates with no growth, 109 with insignificant growth and 225 with significant growth. Overall preliminary results had sensitivity 96%, specificity 95%, positive predictive value 96% and negative predictive value 95%. Preliminary BAL culture results accurately predicted the presence or absence of VAP in 413 (95%) of the BALs performed. Isolates with no growth (preliminary 100 and final 89 or 89%) had greater reliability in predicting the absence of VAP than isolates with insignificant growth (preliminary 109 and final 130 or 83%), whereas no difference was noted in false negatives, while on antibiotics between insignificant and no growth groups.
Conclusions: Preliminary BAL culture results with significant or no growth are highly predictive for similar final results, whereas results with insignificant growth are less reliable, in our data. Isolates with significant or no growth are clinically important and antibiotics should be adapted appropriately or discontinued respectively, before obtaining final results.

P1543 Ventilator-associated pneumonia and modification of initially inappropriate antibiotic therapy according to bronchoalveolar lavage culture results: timing and outcome

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Objectives: To compare the impact on outcome, of early (<7 days of mechanical ventilation) vs. late (≥7 days of mechanical ventilation) modification, according to bronchoalveolar lavage (BAL) culture results, of initially inappropriate antibiotic therapy of patients with Ventilator Associated Pneumonia (VAP).
Methods: In total 159 subjects (95 males (60%) and 64 females (40%),median age 61.25 years, range 21–87) diagnosed as having VAP with quantitative culture results of BAL and receiving initially inappropriate antibiotic treatment were enrolled. Initial antibiotic was inappropriate when at least one developed microorganism was resistant to administered antibiotics and was modified according to BAL cultures results when became available. Parameters recorded included the timing of modification (early vs. late) according to when the VAP episode was developed and the outcome.
Results: Inappropriate initial antibiotic therapy was modified early in 50.94% (81 of 159) and late in 49.05% (78 of 159).Crude mortality was 20.12% (32 of 159). Crude mortality in case of early modification of inappropriate treatment was 4.9% (four of 81) and in case of late modification 35.89% (28 of 78). Apache II score ($t = 1.459$, $P = 0.1450$) and *Pseudomonas* spp. ($P > 0.005$) development did not differ significantly between the two groups (early and late modification of initially inappropriate treatment).
Conclusions: In our data a more favourable outcome of patients with VAP was observed when initially inappropriate empiric antibiotic therapy was modified according to BAL culture results early (<7 days of mechanical ventilation) than late (<7 days of mechanical ventilation).

P1544 Ventilation-associated pneumonia and de-escalation practice, oriented by the results of bronchoalveolar lavage vs transtracheal aspiration cultures

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Objectives: To compare the impact on outcome of Ventilator Associated Pneumonia (VAP) when de-escalate according to bronchoalveolar lavage (BAL) vs. transtracheal aspirations (TTAs) culture results. De-escalation therapy is based on the use of initial large spectrum, first hand therapy, that is reassessed when microbiological data become available in order to reduce to a narrower spectrum therapy.
Methods: A total of 408 subjects, with clinical evidence for VAP, were randomly assigned into two groups A and B. In group A ($n = 208$, males/females = 125/83, median age 61.25 years, range 24–87 years) quantitative cultures (QCs) of BAL were taken whereas in group B ($n = 200$, males/females = 131/69, median age 58.90 years, range 24–87 years) QCs of TTAs. Initial antibiotic therapy, when modified in the light of cultures results, was either stopped if inappropriate or at least one strain resistant to administered antibiotics or changed to another with a narrower spectrum. The outcome in terms of crude mortality rates was assessed in both groups.
Results: Patients diagnosed as having developed VAP in groups A and B respectively were 81% (169 of 208) and 88% (175 of 200). No significant difference in age ($t = 1.33$, $P = 0.183$), Apache II score ($t = 1.459$, $P = 0.145$) duration of mechanical ventilation until samples were taken ($t = 0.511$, $P = 0.609$) or contribution of medical vs. surgical patients ($x^2 = 3.97$, $P = 0.137$) was detected between groups A and B. The BAL and TTAs QCs results permitted de-escalation in groups A and B respectively, in 94% (159 of 169) and 71.41% (125 of 175), whereas therapy remained unchanged in 23.55% (49 of 208) and 37.5% (75 of 200). Overall mortality in groups A and B was 30% (63 of 208) and 53% (105 of 200) ($x^2 = 20.768$, $P < 0.005$). Crude mortality when de-escalation was applied in groups A and B respectively was 20.12% (32 of 159) and 71.41% (125 of 175) ($x^2 = 23.294$, $P < 0.005$) and when therapy remained unchanged 37% (31 of 49) and 40% (45 of 75) ($P > 0.005$).
Conclusions: In our data VAP patients had more favourable outcome when de-escalation was applied t in the light of QCs of BAL rather than TTAs.

P1545 *Pseudomonas aeruginosa* and ventilator-associated pneumonia: impact on outcome and duration of mechanical ventilation

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Objectives: To compare Ventilator Associated Pneumonia (VAP) because of *Pseudomonas aeruginosa* (PA) with VAP because of flora other the PA, in terms of duration of mechanical ventilation and outcome.
Methods: A total of 208 patients [125 males (60%) and 83 females (40%), median age 61.25 years, age range 21–87 years] diagnosed as having developed VAP by quantitative culture results of bronchoalveolar lavage (BAL) were enrolled between September 2001 and September 2003. Responsible for VAP flora as PA or other than PA, duration of mechanical ventilation up to VAP development and up to discharge and outcome were the parameters recorded.
Results: VAP episodes were attributed to one pathogen in 120 episodes (58%) and multiple pathogens in 88 episodes (42%). In total 217 micro-organisms were developed in quantitative cultures of BAL, and 107 (42%) were PA. VAP were attributed to other than PA (OTPA) flora in 106 (51%) and to PA (exclusively or to flora including PA among other strains) in 102(49%) episodes. The median value for APCHE II score in VAP episodes due to PA and due to OTPA flora was 15.57 ± 4.52 and 16.25 ± 4.86 respectively ($t = 1.459$, $P = 0.145$). Crude mortality in VAP due to PA or OTPA was 13.72% (14 of 102) and 16.98 (18 of 106) respectively ($P > 0.005$). Median duration of ventilation I up to development of VAP because of PA or to OTPA was $7.114 = -6.27$ and $9.21 = 7.20$ days respectively ($P > 0.005$). Duration of mechanical up to discharge from Intensive Care in VAP because of PA or OTPA was 8.49 ± 7.63 and 8.13 ± 6.79 respectively ($t = 0.511$, $P = 0.609$).
Conclusions: In our data no difference was detected between VAP because of PA and VAP because of OTPA flora, in terms of duration of mechanical ventilation and outcome.

P1546 Ventilator-associated pneumonia because of glycopeptide-intermediate *S. aureus* in intensive care unit patients: does vancomycin remain clinically effective?

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Introduction: An increasing number of recent reports have suggested that Van was sub-optimal in the treatment of GISA infections. This study looks at mortality of ICU patients with *S. aureus* VAP because of MSSA, MRSA or GISA.
Methods: From 06/97 to 02/03, we extracted the following information prospectively recorded from our database Outcomerea: demographics characteristics, admission category, severity of illness at admission using the Simplified Acute Physiologic Score (SAPS II), hospital mortality. All VAP were diagnosed using protected specimen brush and broncho-alveolar lavage samplings. Van was monitored in all patients (pts). A Cox model with time-dependent covariates was used to measure the impact of *S. aureus* VAP on hospital mortality.
Results: Van administered as a continuous infusion was used alone (21.4%) or in combination with fosfomycin (57.1%), fucidic acid or synercid (21.4%) in GISA VAP. When adjusted on SAPS II and category admission (medical vs. surgical), MRSA pneumonia (OR: 2.50, 95% CI/ 0.6–10.06, $P = 0.20$) and GISA pneumonia (OR: 0.85, 95% CI: 0.14–5.05, $P = 0.85$) were not associated with an increased risk of mortality as compared with MSSA pneumonia pts.
Conclusions: Continuous IV Van therapy with accurate serum level monitoring, achieving a 20–30 mg/L, appears effective in our case-mix for the treatment of VAP because of GISA. Studies comparing Van to new available antibiotics are warranted and should include, both a continuous IV regimen and a monitoring of Van serum levels.

P1547 Incidence, risk factors and mortality of nosocomial pneumonia in intensive care units: a prospective study

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Objectives: To determine the frequency, risk factors and mortality of nosocomial pneumonia (NP).
Methods: Between February 2001 and February 2002 a prospective study was conducted among intensive care units (ICU) patients of the Erciyes University Hospital. Patients from the surgical ICU (SICU) (24 beds), medical ICU (MICU) (nine beds) and burn unit (seven beds) were included. Patients older than 16 years of age were included. Data collection included physical examination findings, APACHE II scores on admission and onset of NP, consciousness, risk factors (intubation, MV, nasotracheal aspiration, presence of nasogastric tube, enteral nutrition, tracheotomy), prior surgery, immunosuppression, prior antimicrobial and antacid or histamine type 2 (H2) blocker therapy, clinical outcome, length of stay in ICU and in the hospital. NP was defined according to the Centers for Disease Control (CDC) Criteria. One hundred and sixty three patients who were admitted to the ICU during the same period but had no bacteriologic or histologic evidence of pneumonia were used as a control group.
Results: Overall, 163 (6.8%) of the 2402 patients developed NP and 75.5% ($n = 123$) of all patients with NP were ventilator-associated pneumonia (VAP). One hundred and sixty-three patients who were admitted to the ICU during the same period but had no bacteriologic or histologic evidence of pneumonia were used as a control group. Coma, COPD, hypoalbuminaemia, mechanical ventilation (MV), tracheotomy, presence of nasogastric tube, nasogastric aspiration, and previous treatment with broad spectrum antibiotic were found as independent risk factors. Crude and attributable mortality were 65 and 52.6%, respectively. The mortality rate was five times greater in the cases (OR: 5.2; CI 95%: 3.2–8.3). The mean length of stay in the ICU and hospital in the cases were longer than control group ($P < 0.0001$).
Conclusions: Patients requiring MV have a high frequency of NP. NP is associated with poor prognosis and increases the length of ICU and hospital stay.

P1548 Three-year surveillance of ventilatory-associated pneumonia in a paediatric intensive care unit

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Background: The aim of the study was to estimate the ethiology, frequency of ventilatory-associated pneumonia in the PICU and the contribution of patient's endogenous flora to the pathogenesis of VAP.
Methods: Overall 177 patients admitted to the PICU between 01-01-2000 and 31-12-2002 and required mechanical ventilation for more than 2 weeks were enrolled to the study. Surveillance cultures of BAL-fluid, throat and rectum swabs were obtained on admission and thereafter once weekly during the whole period of hospitalisation. Isolated strains were identified and susceptibility to antibiotics was determined. The bacterial strains were typed by PFGE.
Results: Forty-nine of 177 patients, included in the study had positive BAL-fluid culture on admission. The pathogens isolated from BAL-fluid at admission to the ward were as follows: *Klebsiella pneumoniae* (13), *Pseudomonas aeruginosa* (10), *Candida* sp. (7), MRSA (3), *Serratia marcescens* (3), *Staphylococcus epidermidis* (3), *Acinetobacter baumanii* (2), *Enterococcus faecalis* (2) and *Streptococcus viridans* (1), *Enterobacter cloacae* (1), *Escherichia coli* (1). In four cases BAL-cultures were polymicrobial. Of 128 patients with negative primary BAL-fluid culture, 57 developed VAP during hospitalisa-

tion. The organisms isolated from BAL-fluid from VAP(+) patients were: *P. aeruginosa* (20), *K. pneumoniae* (18), *Stenotrophomonas maltophilia* (16), *E. cloacae* (7), *S. marcescens* (3), *Acinetobacter* (1), *E. coli* (1), *Enterococcus* (1), MRSA (1). Seven cultures were polymicrobial and four patients developed two episodes of VAP caused by different pathogens during respiratory treatment. The same organisms were isolated from BAL-fluid and throat and/or from rectum swabs in 89 of 95 VAP-patients. Majority of *Klebsiella* and *Pseudomonas* isolates obtained from BALs of different patients had identical PFGE patterns, but isolates of *Enterobacter* and *Stenotrophomonas* were different. PFGE typing confirmed that five cases of VAP were caused by micro-organisms (*K. pneumoniae* 2, *P. aeruginosa* 3) present at the time of admission. The remaining VAPs were due to nosocomial flora.
Conclusions: The majority of infection developed in our PICU was caused by secondary endogenous flora. Our results confirmed close relationship between endogenous flora of individual patients treated in the PICU and development of VAP.

P1549 Antimicrobial resistance of Gram-negative bacilli isolated from patients with ventilator-associated pneumonia in a Turkish university hospital

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Objectives: Ventilator Associated Pneumonia (VAP) is an important cause of mortality throughout the world. The aim of this study is to investigate the antibacterial resistance of gram negative basilli isolated from respiratory samples of patients having VAP.
Methods: The study included 60 *Pseudomonas aeruginosa*, 35 Acinetobacter spp, 28 *Klebsiella pneumoniae* and five *Escherichia coli* isolated from tracheal aspiration and bronchoalveolar lavage samples of patients having VAP. The antibiotic susceptibilities and Extended-spectrum beta-lactamase producing (ESBL) were determined by ETest (AB BIODISK) using Mueller-Hinton agar (OXOID) according to NCCLS recommendations.
Results: The antibiotic resistance of the strains are presented in the Table. ESBL production ratio was 32% for *K. pneumoniae* strains while it is produced by none of *E. coli* strains.

Pathogens ($n = 128$)	MEM %	IPM %	CIP %	CAZ %	P+T %	PM %	CTX %	TOB %	ESBL n(%)
P. aeruginosa ($n = 60$)	77	88	92	92	90	88	100	95	
Acinetobacter. spp ($n = 35$)	91	100	100	100	100	100	100	100	
K. pnemoniae ($n = 28$)	0.3	39	86	75	89	43	71	100	9 (32%)
E. coli ($n = 5$)	0.0	0.0	100	40	100	60	60	80	0 (0.0%)

Conclusions: In our study the most common gram negative bacilli causing lower respiratory tract infections, *P. aeruginosa* and *Acinetobacter* spp were noticed as resistant to many antimicrobials including carbapenems. However, regarding all the strains, carbapenems and cefepime were identified as the most effective antimicrobial agents.

P1550 Nosocomial pneumonia in postsurgical patients in the increased care unit

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Objectives: In the Increased Care Unit of our hospital, severely-ill patients (pts) are often hospitalised because of a failure of postsurgical resuscitation.
Methods: We evaluated 80 pts, (40 men, 40 women), mean age 62.5 ± 19 years. The mean duration of hospitalisation was 5.6 ± 7.7 days. The cause of the surgical intervention was as follows: acute abdomen (20 pts, 25%), ileus (14 pts 17.5%), open lung biopsy (12 pts 15%), abdominal Ca (eight pts 10%), traumatic haemothorax or pneumothorax (six pts 7.5%), pneumonectomy (six pts 7.5%), rupture of concave viscera (four pts 5%), aortal or abdominal aneurysm (four pts 5%), and miscellaneous (six pts 7.5%).
Results: The total mortality was 42.5%. Twelve of 80 pts (15%) developed nosocomial pneumonia with positive cultures of endobronchial secretions in the first 36–72 h of hospitalisation in the Increased Care Unit (eight *Staphyl. aureus*, four *Pseud. aeruginosa*). In 14 of 80 pts (17.5%) pneumonia occurred later and the causative micro-organism was isolated from the culture of endobronchial secretions after the third day of hospitalisation in the Increased Care Unit (eight *S. aureus*, four *Acinetobacter* sp. and 2 *P. aeruginosa*). In the subgroup of pts with early-onset pneumonia (first 3 days), the mortality was 75%, while in the subgroup with late-onset pneumonia (after third day) the mortality was 57.1%. No statistical difference was detected in APACHE II score between the two subgroups (17.8 ± 6.9, 16.5 ± 6.6 respectively). No correlation was found between mortality and age, sex or the cause of surgical intervention.
Conclusions: Our findings indicate that in postsurgical pts that require increased care the development of pneumonia during the first 3 days of their hospitalisation in the Increased Care Unit (indication of colonisation or pre-existing infection possibly unidentified) is related to a more severe prognosis.

P1551 The colonisation with polyresistant strains and bronchopulmonary infectious complications in multiple severe trauma patients

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Objective: The colonisation and microaspiration are supposed to be important factors in the development of ventilator-associated infections. The spectrum of bacteria, isolated from both the oropharynx and the bronchoalveolar lavage fluids of 30 ventilated multiple severe trauma patients in intensive care unit (ICU) were studied in the period of 1–20 days from the onset of the diseases.
Methods: The identification of isolated bacteria was performed by classical methods, and the susceptibility to antibiotics was tested by Kirby–Bauer method according to NCCLS's criteria. The susceptibility to Ciprofloxacin (CIP), Imipenem (IMP), Amikacin (AN), Ceftazidim (CAZ), Cefepim (FEP) was examined. The double-disk synergy test was used to detect extended-spectrum beta-lactamases (ESBL) production.
Results: In the first 4 days of admission, the strains ESBL-producing *K. pneumoniae* resistant to AN, susceptible to CIP and IMP were isolated from the oropharynx in 40% ICU patients, there being no infectious involvement of respiratory tract. Eighty per cent patients who stayed at the hospital for more than 7 days were demonstrated to have mucous membranes colonised additionally with pyoverdin-producing strains *P. aeruginosa*, being resistant to CIP, AN, IMP and susceptible to CAZ and FEP. Starting with the tenth day of the patient's stay in ICU, the microbial spectrum of oropharynx presented the *P. aeruginosa* associated with *K. pneumoniae*, *Acinetobacter* spp. and *Enterococcus* spp. The similar associations were found in the process of microbiological study of bronchoalveolar lavage fluids in patients with the developed ventilator-associated bronchopulmonary infectious complications.
Conclusions: Thus, the study of the rate of colonisation and the bacterial spectrum characteristics of the oropharynx showed that the bronchopulmonary infectious complications in ventilated multiple severe trauma patients may be due to the aspiration of hospital strains, colonising oropharynx early following the patient's admission to the hospital.

Nosocomial infections: nonfermentative Gram-negative bacilli - II

P1552 Changing epidemiology of multiresistant *Acinetobacter baumannii* in intensive care and burns unit patients, Sydney, Australia

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Objectives: Review of multidrug-resistant *Acinetobacter baumannii* (MRAB) incidence in a University teaching Hospital with a major Burns Centre over 4 years 2000–2004. Relationship of number and severity of burns and ICU rebuilding on cross-infection and bacteraemia rates. Impact of external source of MRAB, following Bali Bombing in October 2002 on changing MRAB epidemiology.
Methods: Monthly retrospective review of pharmacy and laboratory data, including susceptibility data. RFLP typing of MRAB isolates.
Results: Longitudinal survey of MRAB incidence will be presented graphically. The original strain of MRAB (clone A – meropenem R, amikacin S) appeared in July 2000 and became the dominant MRAB phenotype. Close correlation existed with numbers of patients with severe burns and MRAB colonisation; cannula infection and bacteraemia. The rate increased during ICU rebuilding when housed in a temporary facility, indicating failures in infection control as a factor in increased infection rates. Transfer to a new facility resulted in reduced MRAB, but burn severity and duration of ICU stay continue to be a predictor for individual patient MRAB infection. From July 2000 to September 2002 there were 52 MRAB bacteraemias. In 56%, bacteraemia (and cannula tip infection) was the first indicator of MRAB colonisation. After the Bali bombing in October 2002, eight victims were admitted to ICU. On admission, 7/8 were colonised with MRAB, with 12 different susceptibility phenotypes demonstrable, including isolates susceptible only to polymyxin. All differed to MRAB clone A. Some of these strain types have now supplanted the original clone A, as dominant MRAB epidemic strains. Genetic relatedness typing presentation and performance is being planned. There has been a resultant increase in demand for polymyxin use for suspected MRAB infection. Environmental swabs have yielded variable results. Faulty mattresses provided a maintained reservoir for MRAB, but alterations have not curtailed ongoing transmission.
Conclusions: MRAB epidemiology is complex, particularly when multiple clones become prevalent. Burns patients are at high risk for colonisation and bacteraemia. Ongoing transmission is affected by multiple factors including ward design, antibiotic use and Burns Unit activity.

P1553 Antibiotic susceptibility of *Acinetobacter* strains isolated in blood cultures of children

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Objectives: To determine the frequency and antibiotic susceptibility of *Acinetobacter* strains in blood cultures in Turkish children.
Methods: Blood culture results of children who had been admitted to Children's Hospital at Ankara University Medical School in 1992–2002 were retrospectively reviewed. Antibiotic susceptibility of *Acinetobacter* strains was determined by disc diffusion method. Intermediate-resistant strains were considered as resistant.
Results: Seventy-six of 1269 (6%) blood cultures were positive for *Acinetobacter* strains. *Acinetobacter* strains constituted 13.8% of blood cultures positive for all Gram-negative organisms. *Acinetobacter baumannii* (68 strains) was the most common serotype followed by *A. lwoffii* (seven strains) and *A. haemolyticus* (one strain). Antibiotic susceptibilities of all *Acinetobacter* strains were as follows: ampicillin 30%, ampicillin–sulbactam 78%, amoxicillin–clavulanate 70%, piperacillin 66%, piperacillin–tazobactam 88%, cephalothin 14%, cefuroxime 39%, cefixime 42%, ceftriaxone 31%, ceftazidime 46%, cefotaxime 53%, cefoperazone 50%, cefoperazone–sulbactam 81%, cefepime 71%, aztreonam 44%, imipenem 100%, meropenem 100%, ciprofloxacin 93%, ofloxacin 90%, amikacin 86%, gentamicin 85%, netilmicin 76%, tobramycin 72%, and trimethoprim–sulfamethoxazole 64%.
Conclusions: Carbapenems, fluoroquinolones, piperacillin–tazobactam and cefoperazone–sulbactam are the most effective agents against *Acinetobacter* strains isolated in blood cultures of children in our hospital. The results of our study demonstrate that antibiotic resistance is an important problem for *Acinetobacter* infections in Turkey.

P1554 Epidemiological characterisation of *Acinetobacter baumanii* isolated in a university hospital

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Objective: The aim of the study was to determinate the epidemiological patterns of *Acinetobacter baumannii* isolates in a tertiary Hospital.
Methods: During the period from 01 January 2000 to 15 November 2003 we performed a prospective study of all medical charts from patients with *A. baumanii* isolates. Identification and susceptibility testing was performed by the VITEK II (Biomerieux) automatised system. A case of nosocomial infection was defined using the criteria established by the CDC (1998, USA).
Results: During this period, 129 *A. baumanii* strains were collected from 115 patients: 60 (46%) were collected from patients hospitalised at medical ward, 43 (33%) isolates from surgery wards and 23 (17%) from the intensive care units patients. The average hospital stay before *A. baumanii* was detected was 37.2 days and the mean age was 61 years old (range 5 days to 92 years). The sources of isolates were as follows: wounds 51 (40%), respiratory specimens 29 (23%), urine 28 (22%), catheters nine (6%), blood seven (5%) and others samples five (3%). Forty of 129 (31%) were nosocomial infections: 12 (30%) urinary tract infections, eight (20%) surgical site infections, eight (20%) skin and soft tissues infections, seven (17.5%) bloodstream (six primary bacteraemia, one catheter-related infection) and five (12.5%) respiratory tract infections. Ninety-nine per cent of the isolates were susceptible to imipenem. Seventy-three per cent were susceptible to amikacin, 56.5% to piperacillin–tazobactam, and 43% to ceftazidime. We did not find outbreaks.
Conclusions: At the moment *A. baumanii* is not yet a health problem in our hospital. However, in order to prevent nosocomial outbreaks and multiresistant strains, surveillance, antibiotic policies and infection control measures should be supported.

P1555 *Acinetobacter baumanii* isolated from surgical patients

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Objectives: *Acinetobacter baumanii* (A.b.) has emerged as an important nosocomial pathogen showing usually an increasing resistance to antibiotics. The purpose of our study was to investigate A.b. strains isolated in our hospital for a 3-year period.
Methods: Beginning January 2001 to November 2003 all strains of A.b. isolated from surgical patients were studied. Isolation of A.b. was based on standard methods, while identification and antibiotic susceptibility testing were carried out using the VITEK System, ATB Expression (BioMerieux, France).
Results: Ninety-five strains of A.b. from 33 surgical patients were isolated. Of the 33 patients 23 were males (69.7%) and 10 were

females (30.3%). Most of the patients (75.8%) were or had been in the ICU when A.b. was isolated. The median time of stay in the ICU prior to the detection of A.b. was 20.2 days (range 2–65). The most common isolation sites were surgical wound infection (46.3%) and blood (21,0%). Most of the patients (60,6%) had undergone surgery and 36.4% were on mechanical ventilation device. Of the antimicrobial agents tested the most active against A.b. strains isolated were carbapenems (meropenem with 14% and imipenem with 19% resistance). Cefepim presented moderated activity (46% resistance) and the same was true for the combination piperacillin + tazobactam (51% resistance). Most of the strains were resistant to piperacillin (69%), amikacin (78%) and ceftazidime (85%).
Conclusions: Colonisation with A.b. occurred mostly in patients who had been in the ICU and in those who underwent surgery. The strains isolated from our hospital were highly resistant posing a serious threat to hospitalised patients.

P1556 *A. baumannii* clonal dissemination in Brazilian intensive care units during 2002

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Objective: To investigate the clonal dissemination of multiresistant *A. baumannii* causing nosocomial infections within and between Brazilian intensive care units, which participated in the MYSTIC Program Brazil 2002.
Methods: Twenty-two *A. baumannii* isolates with the same phenotypic characteristics were collected during 2002 at two centres in São Paulo city (centre nos 1 and 4). Isolates resistant to either meropenem or imipenem were studied. They were analysed by pulsed-field gel electrophoresis (PFGE). SpeI genomic restriction fragments were separated with CHEF-DR III System. Electrophoretic patterns were analysed with GelCompar II v. 2.5 (Applied Maths, Kortrijik, Belgium). Interpretative criteria used were those described by Tenover *et al*.
Results: Eight major clones were identified (A, A1, A2, A3, B, F, G, H). Clone A is constituted of five isolates with indistinguishable patterns, three from centre nos 1 and 2 from centre no. 4, indicating clonal dissemination between centres. Clones A1, A2, A3 are closely related to clone A, probably representing clonal dissemination within centre no. 1. Clone B is possibly related to clone A, also present in centre no. 1. Clones F and G are constituted of two isolates each and are closely related, both present in centre no. 4 indicating clonal dissemination within centre. Clones H and H1 are constituted of three isolates that are closely related, also indicating clonal dissemination within centre no. 1.
Conclusions: Clonal dissemination was detected within (clones A, A1, A2, A3, B, F, G, H) and between centres (clone A). These findings are important when analysing surveillance data, as susceptibility rates may be significantly affected with elevated resistance mainly because of due to clonality.

P1557 One hundred fifty *Acinetobacter* spp. isolates – identifications by API and VITEK systems

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Objective: Comparison of identification of one hundred fifty *Acinetobacter* spp. isolates obtained from 1996 to 1999.
Methods: From May 1996 to January 1999 *Acinetobacter* spp. isolated from clinical and environmental specimens were identified by API 20 NE and VITEK system (bioMerieux). Forty-five isolates (30%) were obtained from wounds, 35 (23%) from blood, 33 (22%) from respiratory system, 17 (11%) from environmental examinations and 19 (13%) from other clinical specimens.
Results: Using API 20 NE we differentiated eight phenotypes of *Acinetobacter* spp. Only one isolate was identified as *A. lwoffii*, 149 were identified as *A. baumanii*. The predominant phenotypes were: phenotype e-0041073 – 46.6% ($n = 70$), phenotype c-0001073 – 36% ($n = 54$) and phenotype g-0041473 – 8.6% ($n = 13$). VITEK system differentiated six phenotypes with predominant phenotype II-30111000004 – 82,6% ($n = 124$). Comparison of phenotypes obtained by API and VITEK system shows that predominant phenotypes were eII-0041073/30111000004 (59 isolates – 39.3%) and cII-0001073/30111000004 (48 isolates – 32%).
Conclusions: During the 4-year period two predominant phenotypes of *A. baumannii* were identified. Endemic outbreak was suspected which prompted us to conduct molecular epidemiology study.

P1558 Bacteraemia due to *Stenotrophomonas maltophilia* strains in children

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Objectives: To evaluate the clinical features, antibiotic treatment and prognosis of *Stenotrophomonas maltophilia* bacteraemia in Turkish children in a university hospital.
Methods: Patients whose blood cultures had been found to be positive for *S. maltophilia* at the Children's Hospital of Ankara University Medical School in 1992–2002 were retrospectively evaluated. Antibiotic susceptibility of *S. maltophilia* strains was determined with disc diffusion method. Susceptibility to ciprofloxacin was also determined with agar dilution method.
Results: Thirty-one of 1269 (2.4%) blood cultures were positive for *S. maltophilia* that constituted 5.6% of blood cultures positive for all Gram-negative organisms. All bacteraemia episodes were hospital-acquired. Twenty-two patients (71%) had an underlying illness. Twenty patients (65%) were receiving antibiotic therapy within the previous week. Septicaemia and pneumonia due to *S. maltophilia* bacteraemia were the most common clinical diagnoses. Ciprofloxacin or trimethoprim–sulfamethoxazole in combination with/without an aminoglycoside were the most common selected antibiotics for the treatment of *S. maltophilia* infections. Only one child died during acute *S. maltophilia* bacteraemia episode. Antibiotic susceptibilities of 31 *S. maltophilia* strains to various antibiotics are as follows; penicillin G 0%, ampicillin 0%, ampicillin–sulbactam 0%, amoxicillin–clavulanate 0%, piperacillin 0%, ticarcillin–clavulanate 68%, cephalothin 22%, cefuroxime 19%, cefixime 32%, ceftriaxone 27%, ceftazidime 21%, cefotaxime 25%, cefoperazone 0%, cefoperazone–sulbactam 55%, cefepime 33%, aztreonam 0%, meropenem 0%, ciprofloxacin 100%, ofloxacin 100%, amikacin 38%, gentamicin 22%, netilmicin 41%, tobramycin 45%, chloramphenicol 25%, doxycycline 91%, and trimethoprim–sulfamethoxazole 85%. Ciprofloxacin susceptibility was determined as 49% by agar dilution method.
Conclusions: Our study demonstrates that although *S. maltophilia* bacteraemia is rare in children, the antibiotic resistance is an important problem in these strains. Tetracyclines, trimethoprim–sulfamethoxazole and fluoroquinolones are the most effective agents against *S. maltophilia* strains isolated from blood cultures of children in our hospital. However, ciprofloxacin susceptibility should be determined by dilution methods. Prompt administration of appropriate antibiotics improves the prognosis of *S. maltophilia* bacteraemia.

P1559 Typing of hospital strains of *Stenotrophomonas maltophilia* by pulsed-field gel electrophoresis

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Objectives: To characterise the epidemiological relationships among *Stenotrophomonas maltophilia* isolates in the surgery and intensive care unit (ICU) of our hospital over a 12-month period in which an increased number of isolates was observed.

Patients and Methods: Majority of the surgery patients were liver transplant recipients, and other surgical and ICU patients with severe underlying diseases, predisposing the drug-resistant infections. *S. maltophilia* strains were isolated from blood, surgical wounds, drainages, BAL, throat and stool. A total of 72 clinical strains were characterised by antibiotype and 54 were characterised by genotype [pulsed-field gel electrophoresis (PFGE) digestion with XbaI]. Identification was confirmed by the API 20 NE system. Antimicrobial susceptibility testing was performed using disc-diffusion methods according to the NCCLS recomendations.
Results: Almost all strains had different genomic patterns in PFGE. Only four patients from ICU were infected or colonised by the same strain. Strains from an individual patient with different antibiotype patterns, or ESBL+ and ESBL−, had the same PFGE pattern.
Conclusions: PFGE revealed the endogenous source of *S. maltophilia* that had infected and colonised all surgical patients, and only few clonal infections or colonisations existing among ICU patients. This finding confirms the selective role of carbapenems' therapy in the risk of *S. maltophilia* infections.

P1560 The *Stenotrophomonas maltophilia* K279a genome sequence project

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Objectives: *S. maltophilia* is an important, emerging, nosocomial pathogen. Isolates are generally highly resistant to beta-lactams, macrolides and aminoglycosides, fluoroquinolones, tetracyclines and chloramphenicol. The threat of *S. maltophilia* comes from its multi-drug resistance, its ability to form biofilms, and so colonise surfaces of medical devices, and its ability to produce virulence factors. We report here preliminary analysis of the genome sequence of a clinical *S. maltophilia* isolates, K279a from Bristol (UK).
Methods: *S. maltophilia* isolate K279a is in the same 16S rRNA subgroup as the type strain, and was isolated in 1995 from a bacteraemia oncology patient. Genomic DNA was produced by the CTAB method, sheared, and ligated into the cloning vector pUC18. Shotgun sequencing was performed and raw sequence data assembled into contigs. Contigs were searched using tBlastn for homologues to database sequences.
Results: Currently, 62 854 reads totalling 44.736 Mb of raw sequence have been performed, giving a theoretical coverage of 99.99% of the genome. There are currently, 413 contigs > 1kb (362 contigs > 2 kb) with a total size of 4.835 Mb. Blast analysis reveals six operons being homologous to known tripartite multidrug efflux pumps; smeDEF, smeABC, homologues of the *Pseudomonas aeruginosa* mexCD and mexEF, and of the *Acinetobacter baumanii* adeABC. A homologue of the *Xanthomonas campestris* rpf cluster, which is involved in quorum sensing and virulence factor production, has been located. This system is unique to members of the *Xanthomonas* group (which includes *S. maltophilia*), which do not produce the quorum-sensing signal, homoserine lactones. A number of putative virulence factor genes have been located.
Conclusions: Determination of the *S. maltophilia* genome sequence will allow knock-out mutagenesis to determine which genes are important for multidrug resistance, biofilm formation and virulence factor production, which together give this bacterium its potential for future threat in the clinical setting.

P1561 Subgrouping of *Stenotrophomonas maltophilia* isolates by 16S rRNA sequence allows prediction of beta-lactam resistance profile

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Objectives: *Stenotrophomonas maltophilia* is an important, emerging, nosocomial pathogen. Isolates are generally highly resistant to beta-lactams, macrolides and aminoglycosides, fluoroquinolones, tetracyclines and chloramphenicol. Isolates from Bristol (UK), have been subgrouped according to 16S rRNA sequence into three: A, B and C. Interestingly, group A isolates are resistant to all beta-lactams because of inducible L1 and L2 beta-lactamases; Group B isolates are resistant to all beta-lactams except later generation cephalosporins because only L1 is inducible; Group C isolates are not resistant to beta-lactams, because they do not express inducible beta-lactamases. The aim of this study was to see if similar groupings of *S. maltophilia* isolates could be found in a collection of isolates from another European country.
Methods: Seven Turkish *S. maltophilia* isolates were used. 16S rRNA sequencing was performed using PCR. Beta-lactamase induction was attempted using imipenem (2 mg/L for 2 h) in nutrient broth grown cultures. Nitrocefin-hydrolysis assays used a spectrophotometer and crude cell extracts. MICs were determined by *E*-test, using Muller–Hinton agar.
Results: From 16S rRNA sequencing, the seven Turkish isolates were divided into four groups, three isolates were identical to Bristol group A, two isolates to Bristol group B and one isolate to Bristol group C. One isolate (group D) had a novel 16 S rRNA sequence. Beta-lactamase induction, coupled to assays of combined L1 + L2 activity (in the absence of EDTA), or L2 activity alone (in the presence of EDTA) confirmed that the Turkish group A, B and C isolates behaved exactly like the equivalent Bristol isolates. Group D isolates have an inducible L1 only. The profile of beta-lactamase inducibility reflected beta-lactam resistance levels; with group B and D isolates having lower MICs of cephalosporins than group A isolates, and group C isolates not being resistant to beta-lactams at all.
Conclusions: We have shown that not all *S. maltophilia* isolates have the same profile of beta-lactamase expression, and so resistance, but the pattern is not random. There is a clear link between 16S rRNA sequence subtype and expression of beta-lactamases. Knowledge of such a link might be important in a clinical setting.

P1562 Expression of putative pathogenicity factors by clinical isolates of *Stenotrophomonas maltophilia*

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Objectives: *Stenotrophomonas maltophilia* is increasingly reported to cause nosocomial infections in the immunocompromised, ventilated patient. However, little is known about its pathogenicity factors. The present study investigates the ability of clinical isolates of *S. maltophilia* to express characters that are considered to be virulence factors in other nonfermenters.
Methods: A total of 92 *S. maltophilia* clinical isolates were investigated. For comparison, 50 clinical *Pseudomonas aeruginosa* strains were included in the study. Haemolytic activity was examined on sheep blood agar, and elastase production on elastin-trishydrochloride agar after incubation of the bacteria for 3 h at 37°C. Susceptibility to the bactericidal activity of serum was examined by determining the survival rates for 3 h in 75% normal human serum. Induction of respiratory burst as a gauge of phagocytosis of the bacteria by neutrophils was measured by the luminol-enhanced chemiluminescence test.
Results: Production of elastase was detected more frequently among *S. maltophilia* isolates (77%) than among *P. aeruginosa* strains (64%), while haemolytic activity could be observed in both species with similar frequencies (100% vs. 98%). *Stenotrophomonas maltophilia* isolates were found to be significantly less often serum resistant (10%) than *P. aeruginosa* strains (30%). In contrast, the level of respiratory burst induction in neutrophils by *S. maltophilia* isolates was significantly higher than that observed by *P. aeruginosa* strains ($P < 0.0001$).
Conclusions: Our results show that, except serum resistance properties, the ability of clinical *S. maltophilia* isolates to express characters regarded as pathogenicity factors is similar (haemolysin, elastase expression) or even higher (no stimulation of neutrophils) than that of *P. aeruginosa*, and indicate a pathogenic potential of *S. maltophilia*.

P1563 Outbreak of *Pseudomonas putida* bloodstream infection

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Objectives: Describe the investigation and control of the outbreak.
Methods: Hospital Sirio-Libanes is a private 250 bed community hospital in Sao Paulo (Brazil), with predominantly surgical and oncologic patients. *Pseudomonas putida* was isolated in blood cultures (BC) from eight patients in 2 days – an outbreak was suspected. Positive BC and bacteria were identified by automated methods (Bactec and Vitek). Strains were genotyped by RAPD using ERIC 2 as primer. A case was any patient with a BC positive for *P. putida*. All first eight cases had tumours and carried a central venous catheter (CVC); therefore the heparin lock solution was suspected. Prefilled heparin syringes were recently bought from a new caterer. All patients charged for heparin lock (since the day the first case received it) who carried a CVC were called in for catheter drawn BC, irrespective of signs/symptoms. After blood drawing, long-term CVCs were locked with amicacin and heparin and oral ciprofloxacin was started. Most patients with positive BC and long-term CVC were treated with IV and local (lock) antimicrobial agents and short-term ($n = 5$) CVCs were removed promptly.
Results: *P. putida* was isolated from one of three valid lots of heparin lock syringes in use and from BC of 32 patients. A total of 154 patients had been charged for the heparin syringe. Thirty non-symptomatic patients with a CVC were called in and 26 attended; seven of 26 had chills and fever after CVC manipulation. Fourteen of 26 nonsymptomatic and 18 symptomatic patients had *P. putida* isolated. Eleven patients had BC drawn from both the CVC and venipuncture – seven had positive BC only from the CVC (five symptomatic) and four had positive BC from both sites (three symptomatic). BC from 10 patients also yielded *Stenotrophomonas maltophilia*. Only five of 27 long-term CVC had to be removed. Genotyping of *P. putida* from patients showed three different band patterns; two of these were also found in heparin isolates to the present moment.
Conclusions: (1) Manipulated prefilled heparin solution plastic syringes were contaminated with *P. putida*; (2) BC collected through the CVC may be preferable to venipuncture in the diagnosis of CVC-related infections; (3) Most long-term CVC (22 of 27) were salvaged with IV associated with lock antimicrobial therapy; (4) The clinical and epidemiological significance of the *S. maltophilia* isolates is unclear and under investigation.

P1564 *Burkholderia cepacia* complex in an Italian cystic fibrosis centre: epidemiology and clinical course of patients infected with different, *Burkholderia cenocepacia* RecA strains

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Objective: Bacteria of the *Burkholderia cepacia* complex (Bcc) are important pathogens in cystic fibrosis (CF) patients (pts). Bcc comprises at least nine species or genomovar. Aim of this study was to assess the epidemiology of Bcc recovered from pts attending the CF Centre at the Gaslini Children's Hospital (Genova, Italy) from 1984 to 2001.
Methods: A total of 195 Bcc isolates were recovered from 75 of 326 (23%) of pts in regular follow up in the study period. The genomovar of all isolates was determined by RFLP analysis of recA gene and confirmed by PCR of recA with genomovar-specific primers. All strains were typed by RAPD analysis. The clinical course of pts infected by different species was determined comparing: infection with *P. aeruginosa* prior Bcc acquisition, changes in lung function (FEV1) and body weight in the 2-year Bcc postacquisition period, mortality in long-term period.
Results: *Burkholderia cenocepacia* (genomovar III) is the predominant species recovered from the CF pts infected with Bcc in the Genoa Centre. Of the other eight species comprising the Bcc, only few isolates belonging to Bc genomovar I, *B. stabilis*, and *B. pyrrocinia* were found. Of the four recA lineages of *B. cenocepacia*, most pts harboured IIIB strains. Patient-to-patient spread of Bcc among CF pts was mostly associated with *B. cenocepacia* strains, in particular with IIIA and IIID recA lineages. Mortality was significantly higher in pts infected with Bcc than in noninfected pts. All deaths were associated with the presence of *B. cenocepacia*. Within *B. cenocepacia*, infection with epidemic strains belonging to lineages IIIA and IIID was associated with higher mortality than with lineage IIIB strains. No significant differences in lung function, body weight and mortality rate were observed between pts infected with epidemic strains belonging to either *B. cenocepacia* IIIA or *B. cenocepacia* IIID.
Conclusions: Our study confirms the prevalence of *B. cenocepacia* among Bcc-infected CF pts and the high percentage of mortality associated with this species. The major role of an epidemic strain belonging to the recently identified recA lineage IIID in spreading Bcc infection among CF pts has been recognised for the first time.

Hepatitis C

P1565 Pegylated interferon-alpha2a and ribavirin in HIV-HCV co-infected patients

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Objectives: Standard interferon treatment of Hepatitis C Virus (HCV) infection in HIV-positive patients had low response rates, with or without ribavirin (RBV). Better outcomes have been obtained with pegylated interferons, although liver toxicity of antiretroviral drugs, immune restoration and metabolic syndromes are major confounding variables in co-infected patients. We started an open, multicentre, prospective study with pegylated interferon-alpha2a (PegIFN) and RBV in co-infected patients not undergoing antiretroviral treatment.
Methods: Inclusion criteria were: absence of antiretroviral treatment (ART) for more than 24 weeks, CD4+ cell count 350/mm^3, HIV-RNA < 30 000 copies/mL, and biopsy-proven chronic C hepatitis. Patients were treated with PegIFN alpha2a 180 μg subcutaneously weekly and RBV 800 mg po daily for 48 weeks. HIV-RNA, HCV-RNA, CD4+ cell count and ALT level were monitored.
Results: As of October 2003, 28 patients (median age: 39 years) started the treatment. HCV non-1 genotype was found in 88% of patients. At 12 and 24 weeks, respectively, 58.3 and 62.5% of patients had virological response (HCV-RNA < 100 copies/mL), and 75% of patients had biochemical response. There was no significant decrease of median CD4+ cell count from the baseline and HIV-RNA did not change significantly. Five patients (17.8%) discontinued treatment because of adverse effects.
Conclusions: A virological response was obtained in more than 50% of patients. These data indicate that administration of PegIFN and ribavirin may be effective in co-infected patients. Treatment of HCV infection without concomitant ART may offer some advantages: avoidance of hepatotoxicity of antiretroviral drugs and no immune restoration syndrome potentially complicating the treatment. Moreover, a CD4+ cell count above 350/mm^3 is associated with a good immune system activity. Limits of our

study were the high prevalence of non-1 HCV genotypes, which is known to be associated with a better response rather than genotype 1.

P1566 Compliance and response to PEG-IFN alpha and Ribavirin combined treatment of a special category of chronic hepatitis C patients, the ex-users of intravenously administered narcotic substances

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Purpose: Evaluation of both compliance and response to PEG-IFN alpha and Ribavirin combined treatment of a special category of chronic hepatitis C patients, the ex users of intravenously administered narcotic substances.
Material and Methods: Sixty-two patients who had stopped using addictive substances about 6 months before initiating combined treatment with PEG-IFN alpha were studied. Among them 57 were men and five were women (average age 31 years old).
Results: As regards this category of patients, compliance with treatment was poor. A 20.9% (13 patients) fully complied with treatment, 29.1% (18 patients) partly complied with it (did not return for the second biopsy and PCR tests), while 46.7% (29 patients) did not follow the treatment recommended. Two of 13 treatment-abiding patients had to interrupt it, because of very serious side-effects, such as severe weight loss and peronial nerve paresis. The S.V.R. of the 13 treatment-abiding patients is assessed to 84.9%.
Conclusions: The intravenous narcotic substances users abide by the recommended treatment to a very limited extent, while they respond to combined PEG-IFN alpha and Ribavirin treatment to a great extent. This greater extent of response is attributed to the patients' young age as well as the prevailing genotype 3.

P1567 Effects of combination treatment with interferon and ribavirin on endothelial cell inflammatory integrins and stress markers in advanced chronic hepatitis C

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Objectives: Individuals with chronic hepatitis C (CHC) progress to cirrhosis and hepatic cancer. Individuals with advanced CHC are coagulopathic and can manifest fibrinolysis. The coagulopathy is a consequence of hepatocytic dysfunction. The fibrinolysis represents a response to local endothelial cell injury. Interferon and ribavirin decrease necroinflammation in chronic hepatitis C with or without virological clearance. The aim of this study was to evaluate the effect of combination therapy with interferon and ribavirin on endothelial cell inflammatory integrins and measures of endothelial stress.
Methods: Immediately prior to the treatment in 53 individuals with advanced CHC, the plasma levels of tissue factor (TF), thrombomodulin (TM), soluble ICAM-1 (s-ICAM-1), soluble VCAM-1 (s-VCAM-1), soluble L-Selectin (s-L-Selectin), the prothrombin time and the activated partial thromboplastin time were determined. The same parameters were assayed at 1, 4, 12 and 24 weeks.
Results: TF and TM levels were very high at baseline consistent with a vascular endothelial stress response. Similarly s-ICAM-1, s-VCAM-1 as well as L-Selectin levels were increased. At 4 weeks after the therapy, a marked reduction in TM, ICAM-1 and VCAM-1 and to a lesser degree TF and L-Selectin levels was observed. This reduction persisted for 24 weeks. No change in measures of fibrinolysis [plasminogen activator inhibitor-1 (PAI-1), total tissue factor pathway inhibitor (t-TFPI), activated tissue factor pathway inhibitor (TFPIa), d-dimers (DD), and fibrinogen levels] occurred.
Conclusions: Based upon these data it can be concluded that: (1) combination therapy with interferon and ribavirin corrects the coagulopathy seen in advanced CHC; (2) reduces endothelial cell injury and/or stress as evidenced by the TF, TM, s-ICAM-1 and s-VCAM-1 levels in plasma; (3) these changes in coagulation occurred without inducing a propagated vascular thrombosis.

P1568 Influence of interleukin-10 therapy on endothelial cell proteins in patients with chronic hepatitis C

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Objectives: Hepatitis C virus (HCV) infects hepatocytes and utilizes the hepatocyte to replicate. In so doing, many hepatocyte activities are shifted from their native state to one reflecting liver cell stress. Thrombomodulin and tissue factor are endothelial cell proteins that are expressed as a result of tissue injury or stress. The aim of this study was determine the effect of interleukin (IL)-10 administrations on levels of thrombomodulin and tissue factor in patients with HCV-related liver disease.
Methods: Forty-three patients with chronic hepatitis C who had failed antiviral therapy were enrolled in a 6-month treatment regimen with SQ IL-10 given daily or thrice weekly. Liver biopsies were performed before and after therapy. The levels of thrombomodulin and tissue factor in plasma and hepatocyte cytosol have been evaluated.
Results: IL-10 led to significant improvement in serum ALT (mean ALT: day $0 = 151 \pm 14$ vs. month $6 = 78 \pm 11$; $P < 0.05$). Hepatic inflammation score decreased by at least two in 17 of 43 patients (mean decrease from 5.7 ± 0.5 to 4.8 ± 0.7, $P < 0.05$) and 14 of 43 showed a reduction in fibrosis score (mean change from 6.1 ± 0.7 to 5.1 ± 0.2, $P < 0.05$). IL-10 caused a decrease in thrombomodulin level both in plasma and cytosol ($P < 0.01$). These changes parallel the improvement in ALT. The levels of tissue factor in plasma and cytosol varied minimally between baseline and after IL-10 therapy.
Conclusions: IL-10 therapy appears to decrease disease activity in patients with HCV-related liver disease. Thrombomodulin may be unique marker of cellular and endothelial stress present in individuals with chronic hepatitis C. This marker might be useful during the clinical course of chronic hepatitis C, as a means of gauging the tissue response to therapy.

P1569 Correlation between endothelial haemostatic markers in patients with chronic hepatitis C

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Objectives: Thrombosis of the small intrahepatic veins has been suggested to trigger liver tissue remodelling. It has been suggested to be quantified by measuring plasma markers, such as von Willebrand factor and thrombomodulin and soluble adhesion molecules. We hypothesized there may exist a correlation between the plasma levels of von Willebrand factor, thrombomodulin, and tissue plasminogen activator antigen (tPAag) as markers of endothelial cell dysfunction and the serum concentrations of soluble adhesion molecules and monocyte chemoattractant protein-1 (MCP-1) in patients with chronic hepatitis C.
Methods: Patients ($n = 117$) with chronic hepatitis C without malignancy, a history of venous thrombosis, or antiviral/immunosuppressive therapy within the last 6 months and matched controls ($n = 115$) were included. To evaluate possible influence of acute phase reaction, reinvestigation was performed after 6 months.
Results: The concentrations of von Willebrand factor, thrombomodulin and tPAag in plasma were significantly higher in patients with chronic hepatitis C as compared with healthy subjects ($P = 0.017$, $P < 0.05$ and $P < 0.001$, respectively), still statistically significant after 6 months and also after adjustment for

established risk factors. The patients also had significantly higher concentrations of soluble vascular cell adhesion molecule-1 (sVCAM-1) and MCP-1 compared with healthy controls ($P < 0.001$ for both comparisons). There were strong correlations between the concentration of soluble intercellular adhesion molecule-1 (sICAM-1) and von Willebrand factor in patients with chronic hepatitis C ($r = 0.64$, $P < 0.001$), between the concentration of thrombomodulin and sVCAM-1 ($r = 0.62$, $P < 0.001$), and between tPAag sVCAM-1 ($r = 0.51$, $P < 0.05$). Furthermore, a negative correlation was observed between the concentration of thrombomodulin and the cell surface expression of CD11b on monocytes and granulocytes in the peripheral circulation ($P < 0.05$ in both cases).
Conclusions: The strong correlation between markers of endothelial dysfunction and soluble adhesion molecules in patients with chronic hepatitis C strengthen the view that an ongoing stress on endothelial cells is present in these patients. This may play a pathophysiological role in the progression of disease.

P1570 The role of antibodies against NS5 protein of hepatitis C virus (HCV) and IgM antibodies against HCV as predictive factors to the treatment with interferon alpha in patients with chronic hepatitis C

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Objectives: Antibodies against NS5 protein of hepatitis C virus (HCV) and IgM antibodies against HCV (anti-HCV IgM) were studied as predictors of response to the therapy with interferon alpha in patients with chronic hepatitis C living in Gomel region, the most affected by Chernobyl catastrophe.
Methods: A study was carried out in 36 patients with chronic hepatitis C who were treated with interferon alpha in Gomel region. The antibodies against NS5 (anti-NS5) and anti-HCV IgM were studied before the course of treatment by means of ELISA test. There were 16 (44.4%) anti-NS5 positive and 19 (52.8%) anti-HCV IgM positive sera samples. Virologic response (undetectable HCV-RNA in serum) was evaluated in 3 months of treatment. Virologic response was detected in 16 (44.4%) of patients, nonresponse – in 20 (55.6%) of patients.
Results: Anti-NS5 before the course of treatment was revealed in 25.0% of responders vs. 60.0% in nonresponders (chi-square = 4.41; $P = 0.036$); anti-HCV IgM – in 31.3% of responders vs. 70.0% of nonresponders (chi-square = 5.36; $P = 0.021$).
Conclusions: Antibodies anti-NS5 and anti-HCV IgM were revealed significantly more frequently in nonresponders to alpha-interferon therapy. Both anti-NS5 and anti-HCV IgM showed predictive value of response to interferon therapy of chronic hepatitis C.

P1571 Evaluation of the total hepatitis C virus core antigen in patients with chronic hepatitis: comparison with quantiplex branched DNA 3.0

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Objectives: The aim of our study was to evaluate the correlation between quantitative total hepatitis C virus core antigen and HCV-RNA levels in patients treated with pegylated IFN-a2b (PEG-IFN) plus ribavirin combination therapy.
Methods: A total of 46 patients aged 20–65 years infected by genotype 1, 2, 3, 4 were enrolled to this prospective study. Patients were anti-HCV positive, pretreatment viral load (bDNA 3.0; Bayer Diagnostics) >20 000 UI/mL with histologically confirmed chronic hepatitis and abnormal activity of aminotransferases lasting at least 6 months before the start of therapy. Patients were treated with PEG-IFN plus ribavirin for 6 months (genotype 2, 3), and for 12 months (genotype 1, 4). HCV-RNA not detectable (cut-off: <615 UI/mL to <3200 copies/mL) at 3 months was defined as early response (ER), at 6 and 12 months as end of treatment response (ETR) for genotype 2–3 and genotype 1–4, respectively. Total HCV Core Ag quantification and HCV-RNA viral load was assessed at baseline and after 3, 6, 9 and 12 months (M3, M6, M9, M12). Total HCV Core Ag quantification was assessed using Ortho track-C assay (Ortho-Clinical Diagnostics, Raritan, NJ, USA) and HCV-RNA was assessed using the Quantiplex brached DNA 3.0 (bDNA 3.0) assay (Bayer Diagnostics, Emmeryville, CA, USA). All assays were performed according to the manufacturer's instructions.
Results: After 6 months, 31 patients (67.4%) were negative for both HCV-RNA and HCV-Ag, 12 (26.1%) positive for both, two (4.3%) HCV-RNA positive but HCV-Ag negative, and one (2.2%) HCV-RNA negative but HCV-Ag positive (1.6 pg/mL). After 12 months, 36 (78.2%) were negative for both HCV-RNA and HCV-Ag, nine (19.6%) positive for both, and one (2.2%) HCV-RNA positive but HCV-Ag negative.
Conclusions: The HCV-Ag test showed a strong correlation with HCV-RNA levels (percentage of concordance ranging from 94 to 98%).

P1572 Rapid and accurate quantitation of different HCV genotypes by optimisation of a new real-time PCR test

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Objectives: Although there are a wealth of information about and numerous assays for HCV RNA detection in serum and plasma, real-time PCR has not yet been applied to viral load measurements.
Methods: We had developed a single step Real Time RT-PCR protocol for accurate quantitation of HCV RNA in plasma samples using the LightCycler and dual hybridisation probes technologies. HCV RNA quantification in clinical samples was based on the amplification of only one 170 000 IU/mL RNA reference standard (Accurun 305, BBI), which was compared with the previously quantified standard curve.
Results: The inter-assay coefficient of variation and the standard deviation of the reference standard was about 3% and 1, respectively, over a period of 8 months. A total of 960 plasma samples were tested and HCV RNA was quantified in 528 samples with levels varying from 5.7×10^1 to 2.52×10^9 IU/mL. The amplicons were removed from the LightCycler glass capillaries, purified from the 5'LC and 3'FL probes, and used for automated sequence analysis (Visible Genetics – Bayer diagn). The genotype was assessed for 112 HCV positive samples: the majority were type 1b (33.0%) and 2 (32.1%), but genotypes 4 and 5 were also identified. The melting temperatures of the probes obtained for each genotype will be compared with those temperatures calculated from the sequence analysis of the amplified targets to verify the feasibility of HCV genotyping by Real Time PCR.
Conclusions: The new HCV quantification method seems to be rapid, accurate, reproducible and able to quantitate all HCV genotypes. HCV typing by melting temperatures study has to be further optimised.

P1573 Genotyping distribution of hepatitis C virus in patients with HCV infection in Northern Greece

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Objectives: The genotypes' distribution of hepatitis C virus in different groups of patients with hepatitis C and its correlation with age, virus load and ways of contamination.
Methods: One hundred and sixty patients with HCV infection – before the commencement of therapy – who were admitted to AHEPA University Hospital between January 2000 and December 2002. HCV-RNA presence was assessed by reverse transcription-PCR (HCV Monitor CA-Roche). Reverse hybridisation test of the amplifications was used for the genotyping (Inno-Lipa/ Innogenetics).

Results: Thirty-four of the patients (21.25%) have been multitransfused, 11 (6.9%) presented with end stage renal disease (ESRD) under hemodialysis, 2 (1.25%) were patients with HIV coinfection, 42 (26.2%) were intravenous drug users (IVDU) and 71 were sporadic cases (SC) of hepatitis C with unknown source of infection. The SC samples were distributed into four groups according to their age: <30, 31–40, 41–50, >50 years. The most common genotype in all groups of patients was 1b (77 of 160 = 48%) followed by genotype 3 (38 of 160 = 23.7%). Genotypes 2 and 4 were less common, with 15 cases of genotype 2 (9.3%) and 10 cases of genotype 4 (6.2%). High level of viraemia >100 000 copies/mL was observed in 127 of 160 samples (79.4%) with the following distribution: genotype 1b: 96.1%, genotype 3: 73.7%, genotype 4: 70%. The lowest viraemia was observed in the other subtypes of genotype 1. The genotype 1b was observed in 71.4% (20 of 28) of the patients older than 50 years and genotype 3 was more frequent in patients younger than 40 years (15 of 43 = 34.8%).
Conclusions: Genotype 1b is the most common in all groups of patients. In particular, for patients older than 50 years it was associated with higher levels of virus load in comparison to other subtypes of the genotype 1. The same observation does not include other genotypes.

P1574 Use of transcription mediated amplification method (TMA), line probe assay (LiPA) and branched DNA (bDNA) assay, to study chronic hepatitis C in a group of intravenous drug users

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Objectives: To study the prevalence of hepatitis C virus (HCV) infection, the distribution of HCV genotype and the correlation of HCV genotype and viral load among patients with Chronic Hepatitis C, in a group of Intravenous Drug Users (IVDU).
Methods: Serum samples from 32 men IVDU, range age 25–40 years, were screened for anti HCV (MEIA HCV 3.0 Axsym; Abbott). Anti HCV positivity was confirmed by an immunoblot assay (INNO-LIA HCV III Innogenetics). HCV RNA was determined by TMA (HCV RNA Qualitative Assay, Versant) in all anti HCV positive sera. TMA is an isothermal autocatalytic target amplification method, very sensitive, which has the potential to detect low viraemia approximately 5 IU/mL HCV RNA. Genotyping was carried out after a short, 2 h PCR procedure in TMA product. PCR-TMA product was used for HCV genotyping, which was carried out by a reverse hybridisation test LiPA (HCV Genotype Assay, LiPA, Versant). Viral load of HCV RNA positive sera was quantified by a bDNA assay (HCV RNA 3.0 Assay, b-DNA, Versant).
Results: Among 32 IVDU 22 had antibodies for HCV (anti HCV) and active viraemia with HCV RNA positive in serum. The distribution of HCV genotypes was 3a in 11 cases, one in eight cases (4-1a, 4-1b) and 2a/2c in three cases.
Conclusions: In the above group of IVDU the prevalence of HCV infection was high (68%) and the predominant genotype was 3a (50%). Viral load among HCV RNA positive patients was 520–1.237×10^6 IU/mL, whereas none of them had evidence of mixed HCV infection.

P1575 Molecular epidemiology of hepatitis C among drug users, correlation with clinical parameters, sexual behaviour and drug-related risk behaviour

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Background: The Hepatitis C virus (HCV) is a single stranded RNA virus exhibiting an important genetic diversity. Determining the genotype is crucial for epidemiological as well as for clinical analysis since HCV genotypes differ in geographic distribution, in response to treatment and according to the route of transmission. The objective of this study was to determine the genotypic variation among drug users in Flanders and to relate the genotype distribution to the characteristics of the population.
Methods: HCV-RNA quantification and genotyping was performed on stored samples from 161 anti-HCV positive injecting and noninjecting drug users. A standardised interview providing information on their socio-demographic status, drug-related and sexual risk behaviour was available for each drug user.
Results: HCV-RNA was present in 152 of 161 samples. The genotype could be determined for 148 cases. Genotype 1 was predominant (48.6%), followed by genotype 3 (41.2%), genotype 4 (8.8%) and genotype 2 (1.4%). In the univariate analysis HCV genotype was related to geographic region, history of IDU, number of sexual partners last year, having a tattoo and the presence of anti-HBc. In the multivatiate analysis having no history of injecting drug use (IDU) was confirmed as a statistically significant predictor for an infection with genotype 1. Predictors for an infection with genotype 3 were found to be the presence of anti-HBc antibodies and a history of injecting drug use. Being tattooed emerged as a statistically significant predictor for an infection with genotype 4.
Conclusions: The prevalence of HCV-RNA among anti-HCV positive drug users was 94%, being considerably higher than the expected 60–80% chronicity rate. Furthermore, the distribution of HCV genotypes in IDU differs significantly from the distribution among non-IDU, genotype 3 being predominant among the former and genotype 1 among the latter. Finally, the results of this study are suggestive for a role of tattooing practices in the spread of HCV among drug users.

P1576 Evaluation of total HCV core antigen detection in haemodialysis patients

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Objective: The aim of this study is to evaluate an immunoenzymatic antigenic detection for early diagnosis of hepatitis C virus (HCV) infection in dialysis patients.
Patients and Methods: We used the enzyme-linked immunosorbent assay (Ortho HCV 3.0 ELISA test System; Ortho-Clinical Diagnostics) for the detection of antibody to HCV in 144 patients with chronic renal failure treated in the haemodialysis department of our hospital. Total HCV Core Antigen was determined by an immunoassay (Track-C, Ortho Total HCV Antigen Assay, Ortho-Clinical Diagnostics) and compared with measures obtained by Cobas Amplicor HCV test version 2.0 (Cobas Amplicor and Cobas Amplicor Monitor HCV, Roche Molecular Systems). All assays were performed according to the manufacturer's instructions. The cut-off value for HCV Antigen was 1.5 pg/mL. The cut-off value for the Cobas Amplicor HCV test was 50 UI/mL and for the Amplicor HCV Monitor test was 600 UI/mL.
Results: The patients with anti HCV antibody-positive (25) showed a global correlation between the HCV Core Antigen and the Amplicor HCV test of 92% (Table 1) and the patients with anti HCV antibody-negative (119) showed a global correlation of 95.8% (Table 2).

Table 1.

	HCV Core Ag Positive	HCV Core Ag Negavtive
PCR HCV Positive	17	1
PCR HCV Negative	1	6
	HCV Core Ag Positive	HCV Core Ag Negative
PCR HCV Positive	0	1
PCR HCV Negative	4	114

Conclusions: There was a good correlation between the HCV-PCR and the HCV Core Antigen. This assay is a new useful test for early detection of HCV infection in this study group.

P1577 Clinical evaluation of the male sexual partners of HCV-infected pregnant women

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To determine the risk of heterosexual transmission of hepatitis C virus (HCV) and to identify other risk factors for HCV seropositivity in heterosexual couples. We have investigated a cohort of pregnant women HCV-Ab +ve.
Materials and Methods: During September 1994 and November 2003, at the Maternity Dpt. in Bergamo 26 308 consecutive subjects, aged 16–47 years (mean 31 ± SD 5 years), were tested for anti-HCV on screening and 728 women (2.8%) were anti-HCV+ve. In those anti-HCV+ve, ALT, HCV-RNA and HCV viral load (and HCV genotype in those viraemic) were determined. 383 women (52.6%) were at their first pregnancy. A previous history of intravenous drug addiction (IVDU) or haemotransfusion of both, patient and partner, was investigated, a clinical evaluation and serum levels of ALT was performed. These determinations were performed, at 24–26w of gestation, at delivery and 6 months after. HIV or HBV coinfection was present in 50 (6.7%) and 14 cases (2%), with two cases of triple infection. HCV genotypes were determined in 479 viremic pregnant women: 1b, 2, 1a, 3a, 4 and indeterminate one accounted for 30, 24, 20, 19, 6 and 1%, respectively. ALT levels were over the ULN in 51% initially, in 5% at the third trimester and in 49% 6 months after delivery. Sexual partners were investigated, to check anti-HCV+vity. Heroin abuse, blood transfusion(s), health care employment, and unknown source were responsible for HCV infection in 35, 17, 6, and 42%.
Results: In all 728 male partners (100%) were checked for HCV+vity and in 160 of them (21.9%) was +ve. Among these 160 +ve partners, 143 (89.4%) were IVDUs, 13 (8.1%) reported haemotransfusion, and four (2.5%) did not report any risk factors.

Heroin Abuse *n* 728 Anti-HCV+	Anti-HCV in 728 Regular Male Sexual Partners	
Pregnant Women	Positive ($n = 160$)	Negative ($n = 568$)
Present ($n = 258$)	128*	135
Absent ($n = 470$)	37** p< 0.0001	433

*All IVDUs. **20 IVDUs, 13 had received at least one transfusion, and four had no apparent risk factor.

Discussion: High rate of anti-HCV+vity (21.9%) was observed in the group of regular sexual partners of the anti-HCV+ve pregnant women. Hundred and forty-three of the 160 anti-HCV+ve men (89.4%) were IVDUs, and 13 (8.1%) received transfusion(s) suggesting that the greatest risk factor for HCV infection is related to the presence of blood-borne risk factor(s) (97.5%) and it makes difficult to distinguish the contribution of sexual activity to the risk of HCV transmission.

P1578 Outcome of pregnancy in hepatitis C virus infection

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In the Western countries the incidence of hepatitis C virus (HCV) infection has steadily been increasing especially among young adults. It is thus likely that an increasing prevalence of HCV infection is also found in pregnant women. To assess the frequency of HCV infection in the metropolitan area of Helsinki selected anti-HCV antibody testing was carried out for pregnant women during the years 1991–1999. Altogether 145 mothers were identified among 44 680 mothers. The frequency of anti-HCV positivity rose from 0.13% in 1991 to 0.43–0.53 in 1997–1999. In early 1990s only 20% of mothers knew about their seropositivity, whereas by the end of the follow-up period almost 70% of mothers knew about their HCV infection already before the pregnancy. Intravenous drug abuse was the major risk factor (71% of cases) for contracting the disease. In 90% of the mothers chronic HCV infection was well under control and in this population the mean serum alanine aminotransferase (ALT) values decreased towards the end of the pregnancy. However, 10% of anti-HCV Ab positive mothers developed intrahepatic cholestasis (odds ratio 16.4) as characterised by itching and elevated serum bile acid levels. The corresponding value in the control pregnancies was only 0.7%. Anti-HCV Ab positive mothers were younger, delivered earlier and gave birth to babies with smaller birth weight as compared with control deliveries. To have a more comprehensive view of the problem of HCV infection during pregnancy randomly selected serum specimens from the Finnish maternity cohort were tested. 2000–5000 serum specimens were tested in selected cohorts (1985, 1990, 1995 and 2000). In 1985, the prevalence was 0.19% and it steadily role to 0.42% in 2000. In the metropolitan area of Helsinki the prevalence was higher being 0.68% and 0.70 in 1997 and 2002, respectively. Our study indicates that there is an increasing problem of HCV infection in pregnant women in Finland. Although most women cope well with their disease during pregnancy there is a subpopulation of mothers who develop cholestasis and their liver status should thus be followed-up carefully. Testing of all mothers for serum anti-HCV antibodies is recommended.

P1579 Prevalence of hepatitis C virus infection and risk factors in an incarcerated population in Iran

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Objective: Hepatitis C virus (HCV) is a leading cause of liver failure and liver transplantation in adults. Identified risk factors for HCV infection include intravenous (IV) drug use, exposure to infected blood products, and intranasal drug use, High-risk sexual activity [multiple sexual partners, history of sexually transmitted disease (STD)], tattooing and skin piercing. The purpose of this study was to determine the prevalence of HCV and high-risk behaviours in drug addiction inmates.
Methods: We conducted a cross-sectional prevalence study of HCV infection in drug addiction inmates who were admitted to a prison in Guilan province, north of Iran, between September 2003 and October 2003. Subjects were asked questions regarding behaviours that might put them at risk for acquiring HCV, and blood was drawn for HCV antibody testing use of an enzyme-linked immunosorbent assay (ELISA ÍÍ) and positive samples rechecked with Western blot.
Results: Four hundred and fifty inmates participate in the seroprevalence study. The mean age of respondents was 34.7 ± 9 SD, (range from 18 to 65) year. Duration of addiction was 9.6 ± 8 SD years and 49% were treacle addiction, 17.3% heroin addiction, 11.5% cannabis addiction and others had addiction to more than one substance. HCV risk behaviours were common in this population: intravenous drug use (15.3%), intranasal drug use (56.2%) and tattoos (55.5%). Two hundred and two study subjects (44.9%) were HCV antibody positive. HCV-positive status was significantly associated with history of intravenous drug use, having had tattoos, duration of addiction, duration of staying in prison, body piercing.

Conclusions: Infection with hepatitis C is endemic in Iranian prisons. Better access to harm reduction strategies is needed in this environment and future studies should address the need for targeted HCV screening and education of prison inmates regarding risks for HCV.

P1580 HCV genotypes in the Slovak Republic

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Objectives: Liver diseases caused by hepatitis C virus (HCV) pose a serious public health problem. HCV presents a group of highly variable strains due to variability in nucleotide sequence between the virus isolates. To indicate effective therapy of HCV infection, it is important to determine not only the presence of active virus in the patient's body, but also to specify the HCV genotype. The object of this study was to determine incidence of HCV genotypes in the patients included into the therapeutic process in the Slovak Republic.
Methods: Altogether 302 patients were chosen for this study, based on their HCV RNA positivity in the serum as detected by PCR method (Cobas Amplicor version 2 test, Roche). Then, the specific genotype and/or subtype of these amplicons was determined by hybridisation to specific probes with genotyping test Inno-Lipa HCV II, Innogenetics.
Results: Of 302 sera tested, HCV genotype 1 was found in 234 (77.5%) cases, genotype 3 in 64 (21.2%) cases and in four (1.3%) positive cases the genotype could not be determined. More detailed analysis revealed 1a subtype in 15 (5,0%), 1b subtype in 203 (67.2%), 1a/1b in five (1.7%) cases, but 11 cases belonging to the genotype 1 were indeterminable subtypes. In the genotype 3, there were 63 (20.9%) 3a subtypes and 1 (0.3%) 3a/3b subtype. As to the age distribution, in the group of patients older than 30 years, the genotype 1 highly predominated with 189 (97.4%) cases, whereas the genotype 3 occurred in four (2.1%) cases only and in one (0.5%) case the genotype could not be determined. In contrast, in the group of patients younger than 30 years, the genotype 3 prevailed with 60 (55.5%) cases followed by 45 (41.7%) cases of the genotype 1 and 3 (2.8%) indeterminable genotypes.
Conclusion: From our study follows that the genotype 1, the most difficult to treat is the most common in the Slovak Republic. However, in the younger population (<30 years, with higher incidence of drug addicts), the genotype 3 prevails.

P1581 Genotypical profile of infection by HCV

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The infection by the HCV is the most important cause of the viral hepatitis, as by the number as by the gravity of its complications, in addition to the possibility of an effective treatment. We have studied the epidemiological characteristics of the infection by HCV in an area about 311 720 people, detecting 314 carriers (prevalence of 1007 by 100 000 inhabitants). The average age is of 41 years old (range 13–79), corresponding the majority to men (76.8%), with an average age of 39.8 years old, lower than the women (44.6). The global genotypical distribution shows the group 1 as the most frequent (64%), following of the 3 (21%) and the 4 (11%), although the genotype changes based on other parameters like the co-infection with HIV. Among HIV+ the most frequent is the 1a (30.8%), following 3a (23.1%), 1b (21.2%), 4c/4d (16.1%), etc. In the other side among population HIV – the most frequent is 1b (50.1%), following of 3a (19.2%), 1a (16.5%), 4c/4d (5.1%), etc. Other genotypes have been in a proportion far below. Globally, in the distribution by sex, 1b emphasises the predominance of the genotype 1b (47.9% in women and 32.8% in men), following of 1a with similar numbers (21.9 and 23.7%). It is only showed significant differences in the genotype 3a in relation to sex, since it is detected in 9.6% of women and 24.1% of men; in four position is 4c/4d with percentage next to 10% in both groups. In relation to the route of infection, the parenteral one was the most frequent (76.6%) mainly in HIV (79.7%), to which is added 3.9% whose route could by sexual, in addition to the previous one. The rest of transmission routes were inferior to 5%. When studying the years since the infection was acquired, we have observed that between the no-HIV group the incidence had not varied, whereas in HIV+ it decreased. With these results we observed the existence of a high prevalence of HCV in our sanitary area, fundamentally in men, related to infection by HIV in half of the cases, mainly parenteral acquisition (with tendency to the diminution in the HIV carriers) showing great predominance the genotype 1, varying in the different populations.

P1582 Distribution of HCV genotypes among patients in São Paulo, Brazil

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Objectives: HCV is a major cause of chronic hepatitis and has become the most chronic blood-borne infection all over the world. It is estimated that 200 million people are currently infected with HCV, and around 3.5 million people contract the virus each year. HCV genotyping is important not only as predictive factor for therapeutic response, but also for epidemiological data, development of vaccines, and other viral biological studies. The main objective of the present study was to determine the HCV genotype distribution in patients assisted at the Hospital das Clinicas – School of Medicine University of São Paulo (São Paulo, Brazil).
Methods: From May to December 2003, the Molecular Biology Laboratory received in its routine basis, 1300 HCV qualitative PCRs (Amplicor, Roche Diagnostic Systems, Brazil), mean 160 tests/month; approximately 60% of these tests are positive. HCV genotyping were performed in 279 samples, mean 35 tests/month, using the commercial kit INNO-LiPA HCV (Innogenetics, N.V., Ghent, Belgium, distributed by Bayer Diagnostics, Brazil).
Results: Of 279 HCV-genotyped patients, 137 (49.1%) were males and 142 (50,8%) were females; the age varied from 21 to 80 years old, 24% were in the age range of 21–40 years old, 57% in 41–60 years old and 19% were over 60 years old. The distribution of genotypes were as follows: Two samples were nontypeable by the method used and were sent to sequencing.

	Genotype											
	1	1a	1b	1a/1b	2	2b	2a/2b	3	3a	4	5	6
No. tests	26	45	133	5	1	1	1	1	63	1	0	0
%	9.40	16.25	48	1.8	0.36	0.36	0.36	0.36	22.75	0.36	0	0

Conclusions: HCV subtype 1b is the most prevalent follow by subtype 3a in the population studied. HCV infection was more prevalent among older patients (>50 years old) and gender was not found as a significant factor.

Sexually transmitted diseases

P1583 Prevalence of *C. trachomatis* infection in the general population and in a sexually transmitted disease centre in the Balearic Islands, Spain

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Objectives: We report a prospective study about the prevalence of genitourinary infections by *C. trachomatis* in the general population and in a group of prostitutes treated in an STD clinic during the year 2002.
Methods: The clinical samples (urethral and endocervical swabs) were inoculated in shell vials of McCoy cell line (Vircell, Spain) and incubated for 48–72 h at 36°C and then stained with a monoclonal antibody against MOP of *C. trachomatis* (MicroTrak, Ireland) using a direct immunofluorescence assay.
Results: Over the study period 1504 clinical samples were studied. Of these 580 (38.5%) were urethral samples from males. *C. trachomatis* was isolated in 19 (3.2%) of these samples. Of the female samples (endocervical) 606 (65.5%) were obtained from the general population, of these five (0.8%) were positive for *C. trachomatis*, and 318 (34.5%) were from a the group of prostitutes. In this group 9 (2.8%) cases of infection by *C. trachomatis* were detected ($P < 0.05$). In all samples studied 33 (2.2%) were positive for *C. trachomatis*. The prevalence of the infection by *C. trachomatis* in the last few years in our geographical area (Balearic Islands, Spain) is as follows: in 1999 men 2.5% and women 0.9%; in 2000 men 2.8% and women 0.6%; and in 2001 men 1.8% and women 1%.
Conclusions: An stable prevalence of infection by *C. trachomatis* is observed in the general population in recent years with a positivity percentage for men statistically higher ($P < 0.05$) to that detected in women. In the high-risk group of the prostitutes a prevalence of 2.8% was detected. This value is very much higher ($P < 0.05$) than that of the general population and similar to detected in men with the clinical diagnosis of urethritis.

P1584 *Chlamydia trachomatis* infection and risk of preterm birth

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A group of 103 unselected consecutive patients presenting for routine prenatal care were examined for *Chlamydia trachomatis* (CT) in the clinic of Obstetrics and Gynecology of Varna city. The aim was to determine whether serologic evidence of CT during pregnancy is a risk factor for preterm delivery (before 37 weeks' gestation). A total of 21 women (20%) were found to be seropositive for IgG antibodies to CT. They were similar to the seronegative women with respect to maternal age, history of preterm birth, obstetric or medical problem, smoking status, history of drug abuse, educational status and psychosocial stressors. The seropositive women were significantly more likely than the seronegative women to have a preterm birth [24% (5/21) vs. 7% (6/82); $P = 0.029$]. The positive predictive value of a seropositive result for preterm birth was 31% (five of 16); the negative predictive value of a seronegative result for preterm birth was 8% (six of 76). In conclusion, women with serologic evidence of CT may be at risk for preterm birth. Further study is required to determine whether serologic testing for CT should be a routine part of prenatal care.

P1585 Prevalence of *Chlamydia trachomatis* in Istanbul among men with urethritis

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Background: *Chlamydia trachomatis* one of the most wide sexually transmitted bacterial diseases. It is also known to cause urethritis. However only small number of studies in Turkey have investigated the causes of urethritis.
Objectives: To determine the prevalence of *C. trachomatis* among men with symptomatic urethritis in Istanbul, Turkey.
Methods: Men with diagnosis of urethritis at the Istanbul Faculty of Medicine from January to November 2003 were screened for *C. trachomatis* by Gene-Probe amplified *C. trachomatis* assay using urethral swabs.
Results: The study enrolled 720 men. The prevalence of *C. trachomatis* was 116 of 720 or 16.1%.
Conclusions: *C. trachomatis* is commonly found in men in Istanbul, Turkey. Further surveys are needed in order to determine the prevalence of other sexually transmitted pathogens in patients with urethritis in Turkey.

P1586 The prevalence of pathogenic micro-organisms in men with nongonococcal urethritis

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Objectives: The goal of the study is to find out the etiologic agents in men with nongonococcal urethritis and search the prevalence of *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Trichomonas vaginalis*.
Methods: This study incudes 200 men with nongonococcal urethritis. *U. urealyticum* and *M. hominis* were diagnosted by using Mycifast 'All-In' test, *C. trachomatis* was diagnosed by direct immunofluorescent test. *T. vaginalis* was diagnosed by the examination of wet preparations.
Results: *C. trachomatis* was isolated in 43.0%, *U. urealyticum* in 27%, *M. hominis* in 1,5%, *U. urealyticum* + *M. hominis* in 6.5%, *C. trachomatis* + *U. urealyticum* in 30.2%, *C. trachomatis* + *U. urealyticum* + *M. hominis* in 2.0%, *C. trachomatis* + *M. hominis* in 8.1%, *T. vaginalis* in 2.5% of patients.
Conclusions: We can conclude that *C. trachomatis* and *U. urealyticum* are important pathogen agents in patients with urethral discharge. Mixed infections are very common, and it is very important to have it in mind for correct and complete therapy.

P1587 Microbiological aspects of male urethritis observed from 1990 to 2002 in a central military hospital, Algiers

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Objectives: To determine the aetiology of the male urethritis observed at the Sexually Transmitted Diseases out patient department of the Central Military Hospital of Algiers. To evaluate the Gonococcal Urethritis (GU) and the Nongonococcal Urethritis (NGU) frequency. To determine also the place of *Chlamydia trachomatis* and other agents of the NGU.
Methods: The patients were addressed to our out patient department by the unit's physician of different military regions or by the university military hospital's specialists. A questioning and a clinical exam were made and a specimen executed. In the laborat-

ory, a direct optic microscopy exam was performed immediately, the specimen was afterwards isolated on the specific medium for *Neisseria gonorrhoeae* (GC agar + Polyvitex), identified with NH gallery (Biomerieux*). We searched for the betalactamase of the NGPP strains by the Cefinase test (Biomerieux*) and the susceptibility to the antibiotics of the strains of *N. gonorrhoeae* was tested by agar diffusion method according to NCCLS. The research of *C. trachomatis* was performed by DIF (direct immunofluorescence) with monoclonal antibodies and ELISA (antigen).The *Mycoplasma* were isolated on the specific medium: *Mycoplasma* DUO (Biorad*) and *Mycoplasma* IST (Biomerieux*). The other aetiological agents were isolated and identified according to the classical methods.

Results: 1705 urethral specimens were analysed in our laboratory. 1143(67%) were positive and 562 (23%) were negative. The repartition of the aetiological agents was: 384 cases (22.52%) of *C. trachomatis*, 369 cases (21.64%) of *N. gonorrhoeae*, and 140 cases (08.21%) of *Ureaplasma urealyticum*, etc. The mean NGPP incidence was 45% between 1990 and 2002, but we have observed a crescent evolution of this incidence during these 12 years (from 12.5% in 1990 to 64% in 2002). We observed also resistances of *Neisseria gonorrhoeae* to cotrimoxazol (81%) and to tetracycline (=1%).

Conclusions: These results show that from 2000, *C. trachomatis* took the place of *N. gonorrhoeae* which was until 1999 the first aetiological agent of the urethritis in our country. Besides, we have observed the decrease of the undetermined aetiology urethritis rate; this is probably due to a better diagnostic and therapeutic taking charge of this disease and the result of the strategy that was adopted since 1999 in the military medium.

P1588 Women prefer self-collection of vaginal swabs which are as effective for the diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* as urine, cervical swabs and clinician-collected vaginal swabs

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Background: *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) are easily transmitted because of high asymptomatic infection rates in women and men. Diagnostic and treatment goals are to screen sexually active men and women using noninvasive samples tested by sensitive and specific assays.

Methods: A total of 1464 women from eight geographically diverse prevalence sites in Northern America were enrolled. They consented to the collection of two cervical swabs (CS) and one clinician-collected vaginal swab (CVS). Patients also self-collected a first catch urine (FCU) and a vaginal swab (PVS) by following a simple set of illustrated instructions. Samples were tested for CT and GC by the APTIMA Combo 2 assay and APTIMA CT and APTIMA GC assays (GenProbe Incorporated, pending FDA clearance). One CS and FCU were tested by the BD ProbeTec Assay (Becton Dickenson). After vaginal swab self-collection women completed a questionnaire to determine ease or difficulty of the collection procedures. Preferences were compared by biological and behavioural factors.

Results: The prevalence of CT and GC were 12.5 and 5.4%; equally determined from PVS or CVS which yielded an equal number or more positive patients than CS or FCU by all the assays. FCU. More than 90% reported vaginal swab self-collection very easy. Preference of a PVS over a pelvic examination or urine collection was 76 and 60%. If a PVS was available to investigate sexually transmitted infections (STI), 94% indicated they would get tested more often. Factors such as age, education, English as a second language, and past experience with an STI did not influence their indicated preference.

Conclusions: This data indicates that PVS tested for CT and GC by the APTIMA assays would enable women to choose this noninvasive specimen and facilitate implementation of screening programmes.

P1589 Comparison of sequence typing and opa-typing for discrimination of *Neisseria gonorrhoeae*

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Objective: To observe the variation in genes targeted by *Neisseria gonorrhoeae* molecular typing methods by applying opa-typing and a recently developed sequence-based method (NG-MAST) to isolates from sexual contacts within known transmission chains. To assess the levels of discrimination and concordance afforded by these methods.

Methods: NG-MAST involves sequencing internal fragments (490 and 390 bp) of two highly polymorphic loci, por and tbpB, which encode an outer membrane protein porin and a component of the transferring-binding protein, respectively. Each unique sequence at por and tbpB was assigned an 'allele number' providing each gonococcal isolate with a two digit allelic profile. Each unique allelic profile was assigned as a sequence type (ST). The NG-MAST results were compared with opa-typing results. Transmission chains were constructed by identifying sexual contacts who had been mutually named at interview.

Results: A total of 28 transmission chains were identified, comprising 13 pairs, 10 chains of three, two chains of five and one chain each of four, seven and eight individuals; 85 isolates from 84 individuals were available for study. 19 of the 28 chains were known to be part of two larger clusters. NG-MAST identified 16 ST's among the isolates from patients in the transmission chains, compared with 24 opa-types. In 13 chains, ST and opa-type were totally concordant. In two chains they were predominantly concordant except for two isolates, which differed in ST between sexual contacts, but only by a single nonsynonymous substitution in por. In 10 chains, the opa-type differed between direct contacts whereas the ST remained consistent. There were three chains in which both opa-type and ST differed between sexual contacts, suggesting transmission of multiple strains.

Conclusions: These results indicate that sequencing the por and tbpB gene fragments provides somewhat less discrimination than analysing the family of opa-genes, but the former procedure is simpler and produces unambiguous data. Both NG-MAST and opa-typing are highly discriminatory typing methods but NG-MAST identifies larger clusters of indistinguishable isolates, whereas opa-typing gives a finer discrimination within the chains and may more often result in isolates from sexual contacts that appear to be distinct.

P1590 Local outbreak of ciprofloxacin-resistant *Neisseria gonorrhoeae* in Finland

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Objectives: After many years of decrease, the number of uncomplicated gonorrhoea cases increased remarkably in Päijät-Häme Hospital District (approximately 200 000 inhabitants) in Finland. The incidence rate in 2002 was 1.46/10 000 inhabitants compared with 0.44 in the whole country. A special feature of this epidemic was that it mostly consisted of ciprofloxacin-resistant isolates. As part of the epidemiological survey, we analysed the susceptibilities of *Neisseria gonorrhoeae* isolates in the years 2001–2003 in this area.

Methods: Fifty-three *N. gonorrhoeae* strains were isolated between 1 January 2001 and 11 September 2003 in the laboratory of clinical microbiology of Päijät-Häme Central Hospital. The specimens were originally cultured on selective agar plates (Oxoid, UK). The identification was based on Gram-staining and positive oxidase reaction followed by confirmation with the Accuprobe *N. gonorrhoeae* idenfication test (Gen-Probe Inc., USA). The MIC-values were determined using the agar dilution method by NCCLS for penicillin, tetracycline, ceftriaxone and ciprofloxacin. Beta-lactamase was tested using the nitrocefin disc method (Ab Biodisk, Solna, Sweden).

Results: Eleven, 34 and five strains were cultured in years 2001, 2002 and 2003, respectively. Resistance rates to penicillin, tetracycline, ceftriaxone and ciprofloxacin varied annually. In the year 2002, the highest number of ciprofloxacin-resistant isolates (85%) was detected. All ciprofloxacin-resistant strains during this 3-year period ($n = 32$) were resistant or intermediate to penicillin (27 beta-lactamase positive, five negative) and tetracycline. Seven of these (19%) showed slightly elevated MIC-values for ceftriaxone (0.5 mcg/mL). According to NCCLS guidelines these are interpreted as nonsusceptible. The first ciprofloxacin-resistant isolate was cultured in December 2001 and was probably imported from Thailand. Due to rapid increase of ciprofloxacin-resistant strains ceftriaxone replaced fluiroquinolones as the drug of choice. After November 2002 no ciprofloxacin-resistant isolates have been found. Contact tracing by interviewing the patients was unsuccessful. Molecular typing will be conducted to analyse the similarity of the *N. gonorrhoeae*-strains.
Conclusions: This observation emphasises the importance of continuous surveillance of antimicrobial susceptibility of *N. gonorrhoeae* isolates to guide the treatment. Therefore, culture should remain the basic laboratory method for the diagnosis of this disease.

P1591 Characterisation of ciprofloxacin-resistant *Neisseria gonorrhoea* isolated in Italy

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Objectives: Antimicrobial susceptibility of *Neisseria gonorrhoea* isolates was monitored as part of a pilot surveillance programme started in April 2003 in Italy. Among 76 gonococci collected in the first 7 months of this study, 21 (27.63%) resulted resistant to ciprofloxacin (CipR). The aim of this study was to characterise and to define the patterns of mutations at gyrA and parC genes in these gonococcal isolates.
Methods: Susceptibility testing was performed by *E*-test method following the manufacturer's procedures. The 21 CipR strains were further differentiated according to serovars and pulsed-field gel electrophoresis (PFGE) profile. Mutations in the two target genes were identified by PCR and direct sequencing of the amplified products.
Results: From April to November 2003, 27.63% of the isolated strains were CipR (21 of 76 *N. gonorrhoea*). Their MICs ranged from 6 to 32 mcg/mL. Six of them were isolated from patients with previous ciprofloxacin therapy. Ten of these 21 were also penicillin resistant (MIC $\geq$ 2 mcg/mL). All the 21 CipR strains, except two, belonged to serovar IB, the remaining were cross-reacting IA/IB. Three different unrelated PFGE profiles were identified. DNA sequencing showed that the most common mutations had amino acid substitutions of Ser91 to Phe, Asp95 to Gly or Ala of the gyrA and Asp86 to Asn, Ser87 to Asn or Arg, Glu91 to Lys of parC. A further mutation, Ala123 to Pro was found in one strain in the parC gene.
Conclusions: The resistance was present among different types of strains, suggesting that the incidence of CipR strains in Italy was not exclusively due to the appearance of a single clone. Cross-resistance between ciprofloxacin and the structurally unrelated penicillin was observed, as already described, in all those strains harbouring the Ser91-Phe mutation in the gyrA gene. As ciprofloxacin is recommended as the first line of therapy for gonorrhoea, the emergence of a significant resistance should be taken into account for the therapeutical choices.

P1592 Antimicrobial resistance of *Neisseria gonorrhoeae*, and emerging fluoroquinolone resistance in Russia

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Background: *Neisseria gonorrhoea* is adept at developing mechanisms of resistance to antimicrobial agents, and there is a continuing need for information on antimicrobial susceptibility patterns. Here we describe the susceptibility of 52 *N. gonorrhoea* isolates to five antibiotics of main groups which using in treatment of gonorrhoea in Russia such as: ciprofloxacin, penicillin, ceftriaxone, erythromycin and tetracycline.
Methods: Isolates of *N. gonorrhoea* were obtained from mail patients with acute urethritis who visited three sexual transmitted disease clinics in Moscow from December 2002 through May 2003. The susceptibility to antibiotics was determined by the agar dilution method using GC-II agar base with IsoVitaleX supplement and twofold dilutions of antibiotics according to NCCLS. For quality control used N. gonorrhoea ATCC 49226. Beta-lactamase production was tested by using nitrocefin disks (Cefinase; BBL Microbiology Systems).
Results: Only one strain of *N. gonorrhoea* was β-lactamase positive, though only 10% of isolates was sensitive to penicillin (MIC90, 8 μg/mL). Resistance to tetracycline was slightly lower, with 23% of strains were sensitive, and 64% of strains were intermediate resistant (MIC = 0.5–1 μg/mL). The most active was ceftriaxone (MIC90 < 0.125 μg/mL) all *N. gonorrhoea* isolates were susceptible. Unexpected result was detection high level resistance to fluoroquinolone, with 56% of isolates being resistant (MIC90 = 32 μg/mL). A 75% of strains were susceptible to erythromycin, and 25% being intermediate resistant (MIC90 = 1 μg/mL).
Conclusions: This study indicates that high level resistance to penicillin in all probability relate with chromosomal resistance, and fluoroquinolone resistance in *Neisseria gonorrhoea* is emerged in Russia. Ceftriaxone and erythromycin were the most active drugs.

P1593 Susceptibilities of *Neisseria gonorrhoea* and *Ureaplasma urealyticum* isolates from male patients with urethritis to several antibiotics including telithromycin

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Objectives: Despite the reports of antibiotic resistance in *Neisseria gonorrhoea* and *Ureaplasma urealyticum* all over the world, there are very few studies for antibiotic susceptibility of these pathogens in Turkey. The goal of the study was to determine the antibiotic susceptibilities of *N. gonorrhoea* and *U. urealyticum* strains isolated in Istanbul.
Methods: The MICs of telithromycin, azithromycin, clarithromycin, erythromycin, gemifloxacin, moxifloxacin, levofloxacin, ciprofloxacin, ofloxacin, norfloxacin, doxycycline and tetracycline against 78 *N. gonorrhoea* and 31 *U. urealyticum* strains which were isolated as causes of urethritis were determined and compared. Additionally, the activities of penicillin and ceftriaxone against *N. gonorrhoea* strains were explored.
Results: The susceptibility rates for penicillin and tetracycline in *N. gonorrhoea* strains were 35.9 and 24.3%, respectively. All gonococcal strains were susceptible to ceftriaxone with very low MICs (MIC90 = 0.008 mg/mL). Quinolone resistance was detected in a single strain of *N. gonorrhoea* (1.3%). Ciprofloxacin was the most active quinolone against *N. gonorrhoea* (MIC90 = 0.008 mg/mL) while gemifloxacin and moxifloxacin were the most active against *U. urealyticum* (MIC90 = 0.25 mg/mL) and the quinolone-resistant *N. gonorrhoea* strain (MICs = 0.25 mg/mL). Telithromycin was highly active against *N. gonorrhoea* and *U. urealyticum* strains (MIC90 = 0.25 mg/mL and 0.12 mg/mL, respectively).
Conclusions: Due to the very low susceptibility rates of tetracycline and penicillin, they should not be the option in empirical treatment of *N. gonorrhoea* infections. There is still no resistance or intermediate susceptibility or even decreased susceptibility against ceftriaxone among gonococci, and ceftriaxone continues to be the first choice antibiotic for treatment of gonococcal urethritis. For co-infections due to *N. gonorrhoea* and *U. urealyticum*, the ketolide telithromycin may be used as a treatment alternative according to our *in vitro* results. This finding has to be supported by further clinical studies. Within the quinolones, ciprofloxacin is still the most effective against *N. gonorrhoea*.

P1594 Occurrence of *Ureaplasma urealyticum* and *Mycoplasma hominis* among immigrant prostitutes and nonselected patients

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Naples, I

Objectives: *Ureaplasma urealyticum* and *Mycoplasma hominis* are frequently isolated by uro-genital tract being associated with different clinical syndromes. They assume a particular epidemiological importance in prostitutes due to their possible sexual transmission. We evaluate the *U. urealyticum* and *M. hominis* prevalence and its possible relationship to behavioural risk factors by a prospective study carried on immigrant prostitutes from sub-Saharian area and nonselected patients with clinical signs of uro-genital infection.
Patients and Methods: Between 1999 and 2002, 195 immigrant prostitutes (average age 26 years) and 227 nonselected patients resident in Naples (average age 35.2 years) underwent a microbiological investigation to detect possible presence of mycoplasms in endocervical swabs possibly associated with other bacterial pathogens. Mycoplasmas' identification, colony count and *in vitro* susceptibility tests to erythromycin (E), josamycin, tetracycline, doxicicline and ofloxacin (OFX) was performed by means of Mycoplasma IST kit (bio-Mérieux); identification of other micro-organisms was based on morphological features and biochemical tests (API system, bio-Mérieux).
Results: Overall, positive samples (>104 UCC/mL) were detected in 100 of 195 (51.3%) and in 75 of 227 (33%) of immigrant prostitutes and nonselected patients, respectively. *U. urealyticum* was isolated in 78 of 100 (78%) of immigrant prostitutes and in 71 of 75 (94.6%) of nonselected patients. The contemporary detection of *U. urealyticum* and *M. hominis* was noted in seven (7%) immigrant prostitutes and in three (4%) nonselected patients. Association of mycoplasms with *Gardnerella vaginalis* was observed in 35% of immigrant prostitutes and 3% of nonselected patients. In the immigrant prostitutes group, detection of mycoplasms was related to the number of daily sexual partners and to the condom use: a number of partners < or >5 resulted in 4 and 30% of positivity, respectively; condom use/not use =3.7%/16% positivity. Susceptibility pattern of mycoplasms ranged between <50% (E, OFX) and >94% (other drugs).
Conclusions: The incidence of mycoplasms in immigrant prostitutes is higher than nonselected patients and correlated to a number of well-defined risk factors. The rate of resistance to conventional drugs remains low.

P1595 Serological screening for sexually transmitted diseases among immigrants in Italy

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Objectives: Immigration has reached big proportions in Western Countries. The poor economic and social conditions of immigrants together with difficulties to access to healthcare institutions and proper sanitary information constitute enormous risk to acquire and/or to transmit sexually transmitted diseases. Within a project aimed to make easy the immigrants' access to social sanitary facilities and to educational and sanitary prevention interventions, we carried on an epidemiological survey among immigrants to evaluate the incidence of the main sexually transmitted diseases in order to realise proper educational programs directed to sexually transmitted diseases prevention.
Patients and Methods: In the period between October 1999 and January 2003, 976 subjects (M/F: 357/605; average age 27 years, range 18–54), referring to the Volunteers Association 'J.E. Masslo', underwent a serological screening directed to detect the following markers: anti-HIV, HBsAg, anti-HCV and MHA-TP. Informed consent was obtained prior performing tests. Most of subjects came from Nigeria (61.4%), the remaining from other sub-Saharian countries and Eastern Europe.
Results: Overall, 161 (16.5%) subjects resulted positive for one serological marker: Forty-one of the 83 positive females (49.4%) were prostitutes.

HIV+ve:	54 (33.5%);	M/F:14/40
HbsSAg +ve:	58 (36%);	M/F:36/22
HCV+ve:	25 (15.5%);	M/F:17/8
MHA-TP+ve:	24 (15%);	M/F:17/7
Fourteen (1.4%) subjects resulted contemporary positive for two markers:		
HIV-HBsAg+ve:	8 (57.1%);	M/F:3/5
HIV-HCV+ve:	1 (7.1%);	M/F:1/0
HIV-MHA-TP+ve:	1 (7.1%);	M/F:1/0
HBsAg-HCV+ve:	1 (7.1%);	M/F:1/0
HBsAg-MHA-TP+ve:	3 (21.4%);	M/F:2/1

Conclusions: The positivity rate of serological markers for sexually transmitted diseases is significantly high among immigrants; prostitution and related factors represent the major risk in this population. Appropriate educational campaigns are mandatory in order to reduce the sexually transmitted diseases transmission.

P1596 Updated profile of infections in premature deliveries in the obstetrics department of a medical university, Gdansk

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Introduction: In the majority of premature deliveries, particularly those ended before 28 Hbd, bacterial infection of a fetal egg is involved. Premature delivery and premature fetal membrane breaking are frequently connected with bacterial vaginosis, UTI, gonococcal and streptococcal cervical infection. However the role of infections caused by *Chlamydia*, *Mycoplasma*, *Ureaplasma*, *Trichomonas* and *Candida* is not fully understood. Recently *Mycoplasma* or *Ureaplasma* or both were isolated from vaginitis as the only potential pathogens responsible for premature delivery.
Objectives: The aim of this work was the epidemiological, microbiologic and clinical characteristics of infections connected with premature deliveries and premature fetal membrane breaking.
Methods: Eighty-seven pregnant patients with premature delivery symptoms and preterm rupture of oocystic membrane were examined and cervical swabs were taken. Cultures and identification were performed by standard methods. For isolation and identification of *Mycoplasma* – *Mycoplasma* Duo tests and A7 Agar (bio-Merieux) were used.
Results: Forty-six patients (53%) had positive cultures. *Escherichia coli* was recovered from 21 cases (46%). In 18 cases *Ureaplasma urealyticum* (39%), three – *Mycoplasma hominis* (0.6%), 11 – *Gardnerella vaginalis* (24%), 11 – *S. epidermidis* (24%), seven – *E. faecalis* (15%) and seven – *Candida albicans* were found. *S. aureus*, *S. agalactiae*, *P. mirabilis*, *Corynebacterium* sp., *Prevotella bivia*, *K. pneumoniae*, *Peptostreptococcus magnus* were isolated in monomicrobial cultures. *M. hominis* or *U. urealyticum* were detected in eight patients as the only potential pathogens.
Conclusions: A 53% of women hospitalised because of imminent premature delivery had endocervical infection. The most frequent pathogen was *E. coli*. Significant number of *Ureaplasma* and *Mycoplasma* infections were detected.

P1597 Incidence of simultaneous chlamydial and *Mycoplasma* infection in women of reproductive age

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Objectives: To determine the incidence of simultaneous chlamydial and *Mycoplasma* infection in women of reproductive age in order to avoid problems influencing conception.

Methods: A total of 1700 cervical and vaginal smears of women in reproductive age who attended the Outpatient Dpt. of our Hospital were tested for the presence of *Chlamydia trachomatis* and *Mycoplasma*. The detection of *C. trachomatis* was performed using the ligase chain reaction method (Lcx, ABBOTT) and *Mycoplasma* identification and sensitivity test was performed using the Mycofast Screening Evolution 2 kit (International Microbio, France).
Results: Of 1700 specimens a percentage of 3.88% were positive for *C. trachomatis*, 24.28% were positive for *Mycoplasma* (21.49% *U. urealyticum*, 2.78% *M. hominis*). Of 66 *Chlamydia*-positive specimens 18 were positive for *Mycoplasma* (27.27%). *M. hominis* isolates showed resistance to Roxithromycin in a percentage of 85.9% and to Ofloxacine 24.5%. *U. urealyticum* isolates showed resistance to Roxithromycine in a percentage of 7.8% and to Ofloxacine 4.3%.
Conclusions: The incidence of simultaneous chlamydial and *Mycoplasma* infection among *Chlamydia* positive patients is high (27.27%). The prevalence of genital *Mycoplasma* is high (24.28%) and the prevalence of resistance to antimicrobial agents is considerable especially for *M. hominis*. Simultaneous chlamydial and *Mycoplasma* infection could be a problem influencing fertility.

P1598 Prevalence of *Mycoplasma* in female genital tract and susceptibility to antimicrobial agents

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Objectives: To determine the prevalence of *Mycoplasma hominis* and *Ureaplasma urealyticum* in vaginal smears of women of reproductive age and their susceptibility to antimicrobial agents.
Methods: A total of 2047 vaginal smears of women in reproductive age who attended the Outpatient Dpt. of our Hospital were tested for the presence of *M. hominis* and *U. urealyticum*. The identification of *Mycoplasma* and sensitivity test were performed using the Mycofast Screening Evolution 2 kit (International Microbio, France).
Results: Of 2047 specimens 497 (24.28%) were positive for *Mycoplasma*. A total of 440 specimens (21.49%) were positive for *U. urealyticum*. From 440 *U. urealyticum*-positive specimens 111 gave positive results in 1/1000 dilution, 127 in 1/10 000 dilution and 202 in 1/100 000 dilution. A total of 57 specimens (2.78%) were positive for *M. hominis* in 1/10 000 dilution. In 48 cases (9.66%) both *U. urealyticum* and *M. hominis* were identified. Among 440 *U. urealyticum* isolates 34 were resistant to Roxithromycin (7.73%), 19 to Ofloxacin (4.31%) and five to Doxycycline (1.13%). Among 57 *M. hominis* isolates 49 were resistant to Roxithromycin (85.96%), 14 to Ofloxacine (24.56%) and two to Doxycycline (3.5%). In 48 cases of simultaneous *U. urealyticum* and *M. hominis* infection resistance to Roxithromycine was 64.5%, to Ofloxacine 18.45% and to Doxycycline 2%.
Conclusions: The incidence of *Mycoplasma* infection in genital tract of women in reproductive age is relatively high (24.28%). Resistance to antimicrobials is high especially for *M. hominis* and could be a therapeutic problem influencing conception and leading to infertility.

P1599 Incidence and *in vitro* sensitivity of genital mycoplasmas to antibiotics

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Objective: The aim of this study was to evaluate the prevalence of genital mycoplasmas, *U. urealyticum* (U.u) and *M. hominis* (M.h), and their antimicrobial susceptibility to six antibiotics [tetracycline (TE), pefloxacin (PEF), ofloxacin (OFX), doxycycline (DO), erythromycin (E), clarithromycin (CLA).
Material and Methods: Vaginal specimens were collected from 967 women (742 natives, 225 foreigners), aged between 16 and 72 years, attending the outpatients' department of gynaecology in our hospital during a 7-month period. Each sample was cultured onto a A7 agar plate and incubated at 37°C for 48 h. The enumeration and susceptibility testing of genital mycoplasmas were determined by use of a commercially available system (*Mycoplasma* system-Liofilchem s.r.l, Italy).
Results: Of 967 women examined, 427 (44.15%) were positive for *M. hominis* and/or *U. urealyticum*. In particular, 359 (84%) yielded isolates of U.u (259 natives, 100 foreigners), seven (1.6%) of M.h (six natives, one foreigner) while 61 (14.3%) presented both species (49 natives, 12 foreigners). The prevalence of U.u among native (37.32%) and foreign (46.95%) women was statistically significant ($P < 0.001$). Almost all strains of U.u were sensitive to DO (98.32%) and PEF (98.05%). Resistance was found to OFX (2.52%), TE (2.24%) and E (1.68%). All strains of M.h were susceptible to TE and fully resistant to E and CLA. Among the 61 patients who were colonised by both species, the most frequent patterns of resistance were as follows: 25 isolates resistant to E and CLA with intermediate resistance to OFX, 18 resistant to E and CLA, four showed resistance to E with intermediate resistance to OFX and CLA and three were resistant to E with intermediate resistance to CLA. It is noticeable that one strain isolated from a patient with mixed infection was fully resistant to OFX, PEF, E, CLA and intermediate resistant to DO and TE.
Conclusions: (1) Not one isolate studied was resistant to all antibiotics tested. Nevertheless, resistance to more than one antibiotic was quite frequent, especially when both genital mycoplasmas were present, (2) The frequency of acquired resistances does not justify modifications in the usual treatment of genital mycoplasma infections.

P1600 Genome differences and detection of pathogenic *Treponema pallidum* subspecies

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Objectives: Genus *Treponema* includes several pathogenic spirochetes (e.g. *Treponema pallidum* subsp. *pallidum* is the causative agent of syphilis, *T. pallidum* subsp. *pertenue* causes yaws). Today's serological tests are negative in early stages of treponemal infection and cannot distinguish between syphilis and yaws. Identification of chromosomal sequences specific for these pathogens can be used for selective PCR diagnostics of seropositive samples of sera from patients suspect of early treponemal infection and for discrimination of pallidum and pertenue subspecies.
Methods: DNA-microarray hybridisation of fluorescent-labelled cDNA obtained by direct labelling of treponemal chromosomal DNA was performed. 31 genes of three strains of *T. pallidum* subsp. *pertenue* have been sequenced. Diagnostics of individual strains is based on PCR reaction.
Results: Chromosomal DNA of *T. pallidum* subsp. *pallidum* strain Nichols was compared with genomic DNA isolated from three different strains of *T. pallidum* subsp. *pertenue* (strain Gauthier, Samoan D, CDC-2). No significant changes have been detected. However, 31 genes with highest/lowest and equal pallidum/pertenue ratio have been selected and sequenced. Altogether, 31 166 bp (2.74% of the genome size) were analysed. No region of extensive sequence heterogeneity was detected. However, 19 different single nucleotide polymorphisms (SNPs) were identified: four SNPs in Gauthier strain, 18 in Samoan D and 19 SNPs in CDC-2. Twelve (of 19) SNPs cause amino acid changes. Characteristic pattern of SNPs for each strain allowed PCR specific detection of these sequence changes. We developed PCR diagnostics to distinguish pallidum strains from pertenue strains and to unambiguously detect pertenue strains. This PCR test is based on a primer 3′-terminal nucleotide perfect match requirement. Two parallel reactions are performed for each exclusive SNP using two primers varying at 3′-end nucleotide position.
Conclusions: Comparative genomics and sequencing of selected genes of closely related treponemes causing different diseases allowed to design sequence specific diagnostic test. To further

prove this diagnostic amplification, this test will be performed on sera samples from seropositive patients.

P1601 Comparative genomics of *Treponema pallidum* ssp. *pallidum* and *Treponema paraluiscuniculi*

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Objectives: Genomes of closely related treponemes, *Treponema pallidum* ssp. *pallidum* (Nichols) and *Treponema paraluiscuniculi* were compared using several techniques of genomics and molecular biology. *T. pallidum* ssp. *pallidum* is the causative agent of a highly invasive human disease syphilis whereas *T. paraluiscuniculi* is not pathogenic to humans.
Methods: DNA microarray technology has been utilised to compare genomic DNA of both treponemes. Microarrays containing all 1039 annotated ORFs of *T. pallidum* subspecies *pallidum* (Nichols) were used. This approach identified genes that are missing or have heterologous sequence in *T. paraluiscuniculi*. To identify genes that are present in addition to gene set of *T. pallidum* ssp. *pallidum*, whole genome fingerprinting has been performed. Moreover, gene expression of 84 *T. pallidum* and *T. paraluiscuniculi* genes has been monitored using real-time RT-PCR approach. Over 60 short chromosomal regions of *T. paraluiscuniculi* have been sequenced to estimate the frequency of nucleotide changes when compared with genome sequence of *T. pallidum* ssp. *pallidum*.
Results: After labelling of chromosomal DNA using random hexamers and hybridisation to the DNA microarray, at least 10 *T. paraluiscuniculi* genes showed significantly lower signals when compared with signals of *T. pallidum* ssp. *pallidum*. These genes were clustered at least in five chromosomal regions in vicinity of tpr regions and code for hypothetical proteins with unknown function. PCR amplification and subsequent restriction analysis of 61% of the *T. paraluiscuniculi* and *T. pallidum* ssp. *pallidum* genomes revealed difference in nine *Bam*H I target sites and four deletions/insertions in *T. paraluiscuniculi* chromosome. Among 84 genes tested with real-time RT-PCR, 12 showed significant differences in gene expression. In general, genes encoding proteins involved in electron transport were less expressed in *T. paraluiscuniculi* and higher expression was observed in genes encoding Tpr proteins. Sequencing of more than 9.7 kb of *T. paraluiscuniculi* genome in short regions dispersed throughout the chromosome revealed, on an average, one nucleotide change per 183 bp.
Conclusions: Observed differences in genome content and composition provide insights into the different pathogenic potential of both treponemes and help to define genetic basis of syphilis.

P1602 An outbreak of primary and secondary syphilis among HIV seropositive patients in Korea

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Objectives: This study was performed to characterise the epidemiology and clinical features of an outbreak of syphilis among HIV sero-positive patients in Korea.
Methods: A retrospective case review of patients diagnosed with primary and secondary syphilis in the period July 1998 to September 2003 was carried out at Seoul National University Hospital in Korea. To estimate incidence, person-years (PYs) of all HIV seropositive patients who visited the hospital in the same period were also calculated every 6 months.
Results: In a 51-month period, 465 HIV-positive patients were followed up at Seoul National University Hospital. 38 cases of primary and secondary syphilis were diagnosed; two cases were primary syphilis and the other 36 cases were secondary syphilis. All cases were men. The incidence of primary and secondary syphilis was 4.10 per 100 PYs during the period. There was no case during July 1998 to December 2001, and then the incidence has risen till September 2003 (5.55 per 100 PYs to 18.80 per 100 PYs). The rate of primary and secondary syphilis was 4.32 times higher among homosexual and bisexual men than heterosexual men (95% CI 1.87–11.17), and 10.92 times higher among patients who did not receive HAART than patients who were receiving HAART (95% CI 5.47–21.79). The incidence of primary and secondary syphilis among patients who were receiving HAART has risen continuously (0.86 per 100 PYs to 15.73 per 100 PYs).
Conclusions: The outbreak of primary and secondary syphilis among HIV-positive patients started in 2002 and has been escalating, especially among homosexual/bisexual men and patients who did not receive HAART.

P1603 Incidence of syphilis in AREA 2 of Madrid during 4 years: a retrospective study (2000–2003)

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Syphilis is a sexual transmitted infectious disease caused by *Treponema pallidum*. This venereal pathology is successfully treated with penicillin but its incidence has increased during the last decade.
Objectives: The aim of this study was to determine the incidence of syphilis diagnosed by serological tests in the Serology laboratory at the Microbiology Department of the H. U. La Princesa in Madrid.
Methods: The diagnosis of syphilis was made using treponemal tests of microhaemagglutination assay for antibodies (MHA-TP) and fluorescent treponemal antibody absorption (FTA-ABS) and nontreponemal test (regain) based on flocculation (VDRL). Sex and nationality of patients were studied. Provenience of serum samples was also searched.
Results: A total of 462 cases of syphilis were found (102, 22.1% during 2000; 91, 19.7% during 2001; 140, 30.3% during 2002 and 129, 27.9% during 2003). 169 (36.6%) were females and 293 (63.4%) males. 417 (90.2%) were Spanish and 45 (9.8%) were foreigners. MHA-TP results were: 342 (74.1%) positives, nine (1.9%) doubtful results, 53 (11.5%) weak positives and 58 (12.5%) negatives. FTA-ABS results were: 110 (23.8%) positive++ and 352 (76.2%) positive+++. VDRL results were: 186 (40.2%) positives and 276 (59.8%) negatives. 228 (49.3%) of the serum samples proceeded from patients attended at hospital, mainly from Departments of Infectious Diseases (78; 34.2%), Dermatology (49; 21.5%), Neurology (41; 17.9%), Psychiatry (14; 6.1%), Internal Medicine (12; 5.2%), Gastroenterology (11; 4.8%), Rheumatology (7; 3.1%) and Ophthalmology (6; 2.6%).
Conclusions: (1) The incidence of syphilis in Madrid has increased during the last years. (2) Syphilis affected more frequently males (63.4%). (3) Approximately 10% of the positive samples belonged to foreigner patients. (4) 40.2% of the cases were secondary syphilis.

P1604 Epidemiology of syphilis in a hospital in Tunis

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Syphilis is an infectious disease sexually transmitted with obligatory declaration and whose pathogenic agent is *Treponema pallidum*. It appears clinically by a succession of phases during which the *Treponema* can be highlighted by direct or indirect methods. The existence of quiet clinical phases during which the diagnosis is not currently possible that by test serologic one of the major difficulties of the tracking of syphilis constitutes. Before accusing a psychiatric cause, we carry out a research of the neurosyphilis systematically. Indeed, this can associate a neurological syndrome being able to lead the complete physical forfeiture or a driving psychiatric syndrome in a true irrational state. Our work concerned 8251 serums and 134 LCR (spinal puncture) coming from patients addressed to the psychiatric hospital of Razi from January 1996 to December 2003 for serology of syphilis and neurosyphilis. Half of the patients

had an age ranging between 40 and 60 years, 70% were unmarried and 80% were without job. Our syphilitic serology comprised each time two reactions: agglutination (VDRL coal) and specific passive haemagglutination (TPHA). The results showed a prevalence of the disease syphilitic serum and neurological at the man (65–70%) what is in conformity with the literature. The prevalence of serum syphilis is higher in the age breaked ranging between 20 and 45 years; it should be counted a few years before the neurosyphilis is not declared. 60–80% of our patients are unmarried (reflexion of a great freedom of manners) and without job; a better social integration could make regress the disease. We noticed that the prevalence decreased as for syphilitic serology (it passed from 7.6% in the 5 years of 1986–1990 and 4.7% of 1991–1995 than finally to 2.9% currently). On the contrary, the prevalence of the neurosyphilis appreciably increased passing from 1.6 to 3.47% in 10 years. The efforts provided in regards national fight against the MST thus seem to give results concluding, although cases of neurosyphilis continue to be reported.

P1605 Sexually transmitted HAV

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Objectives: To determine epidemiological characteristic for HAV infection admission in two Infectious Diseases Department in Italy.
Methods: Review of clinical records for epidemiology and risk factors for patients with HAV infection admitted from January 2000 to November 2003 in the I and II Department of Infectious Diseases, L. Sacco Hospital (Milan, Italy).
Results: In the considered period 71/4325 (1.64%) patients have been admitted for HAV infection (mean age 33.88 $\pm$ 8.517). The rate of admission rose from 1.48% in 2000 to 2.9% in 2003 ($P = 0.0013$). HAV infection among travellers to highly endemic countries decreased from 31.25% in 2000 to 23.33% in 2003. In the same period the male/female ratio raised from 1.28 to 14.0 ($P = 0.0165$). HAV acquired as a food-borne disease was observed in 43 of 71 (60.56%) patients while in 25 of 71 (35.21%) HAV infection was probably acquired through sexual exposure. A cluster of HAV infection among MSM was indeed observed during 2003 with a sharp increase both in admission for HAV infection and sexual exposure. In the last year admission for HAV infection was observed in 19 MSM among 28 total male admissions for HAV (67.85%).
Conclusions: In Italy HAV infection declined in the last decades for the improvement of social-economic and hygienic conditions leaving a growing percentage of young adults unprotected against HAV infection. Vaccine protection for travellers directed toward highly endemic countries is becoming a relatively common practice which can protect against the great majority of HAV infection acquired through the oral route, reported in 65.2% of the national cases (SEIEVA 2002) and in 60.56% in our report. In young unprotected MSM HAV must be considered, as a sexually transmitted disease and the need of HAV vaccine within this risk group must be evaluated for the potential severe evolution of this disease in adults.

P1606 Prevalence of sexually transmitted viruses in Polish commercial sex workers including HIV-seropositive individuals

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Objective: Patients infected with the human immunodeficiency virus (HIV) are often co-infected with other viruses, especially hepatitis viruses. Sera of patients with sexually transmitted diseases (syphilis, gonorrhoea, chlamydiosis, HPV, and HIV infections) and sera from healthy blood donors were investigated for presence of hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis G virus (GBV-C/HGV) transfusion transmitted virus (TTV). High prevalence of TTV infection recently has been reported among blood donors and patients with liver disease. However, as with GBV-C/HGV, the disease potential of TTV and its principal modes of transmission are unclear. To assess possible sexual transmission, of TTV we examined the prevalence of TTV viraemia among female sexual workers in Warsaw. To demonstrate exposure by this route all subjects were simultaneously examined for infections associated with risk behaviours HBV, HCV, HGV/GBV-C.
Methods: Presence of HBV, HCV, HGV and TTV was analysed in plasma samples from 20 HIV1-infected commercial sex workers (CSWs), from 20 HIV-1 uninfected CSWs and from 40 the blood donors. The prevalence of HBV, HCV, HGV, TTV was estimated on the basis of polymerase chain reaction (PCR).
Results: The prevalence of HBV was 0% in blood donors and HIV-1 negative CSWs and 15% in HIV-1-positive CSWs. The prevalence of HCV was 2,5% in blood donors, in HIV-1 negative CSWs was 55 and 40% in HIV-1-positive CSWs. The prevalence of HGV was in these groups is 0, 0 and 5%, respectively. The prevalence of TTV exposure in the STD clinic was statistically significant higher. In a comparison of the subjects with STD vs. those without STD, the prevalence of TTV was, 90% in HIV-1-positive and 65% in HIV-negative CSWs vs. 55% on the basis of PCR results. There was a statistically significant higher prevalence of hepatitis B virus, hepatitis C virus and HGV/GBV-C, exposure in the STD clinic compared with the group who never had received treatment for a STD.
Conclusions: The accumulated evidence indicate that hepatitis C virus can be transmitted by sexual contact but much less efficiently than other sexually transmitted viruses, including transmitted transfusion virus. We suggest that TTV infection have a diverse route of transmission. The prevalence rate of TTV is very high in certain risk groups.

P1607 Is systematic screening of hepatitis C virus (HCV) in sexually transmitted diseases (STD) clinics necessary?

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Developed studies about the sexual transmission of VHC show contradictory results. Although in many of them the prevalence of the infection for VHC is higher than in general population, most of the agencies do not recommend routinely detection of Anti-HCV antibodies in STD's clinics. In La Rioja (Spain), the seroprevalence rate of AntiHCV is 2%.
Objective: To evaluate the rentability of HCV systematic screening in patients who come to our hospital STD clinic.
Material and Methods: All the patients that come to STD clinic in La Rioja Hospital from January 1999 to February 2003 were studied ($n = 803$). The HCV antibodies were detected by means of a conventional ELISA test. In all the positive samples, confirmation was performed by PCR.
Results: Ten of 803 studied samples presented AntiHCV antibodies (1.24%), and in eight of them, RNA determination by PCR was positive.
Conclusions: Our results suggest that people who come to STD clinics do not represent a special risk group for the HCV infection. The systematic serologic study of HCV in this kind of clinics is not effective, as the prevalence is not higher than the prevalence in the general population.

P1608 Clinical evolution of anal and cervical HPV dysplasic lesions in HIV-1 infected patients

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Objective: To evaluate clinical evolution of genital HPV dysplasic lesions in HIV-1 positive subjects.
Methods: Comparative study on clinical evolution of anal and cervical diplasic HPV-related lesions in HIV-1 infected males and females. All HIV+ outpatients attending the STD service with genital dysplasia identified through cytology have been enrolled. High grade resolution anoscopy and colposcopy, Digene Hybrid Capture® HPV DNA test and histology, CD4 T-cell counts, HIV

RNA, antiretroviral treatments were evaluated at baseline and on follow up. Progression (P), Regression (R) and Stable persistent (SP) lesions were evaluated.
Results: Among the 143 HIV+ patients enrolled (116 F and 27 M) 112 cervical and 36 anal dysplasic lesions were observed. On enrolment 56 patients were receiving ART including PI (HAART). Baseline cervical lesions were LSIL in 94.64%, HSIL in 4.4% and negative in 0.8%. Anal lesions were LSIL in 86.11%. In five cases dysplasia was assessed trough high-resolution anoscopy. Digene test was negative in 11.60%, low risk genotypes (LR) were observed in 7.14%, high risk genotypes (HR) in 33.92% and LR/HR in 47.32%. Mean follow up in 104 (70.27%) cases was 581 days (median 378 days). Patients with cervical and anal lesions were comparable for age, baseline mean CD4 T-cell counts, viral load, CDC stage and HPV HR genotypes while higher percentage of non-HAART treatment in the cervical lesions group was recorded ($P < 0.0001$). No differences in clinical evolution were observed in relation to the baseline ART. Progression rate was 11.53%: 10.12% for cervical and 16% for anal lesions (P NS). Time to progression was shorter for anal lesions: median survival time 379 vs. 1394 days in anal and cervical lesions respectively (log rank test $P < 0.0001$). Similar results are obtained if only patients with HAART are considered.
Conclusions: The risk of invasive cancers in HIV+ patients has been related to onchogenic serotypes, persistence and co-infection with several HPV genotypes, baseline CD4 T-cell counts, plasma HIV viral load and ART. We observed a higher rate of spontaneous regression of cervical lesions and a higher progression rate, within 2 years from the diagnosis, in anal than cervical localisations in the presence of comparable baseline values of such parameters suggesting a different natural history related to the anatomical site of the lesions. More extensive studies are needed to verify such data.

P1609 Bacterial vaginosis in patients with genital HPV infection

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Objectives: Human papillomavirus (HPV), particularly HPV 16 and 18, is now recognised as the main cause of cervical cancer. It has been suggested that bacterial vaginosis and altered vaginal flora may be a risk factor for sexually transmitted diseases (STD). The aim of study was relationship between genital HPV infection and bacterial vaginosis.
Methods: This study were performed on 120 women between 21 and 42 years of age and sexually active. Vaginal flora was examined by Gram's stain, standardised scoring criteria (Nugent's score). Bacterial vaginosis was diagnosed if a score was 7–10, a score of 4–6 indicated intermediate vaginal flora and a score of 0–3 normal vaginal flora. In addition, presence of *Lactobacillus* spp. and *Gardnerella vaginalis* was confirmed by culture. HPV DNA type: 16 and 18 was detected using PCR amplification assay (amplification of DNA fragment which encoded E6 gene). Results: In the group of 120 women, 56 had bacterial vaginosis and intermediate vaginal flora, including 10 with HPV16 infection. The remaining 64 patients manifested normal vaginal flora and only 1 had signs of HPV16 infection. None of the patients had DNA HPV18.
Conclusions: The results indicate that women with genital HPV oncogenic type infections are at increased risk for BV.

P1610 Testing for oncogenic HPV in PaP liquid preservative fluid (Tripath Sure Path™) correlates with cytopathology and Digene sampler results

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Objective: Recent approval by the FDA of adjunctive screening for high risk (HR) HPV with PAP testing led us to examine (a) the utility of the Digene HCII HR HPV test on specimens collected by the Digene sampler (DS) vs. residuum from the SurePath™ liquid preservative (LP) after PAP testing (b) correlation of HR HPV test results with PAP cytopathology.
Methods: A total of 320 women consented to the collection of an LP followed by a DS. Samples from group 1 ($n = 106$) were tested unfrozen. Discordant samples were freeze-thawed, retested twice and tested by PCR. Samples from group II ($n = 214$) were freeze-thawed before testing. Patients were considered infected when positive in LP and DS, or positive in one sample two of three times. A volume of 2 mL was used for testing with 90 min denaturation.
Results: In the first group 20 were positive in both samples. Retesting 20 discordants increased the positives to 27 and suggested a benefit of freezing to reduce discordancy. PCR testing did not contribute to discordant analysis. In group II ($n = 214$) 54 positives matched and 13 were discordant. After discordant analysis 63 patients were considered positive. Combining the positives and negatives of both groups the LP detected 92% (83 of 90) and DS 84.4% (76 of 90) of the positives. Normal cytology was observed in 79.2% (182 of 230) of the patients without HR HPV compared with 37.8% (34 of 90) of the HPV infected group. The PAP results for the 58 HPV positives were two carcinomas, 13 HSIL, 31 LSIL, eight ASCUS, and five benign cellular changes (BCC). Abnormal PAP results in the HPV negative group were BCC (29 of 48) with the remainder LSIL or ASCUS.
Conclusions: HR HPV testing on LP identified most of the infected women. Infection with HR HPV correlated with cervical abnormalities.

P1611 Epstein–Barr virus (EBV) and cervix uterine cancer

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Objectives: Present data indicate that EBV may infect human epithelial as well as suggest a specific and active role of the virus in carcinogenesis. Therefore, in this study we aimed to examine whether, apart from human papillomavirus (HPV), also EBV may be associated with cervical intraepithelial neoplasia (CIN).
Methods: The studies were performed on 19 CIN-positive women, aged 21–42 years. Based on cytology screening, in 10 women mild dysplasia (CIN grade I) (subgroup 1) was diagnosed while in the remaining nine women – moderate (CIN grade II), severe dysplasia (CIN grade III) or carcinoma *in situ* (CIS) (subgroup 2). All cervical specimens were tested for the presence of EBV DNA and HPV DNA (Sharp Signal System, Digene). In addition, attempts were made to detect HPV 16 and HPV 18 using other PCR amplification techniques. In parallel, sera of all patients were tested for IgG anti-EA antibodies (ETI-EA-G, DiaSorin), IgG anti-VCA antibodies (ETI-VCA-G, DiaSorin) and IgG anti-EBNA antibodies (ETI-EBNA-G, DiaSorin).
Results: In subgroup 1 all women proved HPV negative. At the same time, in two of them presence of EBV DNA was detected. In turn, in subgroup 2 seven women proved HPV 16-positive and two cases were HPV-negative. In all women of the subgroup presence of EBV DNA was documented. Serum anti-EBV antibodies were detected In all women In both subgroups. In all the cases, the serological pattern typical for past EBV infection was documented.
Conclusions: The results indicate that EBV may be involved in cervix uterine cancer aetiopathogenesis.

P1612 Prospective study on the risk factors associated with cervical dysplasia and cervical carcinoma

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Objective: The aim of the study was to evaluate the risk factors – both infectious and noninfectious-associated with the occurrence of cervical dysplasia and cervical carcinoma.

Material and Methods: The study comprises 250 patients that addressed to gynaecologists for specialised consult, control and/or treatment. The patients have been asked to fulfil an anonymous questionnaire regarding the age of initiation of sexual activity, numbers of pregnancies and/or abortions, sexual behaviour, contraceptive use, number of sexual partners over the years, previous genital infections or other genital pathology known, data regarding smoking habits. The laboratory exams for all patients included Papanicolau tests, cervical bacterial and parasitary cultures, antibody testing for *Chlamydia trachomatis*, Herpes simplex, syphillis, hepatitis B and C and HIV.
The patients were divided in three relatively equal groups as number: group A: without significant pathological changes; group B: with cervical dysplasia in different stages; group C: with cervical carcinoma confirmed both cytologically and colposcopically.
Results: The most significant risk for the occurrence of cervical dysplasia and carcinoma was associated with: the initiation of sexual activity before 16 years of age, one or more pregnancy/abortion before 18 years of age, more than three sexual partners during lifetime, previous sexual transmitted diseases, use of empirical local contraceptives (e.g. vitamin C, quinine) for more than 5 years, low socio-economic status, smoking more than 20 cigarettes per day at the moment of diagnosis. The prevalence of infectious factors were higher in groups B and C than in group A:
– *Trichomonas vaginalis*: 62% in group B, 51% in group C, compared with 12% in group A;
– *Candida albicans*: 47% in group B, 38% in group C, compared with 14% in group A;
– Gram-negative and gram-positive cocci: 29% in group B, 8% in group C, 13% in group A;
– Enterobacteriaceae: 5% in group B, 4% in group C, 5% in group A.
– Coccobacilli: 14% in group B, 12% in group C, 9% in group A
Conclusions: The carcinogenesis of uterine cervix is a multifactorial process involving different factors that intricate and conditions each other. The factors that determine a so-called 'sexual pollution' regard the ambient, sexual habits and the contamination with bacterial, viral and parasitic germs. A primary prophylaxis of cervical carcinoma cannot be conceived without taking into consideration, all these factors.

Diagnostic methods: antibiotic susceptibility

P1613 Direct susceptibility testing of positive Bac T Alert blood culture by disk diffusion and preliminary bacterial identification

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Objectives: The procedure described by the NCCLS for susceptibility testing requires subculture of the broth on agar media. Results are available to clinicians 48 h after positive blood cultures. The outcome of patients with septicaemia depends on early treatment with appropriate antimicrobial agents. This study evaluated susceptibility results obtained by using bacterial pellets and preliminary classification of bacteria based on tube coagulase (TCT) and cytochrome oxidase test (COT). Results for COT and TCT tests are obtained after 15 min and 4.5 h, respectively and are helpful for the choice of antibiotics.
Methods: A bacterial pellet was obtained by centrifugation of an aliquot of culture broth in a serum separator tube. Bacteria were collected from the gel with a cotton swab and suspensions equivalent to 0.5, 1.0 and 2.0 Mc Farland (McF) were made. A colony count was performed in order to ascertain the proper colony density used. Susceptibility testing were done with the three inocula and directly from one drop of broth (nonstandardised inoculum :NSI) on Mueller Hinton agar, Oxacillin Screen and Vancomycin Screen plates. TCT and COT tests were performed with the bacterial pellet obtained after centrifugation. A total of 40 significant positive blood cultures were investigated (1 isolate per patient). 28 Gram-negative (408 antimicrobial agent-organism combinations-AAOC) and 12 Gram-positive (108 AAOC) isolates were analysed. A scoring system was adopted for analysis of discrepancies: 5 points for very major errors (false susceptibility – VME), 3 points for major errors (false resistance – MAE), 1 point for minor errors (MIE) and 0 point for no error.
Results: For the gram-negative isolates categorical agreements of 93.1, 95.8, 93.6 and 94.4% were obtained with the NSI, 0.5, 1.0 and 2.0 McF suspensions, respectively. VME were observed in 1.0% with (NSI, 0.5, 1.0 McF) and 1.2% (2.0 McF). MAE in 0.2% (NSI, 0.5 McF). MIE were observed in 5.6, 2.9, 5.4 and 4.4% with NSI, 0.5, 1.0, 2.0 McF inocula, respectively. For Gram-positive cocci categorical agreements were observed in 87.0% (NSI), 90.7% (0.5, 1.0, 2.0 McF). VME were observed with all suspensions in 0.9% of cases. No MAE was found. MIE were found in 12% (NSI) and 8.3% (0.5, 1.0, 2.0 McF). No discrepancies were observed for TCT and COT tests.
Conclusion: Preliminary susceptibility results could be given to clinicians 24 h earlier, however, certain results need to be confirmed by the NCCLS method.

P1614 Evaluation of the VITEK 2 system for rapid direct identification and susceptibility testing from positive blood cultures

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Objectives: We aimed to evaluate the accuracy of direct inoculation of VITEK2 cards from positive blood culture bottles to achieve rapid bacterial identification (ID) and antibiotic susceptibility testing (AST).
Methods: Positive BacT/Alert blood cultures sampled between 05/2002 and 11/2003 were included. Direct inoculation of bacterial suspensions to VITEK 2 ID and AST cards was made by differential centrifugation of blood cultures. The types of VITEK 2 ID and AST cards inoculated (Gram-negative, staphylococci, streptococci/enterococci) were selected according to the morphology of the organisms on direct Gram-stain. Results of the rapid method were compared with those of the routine methods based on API identification systems and AST by the Kirby-Bauer disc diffusion method according to NCCLS guidelines.
Results: A total of 166 clinically relevant isolates were investigated. Overall, 103 (62%) were correctly identified to the species level. Sixty-three (38%) strains could not be identified due to various reasons (haemolysis, insufficient bacterial pellet, residual traces of charcoal) but no strain was misidentified. The rapid method proved mostly accurate for ID of Enterobacteriaceae (EB) [80/91 (87%) and enterococci (8/10) but not for Gram-negative nonfermenters (GNNF) nor for streptococci and staphylococci (only 20–40% correct ID). Direct susceptibility performed for 103 strains (80 EB, 10 GNNF, 13 staphylococci) and including 1210 antibiotic-isolate combinations gave an overall essential agreement of 93%. Most discordances corresponded to minor errors (4.5%) while major (S by VITEK2, R by routine testing) and very major errors (R by VITEK2, S by routine testing) were found in 0.7 and 1.5% of all organism-antibiotic combinations, respectively. The rates of agreement between the rapid and the conventional methods ranged from 86 to 100% depending on the antibiotics, but there were no clustering of discordances for specific antibiotic-

isolate combinations. The reporting time for the direct testing of susceptibility against the 13 antibiotics for 103 blood culture isolates by the VITEK2 system ranged from 4.5 to 18.1 h.
Conclusion: Direct inoculation is directly applicable as routine method for ID and AST of EB and enterococci, but not for GNNF, staphylococci or streptococci. Compared with conventional methods that require 1 or 2 days, this method can make same-day reporting possible and it may thus permit better patient management.

P1615 Antibiotic susceptibility test on pathogens isolated in ICU using the Uro-Quick system

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Objectives: The Uro-Quick, an automatic instrument widely used for the screening of bacteriuria, was previously employed to detect antibiotic resistance in well-characterised bacterial strains (*J Chemother* **16**, 2004 in press) and in uropathogens. In this study pathogens isolated in ICU of a great Italian hospital during the period September–November 2003 were examined employing the Kirby–Bauer technique for antibiotic susceptibility tests and this usual system was compared with the new rapid Uro-Quick method.
Methods: Antibiotic (in appropriate concentration) was introduced in a vial containing 2 mL of Mueller–Hinton (MH) broth, then 0.5 ml of broth containing 5×10^5–10^6 cells/mL of the strain to test were added in each vial containing the antimicrobial molecules and even in a drug-free vial as control. After 3 and 5 h of incubation (Gram-negative or Gram-positive strains, respectively) the instrument printed the results: no growth and a growth curve like the control is representative of a susceptible and resistant strain respectively. Gram negative strains were tested against ciprofloxacin (CIP), ampicillin (AM), aztreonam (ATM), co-clavulanate (AMC), piperacillin/tazobactam (TZP), ceftazidime (CAZ), cefotaxime (CTX), cefuroxim (CXM), ceftriaxone (CRO), imipenem (IPM), amikacin (AN), gentamycin (GM) and trimethoprim–sulphamethoxazole (SXT), while Gram-positive bacteria against CIP, clindamycin (CM), erythromycin (E), IPM, linezolid (LZD), AM or penicillin (P), rifampicine (RA) GM, streptomycin (S), tetracycline (TE), vancomycin (VA), oxacillin (OXA) and SXT.
Results: The Gram-negative strains tested were 121 and the Gram-positive 144, agreement between the two methods was 93% against Gram-negative (SXT and CTX 98.3%, CIP 97.5%, GM 96.7%, CRO 96%, AN and CAZ 95%, IPM 94%, AM 92%, CXM and AMC 90%, TZP 88%, ATM 84%) and 95% against Gram-positive (AM 100%, LZD 99%, CM, IPM, VA and S 97%, CIP, RA, TE and SXT 95%, E 92%, OXA and P 94%, GM 88.4%) pathogens.
Conclusion: Against major pathogens (staphylococci, enterococci and Enterobacteriaceae) agreement between the Uro-Quick system and the Kirby–Bauer method was always more than 90%. On the basis of the present findings the rapid method appears useful in severe nosocomial infections because the rapid detection (in 3 or 5 h) of antibiotic susceptibility allows a more direct treatment, reduce the empiric therapy and the diffusion of resistant pathogens.

P1616 Comparison of Phoenix, Vitek and Vitek 2 automated identification and susceptibility testing systems

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Objectives: To compare identification (ID) and susceptibility testing results (AST) obtained with the Becton Dickinson PHOENIX System (PH) and the BioMérieux VITEK (V1) and VITEK2 (V2) systems in tests on a challenge set of organisms.
Methods: Strains tested were 125 *S. aureus* (SA), 84 coagulase-negative staphylococci (CNS), 240 Enterobacteriaceae (EN), and 107 nonfermenting Gram-negative rods (NF) comprising 78 *Pseudomonas* spp. (PS), 19 *S. maltophilia* (SM) and 10 *Acinetobacter* spp. (AC). The strains were biased towards those with resistance to various antimicrobial agents. Identification and antimicrobial susceptibility tests were run in the three systems simultaneously. Susceptibility interpretations made by the expert systems were used in the comparisons. Reference identification was by API (BioMérieux) or PCR. Susceptibility discrepancies were assessed against agar dilution MICs determined by NCCLS methods.
Results: Accuracy of identification (%) to species level by PH, V1 and V2 compared with reference identifications was, 98.4, 100 and 98.4, respectively, for SA; 73, 57, and 79 for CNS; 96.3, 95.4 and 96.3 for EN; 96.3, 95.3 and 77.6 for NF. With SA there were up to 1.6% major and 2% minor discrepancies in susceptibility categorisation between systems with 1431 organism–agent combinations tested. With CNS there were up to 9.0% major and 3.2% minor discrepancies for 831 organism-agent combinations tested – discrepancies were most common with oxacillin. For staphylococci there were fewer discrepancies between PH and V2 than between V1 and either V2 or PH. With EN there were up to 4.2% major and 15.6% minor discrepancies in susceptibility categorisation among 3080 organism–agent combinations tested. For NF there were up to 4.2% major and 8.5% minor discrepancies in susceptibility among 503 organism–agent combinations tested.
Conclusions: Identification in all systems was generally reliable for SA, EN and NF (although V2 reported some strains of *Pseudomonas* spp. and *Acinetobacter* spp. as nonfermenters). Identification of CNS to species level was less reliable, particularly with the V1 system. With this collection, which included a high proportion of resistant organisms, there was very good agreement in susceptibility categorisation between systems with SA but less so with other organisms.

P1617 The application of two disc methods and the E-test for detecting extended-spectrum beta-lactamases (ESBL) in clinical strains of *K. pneumoniae* and *E. coli*

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Objectives: The aim of this study was to compare the effectiveness of two disc methods and the *E*test applied for the detection of ESBLs.
Methods: We tested 148 clinical strains of *E. coli* and 78 strains of *K. pneumoniae*. All the strains were examined by using the following tests: double-disc synergy test (DDST) according to Jarlier (cefotaxime, ceftazidime, aztreonam and clavulanic acid), disc test according to Appleton (cefpodoxime, cefpodoxime + clavulanic acid) and also by *E*test (cefotaxime, cefotaxime + clavulanic acid and ceftazidime, ceftazidime + clavulanic acid).
Results: In the case of *K. pneumoniae*, the activity of the ESBLs was detected among 30 strains – both in Jarlier test, Appleton test and the *E*test. Among *E. coli*, four strains were found ESBL-positive in the Jarlier's test. However only three of these strains were ESBL-positive when Appleton test and *E*test were used.
Conclusions: (1) ESBLs must be routinely detected in clinical strains *E. coli* and *K. pneumoniae*. (2) All the methods used for detecting ESBL in *K. pneumoniae* strains are equally effective. (3) Two tests (Appleton test and *E*test) were found to be more useful for detecting ESBLs in *E. coli* strains.

P1618 Sensitivity and cost-effectiveness of different phenotypic methods for the detection and confirmation of extended-spectrum beta-lactamases including an inexpensive novel method

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Objectives: To evaluate the sensitivity and cost-effectiveness of extended-spectrum beta-lactamases (ESBLs) detection and confir-

mation methods in our country, including an inexpensive modification of the original Jarlier's method.
Methods: The sensitivity and cost-effectiveness of Single Disk Diffusion Test (SDDT), Inhibitor Potentiated Double Disk (IPDD), and *E*test, were evaluated in a collection of 326 ESBL-positive organisms isolated in Israel, including *E. coli*, *Klebsiella* spp., *Proteus* spp., and *Enterobacter* spp. In addition, Aproximation Test (AT), a modification of the original Jarlier's method using first and second-generation cephalosporins, was evaluated.
Results: The sensitivity of the different methods varied from 66.9% for SDDT with ceftazidime to 98.8% for *E*test with cefotaxime alone. Several combinations of tests reached a sensitivity of 100%.
Conclusions: IPDD using cefotaxime and ceftazidime, or ceftazidime and cefpodoxime proved to be the best options in our country in terms of sensitivity and cost-effectiveness. However, AT with cephalotin and cefuroxime, an inexpensive method, can be used for the first screening of ESBLs with acceptable results.

P1619 Evaluation of the Etest MBL for detecting metallo-beta-lactamases in carbapenem-resistant *Pseudomonas aeruginosa* isolates

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Objectives: Carbapenem resistance mediated by the acquired metallo-β-lactamases (MBLs) of IMP and VIM types is increasing in several countries particularly among *Pseudomonas aeruginosa* clinical isolates and limits the usefulness of beta-lactams. The purpose of this study was to evaluate the performance of the *E*test MBL, a phenotypic test commonly in use for the detection of metallo-β-lactamases in *P. aeruginosa*, in a region with a high-prevalence of MBL-producing pseudomonads.
Methods: Eighty-eight clinical isolates of *P. aeruginosa* resistant to both imipenem and meropenem isolated during 2 years (2002–2003) from separate patients in the University Hospital of Thessaly were included in this study. The susceptibility of the isolates to various antibiotics including carbapenems was examined by the disk diffusion method. Detection of the genes blaIMP and blaVIM that code for MBLs and have been described in Mediterranean regions was performed by polymerase chain reaction (PCR) using consensus primers for each gene. Finally, all isolates were tested with *E*test MBL strips according to the manufacturer (AB Biodisk, Solna, Sweden).
Results: Fifty-five of 88 isolates showed positive result for the presence of blaVIM by PCR. blaIMP genes were not detected in any isolate tested. The *E*test MBL was indicative of MBL production (EDTA potentiated imipenem activity by ?Ý 8 times) in all but two of the blaVIM positive isolates, while it was positive in additional 21 isolates with a negative PCR reaction. In two isolates Etest MBL was negative while PCR gave a specific PCR product. All isolates that exhibited MIC for both carbapenems of > 64 mg/L had an imipenem/imipenem plus EDTA ratio of >40 and carried blaVIM Cgene, while all discrepancies were observed in isolates with carbapenem MICs of 8–16 mg/L.
Conclusions: The *E*test MBL strips are a quick and easy method to perform in routine clinical laboratories. The discrepancies in the detection of low-level carbapenem-resistant isolates suggest that in such cases the use of this method may require the use of an additional assay, such as PCR.

P1620 Evaluation of BDXpert™ System for interpretation of antibiotic susceptibility testing results for *Streptococcus* species

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Objectives: Antibiotic resistance in *Streptococcus* (STR) species has increased worldwide in the past decade. The NCCLS (USA) and CA-SFM (France) publish AST standards to interpret in vitro susceptibility results. Both standards also include relevant therapy warnings and guidelines for interpreting intrinsic susceptibility/resistance and cross-resistance when appropriate. Such information can be communicated to assist clinicians in selection of optimal therapy for patients with streptococcal infections. The BDXpert System was developed for the BD Phoenix™ System and BD EpiCenter™ to interpret AST test results and communicate appropriate comments recommended by NCCLS or SFM. This study evaluates the functionality of expert rules created specifically for interpreting AST results for STR.
Methods: Comments listed in the NCCLS M100-S13 and CA-SFM Report 2003 were converted into expert rules used in the BDXpert system. Special rules were created to detect the following resistance markers: high-level penicillin resistance (HLPSP) and low-level penicillin resistance (LLPSP) in *S. pneumonia*e (SP), inducible MLSb/efflux (MEFF), constitutive MLSb (MLSb), and resistance to high-level streptomycin (HLSR), gentamicin, and kanamycin (HLKR). Actions and associated messages were implemented as specified in the standard documents. A total of 95 challenge strains possessing these resistance mechanisms were selected based on previously determined genotypic and phenotypic characteristics to evaluate the effectiveness of BDXpert rules. Reports were reviewed by at least two human experts for the accuracy of actions or messages.
Results: MIC values were interpreted correctly according to the breakpoints in the respective standard. Evaluation of 64 SP isolates using both NCCLS and SFM criteria demonstrated correct detection of HLPSP (20), LLPSP (26), MEFF (21), or MLSb (22). When SFM criteria were applied, HLSR or HLKR was also detected in 19 strains. Of 11 *S. pyogenes* strains tested, MEFF and MLSb phenotype were detected in three strains each. Expert messages identifying intrinsic resistance or communicating appropriate therapeutic warning were consistent with AST results and species.
Conclusions: The BDXpert System reliably detects and reports resistance markers in STR. Special messages, including therapy warnings, intrinsic resistance/susceptibility and cross-resistance can be used to communicate timely and accurate information to clinicians for proper antimicrobial therapy.

P1621 Evaluation of cefepime with and without clavulanic acid for the detection of extended-spectrum beta-lactamases in Enterobacteriaceae species

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Background: NCCLS confirmatory extended spectrum beta-lactamase (ESBL) test methods using ceftazidime (CAZ) and cefotaxime with and without (±) clavulanic acid (CA) are recommended only for *E. coli* (EC) and *Klebsiella* spp. (KLs). ESBLs are increasingly reported in other species of Enterobacteriaceae (EN). Detection of ESBLs in all species is critical for therapy and infection control.
Methods: We evaluated the utility of cefepime (FEP) ± 10 mcg/mL CA as an ESBL confirmatory test using TREK frozen microdilution (MB) panels with a FEP range of (0.06–128 mcg/mL) and FEP/CA range of (0.03/10–32/10 mcg/mL). Strains tested were recent US clinical isolates and included 216 ESBL producing (56 from species other than EC and KLs), and 452 non-ESBL beta-lactamase producing strains, characterised for beta-lactamase production by phenotypic, biochemical and molecular methods. A subset of isolates [*Serratia marcescens* ($n = 12$), *K. pneumoniae* ($n = 4$), and *K. oxytoca* ($n = 1$)] which produced an SHV-7 like enzyme was also tested using *E*-test FEP ± CA strips. These isolates were CAZ ± CA positive for ESBL production and negative with FEP ± CA in MB tests.
Results: The FEP ± CA combination was 88% sensitive and 87% specific in the MB tests. The FEP ± CA test detected 21 ESBLs in isolates that were highly resistant to CAZ and co-produced chromosomal or plasmid-mediated AmpC beta-lactamases. There were 59 false-positive results; mainly in EC ($n = 16$), *K. pneumoniae* ($n = 17$), *K. oxytoca* ($n = 17$) and *P. mirabilis* ($n = 4$). The *E*-test FEP ± CA strips detected 10 of the subset of 17 isolates with SHV-7 like ESBLs that were negative with FEP ± CA with MB.

Conclusions: The false-positive results in this study indicated that FEP ± CA should be used cautiously for ESBL detection in EC and KLs. Furthermore FEP ± CA was not reliable for the detection of SHV-7 like ESBLs, especially in MB tests. FEP ± CA was most useful for ESBL detection in EN that co-produce an AmpC beta-lactamase.

P1622 Evaluation of the MicroScan ESBL Plus confirmation panel for Enterobacteriaceae-producing extended-spectrum beta-lactamases

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Objectives: The increasing emergence of Enterobacteriaceae-producing extended-spectrum-beta-lactamases (ESBL) is a cause of concern and their detection is important in the laboratory. We evaluated the MicroScan ESBL Plus confirmation panel (Dade Behring, Sacramento, CA, USA) for the detection of 12 Enterobacteriaceae-producing ESBL species.
Methods: Two-hundred strains were studied: 185 were isolated from patients at our institution, 12 belonged to a well-defined previously published collection of Entrobacteriaceae with different resistance mechanisms, and three were control strains: *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603 and *P. aeruginosa* ATCC 27853. A total of 168 isolates produced ESBL and comprised 135 strains targeted by the NCCLS ESBL test (*E. coli*, *K. oxytoca* and *K. pneumoniae*) and 33 nontarget strains (*Salmonella* spp., *P. mirabilis*, *S. marcescens*, *Enterobacter* spp., and *C. freundii*). In addition, we tested 14 AmpC-hyperproducing (AmpC-h) *E. coli*, 13 K1-hyperproducing (K1-h) *K. oxytoca*, and two AmpC-h *E. cloacae*. Susceptibility testing was performed by the broth microdilution method following the manufacturer's instructions, and readings were performed visually after 18–24 h. of incubation at 35°C.
Results: Sensitivity and specificity of the MicroScan panel for the target bacteria were 97 and 71%, respectively; sensitivity and specificity for nontarget bacteria were 89 and 100%, respectively. Eighteen strains were classified incorrectly: 10 isolates of K1-h *K. oxytoca* and 1 AmpC-h *E. coli* were misidentified as ESBL producers; and seven ESBL-positive isolates were not detected [*E. coli* (1 strain), *K. pneumoniae* (2), *Salmonella* spp. (1), *C. freundii* (1), *E. cloacae* (1), and *S. marcescens* (1)]. In these seven strains, the synergy effect of clavulanic acid was observed in the double-disk synergy test using only the cefepime disk. The chromosomal AmpC-producing micro-organisms might produce high levels of AmpC in addition to ESBL (MICs of cefoxitin >32 mg/L), and the remaining ESBL-producers showed either very high or very low ceftazidime and cefotaxime MICs.
Conclusions: The MicroScan ESBL Plus confirmation panel showed excellent performance in detecting target micro-organisms, although misidentification of a non-ESBL producer as an ESBL producer was frequent with K1-h *K. oxytoca*. Addition of cefepime and clavulanic acid to the panel may significantly improve detection of ESBL-producing strains.

P1623 Evaluation of the ability of Italian laboratories to detect beta-lactam resistance phenotypes in Enterobacteriaceae: a proficiency study by the Associazione Microbiologi Clinici Italiani (AMCLI)

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Objectives: To evaluate, at a countrywide level, the proficiency of Italian laboratories to detect beta-lactam resistance phenotypes, including those mediated by extended-spectrum beta-lactamases (ESBLs), in well-characterised isolates of Enterobacteriaceae.
Methods: On June 2003, 60 clinical microbiology laboratories located in different Italian regions entered this nationwide proficiency study. All participating laboratories received five well-characterised strains of Enterobacteriaceae producing ESBLs (*Klebsiella pneumoniae*/SHV-12, *Klebsiella oxytoca*/SHV-12, *Proteus mirabilis*/TEM-52, *Escherichia coli*/CTX-M-1, and *K. pneumoniae*/SHV-18), and two hyper-producers of chromosomal enzymes (an AmpC-hyperproducing *E. coli*, and a K1-hyperproducing K. oxytoca). In addition three reference strains (*E. coli* ATCC 25922, *E. coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853), were also provided as a blind internal quality control. Each laboratory was requested to use the routine methods for identification and susceptibility testing (AST). Expected results were established by two independent reference laboratories according to the NCCLS guidelines.
Results: Identification to species and genus level was correctly reported in 96.0 and 99.5% of cases, respectively. The performances in AST were different depending on the species and type of ESBL produced. Approximately 30% of laboratories failed to apply NCCLS rules with *Klebsiella* spp. producing SHV-type ESBLs. In these cases, very major errors were mostly related to cefotaxime (18.4%), ceftriaxone (23.4%) and cefepime (26.2%). A higher rate of failure occurred in detecting the ESBL phenotype of CTX-M-1-producing *E. coli* (44.1%) and TEM-52-producing *P. mirabilis* (67.8%). These two strains were incorrectly reported as susceptible to ceftazidime in 44.1 and 55.9% of cases, respectively. On the contrary, the isolates hyperproducing chromosomal enzymes were correctly reported more frequently, although an ESBL-mediated resistance mechanism was erroneously reported in about 15% of cases. Notably only 27 of 60 centres used a confirmatory test for ESBL production.
Conclusions: Detection of ESBLs is still a problem for clinical laboratories. Laboratory methods for AST appear to be performing better when well known enzymes are involved, whereas they fail to report resistance when facing emerging enzymes (e.g. CTX-M-type) or organisms not well recognised as ESBL-producing by current international guidelines (e.g. *P. mirabilis*).

P1624 Characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) undetected by Vitek 2

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Objectives: The aim of this study was to compare different phenotypic methods for detection of methicillin-resistant *Staphylococcus aureus* that appeared methicillin susceptible with Vitek 2 but that carry the *mecA* gene.
Methods: Between November 2001 and June 2003, 771 strains of MRSA were detected by multiplex PCR analysis of *nucA*, *mecA* and 16S rRNA genes. Among these strains, 31 (4%) appeared methicillin susceptible by Vitek 2 susceptibility card AST-P515 (bioMérieux). We compared: (i) disc-diffusion method using oxacillin 5 mg (CA-SFM breakpoint) and cefoxitin 30 mg (inhibition diameter: 27 mm), (ii) agar dilution MICs, (iii) ATB STAPH system (bioMérieux), (iv) standard *E*test procedure. Population analysis profiles method was used to detect heteroresistance level.
Results: Methicillin resistance was demonstrated among 11/31 strains (35%) using agar dilution MICs, 14/31 strains (45%) by disk diffusion method using oxacillin disk, 21/31 strains (68%) using standard Etest, 22/31 strains (71%) using ATB STAPH system, 30/31 strains (97%) by disc-diffusion method using cefoxitin disk. Population analysis profiles method demonstrated extreme heteroresistance among all the strains tested ; 28/31 (90%) were compatible with Tomasz's class 1 (1/108 of the native population), and 3/31 (10%) with class 2.
Conclusions: These data suggest that the MRSA strains undetected by Vitek 2 could be classified as heteroresistant MRSA. PCR detection of *mecA* gene represent the gold standard for methicillin-resistance diagnosis though cefoxitin disk diffusion can actually be considered as the most accurate phenotypic method to detect methicillin resistance among *S. aureus*. However, clinical relevance of these heteroresistant strains remains to be evaluated.

P1625 Bench validation of methicillin resistance detection by Vitek 2 in clinical staphylococci isolates

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Objectives: Test nonselected clinical strains of staphylococci for oxacillin (OX) susceptibility by Vitek2 (VT2) instrument and by Kirby-Bauer (KB) and devise a strategy to solve discrepancies.
Methods: During a month (August 2003) all clinically significant staphylococci isolated in our laboratory, except blood culture isolates, were tested simultaneously by VT2 AST P-523 card and by OX disc diffusion using NCCLS guide lines and M100-S9 interpretive criteria. All discrepancies between the three tests [OX calculated MIC (OXMIC); growth in the presence of 3 mg/L OX + 2% NaCl (OXST); disc diffusion (OXKB)] were resolved by the following complementary tests: induced PBP2′, *mecA* gene by PCR and identification to the species level for coagulase-negative staphylococci (CNS).
Results: There were 196 *S. aureus* (SA) (27 MRSA) and 38 CNS (23 MRSE) tested. The bacterial suspension could be prepared from primary plates for 111 (57%) of SA and for 14 (37%) of CNS. Only two strains needed repetition because of mixed inoculum. 14 of the SA (7%) and eight of the CNS (21%) [*S. capitis* (1), *S. epidermidis* (1), *S. lugdunensis* (1) and *S. saprophyticus* (5)] needed complementary tests. 21 of them were *mecA* gene and PBP2′ negative while the *S. epidermidis* strain was positive for both. Of the 14 SA, four were false MRSA by VT2 and 10 were flagged by the Advanced Expert System because of discordances between OXMIC and OXST. Of the eight CNS, the five *S. saprophyticus* were false MRSE by both OXMIC and OXKB and the *S. lugdunensis* by OXMIC only; one *S. capitis* was flagged because of growth in OXST well, and the last was the MR *S. epidermidis* missed by OXKB. Thus in this routine setting the PPV/NPV of VT2 for detecting methicillin resistance in SA vs. CNS were 87%/100% vs. 79%/100%. By comparison these figures for KB were 100%/100% vs. 67%/91%.
Conclusions: We found the strategy of combining VT2 with OXKB and with induced PBP2′ detection the best way to safely detect mixed inocula, to quickly solve either flagged results on VT2 or discordances between the three tests. It allowed us specially to improve the specificity of methicillin detection in CNS.

P1626 Detection of clinical MRSA strains in routine diagnostic laboratory

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Objectives: The aim of this study was to compare results obtained with two methods of methicillin resistance determination in *Staphylococcus aureus* strains, e.g. the disc-diffusion method and the ATB STAPH 5 (version 2000) method.
Methods: The methicillin resistance of 120 *S. aureus* strains was examined. All strains were isolated from ambulatory or hospitalised patients of the Infant Jesus Clinical Hospital – Centre for Trauma Treatment in Warsaw, from April to July, 2002. Two reference control strains (1. MSSA-PSSA *S. aureus* ATCC 25923, 2. methicillin-resistant *S. aureus* MR 3) were used in the study. Strains were identified biochemically with the automatic ATB Expression system – ID 32 STAPH strips (bioMerieux, Poland) according to the recommendations of the producer. Identification was confirmed with Slidex Staph-Kit (bioMerieux, Poland). Methicillin resistance (MR) was determined with: the disc-diffusion method (according to NCCLS recommendations) using Mueller-Hinton 2 agar (bioMerieux, Poland) and 1 mcg oxacillin discs (OXOID Ltd, England), and the ATB Expression system – ATB STAPH 5 strips (version 2000) according to the recommendations of the producer (bioMerieux, Poland).
Results: A total number of 120 *S. aureus* strains were examined to determine their methicillin resistance with methods mentioned above. In the case of 116 (97%) of cultured strains, consistent results of both methods were obtained. Proper results for control strains were achieved. Thirty clinical strains of *S. aureus* (25% of all examined strains) showed methicillin resistance in the both methods, 86 strains (72%) were indicated as MSSA in the case of four strains (3%), results of the both methods were inconsistent.
Conclusions: High consistency of the results of two methods: the disc-diffusion and the ATB STAPH 5 (version 2000) applied for detection of methicillin-resistant *S. aureus* (MRSA) strains was revealed. The ATB STAPH 5 (version 2000) test seems to be equally efficient for routine determination of MRSA and MSSA strains as the disc-diffusion method, and may be used alternatively.

P1627 Comparison of two methods for screening of MRSA carriers

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Objective: Since isolation frequencies of methicillin-resistant *Staphylococcus aureus* (MRSA) in Switzerland are rising, we set out to find a screening method with optimal sensitivity for MRSA recovery. Among all protocols implemented in this country, the two methods using (1) Mueller–Hinton/oxacillin enrichment broth combined with mannitol-salt agar for subculture (MHO/MSA) and (2) mannitol-salt broth combined with mannitol-salt-oxacillin agar (MSB/MSO) are most frequently used in major laboratories. Although both methods are able to identify MRSA carriers we investigated their ability to identify possible lower levels of MRSA carriage such as during decontamination of patients and other carriers.
Methods: We compared the two methods using 156 clinical samples for MRSA screening. In addition, we challenged their sensitivity *in vitro* at lower levels of MRSA loads (10(5)-10(3) CFU) using 50 well characterised MRSA strains and different incubation periods 24-72 h.
Results: From the 156 clinical samples, MHO/MSA and MSB/MSO isolated 20 (12.8%) and 21 (13.5%), respectively ($P = 1.000$). However, the in vitro studies showed, for a load of 10 (5) MRSA CFU, sensitivities of 46% for MHO/MSA and 100% for MSB/MSO ($P < 0.0001$) after 72 h incubation. At 48 h incubation, isolation rates were 12% for MHO/MSA and 100% for MSB/MSA. Shorter incubation times and lower MRSA loads resulted in even poorer yields for MHO/MSA while MSB/MSO remained highly sensitive.
Conclusions: Both methods appeared to be equally sensitive when applied to clinical samples of untreated MRSA carriers. For low MRSA numbers and shorter incubation times, however, MSB/MSO was significantly superior to MHO/MSA. We also found that MRSA workup after incubation was less tedious in the laboratory with the MSB/MSO method.

P1628 Comparative study of various methods, NCCLS M27-A2, EUCAST and ATB Fungus 2 (bioMérieux) for the in vitro antifungal susceptibility testing of *Candida* sp.

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Objectives and Methods: Reference methods (NCCLS M27-A2 and/or EUCAST E. Dis. 7.1) and the newly commercialised ATB FUNGUS 2 strip (visual reading) were compared for determination of the in vitro susceptibility to Amphotericin B (AMB), Flucytosine (5-FC), Fluconazole (FCA), and Itraconazole (ITR) of 93 Candida sp. clinical isolates (40 *C. albicans*, 15 *C. glabrata*, 11 *C. tropicalis*, seven *C. parapsilosis*, six *C. dubliniensis*, six *C. krusei*, eight other species). Were also added in this study, a comparison between NCCLS and EUCAST methodologies for Voriconazole (VRC), and a comparison between NCCLS micro-dilution and NCCLS macro-dilution methods for AMB.
Results: Overall essential agreement (±2 dilution) between NCCLS and EUCAST was 100, 90, and 86% for 5-FC, FCA, and VRC, respectively; between NCCLS and ATB FUNGUS 2, 99, 94, and 100% for 5-FC, FCA, and AMB; between EUCAST and ATB FUN-

GUS 2, 100, 92, and 88% for 5-FC, FCA, and ITR; between NCCLS micro-dilution and macro-dilution methods for AMB, 98%. MIC50, MIC90, range MIC and modal MIC values were within ±1 dilution for the three methods and all antifungals, except ITR. Among the seven isolates with known mechanism of resistance to azoles, all were correctly detected with EUCAST and ATB FUNGUS 2 methods and all but one with NCCLS. Among the 15 isolates found to be resistant or susceptible dose dependant to 5-FC or FCA by either one of both reference methods, the number of minor, major and very major discrepancies (accordingly to NCCLS breakpoints) found for one method in comparison with the two others were as follows, respectively: 4, 0, 1 by EUCAST; 1, 1, 0 by NCCLS and 2, 0, 0 by ATB FUNGUS 2.
Conclusion: The comparison between NCCLS M27-A2, EUCAST E. Dis 7.1 and ATB FUNGUS 2 showed good essential agreements (>90%) between all methods and for all antifungal agents, except ITR (ATB FUNGUS 2 vs. EUCAST, 88%) and VRC (EUCAST vs. NCCLS, 86%). In particular, for isolates found to be resistant by one reference method, very few major and very major discrepancies were observed. According to this study, both reference methods and ATB FUNGUS 2 show similar performance results.

P1629 Evaluation of a new generation of Sabouraud gentamicine chloramphenicol medium for the isolation of yeasts and moulds in clinical specimens

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Sabouraud gentamicine chloramphenicol 2 (SGC2) is a second-generation selective media recommended for the isolation of yeasts and moulds from clinical samples. The Sabouraud gentamicin chloramphenicol (SGC) was reformulated in order to improve the pigmentation of moulds and the fertility of yeasts.
Objective: The purpose of this study was to compare the performance of SGC2, in terms of fertility, pigmentation and selectivity, with SGC (bioMérieux).
Methods: Ninety-seven micro-organisms (47 yeasts, 21 moulds and 29 bacteria) were tested in parallel on the two SGC media and a reference medium: Sabouraud dextrose for yeasts and moulds and trypticase soya agar for bacteria. Fungi were inoculated using a technique allowing an enumeration after incubation. Yeasts were incubated during 3 days at 37°C and moulds at 25°C and/or 37°C during 7 days according to the media package insert information. Bacteria were inoculated by a semi-quantitative culture method with a 10 μL loop and incubated for 7 days at 37°C. Enumeration, growth intensity, morphology of colony were studied simultaneously for all media after incubation.
Results: On 47 yeasts inoculated, 42 grew on the reference medium. SGC2 enabled the growth of 40 strains whereas 36 strains grew on SGC after 72 h of incubation. For the 21 moulds incubated at 37°C during 7 days, 15 strains showed a growth on the reference medium. SGC2 enabled the growth of 11 strains with the correct morphology. On SGC, nine strains showed a growth but only three gave the expected colouration. At ambient temperature during 7 days, 20 strains grew on reference medium. Sixteen moulds grew on SGC2, among them 15 showed the expected pigmentation, whereas only three of 14 strains were observed with the right aspect on SGC. For the 29 bacteria tested, 2 and 3 strains grew on SGC2 and SGC after 24 h, respectively.
Conclusion: The SGC2 medium is significantly superior to the SGC for the growth of moulds and yeasts, the selectivity for bacteria being equivalent. This new formula demonstrates a good capacity to obtain colonies with a specific morphology, which is very important for the orientation of the diagnosis of fungal infections.

P1630 Detection of hetero-resistant MRSA: Controlled comparison of oxacillin/cefoxitin susceptibility testing by disk diffusion, agar screen, Vitek-2 and BD Phoenix automated systems

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Objectives: To compare the performance of disk diffusion, agar screen and automated systems for detection of hetero-resistant and recent epidemic strains of methicillin-resistant *Staphylococcus aureus* (MRSA) using oxacillin and cefoxitin.
Methods: This study included two sets of strains. The first set comprised MRSA (n = 67), representative major epidemic PFGE types recovered in Belgian hospitals, and methicillin susceptible *S. aureus* (MSSA) ($n = 61$) isolates collected during National Microbiological Surveys conducted in 2001–2003. The second set comprised 'challenge strains' ($n = 31$) (hetero-MRSA) with low oxacillin resistance level (MIC < 16 μg/mL) collected since 1995. Oxacillin susceptibility was tested by oxacillin agar screen method (6 μg/mL) (OAS), oxacillin and cefoxitin disk diffusion methods and by the automated Phoenix and Vitek-2 systems. Oxacillin disk diffusion was determined by two methods according to NCCLS recommendations (oxacillin 1 μg, 35°C for 24 h incubation) (oxa-1) and to French CA-SFM recommendations (oxacillin 5 μg, 30°C for 24–48 h incubation) (oxa-5). Oxacillin and cefoxitin MICs were determined by the agar dilution method. Oxacillin resistance was confirmed by multiplex PCR for *mecA* gene.
Results: The overall sensitivities for oxacillin resistance detection were 97, 94, 93, 89 and 87% for OAS, oxa-1, oxa-5, Phoenix and Vitek-2, respectively. All methods were fully specific (100%). All methods showed sensitivity >99% with epidemic MRSA. In contrast, hetero-MRSA isolates were detected with sensitivity of 90, 81, 81, 65 and 71% for OAS, oxa-1, oxa-5, Phoenix and Vitek-2, respectively. Oxa-5 disk diffusion method had to be interpreted after 48 h incubation because 15% of strains were falsely reported as susceptible after 24 h. Cefoxitin testing showed MIC > 4 μg/mL by agar dilution and by the Phoenix system in 98% of MRSA strains and zone diameter for cefoxitin was <20 mm in 99.5% by disk diffusion.
Conclusions: Cefoxitin testing by disk diffusion and by Phoenix system were the most sensitive and specific methods for oxacillin resistance detection, particularly for hetero-MRSA strains. Automated systems need further optimisation for detection of low-level MRSA strains.

Diagnostic methods: serology

P1631 Comparison of three commercial methods for the diagnosis of pertussis in single serum samples

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Objective: Laboratory diagnosis of pertussis by isolation or identification of the bacteria is hampered by the difficulties of testing early samples taken in the acute phase of the disease; the same happens with the study of seroconversion in paired sera, because is difficult to have an acute, negative sample. The quantification of antibodies against pertussis toxin (PT) in a single serum sample has been suggested as a useful diagnostic approach of pertussis. We have compared three serological methods in their application for the quantification of antibodies against different antigens as diagnostic tools of *Bordetella pertussis* infection in single serum samples.

Methods: We have used serum samples from 18 patients from an outbreak of pertussis in vaccinated children, as well as 79 controls from general population. Both cases and controls had the same age (mean age 6.3 and 6.7 years, respectively), and received the same number of doses (3.7 and 3.5, respectively) and type of vaccine (whole cellular vaccine). All the samples were tested by three commercial methods designed for detecting antibodies against a mixture of PT and filamentous haemagglutinin (FHA) (Mardx, United States), IgG against whole cell antigens (Serion, Germany), and IgG and IgA against PT and FHA in separate determinations (Pertusscan, Sweden). The diagnostic cut off of the assays was established.
Results: According to the cut off provided by the manufacturers, the sensitivity ranged from 27.8% (IgA-TP, Pertusscan) to 94.4% (Serion and Mardx); the specificity varied from 59.5% (Serion) to 89.9% (Mardx), being >95% for all determinations in Pertusscan. By modifying the cut off values, the figures were strongly improved. The sensitivity and specificity values of Serion (>300 units) were 72 and 91%. The corresponding values of Mardx assay (ratio sample absorbance/cut off >2.0) were 94 and 98%. The best figures in Pertusscan were 83% (sensitivity) for and about 90% (specificity) for IgG against HAF and PT (ratio sample absorbance/cut off >1.0).
Conclusions: The use of commercially available serological methods to test single serum samples seems to be useful tools for the diagnosis of pertussis, once the diagnostic conditions of the assays were established. The application of commercial kits will improve the knowledge of the real incidence of pertussis.

P1632 An enzyme immunoassay for measuring specific IgG antibodies to tetanus toxoid

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Objective: In this study, performance characteristics of a tetanus toxoid IgG EIA were investigated.
Methods: Tetanus vaccines utilise a denatured form of tetanus toxin (tetanus toxoid) and the antibody response to these vaccines can be measured by enzyme immunoassay (EIA). In this study, the performance of Binding Site Ltd UK tetanus toxoid IgG EIA is assessed. In addition a correlation was performed against an in-house EIA from Respiratory and Systemic Infections Laboratory, Central Public Health Lab, London.
Results: The kit is calibrated against the NIBSC reference material 76/589. The assay measuring range is 0.01–7.0 IU/mL. The analytical sensitivity of the assay was measured at 0.0093 IU/mL. Intra-assay precision was found to be 5.1% or less by assaying a low, medium and high level sample each 16 times. Inter-assay precision was shown to be 8.8% or less on the same samples measured on three separate occasions. To determine the effect of potentially interfering substances, high and low samples were assayed with and without the addition of a range of standard interference substances, was <5% variation was observed. Linearity was assessed using three positive samples, comparison of the achieved values by linear regression gave values of greater than $R^2 = 0.998$ in each case. A correlation was performed against an in-house EIA from Respiratory and Systemic Infections Laboratory, Central Public Health Lab, London using 71 serum samples. Linear regression showed good correlation with $R^2 = 0.7346$. Using a result of >0.1 IU/mL as indicative of a protective level of specific antibodies, the overall correlation of the two assays was 93.3%
Conclusions: From the results achieved in this study, The Binding Site EIA appears to be a suitable assay for measurement of specific IgG antibodies to tetanus toxoid. The results also correlated well with an existing in-house enzyme immunoassay.

P1633 A new enzyme immunoassay for the measurement of specific IgG antibodies to diphtheria toxoid and correlation with the Vero Cell Assay

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Objective: In this study the performance characteristics of Diphtheria toxoid IgG enzyme immunoassay (EIA), were investigated together with its correlation to the VCA.
Methods: Classically neutralising antibodies to Diphtheria have been measured by an in-vitro toxin neutralisation assay, the VCA. Diphtheria vaccines utilise a denatured form of diphtheria toxin (diphtheria toxoid) and the antibody response to these vaccines can also be measured by (EIA). In this study a new EIA from The Binding Site Ltd UK, was assessed and its results correlated to those of the VCA as performed by the Respiratory and Systemic Infections Laboratory, Central Public Health Lab (London).
Results: The ELISA kit is calibrated, against the NIBSC reference material 00/496. The assay measuring range is 0.004–3.0 IU/mL. Intra-assay precision was found to be between 5.8 and 2.7% by assaying a low (0.06 IU/mL), medium (0.71 IU/mL) and high (2.6 IU/mL) level sample each 16 times. Assay linearity was assessed using three positive samples, comparison of the achieved and expected values by linear regression gave values of greater than $R^2 = 0.99$ in each case. A correlation was performed against the VCA, using 34 serum samples. Linear regression showed excellent correlation with $R^2 = 0.96$. Using a result of >0.01 IU/mL as indicative of a minimum protective level of specific antibodies, the overall agreement of the two assays was 96%.
Conclusions: From the results achieved in this study, The Binding Site assay appears to be an accurate and precise assay for the measurement of specific IgG antibodies to Diphtheria toxoid. It could be considered a possible alternative to the VCA, with the significant advantages of speed, ease of use and adaptability to automation.

P1634 Application of gene recombination and chromatography of bio-affinity for production of the purified *Yersinia enterocolitica* protein antigens – use of the recombinant YopD protein in serodiagnosis of enteric illness and reactive arthritis

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Objectives: *Yersinia enterocolitica* causes a variety of infections in human, including enterocolitis, acute mesenteric lymphadenitis, erythema nodosum and reactive arthritis. Both whole *Yersinia* bacteria and purified lipopolysaccharide (LPS) have been used as antigens for detecting antibodies in sera of patients. However cross-reactions in the ELISA between *Y. enterocolitica* and other pathogens have been described. Therefore high specific antigens, free of lipopolysaccharide, based on *Yersinia* outer membrane proteins (YOPs), or adhesins: Ail, YadA, Invasin or Myf are needed. The aim of this study was to evaluate the usefulness of gene recombination technique using the pET-30 Ek/LIC expression vector for production a 36-kDa YOP called YopD and evaluate of this purified protein as antigen in serodiagnosis of yersiniosis.
Methods: Protein YopD of *Y. enterocolitica* was expressing in *Escherichia coli* BL21 (DE3) using the pET-30 Ek/LIC expression vector. Purification of the expressed enzyme from suspensions of *E. coli* cells treated with Bug Buster™ Protein/Extraction Reagent was accomplished by immobilised metal (Ni^{2+}) affinity column chromatography (His-trap). The IgM, IgG and IgA class antibodies to YopD were measured in 100 serum samples collected from patients suspected for yersiniosis and 100 blood donors. The obtained results were compared with the results of ELISA with

LPS and YOPs isolated from the culture of *Y. enterocolitica* supernatant under calcium deficient conditions, as antigens.
Results: In the case of patients suspected in clinical investigation for yersiniosis most frequently the positive results were obtained in IgG class of antibodies (41.0%). IgM and IgA antibodies were detected in 33.0 and 10.0% serum samples, respectively. In sera obtained from blood donor's antibodies, in all immunoglobulin classes, to YopD antigen were detectable significantly rarely ($P < 0.001$). A very high (94.0–100.0%) specificity and good sensitivity (67.0–80.0%) was displayed by the ELISA with YopD in relation to ELISA with LPS and YOPs antigens. IgA and IgG more frequently were found in sera of adult persons with arthritis and immunoglobulin M in the sera of children with enteric illness.
Conclusions: The recombinant YopD protein purified by chromatography of bio-affinity may be used in serodiagnosis of yersiniosis as a high specific antigen free of *Yersinia* lipopolysaccharides.

P1635

Abstract withdrawn.

P1636 Quantitative detection of anti-*Chlamydia pneumoniae* IgG and IgA antibodies by ELISA: comparison to the microimmunofluorescence test

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Objectives: Serological analysis represents the current routine method for the diagnosis of *Chlamydia pneumoniae* infections because the direct research by PCR can be difficult. Seroepidemiological studies have shown that the detection of specific IgG antibodies in single serum specimens have a limited diagnostic value. Only a significant increase of specific antibody titers in paired sera is accepted as a serological indication of an acute *C. pneumoniae* infection. The microimmunofluorescence assay (MIF), which requires expertise to perform, was considered, for a long time, the 'gold standard' method. MIF leads to variable results, urging the need for more objective test methods. Now, automated enzyme-linked immunosorbent assay (ELISA), which is more standardised, allows to define antibody titers comparable to MIF, but without any subjective bias. The aim of our study was a comparison between two quantitative assay methods: a second generation ELISA-based assay and MIF.
Methods: We selected serum samples during normal laboratory routine from patients with *C. pneumoniae* infection: 131-paired sera were collected from a adult population with high or low respiratory tract infections that showed high serum level *C. pneumoniae*-specific IgA and/or IgG antibodies in MIF (SeroFia, Savyon, IL, USA), and serum samples from 105 apparently healthy children without antibodies (according to MIF) against *C. pneumoniae*, *C. trachomatis*, *C. psittaci* which were used as a control group. *C. pneumoniae*-specific IgA and IgG antibodies were also determined in these two groups of serum samples by a commercial quantitative ELISA test (Cp-Quant Eurospital, I). The tests were performed as recommended by the manufacturer.
Results: The concordance of the results of the MIF and CP Quant for the adult patients was 96% for the IgG determination and 93% for the IgA. All 105 healthy children were negative for both IgG and IgA in ELISA test. There was a very high overall agreement between the results of the two different methods.
Conclusions: The results obtained by ELISA overlap those obtained by a traditional MIF assay and show that the ELISA quantitative test is highly reliable. In addition, the rapidity, the ease of use, the possible automation as well as the easy analytical interpretation independent of the subjective evaluation of the operator, are important advantages. The ELISA quantitative test can be used as an alternative clinical diagnostic test to MIF.

P1637 Evaluation of *Mycoplasma pneumoniae* commercial tests for detection of serum antibodies using PCR as 'the gold standard'

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Aim of the Study: Serological methods are most widely used for diagnosis of *Mycoplasma pneumoniae* (MP) infection, but very few studies have evaluated available commercial tests. We studied commercial MP IgG and IgM EIAs using PCR for detection of MP as gold standard.
Methods: Twenty-nine MP PCR-positive patients (49 serum samples) were included from two prospective studies on acute lower respiratory tract infections (LRTI). Control sera were tested from (i) 20 patients with acute LRTI negative for M by PCR and from (ii) 61 patients with microbiological documented LRTI other than MP, but without PCR exclusion. The different EIAs tested were Platelia (Bio-Rad); SeroMP (Savyon); Serion classic (Virion/Serion); Biotest EIA (Biotest); Ridascreen EIA (r-Biopharm); AniLabsystems EIA (Labsystems); Novum EIA (Novum Diagnostica); Diagnosys EIA (MP products); Genzyme/Virotech EIA; Immuno-Well EIA (Genbio); Immunocard EIA (Meridian). In addition, the complement fixation test (CFT) and Serodia-Myco II agglutination test (Fujirebio) were included.
Results: The sensitivities of IgM EIAs ranged from 7 to 23% in the first 6 days after onset of disease to 29–86% after more than 16 days of illness. IgG EIAs detected seroconversion or a significant rise of IgG titers in 47–63% of the PCR-positive patients. IgM tests with the best results for both sensitivity and specificity were AniLabsystems EIA (86%/92%), Diagnosys EIA (71%/94%) and Serodia-MycoII (80%/87%), whereas other IgM tests failed to achieve satisfactory results for either the sensitivity (below 70% after 16 days) or specificity (below 80%). False-positive EIA IgM results occurred more frequently in the control patients with acute EBV infection. The sensitivity and specificity of the CFT (99%/95%) exceeded those of the commercial IgM tests at all clinical time-points.
Conclusions: Evaluation of the currently available serological EIAs, CFT, and agglutination test for *M. pneumoniae* using PCR as gold standard, showed substantial differences between the performances of the assays.

P1638 Recombinant antigens of *Ureaplasma* spp. as a possible tool for a serological test

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Objectives: *Ureaplasma* spp. (14 serotypes) are frequently isolated from the lower genital tract of men and women, and are related to adverse pregnancy outcome. The development of serological assays for *Ureaplasma* spp. antibodies has been hampered by the

low purity of antigen used in the assays and by the difficulty to obtain large quantities of the antigen. The aim of the present study is to produce recombinant antigens for all serotypes for the development of a serological assay.
Methods: The multiple banded antigen (MBA) of *Ureaplasma* spp. is a surface expressed antigen. It is one of the major antigens recognised during infection and is present in all serotypes. This makes it a suitable candidate for use in serological assays. The coding part of the MBA gene from the serotypes was amplified by PCR. The PCR products were cloned by ligation into a pTrc-His Topo plasmid followed by transformation into *E. coli* competent cells. Expression of the antigens was induced by IPTG addition. The expressed proteins, containing a poly-His tag, were purified and analysed by western blot assay (WBA) using an anti-poly His antibody and by ELISA and WBA using the 14 serotype-specific monoclonal antibodies (MAbs).
Results: Except for serotypes 1, 6 and 13, the MBA was successfully expressed for all serotypes. MBAs of serotypes 2, 3, 4, 5, 8, 9, 10, 12 and 14 were recognised by serotype specific MAbs in ELISA. Moreover, preliminary analysis of the MBA-3 in ELISA using human sera from serotype 3 infected patients has shown promising results.
Conclusions: These results indicate that the purified recombinant multiple banded antigens are promising for de detection of antibodies against *Ureaplasma* spp. serotypes.

P1639 Syphilis Fast, a rapid treponemal test for the serodiagnosis of syphilis

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Background: Syphilis remains a significant cause of morbidity with an estimated annual incidence of 12 million new cases worldwide. Serologic tests play a decisive role in the diagnosis of syphilis. Two types of serologic test are used: nontreponemal and treponemal tests. The main limitation of current treponemal tests is that they cannot be used in situations where a very rapid diagnosis is required. On account of this disadvantage, rapid recombinant antigens based tests have been recently introduced in routine syphilis serology.
Objective: To evaluate a new treponemal test, Syphilis Fast (DIESSE Diagnostica Senese, Italy) for the rapid serodiagnosis of syphilis in comparison with other serological tests. The Syphilis Fast test is a latex agglutination test based on polystyrene particles coated with three immunodominant proteins of *Treponema pallidum* with molecular weight of 15, 17 and 42 kDa, obtained in *Escherichia coli* by recombinant technology.
Patients and methods: A total of 303 sera were analysed by a nontreponemal test, the Rapid Plasma Reagin test (RPR) and the combination of two treponemal tests (Syphilis Fast and Mercia Syphilis Total EIA). Reactive specimens were further confirmed using a fluorescent treponemal antibody absorption test (FTA-Abs), considered as the gold standard in serodiagnosis of syphilis. The study population included immigrants and returning travellers from sub-Saharan Africa, patients with risk behaviour for sexually transmitted diseases and HIV (homosexuals or bisexuals, intravenous drug users, patients with multiple sexual partners), immigrant sex workers, adopted children, pregnant women and patients with neurological symptoms or genital cutaneous lesions.
Results: Agreements between the FTA-Abs and the Syphilis Fast were 98% (297/303, three false-reactive and three false nonreactive results), and between the FTA-Abs and the Mercia Syphilis Total EIA were 97.4% (295/303, eight false nonreactive results). Comparatively, Syphilis Fast was found more sensitive than Mercia Syphilis Total EIA for the detection of syphilis (97.8 vs. 94.2%) and slightly less specific (98.2% vs. 100%).
Conclusions: Syphilis Fast is a rapid, cost-effective, easy-to-use and reliable assay. The results of this study indicate that the Syphilis Fast may be an alternative to other treponemal tests for routine serodiagnosis of syphilis in clinical laboratories.

P1640 A multiplexed syphilis IgM serological test for the diagnosis of *Treponema pallidum* infection

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Objectives: Serological tests for syphilis IgM include FTA-ABS 19S IgM, FTA-ABS immunoglobulin (IgM), and IgM capture ELISA. In recent years, the Western blot (WB) IgM syphilis test has become a popular method to verify syphilis infection. Due to performance limitations of EIA syphilis tests [Schmidt *et al.*, *J Clin Microbiol* (2000) 38: 1279–1282], and the laborious nature of FTA-ABS and WB analyses, IgM serology tests for syphilis are not often performed on adult samples. We developed a multi-analyte immunoassay for antibodies to *Treponema pallidum* in human serum and plasma on the automated, high-throughput, random access Bio-Rad BioPlex 2200 immunoassay analyzer. Using fluorescent magnetic microspheres with discrete spectral addresses, recombinant proteins were immobilised and simultaneously evaluated for the presence of IgG and IgM antibodies. This technology was used to simultaneously detect anti-*T. pallidum* r17 and r47 proteins as markers in each analysis to identify the presence of early infection.
Methods: The BioPlex 2200 syphilis IgM test was compared with various methods including the Meddens m-capture EIA, a modified TPPA, and two WB tests (Mikrogen recomBlot Treponema IgM and Trinity T. pallidum IgM MarBlot™ strip test).
Results: Using adult specimens, the BioPlex 2200 syphilis IgM test had 75.5% sensitivity and 92.3% specificity ($n = 201$) compared with the Meddens EIA. In comparison with a composite of EIA, TPPA-IgM, and Trinity WB (either two or three positives in this three assay panel was interpreted as a positive result), the BioPlex 2200 yielded 89.4 and 97.8% sensitivity and specificity ($n = 186$), respectively (equivocal results removed). In a like comparison using the Mikrogen WB test, the BioPlex 2200 syphilis IgM test yielded 100% sensitivity and 97.1% specificity ($n = 189$). With use of a syphilis performance panel, all samples with an RPR > 1:8 showed a positive result in the BioPlex 2200 test. The Bio-Rad IgM test detected early seroconversion with a longitudinal panel of a patient's sera. Also, a comparison of the Meddens EIA to Trinity WB tests showed only 90.9% sensitivity and 74.0% specificity ($n = 150$).
Conclusions: Current syphilis IgM tests are lacking in clinical sensitivity and/or specificity. The Bio-Rad BioPlex 2200 IgM test for syphilis compares very favorably to a composite analysis by multiple methods. The BioPlex 2200 immunoassay analyzer provides a multiplexed test with testing time and full automation.

P1641 Evaluation of GLP antigen for serodiagnosis of human leptospirosis

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Leptospira is a genus of spirochetal bacteria and the causative agent of leptospirosis, a zoonotic disease with a global distribution. The extensive serovar diversity has been attributed to differences in the structure and composition of lipopolysaccharide (LPS). Much work has focused on the role of leptospiral LPS in immunity. Preparations of leptospiral LPS can elicit protective in

immunity, but this immunity is generally serovar-specific. Among the many antigens isolated from pathogenic *Leptospira*, glycolipoprotein (GLP) is likely to be involved in the pathogenesis of the disease inducing lymphocyte and monocyte activation. The focus of research on protective antigens and better serodiagnostic strategies has shifted toward conserved antigens, which may be able to stimulate heterologous immunity. The identification of leptospiral antigens expressed during infection has potentially important implications for the development of new serodiagnostic and immunoprotective strategies. In this work, we study candidate antigens for serodiagnosis using the ELISA-dotblot reactivity of sera from patients with leptospirosis confirmed by microscopic agglutination test (MAT). ELISA-dotblot analysis was performed using sera from patients with leptospirosis and sera from patients with other diseases as negative control. GLP is identified as a candidate antigen for serodiagnosis of leptospirosis. Seven-day-old culture of *Leptospira interrogans* serogroup Icterohaemorrhagiae was used for preparing the antigen. Serum samples were assayed by ELISA-dotblot using our antigenic preparation and by a microscopic agglutination test (MAT) using 19 serovars. The antigen prepared had aproximately 220 μg/mL of GLP. IgG antibodies didn't show reactivity in all positive sera by MAT. However, on the basis of IgM response, all positive sera by MAT were found to react with GLP. None of the controls were positive. These preliminary observations demonstrate that GLP is a newly identified antigen which is recognised by sera from patients with leptospirosis. These data provide tools to understand the pathogenesis of leptospirosis and the identification of candidate antigens for serodiagnosis and immunoprotection.

P1642 Comparison of three different *Toxocara* ELISA and Western blotting methods in respect of sensitivity and specificity

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Objectives: Human toxocarosis is caused by the larval stage of different *Toxocara* species. Due to the lack of specific clinical signs, the diagnosis is usually based on the detection of anti-toxocaral IgG or IgG + IgM antibodies by indirect ELISA or by Western blot (WB) methods. All these tests utilise the excretory–secretory (ES) antigens obtained from *in vitro* maintained *T. canis* larvae. The home-made tests of different laboratories and commercial ELISAs of different manufacturers vary in their procedural parameters and in their performance characteristics, which can cause difficulties in the comparison and interpretation of the results. The objective of our investigations was to study the outcome of some tests.
Methods: A total of 147 serum samples of patients with clinically suspected *Toxocara* infection were selected on the basis of the results obtained by the home-made ELISA test. The whole range of OD values was covered by these sera (52 negative, 14 borderline and 81 weak to strongly positive samples). The results of our home-made ELISA were compared with two commercial ELISA tests (EIA *T. canis* IgG, Test-Line, Czech Republic and *T. canis* IgG ELISA, Novatec, Germany) and with a WB method (*Toxocara* Western Blot IgG, LDBIO, France). The sensitivity and specificity of each ELISA test were computed in relation to the WB results.
Results: The sensitivity of the home-made, Test-Line and Novatec ELISA was 63, 51, 56%, while the specificity was 95, 100, 100%, respectively. The distribution of the negative and positive results was significantly influenced by the cut-off level of the given test. The best fitting of semiquantitative results was found between the home-made and Test-Line ELISA. The borderline and negative (but close to borderline) ELISA results of the home-made, the Test-Line and the Novatec ELISA were found to be positive by the WB in 84, 96 and 97% of cases, respectively.
Conclusion: The high proportion of WB positive patients with negative-ELISA results suggests that low level antibodies can frequently occur in clinical samples. These data underline the necessity of the confirmation of the negative and borderline ELISA results with WB.

P1643 Evaluation of multiplexed IgG and IgM immunoassays for infectious mononucleosis and Epstein-Barr virus using the Bio-Rad BioPlex 2200 immunoassay analyser

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Objective: Diagnosis of infectious mononucleosis (IM) and clinical assessment of Epstein-Barr virus (EBV) infection involves detection of IgG antibodies to three EBV antigens and IgM antibodies to the EBV viral capsid antigen (VCA) and non-EBV heterophile antigens. Typical identification of these antibodies involves multiple independent EIA and agglutination immunoassays and is not amenable to high throughput analysis. The objective of this study is thus to develop automated high throughput IgG and IgM immunoassays for EBV that generate composite data to differentiate between acute IM and secondary infection.
Method: Using the Bio-Rad BioPlex 2200 immunoassay analyser, which is an automated high throughput multiplex platform, we report the detection of IgG and IgM antibodies against EBV and heterophile antigens and compare our results to those generated by commercially available tests. The assays employ an array of control and antigen-coated fluoromagnetic beads with discrete spectral addresses assigned to each substrate. Results are reported as an index value relative to a four-point calibration curve and are sorted based on the fluorescent emission assigned to each analyte or control bead.
Results: Concordance testing for IgG against the Trinity (TM) EIA-revealed relative sensitivity and specificity values of 100% (217/218) and 95% (38/40), 60% (31/52) and 98% (205/210) and 100% (215/216) and 93% (43/46) for VCA, early diffuse antigen, and nuclear antigen-1, respectively. Concordance testing for VCA and heterophile IgM against the Diasorin ETI EIA and Wampole Laboratories MONO-LATEX (R) test revealed relative sensitivity and specificity values of 99% (107/108) and 97% (86/89), and 100% (11/11) and 96% (94/98), respectively.
Conclusions: In contrast to conventional serological tests, the Bio-Plex 2200 assays offer a rapid method for acquiring composite IgG and IgM data for diagnosing acute IM and identifying EBV infection. The inclusion of control beads in every sample ensures reliable results and the ability to process up to 100 primary sample tubes per hour and access samples continuously offers significant advantages over standard protocols.

P1644 Evaluation of sensitivity and specificity of six combined P24 antigen and HIV antibody screening assays

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Early detection of human immunodeficiency virus (HIV) infection is critical for clinical diagnosis and safety of blood products.
Objectives: To evaluate performance of six HIV-combined antigen–antibody (Ag/Ab) assays: AxSYM HIV Combo, Enzygnost HIV Integral, Genscreen Ag/Ab Plus, Murex HIV Ag/Ab Combo, VIDAS HIV DUO and Vironostika HIV Uniform II Ag/Ab. Two-third generation antibody-only assays (Genscreen HIV 1/2 v.2 and Ortho HIV 1/2 antibody Capture) were also evaluated.

Methods: The specimens for sensitivity evaluation included: 25 seroconversion panels, HIV-1 antigen panel of 31 cell-culture derived, diverse group M subtypes and group O (each isolate tested at 2, 5, 10, 25 pg/mL of P24 Ag) and 669 of HIV-1 or HIV-2 samples collected from various areas of the word. For specificity, 1005 unselected HIV negative samples collected from four French laboratories were used.
Results: For the detection of P24 Ag, Murex and AxSYM showed the best limit detection of P24 Ag (6–8 pg/mL) irrespective of train, VIDAS DUO could detect 12 pg/mL except for some subtypes C strains and the limit detection of Genscreen Plus was 20 pg/mL except subtype F and group O. The Vironostika and Enzygnost that did not detect any of the HIV strains at 25 pg P24/mL. Of 25 seroconversion panels, AxSYM and Murex detected the first positive bleed in 23 and 21 panels respectively, whereas VIDAS DUO, Genscreen Plus, Enzygnost, Vironostika, Genscreen v.2 and Ortho detected the first positive bleed in 12, 10, 6, 6, 5 and 4 panels, respectively. The 553 HIV-1 group M, 6 HIV-1 group O and 110 HIV-2 positive samples were detected by all assays except Genscreen HIV 1/2 v.2 which missed 1 group O, VIDAS DUO missed 1 subtype F, Enzygnost missed 1 subtype C and Genscreen Plus missed 1 subtype B. The specificity for assays were: Ortho 100%; Genscreen Ag/Ab Plus 99.9%; AxSYM Combo 99.8%; Enzygnost 99.7%; Vironostika and Murex Combo 99.6%; VIDAS DUO 99.5% and Genscreen HIV v.2 99.4%.
Conclusions: These results show that the Ag/Ab HIV combined assays present better performance when compared with third generation antibody assays, however, there are significant sensitivity and specificity differences among combination assays.

P1645 Evaluation of sensitivity and specificity of a new generation of Immunocomb II HCV for the detection of anti-hepatitis C virus antibodies

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Objectives: To evaluate a new generation of the ImmunoComb II HCV assay (PBS Orgenics) in comparison with Axsym HCV 3.0 (Abbott Laboratories) for the detection of anti-hepatitis C virus (HCV) antibodies (Ab).
Methods: We have studied three populations of patients; 162 pregnant women, 397 patients with chronic HCV infection and well known genotype (InnoLipa II, Innogenetics); with the following distribution: 224 patients with HCV genotype 1, 33 patients with HCV genotype 2, 88 patients with HCV genotype 3, 40 patients with HCV genotype 4 and 11 patients with HCV genotype 5a; and 224 patients with undetectable anti-HCV Ab. Discordant samples were tested and confirmed with Ortho ELISA HCV 3.0 and RIBA HCV 3.0 (Ortho-Clinical Diagnostics). The rapid test ImmunoComb II HCV assay have six rows; A for specimen diluent, B for washing, C for conjugate, D and E for washing and F for chromogenic substrate.
Results: Results obtained in pregnant women were respectively for Axsym HCV 3.0 and ImmunoComb HCV II: Detectable 2/2, Doubtful 3/2, Undetectable 157/158; in patients with chronic HCV infection: Detectable 395, Doubtful one, undetectable one for ImmunoComb HCV II and in patients with undetectable anti-HCV Ab: detectable 0/1, doubtful 5/2, undetectable 219/221.
Conclusions: The new generation of ImmunoComb II HCV assay has a good sensitivity (99.7%) and a good specificity (100%). This test is easy and quick to perform and results could be obtained in routine laboratories without the need of special equipment or training.

P1646 Western immunoblotting for serologic diagnosis of human candidosis

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Objective: To improve the reliability of the serodiagnosis of human deep *Candida* infection, a western immunoblot analysis was evaluated in a retrospective study.
Methods: Conditions of immunoblot were standardised then sera of different five groups of patients were tested: group 1, healthy blood donors ($n = 40$), group 2, patients with systemic candidosis with *Candida* isolated from at least one blood culture ($n = 58$), group 3, patients with probable or proven candidosis with *Candida* isolated from a sterile site except blood culture ($n = 52$), group 4, patients hospitalised with *Candida* isolation in a peripheral site ($n = 25$) and group 5, patients without isolation of *Candida* but with positive serology with routine techniques ($n = 33$).
Results: After standardisation of the technique, the lecture method was defined with exclusion of nonspecific bands. 52% of patients from the group 2 and 48% from the group 3 were positively detected by immunoblot technique. Only 16% were detected in group 4 and none in group 1 under these conditions. Only 6% of the patients from the group 5, with positive *Candida* serology in routine diagnosis, were detected by immunoblot analysis. Most of patients from this group 5 had a liver transplantation (57%), explaining a probable nonspecific serologic reactivity with routine techniques. Patients with probable or proved candidosis gave then results significantly different from patients without candidosis, the test showing a good positive predictive value (93.2%).
Conclusions: These findings suggest that western immunoblot test could be used as a good, confirmatory method for the detection of *Candida* antibodies in patient serum.

P1647 Advanced serological tests for the diagnosis of African tick bite fever

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Objectives: The serological diagnosis of African tick bite fever (ATBF), a spotted fever group (SFG) rickettsiosis caused by *Rickettsia africae* and an emerging health problem in international travellers to sub-Saharan Africa, is troublesome with many available tests having either poor sensitivity or poor specificity, or both.
Methods: We evaluated the sensitivity and species specificity of a multiple-antigen immunofluorescence assay (IFA) and a multiple-antigen Western blot (WB) assay in detecting antibodies against SFG rickettsiae and *R. africae* in paired serum samples collected from 40 consecutive patients with ATBF. Samples from patients with only nonspecies-specific antibodies detectable were also tested by cross-adsorption assay (CA).
Results: Antibodies against SFG *rickettsia* were detected by IFA in 18/40 (45%) patients and by WB in 40/40 (100%) patients ($P < 0.001$). During the first 2 weeks of illness, the corresponding estimates were four of 40 (10%) and nine of 40 (23%), respectively ($P = 0.03$). By comparing titres and staining characteristics, the multiple-antigen IFA demonstrated specific antibodies against *R. africae* in six of 18 (33%) IFA-positive patients. WB detected reactions to specific protein antigens of *R. africae* in 22/40 (55%) cases. For seven of 13 (53%) patients with only nonspecies-specific antibodies detectable by WB, CA demonstrated specific antibodies against *R. africae*.
Conclusions: Our study demonstrates that WB is significantly more sensitive than IFA when diagnosing consecutive cases of ATBF, also during the acute phase when most patients are likely to present. The species specificity of WB may be further increased by the use of CA in selected cases. The status of IFA as the considered serological reference method in the diagnosis of ATBF should be re-evaluated.

Diagnostic methods: blood culture

P1648 Evaluation of two procedures for direct inoculation of Gram-negative aerobic and facultative anaerobic bacteria from positive BACTEC blood cultures into the Phoenix automated microbiology system

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Objectives: To validate and evaluate the inter-laboratory concordance of two methods of specimen preparation for direct inoculation of PHOENIX with culture suspensions from positive BACTEC blood culture vials, compared with the routine PHOENIX method for ID and AST.
Methods: Multi-centre prospective clinical trial with parallel processing of clinical samples by investigational and reference methods. BACTEC vials were processed within 24 h after positivity. Only aerobic and facultative anaerobic Gram-negative rods were included. Differential centrifugation method (DIFF): bacteria were recuperated through two consecutive centrifugation steps (10 s at 6400 *g*; supernatant 60 s at 20 800 *g*).Gel separation method (SST): bacteria were recuperated through one centrifugation step (2000 *g* for 1 min) using a Vacutainer SST II gel tube. Reference method (REF): standard procedure starting from a pure overnight culture on solid media. ID accuracy was calculated at genus and species level. AST accuracy (category concordance and error rates) was determined for 19 antibiotics.
Results: A total of 226 isolates were obtained, of which 210 could be included in the study. DIFF and SST identified 92.1 and 91.6% of the isolates to the genus level and 90.7 and 90.2% to the species level, respectively; no identification was obtained for 10 (4.8%) vs. 13 (6.1%) isolates. AST concordance of DIFF and SST with REF was 95.4% vs. 94.1%, with only 0.26% vs 0.33% Very Major Errors. Reporting time for ID + AST with DIFF and SST was 10 h, 54 min and 11 h, 21 min vs. REF 11 h, 09 min.
Conclusions: Excellent results were obtained with both inoculum preparation methods for ID and AST testing. No major differences were observed between methods or sites. The short reporting time obtained with direct inoculation may be relevant to improve the management of bacteremic patients.

P1649 Clinical and organisational factors associated with negative blood cultures from general internal medicine wards

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Objective: Blood cultures (BC) help defining pathogen and resistance spectra. At the same time, the benefits of BC results in the management of individual patients – and therefore their cost effectiveness are disputed. We aimed to identify clinical and organisational factors associated with negative BC results for obligate pathogens (OP) for BC from patients in general internal medicine wards.
Methods: We abstracted charts of patients who had at least one BC (blood volume per BC approximately 20 mL) drawn on a general internal medicine ward of our tertiary care hospital in the year 2000. We retrieved and evaluated in a multiple logistic regression the following factors for their association with BC negative for OP: site and time of BC draw; presence of central venous catheter; prior BC taken in the emergency room or intensive care unit; any antibiotic treatment in prior 7 days; intravenous antibiotics in prior 2 days; temperature (value of <40°C; no rise or rise <2°C); leucocyte count (value of <12/nL; no rise or rise <2/nL), C-reactive protein (CRP) level (value of >100 mg/L; rise by >50 mg/L), general and special nursing categories (1 vs. 2 or 3), and prior order written in the chart to draw BC in case of fever. We also evaluated the number of BC within diagnostic episodes (DE): consecutive BC from a single patient taken <3 days apart were attributed to the same DE.
Results: A total of 96 (13%) of 711 BC from 310 patients were positive, 59 (8%) yielded OP. Temperature <40°C [odds ratio (OR) 2.0; 95% confidence interval (CI) 1.0–3.8], any antibiotic treatment in the prior 7 days [OR 2.3; CI 1.2–3.8], no rise of leucocyte count or <2/nL [OR 2.3; CI 1.2–4.2], rise of CRP >50 mg/L [OR 3.1; CI 1.1–9.0], and a low general nursing category [OR 4.0; CI 1.7–9.1] were independently ($P < 0.05$) associated with negative BC for OP. Of the 470 DE, 40 (9%) yielded OP. Of the 156 DE with more than 1 BC, 17 DE yielded OP. Two of the 17 DE yielded two different OP, and three failed to identify the OP in the first BC taken. Obtaining more than three BC within 3 days identified no additional OP.
Conclusions: A good overall clinical state of the patient represented by the lowest general nursing category and an already documented CRP rise >50 mg/L showed the strongest associations with negative BC results for OP. Therefore, rise in CRP should not encourage the draw of BC. Obtaining more than three BC within 3 days identified no additional OP.

P1650 Reducing the rate of blood culture contamination

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Objectives: At our Medical Center, blood cultures are drawn by resident physicians, interns and medical students. More than 20 000 blood cultures are examined at our institution every year, 10% of the samples being positive. About 2.5 of total blood cultures grow bacteria considered a contaminant. At the beginning of 2002, we implemented an intervention programme for 3 months in an attempt to reduce contamination rates in the internal medicine wards, where the largest proportion of blood cultures is taken.
Methods: The infectious diseases team conducted an education campaign on the subject of the technique of blood culture sampling in each of six internal medicine wards. Residents, interns and students who drew blood cultures were asked to sign their full names on the requests accompanying the blood samples, and each one of them was instructed to be responsible for his culture results. A few residents were found to be 'Super-Contaminators' and these were asked particularly to adhere to the proper technique of blood sampling and skin decontamination.
Results: During March, April and May 2002, 1786, 1780 and 1688 blood samples were drawn respectively for cultures in the internal medicine wards. In March, when the intervention program began, the contamination rate was 4.6%, in April the rate decreased to 2.6% and diminished further to 1.8% in May. Disappointingly, but not unpredictably, the rate of contamination increased as soon as the intervention programme ended. Comparing the results of a whole year (a year prior to the intervention period and a year afterwards), we found no difference in contamination rates – 5.65 and 5.36%, respectively.
Conclusions: Our programme intervened on two levels: the first was education regarding the technique of drawing blood cultures; the second was to try and make every physician responsible for his own samples. The efforts to reduce blood culture contamination rates were effective only in the period immediately following the intervention programme. In a hospital, where resident physicians and students collect blood cultures instead of skilled phlebotomists, a continuous effort is needed for the education of the staff.

P1651 Comparison percentage of blood culture (BC) contamination in four hospital units and corrective sampling and processing after training

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Objectives: The aim of study was monitor and improve BC sampling and necessary role of training.
Methods: This study performed in two phases based on TQM training with Focus-PDCA procedure. In the first phase we had the retrospective study for collecting data BC contamination in the forth first month of the year and we calculated the rate of blood contamination between four hospital units (ICU, NICU level I, NICU level II, and pediatric). Then we have initiated additional training of staff. In the second phase is collected blood contamination's data in the forth second month of the year (after training).
Results: Comparative's study between the results of BC contamination before and after training is showed that contamination of NICU I was 2.9% before training and 1.3% after training NICU II was 7.7% before training and 4.2% after training ICU was 5% before training and 1.6% (at), pediatric was 5.2% before training and 3.6% after training. Also it was comprised the results of BC contamination between each month (8 months) for four units.
Conclusions: Cost related to false-positive blood culture results (i.e. contaminant) are associated with 40% higher charges for IV antibiotics and microbiology testing. So training will be more cost efficient. The rate of contamination after training decreased 2–3% in four hospital units.

P1652 Prospective study to evaluate differential time-to-positivity in the diagnosis of port-related bloodstream infection

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Objectives: Intravascular catheters are indispensable in modern-day medical practice. Diagnosis of port related bloodstream infection (PRBI) remains difficult without catheter removal. Clinicians usually avoid removal of the port because reinsertion of a new catheter carries substantial risks. Blot *et al.* described in 1998 a method that evaluated the differential time to positivity (DTP), as determined by a continuous blood culture monitoring system, for qualitative blood cultures drawn simultaneously from the catheter and a peripheral vein, to diagnose catheter related bloodstream infection. However, this method has not been validated in long-term tunnelled catheters. The aim of the present study is to asses the value of DTP for the diagnosis of PRBI using paired quantitative blood cultures as the standard criterion to define PRBI.
Methods: During a 3-year period (June 2000–June 2003) patients with port suspected as being the primary source of fever were studied prospectively. Two qualitative and two quantitative cultures of blood samples were simultaneously obtained from a peripheral vein and through the port. A difference between the peripheral and port blood qualitative culture in time to detection >120 min was used as the cut-off point to diagnose PRBI.
Results: A total of 129 episodes occurred in 119 patients during the study period. Ten patients were excluded of the study because they were diagnosed of pocket-site infection. Forty patients were being treated with antibiotics when blood samples were obtained. Of the 109 episodes, 83 (76.2%) were PRBI and 26 (23.8%) were non-PRBI, as determined by the standard criterion. The median time to positivity of the blood samples for PRBI (462.12 min; range, −50 to 2010 min) was significantly greater than that for non-PRBI (−33.07 min; range, −510 to 450 min); $P < 0.001$. The sensitivity, specificity and predictive values of positive and negative results of DTP compared with quantitative cultures were 85.5, 80.8, 93.4 and 63.6% respectively.
Conclusions: Differential time to positivity is an easy and reliable method to diagnose port related bloodstream infection without removing of the catheter, when compared with quantitative blood cultures.

P1653 Procalcitonin rapid test in the diagnosis of sepsis

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Background: Procalcitonin (PCT) has been suggested to aid in diagnosis, monitoring and outcome prediction in septic patients.
Objective: The aim of the study is to determinate clinical usefulness of rapid semi-quantitative test for detection of PCT (B.R.A.H.M.S. PCT-Q, B.R.A.H.M.S.-Diagnostica GmbH, Germany) in septic patients within 24 h of admission. We analysed 12 medical patients treated in the intensive care unit (six with sepsis, five with severe sepsis and one patient with septic shock). In patients with sepsis PCT levels were: <0.5 ng/mL ($n = 1$); ≥0.5 ng/mL ($n = 1$); ≥2 ng/mL ($n = 3$); ≥10 ng/mL ($n = 1$). In patients with severe sepsis PCT levels were: ≥2 ng/mL ($n = 1$); ≥10 ng/mL ($n = 4$). The patient with septic shock had PCT level ≥10 ng/mL.
Conclusions: Measurement of plasma PCT with semi-quantitative PCT-Q test allow rapid support in the diagnosis of septic patients. However follow-up of PCT during clinical course of illness and its correlation with outcome is still to be determined.

P1654 Usefulness of plasma procalcitonin (PCT) in diagnosis of microbial infection among adults patients

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Objectives: To study the possible discriminative role of procalcitonin (PCT) in differentiating acute fever because of bacterial infection from fever of other inflammatory processes, in patients treated in an internal medicine department.
Methods: We prospectively examined 121 patients with acute fever admitted in our department. Variables recorded included patient demographics and principal diagnosis. PCT, C-reactive protein (CRP), Erythrocyte Sedimentation Rate (ESR) and white blood cell count (WBC) were measured. Blood samples were collected 24–48 h after the presence of fever. The outcome was determined as survivors and non-survivors. Patients were distinguished according to aetiological diagnosis, based on clinical assessment and laboratory results, to those with fever because of bacterial infection (Group A) and those with fever of other inflammatory processes (Group B). Among statistical tests applied were Student's *t*-test and Chi-Square. The ability of PCT to predict patients with bacterial infection was evaluated by performing receiver operative characteristic curves analyses.
Results: Of 121 study patients, 81 (66.9%) had fever because of bacterial infectious and 40 (33.1%) because of other inflammatory processes. The mean plasma concentrations of PCT in patients with bacterial infectious were 3.27 ng/mL (±SD 6.29) while in patients of Group B 0.41 ng/mL (±SD 0.5). Subgroup analysis in Group A, shows that patients with sepsis had higher values of PCT 12.74 ng/mL (±SD 10.48) compared with other patients with bacterial infection ($P = 0.02$). Patients in Group A, had as expected, higher values of ESR ($P < 0.0001$) and CRP ($P = 0.001$). The mean values of WBC did not differ between the two groups, while percentage of neutrophil differ at a significant level ($P < 0.0001$). 81.8% of patients who died had PCT levels higher than 0.5 ng/ml compared with 43.8% of survivors ($p = 0.03$). Predictive accuracy for bacterial infectious expressed as area under the receiver operating characteristics curve was 0.74 for PCT (95% CI 0.65–0.83), 0.75 for ESR (95% CI 0.65–0.84) and CRP 0.69 (95% CI 0.50–100).
Conclusions: PCT can probably be used as a useful tool in the initial work up of patients with fever. Supplemented by other biolo-

gical indicators, PCT can help in make decisions about antibiotic therapy in patients treated in an internal medicine department.

P1655 Should procalcitonin be introduced in the diagnostic criteria for the systemic inflammatory response syndrome and sepsis?

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Objectives: To define whether procalcitonin should be introduced in the diagnostic criteria of sepsis.
Methods: Procalcitonin was estimated in the sera of 105 critically ill patients by an immunochemiluminometric assay. Diagnosis was settled by three types of criteria; (A) the ACCP/SCCM 1992 criteria; (B) the ACCP/SCCM criteria and concentrations of procalcitonin above 1.0 ng/mL as indicative of both systemic inflammatory response syndrome (SIRS) and sepsis and (C) the ACCP/SCCM criteria and concentrations of procalcitonin between 0.5 and 1.1 ng/mL as indicative of SIRS and above 1.1 ng/mL as indicative of sepsis.
Results: Median procalcitonin of patients with SIRS, sepsis and septic shock diagnosed by criteria A was 1.15, 5.49 and 13.75 ng/mL upon presentation of their syndrome respectively. That of patients diagnosed with SIRS, sepsis, severe sepsis and septic shock by criteria B was 2.52, 2.70, 8.30 and 12.95 ng/mL respectively; and that of patients diagnosed with SIRS, sepsis, severe sepsis and septic shock by criteria C was 0.69, 2.64, 8.30 and 12.94 ng/mL. Criteria A identified 53 (50.5%) patients with SIRS, 19 (18.1) with sepsis, one (0.9%) with severe sepsis and 24 (22.9%) with septic shock; respective diagnosis by criteria B were 28 (26.7%), 10 (9.5%), 11 (10.5%) and 27 (25.7%); and respective diagnosis by criteria C were 20 (19.0%), 27 (25.7%), 10 (9.5%) and 27 (25.7%). Sensitivity of concentrations between 0.5 and 1.1 ng/mL was 25.6% for SIRS; and above 1.1 ng/mL 92.8% for sepsis.
Conclusions: Despite the limited diagnostic value of procalcitonin for SIRS, concentrations of procalcitonin above 1.1 ng/mL are highly indicative for sepsis.

P1656 Procalcitonin as a diagnostic aid in osteomyelitis and septic arthritis

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Objective: To evaluate the diagnostic value of Procalcitonin (PCT) in the detection of osteomyelitis (Om) and septic arthritis (Sa) in children.
Methods: PCT, C reactive protein (CRP), erythrocyte sedimentation rate (ESR) and white blood cell (WBC) were measured in children admitted with suspicion of osteomyelitis or septic arthritis. PCT was measured by the PCT-Q Kit, PCT immunochromatography assay, based on monoclonal and polyclonal antibodies against catacalcin. B.R.A.H.M.S. The results were divided into four categories, normal (under 0.5 nn/mL), mildly elevated (0.5–2 nn/mL) high (2–10 nn/mL) and very high value (>10 nn/mL). The diagnosis of Om was made by two of the following diagnostic criteria: (1) presence of purulence of bone, (2) positive bone or blood culture, (3) localized erythema oedema or both or (4) a positive imaging study either on radiography, Tc scintigraphy or magnetic resonance imaging.
Results: A total of 43 children were evaluated. Eleven (25.5%) with the diagnosis of Om, 11 with septic arthritis (25.5%) diagnosed by aspiration of sinovial fluid, six children (13.9%) were diagnosed as soft tissue infection. Transient synovitis or reactive arthritis were diagnosed in another six children (13.9%), three of them (6.9%) were diagnosed later on as juvenile rheumatoid arthritis. Six children have another different diagnosis (13.9%). WBC was higher in Sa compare with Om and other diagnosis: $16.2 \pm 7.3 \times 10^9$/L, $11.8 \pm 4.9 \times 10^9$/L and $13.2 \pm 5.5 \times 10^9$/L respectively ($P = 0.221$). ESR on admission was higher among the children diagnosed with Om compared with those with Sa and other diagnosis (67 ± 37.7 vs. 53 ± 27 vs. 49 ± 37). CRP on admission was higher among the children subsequently diagnosed as Sa compared with those with Om and other diagnosis but the differences were not significant (98.5 ± 76.3 vs. 85.1 ± 93.4 vs. 73.7 ± 95.4). PCT value was mildly elevated in six of 11 patients with Om (66.7%) and only three children with the diagnosis of Sa (33.3%) had a mildly elevated value. Among the children with other diagnosis there were no positive PCT values.
Conclusions: PCT was found to be a good tool in the diagnosis of osteomyelitis but not in septic arthritis. Larger studies are needed to confirm our findings.

P1657 Comparison of the time-to-positivity of hub-blood vs. peripheral-blood cultures as useful tool for diagnosing catheter-related infection

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Objectives: To evaluate the differential time to positivity (DTP) of paired blood cultures drawn simultaneously via the catheter hub and from a peripheral venous site as a diagnostic tool for catheter-related infection (CRI).
Methods: During 2002–03 period, 96 simultaneous hub-blood and peripheral-blood cultures were obtained from patients with suspected CRI. We recorded the DTP between hub-blood and peripheral-blood cultures with an automatic device (BacT/Alert system, Organon Teknika) for detection of blood culture positivity.
Results: The same micro-organism was found in both hub-blood and peripheral blood cultures in 46 (48%) of the 96 episodes of suspected CRI. Of these 46, 37 (80%) were associated with bacteraemic CRI (the DTP was over 2 h), whereas CRI was excluded in the remaining nine. The major micro-organisms involved in bacteraemic CRI were: *Candida* spp. (n=10), coagulase-negative staphylococci ($n = 7$), *Klebsiella pneumoniae* ($n = 6$), *Acinetobacter* spp. ($n = 3$), *Morganella morganii* ($n = 3$), *Staphylococcus aureus* ($n = 2$), *Escherichia coli* ($n = 2$), *Serratia* spp. ($n = 2$), *Stenotrophomonas maltophilia* ($n = 2$), *Enterococcus* spp. ($n = 1$), and *Pseudomonas aeruginosa* ($n = 1$).
Conclusions: Our results show that the measurement of the delay between the positivity of hub-blood and peripheral blood cultures can be a simple and useful tool for diagnosing CRI, and can be proposed for routine practice in hospitals using automatic devices for detection of positive blood cultures.

P1658 Comparison of the VersaTREK to the ESP Culture System II for blood cultures

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Objective: A series of studies were conducted comparing the next generation blood culture instrument, the VersaTREK (VT) to the ESP Culture System II (ESP). These studies were conducted in the laboratories of Trek Diagnostic Systems (TS), Sun Prairie WI, USA and St. Vincent's Hospital (SVH) New York, New York, USA.
Methods: VT 80 ml blood culture bottles and ESP 80 mL blood culture bottles were seeded with suspensions of a variety of micro-organisms. These bottles were incubated in their respective instruments and compared as to the time required to detect a positive culture and the quality of the curves generated. A clinical trial was conducted using specimens routinely collected for blood culture from inpatients at SVH.
Results: The initial seeded studies at TS included 45 different Gram Negative and Gram Positive bacteria, and yeast. Comparing the ESP 80A bottle to the Redox 1 (VT) bottle, isolates in the VT had a shorter time to detection (TTD) than in the ESP with 44 of 45 of the isolates by an average time of 1.08 h. The resulting graph profiles in the VT were equal to, or better than, those

generated in the ESP. Comparisons of the ESP anaerobic bottle to the VT Redox 2 yielded similar results. Seeded studies were conducted at SVH with 69 isolates representing 13 different species of bacteria and yeast. Comparing the two systems, 11 of 13 species were seen to have a faster TTD in the VT than in the ESP. Preliminary data on over 300 samples from an ongoing clinical trial comparing the recovery and TTD from patient specimens has shown the VT to be at least comparable with the ESP for these two criteria.

Conclusions: Seeded and clinical data has demonstrated that the VT is at least comparable with the ESP for blood cultures and has validated the VT for use in the clinical microbiology laboratory.

P1659 Comparison of the effect of delayed entry into two different blood culture systems (Bactec 9240 and BacT/Alert 3D) on culture positivity

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Automated continuously monitoring blood culture systems brought many advantages into laboratory practice such as rapid detection of agents and labor reduction. Delay in the specimen transport is one of the problems that may effect the culture positivity in clinical laboratories. To evaluate the difference between effect of delayed entry into blood culture systems [Bactec 9240 (BD) and BacT/Alert 3D (BA)] on detection of bacterial growth in blood culture media (Bactec 92 F, 93 F and BacT/Alert FA, BacT/Alert FAN), standard inoculums (25 cfu) of micro-organisms frequently isolated from blood cultures; *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Acinetobacter baumannii*, *Haemophilus influenzae*, *Bacteroides fragilis* and *Streptococcus pneumoniae* were inoculated into blood culture bottles which contained 2 mL of sterile human blood. The bottles were cultured using two instruments after they were stored at room temperature and at 35°C incubators for 0, 4, 6, 12, 24 and 48 h. Five sets (60 pairs) of each bacteria were studied for each temperature. All the positive and negative cultures (after 5 days) were recorded and subcultures were performed. None of the cultures were false negative in the first 12 h of delay except two strains (*S. pneumoniae* and *H. influenzae*) in BA, after preincubation at 35°C. At 24 h of delay, false negativity was 14/60 for BD (*E. coli* 4, *A. baumannii* 3, *E. faecalis* 4 *S. pneumoniae* 3), 8/60 for BA (*S. pneumoniae* 8). False negative results were from bottles preincubated at 35°C in BD. At 48 h of delay, false negativity rate was 34/70 for BD (*E. coli* 10, *B. fragilis* 10, *E. faecalis* 6, *S. pneumoniae* 5, *A. baumannii* 2, *H. influenzae* 1), 15/70 for BA (*S. pneumoniae* 9, *A. baumannii* 3, *B. fragilis* 3). For delayed preincubation (24–48 h) false negativity was more common in BD especially for storage at 35°C. BA had difficulty to detect *S. pneumoniae* starting from 24 h preincubation. Failures to detect microbial growth is noticed after 24 h for both systems. This may not be a disadvantage in clinical laboratory practice at institutions where specimen transportation work regularly.

P1660 The diagnosis of brucellosis by use of BACTEC 9050 blood culture system

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Objectives: The diagnosis of brucellosis is generally made when a standard tube agglutination titre of 1/160 or more for anti-Brucella antibodies in the presence of compatible clinical signs and symptoms. In this study we aimed to describe the rate and duration of isolation of *Brucella* spp. from blood culture by using an automated blood culture system (BACTEC 9050).

Methods: Between March and December 2003, 31 adults were diagnosed as brucellosis by means of positive standard tube agglutination (>160) cultures and clinical manifestations. Blood cultures were obtained from the patients whom standard tube agglutination titres were 1/160 or more. Ten millilitres of blood were inoculated in a Bactec Plus aerobic/F bottle and incubated in BACTEC 9050 automated system. The bottles were kept in incubation for 21 days and they were subcultured when the machine signalised the growth. A blind subculture was performed after 21 days for the negative cultures.

Results: 31 patients, all of the patients with positive standart tube agglutination had bacteremia A positive result appeared in 3.5 days (84 h) as mean. Earlier detection were seen in second day and latest one at sixth days.

Conclusion: We concluded that automated BACTEC culture system can isolate *Brucella* spp. in a fast and efficient way. And we suggest at least 7 days in the case of Brusellosis suspicious.

P1661 Modification of 'gold standard' method for the diagnosis of *Chlamydia trachomatis* infection using peripheral blood leucocyte culture

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Chlamydia trachomatis is the most common disease among sexually transmitted diseases world-wide. Evidence of the family character of this infection additionally underlines the importance of the diagnosis of *C. trachomatis* infection especially its concealed, latent forms with asymptomatic course among adults and children as well. Considering the persistence of *C. trachomatis* infection in the organism and knowing that the most accurate method for the diagnosis of this infection in clinical specimens from all sites is the tissue culture technique employing ciclohexiimide treated McCoy cells, so called 'golden standard' we decided to investigate the cytomorphological peculiarities of *C. trachomatis* in the cultures of peripheral blood leucocytes of patients with urogenital chlamydiosis. For this aim the peripheral blood leucocytes of 14 patients infected only by *C. trachomatis* infection were studied using the original method of culturing. The diagnosis of *C. trachomatis* was confirmed by direct fluorescent-antibody technique. *In vitro* morphological inclusions specific for *C. trachomatis* were revealed in the cytoplasm of monocytes-macrophages, segmented neutrophils and even in lymphocytes, especially among children. In all cells these inclusions appeared to be PAS-positive cytochemically stained on glycogen. The use of peripheral blood leucocyte culture enabled us to diagnose the infection of *C. trachomatis* for the first time in 24 patients. Among them were two children at the age of 3 and 6 years and four patients with myelodysplastic syndrome, who applied to us for the estimation of immune reactivity of organism according to the amount of macrophage-lymphocyte rosette formation *in vitro* (Georgia Patent N 1296). In these cases the diagnosis of *C. trachomatis* infection was confirmed by direct or indirect immunofluorescent assays. According to our studies the macrophage-lymphocyte rosette formation was significantly decreased in 85% of patients with urogenital chlamydiosis composing 12–25% instead of $37.2 \pm 2.5\%$ (observed in healthy donors). pointing to the decreasing of the functional activity of immunocompetent cells. Results of our research show that blood leucocyte culture methods can be used for the diagnosis of latent forms of chlamydial infection and for the estimation of the efficacy of treatment and immune reactivity of organism as well.

Community-acquired infections

P1662 Infections due to *Abiotrophia defectiva* and *Granulicatella adiacens*: identification by sequencing of 16S rDNA and clinical manifestations

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Background: The taxonomy of nutritionally variant streptococci (NVS) has recently been modified, including the genus *Abiotrophia* created in 1995 and the new genus *Granulicatella* proposed in 2000. Reported clinical manifestations of NVS infections are endocarditis, septicemia and bacteremia. Isolated cases of keratitis, endophthalmitis, brain abscess, iatrogenic meningitis and osteomyelitis have also been described.
Objectives: Identify by molecular tool the NVS strains isolated from clinical specimens in our clinical laboratory, and correlate the molecular identification with the clinical diagnosis.
Methods: The strains of NVS isolated during the five last years from blood culture or vascular endograft specimen were retrieved from storage (one strain per patient) and identified by partial 16S rRNA sequence analysis. Patient's charts were reviewed and clinical characteristics were reported for each documented infection due to NVS.
Results: NVS were isolated from seven patients during the 5-year period. Identification based on partial 16S rRNA sequence analysis showed two genogroups: *Abiotrophia defectiva* (3) and *Granulicatella adiacens* (4). Clinical diagnoses for the three patients with *A. defectiva* were endocarditis in two, plus septic metastatic arthritis in one of them, and aortic endograft infection in one. For the four patients with *G. adiacens*, clinical diagnosis was febrile neutropenia with bacteremia in all four, plus possible endocarditis in one. Risk factors were malignant hemopathy (3) and solid tumour (1).
Conclusions: In our small serie, *G. adiacens* appears to be associated with bacteremia in febrile neutropenic patients, in contrast to *A. defectiva*, observed in non-neutropenic patients.

P1663 Mediterranean spotted fever: study of 29 cases

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Introduction: Mediterranean spotted fever also known as boutonneuse fever is an estival endemic zoonosis in the Mediterranean region. The dog tick, *Rhipicephalus sanguineus*, does the accidental transmission of *Rickettsia conorii* to men.
Aims: The authors claim to demonstrate the distribution of the disease in a Medicine Department during the period 1993-2003 relatively to sex, age, clinical symptoms, residence, job, animal contacts, seasonal distribution, 'tache noire' location, analytical alterations, complications and treatment.
Methods: Retrospective study of Mediterranean spotted fever between 1993 and 2003 in the Medicine III Department of Coimbra University Hospital.
Results: The peak incidence occurred during summer season, both sexes were equally reached with a predominance on the sixth and seventh decades of life. The 'tache noire' was not observe in all patients and the main clinical manifestations were fever and maculopapular rash involving the palms and soles. The more frequent laboratory alterations observed were: increased AST and ALT, thrombocytopenia and hiponatremia. The common complications were: shock, pneumonia and disseminated intravascular coagulation. Ten of the 29 patients had a positive serological result for *R. conorii*. Treatment with doxycycline was the most frequent adopted.
Conclusions: Generally, boutonneuse fever is a benign disease but, as it can progress to severe ill (shock, pneumonia) and lead to significative increase in mortality, is advisable to maintain a close follow-up of this cases.

P1664 A rare cause of wound infection: *Shewanella putrefaciens*

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Objectives: *Shewanella putrefaciens* is a saprophyte micro-organism distributed commonly in water and soil. Isolates of *S. putrefaciens* from human clinical specimens are uncommon and usually represent colonisation. It causes a wide range of infection from mild cellulitis to life threatening septicemia or endocarditis. We describe a scalp infection caused by *S. putrefaciens* in a healthy man.
Case: A 27-year-old man was admitted to the Department of Neurosurgery of Ankara Training and Research Hospital with wound infection on the scalp after accidentally hitting his head to a rock under the sea 5 days before. After the trauma his wound had been sutured at the emergency room. A purulent discharge had developed 3 days later. On physical examination, there was only a 10 cm length, superficial incision surrounding erythema area with purulent discharge on left parietal localisation. Other physical examination findings were normal. The full blood count showed a minor leucocytosis (14 000/mm^3). The lesion was debrided and resutured. Culture was taken from the lesion. The patient was given empirical antimicrobial therapy with intravenous cefazolin 3 g/day and a protective dressing on the affected region. On the third day from admission and the start of the therapy, wound culture became positive for *S. putrefaciens*. The identification of the isolate was performed using mini API instrument with ID32 E (bioMerieux sa, Marcy-I'Etoile, France). The microorganism was sensitive to ceftazidime, cefepime, cefoperazone, ciprofloxacin, gentamicin, imipenem and aztreonam. The treatment was changed to ciprofloxacin (500 mg bid, p.o.). He responded well to the treatment. He was discharged on the fifth day, still on treatment with ciprofloxacin, which was definitely withdrawn 10 days later.
Conclusion: Although *S. putrefaciens* infection are very rare it should be considered if a history of a marine contact is obtained.

P1665 Pathogens isolated from ambulatory patients with acute upper-respiratory tract disease

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Objectives: The aim of study was to estimate the frequency of bacterial infections in ambulatory patients with acute-respiratory tract disease.
Methods: We examined ambulatory patients from January to March 2003. A pharyngeal swab was taken from each patient. Microorganisms were identified by standard methods. Disc-diffusion susceptibility testing was done according to NCCLS.
Results: We examined 124 patients with acute upper respiratory tract infection aged 6 months to 72 years. (mean age 29.8 years). None of them was treated with antibiotic before microbiological examination. Each patient suffered had a 2–10 days history of upper-respiratory tract disease. A total of 44 (35.5%) pts had bronchitis, six (4.8%) laryngitis, 63 (50.8%) pharyngitis, eight (6.45%) sinusitis. Bacterial aetiology of infection was confirmed in 76 patients (61.3%). In other cases we found normal flora. The most often isolated pathogens were B-haemolytic streptococci (39.2%) susceptible to almost all antibiotics, *S. aureus* (29.4%) majority resistant to penicillin, *H. influenzae* (23.5%) mostly multisusceptible. *S. pneumoniae* we isolated in 6.8% (with one strain resistant to penicillin) and *M. catarrhalis* (1.1%).
Conclusions: (i) B-haemolytic streptococci were the most frequent aetiological agents in ambulatory patients suffering from upper respiratory tract infections. (ii). Most of isolated patogens were multisusceptible. (iii). Patients with negative cultures were suspec-

ted to have viral infections. (iv). High percentage of *S. aureus* may represent colonisation.

P1666 Characteristics of microbiologic profile in cervical samples of pregnant women and nonpregnant healthy young women

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Objectives: This study compares frequency of the most common isolates of genital tract between two groups: pregnant women (in first and third trimester of pregnancy) and nonpregnant healthy young women. Our goal was to define how many of them have colpitis in compare with them who have only colonisation.
Methods: Collected samples, endocervical swabs, were treated according to the standard laboratory procedures. Methods included: direct smear for inflammatory cells, dominant growth of etiologic bacterial agent after standard cultivation, biotyping, determination of antibiotic susceptibility and mycologic identification.
Results: During the 1-year period of investigation 95 pregnant women (each one in first and third trimester of pregnancy) and 102 nonpregnant healthy young women were controlled. All women were in generative age (20–43 years old). The patients with inflammatory cells (>10 per field), clinical symptoms and isolate were considered to have colpitis. The cases with microbiological isolate, epithelial cells in direct smear were considered as colonisation. The most common isolates in pregnant women in first trimester were: *S. agalactiae* (14%), *E. Coli* (11.5%), *Candida* (15.4%), while in third trimester the most common isolates were: *Candida* (32%), *E. coli* (7.6%) and *S. agalactiae* (6.4%). The isolates of nonpregnant healthy women included: *S. agalactiae* (8.8%), *E. coli* (8.8%) and *Candida* (16.6%). We noticed that women with isolated *S. agalactiae* or *E. coli* had deficit of *Lactobacillus* (only 11–13%) in comparison with women without pathogens (50–54%) in both groups. Inflammatory cells were found in 12.7% direct smears of nonpregnant women and in 5% of pregnant women.
Conclusion: In this study the most common isolates were *S. agalactiae* in first trimester of pregnancy. *Candida* was the most frequent in third trimester, which is in connection with elevate of pH in late pregnancy (but only 21.6% of them had conidial forms in direct smears which can be considered as infection). *Lactobacillus* concentracion is severely depressed in women with some other isolates wich can be explained as run for free areas. Amount of inflammatory cells is significantly elevated in nonpregnant women. Such a finding could be both the result of better control in pregnancy and the fact that pregnant women usually have only one partner. A complete evaluation of the cervical swabs in pregnancy should be preventive strategy for useful informations to obstetricians in order to avoid neonatal diseases.

P1667 Respiratory symptoms during leptospirosis: a retrospective study of nine patients

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Objectives: To describe leptospirosis of the lung in a rural area of France (Brittany).
Methods: We retrospectively reviewed medical records and chest radiographs of 34 consecutive patients with serologically confirmed leptospirosis admitted in our institution during years 1992–2002. To be qualified as confirmed leptospirosis, cases had to meet all three following criteria: (i) clinically compatible illness; (ii) presence of thermo resistant antigen or class IgM antibody (ELISA) titre of at least 1:400; (iii) positive microscopic agglutination test with a titre of at least 1:100.
Results: Nine patients (26.5%) had respiratory symptoms on admission. They were eight males and one female with a mean age of 47 years. Symptoms on admission included cough ($n = 4$), shortness of breath ($n = 4$), cyanosis ($n = 2$) and haemoptysis ($n = 1$). Six patients had pulmonary radiographic abnormalities including : (i) diffuse, ill-defined, ground glass density ($n = 3$); (ii) diffuse alveolar opacities ($n = 2$); (iii) small nodular density ($n = 1$). Seven patients reported exposure source including hunting ($n = 2$), fishing ($n = 2$), fresh water swimming ($n = 2$) and canoeying ($n = 1$). All patients had high grade fever on admission with a mean of 40.1°C. Other common features included headache ($n = 4$), vomiting ($n = 3$) and myalgia ($n = 3$). The most common biological abnormalities included elevated liver enzymes ($n = 8$), proteinuria ($n = 7$), lymphopenia ($n = 6$), haematuria ($n = 5$), renal failure ($n = 4$), anaemia ($n = 4$) and elevated neutrophils count ($n = 4$). PaO_2 was measured for three patients while they were breathing room air and was at 32, 55 and 66 mmHg. No patients required ventilation support. Suspected diagnosis on admission included: leptospirosis ($n = 2$), bacterial pneumonia ($n = 2$), intoxication ($n = 1$), influenza ($n = 1$), viral hepatitis ($n = 1$), biliary tract lithiasis ($n = 1$) and rapidly progressive glomerulonephritis ($n = 1$). The first serological testing for leptospirosis was positive for five patients (55%). Serovar could be determined for seven patients, including *L. australis* ($n = 3$), *L. grippotyphosa* ($n = 2$) and *L. ictero-hemorragiae* ($n = 2$). Seven patients were treated with penicillin G or A; two patients received no antibiotics. All patients were cured.
Conclusion: Patients with leptospirosis may present predominantly with aspecific pulmonary symptoms. In these patients, leptospirosis must be suspected when there is a potential exposure to rats, especially in case of high grade fever, myalgia, hepatitis and renal abnormalities.

P1668 Abscesses of skin and skin structures: insights from a randomised study of ertapenem vs. piperacillin-tazobactam

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Objectives: Ertapenem (ETP), a group I carbapenem, was as effective as piperacillin/tazobactam (P/T) in the treatment of complicated skin and skin-structure infections. In this subgroup analysis, we compare the demographics, microbiology, need for surgical intervention, and response to therapy in patients with and without abscesses.
Methods: In a double-blind study with sponsor blinding, adults with complicated skin or skin-structure infections of moderate-severe intensity requiring 7–14 days of parenteral antibiotic therapy were randomised within two strata to receive ETP 1 g once a day or P/T 3.375 g every 6 h. Patients with pressure ulcers or with diabetic or neuropathic lower extremity infections (stratum I) were excluded from this analysis. Results for stratum II patients with and without abscesses were analysed using clinically evaluable patients (EP analysis) and all treated patients satisfying the case definition [modified intent-to-treat (MITT) analysis]. Clinical response was assessed 10–21 days post-therapy test of cure (TOC). Patients with missing assessments were counted as failures in the MITT analysis. Surgical procedures during the first 48 h of study therapy were regarded as part of standard care. Patients requiring extensive debridement or incision and drainage (I + D) after the initial 48 h were considered failures.
Results: A total of 141/281 (50%) EP had abscesses. EP with abscesses did not appreciably differ from those without abscesses in age, gender, race, or severity of infection. The N (%) of EP with vs. without abscess infected by the most common pathogens were: 53 (38%) vs. 50 (36%) for *S. aureus*; 26 (18%) vs. 29 (21%) for haemolytic streptococci; 33 (23%) vs. 40 (29%) for Enterobacteriaceae; 4 (3%) vs. 5 (4%) for *P. aeruginosa*; and 122 (87%) vs. 43 (31%) for anaerobic/microaerophilic bacteria. Extensive debridement or I + D was performed in 75% of EP with abscesses and 49% without abscesses during the 48 h before or after initiation of study therapy. Median duration (range) of therapy in EP was 7 (3–15) and 8 days (5–16) for ETP and P/T when abscesses were present

vs. 9 (5–16) and 9 days (4–17) when absent. Favourable response rates are shown in the Table.

		at TOC assessment			
	Abscess	ETP		P/T	
EP	yes	63/70	90%	66/71	93%
	no	61/73	84%	54/67	81%
MITT	yes	76/97	78%	80/98	82%
	no	78/110	71%	77/108	71%

Conclusions: Response rates to ETP once a day or P/T four times a day were similar for patients with or without abscesses. Anaerobic bacteria were recovered more often when an abscess was present. Early operative intervention was performed in a higher proportion of patients with abscesses.

P1669 Classification of clinically-relevant micro-organisms in nonmedical environments

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Objectives: History and modern research have shown us that the looming crisis of antimicrobial resistance cannot be avoided, but it can be controlled. Our ability to control the rate at which resistance develops and spreads requires an understanding of bacterial adaptation, colonisation and infection. Environmental studies are the first important step in clarifying the evolutionary trends and molecular relationships between strains of community associated bacteria. Data from such studies can provide information used to develop viable long-term strategies for the treatment of infectious disease. The objectives of this study were to collect, isolate and characterise samples of *S. aureus* from areas utilised by midshipman athletes at the United States Naval Academy.
Methods: Environmental samples were collected from 16 indoor and outdoor varsity and intramural athletic areas. These samples were classified in several steps including their gross morphology, determinative biochemical activities and DNA sequences. In addition, the results obtained from environmental samples were correlated with local clinical isolates, and sequence comparison of specific genomic loci was performed.
Results: Observed differences in antibiotic sensitivity, protein A and coagulase production in samples indicated the presence of at least three to five distinct strains of *S. aureus*. Three strains exhibited resistance to oxacillin.
Conclusions: The increasing prevalence of observed antibiotic resistance in athletic facilities is representative of many factors that have complicated recent attempts to model bacterial interaction and evolution in nonmedical environments, but new genetic information and effective evolutionary trend analysis will refine the relationships between such strains. Systematic surveillance and documentation of environmental factors such as weather, frequency of use, and methods of cleaning can then be added to model and eventually control the development and spread of antimicrobial resistance.

P1670 Comparison of azithromycin and amoxicillin for treatment of adult patients with solitary erythema migrans

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Objective: To evaluate the effectiveness and side-effects of azithromycin and amoxicillin for treatment of adult patients with solitary erythema migrans.
Methods: Consecutive adult patients with typical erythema migrans were enrolled in a prospective study on early Lyme borreliosis at the Department of Infectious Diseases in Ljubljana during 1997. Patients receiving antibiotics at their first visit, having clinical evidence of disseminated *Borrelia burgdorferi* s.l. infection, and/or being pregnant were excluded. They were randomised to receive either azithromycin 500 mg b.i.d. for the first day, followed by 500 mg once a day for the following 4 days (AZT) or amoxicillin 1000 mg t.i.d. for the first 5 days, followed by 500 mg t.i.d. for the following 10 days (AMO). Basic epidemiological data were obtained by means of questionnaires. Serum IgM and IgG antibody titre against *B. burgdorferi* s.l. were determined by IFA without absorbtion. Titres of ^{3}1:256 were interpreted as positive. In all patients skin biopsy had been accomplished prior to the institution of antibiotic treatment and specimen cultured in MKP medium.
Results: A total of 133 patients, 77 (57.9%) females and 56 (42.1%) males, aged 16–83 years (median 49) were included in this study. 65 patients were evaluated in AZT group and 68 patients in AMO group. No differences in epidemiological and pretreatment characteristics were present comparing the two groups. Median duration of skin lesions after the institution of treatment was 7 days (1–60) in the AZT group and 7 days (2–180) in the AMO group ($P = 0.325$). During the follow-up of 12 months none of the patients developed major late manifestations of *Lyme borreliosis* but in six patients severe minor manifestations appeared: in two (3.1%) from AZT group and in four (5.9%) included in AMO group. Isolation rates of *B. burgdorferi* s.l. from skin before treatment (25/65 vs. 33/68; $P = 0.319$) and 2–3 months after therapy (0/25 vs. 0/33) were comparable for the two groups. Three (4.6%) AZT group patients and one (1.5%) patient from AMO group reported mild gastrointestinal discomfort ($P = 0.358$).
Conclusions: Treatment of adult patients with solitary erythema migrans with two different antibiotics exhibited equal effectiveness and comparable side-effects. The outcome of borrelial infection after 1 year was favourable in both treatment groups.

P1671 Refractory gingivitis in a patient with osseointegrated implant by the transmission of *Actinobacillus actinomycetemcomitans*

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Objectives: The presence of periodontopathogenic bacteria is a risk factor for peri-implantitis. Microbiological testing of the subgingival flora can be helpful to estimate the risk of implantation failure. Periodontal pathogenes, especially *Actinobacillus actinomycetemcomitans* (A.a.) show a high potential of transmission of family members. The purpose was introducing A. a. transmission in a couple with implantation. We examined the presence of periodontopathogenic bacteria prior the implantation of a 42-years-old patient. The result was negative, a double Restore golden implant was made for her, succesfully. After 10 months, suppuration appear next to the implant.The result of the microbiological cultivation was positive for the A.a. In order to detect the potential source of infection we collected four different samples from the husband. We made cultivation from the dorsum of the tongue, the buccal mucosa, the tonsils and pooled samples of three teeth. A.a. was isolated from all of the places except the dorsum of the tongue.
Methodes: The purpose of the investigation was to study of restriction endonuclease analysis (REA)-types present in the oral cavity of A. a. positive subjects and to study the possibility of transmission of A.a. within families. DNA of A.a. isolates was digested with a combination of the restriction endonuclease PstI and BamHI, after which DNA fragments were separated by agarose gel-electrophoresis.
Results: The result showed only one REA-type is present.
Conclusions: Elimination of the periodontal pathogens from the patient's and partner's oral cavity before administering dental implant treatment may inhibit colonisation by these pathogenes and reduce the risk of peri-implantitis.

P1672 The micro-organisms isolated from sinovial fluid

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Objectives: Infectious arthritis is an infection of sinovial fluid caused by several microorganisms and may cause serious complications including degenaration of the joint if remains untreated. So, early diagnosis and appropriate theurepatic approachment is very important in prevention of complications and sequels. The identification of microorganisms that cause arthritis constitude the basement of appropriate antimicrobial threapy. In that study we aimed to isolate and identify the microorganisms that cause arthritis, from the specimens of sinovial fluids that are accepted to our laboratory.
Method: We analysed 189 sinovial fluid specimen sent from several wards for identification of infectious agent that cause arthritis, from May 2002 to September 2003. Each material is stained in either Gram or Ehrlich–Ziehl Neelsen methods. All of the specimen were inoculated to human blood agar, EMB Agar, Leuwenstein-Jensen and Sabourad media and also incubated automated-calorimetric BacT/Alert microbial detection system (bioMeriux, France). Growing Gram-positive and Gram-negative bacteria were identified by using VITEC and API identification system. The yeast form microorganisms were identified by using germ tube test and API ID 32 C system lactophenol cotton blue stain is used to identify the filamentous fungal elements.
Results: We isolated the infectious agent in 103 of 189 sinovial fluid specimen that sent to our laboratory. The frequecy of isolated microorganisms are in that order; *Staphylococcus aureus* 53 (51.47%), coagulase-negative *Staphylococcus* 20 (19.43%), *Streptococcus* spp. seven (6.79), *Acinetobacter calcoaceticus* four (3.88%), *Brucella mellitensis* three (2.91%), *Pseudomonas aeruginosa* three (2.91%), *Klebsiella pneumonia* three (2.91%), *Proteus mirabilis* two (1.94%), *Enterococcus faecium* one (0.97%), *Aerococcus viridance* one (0.97 %), *Salmonella schutmuelleri* one (0.97%), *Enterobacter aerogenes* one (0.97%), *Alcaligenes xylosus* one (0.97%), *Mycobacterium tuberculosis* one (0.97%), *Candida tropicalis* one (0.97%), *Aspergillus fumigatus* one (0.97%). According these results; *Staphylococcus* spp. are the most common (71%) infection agents in infectious arthritis. We determined the methicillin resistance in 29 (39.7%) of the isolated staphylococcus strains (18 of them are MRSA and 11 are MRCNS)
Conclusions: It will be suitable to initiate the empirical theurapy towards to *Staphylococcus* strains especiallly having methicillin resistance regarding to underlying disease and patients' clinical situations.

P1673 Charasteristics of cutaneous anthrax in Turkey

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Objective: To evaluate the epidemiologic and clinical characteristics of 53 adult cutaneous anthrax cases.
Methods: The charts of the patients, who had been hospitalised between 1992 and 2003 in the Infectious Diseases and Clinical Microbiology Department of Ankara Numune Education and Research Hospital, were reviewed.
Results: The mean age was 47 and 36% were female. The most common professions were farmers (59%), butchers(20%), and housewives (17%). The mean incubation period was 8 days. Most of the cases (60%) exposed to bacteria when butchering sick animals. Sixteen patients used antibiotic before addmission to hospital (30%). The most common effected site of lesions were hand (38%) and finger (32%). Other sites were arm (8%), eyelid (8%) and neck (4%). Dissemination of lesion was seen in 28% of patients. Gram stain was positive in nine cases and culture was positive in five cases for *Bacillus anthracis*. All except one patient discharged and treated with penicillin and ciprofloxasin. One patient with disseminated lesion on neck died, although steroid was used with antibiotic.
Conclusion: Cutaneous anthrax is still a public health problem in Turkey. Cutaneous anthrax should be considered in any patient with a painless ulcer with vesicles, oedema and a history of exposure to animals or animal products. Penicillin and ciprofloxacin were effective in treatment of anthrax.

P1674 Prevalence β-haemolytic streptococci from throat swabs in health care area from Pontevedra, Spain

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Objectives: β-haemolytic group A, and G *Streptococcus* are a major cause of upper-respiratory infection such of pharyngitis and tonsillitis. We studied the prevalence of β-haemolytic group A, C and G *Streptococcus* in pharyngotonsillitis in our area.
Material: All throat swabs collected from our health care area, including paediatric population, during 11 months of 2003 were plated to blood agar in aerobic athmosphere and CNA medium in anaerobically conditions and incubated at 35°C during 48 h. Testing for Lancefield's Group was realised by Streptex (Remel Europe Ltd, UK). In addition to culture, rapid detection of group A *Streptococcus* was performed in 326 throat specimens (Abbot Testpack Plus StrepA).
Results: From January 2003 to December 2003 a total of 1411 throat swabs from patients with pharyngotonsillitis were studied. β-haemolytic streptococci were isolated in 270 (19%) of them: 114 (8%) from group A, 115 (8%) from group C and 32 (2.3%) from group G. *Streptococcus* group B were isolated in 9 (0.6%) Table 1. Only 49 (15%) from 326 rapid test of group A antigen were positive, and the rest ones wich were negative showed in the culture other β-haemolytic streptococci (group C and G) and normal flora of the upper respiratory tract.
Conclusions: These results show a similar prevalence of *Streptococcus* group A (8%) and *Streptococcus* group C (8%) and lower prevalence of *Streptococcus* group G (2.3%) in studied patients with pharyngotonsillitis. The results of the rapid test, only has been useful in the 15% of the cases studied (326). Then, the prevalence of another β-haemolytic nongroup A support the convenience of use culture besides rapid test to study the aetiology of pharyngotonsillitis.We also want to detach the importance of anaerobically conditions to recover other β-haemolytics nongroup A.

P1675 Acute bacterial sinusitis: 43 cases of complications

D. Stoll and the ORLI group

Objectives: To describe the observed complications (C) of acute bacterial sinusitis in France, the underlying diseases (or conditions), the pathogens involved, the surgical and medical management, and the prognosis.
Methods: Prospective study conducted by six French hospitals between November 2001 and March 2003 in patients older than 15 years and hospitalised with a diagnosis of a complicated community-acquired, presumed bacterial sinusitis. The clinical, radiological and bacteriological data were collected with documentation of the surgical and medical management. A descriptive statistical analysis was performed.
Results: This is the first prospective study performed in France in this area, with a collection of 43 cases of C. They were: ocular ($n = 15$), meningo-encephalic ($n = 16$, of which 12 cases of meningitis), sub-cutaneous ($n = 8$) or combined ($n = 4$). No risk factor (tobacco consumption, diabetes mellitus, use of corticosteroids, viral infection) was present. When an antibiotic treatment was prescribed before the onset of the C, it was in adequation with the French guidelines. The C were noted after a pan sinusitis (involvement of all sinuses) in the majority of the cases, or after a frontal or sphenoid sinusitis. The responsible pathogens were identified in 53% of the cases, mainly *Streptococcus* spp., with or without combination to anaerobic bacteria. In the meningitis cases, *S. pneumonia*e was the main causative pathogen. The medical treatments prescribed were in accordance with the recommended

standards. Conversely, the surgical management was more heterogeneous. Cure was obtained in 60% of the cases with a mortality rate of 2%. Sequels, essentially ocular, were not rare. Successive C could occur, indicating the need for a prolonged clinical and radiological follow-up.
Conclusions: In adults, severe C following bacterial sinusitis are still observed, in spite of an adequate antibiotic treatment and a susceptibility of the pathogens to the usual antibiotics, with a high morbidity and a residual mortality. Obtaining the complete disappearance of the C is unrealistic, however the present low rate can be expected to be maintained by an adequate antibiotic treatment, in terms of choice of the drug and respect of the treatment duration. A better standardisation of the surgical management is warranted.

P1676 Distribution of sites of infection and T-types of *β*-haemolytic streptococci group A during a period of hyperendemicity in a Danish region

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Objectives: The peak incidence of infections due to *β*-haemolytic streptococci group A (GAS) is during the winter. In the beginning of 2003, we became aware of more GAS infections than expected, which coincided with many reports of impetigo from general practitioners (GPs). In order to ascertain the problem we undertook a prospective survey of GAS infections during February and March 2003 in the Danish County of North Jutland.
Materials and methods: The Department of Clinical Microbiology, Aalborg Hospital provides diagnostic bacteriology to the County of North Jutland (approximately 500 000 inhabitants) and serves around 300 GPs and seven hospitals. Information related to positive cultures was obtained from GPs and hospital physicians. T-typing was done by the *Streptococcus* Unit, Statens Serum Institut, Copenhagen. Retrospective data was retrieved from the department's laboratory information system (ADBakt, Autonik, Sweden).
Results: During February–March 2003, we observed a total of 421 GAS infections (approximately 90/100 000), an increase of 75% compared with the same two months in 2002 and 60% above the average for these 2 months during the previous 6 years. Preschool children accounted for 48%, the age group 7–14 years 20%, young aged 15–24 years 2%. A second peak incidence occurred in the age group of 30–39 years (11%). The skin was the most common site of infections (36%), followed by the middle ear (24%) and throat (23%). Perianal streptococcal infections (PASI) came fourth in line (6%). We diagnosed 26 cases of PASI (12 boys, eight girls and six adults); most children were below 5 years of age. The predominant T-types were T1 (47%) and T28 (20%). T1 was more prevalent in middle ear (64%) than throat (51%) and skin infections (37%), whereas T28 was more prevalent in PASI (50%) than skin infections (25%), middle ear (12%) and throat (11%).
Conclusions: During a period of hyperendemicity of GAS infections we found two T-types to be predominant and the clinical spectrum seemed to be linked to the particular T-type: otitis media to T1, and skin infections (incl PASI) to T28. We have previously experienced a community outbreak of PASI (*Pediatr Infect Dis J* (2003) **22**, 105–109), and the current observations underline that PASI continues to be a clinical problem possibly linked to the high prevalence of T28.

P1677 Assessment of documentation of markers of sepsis and appropriateness of antimicrobial prescribing in an acute medical admission unit prior to and following the introduction of a sepsis stamp

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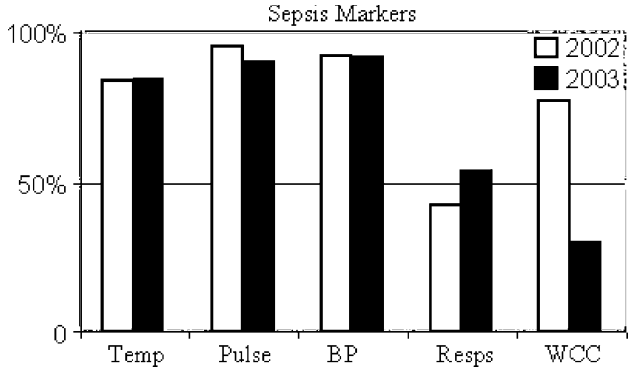
Objectives: To assess the quality of recording of markers of sepsis prior to commencement of antimicrobial therapy and adherence to local antibiotic guidelines in an acute medical receiving unit prior to and following the introduction of a sepsis stamp in the clinical notes.
Methods: Data was gathered from case notes of all patients admitted to a medical receiving unit and receiving antibiotics within 24 h of admission over a fixed period of time in winter 2002. The study was repeated in winter 2003 following the introduction of a clinical stamp for recording markers of sepsis, which was included on the clerking sheet for all patients, the same data was collected from this second group of patients and the results compared. The presence or absence of sepsis and its severity was assessed using a scoring system based on a relevant clinical history plus two or more of the following (one point for each positive), temperature >38°C or <36°C, pulse >90/min, respiratory rate >20/min, white cell count <4 or >12, blood pressure systolic <90 mmHg or diastolic <60 mmHg. Total score <2, no sepsis; 2, sepsis, >2, with evidence organ dysfunction e.g. confusion, hypoxia, severe sepsis; >2, with systolic BP <90 mmHg refractory to fluid replacement, septic shock. The appropriateness of the antibiotic prescribed and route was assessed based on local antibiotic policy.
Results: A total of 629 patients were included in the initial study in 2002 and 200 so far in 2003, which is still ongoing. The mean ages in the two groups were 66.3 years and 60.1 years, respectively, with a male:female ratio of 43:57 and 47:53. The recording of sepsis markers in the two groups is shown in graph 1. The stamp itself was fully completed for 8% of the patients, partially complete in 9.5% and ripped out, ignored or covered in 82.5%. Patients (50%) did not meet sepsis criteria in the first study and 64% in the second with 64.5 and 60.4% of patients being prescribed antibiotics via the intravenous route. The choice of antibiotic was in accordance with local guidelines in 55% of patients in the initial study and in 49% in the follow-up.



Conclusions: Recording of sepsis markers is poor particularly with regard to respiratory rate and white cell count. Introduction of a sepsis stamp did not improve this or appear to influence antibiotic prescribing practice. Intravenous antibiotics are often prescribed inappropriately and although antibiotic guidelines are available they are often not used.

P1678 Levofloxacin experience in a general hospital

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Objective: To analyse the clinical profile and the outcome of patients treated with levofloxacin (LF) in our hospital.
Method: Retrospective and descriptive study of all the in-patients treated with LV oral or intravenous in our centre from September 2001 to April 2002. We used a general database (without the patient from emergency, critical care, paediatry and gynaecology).
Results: We used LF in 225 patients. Mean age 67 ± 15 years (range 24–99). Gender, 68% male, comorbility, 35% suffered from chronic obstruction to airflow (COAF), 26% diabetes and 6% HIV

infection. We detected 19% of penicillin allergic patients. There were previous treatment with other antibiotic in 30%, that antibiotic was 50% a β-lactamic. In previous months 19.3% suffered from upper respiratory-tract infection. Up than 80% of LF was indicated by respiratory infection, which were pneumonia in 45%, no pneumonia in 37% (60% COAF exacerbated), 6% sinusitis, 2% urinary tract infection, 2% abdominal infections and 1% skin infection. Bacteria isolated were obtained in 41 cases (19%): *E. coli* (seven cases), *Pseudomonas* (six cases) and *S. pneumonia*e (five cases). Respiratory insufficiency was present over 50%. In 44 patients intravenous therapy was initiated and followed during a mean time of 2.7 ±3 days and was completed orally during a mean time of 7.2 ± 4.1 days. A second antibiotic was associated in 18% of cases, mainly a β-lactamic. LF was suspended in 17%: 5% by worsening, 7% by side-effects, 4% by resistance. A total of 17 patients died (7.6%) and two needed critical care. Side-effects: six patients (2%) suffered from diarrhoea, five from nausea and two rash. Serum kalium was touched on 25 cases (11%) without clinical relevance.

Conclusions: In our area LF is an antibiotic used commonly in respiratory infections and as unique drug. It is specially used in penicillin allergic patients. Normally the treatment is started by intravenous way switching quickly to treatment by mouth with good tolerance.

P1680 Characterisation of *Staphylococcus xylosus* strains isolated from humans clinical material

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Objectives: Staphylococci are common inhabitants of skin, skin glands and mucous membranes of mammals and birds. Certain *Staphylococcus* species are found as aetiological agents of a variety of human and animal infections as well. *S. xylosus* and *S. equorum* represents two phylogenetically and biochemically close species. Although *S. xylosus* is usually only transient on humans and is primarily acquired from domestic animals and their products it has been isolated from clinical material, mainly from urinary-tract infections. It is difficult to distinguish *S. xylosus* and *S. equorum* on the base of the biotyping. *S. equorum* was isolated from animal sources, but it has not been isolated from human clinical specimens yet.

Methods: A group of 18 presumptive *S. xylosus* strains isolated from human clinical specimens and reference strains (*S. xylosus*, *S. equorum* and *S. gallinarum*) were analysed by biochemical tests and ribotyping. Biochemical properties were tested by API Staph and ID 32 Staph kits and by conventional tests. Ribotyping was done with *Eco*RI and *Hin*dIII restriction enzymes and a probe complementary to 16S and 23S rRNA from *E. coli*.

Results: Phenotypic data of key tests corresponded with species description of *S. xylosus* except for one intermediate strain *S. xylosus*/*S. equorum*. Some results obtained for acid production were different from *S. xylosus* description, but identification to the species level was acceptable. Ribotyping with *Eco*RI divided tested strains into two groups, the *S. xylosus* group and smaller group which, as we supposed, belongs to the *S. equorum* species. This group contained of two reference strains and four clinical isolates. Group *S. equorum* was clearly distinguished also with *Hin*dIII, but *S. xylosus* group was divided into three smaller groups by using *Hin*dIII. Results of ribotype profiles did not correspond with biochemical characterisation of the isolates.

Conclusions: There were identified 17 *S. xylosus* strains and one intermediate strain *S. xylosus*/*S. equorum* by biochemical tests. Contrary to biotyping, the colony size on the P agar and the ribotyping with both used restriction enzymes, showed, that four strains from tested series represent *S. equorum* species. This is the first case of occurrence *S. equorum* in human clinical specimens. Ribotyping with *Eco*RI and with *Hin*dIII showed heterogeneous ribotype profiles. These results imply that this method could be suitable for intraspecies characterisation of *S. xylosus* and *S. equorum*.

P1681 *Staphylococcus lugdunensis*: an overlooked but easy identifiable, frequent cause of abscesses in general practice

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Objectives: *Staphylococcus lugdunensis* (Sl) is a coagulase-negative *Staphylococcus* known as a rare cause of severe infections. As a consequence of two serious infections we decided to optimise identification of Sl and make a comprehensive epidemiological analysis of the Sl infections in Viborg County (population: 235 000), Denmark.

Methods: All specimens send to department of Clinical Microbiology, Viborg County from July to December 2002 were included. Specimens were inoculated on Columbia agar plates containing 5% sheep blood. Suspected staphylococci were identified by catalase, slide coagulase test, colony phleomorphism, β-haemolysis, and examined for a characteristic 'Eikenella-like' smell after 2 days of incubation. Strains suspected to be Sl were examined with API-ID-32-Staph. All cases positive with Sl were followed up by a telephone interview with the general practitioners or by reading the hospital records.

Results: When cultured on sheep blood agar, Sl develops a characteristic smell on the second day of culturing. This smell together with β-haemolysis and colony phleomorphism is very helpful as a first step screening for the presence of Sl in the routine cultures. The total incidence of Sl infection in Viborg County was 4/10 000 per year. Sl infections were three times more common in general practice than in hospitals. Sl was found in 13% of all microbiologically examined abscesses with bacterial growth from general practice. Most patients were otherwise healthy. All patients needed antibiotics and/or surgical treatment. Most of the infections were abscesses, and 70% of them were monocultures. The localisation of the infections seems to be age dependent.

Conclusions: Calculated from our incidences 3–5% of the otherwise healthy population will be Sl infected at least once during their life span, and our findings are most probably a low estimate. Sl is an important and common cause of abscesses. It should be looked for and identified in all bacteriological examinations. Furthermore the microbiologists should make all general practitioners and hospital physicians acquainted with its name, the pathology and the risk of serious complications.

P1682 Frequency and clinical spectrum of *Staphylococcus lugdunensis* infections – a French national survey: preliminary results

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Objectives: To evaluate frequency and clinical spectrum of *Staphylococcus lugdunensis* infections in French general hospitals, over a 2-year period.

Methods: The ColBVH study group is a French network of 196 nonteaching sentinel hospitals. From January 2002 to December 2003, all correspondent laboratories of the group were asked to declare via our website all cases of *S. lugdunensis* infection. Identification methods and susceptibility testing were performed as routinely by trained microbiologists. Clinical data were recorded in view to describe the pattern of infections and to establish precise clinical relevance of the isolates.

Results: A total of 94 cases of *S. lugdunensis* infection, corresponding to 91 patients (56% males, median age 56 years), have been recorded from 32 unlinked hospitals. The origin of the isolates are skin and soft tissue (41), haemocultures (14), urine (nine), bone or joint fluid (nine), surgical wound (seven), i.v. catheter or prosthetic material (seven), various other sites (seven). *S. lugdunensis* was isolated as a sole agent in 81% of cases. All but three cases have been confirmed as infections. Soft tissue abscesses (26) and skin infections (19) are predominant (48% of the overall). The other types of infection are: septicaemia (11), urinary-tract infections (eight), bone and joint infections (seven), surgical wound infections (five), prosthetic material infections (five), endocarditis (one), i.v. catheter-related infections (one), miscellaneous (five).

The infection is nosocomially acquired in 31% of cases and associated with foreign device in 21%, mainly orthopaedic prosthetic material (nine cases). 28% of the isolates are β-lactamase positive and 2% are methicillin-resistant.
Conclusions: *S. lugdunensis* is now well recognized as a pathogen in superficial, but also deep serious infections. However, it may be difficult for the laboratory to establish relationship between isolation of *S. lugdunensis* and infection, as the bacteria is part of normal human flora, and may be isolated as colonising organism. Our survey was conducted to evaluate the frequency of true *S. lugdunensis* infections, and clinical data were collected and analysed in this aim. The preliminary results confirm the wide range of clinical patterns and emphasise the predominance of skin and soft tissue infections. Further investigations are actually conducted (i) to review the patients medical records by and independant physician, (ii) to search for virulence factors in the strains collected.

P1683 Changes in management and outcome of *Staphylococcus aureus* bacteraemia 1996–2003

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Objectives: *Staphylococcus aureus* is a common and important microorganism in modern medicine associated with increasing antibiotic resistance. Mortality of *S. aureus* bacteraemia (SAB) has been reported to be as high as 30%. The aim of this study was to evaluate the management, outcome and predictive factors of SAB between 1996 and 2003. The study also investigated the impact of the introduction of an infectious diseases (ID) consult service in 2001.
Methods: All in-patients in the Cantonal Hospital St Gallen with an episode of SAB between April 1996 and June 2003 were included. Exclusion criteria were: polymicrobial bacteraemia; only one positive blood culture bottle without other signs of infection; if SAB was not treated because of a terminal underlying disease, or if the patients record was not available. We reviewed retrospectively the patients records for demographic data, clinical course of infection, underlying conditions, focus of SAB, treatment, complications and outcome. Treatment was considered to be adequate if it was in accordance with the written guidelines of the ID service.
Results: Among 204 patients with SAB (136 community-acquired; 68 nosocomially acquired) 106 were cured, 41 (20%) died, nine experienced a relapse, and 19 had persistent signs of infection. In 38 cases no outcome information was available. Risk factors for mortality were sepsis or septic shock at presentation ($P < 0.00001$) and age >65 years ($P = 0.04$), but not inadequate treatment. In univariate analysis, inadequate choice of antibiotics ($P = 0.02$) and inadequate length of treatment ($P < 0.001$) were significant predictors of mortality. After the introduction of an ID consult service the proportion of patients with inadequate treatment was significantly lower ($P = 0.03$). Despite the improved management, mortality was higher after the introduction of ID consultations. However, we observed an increase in severity of disease and underlying conditions during the same period. There was also an increase in catheter related SAB ($P < 0.001$).
Conclusions: An increasing trend in severe cases of SAB was observed over time. Improved management of SAB (adequate selection of antibiotic drugs and appropriate length of therapy) were associated with a better treatment outcome and with the introduction of an ID consult service.

P1684 Clinical aspects and risk factors for mortality of *Staphylococcus aureus* sepsis in a Swiss tertiary Centre

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Objectives: To analyse the clinical, therapeutic and microbiological aspects of *S. aureus* (SA) sepsis occurring at the University Hospital Basel, with an emphasis on risk factors for mortality.
Methods: We retrospectively analysed the charts of 308 patients, hospitalised between 1998 and 2002, who had a systemic inflammatory response syndrome and at least one positive blood culture for SA. Bacteraemia due to MRSA was not analysed separately since there were only six episodes (2%).
Results: We found 150 patients with community-acquired and 158 with nosocomial SA sepsis. 78 patients (52%) with community-acquired infection had primary bacteraemia. The most common focus for nosocomial SA sepsis was i.v. catheter-related infection (96 episodes or 61%). There were 52 patients (17%) with endocarditis. An infectious diseases (ID) specialist was consulted in 82% of all cases. Empiric and definite antibiotic therapy were correct in 77 and 88% of all episodes, respectively, according to the Sanford Guide to Antimicrobial Therapy or to our written hospital guidelines. The mean time between drawing blood cultures and starting correct antibiotic therapy was 10.7 h. The most common empiric therapy was amoxicillin/clavulanate; the most common definite therapy was flucloxacillin. 31 patients (10%) developed septic shock, 70 (23%) had acute renal failure, and 73 (24%) developed secondary infectious foci. ICU-care because of sepsis was necessary in 66 patients (24%); 20 (6%) required mechanical ventilation. Crude in-hospital mortality was 20%, the mean time from the first positive blood culture to death being 8 days. In multivariate analysis, significant risk factors for mortality were age, alcoholism, chronic obstructive pulmonary disease, primary SA bacteraemia, septic shock, and acute renal failure. Factors significantly associated with a better prognosis were i.v. catheter-related infection as the source of bacteraemia and consultation of an ID specialist.
Conclusions: Despite adequate antibiotic therapy and the progresses of intensive care medicine, SA sepsis remains a serious infection with a high mortality. Notably, consulting an ID specialist decreases the in-hospital mortality.

P1685 Microbiological results from a randomised, open-label study of ertapenem vs. piperacillin/tazobactam for the treatment of community-acquired intra-abdominal infections requiring surgery

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Objective: We compared the microbiological response rates of ertapenem (ERT, a once-a-day parenteral group one carbapenem) to that of piperacillin/tazobactam (P-T) in the treatment of IAI requiring surgical intervention.
Methods: In an open-label, multicentre trial, 370 adults with clinical and/or radiographic evidence of IAI who required surgery were randomised to ERT 1 g or P-T 13.5 g daily for 4–14 days. Appropriate specimens for aerobic and anaerobic cultures were obtained at baseline and at the time of clinical failure if feasible. Blood cultures were also collected throughout the study when clinically indicated. The primary endpoint was the clinical efficacy in microbiologically and clinically evaluable patients 2 weeks post-therapy [test of cure (TOC)]. Following the initial analyses, a *post hoc* re-analysis was conducted after correcting for errors and missing-data. Overall microbiological response required documented or presumptive eradication of all baseline intra-abdominal and/or blood pathogen(s) in order to be favourable. Reported here are the secondary endpoints of the post-hoc analysis examining the overall microbiological response rates and the clinical response rates by baseline intra-abdominal or blood pathogen in microbiologically and clinically evaluable patients.
Results: Baseline characteristics, median treatment duration (6 days), and clinical response rates at TOC (>89%) were comparable in both treatment groups. 75.6 and 69.5% of all treated patients in the ERT and P-T groups, respectively, had at least one pathogen identified at baseline from IAI or blood cultures. The large majority of baseline isolates, other than the infrequently encountered isolates of *P. aeruginosa* and enterococci, were susceptible to both study regimens. In the *post hoc* analysis, the

overall microbiological response rates in the evaluable patients at TOC were 95.2% for ERT and 93.2% for P-T [difference 1.9; 95% CI (−4.3, 8.6)]. Clinical response rates in evaluable patients at TOC for the most common baseline pathogens are shown below.

	Treatment Group	
	Ertapenem n/N+(%)	P-T n/N+(%)
Gram-negative aerobic bacilli	97/105(92.4%)	94/101(93.1%)
• *E. coli*	80/88(90.9%)	80/87(92.0%)
• *K. Pnemoniae*	10/12(83.3%)	4/4(100.0%)
• *K. oxytoca*	7/7(100.0%)	2/2(100.0%)
• *Proteus mirabilis*	5/5(100.0%)	4/4(100.0%)
• *P. aeruginosa*	11/11(100.0%)	5/6(83.3%)
Anaerobes	22/25(88.0%)	31/34(91.2%)
• Bacteroides spp	19/21(90.5%)	24/27(88.9%)
• Gram-positive Anaerobes	7/8(87.5%)	16/17(94.1%)
Gram-positive aerobic cocci	38/42(90.5%	29/32(90.6%)

Conclusion: The favourable microbiological response rates in microbiologically evaluable patients treated with ertapenem were similar to that of patients treated with piperacillin/tazobactam.

P1686 Ertapenem vs. piperacillin/tazobactam for the treatment of intra-abdominal infections requiring surgical intervention (OASIS-1): results of a prospective, randomised, open-label study

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Objective: We compared the efficacy and safety of ertapenem (a once-a-day parenteral group 1 carbapenem) to piperacillin/tazobactam for the treatment of IAI requiring surgical intervention.
Methods: In a prospective, open-label, multicentre trial, 370 patients aged 18 years who had clinical and/or radiographic evidence of IAI were randomised to either group A ($n = 180$): ertapenem 1 g daily (i.v. or i.m.) or group B ($n = 190$): P-T 3.375 g Q6 h or 4.5 g Q8 h. Surgical intervention included open or laparoscopic surgery, and/or percutaneous intervention. The recommended treatment duration was 4–14 days. The primary endpoint was the clinical efficacy in microbiologically and clinically evaluable patients 2 weeks post-therapy [test of cure (TOC)]. Following the initial analyses, a *post hoc* re-analysis of the data was conducted after correcting for errors and missing-data in the prior evaluability determinations regarding nonstudy antimicrobials following study therapy and follow-up data. Reported below are the results of the *post hoc* analysis.
Results: Randomised patients [65.4% (242/370)] were protocol evaluable at TOC. Baseline demographic and disease characteristics of patients in both groups were generally similar. The most common baseline pathogen isolated in both groups was *E. coli* (A = 54.4%; B = 51.6%) while each of the others were isolated in <10% of patients with the most prevalent one being *B. fragilis* (A = 8.9%; B = 9.5%). Median days of therapy in all treated patients were 6 days for both groups. Results on clinical cure rates in protocol evaluable patients are included in table below. The most common adverse events (AEs) in both groups were pyrexia, diarrhea, vomiting and elevations of aminotransferase levels. Discontinuation of therapy due to drug-related clinical AEs was similar in both groups [1.7% (A), 1.6% (B)]. Six patients (3.3%) in group A and three patients (1.6%) in group B were reported to have a serious AE which was possibly, probably, or definitely related to study drug.

	Treatment Group			
	Ertapenem		Piperacillin/Tazobactam	
Time point	Primary	Post-hoc	Primary	Post-hoc
DCOT	129/132(97.7%)	131/131(100.0%)	117/121(96.7%)	117/122(95.9%)
TOC	107/119(89.9%)	116/124(93.5%)**	107/114(93.9%)*	110/118(93.2%)**
LFU	90/102(88.2%)	112/120(93.3%)	91/99(91.9%)	105/114)(92.1%)

*95%CI[-3.9(-11.5,3.4)]
**95%CI[0.3(-6.4,7.2)]

Conclusions: These data demonstrated that ertapenem 1 g once a day was as effective as piperaciilin/tazobactam for the treatment of IAI requiring surgical intervention. Ertapenem was generally well tolerated with comparable AE and discontinuation rates.

P1687 A prospective randomised trial comparing cefepime plus metronidazole and imipenem-cilastatin for the treatment of intra-abdominal infections

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Objectives: The objectives of the study were to do a comparison of the efficacy of parenteral cefepime plus metronidazole and imipenem-cilastatin for the treatment of serious intra-abdominal infections in adult patients. They were also to obtain in-vitro susceptibility data of pathogenic bacteria that cause intra-abdominal infections.
Materials and methods: This was a prospective, randomised, comparative open study, conducted in a tertiary care hospital.
Patients: The patients included in the study were adults with a clinically confirmed diagnosis of intra-abdominal infections who received either cefepime 2 g intravenously every 12 h in addition to metronidazole 500 mg every 8 h or imipenem–cilastatin 500 mg intravenously every 6 h.
Outcome: Complete clinical response was defined as the absence of pretreatment signs and symptoms of infection. Failure was defined as lack of changing or worsening of pretreatment signs and symptoms of infection. Surgical infection management was determined by the patients' surgeon.
Results: A total of 122 patients were included in the study and 121 were evaluable (ITT). Sixty patients (33 men) received cefepime plus metronidazole and 52 (87%) of them were cured; while 44 (72%) of 61 (27 men) patients in the imipenem–cilastatin arm were cured ($P = 0.015$). There were five failures in the cefepime plus metronidazole arm and 16 failures in the imipenem–cilastatin arm. Microbiological eradication was established in similar proportions in both study groups (43 cefepime plus metronidazole and 38 in the imipenem–cilastatin).
Conclusions: The results of the present study showed that cefepime plus metronidazole was more effective than imipenem–cilastatin in the treatment of intra-abdominal infections.

P1688 Microbiology and empirical treatment of pyogenic liver abscesses: analysis of 105 cases

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Objectives: The aim of the present study was to analyse the microbiology of pyogenic liver abscesses (PLA) and to propose a more effective empirical antibiotic treatment.
Methods: Multicentre and retrospective review of the PLA diagnoses and treated in six Spain's hospitals during the year 1997–2003 were studied. Statistical analysis was performed with the SPSS software package.

Results: A total of 105 patients with PLA were managed. Cultures from blood and/or abscess cavity were performed in 72% (79/105). In patients in whom culture/s were obtained, bacteria were isolated in 94% (74/79). Gram-positive bacteria only were isolated from 16% (17/79), Gram-negative bacteria only from 26% (27/79), anaerobic bacteria only from 6% (six of 79) and polymicrobial infection was present in 23% (24/79). Sensibilities of isolate bacteria to usual empirical antibiotic treatment were studied. Recovered bacteria in patients with PLA were sensible to third-generation cephalosporin with metronidazole in 77% and presented intermediate sensibility in 8%; to imipenem in 88% (intermediate: 10%); to ampicillin with gentamicin and metronidazole were 75% (intermediate: 21%); to amoxicillin–clavulanete were 67% (intermediate: 21%) and to piperacillin–tazobactam were 70% (intermediate: 26%). Recovered bacteria presented more sensibility to imipenem than to: amoxicillin-clavulanete ($P = 0.03$; RR = 3.4), piperacillin–tazobactam ($P = 0.009$; RR = 3) and ampicillin with gentamicin and metronidazole ($P = 0.05$; RR = 2.3). Analysing bacterial sensibility to levofloxacin with amoxicillin–clavulanete was 97% (intermediate: 2.7%). Isolated bacteria have more sensibility to levofloxacin with amoxicillin–clavulanete than to all the other studied empiric treatment: cephalosporin with metronidazole ($P < 0.01$; RR = 10.8), imipenem ($P = 0.02$; RR = 5), ampicillin with gentamicin and metronidazole ($P < 0.01$; RR = 11.6), amoxicillin–clavulanete ($P < 0.01$; RR = 17.4) and piperacillin–tazobactam ($P < 0.01$; RR = 15.3).
Conclusions: Recovered bacteria in PLA show better sensibility to empiric treatment with Imipenem than amoxicillin–clavulanete and piperacillin–tazobactam. The best theoretic empiric treatment to PLA in this series is levofloxacin with amoxicillin–clavulanete. Additional investigations should be carried to confirm the usefulness of levofloxacin with amoxicillin-clavulanete.

P1689 Pyogenic liver abscess. A multicentre experience in management

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Objectives: The aim of the present study was to examine our experience over pyogenic liver abscess (PLA).
Methods: Multicentre and retrospective review of the PLA diagnosed and treated in six Spain hospitals during the year 1997–2003 were studied. Statistical analysis was performed with the SPSS software package.
Results: A total of 105 patients with PLA were managed. PLA was more common in males (65%). The median age was 66 years (range 28–91). Concomitant medical problems included diabetes mellitus in 20 patients, neoplasm in four and transplantation in two (one kidney and one liver). Most patients presented with non-specific clinical and biochemical features. Preadmission, patients were symptomatic for a median 17 days, with the most common symptoms and signs being fever (84%) and abdominal pain/tenderness (67%). A raised ESR was the most common laboratory found in about 86%. Ultrasonography was not as sensitive as computed tomographic scans in detecting abscesses. Single lesions were found in 70 patients, multiple lesions in 35. PLA occurring more frequently in the right hepatic lobe (68%). The microorganism responsible was identified in 74 (71%) of the cases, with enterobacteria being the greatest number isolated. 52% of the positive abscess cultures were polymicrobial. *Escherichia coli* was the most common aetiological agent detected in cultures of blood and abscess aspirates. Abscesses was classified by the presumed route of hepatic invasion: (i) biliary tree (39, 37%), (ii) portal vein (11, 9.5%), (iii) hepatic artery (11, 9.5%), (iv) direct extension from contigous focus of infection (9, 8.6%) and (v) cryptogenic (35, 33%). All patients were treated with intravenous antibiotics. The most commonly used antibiotic combination was a cephalosporin 3a G with metronidazole. Sixty-seven (63%) had both antibiotics and radiologically guided percutaneous catheter drainage. All these patients had abscesses with diameters measuring 2 or more cm. Only 15 (14%) need surgery; 10 because another illness who required surgery, four because of deterioration despite antibiotics and drainage and two because of failure of percutaneous drainage. The case fatally rate was 9.5% (10).
Conclusions: PLA require a high index of suspiction for early diagnosis. Imaging techniques provide the main support in both the diagnosis and treatment. When appropiate theraphy in the form of antibiotics in combination with percutaneous drainage is administered, morbility and mortality is low.

P1690 Two vs. four weeks ceftriaxone + metranidazol treatment in percutaneously drained pyogenic liver absecess: preliminary report

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Objectives: To evaluate the efficacy of 2 week vs. 4 week antibiotic treatment in percutaneously drained liver abscess.
Methods: This prospective randomised controlled study was conveyed on patients with limited number of pyogenic liver abscess (<3), which can be drained by percutaneous techniques, and infected cyst hydatid. The diagnosis was confirmed by USG, CT, Gram-stain examination and cultivation of drainage sample. Coexixtance with biliary obstruction and primary amoebic liver abscess were exclusion criteria. All patients received ceftriaxone (1 g b.i.d) + metranidazol (500 mg q.i.d). In the first arm of the study (*n*: 5), therapy was continued for 4 weeks, in the second arm (*n*: 7), therapy was discontinued at the end of the second week. Failure was defined as inadequate drainage, responsiveness to therapy, microbial resistance and need for open surgical intervention. Repeat ultrasonographies were performed at 48, 96 h after drainage procedure, 1, 3, 9 and 12 months, thereafter. All of the patients were followed-up for at least 12 months.
Results: The groups were comparable regarding to the number, diameter, and localisation of abscesses. All of the patients except one were male. The duration of illness and convalescence period did not differ between groups. The fever subsided after percutaneous drainage and antibiotic therapy, and subsidence did not differ between groups. The cure rate was %100 in all of the patients.
Conclusions: Despite the limited number of patients in the study, it can be concluded that, 2 week period of antibiotic treatment is effective as much as 4 week period, in adjunctive antibiotic of pyogenic liver abscesses which are drained percutaneously.

P1691 Micro-organisms isolated from exit site of peritoneal catheters in patients submitted to chronic ambulatory peritoneal dialysis

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Chronic ambulatory peritoneal dialysis (CAPD) is an alternative to haemodialysis for patients with chronic renal failure and has been a remarkable breakthrough in the management of these patients. Many patients develop infection and its origin in most cases appears to be contamination of the catheter by common skin organisms.
Aim: The aim of this study was to know the microorganism that infected the skin exit site of the catheter in patients submitted to CAPD during a 4-year period.
Material and methods: A total of 817 samples obtained from 130 patients with suspicion of infection were processed. Skin swabs from the exit site of peritoneal catheter were inoculated into agar and chocolate plates and thioglicolate medium and incubated at 350°C in O_2 and CO_2 atmospheres, respectively, for 48 h. Microorganisms were identified and susceptibility performed using conventional methodology.

Results: A total of 704 samples (86.1%) were positive and 113 were negative (18.4%). The microorganisms isolated were as follows: 373 *Corynebacterium* sp. (52.9%), 149 *Staphylococcus epidermidis* (21.1%), 39 other coagulase-negative *Staphylococcus* (5.5), 26 *S. aureus* (3.6%), 20 *Escherichia coli* (2.8%), 28 other Enterobacteriaceae (3.9%), 26 *Pseudomonas aeruginosa* (3.6%), 16 yeasts (2.2%), six *Streptococcus* sp. (0.8%), and 10 other microorganisms (3.6%). In 55 patients more than one microorganism was identified as cause of infection.
Conclusions: A great percentage of samples obtained from patients with suspicion of infection in the catheter insertion site yielded a positive growth of a microorganism. More than 75% of the microorganisms isolated were related to those bacteria commonly found in the skin (*Corynebacterium* sp. and *Staphylococcus* sp.).

P1692 Vertebral osteomyelitis in patients with HIV-infection

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Objetives: To study the epidemiologic, clinical and diagnostic features, and treatment and prognosis of vertebral osteomyelitis (VO) in patients with HIV infection.
Patients and methods: Multicentre, prospective, transverse and descriptive study of 447 cases diagnosed of VO between January 1983 and October 2003. Inclusion criteria: (i) spinal inflammatory pain, or fever and spinal pain on physical examination and (ii) imaging findings compatible with VO and (iii) aetiologic diagnosis.
Results: Twenty patients of the total cases of VO (4.5%) were diagnosed of HIV infection. Seventeen were male (85%) and the mean age was 31.6±6 years. All patients were drug addicts. Primary infection foci: skin five (25%), lung three (15%), gastrointestinal tract two, cardiovascular system two, osteoarticular two. In six patients no previous infection was found. Previous bacteraemia: four cases (20%). vertebral level affected: two cervical (10%), seven dorsal (35%) and 11 lumbar (55%). The mean duration of symptoms prior to diagnosis was 82.75Ó175.2 days. Clinical features: fever 17 cases (85%), inflammatory pain 16 (80%), constitutional symptoms 11 (55%), neurological deficit eight (40%). Blood cultures were positives in five of 10 cases (50%), bone biopsy in eight of 12 (67%), and adjacent infectious foci cultures in 100% (14/14). Causal agents isolated: *Staphylococcus aureus* 10 (50%), *Mycobacterium tuberculosis* six (30%), *Salmonella* spp. two (10%), *Streptcoccus pneumoniae* one and polymicrobial aetiology one. Fifteen patients (75%) had involvement of two or more vertebral bodies, 13 (65%) paravertebral masses, nine (45%) epidural abscesses and four (20%) psoas abscesses. Eight cases (40%) required surgical treatment. Four patients (20%) showed therapeutic failure (two medical and two surgical failures). Five cases (25%) had severe functional sequelae.
Conclusions: Previous bacteraemia or infection are frequent. VO in HIV patients is caused by a variety of organisms. These results strongly suggest the necessity of aetiological diagnosis. Diagnostic yield of microbiological techniques is very high. Paravertebral and epidural abscesses are frequent.

P1693 Imipenem-cilastatin treatment of osteoarticular infections caused by multiresistant Gram-negative rods

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The emergence of multidrug resistant Gram-negative bacilli susceptible to hardly any β-lactam compound has led to infections close to a therapeutic dead end. In such circumstances, imipenem–cilastatin (I-C) is often the only remaining therapeutic option. We report our experience with the prolonged administration of high-doses of I-C in the treatment of osteo-articular infections with bacteria resistant to other β-lactam agents (or fourth generation cephalosporins in 14 cases). Our retrospective study over 7 years included 29 patients with septic arthritis ($n = 3$) continuous osteitis ($n = 6$), septic nonunion ($n = 12$) and prosthetic joint infections ($n = 8$). Treatment included an extensive surgical debridement and postoperative combination antibiotherapy with intravenous I-C and aminoside (54%) and/or fluoroquinolones (46%) and/or phosphomycin (29%). Associated microorganisms requiring yet additionnal antimicrobial agents were associated in 17 (59%) cases. I-C was maintained for an average of 46 days (extremes 21–90), at an average dose of 3.8 g/day (extremes 2–6). The bacteria warranting I-C were cephalosporinase hyperproducing *Enterobacter cloacae* (38%), extended spectrum β-lactamases producing enterobacteria (31%), *Pseudomonas aeruginosa* (21%) and/or *Acinetobacter baumanii* (21%). Early outcome was favourable in 24 patients (82%). Two patients relapsed with the bacteria requiring I-C, failure and two failed to negate succion fluid cultures: one was discharged with no change in his condition, one agreed to a leg amputation and the third died of candidemic septic shock while still bacteriologically positive. Repeated secondary colonisation and infection with yeasts led to a monitoring of yeast load. per os amphotericin B and immediate treatment of urinary colonisation prevented further systemic candidal infections. No other tolerance incidents were noted. Acquired resistance occurred only once in a *P. aeruginosa* isolate while imipenem–cilastatin was chosen to cover an ESBL producing *Escherichia coli*. Secondary treatment with ceftazidime was then successful in eradicating *P. aeruginosa*. I-C has been widely used for the treatment of mixed flora infections as a wide spectrum antibiotic. We report good tolerance of high posology long-term administration in documented osteoarticular indications if yeast colonisation is properly monitored, and eradication rates are comparable with those reported in infections with susceptible bacteria.

P1694 *Staphyloccocus aureus* vs. Gram-negative bacilli pyogenic vertebral osteomyelitis. A clinical, biological and radiological comparative study

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Objectives: Our aim was to define the possible clinical, biological and radiological differences between the pyogenic vertebral osteomyelitis (PVO) produced by *S. aureus* (SAVO) and Gram-negative bacilli (GNBVO), in order to identify an 'aetiological predictor profile' that allows us to initiate empirical treatment when the aetiological agent causing the PVO has not been able to identify.
Methods: We reviewed a large series of 208 cases of PVO diagnosed, treated and followed up homogenously in two tertiary care centers. Of the total of the sample 83 cases (39.9%) and 41 (19.7%) were SAVO and GNBVO, respectively. The clinical, biological and radiological features were compared for these two groups. Coagulase-negative *Staphylococcus* and polimicrobial PVO were excluded.
Results: *E. Coli* was the most common isolated bacterium trough the GNBVO group followed by *Pseudomona aeruginosa* (16 and 11 cases, respectively). Meticilin-resistant *S. aureus* (MRSA) was found in 21 cases (25.3%) and it was related with previous spinal surgery ($P = 0.05$). The mean age was higher in the GNBVO patients than in those with SAVO (57.93 ± 14.64 years vs. 50.00 ± 16.45 years) ($P = 0.01$). A previous either urinary or gastrointestinal infection was more frequent in the GNBVO group whereas a cutaneous or a soft tissue infection was found to be significatively more frequent in the SAVO group ($P = 0.001$). We could not find other relevant differences among the other features analysed. The age and a previous infection remained statistically different after the multivariant analysis.
Conclusion: GNBVO represents an important percentage of PVO. In postsurgical PVO, MRSA incidence is considerable. A higher age and a urinary or gastrointestinal infection preceding the PVO

was significaly related with a GNB aetiology. Therefore, in this case empirical antimicrobial treatment should cover GNB when PVO is diagnosed and the aetiologic agent has not been able to be identified.

P1695 Characterisation of *Salmonella paratyphi* A strains with reduced susceptibility to quinolone isolated from an outbreak in Korea

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Objective: Infections with *Salmonella paratyphi* possessing reduced susceptibility to quinolone may compromise the effectiveness of ciprofloxacin therapy that has been regarded as the drug of choice for paratyphoid fever in Korea before. The purpose of this study was to examine the antibiotic susceptibility and to characterise the resistance mechanism of the *S. paratyphi* A isolates with decreased susceptibility to quinolone isolated from an outbreak in Korea, and to determine if the isolates were genetically similar or clonal.
Methods: A total of 10 clinical isolates of *S. paratyphi* A, nine from an outbreak and one as control were used. Antibiotic susceptibility of *S. paratyphi* A to ampicillin, cephalothin, ceftriaxone, gentamicin, tetracycline, chloramphenicol, nalidixic acid, ofloxacin, ciprofloxacin and moxifloxacin were determined by microdilution broth test. The mutations that are responsible for quinolone resistance in *gyrA*, *gyrB*, *parC*, and *parE* genes of *S. paratyphi* A were investigated using PCR amplification and DNA sequencing of the quinolone resistance determining regions. Reserpine, a known efflux pump inhibitor, was used to determine whether an efflux-mediated mechanism contributed to reduced susceptibility to quinolone. Pulsed-field gel electrophoresis were performed to examine the clonal relatedness of the isolates.
Results: Among seven patients, three with ciprofloxacin therapy showed no clinical responses and required retreatment with ceftriaxone. Four patients treated with ceftriaxone as initial empirical therapy had successful clinical reponses. All isolates were sensitive to ampicillin, cephalothin, ceftriaxone, gentamicin, tetracycline, chloramphenicol. The MICs of the quinolones of the clinical isolates with reduced susceptibility to quinolone exhibited as follows: nalidixic acid >1024 mg/L, ofloxacin 2 mg/L, ciprofloxacin 2 mg/L, moxifloxacin 2 mg/L. The sequence of QRDR of the *gyrA* gene had a single mutation at position 83 (from TCC to TTC:from Ser to Phe), but no mutations were found in the *gyrB*, *parC*, and *parE* genes. Reserpine did not effect the MICs for ciprofloxacin, nalidixic acid, ofloxacin and moxifloxacin. PFGE analysis revealed identical patterns in all isolates.
Conclusions: The sequence of the QRDR of *gyrA* in *S. paratyphi* A strains with reduced susceptibility to quinolone which were isolated from an outbreak had a single mutation at the Ser-83, and no mutations were found in the *gyrB*, *parC* and *parE* genes.

P1696 Rise in isolation rates and nalidixic acid resistance in *Salmonella hadar* due to common strains among poultry and humans

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Objectives: Recently, the isolation rates of *Salmonella hadar* have placed it among the major foodborne serotypes in Greece. Whilst there was no resistance to clinically important drugs, such as cefotaxime and ciprofloxacin, resistance rates to other drugs, including tetracycline and nalidixic acid, were high. Our aim was to study the resistance patterns and the genotypic diversity among human and animal isolates of this serotype during 1998–2001, and investigate the possible relationship of the resulting chromosomal types with specific resistance phenotypes.
Methods: The study sample consisted of all human isolates from 1998 to 2001 (43 in total: 2, 4, 14 and 23 from 1998, 1999, 2000 and 2001, respectively) and all animal or animal feed isolates from 2000to 2001 (19 in total: two and 17 from 2000 and 2001, respectively). Their susceptibility to 17 antimicrobial drugs [ampicillin (Am), amoxicillin/clavulanic acid (Amc), ceftazidime (Caz), cefotaxime (Ctx), amikacin (An), gentamicin (Gm), kanamycin (K), netilmicin (Net), streptomycin (S), tobramycin (Tm), chloramphenicol (C), tetracycline (Te), sulphonamides (Sss), sulphomethoxazole/trimethoprim (Sxt), trimethoprim (Tmp), ciprofloxacin (Cip), nalidixic acid (Na)] was tested on Mueller-Hinton agar by a disc-diffusion assay according to NCCLS guidelines. PFGE of XbaI-digested total DNA was performed in 1% agarose/0.5x TBE, in a CHEF DRIII apparatus.
Results: All isolates were susceptible to Caz, Ctx, An, Gm, Net, Tm, Cip. Resistance rates to other drugs ranged from 2% (C, Sss, Sxt, Tmp) to 95% (Te). Resistance to nalidixic acid was at 90%. The dominant resistance phenotypes among human isolates, NaSTe and AmNaSTeAmc, ranged from 0 and 50% in 1998, to 78 and 13% in 2001. PFGE-distinguished three types (A–C), with eight subtypes in type A. The majority of human isolates belonged to subtypes A4 (40%), A2 (23%) and A3 (21%), predominantly associated with isolates expressing resistance phenotypes NaSTe (100%), NaSTe (70%) and AmNaSTeAmc (56%), respectively. The majority of animal isolates belonged to subtypes A4 (79%) and A2 (11%), to which belonged all isolates with resistance phenotypes NaSTe and KNaSTe, respectively.
Conclusions: The recent rise in *S. hadar* from human infections was reflected by its rise amongst poultry isolates. Nalidixic acid resistant *S. hadar* strains causing human gastroenteritis were indistinguishable, by PFGE, from poultry strains.

P1697 Investigation of clinical and laboratory findings of antibiotic-associated diarrhoea

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Objectives: The purpose of this study is to investigate the clinical and laboratory findings including the spesific culture for *C. difficile* and toxin detection in stool specimens of patients with antibiotic associated diarrhoea (AAD).
Methods: Between June 1998 and December 2003, 288 patients were hospitalised with the diagnosis of AAD in our hospital. We noted the patients' age, gender, history of antibiotic usage, symptoms, physical findings (fever and degree of dehidratation), laboratory findings, and therapy costs. Direct microscopic examination and routine culture of stool specimens were performed in all patients. Toxin detection and spesific cultures could have been performed only in 88 of the patients. Detection of toxin A was performed by ELISA and we used cycloserin-cefoxitin-fructose-agar for isolation of *C. difficile*.
Results: Of the patients, 162 (56.2%) were male and 126 (43.8%) were female. Their mean age was 40 $\pm$ 3.0 years and mean duration of hospitalisation was 3 $\pm$ 1.0 days (minimum 1–maximum 7). Most common symptoms were bloody diarrhoea (99.3%), tenesmus and/or abdominal discomfort (61.1%), and vomiting (37.5%). Mild to moderate dehidratation (108 patients 36.8%), severe dehydrataion (two patients), and fever (one patient) were main clinical findings. The patients with severe dehydratation have suffered from colon cancer and the antibiotic therapy was initiated in the hospital. Rest of the patients had no comorbid factors and all of them had a history of previous oral outpatient antibiotic usage. The time interval between antibiotic use and the onset of symptoms was 7 days in most of the patients (86%); mean time was 9 $\pm$ 1.0 days. Sulbactam ampicillin was the most common agent noted (76%). Neither toxin A positivity nor *C. difficile* isolation from the stool specimens were obtained. We also compared the costs of these diagnostic procedures and therapeutic approaches and saw that toxin A detection and spesific culture for *C. difficile* resulted an increase of 72 Euro cost per patient.
Conclusions: In our study, we observed that in most of the patients with AAD, the disease had begun 7 days after the antibiotic use and symptoms had been resolved within 3 days. Patients recovered before the results of toxin tests and stool cultures were available and these procedures increased the cost of therapy.

P1698 Serotyping and susceptibility testing of *Shigella* isolates during summer outbreak of 2003 in an Iranian hospital, Tehran

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Objectives: The aim of this study was to determine serotyps and susceptibility testing of *Shigella* isolates in Milad Hospital during summer of 2003.
Methods: From June to October 2003 in total 669 stools and rectal swabs from patients admitted to clinics of milad hospital were sent to microbiology laboratory. All specimens were examined microscopically and inoculated into bacteriological culture media for isolation of enteropathogens. All isolated bacteria confirmed by biochemical methods and isolated *Shigella* species seotyped by antisera (Bahar Afshan Company). Susceptibility testing performed my disc-diffusion method as recommended by National Committee for Clinical laboratories Standards (NCCLS M2-A7, M100-S12).
Results: Of 669 specimen 55 specimens yielded *Shigella* species In our study *S. sonnei* was the most prevalent serotype and accounted for (76.3%) of all isolates. The second prevalent serotype was *S. flexneri* with seven (12.2%) isolates followed by *S. boydii* and *S. dysenteriae* each three (5.45%) isolates. The majority of patients were children under 14 years old (85.5%). The mean age of patients was 9.8 years (SD ±16.3). Microscopical examination showed leucocyte and erythrocyte in 80% of cases. More than 80% of isolates were resistant to ampicillin, trimethoprime–sulphametoxazole and tetracycline. The rate of resistance to nadixic acid ciprofloacin cefotaxim, ceftrixone and amikacin were 11.7, 2, 2 and 9%, respectively.
Conclusion: It is concluded that *S. sonnei* was the prevalent serotypes isolates in our hospital and there was a higher rate of resistance to ampicillin, trimethoprime–sulphamethoxazol and tetracycline among isolates of *Shigella* serotypes in our hospital.

P1699 Characterisation, genomic location and distribution of the cytolethal distending toxin genes in Shiga toxin-producing *Escherichia coli* strains of non-O157 serogroups

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Cytolethal distending toxins (CDTs) are a novel family of protein toxins which interfere with the cell cycle of a broad spectrum of mammalian cells and are produced by multiple pathogens. In this study we used polymerase chain reaction (PCR) to target various cdt alleles among 340 Shiga toxin-producing *E. coli* (STEC) human isolates (130 eae-positive and 210 eae-negative) belonging to 100 different non-O157 serotypes. cdt was identified in 17 strains which belonged to serotypes O73:H18, O91:H21, O113:H21, and O153:H18, and were all eae-negative. Southern blot hybridisation and sequence analysis demonstrated that in STEC of serotypes O73:H18, O91:H21, and O113:H21 cdt is located on the chromosome and is closely related to cdt-V from STEC O157:H-. In contrast, in STEC O153:H18, cdt is on a large plasmid and is identical to cdt-III from necrotoxigenic *E. coli*. cdt-I, cdt-II and cdt-IV alleles were not identified. Supernatants of each of 17 cdt-positive STEC progressively distended and subsequently disintegrated Chinese hamster ovary (CHO) cells. No CDT activity was detected by the CHO assay in any of the remaining 323 STEC strains that were PCR-negative for cdt-I, cdt-II, cdt-III, cdt-IV and cdt-V suggesting that no additional cdt allele undetectable by the PCR approaches used occurred among the STEC strains investigated. Our data demonstrate that two different cdt alleles with different genomic locations encode biologically active CDT in STEC of particular non-O157 serotypes. Among eae-negative STEC, cdt was associated with disease (haemolytic uraemic syndrome or diarrhea) vs. asymptomatic infection ($P = 0.003$) suggesting that CDT might contribute to the pathogenicity of these organisms.

P1700 *Vibrio cholerae* in the south-east of Iran

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Objectives: Cholera continues to be an important public health problem among many poorer communities, despite the bacteriology and epidemiology of the disease having been described over a century ago. In order to determine the prevalence of *Vibrio cholerae* in the southeast of Iran, this study was performed.
Methods: A total of 2466 patients with watery diarrhoea were referred to the all hospitals related to Zabol Faculty of Medicine, Sistan-Blouchestan province, situated in the south-east of Iran, during 3 years (July 1998–October 2000).
Results: *V. cholerae* strains were isolated from 193 samples (7.83%). All ages and social and economic strata were affected. Twenty-seven per cent of all isolates were from children under the age of 5 years. Among these patients (116 males and 77 females), only 19 cases live in an urban area (9.8%). A total of 143 cases with cholera had been referred from rural area (74.1%), and the remaining 31 subjects came from neighbour country, Afghanistan (16.1%). Eighty-one of these patients were among Afghan population (41.9%). Of these 193 isolates, 179 were found to be *V. cholerae* O1 Ogawa strain (92.7%) and 14 were confirmed to be *V. cholerae* O139 (7.2%). Inaba and Hikojima sero-subtypes did not found in our investigated patients. A total of 130 were inpatients and the remaining 63 were outpatients. Six of these patients (3.11%) had died because of the severity of their disease, severe dehydration and electrolytes imbalance.
Conclusions: Priorities for cholera control remain public health interventions through improved water and sanitation, improved surveillance and access to healthcare facilities, and further developments in appropriate vaccines.

P1701 Neonatal septicaemia in dogs by *Klebsiella pneumoniae*

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Objectives: An outbreak of septicaemia in young dogs in a kennel caused by a multiresistant strain of *Klebsiella pneumoniae* is described.
Methods: Forty-five pups from 10 litters of 'Pointer' breed were affected by severe respiratory signs and septicaemia about 3 days after birth. The mortality rate of 100% was observed. The bitches were healthy and they were usually fed with foodstuffs from a hospital's dining room. At necropsy the organs of the animals displayed haemorrhagic lesions. Samples of spleen, liver, kidney, lungs and intestines of 10 pups and faecal and pharyngeal swabs collected from the bitches were cultured on sheep blood agar, McConkey (McC) agar, and brain–heart infusion agar. The organisms were identified by a commercial micromethod (api-20E system) and biochemical tests. The strains were tested for susceptibility to 16 antimicrobics by disc agar diffusion method and to genotyping by random amplified polymorphic DNA (RAPD) analysis.
Results: Gram negative rods, nonhaemolytic, lactose-positive on McC were identified as *K. pneumoniae* and were isolated from all the organs of the puppies and from the swabs collected from the bitches. The isolates exhibited the same antibiotype: resistant to ampicillin, amoxicillin/clavulanic acid, azlocillin, bacitracin, ciprofloxacin, gentamicin, metronidazole, norfloxacin, cotrimoxazole, sulphonamide, streptomycin, oxacillin, tetracycline and susceptible to imipenem and cefuroxime. The strains isolated from the lungs and liver of the pups and from faecal and pharyngeal swabs of the bitches displayed the same RAPD profile.
Conclusions: *K. pneumoniae* is a common pathogen of humans, usually responsible for hospital-acquired infections. In dogs *K. pneumoniae* is rarely detected and generally it is not associated with severe clinical signs. As the dogs were fed with foodstuffs from a hospital's dining room, the canine isolates might have a

food-borne origin. The authors hypothesise that *K. pneumoniae* colonised the oropharynx of the bitches, and it was transmitted to the puppies at the birth. Subsequently, the infected puppies developed a severe and fatal septicaemia.

Quinolones in vitro studies

P1702 Lower levels of *in vitro* nonsusceptibility to Moxifloxacin compared with other fluoroquinolones in Canadian clinical isolates of *Streptococcus pneumoniae*

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Objectives: Increasing fluorquinolone resistance in *Streptococcus pneumoniae* (SPN) and concurrent treatment failures have led to concerns about the activity of this class of antimicrobials. We analysed the in vitro activity of commonly used fluoroquinolones against clinical pneumococcal isolates from Canada to determine the evolution of resistance to these agents.
Methods: SPN isolates were collected by the Canadian Bacterial Surveillance Network, which is comprised of private and hospital-affiliated laboratories from across Canada. Laboratories were asked to collect a defined number of consecutive clinical isolates followed by all sterile site isolates of SPN. In vitro susceptibility testing was performed by broth microdilution using NCCLS guidelines. Resistance to ciproflocacin was based on a MIC of $\geq$4 μg/mL. Genetic mutations in the *par*C and *gyrA* loci were examined by molecular methods.
Results: A total of 13 408 clinical isolates, including all patient age groups, were obtained from across Canada between 1996 and 2002. In vitro susceptibility data are listed in the table below. There was a significant increase in nonsusceptibility to all fluoroquinolones, except moxifloxacin, from the year 2000 to 2002 ($P < 0.005$). Fewer isolates were nonsusceptible to moxifloxacin than ciprofloxacin or levofloxacin ($P < 0.05$) in the year 2002. A total of 37 isolates not susceptible to levofloxacin were susceptible to moxifloxacin. Sequence analysis of *parC* and *gyrA* from 16 of these isolates revealed that 14 (87.5%) and seven (43.8%) contained point mutations in either *parC* or *parC* and *gyrA*, respectively.

	Year (% isolates NS)						
Antimicrobial	1996	1997	1998	1999	2000	2001	2002
Ciprofloxacin	0.76	1.81	1.78	1.63	1.41	2.39	2.68
Levofloxacin	0.24	0.71	0.34	0.47	0.97	1.42	2.17
Moxifloxacin	0	0.51	0.21	0.38	0.92	1.01	1.38
Gatifloxilin	0.28	0.51	0.27	0.38	0.92	1.24	1.96

Conclusions: Oxifloxacin, which preferentially targets *gyrA*, appears to be associated with lower levels of in vitro nonsusceptibility at present, compared with *par*C targeted agents. With increasing nonsusceptibility to all other fluoroquinolones tested from 2000 to 2002, moxifloxacin has not had a significant increase in nonsusceptibility. These findings may be explained by the improved activity of moxifloxacin against isolates with *parC* mutations, and possibly *parC*/*gyrA* double mutations. As the number of SPN isolates in Canada with *parC* mutations increases, the utility of *gyrA* targeted agents may become increasingly important.

P1703 Comparative bactericidal activity of moxifloxacin, gatifloxacin, garenoxacin, and levofloxacin at concentrations simulating Cmax and 1/2Cmax

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Objectives: The fluoroquinolones are bactericidal agents frequently used in the treatment of respiratory tract infections. Here, we compared the killing kinetics of moxifloxacin (MXF), gatifloxacin (GAT), garenoxacin, (GAR) and levofloxacin (LEV), against fluoroquinolone susceptible and resistant *Streptococcus pneumoniae* (Sp) at achievable serum concentrations.
Methods: Fifteen strains of Sp., five fluoroquinolone (FQ) susceptible, six parC mutants (low level FQ resistance) and four parC/gyrA mutants (high level FQ resistance) were evaluated for their bactericidal activity in Mueller–Hinton broth supplemented with 5% lysed horse blood. Cultures were inoculated at a density of 1×106 CFU/mL, incubated at 35°C, and examined for viable growth at 0, 1, 2, 4, 6, 12, and 24 h after exposure to the test antibiotic at concentrations simulating Cmax or 1/2Cmax. Serum concentrations of MXF, GAT, GAR, LEV (500 mg), and LEV (750 mg), adjusted for protein binding, were 1.75, 3.2, 1.75, 3.7, and 5.8 μg/mL. Protein binding of MXF, GAT, GAR, and LEV were taken to be 50, 20, 75, and 30%. PCR was used to amplify parC and gyrA and mutations were detected by DNA sequencing.
Results: All fluoroquinolones were highly bactericidal against susceptible strains of *S. pneumoniae* at both Cmax and 1/2Cmax concentrations. Typically, a 3-log drop in CFU was observed at 4–6 h and complete eradication was achieved at 12 h. For parC mutants, MXF and GAR consistently achieved complete bactericidal eradication in 12 h at Cmax and 12–24 h at 1/2Cmax. Regrowth was observed in two of six strains exposed to LEV(500) and one strain exposed to GAT at Cmax. DNA sequencing revealed the selection of a gyrA mutation in each strain. At 1/2Cmax, regrowth was observed in two strains with LEV(500) and one strain with LEV(750). In high level FQ resistant parC/gyrA strains MXF and GAR achieved a 3 log kill in two strains and were bacteriostatic in two strains at Cmax. At 1/2Cmax, MXF and GAR were bacteriostatic. LEV and GAT had growth characteristics comparable with the growth control at both Cmax and 1/2Cmax concentrations.
Conclusions: All the fluoroquinolones are extremely active against susceptible *S. pneumoniae*. Superior killing kinetics were observed with the MXF, GAT, and GAR compared with LEV against low level (parC) fluoroquinolone resistant *S. pneumoniae*. Only MXF and GAR achieved bactericidal activity against high level (parC/gyrA) fluoroquinolone resistant *S. pneumoniae*.

P1704 Comparative *in vitro* activity of fluoroquinolones against clinical isolates of *Streptococcus pneumoniae*

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Objectives: Fluoroquinolones are recommended for the treatment of community-acquired pneumonia because increasing multidrug resistance in *Streptococcus pneumoniae*. The incidence of fluoroquinolone resistance in clinical isolates of *S. pneumonia*e is relatively low. The purpose of this study was to determine the local fluoroquinolone susceptibility of *S. pneumonia*e.
Methods: Ninety-five nonduplicate, clinical isolates of *S. pneumonia*e were collected from January to November 2003 at Basurto Hospital in Bilbao. Agar dilution MICs were determined for levofloxacin (Lev), sparfloxacin (Spx), gatifloxacin (Gat), moxifloxacin (Mox), and gemifloxacin (Gem) using Mueller–Hinton agar with 5% horse blood. Isolates were examined for evidence of efflux mechanism by determining MICs to ciprofloxacin (Cip) in the presence and absence of reserpine (10 mg/L).
Results: The MIC distribution, in mg/L, of the 95 strains is summarised in the Table. The MIC90 of ciprofloxacin, levofloxacin,

sparfloxacin, gatifloxacin, moxifloxacin, and gemifloxacin was (in mg/L): 2, 1, 0.5, 0.5, 0.25 and 0.06, respectively. Of the four *S. pneumonia*e isolates with ciprofloxacin MIC!Ý 4 mg/L, three isolates showed a a fourfold or greater decrease in the ciprofloxacin MIC in the presence of reserpine. The fourth ciprofloxacin-resistant isolate was uniformly resistant to levofloxacin, sparfloxacin, gatifloxacin, moxifloxacin, and gemifloxacin.

	CMI(mg/L)										
Agent	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16
Cip						16	53	22	3		1
Lev					1	30	61	2			1
Spx				12	63	19					1
Gat				21	56	17				1	
Mox			16	58	20				1		
Gem	7	49	37		1		1				

Conclusions: Overall, the data indicated that all the fluoroquinolones are a valuable compounds for the treatment of *S. pneumoniae* infections. Of the fluoroquinolone tested, gemifloxacin was the most active agent.

P1705 The relationship between the mutant prevention concentration and killing by ciprofloxacin and levofloxacin for clinical isolates of *Pseudomonas aeruginosa*

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Objectives: Resistant subpopulations are recovered from large susceptible cultures when exposed to the MIC. For fluoroquinolones (FQ) the mutant prevention concentration (MPC) represents a threshold required to inhibit resistant first-step resistant mutant present when 10 (10) cells are tested. We tested two strains of *Pseudomonas aeruginosa* (PA) using MPC-based experiments at MIC and MPC concentrations to determine the killing of PA by ciprofloxacin (C) and levofloxacin (L).
Method: For each experiment, cultures were grown to late log phase, centrifuged and resuspended in 4 mL of Mueller–Hinton broth and 1-mL aliquots were transferred into four flasks containing 500 mL of broth and MIC or MPC drug concentrations. Growth was sampled over 24 h on drug-free media and plates containing 2 and 4 μg/mL of C and L.
Results: For strain 1 at MIC, -0.1 to -0.74 log reduction (LR) was seen for C and L at 4 h. All cultures demonstrated regrowth by 10 h. A +0.52 to +1.11 log regrowth was seen for all cultures sampled at 24 h. Mutant growth was observed for four cultures at all times tested. At MPC, -1.96 to -2.74 and $\geq$3 LR occurred by 2 h and 8 h. At 24 h -5 to -6 log reduction was observed. No mutant growth was seen with any C treated culture beyond 2 h. No mutant growth was seen with L at 24 h. For strain 2 at MIC -1.69 to -2.3 LR was seen by 6 h. At 24 h no growth reduction was observed. Mutant growth was observed beyond 10h for all cultures tested. At the MPC $\geq$2 LR was observed for all cultures by 2 h. At 24 h -4.5 to -6.3 LR was observed. No mutant growth was observed with any culture after 2 h. At the MIC concentration $\geq$+5 logs of mutant growth was seen at 24 h.
Conclusions: MIC concentrations do not inhibit first-step resistant FQ resistant mutants present in large wild-type susceptible bacterial cultures. Mutant subpopulations proliferate at the MIC. MPC treated cultures resulted in rapid bactericidal killing of both wild type and resistant cells. No mutant growth was seen at 24 h in any MPC treated culture.

P1706 Activity of moxifloxacin against 12 selected clinical *B. fragilis* strains compared with seven other agents investigated by time-kill kinetics

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Objectives: The aim of the present study was to assess *in vitro* the killing activity of moxifloxacin, garenoxacin, gatifloxacin, levofloxacin, clindamycin, imipenem, piperacillin/tazobactam, and metronidazole against 12 selected clinical *B. fragilis* strains.
Methods: MIC values were determined by E-test for all strains and antibiotics. The killing activity of the various antimicrobials was assessed *in vitro* employing concentrations of 1/2, 1, 2, and 4$\times$ MIC established in 2.5 mL of appropriately supplemented Brucella broth and a final inoculum of approximately 1.5×10^7 CFU/mL. At 0, 2, 4, 6, 12, and 24 h after incubation aliquots were obtained and plated. After appropriate incubation CFU were counted. All experiments were carried out in an anaerobic chamber.
Results: MIC ranges (mg/L) were: moxifloxacin, 0.25 to >32; garenoxacin, 0.125–8; gatifloxacin, 0.5 to >32; levofloxacin, 0.75 to >32; clindamycin, 0.03 to >256; imipenem, 0.125 to >32; piperacillin/tazobactam, 0.125 to >256; metronidazole, 0.25 to >256. Moxifloxacin was bactericidal against most of the strains investigated after 24 h of incubation at 2–4$\times$ MIC. All other antimicrobial agents except clindamycin also showed bactericidal activity against most of the strains investigated.
Conclusions: The bactericidal activity of moxifloxacin against selected *B. fragilis* strains shown in this study warrants further investigation into the use of moxifloxacin as an agent to treat anaerobic and aerobic/anaerobic mixed infections, respectively.

P1707 Selection of resistance in *Pseudomonas aeruginosa* and *Acinetobacter* spp. by levofloxacin and ciprofloxacin alone and in combination with β-lactams and an aminoglycoside

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Objectives: To evaluate the ability of levofloxacin (LVX) and ciprofloxacin (CIP) alone and in combination with ceftazidime (CAZ), cefepime (CPM), imipenem (IMI), piperacillin/tazobactam (PTZ) and amikacin (AMK) to select resistant mutants of *Pseudomonas aeruginosa* and *Acinetobacter* spp.
Methods: Clinical strains of *P. aeruginosa* ($n = 5$) and *Acinetobacter* spp. ($n = 5$) susceptible to the drugs used in the study were assayed. MICs were determined by the broth microdilution technique before and after five serial passages on agar plates containing a gradient from 0 to a chosen maximum of each antibiotic alone or of LVX or CIP plus a β-lactam or AMK. Data were expressed as ratio between the final MIC value and the starting one. For antimicrobial combinations, values obtained by a checkerboard assay were considered as the initial MIC. Moreover, the frequency rate of mutations induced by antibiotic concentrations equal to the resistance breakpoints were calculated for each antibiotic alone and in combination.
Results: The medians of ratio between MICs of single antibiotics were: 256 for AMK, 128 for CAZ, 64 for LVX, 32 for CIP, IMI, and PTZ and 16 for CPM, thus generally giving MIC values above the established breakpoints for resistance. When antimicrobial combinations were used, the median of ratios ranged from 8 to 64 for LVX and from 4 to 32 for CIP. For *Acinetobacter* spp., medians of MIC ratios were 512 for LVX, 64 for CIP and PTZ, 32 for CPM, eight for IMI and four for AMK and CAZ. Medians of MIC ratios for combinations ranged from 8 to 128 for LVX and from 2 to 8 for CIP. When compared with the NCCLS breakpoints for resistance a notable decrease in selecting resistant strains was observed for combinations in respect to single antibiotic, with no strain found resistant to LVX and CIP after serial passages. Similar

results were observed for *Acinetobacter* spp., where one LVX-resistant strain was selected by LVX + IMI and one CIP-resistant strain was selected by combination of CIP with AMK, IMI, CAZ, and CPM. Frequencies of mutations were higher for antibiotics alone than for combinations. Combinations giving the highest rates were CIP + AMK and CIP + PTZ, for which the frequency of mutations for *P. aeruginosa* was 2×10^{-8} .
Conclusion: Use of combinations of fluoroquinolones with β-lactams and amikacin reduces the risk for *in vitro* selection of resistant *P. aeruginosa* and *Acinetobacter* spp.

P1708 Mutant prevention concentrations of ciprofloxacin and enrofloxacin against *Salmonella enterica in vitro*

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Objectives: Fluoroquinolones are the antibiotics of choice for treating human cases of salmonellosis, but strains can rapidly become resistant, mainly via mutations in gyrA. The mutant prevention concentration (MPC) has been defined as the lowest concentration of antibiotic to inhibit the emergence of mutants from 1010 CFU. Previous workers have hypothesised that the MPC as determined *in vitro* should minimise the emergence of resistance *in vivo*. The aim of this study was to investigate the mutant prevention concentrations of ciprofloxacin and enrofloxacin *in vitro* against a panel of *Salmonella enterica*. The panel included the following types: (i) fully antibiotic sensitive strains, (ii) laboratory generated MAR phenotype mutants (cyclohexane resistant and *c*. fourfold increase in resistance to unrelated antibiotics such as, β-lactams, chloramphenicol quinolones and tetracycline), (iii) wild type MAR phenotype strains, (iv) *gyrA* mutants and (v) *gyrA* mutants with the MAR phenotype of *Salmonella enteritidis* ($n = 5$) and Typhimurium ($n = 5$). Methods. MICs were determined by the BSAC agar dilution method. MPCs were determined by applying 10^{10} CFU of respective strains to serially diluted antibiotic containing plates that were incubated at 37°C for 24 and 48 h. The MPC was recorded as the lowest concentration of antibiotic to prevent the emergence of any mutants at 24 h. Results. The MICs, MPCs and MPC/MIC ratios for ciprofloxacin and enrofloxacin for the different groups of *Salmonella* are shown in Table 1. All MPC/MIC ratios for strains excluding MAR phenotype mutants without mutations in *gyrA* were in the range 2–16. The MPC/MIC ratios for the MAR phenotype mutants without mutation in *gyrA* were in the range 2–64.
Conclusions: It was of interest to note that the MAR phenotype mutants which lacked mutations in *gyrA* tended to have higher MPC/MIC ratios than the other strains. Based on an MPC of 16 times the MIC, *in vivo* levels of 0.5 μg/mL of ciprofloxacin or enrofloxacin should be sufficient to prevent the development of fluoroquinolone resistance in fully sensitive *Salmonella* strains.

Table 1. MICs and MPCs of ciprofloxacin and enrofloxacin against *Salmonella*.

Resistance type	No. tested	MICμg/mL for-		MPCμg/mL(x MIC)for-	
		Ciprofloxacin	Enrofloxacin	Ciprofloxacin	Enrofloaxin
Sensitive	2	0.03	0.03		0.5(16)
MAR lab[a]	2	0.13	0.06–0.13		
MAR WT[b]	2	0.13–0.25	0.13–0.25		
gyrA	2	0.5	0.5		
gyrA+MAR WT	2	0.5–2	1.2		

[a]MAR phenotype strains generated in the laboratory by passage with chloramphenicol or tetracycline.
[b]Wild type strains with a MAR phenotype.
[c]*gyrA* +, strains with a mutation in *gyrA*.

P1709 Comparative *in vitro* resistance of the new fluoroquinolones among aerobic Gram-negative bacilli-resistant to amikacin in a hospital setting: a 3-year study

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Objective: To assess the rate of resistance to fluoroquinolones in aerobic Gram-negative bacilli (GNB) that is resistant to amikacin and to determine if the fluoroquinolone resistance has increased overtime with the use of this new medications.
Methods: From January 2000 to December 2002 aerobic GNB isolates resistant to amikacin at the San Juan VAMC were prospectively collected and tested for suceptibility to three fluoroquinolones (levofloxacin, gatifloxacin and moxifloxacin). Percentage of resistance was calculated per year of study and changes in resistance overtime were evaluated with Chi-Square test.
Results: Fluoroquinolones resistance was analysed for the following bacterias: *P. aeruginosa*, *E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*, *P. mirabillis*, *Morganella* and *Proteus*. *E. coli* overall resistance was significantly higher to moxifloxacin (55.9%), gatifloxacin (55.9%) and levofloxacin (52.3%). *P. mirabilis* was more susceptible to levofloxacin (16.8%resistance) than to the other quinolones (gatifloxacin 21.4%, moxifloxacin 26.9%). *P. aeruginosa* showed a very high resistance to levofloxacin (69.5%), moxifloxacin (77.5%) and gatifloxacin (72.9%). Isolates of *Klebsiella* sp. had lower resistance to levofloxacin (19.4%) and gatifloxacin (18.8%) than to moxifloxacin with resistance of 29.6%. *Serratia* isolates were significantly more susceptible to levofloxacin with only 7.0% resistance. The other quinolones showed higher resistance to *Serratia* with gatifloxacin (15.0%)and moxifloxacin (20.7%). *E. coli* and *P. mirabilis* showed a statistically significant increase in resistance overtime to all three fluoroquinolones. Resistance to *Citrobacter*, *Enterobacter*, *Morganella* and *Providencia* remained unchanged through years on study.
Conclusions: The resistance to amikacin among GNB in our hospital setting has required the use of alternative drugs. The new quinolones have been used as an alternative in patients with resistance to aminoglycosides but also emergence and evolution of quinolones resistance has affected their use over the years. In our study this was noticed especially on *E. coli* and *P. mirabilis* with a significant increase in resistance overtime. Some species including *E. coli*, *P. aeruginosa*, and *Providencia* showed high resistance (>50%) to the three quinolones tested. Species like *Serratia*, *Klebsiella* and *Proteus* had lower resistance so in this species the use of quinolones could be a useful alternative.

P1710 Baselining levofloxacin activity against Gram-negative organisms collected in five European countries: first results from the 2003 GLOBAL Surveillance

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Background: Gram-negative organisms are common aetiological agents in infections among inpatients and outpatients including complicated (cUTI) and uncomplicated (uUTI) urinary tract, bloodstream, and respiratory infections. Fluoroquinolones such as levofloxacin (LEV) provide an important therapeutic option to treat patients with Gram-negative infections. The GLOBAL Surveillance 2003 surveyed antimicrobial susceptibility among Gram-negative pathogens in five European countries to obtain a current perspective of susceptibility and enable future tracking of changes in susceptibility that may occur.
Methods: A total of 2430 Gram-negative isolates were isolated from patient specimens (cUTI, uUTI, blood and respiratory) at hospital laboratories in five European countries (Belgium, France, Germany, Italy and Spain). Isolates were centrally tested by NCCLS broth microdilution (M7-A6, 2003) against LEV and relevant clinical comparator agents for each infection site. MICs were interpreted as susceptible (S), intermediate, or resistant using the NCCLS published interpretive criteria (M100-S13, 2003).
Results: Overall among Enterobacteriaceae (EB) collected from UTI, LEV showed consistent activity with 75.0–92.8% S among isolates

collected from cUTI and 69.6–97.6% S among isolates collected from uUTI. *Escherichia coli*, the most common EB isolate causing cUTI, showed regional variation with >80% S in Belgium, France and Italy, 76.3% S in Germany and 61.3% S in Spain. Among *Klebsiella* spp. from cUTI, LEV S was 88.9% in Belgium, 87.5% in France, and 91.3–97.7% in Germany, Italy, and Spain. LEV activity against *Pseudomonas aeruginosa* ranged from 47.3% S among isolates from cUTI to 69.6% S among isolates from uUTI. LEV S among EB isolates collected from bloodstream infections ranged from 79.7% among *Enterobacter* spp. to 95.8% among *Proteus mirabilis*. LEV showed potent activity against EB collected from respiratory sources with a range of 82.6% S among *E. coli* to 100% S among *Enterobacter* spp., with limited geographic variation.
Conclusions: Overall LEV was active against all EB tested, irrespective of the specimen source. The longitudinal tracking of antimicrobial susceptibility through initiatives such as the GLOBAL Surveillance is important to identify changing trends and help guide antimicrobial use.

P1711 *In vitro* activity of three different fluorquinolones alone, or in combination against *Pseudomonas aeruginosa*

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Introduction: Ciprofloxacin is usually perceived to have the greatest activity against *Pseudomonas. aeruginosa*, however, other fluoroquinolones also possess *in vitro* activity against this pathogen and levofloxacin has recently been reported as having, at least, the same activity as ciprofloxacin. Owing to its high-level resistance to many antimicrobials and, because of its ability to develop resistance during therapy, the existence of other therapeutic options, alone or in combination, is worthy.
Objectives and methods: The purpose of this study was to evaluate the *in vitro* activities of different fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin) against *P. aeruginosa* isolates and to compare the synergic effects of these fluorquinolones in combination with other potent antipseudomonal agents (aminoglycosides, ceftazidime and piperacillin-tazobactam). MICs were performed on 150 isolates of *P. aeruginosa* obtained from the Clinical Microbiology Laboratory of our Hospital. MICs of the fluorquinolones were determined by Etest, and the synergistic effect by a dilution method according to the NCCLS recommendations.
Resusts: We did not find any statistically difference in the activity against the clinical isolates of *P. aeruginosa* among ciprofloxacin and levofloxacin (69% of strains showed susceptibility to ciprofloxacin vs. 68% to levofloxacin). Moxifloxacin was the less active fluorquinolone (58% of strains were susceptible to moxifloxacin). Isolates obtained at ICU showed the highest rate of resistance. Ciprofloxacin, levofloxacin and moxifloxacin all demonstrated equivalent synergic effects against *P. aeruginosa in vitro*.
Conclusions: No significant differences were found in rates of susceptibility of *P. aeruginosa* to ciprofloxacin and levofloxacin. Both fluorquinolones and moxifloxacin showed to have synergic activity against *P. aeruginosa* when tested in combicnation with other antipseudomonal agents.

P1712 Effect of quinolones on *Plesiomonas shigelloides*

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Objectives: *Plesiomonas shigelloides* is recognised as one of the members among the expanding group of water and foodborne pathogens. In the presented study the effect of subinhibitory concentrations (sub-MICs) (1/4, 1/8, 1/16 of the MICs) of five quinolone antibiotics (enoxacin, ciprofloxacin, norfloxacin, ofloxacin, pefloxacin) on two *P. shigelloides* strains (H-human, W-surface water) was studied. It has been known that sub-MICs of antibiotics do not kill bacteria however they may have impact on their pathogenic potential.
Methods: The properties associated with putative pathogenic factors of this species were evaluated *in vitro* using tests for hydrophobicity, motility, lipase activity, sensitivity to hydrogen peroxide and quorum sensing.
Results: Quinolones reduced hydrophobicity demonstrated by adherence of bacteria to xylene at all sub-MICs tested in strain H (with the exception of enoxacin at 1/8 and 1/16 of the MIC) and at two sub-MICs (1/4 and 1/8 of the MICs) in strain W (with the exception of norfloxacin at 1/8 of the MIC). The strains after effect of some quinolones changed surface hydrophobicity towards the hydrophilic state. Motility of the strains treated with quinolones was in the majority of cases enhanced. Only ciprofloxacin and norfloxacin at 1/8 and 1/16 of the MICs in strain H and norfloxacin at 1/4 of the MIC in strain W reduced motility. Lipolytic activity of antibiotic treated strains was only slightly modified. Quinolones increased sensitivity of *P. shigelloides* to hydrogen peroxide with the exception of enoxacin and ofloxacin at all sub-MICs in strain H and norfloxacin (all sub-MICs) and ciprofloxacin (one sub-MIC) in strain W. The tested strains did not possess short chained acylated homoserine lactones (AHLs) and quinolones did not evoke their production.
Conclusions: The experiments indicate that sub-MICs of quinolones modified putative virulence factors of *P. shigelloides*. The question of pathogenetic relevance of the modifications of bacteria after treatment with quinolones deserves further studies.

P1713 Comparitive in vitro activities of ciprofloxacin, ofloxacin and levofloxacin against nonfermentative bacteria isolated from nosocomial infections

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Objective: To compare the *in vitro* activities of new broad spectrum (levofloxacin, LEV) and previously developed (ciprofloxacin CIP, ofloxacin OFX) quinolones against clinical isolates of nonfermentative bacteria.
Materials and methods: A total of 228 nonfermentative bacteria (107 *P. aeruginosa*, 86 *A. baumanii*, 26 *S. maltophila*, nine *B. cepecia*) recovered from clinical isolates between January 2001 to January 2002 were studied. All isolates were identified by standart methods and kept frozen at −20°C until use. Antibiotic powders were provided by their respective manufacturers (CIP Bayer, OFX and LEV Aventis). MICs were determined by agar-dilution method on Muller–Hinton agar and results were interpreted according to the recommendations of NCCLS. Control strains were *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922. MIC50 and MIC90 values are summarised in Table 1.

Table 1. MIC 50 and MIC 90 values of non-fermentataives bacteria

	MIC(mg/L)				
Bacteria(n)	Antibiotic	MIC 50	MIC 90	Range	Susceptibility %
P. aeruginosa(107)	CIP	0.25	16	<0.25–>16	74
	OFX	1	32	<0.5–>32	47
	LEV	1	32	<0.5–>32	73
A. baumanii(86)	CIP	16	16	0.25–>32	8
	OFX	8	16	<0.5–>32	8
	LEV	16	32	<0.5–>32	9
S. maltophilia(26)	CIP	2	4	1–>16	12
	OFX	1	1	1–>32	96
	LEV	1	4	1–>32	81
B. cepecia(9)	CIP	2	16	1–>16	22
	OFX	2	8	<0.5–8	89
	LEV	4	32	1–32	11

Results: CIP was the most active compound tested againts *P. aeruginosa*, while levofloxacin and ofloxacin showed the best *in vitro* activity against *S. maltophilia* and *B. cepecia*. We also found high MIC values for all quinolones tested against *A. baumanii.*
Conclusions: The nonfermenters are usually resistant to acceptable doses of many antibiotics. So the nosocomial infections caused by these bacteria are frequently difficult to treat. According to our results CIP is still the most effective quinolone against *P. aeruginosa* . Levofloxacin and ofloxacin could become therapeutic options for treating infections due to *S. maltophilia* and *B. cepecia*.