Infection in the immunocompromised host (except HIV)

P679

Comparison of cytomegalovirus viral load measured by real-time PCR with pp65 antigenemia for the diagnosis of cytomegalovirus disease in solid organ transplant recipients

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Objectives: Cytomegalovirus (CMV) infection is an important cause of morbidity in solid organ transplant (SOT) recipients. Currently, the antigenemia assay is widely used to detect this infection, although its success is being questioned to a great extent nowadays. The aim of our study is to compare a quantitative real-time PCR to measure CMV DNA to the antigenemia assay, for the diagnosis of CMV disease.
Methods: We prospectively processed 1198 samples (plasma and peripheral blood leukocytes, PBMC), which belonged to 158 SOT recipients (52 liver, 89 kidney and 17 heart). After transplantation the samples were collected weekly during the first month, fortnightly during the second month, and then once a month and any time when was clinically indicated. In every sample the detection of the pp65 antigen in PBMC was carried out, as well as the quantification of CMV DNA by real-time PCR (Light Cycler, LC-PCR). For this process, FRET probes, which detect a 254 pb fragment from the CMV gB gene, were used. The dynamic range of the LC-PCR was 500–5.10^7 copies/ml plasma and from 62 to 610^6 copies/10^6 PBMC.
Results: The antigenemia assay detected 15.78% (189 out of 1198) positive samples, plasma LC-PCR 18.28% (219 out of 1198) and PBMC LC-PCR 19.53% (234 out of 1198). The antigenemia assay detected 25 positive samples from 21 patients with LC-PCR negative; these patients were asymptomatic and the levels of antigenemia were below 15 cells/2×10^5. Of the 158 SOT patients, 22 (13.9%) developed CMV disease. All these patients had CMV DNA load detected by LC-PCR. Nevertheless, the antigenemia assay was negative in one patient with gastrointestinal CMV disease and in the only patient with relapsing CMV disease. LC-PCR was slightly more sensitive and specific than antigenemia assay (S: 100% versus 91%, E: 67% versus 57%, VPP: 33.8% versus 26.5% and VPN: 100% versus 97.5%. The peak of CMV DNA load and the levels of antigenemia in asymptomatic patients (antigenemia: 12 cells/2×10^5, plasma LC-PCR: <500 copies/ml and PBMC LC-PCR: <62 copies/10^6 cells) were significantly lower than in symptomatic patients (antigenemia: 112 cells/2×10^5, plasma LC-PCR: 2.5×10^4 copies/ml and PBMC LC-PCR 3.5×10^4 copies/10^6 cells.
Conclusions: Detection of CMV DNA load by LC-PCR assay seems to be superior to the antigenemia assay in the early diagnosis of CMV disease in SOT recipients and may be more useful to monitor the risk of CMV disease development.

P680

Incidence of acquired toxoplasmosis in seronegative recipients of solid organ transplant

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Objectives: The aim of this study is to evaluate the frequency of seroconversion for toxoplasmosis in seronegative recipients (R-) of solid organ (heart and/or lung) transplant with seronegative (R-/D-) or seropositive donors (R-/D+). We also assess the efficacy of chemoprophylaxis with pyrimethamine + sulfamethopiraxine for 2 months.
Patients and methods: Sixty-three patients that were seronegative for toxoplasmosis before transplantation were followed-up at different intervals (from 2 months to 10 years). Further, the patients that were R(/D+ were tested at the end of chemoprophylaxis. All serum samples were tested for IgG and IgM antibodies with the ELISA: ETI-TOXOK IgG, ETI-TOXOK IgM (DiaSorin, Saluggia, Italy) and ELFA tests: VIDAS TOXO IgGII (BioMerieux, Marcy l'Etoile, France). In all suspected seroconversions we also performed IgM-ISAGA and VIDAS TOXO IgG AVIDITY test (BioMerieux Marcy l'Etoile France and ETI-TOXOK IgA (DiaSorin Saluggia, Italy).
Results: The frequency of seroconversion was 23.8%. Six patients out of 34 R-/D- (17.6%) without chemoprophilaxis followed up from 2 to 177 months seroconverted. Nine out 29 R(/D+ (31.6 patients with a follow up from 2 to 166 months seroconverted after chemoprophilaxis interruption. The difference in the number of seroconversions was not statistically significant (P = 0.34 Yates corrected Chi-square). In almost all patients who seroconverted the symptoms were absent or mild. Severe disease was only seen in a patient who was positive before transplantation and strongly immunosuppressed when reactivation occurred.
Conclusion: In our study, the overall frequency of seroconversion was 23.8%. There was no difference between matched and mismatched recipients as to the frequency of seroconversion, which therefore would not be related to donor seropositivity. In R-/D-, however, seroconversion was asymptomatic like in non-immunosuppressed patients and occurred later than in the R-/D+. No patients seroconverted during chemoprophilaxis. Patients should be tested immediately before transplantation and the donor as soon as possible, to avoid unnecessary prophilaxis. All positive recipients should be tested if symptoms of infection are present.

P681

Changing trends of bacteraemia in patients with cancer: analysis of 2080 quantitative blood cultures during 1998 and 2004

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Background: The severity of bacterial bloodstream infections (BSI) appears to be related to quantitative bacterial levels (organism load), especially in neutropenic cancer patients. We sought to determine the trends in quantitative patterns of BSI caused by various bacteria in patients receiving care at our comprehensive cancer centre.
Methods: Only one blood culture per patient culture was included in this retrospective analysis of all consecutive blood cultures processed by Dupont Isolator 10 System. Low-grade (<10 colony forming units/millilitre (CFU/ml) and intermediate-grade (11–100 CFU/ml) are grouped in low-bacterial load (LBL); moderate-grade (101–500 CFU/ml), and high-grade (>500 CFU/ml) were high-bacterial load (HBL) BSI.

Results: During 1998, 73% of 1055 and 2004, 82% of 1025 BSI were caused by gram-positive bacteria (GPB) with the most frequent being coagulase-negative staphylococcus (CoNS), 33% and 50% and *S. aureus*, 9% and 6%, respectively. In gram-negative bacterial (GNB) BSI, Enterobacteriacae were common (73% and 56%) followed by non-fermentative (NF)-GNB (37% and 44%); *Escherichia coli* (24% and 24%) and *P. areuginosa* (17% and 19%) being the most common GNB species isolated during 1998 and 2004, respectively. A significant increase in the number of *S. maltophilia* bloodstream infection was noted during 6-year study interval (6% in 1998 vs. 16% in 2004; $P < 0.01$). Compared with GPB infections a significant proportion of GNB bacteremia were high-grade (18% vs. 39% in 1998, and 7% vs. 44% in 2004; $P < 0.001$). In contrast to 1998, in 2004 the non-Pseudomonas NF-GNB including *S. maltophilia* and *Acinetobacter* species were significantly associated with HBL compared to *P. areuginosa* infection (47% vs. 23%; $P = 0.05$). Similarly, HBL associated with *S. aureus* (50%) and *Streptococucs* species (35%) vs. CoNS (13%; $P < 0.0001$) during 1998 was not noted during 2004 (22% *S. aureus*, 20% *Streptococcus* species vs. 21% CoNS; $P > 0.5$). This was due to a significant increase in CoNS HBL BSI in 2004 (21% vs. 13% in 1998; $P < 0.01$).
Conclusions: A high proportion of GNB were HBL compared to GPB BSI. In 2004, the overall significant rise in *S. maltophilia* bacteremia was also accompanied by more HBL infections.

P682

Infections complicating unrelated donor cord blood stem cell transplantation: 281 episodes in recipients of 100 CBST at a comprehensive cancer centre (1996–2005)

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Background: Infections remain a serious complication in patients undergoing allogeneic SCT. We sought to determine infectious complications in patients receiving cord blood stem cell transplantation (CBSCT) at our institution.
Methods: This retrospective analysis was performed after obtaining approval from Institutional Review Board. Records of all 97 recipients of CBSCT were evaluated using a standardized data retrieval mechanism. All values are presented as median ± s.d.
Results: Among 59 pediatric and 38 adult patients age was 7 ± 5 years and 33 ± 12 years, respectively. Ninety-four (97%) of 97 patients had underlying haematologic malignancy, acute lymphocytic leukaemia was common (40 [43%] of 94) followed by acute myelogenous leukaemia (26 [28%] of 94). Myeloablative conditioning regimen was given in 40 (40%) patients; engraftment occurred 24 ± 9 days following transplant. In 20 patients (21%) failure was either due to primary non-engraftment or refractory leukaemia. Seventy percent had graft-versus-host-diseases (GVHD), median time from transplantation and diagnosis of GVHD was 18 ± 39 post-CBSCT days. The infections complications are shown in the table below. The overall mortality was 66% following CBSCT (median 147 ± 214 days and range, 10–993 days), in 20 (31%) of 64 patients who died, death was associated with an infection-related complication.
Conclusions: Interestingly, nearly a quarter of systemic bacterial and viral infections were noted after 100 days post-transplantation. Whereas, most systemic fungal infections in these severely immunosuppressed patients including those with GVHD occurred within 30 days of receiving unrelated-donor CBSCT.

Episodes(%)	0-30	31-100	>101	Overall
	[Days Following CBSCT]			
Bacterial	54 (19)	19 (7)	21 (7)	94 (33)
Viral	31 (11)	35 (12)	25 (9)	91 (32)
Fungal	12 (4)	11 (4)	1 (0.5)	24 (9)
FUO	26 (9)	8 (3)	5 (2)	39 (14)
Sepsis	8 (3)	1 (0.5)	1 (0.5)	10 (4)
Mycobacterias	0 (0)	3 (1)	1 (0.5)	4 (2)
CDI*	8 (3)	5 (2)	6 (2)	19 (7)
Total	**139 (49)**	**82 (29)**	**60 (21)**	**281**

*CDI= clinical document infections

P683

A risk profile for invasive aspergillosis in liver transplant recipients

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Objectives: Given the high incidence (1.5–10%) of invasive aspergillosis (IA) after liver transplantation and the associated mortality, prophylaxis would seem a reasonable approach. The purpose of this investigation was to determine the influence and significance of risk factors for IA for patients in our transplantation centre.
Methods: We analysed retrospectively all clinically relevant data from patients who underwent liver transplantation at the Transplantation Centre of the University Hospital Heidelberg (Germany) between Dec 2001 and Dec 2004 by including all data into a specifically designed data base. IA was defined according to the EORTC/BMSG criteria. Univariate analysis and logistic regression were performed to assign the influence of each assumed risk factor.
Results: A total of 195 liver transplantations were performed in 170 patients, and 39 patients died within the study period. 15 patients developed IA (7.7%) and13 of these patients died within 4 weeks after initial diagnosis of aspergillosis, which is one third of all patients who died after liver transplantation. Univariate significant factors were re-transplantation ($p = 0.006$), CMV-infection ($p = 0.006$), high urgency transplantation ($p = 0.024$), dialysis ($p = 0.001$), renal insufficiency ($p = 0.05$) and leukopenia ($p = 0.002$). Multivariate analysis shows an independent influence of CMV-Infection [OR = 0.213 (95%CI 0.065–0.697)] and dialysis [OR = 0.097 (95%CI 0.024–0.395)].
Conclusion: The investigation showed a rate of 7.7% infections, which is within the range of published data. According to the data, antifungal prophylaxis should be given to liver transplant patients with renal insufficiency, requirement for dialysis, organ failure and CMV–Infection to avoid IA. Furthermore an additional focus should be the prevention of CMV-infections.

P684

Antifungal prophylaxis with caspofungin in high-risk liver transplant recipients: a non-comparative, open-label prospective clinical trial

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Objective: There is significant morbidity and mortality related to invasive fungal infections (IFI) in patients undergoing

orthotopic liver transplant (OLT) recipients. Prevention remains elusive, especially for IFI caused by moulds. The aim of this study was to evaluate the efficacy and safety of caspofungin as prophylaxis for IFI in the subset of OLT recipients at high risk of developing IFI (study sponsored by GESITRA, funded by MSD Spain).

Methods: A prospective, non-comparative, open label trial was conducted in adults undergoing OLT who received caspofungin prophylaxis as soon as they met 2 of the following criteria: renal insufficiency (serum creatinine >2 mg/dl) or dialysis, retransplantation, fungal colonization, high transfusion requirements, biliodigestive anastomosis or reintervention. Caspofungin therapy (70-mg load followed by 50 mg daily) typically continued for a maximum of 21 days or until patient met a predefined endpoint. A successful treatment outcome was defined as the absence of breakthrough IFI (proven or probable per EORTC/MSG criteria) during the first 100 days after the onset of caspofungin in the absence of premature discontinuation of prophylaxis because of toxicity or lack of efficacy.

Results: An interim analysis was performed on the first 15 patients enrolled in the study (enrollment target: 70 patients). No IFI was seen during caspofungin therapy or during the predefined 100-day follow-up period post-discontinuation of study therapy. One (7%) patient experienced a Candida surgical wound infection with no histopathological evidence of invasive infection. Thus, the overall incidence of documented IFI was 0/15 (0%). Neither patient developed a possible IFI. Six (40%) patients had to lower the dose of caspofungin to 35 mg daily and 2 patients interrupted prophylaxis transiently due to OLT-related altered liver function tests. Caspofungin-related adverse events were only reported in one (7%) patient with increasing serum amino transferase levels which led to caspofungin discontinuation after 16 days of therapy. Caspofungin was otherwise well tolerated. Patient survival at 100 days post-discontinuation of caspofungin was 80% (12/15). Three patients died because of caspofungin-unrelated, OLT-related complications. Overall, the favourable response rate was 93% (14/15).

Conclusion: The results of this interim analysis suggest promise for the prophylactic use of intravenous caspofungin in high risk OLT recipients.

P685

Effect of quinolone prophylaxis on resistance in afebrile neutropenic patients – systematic review

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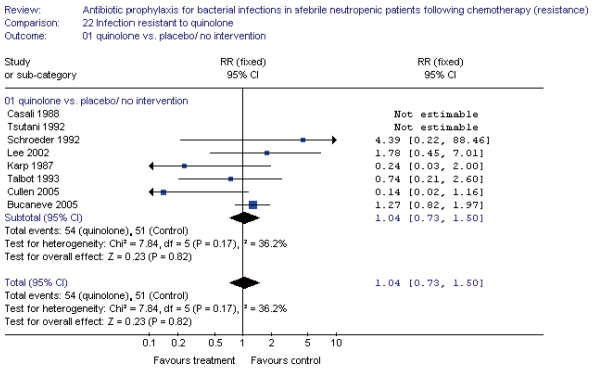
Objectives: To evaluate the effect of quinolone prophylaxis on emergence of resistant bacteria in neutropenic patients following chemotherapy for malignancies.

Methods: Systematic review and meta-analysis including randomized controlled trials (RCTs) comparing quinolone prophylaxis with placebo or no intervention (control), or another antibiotic, to prevent bacterial infections in afebrile neutropenic patients. Search was conducted until 2005. Outcomes assessed were incidence of colonizing resistant bacteria at baseline, during treatment and at the end of prophylactic therapy and incidence of resistant bacteria causing infection at the end of the study period. Relative risks (RR) with 95% confidence intervals (CIs) were estimated and pooled.

Results: Our search yielded 57 trials; 22 compared quinolones to control and 35 compared quinolones to other antibiotics. Data on colonization resistance could be extracted from 28 trials (48%), since only these trials conducted microbiological surveillance. When compared to control, there was a statistically nonsignificant increase in resistant colonizing bacteria (RR 1.68; 95% CI 0.71–4.0). In trials comparing quinolones to trimethoprim-sulfamethoxazole (TMP-SMZ), quinolones caused less development of colonizing resistant bacteria to quinolones in the quinolone arm than TMP-SMZ in the TMP-SMZ arm (RR 0.52; 95% CI 0.39–0.69). Data on baseline resistance of colonizing isolates to quinolones and colonization resistance during the trial were too scarce to analyse. When quinolone treatment was compared to control, there was no difference in the number of patients developing infections with resistant pathogens (RR 1.04; 95% CI 0.73–1.5, Figure).



Conclusions: Our systematic review shows that patients treated with quinolones may develop more colonizing resistant bacteria, compared to control. There is no difference in the number of infections resistant to quinolones. As overall mortality is reduced by quinolone prophylaxis, the danger of resistant organisms is probably much smaller than the gain. Less than half of the trials included in the review conducted microbiological surveillance and only a few reported baseline resistance to quinolones. Centres implementing antibiotic prophylaxis and future trials should assess resistance development more rigorously.

P686

The complications associated with use of peripherally inserted central catheters in cancer patients in Northern Ireland

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Objectives: In view of the increasing use of peripherally inserted central catheters (PICC) for venous access among several populations, including cancer patients, it is valuable to continuously review adverse outcomes associated with their use. We aim to describe the complications attributable to PICC use, up to 12 months following insertion, among adult patients receiving treatment for solid tumours in Northern Ireland.

Methods: A retrospective review was conducted of computerised and paper records of all patients who had a PICC inserted by the specialist PICC team in a regional cancer centre in Belfast between 01 January 2003 and 31 December 2003. Outcomes, complications, fate of PICCs and reinsertion events were followed for 12 months for all patients included. Disease and complication definitions used were based on clinical diagnoses made by the specialist PICC team based on clinical presentation, radiological, histological and laboratory findings.

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Results: During the defined period, 189 patients had a PICC placed. This group had mean age of 55 years (range 20–84); 124 (66%) were female. The most common primary tumour sites were upper gastrointestinal (n = 45, 24%), lower gastrointestinal (n = 45, 24%) and breast (n = 40, 21%). Sixty-eight patients (36%) had complications associated with the PICC; 42 (22%) underwent PICC removal as a result of these complications, 25 of whom required a new PICC to be placed. The most commonly observed complications were regression (lengthening) of the PICC (n = 19, 10%), exit-site infection (n = 14, 7%), mechanical phlebitis (n = 9, 5%) and systemic infection (n = 8, 4%). By the end of the follow-up period, 95 (50%) of patients had survived, 89 (47%) had died as a result of malignancy and 3 (2%) had died of other causes. Of note, none died as a direct result of PICC complications.
Conclusions: PICC use in this population appears to be a relatively safe access means, with no attributable mortality. The infection rate was lower than is often observed with other access devices and few complications, overall, were serious.

P687

Prospective study of viral infection and nephropathy in paediatric renal transplant recipients

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Objectives: Viruses with renal tropism, such as BKV and parvovirus B19, have been associated with allograft nephropathy, but the role of viral infection in acute rejection and posttransplant chronic nephopathy is controversial. Aim of the study was to prospectively investigate the prevalence of viral sequences in kidney biopsies obtained from a series of children submitted to kidney transplantation in the period 2000–2004 in a Single Centre. Virological data were correlated with clinical and histological findings.
Methods: Biopsies were performed because of renal dysfunction in 8 patients or for follow-up observation at 1 year after transplantation (T1) in 53 patients. Samples were analysed for the presence of DNA of EBV, CMV, HHV6, HHV8, BKV, and parvovirus B19 by a sensitive real-time PCR technique and the results were correlated with histological analysis, performed according to the Banff 97 guidelines. Patients (26 females and 27 males, mean recipient/donor age 12.1 ± 8.3/11.9 ± 5.3 years) received immunosuppressive therapy consisting in basiliximab, steroids, cyclosporin, MMF during the first 6 months after transplantation, followed by basiliximab, steroids, and FK506 ± MMF. Mean follow-up period was 27.2 ± 6 months.
Results: Viral DNA was detected in 5 out of 8 biopsies from children with allograft dysfunction, including 2 cases of BKV infection associated with tubulo-interstitial nephropathy, 3 case of parvovirus B19 infection associated with thrombotic microangiopathy, acute vascular rejection, and chronic nephropathy, respectively. Fifty out of the 53 T1 biopsies were suitable for histological and virological analyses. Viral sequences were detected in 29 out of 50 specimens (15 HHV6, 12 parvovirus B19, 3 CMV, 9 BKV, 10 EBV). No significant differences in the prevalence of viral DNA sequences and type of viral infection were observed among cases with normal histology (20/34, 58%), acute or borderline rejection (2/3, 66%), or chronic nephropathy (7/13, 53%). Kidney function at 1 and 2 years post-transplantation was not significantly different between patients with positive virological results and those with negative biopsies.
Conclusions: In our series, parvovirus B19 e BKV are associated with posttransplant nephropathy and virus-associated nephropathies are the most common causes of renal dysfunction at 1-year following transplantation.

P688

Trend towards reduced burden of proven/probable invasive fungal infections in adult non-allo-HSCT neutropenic patients with acute leukemia

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Background: In leukemic patients invasive fungal infections (IFI) are associated with severe morbidity and high mortality (30–90%). Moreover, IFI may have a negative impact on treatment and outcome of leukemia. Recent progresses in diagnosis and management of IFI may have improved outcome.
Objectives: To evaluate the morbidity and mortality of proven/probable IFI in adult non-allo-HSCT neutropenic patients with acute leukemia.
Methods: Clinical, radiological, and laboratory data were prospectively collected in consecutive neutropenic patients with acute leukemia (2002–2005). Antifungal prophylaxis was not used routinely. IFI were classified as proven, probable, or possible (EORTC-BAMSG). Proven/probable IFI cases were compared to controls (no IFI).
Results: 157 neutropenic episodes (71 induction chemotherapy, 64 consolidation, 2 auto-HSCT) occurred in 86 patients (73 AML, 13 ALL). Median age was 57 yr. (range 19–77). IFI was proven/probable in 26 cases (14 aspergillosis, 14 candidiasis, including 2 mixed IFI) and possible in 28. Proven/probable IFI occurred during induction chemotherapy in 69% of cases. At admission neutropenia was present in 50% of IFI cases compared to only 18% of controls ($p < 0.05$). Median days of in-hospital neutropenia were 25 (range 16–71) vs. 20 (range 7–59), respectively ($p < 0.05$). Antifungal therapy was started after a median of 3 days (range 0–13) after fever onset. Initial antifungal therapy consisted of ampho B-deoxycholate (n = 16), voriconazole (n = 5), caspofungin (n = 3), fluconazole (n = 2). Criteria for proven/probable IFI were met a median of 12 days (range 1–51) after fever onset. Morbidity and mortality in IFI cases and controls are shown in Table 1.

	Proven / probable IFI n=26	Controls (no IFI) n=103	*P* value
Median days of hospital stay (range)	43 (26-90) *	32 (16-68)	< 0.05
Median days between recovery from neutropenia and next chemotherapy (range)	15 (11-45)	11 (1-53)	< 0.05
Patients requiring ICU			
Overall	4 (15%)	9 (9%)	NS
Because of IFI	2 (8%)	0	
Overall deaths	3 (11.5%)	2 (2%)	NS
Deaths due to IFI	1 (4%)	0	

* 4 patients had thoracic surgery

Conclusions: In adult non-allo-HSCT patients with acute leukemia and neutropenia, proven/probable IFI were associated with moderate morbidity (low rate of ICU admission, minimal delay of initiation of next chemotherapy) and low attributable mortality (4%) despite prolongation of hospital stay, suggesting a trend towards reduced burden of IFI.

P689

Prevalence of HHV6 and parvovirus B19 in transplant recipients liver tissue: clinical outcomes and viral correlates

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Objectives: To evaluate prevalence and correlates of HHV6 and Parvovirus B19 (B19) infection in liver and blood from patients (pt) undergoing to liver transplant.
Methods: HHV6-DNA and B19-DNA were investigated in liver biopsies (LBP) and peripheral blood mononuclear cells (PBMC) of 30 pts at time of liver transplantation. Viral copy number were calculated out of 10^6 LPB-cells or PBMCs; cell number was evaluated by detection of beta-globin gene by an in-house method, using a Real Time technique (ABI Prism 7900). All pts were studied during follow up (FU); 2 pts were re-transplanted.
Results: HHV6 was found in LBPs in 22 out of 30 pts (73%) with a wide range of amount (6–29728 copies/10^6 cells). 27% out of 30 pts were concordant negative in PBMCs, 17% were concordant positive, but in 56% HHV6 was found only in liver tissue; no correlation among LBPs and PBMCs viral content was found. B19 was detected in 5 out of 30 LBPs (17%), always in association with various amount of HHV6. No correlation was found with HbsAg, HBV-DNA and HCV positivity. CMV-pp65 antigenemia was positive in 5 pts, HHV6 all positive too. During an 8 month FU (median, range 1–15) 7 pts died, 2 HHV6 LBP-negative and 5 LBP-positive. 2 Pts underwent to re-transplantation. The first pt was HHV6 LBP-positive (38 cps) at the time of transplant, LBP-positive (131 cps) at the 2° transplant, and always PBMCs-negative; also PBMCs from donors were always negative. He developed liver failure with cholestasis 14 days after (147,458 cps in PBMCs, 370,312 cps in LBP); in both LBPs and PBMCs, HHV7 and HHV8 were negative, and CMV and EBV were low positive. He was treated with cidofovir, with resolution of cholestasis and 409 cps in PBMCs 10 days after. A second pt was HHV6 and B19 LBP-positive before transplant, HHV6 and B19 LBP negative at second transplant, but his second donor was B19 plasma-positive. 10 days after he developed liver failure with cholestasis and was B19 LBP-positive and plasma-negative. HHV6, HHV7, HHV8 were all negative. 16 days after was still alive, but suffering for neuro-ischaemic damage.
Conclusions: HHV6 infection is common in pts undergoing to liver transplantation, often without blood viremia. Pts- or donor-PBMCs screening is then useless. B19 infection is less prevalent, but his closed association with HHV6 infection needs to be clarified. Donor-LBP analysis and clinical and virological long term outcome of these pts, even in the case of liver failure, have to be studied.

P690

Nocardiosis: a retrospective study of 41 French cases

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A retrospective study of Nocardia infections was achieved among 109 French regional hospitals. In the period from 2000 to 2003, 41 cases were collected and analysed on the basis of a closed detailed questionnaire. Molecular identification showed that 44% of strains were *N. asteroides* complex, 27% were *N. farcinica*, 15% were *N. nova*, 9% were *N. brasiliensis*, and 5% were *N. otitidiscaviarum*. The most common factor that predisposed individuals to nocardial infection was therapy by steroids (24%), followed by cancer (19%), COPD (14%), AIDS (12%), or solid organ transplantation (7%). No predisposing factor was observed for 24% of the patients. Nocardiosis occurred more commonly in the second part of the life, with 70% of the patients between the ages of 50 and 90 years of age. The sex ratio was 1.4. The most common clinical form was pulmonary disease (51%), followed by cutaneous (41%), and cerebral disease (17%). Bacteria were found on direct smear in 65% of the patients and in pure culture in 73% of the samples. After treatment, among the valuable patients (n = 28), 82% were cured and 7% showed a recurrence and 11% died with a mortality directly attributed to nocardiosis.

P691

Kluyvera species as opportunistic pathogens in paediatric cancer patients

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Kluyvera a relatively newly described genus in the *Enterobacteriaceae* family is considered as an infrequent opportunistic pathogen occurring in immuosuppressed as well as immunocompetent hosts. In the current work we studied retrospectively, infections caused by *Kluyvera* species in immunocompromised cancer patients admitted to the Pediatric Department of the National Cancer Institute, Cairo University, from January 2000 to December 2003. Out of 6309 cultures reviewed during the study period, *Kluyvera* species were isolated from 28 (0.4%) of the studied cases. The organism was isolated by routine microbiological methods, identification to the species level and antimicrobial susceptibility were carried out using Sensititre AP80 auto-identification plates (AccuMed International Ltd, West Sussex, UK). *Kluyvera* cryocrescens was isolated in 27 cases while *K. ascorbata* strain was documented in only one case, contrasting the published data where *K. cryocresens* is mostly isolated from the environment. In the current series, the underlying disease was a haematologic malignancy in 67.9% of cases and a solid tumour in 32.1% of cases. Most of the patients were suffering from diarrhoea and fever. The organism was isolated from the stools of 21 cases, from the blood of 5 febrile neutropenic patients, from the wound discharge of one patient and from the nasal swab of another. As regards the antimicrobial susceptibility of the isolates, increased susceptibility was recorded for amikacin and imipenem (82.1%), while ciprofloxacin and gentamycin were active in 55% and 50% of cases respectively. On the other hand, the organism seemed to be very resistant to ampicillin, cefazolin and cefuroxime as well as other third generation cephalosporins. Clinically, all reported episodes of infection with *Kluyvera* were prolonged despite treatment requiring at least 7 days of adequate antimicrobial therapy. The infection was fatal in one case. Although *Kluyvera* species are rarely diagnosed as opportunistic pathogens, yet they should be searched for in the context of febrile episodes in the immunocompromised patients. The presence of diarrhoea is an important predictor of the infection, especially if the course of the illness is prolonged or recurrent. The isolation of *K. cryocrescens* rather than *K. ascorbata* may point to the importance of environmental sources of infection, hence the importance of prompt food hygiene in order to avoid this potentially fatal infection.

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P692

Risk factors for pulmonary aspergillosis in patients with cancer and pneumonia

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Objectives: To identify the risk factors for IPA in cancer patients with pneumonia.
Methods: Prospective study of all patients with cancer admitted in Oncology or Hematology Departments that had a pneumonia. Study period: from November 2002 to February 2005. Univariate and multivariate analysis of risk factors for IPA.
Results: One hundred and nine pneumonias in 93 patients were included. Median age was 57 years (18–84), and 59.5% were males. Eighty-one (75%) had an hematological cancer and 22 (20%) were blood stem-cell transplantation (BSCT) recipients, 50% allogeneic. Sixty-one patients (59%) had neutropenia at pneumonia diagnosis, that was prolonged in 36% of the cases, and deep in 39%. Seventy two episodes (66%) were community-acquired. 30% of patients had another chronic disease different from the neoplasm. 29% had received previous prolonged steroid therapy. 26% had received previous antibiotic therapy, and 18% quinolones. There were 13 episodes of invasive pulmonary aspergillosis. Diagnosis was possible in 9 cases (69%), probable in 1 (8%) and definitive in 3 (23%) according to the EORTC criteria. There were no episodes of IPA between patients with solid cancer, thus we achieved the risk factors analysis only in hematological patients. Independent risk factors for API selected by multivariate analysis were: permanent catheter (RR 6.3; 1.9–21), previous therapy with quinolones (RR 3; 1.1–7.8), prolonged neutropenia (RR 6.2; 1.4–26.3) and deep neutropenia (RR 5.6; 1.3–23.8), allogeneic BSCT (RR 3.9; 1.6–9.9), chemotherapy with purine agonists (RR 3.6; 1.4–91) and cancer induction chemotherapy (RR 0.18; 0.03–1.2); Of these, were independent factors in multivariate analysis: previous therapy with quinolones (RR 24; 1.8–316), allogenic blood stem-cell transplantation (RR 79.5; 2.4–2654), chemotherapy with purine agonists (RR 46.4; 3.2–631) and prolonged neutropenia (RR 11; 1.3–95).
Conclusions: Risk factors for IPA in patients with haematological cancer and pneumonia are previous therapy with quinolones, allogeneic BSCT, chemotherapy with purine agonists and prolonged neutropenia. In patients with solid cancer the incidence of IPA is null in this study.

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Pneumonia risk factors in patients with febrile neutropenia

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Objectives: To analyse risk factors of pneumonia in patients with cancer and febrile neutropenia.
Methods: Prospective study of all patients with cancer and febrile neutropenia admitted in Oncology or Hematology Departments. Study period: from November 2002 to February 2005. Analysed variables: demographics, kind of cancer, stage and therapy for cancer, another inmunosuppresive factor, previous antimicrobial therapy. Univariate and multivariate analysis of risk factors for pneumonia.
Results: Three hundred forty seven cases of febrile neutropenia were included, of which 61 had a pneumonia. Median age was 43.2 years, and 36 (59%) were males. Fifty three (87%) were patients with haematological malignancies, and 14 (23%) were blood stem-cell transplantation recipients. Risk factors for pneumonia in multivariate analysis were: previous steroid therapy (RR 2; IC95%: 1.1–3.6) and induction/consolidation chemotherapy for cancer (RR 2.3; IC95%: 1.1–5.3). In patients with haematological malignancies risk factors in multivariate analysis were previous steroid therapy (RR 2.4; IC95%: 1.1–5.5) and induction/consolidation chemotherapy for cancer (RR 2; IC95%: 1.1–3.6); In patients with solid cancer risk factors in multivariate analysis were previous antimicrobial therapy (RR 11.5; IC95%: 2–73). In patients in induction/consolidation chemotherapy for cancer, the only risk factor selected by multivariate analysis was previous therapy with quinolones (RR 2.4; 1.1–5.6).
Conclusions: Independent risk factors for pneumonia in patients with febrile neutropenia and haematological malignancies are previous steroid therapy and induction/consolidation chemotherapy for cancer. In patients with solid cancer, the only independent risk factor is previous antimicrobial therapy. In patients in induction/consolidation chemotherapy for cancer, it is previous quinolones therapy.

P694

Once daily outpatient quinolone monotherapy for low-risk febrile neutropenic cancer patients

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Background: Low-risk febrile neutropenic patients (FNP) are often treated as outpatients with oral antibiotics (usually combination regimens e.g. ciprofloxacin+amoxicillin/clavulanate). The availability of newer generation, broad-spectrum quinolones (gatifloxacin, moxifloxacin) with long half lives has made it possible to consider once daily monotherapy in this setting. If found to be safe and effective such therapy might be more cost-effective and convenient than current regimens.
Objectives: To evaluate the feasibility of outpatient, quinolone (moxifloxacin) monotherapy in low-risk FNP.
Methods: A pilot study of oral/parenteral moxifloxacin (400 mg/d) as monotherapy in low-risk FNP, (patients were identified using published clinical criteria and the MASCC risk index). Standard definitions for fever (101 °F), neutropenia ($\leq$500 PMN/mm^3), and response or failure were used, based on published criteria. All patients were treated as outpatients in our ambulatory treatment centres. Daily follow up was done (clinic visit on day 1 and 2, and telephone call at home days 3–6. End of treatment visit, usually on day 7).
Results: Fifteen valuable patients have been enrolled thus far, including 12 women and 3 men with a median age of 42 years. Sarcoma (9) and breast cancer (4) were the most common underlying malignancies. Ten patients (66%) had severe neutropenia (ANC$\leq$100/mm^3). Episodes consisted of unexplained fever (9–60%), and documented infections (6–40%), including 4 episodes of bacteremia, 3 coagulase-negative staphylococci and 1 *Mycobacterium fortuitum*) and two of skin/skin structure infection. All 15 patients (100%) responded to outpatient monotherapy with oral moxifloxacin. The median time to defervesce was 3 days, mean duration for recovery from neutropenia was 4 days, and the median duration of therapy was 7 days. No toxicity was observed, and no hospital admissions were required.
Conclusions: Our preliminary data suggest a role for quinolone monotherapy in carefully selected, low-risk, febrile neutropenic patients. This approach needs to be fully evaluated in prospective randomized trials.

P695

Empiric monotherapy with cefepime in standard-risk, paediatric, febrile neutropenic patients

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Background: The management of febrile neutropenic patients is evolving. Low-risk febrile neutropenic patients are often treated as outpatients. Standard, and high-risk patients generally receive hospital-based, parenteral antibiotics. High-risk patients remain hospitalized for the entire febrile episode. Many standard risk patients stabilize quickly and probably can be discharged early, on parenteral/oral antibiotic regimens. All these strategies are easier to implement in adults, but are less well studied in pediatric cancer patients.
Objectives: To assess the efficacy and safety of cefepime monotherapy in standard-risk paediatric febrile neutropenic patients, and the feasibility of outpatient therapy in patients meeting preset criteria for early discharge.
Methods: Prospective clinical trial. Initial risk assessment was performed to identify standard-risk patients who received parenteral cefepime monotherapy in hospital. febrile neutropenic patients meeting preset criteria after 48–72 h were discharged on oral ciprofloxacin + azithromycin. Efficacy, safety, and length of stay on cefepime were assessed, as was the feasibility and success of early discharge on oral therapy.
Results: 105 standard-risk febrile neutropenic patients were enrolled (female 55%, male 45%) with a median age of 9 y. 76% had a solid tumour (primarily Ewing sarcoma or osteosarcoma) and 24% had a haematologic malignancy (mainly ALL). Median ANC at enrollment was 99/mm^3. Breakdown of infection was unexplained fever (71%), bacteremia (15%), other infection sites (14%). Response to cefepime monotherapy was 81% and overall response (with modifications) was 99%. No drug related toxicity was observed. Average length of stay in hospital was 3 days. Fifty-six percent of febrile neutropenic patients received oral, outpatient therapy with an average duration of 3 days. However, an additional 17% met the clinical criteria for early discharge, but chose to remain hospitalized due to logistical reasons.
Conclusions: Cefepime monotherapy is safe and effective in standard-risk pediatric febrile neutropenic patients. Early stabilization and discharge on oral therapy is possible in more than 50% of such patients.

P696

Urinary tract infections after renal transplantation: bacterial isolates and resistance patterns

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Objectives: To evaluate the prevalence of urinary tract infections (UTIs) after renal transplantation, to analyse the risk factors associated with post-transplant UTIs and to identify the bacterial agents and their resistance to antimicrobial agents.
Methods: A retrospective study was conducted in 130 adult (M = 91, F = 39) renal transplant recipients. There were 75 cadaveric and 55 living kidney grafts. The main immunosuppressive regimen consisted of methylprednisolone, mycophenolate mofetil, cyclosporine A and basiliximab (n = 101). UTI was defined as the detection of both bacteriuria and pyuria according to Kass criteria. Identification of microorganisms and susceptibility testing were performed using the Vitek 2 automated system (bioMerieux, France). Statistical analysis was performed using the SPSS 10.0 statistical package.
Results: The incidence of UTIs during the early post-transplant period (first trimester) was 13.8% (18/130 patients) while in the late post-transplant period (after the fourth month) was 12.3% (16/130 patients). Five patients developed UTIs during the early and late period. The results are reported in the table. Resistance rates to commonly used drugs like ceftazidime (CAZ), amikacin, ciprofloxacin (CIP), imipenem (IMP) and piperacillin/tazobactam were 98%, 87%, 99%, 84% and 98% respectively for *A. baumannii* and 60%, 46%, 71%, 57% and 45% respectively for *P. aeruginosa*. *E. coli* isolates presented 25% resistance to amoxicillin/CA and CIP, 27% to trimethoprim/sulfa, 20% to CAZ but all isolates were susceptible to IMP. Among staphylococci, *S. aureus* was 63% resistant to oxacillin (OX), 53% to clindamycin (CC) but susceptible to glycopeptides while *S. epidermidis* was 10% resistant to teicoplanin (TEC), 55% to CC, 95% to OX and all isolates were susceptible to vancomycin (VA). The degree of resistance to Gentamycin-HL for *E. faecalis* was 32%. All isolates were sensitive to VA & TEC. According to statistical analysis of our data significant risk factors for post-transplant UTIs were cadaveric graft ($p < 0.001$) and the immunosuppressive regimen ($p < 0.05$).

Microorganisms	Early post-transplant period	Late post-transplant period
	n=21 (%)	n=29 (%)
E.coli	5 (23.8)	10 (34.5)
Klebsiella pneumoniae	2 (9.5)	3 (10.34)
Klebsiella oxytoca	2 (9.5)	-
S.aureus	1 (4.8)	-
S.epidermidis	1 (4.8)	-
Enterobacter cloacae	1 (4.8)	6 (20.7)
Serratia marcences	1 (4.8)	-
A.baumannii	1 (4.8)	-
P.aeruginosa	-	2 (6.9)
Pr.mirabilis	-	1 (3.5)
E.faecalis	-	3 (10.3)
Candida albicans	2 (9.5)	-
Candida non albicans	5 (23.8)	4 (13.8)

Conclusions: The prevalence of UTIs was higher in the early post-transplant period. Gram-negative rods followed by *Candida* spp. were the most common cause of UTIs. Significant risk factors for post-transplant UTIs were both cadaveric allograft and the immunosuppressive regimen. More judicious selection of antibiotics in order to reduce resurgence of multi-drug-resistant isolates is required.

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Seroepidemiology of *Bartonella henselae/quintana* in immunocompromised patients (human immunodeficiency virus infected/end-stage renal disease patients)

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Objective: *Bartonella* infection is not easily diagnosed, especially when it presents with nonspecific symptoms, such as fever. Since immunocompromised patients are a potential risk group of infection, the aim of this study was to determine the seroprevalence of *Bartonella henselae/quintana* among two risk groups (HIV/ESRD patients) and in a control group.
Methods: Between October 2004 and April 2005, antibodies to *Bartonella* spp. were determined using an immunofluorescent test (Focus, kit) in 253 HIV patients (186 men, 67 women, mean age 45 ± –5 yr) and in 73 haemodialysis patients (41 men, 32 women, mean age 58 ± 5 yr). The epidemiologic study included sex, age, contact with cats, lymphadenopathy, antiretroviral treatment, chemoprophylaxis versus, CD4 lymphocyte count, symptoms associated with *Bartonella* infection.
Results: Of the 253 HIV patients, 103 (41%) were seropositive for *Bartonella henselae* IgG antibodies. Titres 1/64, 1/128, 1/256, 1/512, 1/1024 were found in 17% (44/253), 12% (31/253), 7% (18/253), 4% (9/253), ~1% (1/253), respectively. Fifteen (6%) subjects had IgG antibodies to both *Bartonella* species at a titre 1/64. Only two patients had IgM antibodies at a titre 1/20 and 1/40. The IgG

titres to these patients were respectively 1/512 and 1/1024. No relationship between *Bartonella* seropositivity and CD4+ cell counts was found when HIV patients were analysed. All HIV patients were receiving antiretroviral therapy (HAART) and four were under chemoprophylaxis. Of the 73 ESRD patients, 16 (21%) were seropositive for *Bartonella henselae* IgG antibodies. Titres 1/64, 1/128, 1/256 were found in 13% (10/73), 2% (2/73), ~1%, respectively. Seropositivity against *Bartonella quintana* was not determined. None of both study groups reported animal exposure before serologic test was performed. No symptoms or signs were present during the study period. The seropositivity in the healthy population in Northern Greece as it shown in previously published data relives statistically significant differences only with HIV patients, who present higher seropositivity at the respective levels of IgG. No difference was found in the serology among ESRD patients and healthy ones.
Conclusion: Our data indicate that *Bartonella henselae* infections are most common among immunocompromised patients, especially HIV infected. Therefore, *Bartonella henselae* should be considered in the differential diagnosis of opportunistic infections in HIV disease.

P698

Tobramycin once vs. three times a day given with penicillin G to cancer patients with febrile neutropenia: a prospective randomised multicentre trial

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Objectives: Based on low levels of antimicrobial resistance penicillin G (PG) and an aminoglycoside (AG) are the standard therapy in febrile neutropenia (FN) in Norway. However, so far no prospective randomized trial has evaluated this regimen. The antimicrobial effect of one dose of AG is a best 12 hours (halflife and postantibiotic effect). PG is a poor antibiotic in Gram negative sepsis. When PG is the beta-lactam, is it safe to give the AG once daily to patients without granulocytes to fight an infection?
Methods: The population consisted of adult cancer patients with fever and neutropenia.Patients were randomized to tobramycin 6 mg/kg once or three times a day. All received PG. Once the first dose was given, further antibiotic therapy was up to the doctor's discretion. Success was regress of signs of infection without modification of the antibiotic regimen.
Results: 210 patients were randomized. 145 patients are included in the final analysis. 130 of those had either lymphoma or leukaemia. No statistical difference was found between the ''x1'' and ''x3'' groups in any of the analysis. 39% of the patients were successfully treated. No patients died from the initial infection. Modifications were unusual before the third day. 24 patients had a positive blood culture (9 Gram negatives and 17 Gram positives), and 29% of those had a successful outcome. Creatinine elevations were modest and within the normal range.
Conclusion: In febrile neutropenic patients in Norway, empiric therapy with penicillin G and an aminoglycoside given once daily is safe as long as the regimen is modified depending on the clinical condition of the patient.
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P699

Enterococcal bacteraemia in patients with haematological malignancies

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Objectives: To determine frequency of isolation of enterococci from blood, risk factors Enterococcal bacteremia (BE), sources BE, outcome BE, antimicrobial susceptibility.
Materials and methods: BACTEC 9050 and Bact/ALERT 3D automated blood culture systems were used. Decisions were also made in accordance with the definitions published by the Centers for Disease Control and Prevention. Risk factors, sources, outcome of BE were studied in 226 monomicrobial episodes at 157 adult patients (1997–2002). Frequency of isolation of enterococci from blood was studied within 8 years (1997–2004). Antimicrobial susceptibility testing was performed with the Vitek-2 System, ATB Expression (1997–2004).
Results: *Enterococcus* spp. represented 7% (16/226) of all isolates from blood during 1997–2002. There were basic risk factors: acute leukaemia (62.5%), neutropenia (<500/mm^3) (87.5%), vascular catheter (100%), previous antimicrobial and corticosteroids therapy for a long time (100%, and 37.5%, respectively). There were basic sources of infection: gastrointestinal tract (75%), respiratory tract (18.8%), urinary tract (6.3%). Mortality due to BE has made 31.3% (all cases – sepsis). Last two years (2003–2004) *Enterococcus* spp. represented 13.5% (12/89) of all isolates from blood: *Enterococcus faecium*–50% (6/12), *E. faecalis*–33.3% (4/12), *E. durans* and *E. gallinarum* 8.3% each. ''*In vitro*'' antimicrobial susceptibility (1997–2004) was: to ampicillin 42.9% (12/28), to gentamicin 35.7% (10/28), to linezolid 100% (12/12), to teicoplanin 75% (21/28), to ciprofloxacin 0% (0/28), to levofloxacin 32.1% (9/28), to erytromycin 0% (0/28), to nitrofurantoin 67.9% (19/28), to tetracyclin 78.6% (22/28), to chloramphenicol 35.7% (10/28), to rifampicin 32.1% (9/28). Vancomycin-resistant strains were: *E. faecium* (1/6), *E. durans* (1/1), *E. gallinarum* (1/1).
Conclusion: Frequency of BE is growing up. Overall, BE represented 8.9% (28/315) of all isolates from blood (1997–2004). Linezolid is the most active drugs against *Enterococcus* spp.

P700

Aetiology of infections and antimicrobial resistance in patients treated for cancer at a cancer institute in Slovakia. Results from June 1999 to May 2003

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Objectives: To determine the etiology of infections and antimicrobial resistance in cancer patients.
Methods: Retrospectively conducting study in patients undergoing cancer treatment and i.v. antibiotics treatment in the same time. Our inclusion criteria meet 456 patients. We documented following data for stratification: exposure to antimicrobial agents, type of antineoplastic therapy, antibiotics, microorganisms from diagnostic cultures, results of susceptibility testing, clinical outcome.
Results: *Enterobacteriaceae* were the predictor of the death ($P < 0.01$) not the predictor of infection. *Enterococcus faecalis* was more often present in patients after surgery than in patients after chemotherapy ($P < 0.0001$). *Staphylococcus aureus* was present significantly more often ($P < 0.05$) in patients after

radiotherapy than in patients after surgery. Resistance to gentamicin ($P < 0.002$) and tetracycline ($P < 0.0004$) in *Enterococcus faecalis* was higher in patients after surgery and resistance to tetracycline ($P < 0.01$) was higher in patients after radiotherapy than in patients undergoing chemotherapy. Resistance to cephalotin ($P < 0.008$) and gentamicin ($P < 0.01$) in *E. coli* was higher in patients after surgery than in patients after chemotherapy. Resistance to ceftazidím ($P < 0.002$) in *Pseudomonas aeruginosa* was higher in patients after surgery than in patients undergoing chemotherapy. The broadest resistance was in patient with cancer of cervix uteri. Highest resistance in *E. coli* was to ampicilin ($P < 0.01$) and ciprofloxacin ($P < 0.02$) and highest resistance in Stapylococcus spp. was to cotrimoxazol ($P < 0.03$), erytromycin ($P < 0.01$), chloramphenicol ($P < 0.009$) and spiramycin ($P < 0.01$).

Conclusion: Results may contribute to the evaluation of infection control programs and the development of effective strategies in hospitals. Recognition of aetiology factors and resistance to antimicrobial agents based on truthful database can help better organize therapeutic and prophylactic strategies with particular antimicrobial agents.

P701

Interest of Candida antigen and antibody ELISA for hepatosplenic candidiasis diagnosis

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Objectives: The aim of this study was a retrospective evaluation about the interest of Candida antigen and antibody Platelia® ELISA kits for the diagnosis of hepatosplenic candidiasis in patients of haematology with or without hepatosplenic candidiasis.

Methods: Sera of patients from haematology wards from the Hôpital Edouard Herriot (France) have been routinely tested by Candida antibody and/or antigen Platelia® ELISAs between May 2004 and September 2005. The ELISAs have been performed following the recommendations of the manufacturer. Positive results for antibody and antigen tests were obtained with values superior to 10 UA/ml and to 0.5 μg/ml, respectively. Probable hepatosplenic candidiasis have been defined in neutropenic patients with fever resistant three days to antibiotics and with an positive abdominal CT scan or ultrasonographic imaging showing hepatic lesions, according to the international classification of the EORTC.

Results: Results of 521 sera from 161 haematology patients have been collected between May 2004 and September 2005. 416 sera have been tested by Candida antibody ELISA, 510 by Candida antigen ELISA, 404 being tested by the both techniques. Antibody serum results were positive for 19 sera, intermediate (5–10 UA/ml) for 29 sera and negative for 368 sera. Antigen serum results were positive for 65 sera, intermediate (0.25–0.50 μg/ml) for 11 sera and negative for 434 sera. Among the 161 patients, positive results in antibody and antigen serum detection were obtained for 10 and 31 of them, respectively. Fifteen patients were suspected to have a hepatosplenic candidiasis following clinical and imaging parameters. Significantly positive antibody or antigen ELISA results were obtained for 13 of these patients ($p < 1.10–5$), 11 with positive antigen detection, two with positive antibody detection. For the two patients negative for the both ELISAs, tests have been performed on only one serum for the first patient, decreasing the sensitivity of the detection, and on 12 sera for the second patient. Data about the 28 other patients with positive Candida antibody or antigen results have to be examined and discussed.

Conclusion: Follow-up of Candida antibody and antigen detection by ELISA for neutropenic febrile patients in haematology may be good predictive parameters for the diagnosis of hepatosplenic candidiasis in complement of the abdominal CT scan or ultrasonographic imaging.

P702

Cefepime therapy is effective for children with febrile neutropenia

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Infectious complications are important causes of morbidity and mortality in children receiving chemotherapy for malignant diseases. The aim of this study was to evaluate the clinical effectiveness of cefepime in immunocompromised children. Cefepime was used in 93 febrile events diagnosed in 49 patients as empiric antibacterial therapy alone or in combination with aminoglycoside. The mean age was 10 years 4 month (10 mo–19 y), and the male:female ratio was 1:1.2. Blood samples for microbiological processing were taken from each patient with granulocytopenia (<500 cells/mm^3) and fever (>38° C), prior to the start of any antibiotic therapy. In 21 cases (23%) the infection was documented by blood culture, in 30 cases (32%) the infection was documented clinically and 42 cases (45%) were considered as fever of unknown origin. Cefepime was used in a mean dose of 86 (50–100) mg/kg/day, for a mean of 6 (2–24) days, b.i.d. Duration of the neutropenia was 6.8 ± 4.4 days. In 61 cases granulocyte colony-stimulating factor was administered. In 86% of the cases (80/93) cefepime was combined with aminoglycosid antibiotics. The success rate of the cefepime therapy was 75% (70/93). In the other cases changes of the antimicrobal therapy was necessary, and two patients died (1 progression of the disease, 1 sepsis). No significant severe side effects could be detected. In summary, our results demonstrate, that cefepime is effective and safe treatment for febrile episodes in neutropenic children with malignancies.

Antibacterial susceptibility studies–I

P703

A comparison study of amoxicillin-clavulanate and azithromycin efficacy in treatment of acute sinusitis

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Background: Acute bacterial sinusitis is an acute infection of the paranasal sinuses. Approximately 30 million Americans develop acute bacterial sinusitis annually, resulting in an estimated 25 million physician visits. Shorter courses of antimicrobial therapy (3 to 5 days) tend to increase patients compliance, decrease adverse events, decrease the emergence of resistant strains, and reduce cost. We compared the efficacy and safety of azithromycin regimens, 250 mg/day once daily for 5 days to the efficacy and safety of amoxicillin-clavulanate regimen of 500 mg 3 times daily for 10 days.

Methods: A total number of 80 subjects with clinically and radiologically documented acute bacterial sinusitis were treated

with (azithromycin 40 subjects, amxicillin-clavulanate 50 subjects).
Results: Mean age 32.7 years, male 60%, female 40%, the most common symptoms were headache and paranasal discharge, maxillary sinus was more infected 49%. Clinical success rates were among subjects at the end of therapy (azithromycin 53%, amoxicillin-clavulanate 90%). Subjects treated with amoxicillin-clavulanate reported a higher incidence of treatment-related adverse events (15%) than azithromycin (7%, $p > 0.005$). Gastrointestinal discomfort was the most frequent treatment related adverse effect.
Conclusion: For subjects with clinically and radiologically documented acute bacterial sinusitis azithromycin given in a 250 mg dose once daily for 5 days was not shown to be as efficacious as amoxicillin-clavulanate 500 mg 3 times/day for 10 days.

P704

In vitro activities of various piperacillin and sulbactam combinations against bacterial pathogens isolated from intensive care units in Taiwan: SMART program 2004 data

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Objective: We investigated the *in vitro* activity of various piperacillin and sulbactam combinations against commonly encountered gram-negative bacteria in Taiwan.
Methods: Antimicrobial susceptibility testing was performed using the agar dilution method against 1030 bacterial isolates recovered from intensive care units of nine hospitals. Sulbactam was added to piperacillin either at a fixed concentration of 4 and 8 mg/ml or with a ratio of 1:2 and 1:4.
Results: Piperacillin-sulbactam with a ratio of 2:1 or a fixed 8 mg/ml of sulbactam had better activities against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Serratia marcescens* than other piperacillin-sulbactam combination regimens. For *Pseudomonas aeruginosa*, piperacillin-sulbactam (2:1 or 4:1) had MIC90s of 64 mg/L (>90% susceptibility) compared with 64 mg/L for cefoperazone-sulbactam (68% susceptibility) and 128 mg/L for piperacillin-tazobactam (82% susceptibility). For *Acinetobacter baumannii*, both piperacillin-sulbactam (either 2:1 or a fixed 8 mg/L of sulbactam) and cefoperazone-sulbactam were most potent agents. Adding sulbactam to piperacillin resulted in increased susceptibility rates among piperacillin-resistant *P. aeruginosa* (53–57% in either 2:1 or 4:1 ratios) and *A. baumannii* (38%–46% in either 2:1 or a fixed 8 mg/L of sulbactam) isolates, respectively.
Conclusions: Results of susceptibility tests with piperacillin-sulbactam are method-dependent. Piperacillin-sulbactam combinations possessed better *in vitro* activities than piperacillin alone or piperacillin-tazobactam against *P. aeruginosa* and *A. baumannii*.

P705

Hetero-glycopeptide-intermediate *Staphylococcus aureus*: prevalence and antibiotic susceptibilities in long-term care facilities from the Flemish part of Belgium

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Long-term care facilities (LTCF) are a main source of methicillin-resistant *Staphylococcus aureus* (MRSA) import into acute-care facilities (ACF). In this multi-centre study, we looked for hetero glycopeptide-intermediate *Staphylococcus aureus* (hGISA) among MRSA isolates from LTCF patients and tested all isolates, including the hGISA for susceptibility to established and newer antibiotics. Ten ACF sites located in the Flemish part of Belgium screened LTCF patients for MRSA upon admission. A total of 238 MRSA isolates was collected and sent to a central laboratory for testing. The Etest macromethod (Walsh et al., JCM 2001) was used to identify potential hGISA phenotypes. A 2 McF inoculum was tested on Brain Heart Infusion Agar (BD, USA) with vancomycin (VA) and teicoplanin (TP) Etest strips. Plates were incubated at 35 °C in air and read at 48 h. A non-GISA (ATCC 43000) and hGISA reference strain (ATCC 700698/Mu3) were used for quality control. Isolates with MIC values ≥8 μg/mL for both, VA and TP, or ≥12 μg/mL for TP were interpreted as potential hGISA and were sent to a reference laboratory (Bristol University, UK) for population analysis profile (PAP)-testing. Of the 238 MRSA isolates, 24 (10%) were identified as potential hGISA and 6 (2.5%) were confirmed as hGISA by PAP-testing. Susceptibility data were obtained by the Etest standard method. MIC90s of vancomycin, minocycline, trimethoprim/sulfamethoxazole, mupirocin, fusidic acid, linezolid and daptomycin for the total sample of 238 MRSA isolates were 1, 0.25, 0.1, 0.4, 0.2, 1.75 and 0.4 mg/L, respectively, while tigecycline had a MIC90 of 0.25 mg/L for the 24 isolates identified as potential hGISA. hGISA are present in low numbers in LTCF and consequently in ACF. Current antibiotics used for MRSA are still valuable and the newer agents look promising.

P706

Uncomplicated urinary tract infections, what about fosfomycin and nitrofurantoin in 2006?

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Since 2000 we assessed the prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated urinary tract infections. A total of 13,251 strains of *Enterobacteriaceae*, 2638 of *Enterococci* and 1461 of *Staphylococci* were studied. *In vitro* results of 7 antibiotics were analysed using the disk diffusion method: Amoxicillin (AMX), Amoxicillin-clavulanate (AMC), Cotrimoxazole (SXT), Nalidixic acid (NA), Ofloxacine-Norfloxacine (NOR), Fosfomycin (FOS) and Nitrofuradoin (FT). Including all genus of bacteria in the aim of an empiric treatment, AMX was the antibiotic the less active with only 50.2% of susceptible strains, followed by SXT = 65.5%, AMC = 67.2%, NAL = 74.9%, NOR = 83%, FT = 83.5%, FOS = 91.1%. Nitrofurantoin, and Fosfomycin were at least equals to Norfloxacine/Ofloxacine, these 3 compounds were *in vitro* the most potent drugs. Fluoroquinolones still the class of antibiotic the most prescribed in UTI in our hospital very far from the 2 others antibiotics. As group 1 of Enterobacteria represent 10,113 strains (76% of the panel) with a prevalence of more than 98% of *Escherichia coli* we looked this group more carefully to support our conclusions. FOS = 98.3%, NOR = 89.2%, FT = 86%, NAL = 84%, AMC = 75.4%, SXT = 73.3%, AMX = 53.5% of susceptibility. French and European guidelines concerning the best empiric treatment of UTI includes Fluoroquinolones, Nitrofuradantoin and Fosfomycin. Worldwide fluoroquinolones suffered from about 10% or more of resistance in Enterobacteriacae of group 1 and as Quinolones of ''first generation'' ranged from 15 to more than 20% of resistance, may be the prudent use of antibiotics moved us to really use in clinical practice others antibiotics with proven good *in vitro* and *in vivo* activities for UTI infections.

P707

Antimicrobial activity of linezolid and other agents against nosocomial pneumonia *Streptococcus pneumoniae* isolates

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Objectives: Nosocomial pneumonia is the leading cause of death in patients with hospital-acquired infections. *Streptococcus pneumoniae* is increasingly associated with outbreaks of pneumonia in nosocomial and tertiary care settings. The emergence of antibiotic resistance in *S. pneumoniae* complicates treatment interventions. This study investigated the antimicrobial activity of 14 antibiotics vs nosocomial pneumonia *S. pneumoniae* isolates collected globally.
Methods: Microbroth dilution MICs (expressed in mcg/mL) and their interpretation followed Clinical and Laboratory Standards Institute (USA) guidelines.
Results: Antibiotic resistance rates among the pneumococcal isolates were: penicillin 16%, imipenem 3%, levofloxacin 1%, gemifloxacin 1%, cefuroxime 29%, cefotaxime 1%, ceftriaxone 2%, erythromycin 18%, tetracycline 21%, trimethoprim-sulfamethoxazole 31%. All isolates were susceptible to linezolid, vancomycin, and cefepime.

Antibiotic	No. Isolates	MIC_{50}	MIC_{90}	Range
Linezolid	109	1	2	<=0.125-2
Vancomycin	109	<=0.25	0.5	<=0.25-0.5
Penicillin	110	0.03	2	<=0.015-4
Imipenem	107	0.015	0.5	0.004-1
Levofloxacin	107	1	1	0.5-8
Gemifloxacin	103	0.03	0.06	0.015-0.5
Cefuroxime	103	0.125	8	<=0.03-16
Cefotaxime	107	0.03	1	0.004-4
Ceftriaxone	109	0.06	2	<=0.015-4
Cefepime	107	0.06	1	0.008-2
Erythromycin	109	0.125	8	<=0.06->=8
Tetracycline	103	0.25	32	0.125->32
Trimethoprim-sulfamethoxazole	103	0.5/9.5	8/152	0.125/2.4-16/304
Piperacillin-tazobactam	107	0.03/4	4/4	0.008/4-8/4

Conclusion: This study confirms a high rate of antibiotic resistance among pneumococci. Linezolid, vancomycin and cefepime are the most consistently active agents vs strains of *S. pneumoniae* causing nosocomial pneumonia.

P708

Low-level resistance to ciprofloxacin in *Salmonella* strains isolated from humans in Belarus

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Objectives: To estimate minimum inhibitory concentration (MIC) of ciprofloxacin for *Salmonella* strains.
Methods: In research were included 42 *Salmonella* strains of different serotypes (14 – *S. Enteritidis*, 16 - *S. Typhimurium*, 4 – *S. Hadar*, 3 – *S. Isangii*, 1 – *S. Virchow*, 1 – *S. London*, 1 – *S. Java* 1 – *S. Derby*, 1 - *S. Stanleyville*) isolated from patient with salmonellosis in 2003–2004 years in Belarus. The revealing of antimicrobial susceptibility to ampicillin (A), chloramphenicol (C), nalidixic acid (N), ciprofloxacin (P), tetracycline (T), gentamicin (G), trimethoprim/sulfamethoxazole (R), cefotaxime (F) was checked by the disk diffusion method following the recommendations of the NCCLS. Determination of ciprofloxacin MIC was checked by the broth dilution test (micro method). The terminal concentrations of ciprofloxacin from 0.008 to 4 mg/L were used. For the control of quality parallel determined the ciprofloxacin MIC for the *E. coli* ATCC 25922 and *S. aureus* ATCC 29213. The interpretation of the results is made following standard NCCLS guidelines (R–MIC ≤ 4 mg/L, I–MIC = 2 mg/L, S–MIC ≥1 mg/L). Low-level resistance to ciprofloxacin was taken as MIC 0.25–1.0 mg/L.
Results: By the using of disc diffusion method all strains was susceptible to ciprofloxacin, 23 strains (54.8%) was resistant to nalidixic acid, eight strains (seven *S. typhimurium* and one *S. london*) has multiply resistance (antimicrobial pattern ACNTGRF). The range of ciprofloxacin MIC of 42 tested strain was from 0.03 to 1.0 mg/L. The highest levels of MIC (0.5–1 mg/L) have six strains of *S. typhimuriun* (antimicrobial pattern ACNTGRF), three strains of *S. enteritidis* (antimicrobial pattern NT) and strain *S. london*. The low-level resistance to ciprofloxacin was revealed in 20 strains of salmonella, for the 19 from them (95%) diameter of grows inhibition for nalidxic acid was 6–13 mm (category R – resistant). Was not revealed any strains with lowed susceptibility to ciprofloxacin amount 19 strains, sensitive to nalidixic acid. Values of MIC of control strains were corresponded to reference values.
Conclusion: From almost all of *S. typhimurium* strains with multiply antimicrobial resistance, formally maintaining susceptibility only to fluoroquinolones, was determined the low-level resistance to ciprofloxacin (MIC 0.5–1 mg/L). In case of need to treat the infections, caused with similar strains, it is advisable to change the dosage of fluoroquinolones, or administer the reserve antibiotics.

P709

The influence of UV radiation on the antibacterial activity of ciprofloxacin in aqueous solutions

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Objective: The strong antibacterial activity of fluoroquinolones has led to their large-scale use in medicine. However, effectiveness of the antibacterial agents depends on conditions in which they are kept. The aim was to determine how dose and lambda of the UV radiation influence on the fluoroquinolones antibacterial activity in respect of *Staphylococcus aureus*.
Methods: Ciprofloxacin (''Ciprobay'',''Bayer'') was used. *Staphylococcus aureus* ATCC 21027 was chosen as a test-culture. The samples of cfqH with different starting concentration were irradiated by light (lambda = 254 nm, lambda = 366 nm) during different times. The method of the consecutive double serial dilutions was used for determination of cfqH MIC before and after irradiation.
Results: MIC of the nonirradiated sample was 0.25 μg/ml. During increasing time of the exposure to radiation (lambda = 254 nm) MIC was growing pro rata and was 20 μg/ml after 20 min of irradiation. At the same time we studied the influence of the light with lambda = 366 nm on the antibacterial activity of cfqH. MIC was found to be greatly less than one after lambda = 254 nm irradiation and remained the same long time. Also dependence MIC of the starting concentration of cfqH solution was found. Antibacterial activity of cfqH depends on dose and lambda of the light and starting concentration of cfqH solutions undergone irradiation. CfqH effectiveness decreased considerably after 10 min irradiation lambda = 254 nm and 60 min lambda = 366 nm. At the same time MIC noticeably decreased during increasing starting concentration of the samples.
Conclusions: The features of the kinetic behaviour curves of cfqH under irradiation correlate with MIC changes. The possible mechanism of cfqH inactivation was discussed.

P710

In vitro activity of linezolid against bacteroides and prevotella recovered from soft tissue infections

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Objectives: Bacteroides (Bact) and Prevotella (Prev) are the two major anaerobic bacteria recovered from soft tissue infections (STI) at our institution. We reported earlier an excellent Lin activity against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), *Corynebacterium jeikeium* (Cjk) that are frequently involved in STI. Our objective was to conduct an *in vitro* activity of Lin against a number of Bact and Prev recovered from STI (75% diabetics) in an attempt to gain extra information on Lin activity against Bact & Prev.
Methods: STI samples collected in anaerobic transport tubes were processed within few hrs on 3 pre-reduced anaerobic blood agar plates as described by conventional methods. After 48-h of anaerobic incubation bacteria were identified by API-20A system (Bio-Merioux) and other differential tests. Lin activity was tested by E-test strip (AB-Bidisk) using pre-reduced anaerobic *Brucella* blood agar plates incubated anaerobically at 37 °C for 48 hrs. *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741 were used as controls.
Results: A total of 232 isolates of Bact(n-141) and Prev(n-91) were exposed to Lin. Of Bact there were 65 *B. fragilis*; 26 Bacteroides spp.; 18 *B. vulgatus*; 22 *B. ovatus/thetaiotaomicron* and 10 *B. distasonis*. Of Prev, there were 56 *P. bivia*; 22 *P. melaninogenica/oralis* and 13 *P. intermedia/disiens*. In total 141 Bact isolates tested, the minimal inhibitory concentraion (MIC) values of ≤2 μg/ml (susceptible category), 3–4 μg/ml (intermediate category) and ≥6 μg/ml (resistant category) were exhibited in 64 (45%), 60 (42%) and 17 (12%), respectively. A total of 55 (39%) susceptible Bact strains exhibited MIC of 1–2 μg/ml, whereas all Bact resistant strains had an MIC of only 6 or 8 μg/ml. Of the total 91 Prev isolates tested, MIC of ≤2, 3–4, and ≥6 μg/ml were obtained in 73 (80%), 14 (15%) and 5 (5%), respectively. All the latter 5 Prev strains, had an MIC of 6 μg/ml only.
Conclusion: In general, considering an MIC value of ≤2 μg/ml as the susceptible cut-off line, there was a total of 134/432 (58%) susceptible strains of Bact & Prev. An additional intermediate MIC value of 3–4 μg/ml was obtained in 73 strains (31%). This may increase Lin activity and make this oral agent an alternative therapeutic option in 2 ways (i) to treatment the outpatients who normally suffer from diabetic soft tissues infected with multiple organisms such as MRSA, VRE, Cjk, Bact and Prev, and (ii) to use Linezolid when the patient develops an allergy to vancomycin.

P711

In vitro activities of cefepime, cefpirome and ceftazidime in combination with amikacin or ciprofloxacin against *Pseudomonas aeruginosa*

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Objectives: *Pseudomonas aeruginosa* continues to be a major nosocomial pathogen which is a leading cause of mortality, particularly among patients with immunosuppression, malignancy, cystic fibrosis, burns and traumatic wounds. Since *P. aeruginosa* infections often progress rapidly in these patients, optimal results can only be achieved when timely administration of an effective antimicrobial therapy is ensured.
Results and methods: With this purpose the *in vitro* activities of cefepime, cefpirome and ceftazidime alone and in combination with amikacin or ciprofloxacin were assessed in clinical isolates of *P. aeruginosa*. The minimum inhibitory concentrations (MIC) of these antibiotics were determined by microbroth dilution technique, and according to the MIC values, 95% of the strains have been found susceptible or intermediate susceptible to amikacin, 77% to cefepime, 68% to cefpirome, 59% to ceftadizime and 46% to ciprofloxacin. *In vitro* activities of these antibiotics in combinations with amikacin and ciprofloxacin against 15 *P. aeruginosa* strains have been studied by time kill curve method.
Conclusion: Combinations with amikacin have been found more synergistic than those where ciprofloxacin was involved. When amikacin was combined with cefepime, cefpirome or ceftazidime synergistic interactions were observed most frequent with ceftazidime (93.3%). The combinations with highest bactericidal interactions were the ones where cefpirome was involved. No antagonism was observed with any combination.

P712

Antimicrobial resistance of *Streptococcus pneumoniae* and *Haemophilus influenzae* in Lithuania

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Streptococcus pneumoniae (SP) and *Haemophilus influenzae* (HI) are still the most important respiratory pathogens.
Objective: The aim of this study was to determine antimicrobial resistance of SP and HI isolated from clinically significant specimens in Vilnius University Children's Hospital.
Material and methods: A total of 220 SP and 435 HI strains isolates were examined during the period 2000–2004. Isolation and identification were performed according to conventional microbiological methods. SP susceptibility tests to penicillin (PEN) and erythromycin (ERY) and HI susceptibility to ampicillin (AMP), cefuroxime (CXM), cefotaxime (CTX) and azithromycin (AZI) were performed using the disc-diffusion method according to the NCCLS recommendations.
Results: The overall resistance (%) of SP to PEN was 1.9 (4.1, 0, 5.7, 0, 4.8), to ERY 9.2 (6, 7.3, 8.6, 3.4, 14.5) and the resistance of HI to AMP was 8 (8.3, 3.4, 7.6, 7.3, 11.8), to CXM 5.4 (5.7, 3.6, 6.7, 4.5, 5.9) and 0% for CTX and AZI.
Conclusions: These data showed high sensitivity of SP and HI strains to the tested antibiotics. All HI strains appeared to have stable sensitivities to CTX and AZI.

P713

Cefepime alone or in combination with clavulanic acid, gentamicin, and tobramycin against clinical isolates of methicillin-resistant *Staphylococcus aureus*

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Objectives: To further evaluate cefepime (CPM) alone or in combination with clavulanic acid (CA), gentamicin (GEN), or tobramycin (TOB) against clinical strains of MRSA. We have previously demonstrated synergistic activity with combinations of CPM and GEN, TOB, or sulbactam (SUL) against MRSA.
Methods: 50 clinical MRSA isolates were screened by microbroth dilution for susceptibility (S) to CPM, GEN,TOB, CA, as well as CPM combined with CA 1:1 (CPM-CA), CPM combined

with GEN 1:1 (CPM-GEN), and CPM combined with TOB 1:1 (CPM-TOB). 10 clinical isolates were evaluated by time-killing curve analysis of CPM, GEN, TOB, CA, CPM-GEN, CPM-TOB, and CPM-CA at 0.5, 1, and 2× the MIC using a starting inoculum of 1×10^6 CFU/mL. Synergy is defined as >2.5 log kill, additivity is defined as <2.5 but >1 log kill, and indifference is defined as + or –1 log kill.

Results: MRSA S to CPM, CA, GEN, TOB, CPM-CA, CPM-G, and CPM-T are reported as follow [MIC50 (range) in mg/L]: 32 (1–64), 64 (32–64), 0.5 (0.13–32), 1 (0.25–32), 32 (4–64), 0.5 (0.25–32), 2 (0.5–64). MBC50 (range) in mg/L for CPM, CA, GEN, TOB, CPM-CA, CPM-G, and CPM-T are 32 (1–64), 64 (32–64), 1 (0.5–32), 2 (0.5–32), 64 (4–64), 1 (0.25–64), 3 (0.5–64). 12% of the isolates demonstrated a decrease in the MIC when CA was added. However, all of the clinical isolates demonstrated a decrease in the MIC when GEN was added (100%) while 54% showed a decrease when TOB was added. Time-killing curve analysis of the clinical isolates showed no difference in log kill for 8 of the organisms tested with CPM and CPM-CA. One organism demonstrated synergy and one showed additivity at 24 h when tested with CPM and CPM-CA. Time-killing curve analysis of the same isolates also showed synergy at 24 h for 2 organisms when tested with CPM-GEN or CPM-TOB. However, early synergy was noted at 4 or 8 hours in 6 organisms.

Conclusions: A majority of the clinical isolates reported an elevated MIC to CPM. As opposed to SUL previously reported, the addition of CA did not appear to enhance the S of those organisms. In addition, time-killing curve analysis primarily showed indifference with the CPM-CA combination, suggesting that CA may not have a beneficial effect as SUL. However, susceptibility is enhanced in all and more than half of the clinical isolates with the addition of GEN and TOB, respectively. The combinations, CPM-GEN and CPM-TOB might warrant further investigation.

P714

In vitro activities of daptomycin, linezolid, and quinupristin-dalfopristin against clinical isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis* from Germany

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Objectives: Drug resistance in Gram-positive pathogens has become an increasing problem over the last two decades. Methicillin (oxacillin)-resistant isolates of *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (MRSE) are now major nosocomial pathogens worldwide. New antibacterial agents such as daptomycin (D), linezolid (L), and quinupristin–dalfopristin (Q–D) have been developed to address these problems. The objective of this study was to evaluate and compare the *in vitro* activity of D, L, and Q–D against recent clinical isolates from various places in Germany.

Methods: A total of 164 isolates including MSSA (n = 29), MRSA (n = 50), MSSE (n = 29), and MRSE (n = 56), obtained from various multicentre studies conducted between 2001 and 2004 were tested. Minimum inhibitory concentrations (MICs) for D, L, Q–D, vancomycin, teicoplanin, ciprofloxacin, and oxacillin were determined by the broth microdilution procedure according to CLSI (NCCLS) guidelines.

Results: MIC50/90 values for D, L, and Q–D were 1/1, 2/2, and 0.5/0.5 mg/L against MSSA, 1/1, 2/2, and 0.5/1 mg/L against MRSA, 1/1, 1/1, and 0.5/0.5 mg/L against MSSE, and 1/1, 1/1, and 0.5/0.5 mg/L against MRSE. No significant differences in the success rates between D, L, and Q–D were observed for MSSA (100%, 100%, 100%), MRSA (100%, 94%, 98%), MSSE (100%, 100%, 100%), and MRSE (100%, 100%, 98%), respectively. Vancomycin and teicoplanin were active against all staphylococci. Based on MIC90 values, however, vancomycin was fourfold more potent against MSSE and MRSE than teicoplanin. 98% and 68% percent of the MRSA and MRSE strains were resistant to ciprofloxacin, respectively.

Conclusion: D, L, and Q–D each demonstrated potent *in vitro* activity against *S. aureus* and *S. epidermidis* including methicillin (oxacillin)-resistant isolates.

P715

In vitro antichlamydial activity of moxifloxacin versus a range of antimicrobial agents

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Objectives: To test the *in vitro* activity of moxifloxacin (MXF) versus doxycycline (DOX), levofloxacin (LFX), clarithromycin (CLAR) and erythromycin (ERY) against *C. trachomatis*.

Materials: 30 strains of *C. trachomatis* (10 typed and 20 untyped clinical isolates from urethral swabs of male patients with non-gonococcal urethritis) were tested. Susceptibility testing was performed in plated LLC-MK2 cells in supplemented Eagle's minimum essential medium. Plates were inoculated with chlamydiae that yielded 5×10^3 inclusion forming units (IFU)/mL. Cultures were stained with fluorescein-conjugated monoclonal antibody specific for the chlamydial lipopolysaccharide genus-specific antigen. All antimicrobial agents were obtained as powders and solubilized according the instructions of manufacturers. Minimum inhibitory concentration (MIC; lowest concentration preventing >90% chlamydial inclusion detection compared with the drug-free control) and minimum bactericidal concentration (MBC; lowest antimicrobial concentration resulting in >90% reduction of inclusion) were determined.

Results: The results are shown in the Table. Of the fluoroquinolones, MXF was more active than LFX. Of the macrolides, CLAR was more active than ERY. MXF had the best bactericidal activity of all the compounds tested.

Antibiotic	**MIC range** (µg/mL) (n=30)	**MBC range** (µg/mL) (n=30)
MXF	0.03–0.06	0.03–0.06
DOX	0.03–0.06	0.06–0.125
LFX	0.25–0.5	0.25–0.5
CLAR	0.015–0.03	0.03–0.125
ERY	0.25–1	0.5–2

Conclusions: These data confirm the high activity of MXF versus *C. trachomatis*. Therefore, MXF is likely to be a suitable treatment for infections where *C. trachomatis* is a potential pathogen.

P716

Time-killing of viable *Staphylococcus* spp. against *Helicobacter pylori* clinical isolates

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Objective: To determine the inhibitory effect of 2 viable *Staphylococcus* strains against 4 *H. pylori* clinical isolates by a time-kill assay.

Methods: *H. pylori* strains were isolated from 4 gastric biopsies. They were plated on blood and Pylori agar and incubated under microaerobic atmosphere at 37 °C for 3–5 days. Identification was made by Gram stain and the presence of oxidase, catalase

and urease and susceptibility pattern was performed by disc diffusion for amoxicillin and tetraciclin and E-test for metronidazole and clarithromycin. Virulence factors (cagA and vacA genes) were also studied by PCR. Two Staphylococcus strains (1 *S. auricularis* and 1 *S. epidermidis*) were isolated from blood cultures. Culture supernatants were obtained by centrifugation (10 min at 13,000 rpm), filter-sterilized (0.45 micron pore size filter) to eliminate the presence of viable cells, lyophilized by freezed-drying and concentrated at 1000 mg/ml in PBS. Bacterial viability between *Staphylococcus* strains and *H. pylori* clinical isolates was estimated by a time-kill assay. Tubes were prepared with BHI supplemented with bovine foetal serum and *H. pylori* after a 3 MacFarland suspension. Viable *Staphylococcus* (0.5 MacFarland suspension) or lyophilized *Staphylococcus* were then added and also amoxicillin to one of the tubes. At the initial time of inoculation and at various times thereafter (1 h, 2 h, 6 h, 24 h), the number of viable CFU remaining were determined by performing serial dilution bacterial colony counts on Mueller–Hinton agar with 5% of horse blood, 10 mg/l of vancomycin and 10 mg/l of anfotericin B. Plates were incubated under microaerobic atmosphere at 37 °C for 5–7 days.

Results: We obtained different patterns of inhibition after 24 hours of incubation. Two *H. pylori* isolates were inhibited by the viable Staphylococcus and not by the lyophilized *Staphylococcus*. Two *H. pylori* isolates were inhibited only by one of the viable *Staphylococcus* and not by the lyophilized or the other viable *Staphylococcus*, even though in the last case the number of *H. pylori* colonies did not decreased as much as the amoxicillin curve.

Conclusions: (1) Viable *Staphylococcus* was able to inhibit *H. pylori* growth whether the cell-free supernatant was not. (2) *H. pylori* isolates had different susceptibility patterns so it seems that resistance is not important for *Staphylococcus* inhibition effect. (3) Further studies are needed in order to know the clinical applications of these results.

P717

IBC-1 survey in *Acinetobacter baumannii* isolates in a tertiary care hospital in Thessaloniki, Greece

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Objectives: IBC-1 is a beta-lactamase conferring high level resistance to ceftazidime and inhibited mainly by imipenem and to a lesser extent by inhibitors. bla IBC-1 gene is located in the variable region of class 1 integron, In111, constituted of gene cassettes also encoding the aminoglycoside modifying enzymes AAC(6')-Ib and ANT(3()-Ia as well as dihydrofolate reductase I. This study aimed to investigate the possible distribution of IBC-1, which was first identified in our hospital in an *Enterobacter cloacae* strain, in *Acinetobacter baumannii*.

Methods: From June 2001 to July 2002, 57 clinical *A. baumannii* isolates resistant to ceftazidime and susceptible or intermediately resistant to imipenem were collected and investigated. Identification and susceptibility testing were performed using the Vitek2 automated system (bioMerieux, France). Susceptibilities to amoxicillin/clavulanate (AMC), piperacillin/tazobactam (TZP), ticarcillin/clavulanate (TCC), gentamicin (GM), tobramycin (TOB), amikacin (AN) and trimethoprim/sulfomethoxazole (SXT) were also determined. In order to investigate the presence of bla IBC-1, a 400-bp internal fragment of the gene was amplified, corresponding to the segment 51–450 of the published sequence. *E. cloacae* HT9 was used as positive control.

Results: Out of the 57 isolates 37 were susceptible to imipenem and 20 presented intermediate resistance. All isolates were resistant to ceftazidime. Inhibitor combinations were active only in 3 cases. Resistance to inhibitor combinations was demonstrated for 75.4%, 33.3% and 87.7% for AMC, TZP and TCC respectively. A high percentage of intermediate resistance was reported for TZP (63.1%). Resistance rates for GM, TOB, AN and SXT were 35%, 43.8%, 75.4% and 75.4%, respectively. No specific resistance phenotypes were observed suggesting that there were no epidemic clusters of infection in our hospital. No IBC-1 producing strains were observed since PCR results were negative in all cases tested.

Conclusion: The present study indicates that IBC-1 is not a problem in *A. baumannii* strains in our region. But since a specific geographical distribution has been reported for beta-lactamases and since the beta-lactam selective pressure facilitates the spread of these genes and their establishment in pathogenic microorganisms, a continuous survey for IBC producers in the hospitals of our area is warranted.

P718

Antibacterial activity of fractions of *Quercus infectoria* (nut galls) against enterohaemorrhagic *Escherichia coli*

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Objectives: Stimulation of Verocytotoxin (VT) production due to the use of subinhibitory concentrations of quinolones for patients with diarrhoea caused by enterovirulent organisms including enterohaemorrhagic *Escherichia coli* (EHEC) has been reported. The aim of this study was to analyse the effects of semi-purified fractions of *Quercus infectoria* nutgalls, which has been previously reported as effective against these organisms, on the production of VT.

Methods: Dry powder of crude ethanol extract is further fractionated in quick column chromatography using gradient solution with chloroform of increasing polarity by methanol. Active fractions were screened against different strains of EHEC including two strains of *E. coli* O157: H7, *E. coli* O26: H11, *E. coli* O11: NM, and *E. coli* O22. *Escherichia coli* ATCC 25922 was used as a reference strain. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by broth microdilution method using Mueller–Hinton broth and subculturing method onto fresh Mueller–Hinton agar, respectively. The inhibitory effects of the active fractions on the production of VT were tested for their cytotoxicity activity in a Vero cell assay system and reversed passive latex agglutination.

Results: Three major fractions (fraction Qi 2, fraction Qi 3, and fraction Qi 4) showed good antibacterial activity against all strains of enterohaemorrhagic *Escherichia coli*. The fractions Qi 3 and Qi 4 were demonstrated to be highly effective against *E. coli* O157: H7 with the MIC values of 28 and 56 μg/ml and the MBC values of 56 and 112 μg/ml, respectively. Further study showed that subinhibitory concentration of both fractions significantly depressed the VT production, both VT1 and VT2.

Conclusion: Both fractions, Qi 3 and Qi 4, of Quercus infectoria were proved to be very active against EHEC and depressed the VT production. Tannin has been reported to be main the constituent of this plant. It is now being further purified and identified using NMR spectra and mass spectrometry for potentially bioactive components to provide alternative treatment for EHEC infection.

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P719

In vitro synergy between glycopeptides and carbapenems against methicillin-resistant *Staphylococcus aureus*

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Objectives: The aim of this study is to investigate the synergy between glycopeptides-carbapenems, glycopeptides-cefepime against methicillin-resistant *Staphylococcus aureus* and whether there is any dispreancy between them.
Methods: A total of 30 MRSA isolates were studied. None of the isolates had reduced susceptibility to glycopeptides. E-test was performed to investigate the synergy of vancomycin-imipenem (VA-IPM), vancomycin-meropenem (VA-MEM), teicoplanin-imipenem (TEC-IPM), teicoplanin-meropenem (TEC-MEM), vancomycin-cefepime (VA-FEP), teicoplanin-cefepime (TEC-FEP) combinations.
Results: Results are shown in the Tables 1 and 2. Synergy was detected in 19, 25 of 30 strains for the VA-IPM and VA-MEM, TEC-IPM and TEC-MEM combinations, respectively. VA-FEP synergy was detected in 19, TEC-FEP synergy was detected in the 21 of the isolates.

Table 1. FIC indices of beta-lactam and glycopeptide combinations

	Mean FIC index	Synergy FIC≤0.5	Additive FIC=0.5-1	Indifference FIC=1-2	Antagonism FIC≥2
VA-IPM	0.32	19	0	11	0
VA-MEM	0.39	19	0	11	0
VA-FEP	0.41	19	0	11	0
TEC-IPM	0.31	25	0	5	0
TEC-MEM	0.32	25	0	5	0
TEC-FEP	0.39	21	0	9	0

Table 2. The distribution of the various types of combinations among MRSA strains

Synergistic against	Number of strains
All combinations	15
None of the combinations	5
Glycopeptide-carbapenem only	4
Glycopeptide-cefepime and Teicoplanin-carbapenem	4
Teicoplanin-carbapenem only	2
Total	**30**

Conclusion: Glycopeptides have been used in the treatment of methicillin-resistant *Staphylococcus aureus* infections for the last 30 years. Tissue penetration of the glycopeptides is usually questionable when serious infections such as infective endocarditis are the case. Combination therapy may offer new therapeutic approaches in the treatment of such infections since *in vitro* studies provide promising results.

P720

Susceptibility rates of co-trimoxazole, cefepime, imipenem, meropenem against ESBL-producing and non-producing *E. coli* and *Klebsiella* strains

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Objectives: The number of antibiotics to be used in the treatment of infections caused by extended spectrum beta-lactamase (ESBL) producing bacteria is limited. The aim of this study is to determine the minimal inhibitory concentrations (MIC) and susceptibility rates of ESBL producing and not producing *E. coli* and *Klebsiella* strains against co-trimoxazole, cefepime, imipenem and meropenem.
Methods: One hundred and twenty-eight (48.2%) of 263 *E. coli* strains and 58 (31.1%) of 176 *Klebsiella* strains isolated from the hospitalized patients in 2001 were GSBL producers. A total of 69 *E. coli* isolates (36 ESBL negative and 33 ESBL positive) and 57 *Klebsiella* isolates (34 ESBL negative, 23 ESBL positive) were included to this study. MIC values of the strains were determined by agar dilution method according to the CLSI criteria.
Results: MIC values and susceptibility rates obtained in this study are shown in the Table. MIC values of co-trimoxazole and cefepime against ESBL positive strains are higher than the ESBL negative ones. MIC values of carbapenems were similar against both the ESBL negative and positive bacteria.

Table. MIC values (mg/L) and susceptibility rates of *E.coli* and Klebsiella against the tested antibiotics

	E.coli		Klebsiella	
	ESBL negative (n=36)	ESBL positive (n=33)	ESBL negative (n=34)	ESBL positive (n=23)
Co-trimoxazole				
MIC_{50}	0.25	1	0.25	1
MIC_{90}	0.5	2	0.5	2
MIC Range	0.5-16	0.5-16	0.5-16	0.5-16
Susceptibility rate (%)	78	46	73	42
Cefepime				
MIC_{50}	0.06	16	0.12	16
MIC_{90}	0.12	32	0.12	32
MIC Range	0.06-0.2	2-64	0.06-0.5	2-64
Susceptibility rate (%)	100	12	100	9
Imipenem				
MIC_{50}	0.06	0.12	0.12	0.25
MIC_{90}	0.12	0.12	0.5	0.5
MIC Range	0.06-1	0.06-1	0.06-1	0.06-1
Susceptibility rate (%)	100	100	100	100
Meropenem				
MIC_{50}	0.06	0.06	0.06	0.06
MIC_{90}	0.06	0.06	0.06	0.06
MIC Range	0.06-0.12	0.06-0.12	0.06-0.12	0.06-1
Susceptibility ate (%)	100	100	100	100

Conclusion: All the strains tested were found to be susceptible to imipenem and meropenem. Carbapenems seem to be appropriate choices in the treatment of infections caused by ESBL producing bacteria based on the *in vitro* susceptibility data.

P721

Comparison of the minimal inhibitory concentration values of vancomycin and teicoplanin against staphylococci with the results obtained 7 years ago

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Objectives: *Staphylococci* are frequently isolated from community and hospital acquired infections. Vancomycin has been used for the methicillin-resistant staphylococcal infections confidentially for many years. The aim of this study is to determine the MIC values of vancomycin and teicoplanin against staphylococci and to compare the results with the MIC values obtained from the same laboratory 7 years ago.
Methods: The isolates included in this study were collected from samples obtained from different patients. The distributions of the isolates were as follows; 76 MSSA, 77 MRSA, 79 MS-CoNS, 78 MR-CoNS.
Results: MIC values obtained in this study are shown in the Table. MIC50 and MIC90 values for vancomycin against MSSA,

Table. MIC values of vancomycin and teicoplanin against staphylococci (μg/mL)

	Vancomycin			Teicoplanin		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
MSSA (n=76)	1	2	0.5-2	0.5	1	0.125-4
MRSA (n=77)	1	2	0.5-2	2	2	0.25-4
MS-CoNS (n=79)	1	2	0.25-2	1	4	0.125-4
MR-CoNS (n=78)	1	2	0.5-2	2	4	0.125-4

MRSA, MS-CoNS, MR-CoNS were found to be similar with the values obtained 7 years ago. MIC50 values of teicoplanin against all strains increased. MIC90 values of teicoplanin against MSSA, MRSA and MS-CoNS were similar with the results of the previous study. Only the MIC90 value against MR-CoNS increased.
Conclusion: Absence of staphylococcal isolates either with reduced susceptibility or resistant to glycopeptides in our study is a pleasant result.

P722

Serotypes and quinolone resistance in *Salmonella enterica* isolated from Greek diarrhoeal patients

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Objective: To determine the serotype distribution and the *in vitro* activity of nalidixic acid and ciprofloxacin of 825 *Salmonella enterica* strains isolated from diarrhoeal patients in Crete, Greece.
Methods: A total of 825 *Salmonella enterica* strains isolated from faecal samples were tested. Identification was made according to standard microbiological procedures and serotyping was performed with commercial antisera by the slide agglutination method. Antimicrobial susceptibility testing was checked by the disk diffusion method. The MICs of nalidixic acid and ciprofloxacin were determined by the use of the E-test and were interpreted according to the NCCLS guidelines.
Results: A total of 29 serotypes were identified. *Serotype Enteritidis* was the most prevalent (563 strains or 66%) followed by serotype *Typhimurium* (147 strains or 17.2%). Infantis (3.2%) and Newport (3.2%) were the next most frequent serotypes. All isolates were sensitive to ciprofloxacin but forty-one (4.8%) of them were resistant to nalidixic acid. All nalidixic acid-resistant strains (34 belonging to serotype *Enteritidis* and 7 belonging to other serotypes) exhibited decreased ciprofloxacin susceptibility (0.125–0.75 μg/ml).
Conclusion: Reduced susceptibility to ciprofloxacin found in 4.8% of salmonellae in our area is a cause of concern.

P723

Co-trimoxazole resistance in urinary tract infection agent *E. coli* in 1995 and 2005: a multi-centre study in Ankara, Turkey

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Objectives: Detection of co-trimoxazole resistance in UTI agent E. coli and its distribution in different parameters have been aimed in this study.
Methods: The study was performed in 1995 and 2005, and 382 and 510 UTI agent *E. coli* strains were included, respectively. The strains were identified by standard laboratory techniques in Hacettepe University, Faculty of Medicine (HUFM), Gulhane Military Medical Academy (GMMA), Ankara Numune Government Hospital (ANGH), Dr. Sami Ulus Pediatric Hospital (SUCH) and Refik Saydam National Hygiene Center (RSNHC). There were no record about gender, age or being outpatient/inpatient in 1995, but these parameters were recorded in 2005 with an exception of the age of 44 patients. Antibiotic susceptibility tests were performed by microdilution method according to National Committee for Clinical Laboratory Standards criteria in RSNHC laboratory, trimethoprim (Serva-37049) and sulphamethoxazole (Sigma S-7507) were used and *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922 were included for quality control. Chi-square and Fishers Exact test was done for statistical analysis by SPSS 10.0 for Windows.
Results: The resistance percentage, MIC50 and MIC90 in 1995 were 75.1%, 32/608 and >32/608, respectively and in 2005 were 55.5%, 32/608 and >32/608, respectively. The distribution of number of the strains and resistance percentages according to the hospitals and years are shown in Table. There was a reduction in resistance percentages in every hospital and for the total from 1995 to 2005 and the differences were statistically significant with exception of HUFM and GMMA. On the other hand there was no statistically significant difference among the hospitals in each year. The resistance percentages for children (n = 208) was 61.1% and adult (n = 258) was 51.2%, and the difference was significant ($p < 0.05$). For gender, the resistance was 53.7% for female (n = 380) and 60.8% for male (n = 130), and for outpatient (n = 400) it was 55.3% and inpatient (n = 110) 56.4%, and the differences were not significant (p>0.05) (p>0.05).

Hospitals	Number of strains in 1995	Resistance % in 1995	Number of strains in 2005	Resistance % in 2005	p value
HUFM	121	66.1	98	56.1	p=0.13(p>0.05)
GMMA	3	66.7	104	52.9	p=0.55(p>0.05)*
ANGH	43	76.7	92	50.0	p=0.003(p<0.05)
SUPH	135	79.3	119	62.2	p=0.003(p<0.05)
RSNHC	80	81.3	97	54.6	p=0.009(p<0.05)
Total	382	75.1	510	55.5	p=0.000(p<0.05)
p value		p=0.08(p>0.05)		p=0.461(p>0.05)	
*Fishers exact test					

Conclusion: Co-trimoxazole was frequently the treatment of choice for UTIs as well as other infections from the early 1980s, in Turkey. Increased resistance rates led to its therapeutic failure and subsequent limited usage. The resistance percentages in this study showed statistically significant reduction from 1995 to 2005 which is thought to be a consequence of reduced rate of use.

P724

Profile and phenotypes of resistance of microorganisms from pregnant women with asymptomatic bacteriuria in Russia

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Background: Asymptomatic bacteriuria (ASB) is common during pregnancy. But their adequate empirical antimicrobial treatment is possible only if resistance of the UTI pathogens in region is well established.

Methods: During the 2002–2003 prospective epidemiological study involved 132 pregnant women aged 16 to 43 years with ASB (>105 CFU/ml) at 6 Russian community health-care centres in Moscow, Smolensk, Volgograd and St-Petersburg. After re-identification, MICs for 7 of antimicrobials were determined by agar dilution method and interpreted using NCCLS/CLSI 2003 guidelines.
Results: 129 urine isolates with ASB were collected. *E. coli* was the predominant pathogen (65.1%), followed by *Klebsiella* spp. (9.3%), *Enterobacter* spp. (8.5%), *P. mirabilis* (6.2%), *Staphylococcus* spp. (3.1%) and *Enterococcus* spp. (2.3%). Antimicrobial resistance rates of *E. coli* were as follows: ampicillin–29.8%; co-trimoxazole–12.0%; gentamicin–6.0%; cefuroxime–4.8%; amoxicillin/clavulanate–3.6%; nitrofurantoin–3.6% and fosfomycin–0%. The most active antimicrobials (sensitivity 91–100%) against *Klebsiella* spp. were cefuroxime, cefotaxime, gentamicin and fosfomycin; against *P. mirabilis*–amoxicillin/clavulanate, cefuroxime, cefotaxime, gentamicin and fosfomycin; against *Enterobacter* spp. – cefotaxime, gentamicin and fosfomycin.
Conclusions: The main problem with uropathogens in Russia is a high level of *E. coli* resistance to aminopenicillins and co-trimoxazole. These antimicrobials should no longer be used as drugs of choice in the treatment of ASB. The most active antimicrobials against non-*E. coli Enterobacteriaceae* isolates were cefotaxime, gentamicin and fosfomycin.

P725

Activity of moxifloxacin against the urogenital mycoplasmas *Ureaplasma* spp. and *Mycoplasma hominis*

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Objectives: Urogenital mycoplasmal species, *Ureaplasma* spp. and *Mycoplasma hominis*, play etiological roles in some urogenital diseases in men and women. Antimicrobials potentially active vs mycoplasmas are tetracyclines, macrolides and related antimicrobials and fluoroquinolones. Among fluoroquinolones, the most recent products present the highest activity and are, with ketolides, the only bactericidal agents against mycoplasmas. Moxifloxacin activity against human mycoplasmas has been studied but with a relatively low number of strains and no bactericidal testing. Our objective was to extend the study of the *in vitro* activity of moxifloxacin to a larger number of strains of *Ureaplasma* spp. and *M. hominis*, to compare it to other fluoroquinolones and non-related antimicrobials, and to determine the minimal bactericidal concentrations (MBCs) of moxifloxacin against 12 strains of *Ureaplasma* spp. and *M. hominis*.
Methods: Four ATCC strains, 52 clinical isolates of *Ureaplasma* spp. and 52 clinical isolates of *M. hominis* isolated between 2003 and 2005 at Pellegrin Hospital, Bordeaux, France, were studied. Among the clinical isolates, 22 and 20 were resistant to doxycycline (DOX-R). MICs and MBCs were determined as previously described.
Results: Doxycycline was the most active, with a MIC90 of 0.25 μg/mL vs Ureaplasma isolates susceptible to tetracyclines while the 3 newer fluoroquinolones moxifloxacin, gemifloxacin and garenoxacin were most active vs the DOX-R isolates. Moxifloxacin, gemifloxacin, and garenoxacin had similar MICs (MIC90s 0.5–1 μg/mL) while the MIC90s of gatifloxacin, levofloxacin and ofloxacin, ranged from 1 to 2 μg/mL. Against *M. hominis* isolates, moxifloxacin and gemifloxacin were the second most potent behind garenoxacin with a MIC90 of 0.06 μg/mL. Gatifloxacin, levofloxacin, and ofloxacin had higher MICs than those of moxifloxacin. Except for one *Ureaplasma* spp. isolate with increased fluoroquinolone MICs, moxifloxacin was the only fluoroquinolone to be bactericidal against the 11 fluoroquinolone-susceptible isolates tested, with MBCs ranging from 0.12 to 0.5 μg/mL.
Conclusion: Of 6 quinolones tested, moxifloxacin had good activity vs 108 strains of *Ureaplasma* spp. and *M. hominis* with a MIC90 of 1 μg/mL. It was bactericidal against almost all isolates tested (MBC value of 0.5 μg/mL) inhibiting 11 of the 12 isolates studied. Therefore moxifloxacin could be useful as a treatment for pelvic infections due to *Ureaplasma* spp. and *M. hominis*.

P726

Activity of moxifloxacin against *Mycoplasma genitalium*

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Objectives: *Mycoplasma genitalium* is a genital mycoplasma extremely difficult to isolate by culture. Development of PCR assays showed a strong association of *M. genitalium* with non-gonococcal urethritis in men and cervicitis and endometritis in women. Fluoroquinolones rank among the few antimicrobials bactericidal against mycoplasmas. The activity of moxifloxacin has already been determined against human mycoplasmas, but only against 7 isolates of *M. genitalium*. Our objective was to extend the study of the *in vitro* activity of moxifloxacin to a larger number of strains of *M. genitalium*, in comparison with that of other fluoroquinolones (ofloxacin, levofloxacin, gatifloxacin, gemifloxacin, and garenoxacin) and to doxycycline and erythromycin.
Methods: Fourteen strains of *M. genitalium*, 7 ATCC strains, 2 French and 5 Danish clinical isolates, were studied. Determination of MICs and MBCs was carried out as previously described for each isolate.
Results: Erythromycin was the most active molecule with a MIC90 of 0.015 μg/mL. Activities of moxifloxacin and doxycycline were similar with MIC90s of 0.12 μg/mL. Garenoxacin, gemifloxacin, and gatifloxacin had similar MICs (MIC90s of 0.25 μg/mL) while the MIC90s of levofloxacin and ofloxacin, ranged from 1 to 2 μg/mL. The lowest MBCs against *M. genitalium* isolates were obtained with erythromycin, followed in decreasing order by those of moxifloxacin (MBC90, 0.25 μg/mL), garenoxacin (MBC90, 0.5 μg/mL), gemifloxacin, gatifloxacin and doxycycline (MBC90, 1 μg/mL), and lastly by levofloxacin and ofloxacin (MBC90, 4 μg/mL).
Conclusion: Among the 6 fluoroquinolones tested, including newer compounds, moxifloxacin offered the best activity against 14 strains of *M. genitalium*, with a MIC90 of 0.12 μg/mL. Moxifloxacin appeared to have bactericidal activity against all the isolates with a MBC90 of 0.25 μg/mL, significantly lower than the breakpoints accepted for this antimicrobial.

P727

Emergence of extended-spectrum beta-lactamases in a university hospital, Hamburg, Germany in 2004

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Objectives: Extended-spectrum beta-lactamases (ESBLs) are a major problem in the treatment of gram-negative infections with cephalosporins, due to the hydrolytic inactivation of third generation cephalosporins. Thus, this study aimed at investigating the occurrence of ESBLs in the largest university hospital in Hamburg, Germany.

Methods: Fifty ESBL-producing strains were collected at the University Hospital Eppendorf in 2004 (total ESBL prevalences: *Klebsiella pneumoniae*: 1.9%, *Escherichia coli*: 0.8%, *Klebsiella* oxytoca: 0.8%). The MICs of the following antibiotics were determined by micro broth-dilution technique using Micronaut-S plates: ampicillin (AMP +/− sulbactam, SUL), amoxicillin + clavulanic acid (AMOXI + CLA), piperacillin + SUL or + tazobactam (PIP + SUL, PIP + TAZ), cefuroxime (CEFU), cefoxitin (COX), cefotaxime (CTX +/− CLA), ceftriaxone (CRO), cefepime (CPM), ceftazidime (CAZ +/− CLA), imipenem (IMP), meropenem (MER), ertapenem (ERT), gentamicin (GEN), tobramycin (TOB), amikacin (AMIK), ciprofloxacin (CIP), moxifloxacin (MOX), doxycycline (DOX) and cotrimoxazol (COT). Breakpoints were applied according to the Clinical and Laboratory Standards Institute (CLSI).

Results: All isolates are resistant to AMP (100%), while 76% and 78% are resistant to the AMP + SUL and AMOXI + CLA combinations, respectively. Only 8% of all strains were resistant to PIP + TAZ. Regarding the cephalosporins: 26% resistant to CAZ, 24% to CPM, 6% to COX, 90% to CEFU, 84% to CRO and 70% to CTX. Carbapenems are clinically susceptible in 98% of all isolates. There is one strain which is resistant to IMP and ERT - intermediate to MER. 38% are resistant to DOX. 62% are resistant to CIP, but only 48% to MOX. 66% are not affected by GEN. TOB and AMIK are more potent in the group of aminoglycosids (TOB 18% and AMIK 12% resistant). For COT 80% were resistant. Comparing the results for CAZ and for CAZ + CLA for 96% an ESBL phenotype could be confirmed.

Antibiotic	% susceptible (n)	% intermediate (n)	% resistant (n)
AMP	0 (0)	0 (0)	100 (50)
AMP + SUL	24 (12)	6 (3)	70 (35)
AMOXI + CLA	28 (14)	26 (13)	46 (23)
PIP + TAZ	92 (46)	6 (3)	2 (1)
CEFU	2 (1)	4 (2)	90 (45)
COX	90 (45)	4 (2)	6 (3)
CTX	8 (4)	22 (11)	70 (35)
CRO	8 (4)	8 (4)	84 (42)
CPM	60 (30)	16 (8)	24 (12)
CAZ	52 (26)	22 (11)	26 (13)
IMP	98 (49)	0 (0)	2 (1)
MER	98 (49)	2 (1)	0 (0)
ERT	98 (49)	0 (0)	2 (1)
GEN	32 (16)	2 (1)	66 (33)
TOB	72 (36)	10 (5)	18 (9)
AMIK	72 (36)	16 (8)	12 (6)
CIP	38 (19)	0 (0)	62 (31)
MOX	42 (21)	10 (5)	48 (24)
DOX	44 (22)	18 (9)	38 (19)
COT	20 (10)	0 (0)	80 (40)

Conclusion: Compared to other hospitals the prevalence of ESBLs is very low in the University hospital of Hamburg, Germany. Nearly all infections (98%) could be treated successfully with carbapenems. One strain has shown very high MICs to carbapenems which seems to be caused by a carbapenemase. Fortunately, this isolate has retained susceptibility to CIP, MOX and DOX.

Identification and antifungal susceptibility testing for yeasts and moulds

P728

Candida krusei: the most frequent *Candida* non-*albicans* species isolated from superficial candidosis in Romania

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Introduction: Our previous experience indicated as possible a different profile of the incidence of *Candida* spp. implicated in superficial infections in Romania.

Objectives: To compare the incidence of the most encountered *Candida* spp. oral isolates in Romania and worldwide.

Premise of the study: The epidemiology of *Candida* spp. could present significant variation in different geographic sites, due to specific ''pool of yeasts'' in a certain population.

Materials and methods: 210 oropharyngeal samples were plated onto Sabouraud Dextrose Agar for 48 hours and incubated at 37° C. The identification of the isolated yeasts was made using morphological features, Germ tube tests and automatic API System ID 32 C (BioMerieux, France). The results of the study were compared to those of two Romanian previous studies (from 2001 and 1968) and Artemis Global Study (2001).

Results: From all identified isolates, 58% were *Candida albicans*; 8% *C. krusei*; 7.14% *C. glabrata*; 3.8% *C. kefyr* and 2.8% *C. tropicalis*; other species representing each under 1%. We noticed that along 37 years, the incidence of *Candida albicans* was relatively stable. Thus: in Romania 59% (1968); 63% (2001) and 58% (2005) and worldwide 63% (2001). In the same period of time, the incidence of *C. krusei* in Romania was rather high (12.9% in 1968; 13.5% in 2001 and 8% in 2005) compared with a mere 2.5% in the global study (2001). The increased incidence of *C. krusei* in Romania along almost 40 years made us to conclude: (1) this could be considered a feature of the yeast population in our country, aspect with relevance for the therapeutic strategies (2) the species selection due to antifungal therapy could not explain this status as being present from 1968, a moment before the ''modern antifungal age.''

P729

Variation of galactomannan level during 24-hour period in haematooncological patients with/without invasive aspergillosis

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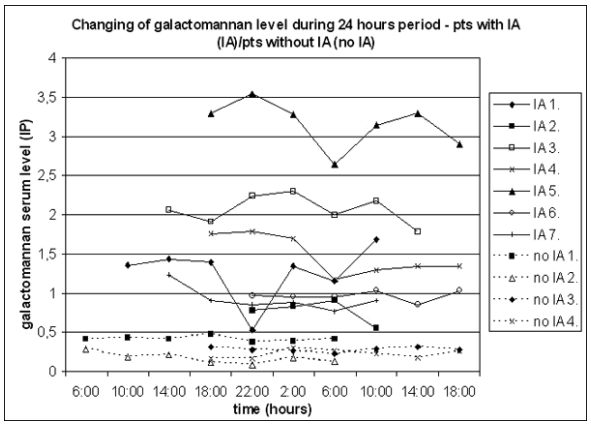
Objective: Detection of galactomannan (GM) serum level by EIA is useful marker for diagnosis of invasive aspergillosis (IA) in haematooncological patients (pts). We evaluated possible affection of sample positivity by GM serum level variation during 24 hours period.

Methods: GM serum levels have been measured in 11 pts with hematological malignancy in 4 hours intervals during 24 hours. Platelia Aspergillus EIA® kit has been used for detection of GM in serum. Results have been expressed as index of positivity (IP) with cut off 0.5, 1.0 and 1.5 considered as positive. The difference between the highest and lowest IP achieved during the 24 hours period in each patient has been calculated.

Results: Mean IP in 7 pts (43 serum samples) with probable/proven IA was 1.6 and mean difference between the highest and

lowest IP achieved during the 24 hours period was 0.6 (0.17–1.15). If IP 0.5 has been chosen as the cut off any sample has not been negative. If IP 1.0 have been chosen as the cut off 14 samples (32%) in 4 pts have been considered as negative. And finally if IP 1.5 has been chosen as the cut off 26 samples (60%) in 5 pts have been considered as negative. Mean IP in 4 pts (27 serum samples) with possible IA was 0.27 and mean difference between the highest and lowest IP achieved during the 24 hours period was 0.14 (0.09–0.2). If the lowest IP 0.5 has been chosen as the cut off any sample has not been positive.



Conclusion: We found significant variation of serum GM concentration in patients with probable/proven IA during 24 hours period. This variation can lead to negative results of Platelia Aspergillus EIA® test if just one serum sample per a day is obtained and IP 1.0 or 1.5 is used as cut off. But if lower cut off (IP 0.5) is used (as approved by FDA and in our lab is used) daily variation of GM level does not influence positivity of samples and taking one sample per a day is sufficient.

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P730

Laboratory diagnosis of pulmonary aspergillosis in tuberculosis patients

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Objective: To analyse the results of mycological and immunological laboratory investigations of pulmonary tuberculosis (TB) patients observed during 2003–2005 in the TB clinic.

Methods: Culture (Sabouraud chloramphenicol agar, Bio-Rad Labs.) and microscopy of bronchoalveolar lavage, sputum, materials from lung and pleural cavities, lung tissue samples (materials of biopsies and resection), specific identification of isolated mold fungi strains (conventional methods, identification media Czapek agar); detection of circulating Aspergillus galactomannan (GM) antigen in the serum using agglutination technique: test Pastorex® Aspergillus, Bio-Rad Labs.

Results: During 2003–2005 we investigated diagnostic materials from 467 pulmonary TB patients, which received treatment. According to conventional criteria, colonization of the lower respiratory tract with fungi from genus *Aspergillus* was detected in 47 (10.1%) patients. In five patients *Aspergillus* sp. (*A. fumigatus* in three cases, *A. flavus*, *A. restrictus*) was isolated from the resection materials of the lung cavity and in two patients *A. fumigatus* was isolated from the materials of bronchial biopsies. Aspergillus GM antigen detection was made in 68 patients from risk group for invasive pulmonary aspergillosis (positive culture and/or clinical and radiographic data). Aspergillus GM antigen was detected in four patients; in three of them positive results were confirmed by positive culture (materials of resection or biopsies).

Conclusions: An urgent problem of clinical physiology is diagnosis of secondary aspergillosis in pulmonary TB patients. It is advisable to perform a complex laboratory examination of TB patients (mycology and immunology studies) for successful differential diagnosis of invasive pulmonary aspergillosis. High level of specific diversity of fungi from genus *Aspergillus* (11 species) colonizing respiratory tract in TB patients was revealed.

P731

Identification of *Malassezia* isolates of patients with pityriasis versicolor by using PCR-restriction enzyme method

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Introduction: The member of the genus *Malassezia* are the causative agents of the skin disease pityriasis versicolor. There are evidences that these yeast may involve in some other dermatological disorders such as seborrheic dermatitis and atopic dermatitis. The recently finding shows various *Malassezia* species can cause the diseases and molecular biological methods are the most reliable way for identification of the isolates. The aim of the present study is identification and determination of frequency of *Malassezia* isolates in Iranian patients with pityriasis versicolor by using PCR-restriction method.

Methods: 83 patient's isolates of *Malassezia* were isolated on modified Dixon agar. Genomic DNA was extracted by glass-beads-phenol chloroform method and purified by alcohol precipitation. In all samples 28srDNA regions was PCR-amplified, digested by the restriction enzyme CfoI and electrophoresed on 1.8% agarose gels. Identification was carried out according the different electrophoretic patterns after RFLP.

Results: The most frequent isolate was *M. globosa* (63.85%), followed by *M. furfur* (28.9%), *M. sympodialis* (3.61%), *M. restricta* (2.4%) and *M. slloffiae* (1.2%), respectively.

Conclusion: 1. PCR-RFLP is an accurate, rapid and straight forward method for differentiation of *Malassezia* species. 2. The most frequent causative agent of pityriasis versicolor in Iran should be *M. globosa*.

P732

Haemolytic activity of *Candida albicans* and *C. dubliniensis* isolated from the oral cavity of immunocompromised patients

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Objectives: The production of haemolysins is virulence factor of several pathogenic bacterial species, but haemolytic activity of clinical important polymorphic yeasts, *Candida albicans* and *C. dubliniensis* is poorly investigated. Here we present study on 66 strains of *C. albicans* isolated from the oral cavity of immunocompromised patients (HIV+ and leukaemia), and *C. dubliniensis* strains from oral cavity of HIV+ patients, and all strains were compared with *C. albicans* from the oral cavity of healthy donors (commensal microflora).

Method: The haemolytic activity was investigated according to the method described by Luo et al., J. Clin. Microbiol. 39 (2001) 2971–2974, where the hemolytic zones (expressed as hemolytic index) around yeast inoculum (10–8 blastospores/mL) were

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measured on Sabouraud 3% (m/V) glucose agar with addition of 7% sheep blood after 48 h at 37 °C under 5% CO^2. Hemolytic index was calculated as ratio between diameters of haemolytic zones and diameters of colony, which was larger than 1 (1 indicates no haemolytic activity).
Results: All *C. albicans* and *C. dubliniensis* strains isolated from immunocompromised patients showed statistically higher hemolytic index ($p < 0.05$) than commensal isolates of *C. albicans*. There were no differences between strains isolated from immunocompromised patients.
Conclusion: The expression of haemolytic activity of *C. albicans* and *C. dubliniensis* could be possible virulence factor, and haemolysis could promote invasiveness of opportunistic commensal flora, especially in the group of immunocompromised patients.

P733

Candida albicans panuveitis and an uneven therapeutic ground, passing through all available and potentially effective antifungal drugs: fluconazole, liposomal amphotericin B, caspofungin, and voriconazole

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Background: Candida endophthalmitis is a severe function-threatening infection, more frequent in immunocompromised patients.
Case report: A patient (p) with insulin-dependent diabetes mellitus was admitted due to a sudden vision loss at right. After a diagnosis of *C. albicans* panuveitis, i.v. fluconazole (400 mg/d) was started. Since a worsening of ophthalmologic-fluorangiographic picture paralleled the appearance of amblyopia, 10 d later liposomal amphotericin B (lAB) at 3 mg/kg/d replaced fluconazole and after 14 d a remarkable reduction of exudates was achieved. lAB was stopped after 17 d to renal insufficiency (creatinine 2.11 mg/dL, azotemia 1.32 g/dL, compared with normal values at baseline) and i.v. caspofungin was administered at standard dosage. After 48 d of caspofungin therapy (50 mg/d) delivered on Day-Hospital basis, active foci were still present at fluorangiography and visual acuity recovered up to 2/10, while renal impairment disappeared. Finally, i.v. voriconazole (400 mg/d) was started and continued for 23 d; after this last course, our p obtained a complete resolution of exudates at ophthalmoscopic–fluorangiographic study and visual acuity rose to 6/10. Oral voriconazole (400 mg/d) was continued for 3 weeks upon discharge.
Discussion: The rationale of antifungal therapy of Candida endophthalmitis is limited by the unfavourable kinetics of several compounds and the absence of controlled trials, so that most informations come from small series and anecdotal reports. While fluconazole may be limited by its reduced activity on some non-albicans Candida, lAB is the standard of care (administered by intraocular and/or systemic route), but isolated failures were reported. The endovitreal penetration of caspofungin is under investigation (although favourably treated p are described) while voriconazole (either as systemic or local injection agent) led to preliminary, satisfactory results. Our p with a severe Candida endophthalmitis received all the four available antimycotic agents effective against *C. albicans*, but experienced disease progression during fluconazole and probably long-term caspofungin administration, while the initially favourable lAB response was hampered by reversible kidney function anomalies, and voriconazole proved safe and effective in leading to a complete cure and a favourable recovery of visual acuity. Further, controlled studies are needed, to trace some therapeutic guidelines of *Candida panuveitis* in patients at risk.

P734

AIDS-associated *Cryptococcus laurentii* meningoencephalitis resistant to amphotericin B, apparently prompted by a cured *Cryptococcus neoformans* disease

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Objective: *Cryptococcus laurentii* is a rare pathogen among immunocompromised patients (p), with <20 described episodes and only 1 report of possible association with *C. neoformans*. An exceedingly rare case of dual HIV-associated *C. neoformans* followed by *C. laurentii* meningoencephalitis is presented and discussed on the ground of the literature which reports only 2 cases of HIV-associated *C. laurentii* infection.
Case report: A 34-y-old man HIV-infected for 8 years was lost to follow-up until his admission due to fever and headache. Lumbar puncture led to microscopical recognition of cryptococci, with both culture examination and capsular antigen search confirming a *C. neoformans* meningoencephalitis, with yeasts testing susceptible to all antifungals; a moderately advanced immunodeficiency concurred (CD4+ count 151/μL). Liposomal amphotericin B (lAB) was promptly started at 3 mg/kg/day with immediate benefit, but our p self-discharged after 12 d, and did not undergo antimycotic-antiretroviral therapy, until a subsequent hospitalization occurred 14 weeks later, owing to the same signs-symptoms, associated to the isolation of *C. neoformans* from both CSF and blood, positive antigen search and a persisting sensitivity to all antifungals. Negative CSF–blood cultures were achieved after 28 days of lAB, but 5 weeks later a novel relapse of meningoencephalitis occurred despite HAART and a maintenance weekly lAB. An unexpected, isolated CSF *C. laurentii* infection was documented with a surprising resistance to AB, while all mycological searches for *C. neoformans* (CSF–blood–urine cultures, capsular antigenemia) proved negative. High-dose fluconazole was started and HAART continued: negative *C. laurentii* microscopy-culture CSF assays were obtained after 43 d, while a significant immune recovery concurred: CD4+ count 256 cells/μL.
Conclusions: Clinicians facing HIV-infected p should consider that cryptococcosis may still occur, especially when HIV disease is missed-neglected. A dual, subsequent infection by *C. neoformans* and *C. laurentii* is possible, although very infrequent event, so that the eradication of *C. neoformans* does not guarantee that another *Cryptococcus* spp. infection could occur subsequently, although an apparently effective therapy was used. Susceptibility studies are mandatory for *C. laurentii*, due to its unpredictable sensitivity profile. Further investigation is needed to establish whether antimycotic therapy directed against cryptococcosis my help select resistant *C. laurentii* strains.

P735

Outbreak of neonatal candidiasis: drug susceptibility and molecular epidemiology of the isolates

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Objectives: Invasive candidiasis in neonates has become an increasing problem over the past decade in Neonatal Intensive Care Units (NICUs); it is a relatively common cause of late onset

sepsis associated with a high mortality. Although there is growing evidence that candidemia develops primarily as a consequence of endogenous colonization, hospital outbreaks of Candida infection are not uncommon. During a three-months period (August–October 2005), four invasive candidiasis occurred in neonates in NICU of the S. Matteo hospital of Pavia. The study was focused on the species involved, their *in vitro* antifungal susceptibility and molecular typing to determinate clonal relatedness.

Methods: Isolates were identified by standard morphological and biochemical methods. MICs of amphotericin-B, itraconazole, fluconazole, ketoconazole, 5-fluorocytosine, voriconazole and caspofungin were determined by Sensititre YeastOne colorimetric antifungal panel plates according to CLSI document M27-A2. The strains were examined for molecular relatedness by PCR fingerprinting employing random amplification of polymorphic DNA (RAPD) assay and amplification of transposable intron region in the 25S rRNA gene.

Results: A total of 14 isolates were obtained from blood, CVC, urine, rectal and cutaneous swabs of 8 neonates in NICU during a three months period. 3/8 patients developed a *C. albicans* bloodstream infection. All the isolates were sensitive to the antifungals tested. The genotyping of the transposable intron region of the colonizing and infecting *C. albicans* strains showed that 12 isolates, including the three blood-isolates, belonged to the same genotype (A) and two isolates to another genotype (B). These results were RAPD confirmed.

Conclusions: These data confirm the epidemic spread of a *C. albicans* strain in the NICU of our hospital. This study represents the first report of a *C. albicans* outbreak in our neonatal setting. The genotypic analysis allow us to understand the epidemiology of *C. albicans* isolates in the NICU.

P736

A new *in vitro* model system to study antimycotic drugs

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Objective: To help develop an *in vitro* bioassay to test antimycotic drugs that more closely reproduces the *in vivo* situation. To this purpose we are working on an experimental model consisting in the infection by *Candida albicans* of human epithelial and mesenchymal cells cultured in the 3-dimensional context of collagen gel.

Methods: We used the oropharyngeal squamous carcinoma HSCO cell line established in our laboratory and the HECV endothelial cell line (ATCC) as a broad representative of mesenchymal cells. These cells were embedded in type I collagen gel. After the cells were grown the gels were infected with 1×10^4 particles/ml of *Candida albicans*, previously isolated from an immunocompromised patient and notable for being sensitive to amphotericin B (AmB) and resistant to ketoconazole (Kc). There were 3 experimental groups: (1) Candida alone; (2) Candida with HSCO cells and (3) Candida with HECV cells. After 24 hours from infection these cultures were treated with AmB and Kc at the concentration of 10 μg/ml. Candida particles were counted 24 hours later by the trypan blu-exclusion test.

Results: We first evaluated the effects of the human cells on the survival of Candida. In the co-culture with HSCO cells the growth of Candida increased up to 100% over the control group. In the co-culture with HECV cells the proliferation of Candida was significantly inhibited ($p < 0.0001$ compared to control). Treatment with AmB consistently inhibited the growth of Candida cells ($p < 0.0007$), irrespective of the presence or absence of HSCO and HECV cells. With respect to treatment with Kc, candida cells were: resistant in group 1, highly sensitive in group 2 ($p < 0.0001$ compared to group (1) and resistant in group 3.

Conclusion: The present results indicate that the sensitivity of Candida to the tested drugs depended largely on the infected human cells. In particular, the co-culture with HSCO cells, but not with HECV cells, significantly increased the sensitivity of Candida to Kc. The proposed model offers distinct advantages over standard procedures, and is expected to advance means to assay antimycotic drugs, serving as a complementary approach to MIC tests. (Research supported by Pfizer Italia).

P737

EFISG survey of current practices in clinical mycology laboratory services in Europe

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Objectives: Mycology laboratory services are essential to the diagnosis of mycoses. New methods (antigen detection, molecular detection or identification, antifungal susceptibility testing [AFST]) are available but it is not known to what extent they are used.

Methods: A questionnaire was sent to the clinical microbiologists members of ESCMID.

Results: 171 questionnaires were returned (64% from University affiliated hospitals) with a mycology lab for 96% of them, separated from the bacteriology lab in 38%. The following tests are offered: direct examination (KOH 90%, brightener 52%, GMS 37%), culture for yeasts (99%), moulds (97%), dimorphic (65%), dermatophytes (82%), conventional identification (for yeasts 96%, moulds 85%), mostly with commercial biochemical systems (98% for yeasts). Molecular identification (sequencing) is available in 24% of labs for yeast, and in 21% for moulds. Antifungal susceptibility testing is done in 94% of the labs, mostly for clinically significant isolates only, using E-test in 42%, NCCLS-CLSI methods in 24%, disk diffusion in 10%, and Eucast methodology in 4%. Fungal serology is carried out in 72% of the labs (*C. neoformans* antigen 59%, *Candida mannan* 26%, antimannan 14%, beta-D glucan 1%, Aspergillus galactomannan in 52%). Molecular tests for fungi are carried out in 29% of the labs, for detection in 18%, for identification in 21%. Antifungal drug levels are offered in only 9% of the labs.

Conclusion: This survey of practices in European clinical mycology laboratories reveals the level of use of recently introduced methods (AFST, antigen or molecular detection and/or identification tests), and will be useful to EFISG and others for the planning of teaching activities or clinical studies.

P738

Comparison of the Sensititre YeastOne colorimetric antifungal panel with reference M38: a method for testing susceptibility *Aspergillus* spp. to caspofungin

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Objective: To compare the *in vitro* activity of Caspofungin (C) by Sensititre YeastOne® method (S) with that of microdilution

reference method (MD) (CLSI, formerly NCCLS M38-A) against *Aspergillus* spp.

Material and methods: The *in vitro* activity of C has been tested against 15 clinical isolates of Aspergillus (5 *A. flavus*, 4 *A. niger*, 3 *A. glaucus*, 2 *A. fumigatus*, 1 *A. terreus*) by the Sensititre Yeast One® colorimetric and the M38-A reference methods. The Sensititre YeastOne® was performed following the manufacture's instructions (range concentrations 0.008–16 mg/l). The MD reference susceptibility testing was performed following the CLSI M38-A document, using drug dilutions from 0.03 to 16 mg/l. Inoculum suspensions for both methods were prepared recovering the conidia from a 7-day culture growth on Potato Dextrose Agar at 35 °C (final inoculum: 0.5–5 × 10^4 CFU/ml). MECs (Minimal Effective Concentrations) readings were performed at 48 h for MD method following the published by Kurtz et al. (1994, AAC 38: 1480–1489). MECs and MICs readings were performed at 48 h. for S method. The MEC50, MEC90 and range of MEC were calculated for both method; the MIC50, MIC90 and range of MIC were calculated for S method. The level of agreement (percentage of MECs and MICs pairs within a ±2 dilutions range) between S and MD were calculated. *C. krusei* 6258 and *C. parapsilosis* 22019 were used as Quality Control strains calculated.

Results: MICs and MECs are shown in Table 1. The level of agreement between MECs of S and MECs of MD was 60% and between MICs of S and MECs of MD was 6.6%. The MECs (mg/l) were <1 mg/l for all strains with both methods. The Quality Control strains were within the published range.

	MIC50	MIC90	Range μg/ml	MEC(50)	MEC(90)	Range μg/ml
MD	--	--	--	0.06	0.12	0.03-0.25
S	>16	>16	≤0.008->16	0.016	0.016	≤0.008-0.03

Conclusions: 1. The reading for a change in color (MIC) in Sensititre YeastOne® method does not appear to be a suitable alternative procedure for Caspofungin susceptibility testing of *Aspergillus* spp. 2. The readings of the MECs in Sensititre YeastOne® method appear to be a suitable alternative procedure for Caspofungin susceptibility testing of Aspergillus. 3. Further studies, with more isolates and species are needed.

P739

Efficacy of anidulafungin against *Candida albicans* in candidaemia and other forms of invasive candidiasis

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Objectives: Although several antifungal agents are currently available for the treatment of candidemia/invasive candidiasis (C/IC), recent clinical trials have failed to demonstrate a significant impact of newer agents on response and survival. Anidulafungin (ANID) has potent activity *in vitro* and in animal models and is fungicidal against Candida. A recent clinical trial demonstrated superior efficacy of ANID over fluconazole (FL) in patients with C/IC. The majority of the patients in this study were infected with *C. albicans* (CA); these patients are the focus of this analysis.

Methods: ANID (100 mg/d iv) was compared with FL (400 mg/d iv) in a randomized double-blind study in patients with clinical signs of C/IC and positive cultures from blood or another normally sterile site. The primary endpoint was global response at the end of IV therapy (EOT), which required improvement of symptoms and eradication (ERAD) of Candida. Organisms were sent to a reference lab for confirmation of identity and MIC determination (CLSI method).

Results: 135 patients (ANID: 74, FL: 61) had baseline infection with CA as a single pathogen. Only 1 patient in each group had a FL non-susceptible isolate at baseline. The global success at EOT was 81% for ANID and 62% for FL ($p < 0.05$). The eradication rate at EOT was 95% for ANID and 81% for FL ($p < 0.01$). The rate of CA persistence at EOT was 2.5% for ANID and 13% for FL ($p < 0.05$).

Conclusion: In patients with C/IC caused by CA, ANID was superior to FL in terms of global and microbiological success rates. Resistance to FL, the comparator, did not contribute to the difference in outcome in patients with CA.

P740

Ability of Spanish clinical laboratories in diagnosing *Candida krusei* infections and in monitoring susceptibility testing. Results of the SEIMC Quality Control Program

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Objectives: To know the capacity of Spanish laboratories in identifying *C. krusei* and in performing susceptibility tests to antifungals.

Methods: As a part of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) External Quality Control Program (QCP) in Mycology, the same yeast strain was sent to an average of 200 laboratories in two separate shipments in 1998 and 2005. Results of identification and susceptibility testing were analysed in comparison with those provided by a reference laboratory. As expected, the *C. krusei* strain was characterized by its intrinsic resistance to fluconazole (FLU).

Results: In the first shipment (1998), the percentage of participation of laboratories was 84.2%, and 72% of them correctly identified the yeast strain at species level, 8.0% reported only genus Candida and the remaining 20% gave a discordant identification. The main methods and commercial brands used in identification were the biochemical systems API 20C AUX and API ID 32C (bioMérieux); the last one got the 100% of correct identifications. Susceptibility testing was performed by 40.7% of participants, and discordant results (susceptible to FLU) were reported by 47.5% of those sending data. In their commentaries, only 2.7% reported explicitly the inherent intrinsic resistance to FLU of this yeast. On the other hand, the percentage of participation was 89.9% in the 2005 control, being the correct species identification 92.1%. Only 2.8% of the centres identified the strain at genus level, and the percentage of discordant identifications dropped to 5.1%. Again, the API galleries were mostly used, and API ID 32C offered a complete agreement in the identification. The 64.5% of laboratories performed susceptibility tests to antifungals, 72.7% reported resistance to FLU, and 25.7% explicitly declared such intrinsic characteristic in their comments.

Conclusion: An increased percentage of participation was observed along the study period, as well as in the correct identification of the *C. krusei* strain, the number or laboratories performing susceptibility tests, and in the right interpretation of their FLU results. Altogether, these results show the need for implementing training programs in Mycology in the clinical laboratories. Also, they underline the potentiality of the SEIMC QCP as a tool in the continuous education of professionals, since the analysis of the results is followed with a revised update on the subject of the control.

P741

FungaLit-multi-parameter bioassay for antifungal compounds mode-of-action studies

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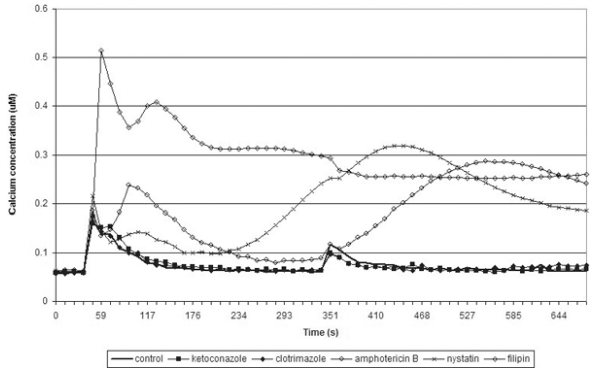
Objective: The objectives of this study were to develop a method for exploring mode-of-actions of antifungal compounds using FungaLit (a novel bioassay enabling analysis of Ca^{2+} signalling using fungi transformed with the recombinant aequorin gene) and to analyse a link between parameters of Ca^{2+} response and mode-of-actions of antifungal compounds.
Methods: Seven antifungal compounds were tested at the concentrations from 10 μM to 50 μM. Each compound was firstly added to the aequorin transformed fungi and the luminescence was monitored for 4.5 min (Stage 1). Subsequently the fungi were subjected to a hypo-osmotic shock and the luminescence was monitored for further 4.5 minutes (Stage 2). For each stage FungaLit calculated a range of parameters characterising Ca^{2+} response including: rise time (RsT), amplitude (A), length of transient (LT), number of increases, final resting level (FRL), recovery time (RT), and total calcium released (TC).
Results: The results obtained (Figure 1) showed that all antifungal compounds tested affected Ca^{2+} signalling. Ketoconazole and clotrimazole caused only small decrease in the A of the Ca^{2+} response indicating that these compounds blocked some Ca^{2+} channels but did not have a chronic affect on fungi at the concentrations tested. Amphotericin, filipin and nystatin triggered strong increases in the RT, TC and FRL indicating that these compounds permeabilized the plasma membrane and were highly toxic to fungi. Filipin caused 4 times increase in the A of the Ca^{2+} response suggesting that it affected Ca^{2+} channels. Nystatin triggered three consequent Ca^{2+} increases during Stage 1 indicating that this compound caused Ca^{2+} release from the internal store. Ciclopirox caused increases in the RsT, LT and also FRL and RT suggesting that it affected both Ca^{2+} channels and Ca^{2+} carriers though its effect on the membrane was limited. Fluorocytosine increased the RsT, FRL and RT during Stage 1 but no long term elevation in Ca^{2+} was observed.



Conclusions: FungaLit was successfully used to analyse effects of antifungal compounds on Ca^{2+}. All different types of antifungal compounds tested caused Ca^{2+} increases of different profiles. Based on this initial study it was possible to suggest that FungaLit could be used for creation of Ca^{2+} profiles of antifungal compounds with different mode-of-actions and subsequently these profiles could be used for *in vitro* testing and optimisations of the current and novel antifungal compounds.

P742

Fungaemia at a tertiary-care hospital: species distribution, antifungal susceptibility and antifungal therapy

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Objectives: Few data are available regarding the susceptibility of Belgian yeast isolates to commonly used antifungal agents. Both species identification and antifungal susceptibility data are routinely used to guide antimicrobial therapy in episodes of candidemia. The aim of this study was to determine the susceptibility to seven antifungal agents for all yeasts isolated from blood cultures during a one year period and to review the species distribution and the antifungal therapy given.
Methods: From June 2004 until June 2005, the first yeast isolate from each fungemic episode was collected and stored at –70 C. Susceptibility testing was performed using Sensititre YeastOne plate according to the instructions of the manufacturer. Antifungal drugs tested were fluconazole (FZ), amphotericin B (AB), voriconazole (VZ), caspofungin (CA), flucytosine (FC), ketoconazole (KZ) and itraconazole (IZ). Antifungal usage data for each patient with fungemia were obtained from the Hospital Pharmacy.
Results: A total of 62 isolates from 60 patients were collected. 33% of the isolates were non-albicans species (11 *Candida glabrata*, 5 *Candida parapsilosis*, 1 *Candida krusei*, 1 *Candida lusitaniae*, 1 *Candida tropicalis* and 1 *Saccharomyces cerevisiae*). The susceptibility results (MIC range; MIC90s (mg/L)) for all isolates tested were as follows: FZ (<0.008–64; 16), AB (<0.008–1; 1), VZ (<0.008–1; 0.25), CA (<0.008–0.5; 0.12), FC (<0.03–4; 0.12), KZ (<0.008–16; 0.5) and IZ (<0.008–>16; 1). All *C. albicans* isolates were fully susceptible to FZ. 91% of the *C. glabrata* isolates showed decreased susceptibility (MIC >8 mg/L) to FZ, with one resistant isolate (MIC = 64 mg/L). VZ and CA inhibited 100% of the isolates at ≤1 mg/L. VZ was less active against *C. glabrata* isolates with decreased susceptibility to FZ compared to FZ susceptible isolates. FZ was used in 75% of fungemic patients. CA was the second most commonly used antifungal therapy (11.7% of the patients). In four patients therapy was switched from FZ to CA, two to four days after isolation of the yeast from the blood culture.
Conclusion: *C. albicans* was responsible for 67% of all fungemic episodes and remained fully susceptible to FZ. Decreased susceptibility to FZ was only observed for *C. glabrata* and *C. krusei*. CA was the compound with the highest *in vitro* activity.

P743

A new *in vitro* kinetic model to study the pharmacodynamics of antifungal agents: Comparison of the activity of voriconazole and amphotericin B administered alone and in combination against *Candida albicans*

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Objectives: It has previously been shown that preexposure of *Candida albicans* (CA) to fluconazole reduces the fungicidal effect of amphotericin B (AMB). The aim of the present study was to investigate whether a similar inhibitory effect was seen between voriconazole (VOC) and AMB in a new *in vitro* kinetic model.
Methods: To a starting inoculum of 10000 CFU/mL of *C. albicans* CCUG 32723 (Culture Collection, University of Göteborg, Sweden; MICs VOC: 0.004 mg/L, AMB: 0.25 mg/L) in sterile broth, antifungal drugs (VOC: 5 mg/L, AMB: 2.5 mg/L)

were administered and placed at 35 degrees centigrade. Antifungal containing medium was eliminated from the culture vessel and replaced by fresh medium with a peristaltic pump at a flow-rate adjusted to obtain the desired half-life of the drugs. A computer controlled infusion pump compensated for the agent with the longer half-life (VOC: 6 h, AMB: 24 h). Repeated sampling for viable counts was made. A magnetic stirrer ensured homogenous mixing. To verify the kinetics, vancomycin was used to simulate VOC and AMB regimens, respectively, and samples for drug-concentrations were measured on an Abbot Architect ci8200 analyser (Abbot, Sweden). Each regimen was performed in triplicate and at each experiment, there was one unexposed control.

Results: The mean half-life when vancomycin were used to simulate one dose of VOC was 6.4 h. The mean half-lives when vancomycin were used to simulate three repeated doses of AMB were 23.5, 27.2 and 21.7 h, respectively. With VOC treatment only, viable counts (mean ± SD) were 4.02 ± 0.09, 4.79 ± 0.20 and 5.41 ± 0.55 log 10 CFU/mL at 3, 24 and 48 h, respectively. After treatment with AMB or AMB + VOC simultaneously, viable count was <1 log 10 CFU/mL at 3 h. With VOC treatment at 0 h followed by AMB at 8 and 32 h, fungal growth at 8 h, before administration of the first dose of AMB, was 5.00 ± 0.19 log 10 CFU/mL. The viable count increased to 5.61 ± 0.29 log 10 CFU/mL at 32 h before administration of the second dose of AMB which only resulted in a limited reduction to 4.70 ± 0.21 log 10 CFU/mL at 54 h.

Conclusion: The pharmacokinetic parameters in the kinetic model were close to target values. Pre-treatment of CA with VOC inhibits the fungicidal effect of AMB. The duration of this inhibitory effect, possible dose-dependency and whether the inhibition is observed when CA is resistant to VOC need further investigation.

P744

Is terbinafine a good antifungal drug to combine with voriconazole or caspofungin? Study of the activity of double combinations against invasive clinical isolates of *A. fumigatus*

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Objectives: Treatment of invasive aspergillosis is complicated by high toxicity and poor response. Double-drug combinations are promising, but *in vitro* studies are necessary. We studied the activities of voriconazole (VCZ, Pfizer), caspofungin (CAS, MSD) and terbinafine (TERB, Novartis) alone and combined against clinical isolates of *A. fumigatus* in order to evaluate the potential of the combination of TERB with the other two antifungal drugs.

Methods: We studied 10 strains of *Aspergillus fumigatus* from 10 hospitalized patients with proven invasive aspergillosis (Ascioglu, CID 2002). Drug combinations were tested by using the guidelines presented in document NCCLS M-38A, as modified for a broth microdilution checkerboard procedure. The MIC for each drug was defined as the lowest concentration of antifungal drug that produced a complete visual inhibition of fungal growth. Interactions between the drugs were studied by calculating the fractional inhibitory concentration index (FICI) and interpreted as synergy (FICI < 0.5), antagonism (FICI > 4) and indifference (FICI between 0.5 and 4). We calculated the MIC instead of the MEC for CAS in order to compare the results with VCZ and TERB.

Results: The MIC90s and ranges of VCZ, CAS and TERB, in microg/ml, were: 1 (0.25–1), >4 and 4 (2– > 4) respectively. VCZ was the most active antifungal drug presenting the lowest MICs. The combination VCZ-TERB proved to be synergistic in 90% of the strains (FICIs between 0.031 and 0.122). The combination VCZ-CAS presented synergy in 10% of the strains (FICI 0.251), and the combination CAS-TERB was indifferent for all strains. Antagonism was not present in any strain or combination. The strain that showed indifference in the combination VCZ-TERB, also presented indifference in the other two combinations.

Conclusions: The *in vitro* combination of terbinafine with voriconazole is synergistic against clinical strains of *Aspergillus fumigatus*. *In vivo* studies are warranted.

P745

In vitro activity of fluconazole and voriconazole against invasive *Candida albicans* isolates in patients with candidaemia from 2001–2005 in Hong Kong

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Objectives: Candidaemia is one of the commonest nosocomial blood stream infection and is associated with high mortality. Antifungal treatment ranges from conventional amphotericin B to the new echinocandins. Azole agents provide an alternative choice where oral formulation is available. Fluconazole and voriconazole has been used in the treatment of candidaemia for several years, thus, it is important to monitor for the trend of antifungal resistance. In this study, we aimed to investigate the *in vitro* activity of fluconazole and voriconazole against invasive *Candida albicans* isolates in patients with candidaemia from 2001 to 2005 in Hong Kong.

Methods: Non-duplicate *Candida albicans* isolates collected from candidaemic patients from 2001–Oct 2005 were tested against fluconazole and voriconazole in accordance to the NCCLS (CLSI) standard M44-A by disk diffusion method. Fluconazole susceptibility results were interpreted according to NCCLS (CLSI) criteria. Voriconazole susceptibility results were interpreted according to Pfaller et al. (Pfaller MA, Boyken L, MesserSA, Tendolkar S, Hollis RJ, Diekema DJ. Comparison of Results of Voriconazole Disk Diffusion Testing for Candida Species with Results from a Central Reference Laboratory in the ARTEMIS Global Antifungal surveillance Program. J Clin Microbiol 2005; 43, 5208–5213). Tests were done in duplicates.

Results: A total of 73 *Candida albicans* isolates were collected over the 5-year period. Among which, 12 isolates were collected from 2001, 15 isolates from 2002, 17 isolates from both 2003 and 2004, 12 isolates from 2005 (up to and including October 2005). Only two isolates (2.7%): one from 2003, one from 2004, showed resistance to fluconazole. None of the isolates showed resistance to voriconazole.

Conclusion: Fluconazole and voriconazole are active against invasive *Candida albicans* isolates. They remain as appropriate choices for patients who can be considered for oral azole agents. For fluconazole, even after many years of extensive usage, resistance rate remain low.

P746

The effect of aminocandin compared to caspofungin and micafungin

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Objective: Aminocandin (AC) is a new echinocandin undergoing pre-clinical and clinical development. Recently, *Candida*

parapsilosis isolates that are resistant to caspofungin (CAS) have been identified. The purpose of this study was to determine whether AC inhibits CAS-resistant *C. parapsilosis* in a dose dependent manner, and to compare the effect of AC relative to CAS or micafungin (MFN) on the growth rate of the fungal isolate.

Method: One CAS-susceptible *C. parapsilosis* strain (MIC of 0.5 micrograms/ml) and one strain with an elevated CAS MIC (64 micrograms/ml) were grown in the presence of various concentrations representing 0.5, 1, 2 and 4 times the MIC of AC against each isolate. At predetermined time points (1, 2, 4, 8, 12, 24, 36 and 48 hours) an aliquot was removed and the optical density (OD) read using a spectrophotometer at 420 nm wavelength. Graphs were plotted as OD against time. Growth curves of CAS and MFN at corresponding concentrations were determined as comparators.

Results: AC and CAS exerted similar inhibitory effects on the CAS-susceptible strain; 1 microgram/ml (1 × MIC) showed retardation of growth, with recovery of cells by 48 hours. In contrast, AC (1 × MIC) showed twice the inhibitory effect of CAS on the CAS-resistant strain at 48 hours. Comparable inhibitory effect by MFN on both strains was attained only with concentrations of 4 micrograms/ml.

Conclusion: Our data show that AC demonstrates potent antifungal activity against *C. parapsilosis* isolates including those known to have elevated CAS MICs and may indicate that subtle *in vitro* differences in the activity of different echinocandins may exist. The clinical implications of these findings are yet to be determined.

P747

Antifungal susceptibility testing of dermatophytes isolated from a worldwide Tinea capitis clinical trial

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Objective: Tinea capitis is an infection of the hair and scalp predominantly affecting children worldwide. The objective of this study was to profile the susceptibility of dermatophyte isolates, obtained from paediatric patients enrolled in a worldwide Tinea capitis study, to commonly used antifungal agents.

Method: Following identification, isolates obtained at baseline visits (n = 544) were tested against voriconazole (VOR), fluconazole (FLU), and griseofulvin (GRIS) using the CLSI M38-A standard for dermatophytes developed at the Center for Medical Mycology.

Results: The most common isolate from the US and Puerto Rico was *Trichophyton tonsurans* (n = 474), followed by *Microsporum canis* (n = 19). Other isolates from the US included *M. gypseum* (n = 2) and *T. mentagrophytes* (n = 1). The most common dermatophyte isolated from Central and South America was *M. canis* (n = 37), followed by *T. tonsurans* (n = 1). The predominant isolate from India was *T. violaceum* (n = 7); others included *M. canis* (n = 2) and *T. tonsurans* (n = 1). Importantly, there was no difference noted in MIC among dermatophyte species from various geographical areas.

Table 1. MIC data (in micrograms/ml)

	MIC Range	MIC_{50}	MIC_{90}
Voriconazole	0.002-0.06	0.015	0.03
Fluconazole	0.25-16	4.0	8.0
Griseofulvin	0.125-2.0	0.5	1.0

Conclusions: VOR demonstrated more potent antifungal activity than either FLU or GRIS, suggesting that VOR could be a useful antifungal to treat infections caused by dermatophytes.

P748

In vitro activities of caspofungin and four other antifungal agents against *Trichophyton* spp. isolates

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Background: In recent years the number of infections caused by dermatophytes has increased considerably. Pharmacotherapy for these infections still remains a problem as the necessity of long time appliance of antifungal agents. Caspofungin is a new antifungal agent that acts on the cell wall by inhibiting glucan synthesis. A standardized reference method for dermatophyte *in vitro* susceptibility testing and reproducible, clinically relevant method for caspofungin has not been fully established. The aim of this study was to evaluate the *in vitro* activity testing method of caspofungin and other antifungal drugs against clinical isolates of *Trichophyton* spp. from Ankara-Turkey.

Methods: A total of 172 clinical isolates of *Trichophyton* spp. (136 *T. rubrum*, 29 *T. mentagrophytes*, and 7 *T. tonsurans*) were tested. The antifungal activities of caspofungin, fluconazole, itraconazole, terbinafine, amphotericin B were determined by broth microdilution tests performed mainly according to the NCCLS standards for filamentous fungi using RPMI 1640 as the test medium. Microscopic and macroscopic minimal effective concentration (MEC) values for caspofungin and MIC values for other drugs were determined after 5–7 day incubation.

Results: Microscopic and macroscopic MEC values were mostly similar for caspofungin at all test conditions (100% agreement for *T. tonsurans*, 1.5% agreement for *T. rubrum* and 79% agreement for *T. mentagrophytes*). Caspofungin was more active against *T. rubrum* (MIC50, 0.06 μg/ml and MIC90, 0.5 μg/ml) than the others (MIC50, 0.25 μg/ml and MIC90, 1 μg/ml). Terbinafine was significantly more active (MIC50, 0.002 μg/ml and MIC90, 0.008 μg/ml) than the other drugs ($p < 0.01$). Fluconazole was the poorest antifungal agent as 3 of *T. rubrum* and 3 of *T. mentagrophytes* have MIC values ≥64 μg/ml.

Conclusions: This study showed that Caspofungin has a good *in vitro* activity against dermatophytes. For methodology; microscopic determination is not an option to assess the MECs of caspofungin for *Trichophyton* spp. Standard antifungal susceptibility procedure should be urgently developed to establish reliable resistance profiles of antifungal agents against dermatophytes and to demonstrate the affects of caspofungin against all molds.

P749

Update on *Candida albicans* antifungal resistance trends among hospitalised patients in a tertiary hospital in Greece

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Objectives: *Candida albicans* is the main aetiological agent of fungal infections in hospitals worldwide. Selection pressure due to the continuous exposure to antimycotic agents has changed

the susceptibility patterns of these isolates so that continuous monitoring is required. In the present study, we examined the *in vitro* susceptibilities of *C. albicans* isolates representing predominantly invasive forms of candidiasis.
Methods: Seventy-five *Candida albicans* clinical isolates obtained from a respective number of patients hospitalised during last year at the AHEPA University Hospital in Thessaloniki, Greece were examined. Samples were cultured on Sabouraud's glucose, supplemented with chloramphenicol, agar slants (30 °C). Yeasts were identified by ID YST identification system (Vitek II, bioMerieux, Marcy l' Etoile, France) with higher than 99% probability. Antifungal susceptibility testing to caspofungin, amphotericin B, voriconazole, itraconazole, ketoconazole, fluconazole and 5-flucytocine was performed using the E-test method (AB Biodisk, Sweden) in accordance to the manufacturer's instructions. CLSI interpretive breakpoints (document M27-A2) and tentative MIC's for the newer antifungal agents were applied.
Results: Sources of isolation were blood (6.6%), bronchoalveolar lavage specimens (37.3%), wound sites (9.3%), sputum (20%), central venous catheters (7%) and normally sterile fluids (19.8%).

Antifungal agent	*MIC range (mg/L)*	*%Susceptible*	*%Intermediate*	*%Resistant*
AmphotericinB	0.047-2	91.9 (*n*=71)	-	5.2 (*n*=4)
Caspofungin	0.016-0.25	100 (*n*=75)	-	-
Itraconazole	0.002-32	68 (*n*=51)	5.2 (*n*=4)	26.6 (*n*=20)
Vorlconazole	0.012-32	83.9 (*n*=63)	-	16 (*n*=12)
Ketoconazole	0.006-32	76 (*n*=55)	-	24 (*n*=18)
Fluconazole	0.25-256	80 (*n*=60)	-	20 (*n*=15)
5-flucytocine	0.023-32	98.6 (*n*=74)	-	1.4 (*n*=1)

Conclusions: Our data confirms previous reports regarding the increasing rates of resistance and cross-resistance to, and between older azoles. Despite the encouraging antifungal potential of voriconazole a significant percentage of resistant isolates is reported. Finally, the recently introduced caspofungin appears to have excellent *in vitro* activity against these isolates.

Virology - I

P750

Subclinical reactivation of VZV in patients with tick-borne encephalitis

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Objectives: Herpesviruses have the very well known ability to establish the latency in different compartments of human organism. To date mechanism of reactivation of these viruses especially in neural tissue is poorly understood. Aim of our study was to evaluate the presence of herpesviruses in cerebrospinal fluid (CSF) of patients with meningoencephalitis from other than herpetical origin.
Methods: Forty patients (all patients hospitalised in season 2004–2005) with dimission diagnosis tick borne encephalitis (TBE) were tested for presence of herpes simplex virus 1 (HSV1), herpes simplex virus 2 (HSV2), varicella zoster virus (VZV) and human herpesvirus 6 (HHV6) DNA in CSF by nested PCR. TBE diagnosis was established on the presence of IgG and IgM antibodies in serum by ELISA, aseptic inflammation in CSF and history of tick bite. Detection of the PCR product was done by agarose gel electrophoresis with etidiumbromide staining. Clinical data including standard laboratory tests and results of neuroimaging methods were collected prospectively.
Results: Three patients were positive for VZV DNA in CSF. These patients had no recent history of herpes zoster and neither rash nor neuralgia located in the dermatome was observed during hospitalisation. There was no significant difference in age, length of febrile period, severity of neurological sequelae, length of hospitalisation, and in laboratory test results including peripheral blood cell count, CSF cytology, proteinorhachia and glycorhachia. Although none of them was treated by acyclovir, outcome of these patients was identical as in non-positive patients. Tests for HSV1, HSV2 and HHV6 were negative in all patients.
Conclusion: We have probably observed a subclinical reactivation of VZV patients. Such findings decrease the value of PCR as a single test for establishing the diagnosis of VZV encephalitis and emphasize the need for sensitive antibody assays. Supported by 1166/2005-IV-GA UK.

P751

Epstein-Barr virus and p53 protein expression in gastric carcinoma without *Helicobacter pylori* infection

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Objectives: Role of Epstein-Barr virus (EBV) in neoplastic transformation of gastric epithelial cells remains unclarified. Therefore, in this study we investigated p53 expression in gastric carcinoma (GC) in patients with or without EBV infection.
Methods: The studies were performed on 26 patients, aged 42–64 years in whom advanced moderately differentiated GC was diagnosed by histological method. Moreover, presence of *Helicobacter pylori* in the studied material was excluded using the urease test and Genta staining method. In parallel, in selected cases in the GC material EBV-encoded small RNA (EBER) particles were detected using *in situ* hybridization (ISH). Expression of p53 protein was analysed using immunohistochemistry.
Results: The results of EBER tests in the material of GC in the selected patients permitted to distinguish 12 patients with documented presence of EBER particles in the cell nuclei of GC cells forming the EBV-positive group of GC patients and 14 patients without EBER forming the group of EBV-negative GC patients. In the group of EBV-positive GC in 8 (66.6%) patients nuclear expression of p53 protein was disclosed and in the group of EBV-negative GC expression of p53 protein was noted in 9 (64.3%) cases. No significant differences were detected in the frequencies of p53 protein expression in the two studied groups.
Conclusions: The results permit to conclude that abnormalities in p53 in GC are independent of EBV infection.

P752

Cytomegalovirus and Epstein-Barr virus in ulcerative colitis and Crohn's disease

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Objectives: Currently, Epstein-Barr virus (EBV) as well as Cytomegalovirus (CMV) are thought to be able not only to infect epithelial cells but also to play a significant role in carcinogenesis. Therefore, in this study we aimed to examine whether CMV and/or EBV may be associated with non-specific colitis (ulcerative colitis and Crohn's disease) and, moreover, whether testing of tissue samples of ulcerative colitis and Crohn's disease can detect disturbed expression of p53.
Methods: The studies were performed on 24 patients, in whom histological evaluation of tissue samples disclosed ulcerative colitis (16 patients aged 24–60 years) or Crohn's disease (eight patients aged 20–50 years). DNA extracted from the samples of ulcerative colitis and Crohn's disease was tested for the presence of CMV DNA using nested PCR and EBV DNA using PCR-ELISA. Moreover, in the tissue samples EBV-encoded small RNA (EBER) particles were detected using *in situ* hybridization (ISH) and expression of p53 protein was analysed using immunohistochemistry.
Results: In the group of patients with ulcerative colitis presence of CMV DNA was identified in six cases (37.5%), while presence of EBV DNA and positive reaction for EBER was documented in 10 cases (62.5%). In three of the patients both CMV DNA and EBV DNA were present. In turn, in the group of patients with Crohn's disease CMV DNA was identified in only two cases (25%), while presence of EBV DNA and positive reaction for EBER were disclosed in five cases (62.5%). In two of the patients both CMV DNA and EBV DNA were present. In none of the cases could nuclear expression of p53 protein be demonstrated.
Conclusions: CMV and EBV infections may accompany ulcerative colitis and Crohn's disease but this does not seem to induce expression of p53 protein.

P753

Cytomegalovirus infection in recipients of liver transplant in Ireland

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Objectives: Cytomegalovirus (CMV) remains the single most important infection complication after liver transplant. The incidence of CMV infection post-orthoptic liver transplantation (OLT) ranges between 25% and 80%. However, no data have as yet been published from Ireland.
Methods: We conducted a three-year retrospective audit of laboratory and clinical records of patients diagnosed with CMV infection. All pre-OLT CMV serological investigations and post-OLT CMV screening from the National Liver Transplant unit (NLTU) are referred to the National Virus Reference Laboratory (NVRL). CMV susceptible or CMV mismatch recipients receive 3 months of CMV prophylaxis post-operatively. Patients suspected of CMV infection are screened with either CMV antigenaemia or PCR. Response to anti-viral therapy is monitored with either antigenaemia or PCR.
Results: Of 133 transplant recipients, 11 (8%) were diagnosed with CMV infection. Seven (63%) occurred more than 3 months after OLT when prophylaxis had ceased. Nine had received prophylaxis: two, ganciclovir and seven, valganciclovir. Four transplants were CMV donor positive /recipient positive (D+/R+); four were D+/R(; one was D(/R(; and one was D(/R+. Six patients were diagnosed on the basis of antigenaemia alone (Mean=13; range 1–77/200,000 PMN). Three were diagnosed initially on antigenaemia (range 3 to >200/200,000) and subsequently confirmed with CMV PCR [Mean=50,000; range 600 to >100,000 copies per ml (cpm)]. Two were diagnosed on the basis CMV PCR alone (Viral loads 1570 and 1410 cpm). Six patients were treated for symptomatic CMV disease. Of these, three received IV ganciclovir, two received oral valganciclovir, and one received oral ganciclovir. No cases of ganciclovir resistance were recorded. None of the patients died. 1003 samples from 221 patients were received for CMV antigenaemia and 51 samples from 39 patients for CMV PCR during the study period. There was a marked increase in the number of requests for CMV PCR (6 in 2003; 18 in 2004; 27 in 2005) and a less striking decrease in the number of requests for CMV antigenaemia (405 in 2003; 314 in 2004; 284 in 2005).
Conclusions: CMV infection remains a significant cause of post transplant morbidity in our liver transplant population, affecting 8% of recipients, the majority of which occurred after antiviral prophylaxis was stopped. This presentation will focus on the risk factors and clinical presentation of CMV infection in our population.

P754

Incidence of primary CMV infection during pregnancy and its transmission rate to the foetus and newborn

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Objectives: To evaluate the incidence of primary CMV infections during pregnancy, and to evaluate the transmission rate to the foetus according to the gestational age at which the maternal infection occurred.
Methods: 9864 unselected pregnant women were included. All pregnant women were tested serologically for detection of CMV-antibodies (IgG and IgM) at the first prenatal visit and at delivery. Primary CMV infection during pregnancy was diagnosed when IgG antibodies appeared in previously seronegative women, or when high IgM antibodies with rising IgG levels where found in the beginning of pregnancy. For each woman the gestational age at which the infection occurred during pregnancy was estimated. This evaluation was based on the dates of the last negative serology and the first positive serum sample and the serological profile. In the neonate CMV urine culture was performed to diagnose congenital infection. In the foetus, congenital infection was diagnosed by CMV isolation and/or by histological examination.
Results: Serological screening showed evidence of past infection in 5595 women (57%); 4049 (41%) women had no antibodies in their first serum sample, and 220 (2%) women had both IgG and IgM antibodies when first tested during pregnancy. Primary CMV infection was diagnosed in 63 pregnancies: being 51 seroconversions during pregnancy and 13 women with a serological profile in the beginning of pregnancy highly suggestive of primary infection. Four women decided not to continue the current pregnancy before any investigation could be performed. Of the remaining 59 women, 28 (47%) delivered a congenitally infected infant. Mean gestational age at infection was 23 ± 9 weeks in the group of women who delivered a congenitally infected infant and 21 ± 11 weeks when the maternal infection did not result in a congenitally infection. Transmission rate was 38% in the first trimester; 52% in the second trimester and 50% in the third. Two children were born with symptomatic disease, leading to death in one of them. Two other pregnancies were interrupted after a prenatal diagnosis: histological examination of these two infected foetuses showed inclusion bodies in multiple organs.

Conclusion: Primary CMV infection occurred in 0.63% of the pregnant women. Transmission to the foetus occurred in 47% of the women with primary CMV infection and was independent of the gestational age at which maternal infection took place.

P755

In situ PCR and in situ RT-PCR to distinguish productive and non-productive human cytomegalovirus infection in blood leukocytes

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Objective: Reactivation or primary human cytomegalovirus (CMV) infection is an important cause of morbidity and mortality in transplant recipients and HIV patients. Diagnostic methods, which correctly assess viremia, including pp65-antigenemia (pp65-ag) and PCR, are recommended in patients exposed to CMV disease. Detection of pp65-ag in nucleus of leukocytes is a marker for productive infection. PCR enables to amplify latent as well as replicating viral DNA sequences. We presented two *in situ* methods that can be helpful in discriminating between latent and productive infection – *in situ* PCR and *in situ* reverse transcription PCR (RT-PCR). These methods can be helpful in explaining the discrepancies with the PCR and pp65-ag results, when the antigen is found in the cytoplasm of leukocytes.
Methods: Leukocytes were obtained from 34 blood samples of 26 patients after bone marrow transplantation. For pp65-ag monoclonal antibodies NCL-CMV pp65 were used. Two pairs of primers for gB were used to amplify 20 or 100 ng DNA isolated from one million of leukocytes. Absence of inhibitors of Taq polymerase was checked. *In situ* PCR and *in situ* RT-PCR were carried out with primers for genes coding gB and pp65 and nucleotides labelled with DIG or fluoresceine.
Results: The results of *in situ* PCR and *in situ* RT-PCR were compared with those obtained by PCR and pp65-ag. Using PCR, viral DNA was detected in 26 materials, two samples were negative and in the remaining six samples the results were unreadable due to the presence of polymerase inhibitors. Productive infection was recognized in seven samples in which pp65ag, PCR and *in situ* PCR were positive and also transcripts for late viral genes (gB) were detected. In 20 samples, in which the *in situ* RT-PCR results were negative, the presence of DNA and pp65 ag localized in cytoplasm could probably be due to phagocytosis. Among seven pp65-ag negative materials, the latent form of CMV was confirmed in five samples on the basis of CMV DNA presence in the nucleus and absence of viral transcripts using *in situ* methods.
Conclusion: On the basis of DNA detection in nucleus or cytoplasm of leukocytes, and suitable transcripts confirmed by *in situ* methods, we allow distinguish between latent and productive infection in 12 materials. In six samples, in which it was impossible to carry out PCR because of the presence of polymerase inhibitors, *in situ* methods played a crucial role in proper diagnosis.

P756

A real-time PCR assay to detect human cytomegalovirus DNA in whole blood of solid organ transplant patients: optimal threshold for guiding pre-emptive therapy

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Cytomegalovirus (CMV) remains the most important pathogen of transplant recipients. The pathogenesis of CMV disease has been extensively studied and evidence suggests that CMV blood load is associated with disease development. The use of pre-emptive therapy and the successful treatment of established infection partially rely on a sensitive and reliable diagnostic test to predict and detect disease early enough for prompt prophylaxis and to guide the duration of treatment. This study evaluated a Real-Time PCR assay (affigene CMV trender, Sangtec Molecular Diagnostics, Sweden) to quantify CMV load in whole blood specimens from solid organ transplant patients in comparison with antigenemia pp65 assay. The purpose was to compare the thresholds of CMV load assessed with this assay and with the antigenemia assay to guide preemptive therapy for CMV disease prevention. We tested 182 serial blood samples from CMV-infected patients, namely from eight liver, eight heart and seven intestinal transplant recipients. DNA was measured using primers and probes located in the Major Immediate Early Gene. CMV DNA (n.copies/mL of whole blood) was calculated using a standard curve with synthetic DNA in concentrations of 13 to 8×10^6 copies/μL. The detection limit of the method was 400 copies/mL of whole blood (WB). CMV viremia was monitored weekly during the first 2 months after transplantation, every two weeks for the next 3–4 months, monthly until 6 months and then every 3 months after transplantation. The viral load obtained by Real-Time PCR assay closely paralleled that obtained with the antigenemia test. Both the Real-Time PCR and the pp65-antigenemia assay detected all cases of disease at medians of 2 and 15 days before the onset of symptoms, respectively. Monitoring asymptomatic CMV infection to guide pre-emptive therapy for solid organ transplant recipients showed that peak DNA levels were $<10^3$ copies/mL of WB before therapy and the antigenemia test was negative. The beginning of pre-emptive therapy was associated with DNA levels from 6500 to 7500 copies (median values) and the peak antigenemia (median values) between 25 and 30 positive cells/2×10^5 polymorphonuclear leukocytes, respectively. Successful treatment was associated with a >90% reduction in DNA viral load and completely negative antigenemia assay, we have obtained nearly always results under the threshold of 400 copies/mL.

P757

Evaluation of a real-time NASBA assay for the detection of herpes simplex virus type 1 and 2

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Objectives: In this study, the performances of the HSV 1/2 reagents, based on NASBA amplification and real-time detection with molecular beacons, were evaluated for the detection of HSV1 and HSV2 infections.
Methods: HSV DNA is isolated using the NucliSens® miniMag extraction. An internal control is added to the sample prior to nucleic acid extraction. The assay is designed to detect in a single tube, with the same primer set and a three-label approach, the internal control and both HSV1 and HSV2 DNA by targeting the POL–gene region. Amplification reactions were performed in a NucliSens® EasyQ Analyser allowing real-time detection.
Results: Using serial dilutions of *in vitro* DNA, a 95% hit rate of the NucliSens® EasyQ HSV 1/2 assay was found to be 87 copies for HSV1 and 86 copies for HSV2 in isolation. The results obtained with the QCMD panel 2005 showed that HSV genotypes were correctly identified down to 580 Geq/ml. In addition, HSV genotypes were detectable in positive clinical samples (CSF, labial and genital swabs). Interestingly, one co-infection HSV 1+2 was detected in a CSF sample, which was not detected

by home brew PCR. No cross-reactivity was observed with VZV, EBV, CMV, HHV6, enterovirus, coxsackievirus and echovirus.
Conclusions: The data showed that the HSV 1/2 reagents provide a sensitive and specific qualitative assay for the detection of HSV1 and HSV2 viruses in a single reaction within 3 hours. It provides a valuable alternative to the classical cell culture method for the clinical management of patients with HSV infections.

P758

Detection of herpes simplex virus in cerebrospinal fluid using real-time PCR

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Objective: To evaluate the performance of the Light Cycler HSV 1/2 Detection Kit (Roche) in 124 cerebrospinal fluid (CSF) samples from adult patients.
Methods: 124 CSF samples from adult patients admitted to our hospital since May 2004 to September 2005 were studied. Thirty-five of them showed a typical glucose, protein and cell count pattern of viral infection. After specific cultures, 100 μl were separated and frozen ((80°C) until further study. We performed nucleic acid extraction with the High Pure Viral Nucleic Acid Kit (Roche) following the manufacturer's recommendations. An internal control was included in each sample. The Light Cycler HSV 1/2 also includes a positive control which, after amplification shows two melting temperature picks; 54°C and 66.5°C (corresponding to HSV-1 and HSV-2 respectively). We also analysed four cell cultures infected with HSV (two with HSV-1 and two with HSV-2) to assess the whole process of extraction and real time PCR.
Results: One of the samples yielded a positive result to HSV-2. It's biochemical characteristics and the clinical pattern of the patient were compatible with a viral meningoencephalitis. The four infected cultures were also positive. The rest of the samples yielded negative results but all of them showed amplification of the internal control. The whole process (extraction and amplification-detection) took approximately 2 hours.
Conclusions: (1) The presence of HSV in CSF samples in our hospital is rare. (2) The HSV 1/2 Detection Kit allows rapid detection and differentiation of HSV-1 and HSV-2 in CSF samples and so can be a helpful tool in management of central nervous system infections.

P759

BK polyoma virus replication in *de novo* renal transplant recipients: Results of a prospective study

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Objectives: BK polyoma virus has been associated with the development of renal allograft interstitial nephritis, which may lead to chronic allograft dysfunction. This study evaluated prospectively the replication of BK polyoma virus in renal transplant recipients.
Materials: Thirty-two *de novo* renal transplant recipients of median age 48.5 years old were studied for mean follow up period of 36 weeks. Plasma and urine samples were collected at three monthly intervals after renal transplantation and examined for BKV with qualitative and quantitative real – time polymerase chain reaction (PCR). All recipients received anti – interleukin-2 receptor monoclonal antibody and triple immunosuppression regimen with calcineurin inhibitor (cyclosporine, n = 19, or tacrolimus, n = 13), mycophenolate mofetil and oral corticosteroids. A total of 340 samples were examined (170 and 170 urine plasma).
Results: BKV was detected in 53 (31.2%) urine and 30 (17.6%) plasma samples in 22 (22/32, 68.8%) and 19 (19/32, 59.4%) patients respectively. Positive samples for BKV simultaneously in urine and plasma were observed in 22 cases (22/170, 12.9%). Quantification of positive urine samples identified 18 with viral load > 10,000 copies/ml which were collected from 10 individuals. Quantification of positive plasma samples showed that two patients had viral load above 10,000 copies/ml that also had high viral load in urine. Relative risk of BK viraemia was 5-fold in recipients who had developed BK viruria (R.R. 5, 95% confidence interval 3.3–9.7). Median time of onset of viruria was 62 days after transplantation and of viraemia 72 days following transplantation. A peak of viraemia and viruria with high viral load in urine was noted 3 months after renal transplantation. Statistical analysis of the immune suppressive regimens revealed that incidence of BK viruria was significantly higher in patients who received tacrolimus compared to cyclosporine ($p = 0.005$). Statistical analysis of serum creatinine between positive and negative cases in plasma and urine of BKV revealed no significant difference or impairment of renal allograft function.
Conclusion: BK viruria precedes BK viremia. High viral load in plasma was accompanied in all cases with high viral load in urine, which suggests that viruria is a risk factor of the development of viraemia. Significant relation was observed between positive urine samples for BKV and tacrolimus immunesuppressive treatment.

P760

JC virus genotype distribution differs between healthy and immunocompromised Irish individuals

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Background: JC virus (JCV) belongs to the family of human polyomaviruses. Serological evidence suggests that JCV infects more than 70% of the human population. JCV urinary excretion in immunocompetent adults varies between 20% and 50%. Amplification and sequencing of JCV from urine has allowed a distinctive map of the distribution of JCV genotypes worldwide.
Objective: To define the frequency of JCV urinary excretion and genotype distribution in Ireland.
Methods: Urines from 121 healthy individuals and from 94 immunocompromised individuals (HIV positive patients and rheumatoid arthritis patients) were collected. JCV DNA was detected using the polymerase-chain reaction (PCR) with subsequent nucleotide sequencing of a fragment of the major capsid protein (VP1).
Results: JCV was detected in 20.7% of healthy individuals and was found more often in the urine of HIV positive patients (54.2%; $p < 0.001$) and rheumatoid arthritis patients (54.4%; $p < 0.001$). In healthy Irish individuals genotype 1 was the predominant genotype in 62.5%, followed by genotype 4 in 16.7% and genotype 2 in 12.5%. Genotype 2 was significantly more often isolated from the urine of immunocompromised patients (55%, $p = 0.001$). Among type-1 viruses more than 80% belonged to subtype 1A. The only subtype found among genotype 2 strains in healthy Irish individuals was subtype 2B, whereas several other genotype 2 sequences were detected in

immunocompromised individuals. Among rheumatoid arthritis patients a new variant of both subtype 1A and subtype 2B was amplified.
Conclusion: The pattern of genotype distribution among healthy Irish individuals is similar to data reported from other European countries. The finding of significant genotype differences between urinary JCV isolates from healthy and immunocompromised individuals suggests that JCV could undergo genetic changes in the coding region in the pre-PML immunocompromised state. As similar nucleotide changes were observed previously in JCV strains from HIV associated PML brain, they might contribute to the disease causing potential of the virus.

P761

Prevalence of human papillomavirus genotypes in genital samples

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Objectives: Infection by human papillomavirus (HPV) is the main cause of genital cancer and recent evidence suggests that Human Immunodeficiency Virus (HIV)-infected patients have an increased frequency of HPV infection. Genital HPV types have been subdivided into high-risk and low-risk genotypes depending on their presence in genital carcinoma, therefore we studied the prevalence of HPV types in the genital samples of 377 patients, 126 of whom were co-infected with HIV.
Methods: 377 patients were included, 209 men (mean age 36 years) and 168 women (aged 26–36). Only six women were HIV-coinfected. The presence of HPV DNA was assessed by polymerase chain reaction (PCR) using consensus primers MY09 and MY11 from the L1 region. HPV genotyping was carried out using a reverse hybridization line probe assay of the amplified product (INNO-LiPA. Innogenetics). Amplification of a fragment of the β-globin gene was performed as an internal reaction control. HPV genotypes 16, 18, 31, 33, 45, 51, 53, 56, 58, 59 66 and 68 were considered as high-risk oncogenic.
Results: HPV-DNA was detected in 149 samples, 67 of which corresponded to HIV coinfected patients ($p < 0.001$). The distribution of HPV genotypes was similar in HIV-positive and HIV-negative patients. HPV 6 was the most common genotype (27.36%) and most prevalent high-risk HPV genotype was 16, in 36 (18.95%) cases. The incidence of HPV type 18 was very low (only two cases). Infection with high-risk HPV was detected in 45.26% of the cases, but there was no significant difference between the two groups. The majority of the HPV-DNA-positive samples contained a single HVP genotype, although 21 of the non HIV-coinfected patients and 15 of the HIV-coinfected patients had mixed infection. HPV could not be typed in 18 cases with the probes used. Total inhibition of the PCR was observed in 13 samples.
Conclusion: The prevalence of HPV infection was 40%. The presence of HPV was significantly more frequent in the patients coinfected with HIV ($p < 0.001$). Half of the patients were infected by a high-risk oncogenic HPV genotype.

P762

Prevalence of human papillomavirus types in cervical samples from HIV-infected women

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Objectives: The aim of this study was to evaluate the distribution of human papillomavirus (HPV)-DNA and simultaneous human immunodeficiency virus (HIV)-related nucleic acids in cervicovaginal secretions of 173 known HIV-1-seropositive women.
Methods: Paired blood and cervicovaginal lavage samples were collected from each women. Gynecologic examination was performed in all participants. HPV DNA was detected in cervicovaginal lavages by using MY09/11 L1 polymerase chain reaction (PCR). HPV typing was performed by E6/E7 nested PCR. Proviral HIV-1 DNA, cell-associated, and cell-free HIV-1 RNA in cervicovaginal secretions were quantitatively evaluated by competitive PCR and reverse transcription PCR.
Results: HPV-DNA in cervicovaginal secretions was detected in 45% (78 of 173) of the women; 23% (18/78) was infected by more than two types. HPV 6/11 was the most prevalent genotype and was observed in 23/173 (13.3%), followed by HPV 18 and 31. The rate of HPV infection was related to severity of cytologically diagnosed squamous intraepithelial lesions. Proviral HIV-1 DNA, cell-associated, and cell-free HIV-1 RNA were detected in 41.6% (72 of 173), 33.5% (58 of 173), and 32.4% (56 of 173) of cervicovaginal samples, respectively, of participants. Out of the 78 women positive by PCR for HPV-DNA, 27 (34.6%) were positive for all HIV-related nucleic acids in cervicovaginal secretions, while 35 (44.8%) and 29 (37.2%) were positive for HIV-1 DNA and cell-associated RNA, respectively.
Conclusion: Our results confirm that HIV infection may strengthen the effect of HPV at cervical level.

P763

Physical status of HPV-16 in patients with LSIL or cervical carcinoma

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Background: Infection with high risk human papillomavirus (HPV) has been associated with cervical carcinoma. Integration of viral DNA into host genome is essential for carcinogenesis. The HPV genome integration usually disrupts E2 gene, leading to overexpression of E6 and E7 oncogenes.
Objectives: To assess the viral integration status of HPV-16 in women with low-grade squamous intraepithelial lesions (LSIL) in comparison to cervical cancer patients making use of multiplex PCR.
Materials and methods: Fifty-three women with confirmed HPV-16 infection were examined; 30 of them with LSIL and 23 with cervical cancer. DNA was isolated from cervical secretions with Genomic Mini Kit (A&A Biotechnology). HPV-16 DNA was detected with the use of PCR with SPF10 as primers and by means of hybridization (INNO-LiPA, Innogenetics). The E2 and E6 genes of HPV-16 were detected by multiplex PCR using two pairs of primers. The PCR products were separated electrophoretically in agarose gel and the densitometric analysis was performed using Bio-Rad Quantity One software.
Results: The E2 and E6 sequences of HPV-16 were detected in 49 (92%) women. HPV-16 E6 was found in all specimens obtained from patients with cervical cancer. In this group of women, it was possible to amplify the E2 region in 83% of cases. Thirty-four percent of patients exclusively harboured the episomal form (E2/E6 ratio = 0) whereas in the remaining group of women a mixture of episomal and integrated forms of viral DNA (1> E2/E6 ratio >0) were found. In the women with LSIL the episomal form dominated (93%, with E2/E6 ratio $\geq$ 1). In two cases the episomal and integrated forms of HPV-16 were detected simultaneously.
Conclusion: HPV-16 integration occurs in a subset of LSILs, not only in the cervical cancer specimens. The assessment of HPV-16 status would be a helpful complementary tool for cytological study in cervical screening to identify women at risk of developing high-grade squamous intraepithelial lesions and cervical cancer.

P764

Prevalence of HPV in Lithuania according to data based on PCR technique

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Background: Human genital papillomavirus (HPV) is common sexually transmitted pathogen and infection with some types of it reportedly was associated with cervical intraepithelial neoplasia (CIN) in women. The risk factor for the development of cervical intraepithelial neoplasia is significantly increased by persistent infection with high-risk HPV genotypes (16, 18, 31 et al). HPV can cause skin and mucosa wartes.
Objective: The aim of present study was to determine HPV prevalence in the Lithuania population.
Methods: Polymerase chain reaction (PCR) for HPV diagnostics was successfully introduced in Biomedical Research Centre in 2001. Patient's samples have been collected from various private clinics of Lithuania. Urethral samples were taken with a dacron swab placed into urethra 2–3 cm in males (M), and vaginal samples were taken from the endocervical region in women (W). During 2001–2005 years 3204 samples for HPV have been analysed. DNA was extracted from clinical samples by using rapid in-house procedures. The presence HPV was determined by using in house PCR with specific primers of E1 gene. The positive samples were reamplified with specific primers for HPV genotypes 16 and 18. The quality of DNA was tested by PCR using primer for human B-globin gene. Amplified PCR products were separated in 2% agarose gel and detected by ethidium bromide staining.
Results: In total, 3204 patients were included into the study: 1811 (57%) women and 1393 (43%) male with signs of urethral discharge and/or urethral discomfort and lower urinary tracts symptoms. We detected that 28% (884/3204) of samples were positive for common HPV (W samples consisted of: 36% (658/1811), and M samples consisted of 16% (226/1393)). The 592 (549 W and 43 M) HPV positive samples were reamplified for 16 and 18 HPV types. The high-risk HPV genotypes were identified as follows: 16-HPV – 26% (151/592 /W: 26% (145/549), M: 14% (6/43)/, and 18-HPV – 6% (37/592) /W: 7% (34/549), M: 7% (3/43)/. Two patients were infected with both high-risk HPV types.
Conclusion: Molecular technique used for HPV detection is simple, economic, rapid and reliable for screening samples and providing information for prevention cervical cancer. The prevalence of HPV in our study according to data based on PCR technique was 28%. We have made conclusion that 32% of patients were infected with high-risk HPV genotypes.

P765

Focal epithelial hyperplasia disease in Panares Indians from Bolivar state, Venezuela

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Focal Epitelial hyperplasia (FEH) is common in native Indians of South and Central America, specially in children and young population; in adults it is rarely disease. FEH presents an exophytic lesions of the oral mucosa and may be rather easily confused with papilloma or condyloma. The aim of this study was the detection of HPV in FEH lesions from Panares Indians of Bolivar State, Venezuela.
Materials and methods: We present 13 children (mean age = 7 years) and 15 adults (mean age = 38 years), who were diagnosed of FEH. This Indian population is a very close community from the Maniapure, Bolívar State. PCR wit HPV type – specific consensus primers followed by analysis of the restriction fragments length polymorphism were used.
Results: All the samples of the mucosal lesions stained with haematoxylin and eosin shows papillomatosis, acanthosis, hyperkeratosis and parakeratosis of the epithelium. A sparse mononuclear inflammatory cell infiltrate was present in the chorion. These findings are compatible with FEH disease. The 82% (23/28) of the patients had HPV. The genotyping assay indicated the presence of HPV 13 (82.6%) and HPV 32 (17. 4%) in this population. Co-infection with more than one HPV type was not found.
Discussion: FEH is very common in this Indian population without gender or age predilection. In this population FEH is not a limited disease and will be related with a genetic predisposition conditions of the Panares Indians. In some cases the patients had a functional problems but they does not received any treatment.
Acknowledgement: Grant of FONACIT S1-2000000643.

P766

Gen tax evidence of HTLV-1 co-infections in HIV-positive patients in a hospital in Bilbao, Spain

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Background: Human T-cell leukaemia virus type I (HTLV-I), is the pathogenic agent of adult T-cell leukaemia-lymphoma (ATLL) and HTLV-1 associated myelopath/tropicalspastic paraparesia (HAM/TSP). In HIV infected patients have been shown to be frequent, probably in consequence of their similar modes of transmission. The gen tax of a human T-cell leukaemia virus type 1 (HTLV-1) induces the expression of several cellular genes that are involved in T cell activation and proliferation. The aim of this study was to analyse HTLV-I proviral DNA presence in HIV infected patients of Bilbao using primers of the region tax of the HTLV-1.
Materials and methods: We analysed peripheral blood mononuclear cells (PBMCs) from 100 patients samples HIV infected patients (EIA, and confirmed with Western blot). These patients were treated with antiretroviral therapy. A conventional PCR and a real time PCR assay using SYBR Green were established. Both assays were designed using primers from tax gene (156 bp) of HTLV-I. The real-time PCR assay reliably detected a single copy of HTLV-I proviral genome in DNA from 1 × 106 PBMCs. For conventional PCR we also included in each sample the HLA DQ alpha gene (242 bp) to check the presence of PCR inhibitors and the quality of DNA extraction. As positive control we used HTLV-I infected MT-2 cell line, which had integrated stable tax gene. Cells were incubated at 37 °C in a 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% heat inactivated Bovine foetal serum (FBS), penicillin and streptomycin.
Results: All the samples analysed had presented HLA DQ alpha gene amplicon indicating that PCR had been carried out under good conditions. In five of the HIV infected patients the band corresponding to the gene tax (156 bp) of the HTLV-1 was detected. In the real time PCR's the MT-2 cells were in use for obtaining a standard melting curve with a melting temperature of 88 C (±1 C). The melting temperature of nine PCR samples from HIV infected patients was similar to the one obtained in the standard melting curve but only five of the samples had been reported as positive when conventional PCR was performed.
Conclusion: The results of this study suggest that both assays could be used to diagnostic HTLV-1 infection in samples of HIV infected patients. Besides, the results also suggest that real time PCR is faster and has more sensitivity fact the conventional PCR but further studies are needed.

Hepatitis

P767

Virologial and clinical aspect of HBV silent infection in patient with chronic hepatitis C

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Occult HBV infection was tested in 89 HBsAg negative consecutive patients with chronic hepatitis C (CHC) at the time the diagnosis was first made by liver biopsy (LB). For each patient three compartments (plasma, a small fragment of LB and six millions of Peripheral Blood Mononuclear Cells-PBMC) were explored for presence of HBV-DNA by PCR; three sets of specific primers for HBV core, surface and x region were used. Of the 89 enrolled patients, 45 were anti-HBs/anti-HBc negative (Group 1), 18 anti-HBs/anti-HBc positive (Group 2) and 26 anti-HBs negative/anti-HBc positive (Group 3). Clinical and virological characteristic of these patients are reported in the Table. *n° of patients with HBV-DNA also in plasma; A: three cases with cirrhosis; B: six cases with cirrhosis; C: staging according Ishak.

	Group 1	Group 2	Group 3
N° of cases	45	18	26
Age, yrs, median (range)	48 (23-68)	47 (27-63)	51 (34-66)
with HBV-DNA in liver	5 (1*)	2 (0*)	7 (2*)
with HBV-DNA in PBMC plus liver	0	8 (1*)	11 (5*)
with HBV-DNA in PBMC	0	1 (1*)	3 (2*)
with 2 or more PCR pos in at least compartment	5 (11%)	11 (61.1%)	21 (80.8%)
HAI ≥7: HBV-DNA pos HBV-DNA neg	2/5 (40%) 15/40 (37.5%)	6/11 (54.5%) 4/7 (45.5%)	15/21 (71.4%) 0/5
staging[C] 5-6:HBV-DNA pos HBV-DNA neg	0/5 12[A]/40 (30%)	2/11 (18.2%) 3/7 (43.8%)	14[B]/21 (66.7%) 0/5

Conclusion: Isolated anti-HBc in CHC cases may predict occult HBV infection, since the detection of HBV-DNA in the liver and PBMC using specific primers for core, surface and x regions identify in up to 81% of these patients occult HBV infection: in these the presence of HBV-DNA correlates with severe fibrosis. The detection of HBV-DNA in plasma is of no value to detect occult HBV infection.

P768

Fulminant hepatitis due to hepatitis B virus

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Objective: Fulminant hepatitis (FH) is the most severe form of viral infection of the liver. Our aim was to correlate the demographic, clinical and laboratory data with the evolution of the disease.
Methods: A retrospective study of 18 patients with FH admitted in the Infectious Diseases Hospital, Iasi, between January 2000 and June 2005.
Results: From the 18 patients (12 men, 6 women) with FH, 15/18 immunocompetent, aged between 4 and 53 years (median 17 years) the aetiology was established in 14 cases. The hepatitis B virus (HBV) was involved in 11 cases (from 553 acute viral hepatitis B – incidence 1.98%), the co-infection B+D in two cases and the hepatitis A virus in one case (from 4313 cases – incidence 0.02%). The HBs antigen was absent in three cases, the anti-HBe antibody were present in four cases and HBV-DNA was detected at low levels (<5000 copies/ml) in two patients who were tested in the first day of coma. The encephalopathy appeared at 8.3 days from the first symptoms and 4.63 days from the jaundice. The mean duration of coma was 4.34 days. An influenza-like syndrome was more frequent ($p < 0.02$) during the onset of FH due to HBV compared with non-fulminant cases. The laboratory findings showed hiperleukocytosis (>10,000 l/mm^3) with a normal erythrocyte sedimentation rate, peak ALAT 20–30 times higher than the normal value, total bilirubin levels up to 350 mg/l and a protrombin index below 30% in all patients. The administration of antivirals (IFN or/and lamivudine) after the installation of coma had no results (six treated patients / six deaths). The lethality rate was 90.9% in HBV FH.
Conclusion: The hepatitis B virus was the most frequent cause of FH, a rare disease with a poor prognosis, more common in young males; the administration of antiviral drugs did not improve the survival rate.

P769

Acute/recent HCV infection in seronegative blood donors and IDUs, viral replication kinetics, immune response and disease outcome

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Objectives: Aim of the study was to reveal acute/recent HCV infection at the very early stage of infection in seronegative blood donors and Injecting Drug Users (IDUs) to observe the clinical course, viral replication kinetic, immune response and disease outcome.
Methods: A prospective 24 months follow-up study was performed in two different sub-groups: blood donors and Injecting Drug Users (IDUs). Namely, ELISA negative 3000 blood donors and 1000 IDUs were investigated on HCV RNA by qualitative PCR method. A pool of six blood specimens was applied for blood donors' testing and a pool of five blood specimens for IDUs. Further testing on individual samples was performed only for PCR positive pools. HCV antibodies were detected by ELISA and RIBA. HCV RNA (Qualitative and quantitative) by RT- PCR (Roche). HCV genotype- by Inno-Lipa. Detection of HCV RNA level (Viral load) was performed weekly for 6 months and then monthly. Cytokines were induced in whole peripheral blood cells using HCV core peptides and tested by ELISA.
Results: We revealed totally 11 patients with acute/recent HCV infection: five from blood donors, seven from IDUs. Among them four were symptomatic and seven asymptomatic. In all patients with acute/recent infection viremia was detectable 2 weeks after inoculation, it increased very rapidly and reached a peak titre by week 4. The viral titre was remarkably stable for the next 5–6–7 weeks, falling only two or three fold by week 9. After week 10 the viremia rapidly decreased: 1 >4 logs or 1 >5 logs by week 12 and it became either undetectable (<102 GE/ml) by weeks 14–15–16 (viral clearance), or virus was not eliminated and viral titre persisted in all follow up period (chronic infection). Among 11 subjects: two had genotype 1a, 5 -1b, 2-2a/2c and 2-3a. Out of 11 patients 3 cleared the virus, (two were symptomatic: 1-genotype 1b, 1 genotype 2a/2c and one asymptomatic- genotype 1a), while 8 developed chronic infection. In three subjects who cleared virus two had 1st type cytokine response (IL-2 and IL-12), in seven patients, out of eight who developed chronic infection cytokine synthesis was shifted towards Th2 response (IL-4 and IL-10).

Conclusion: There was some relationship between high viral titre and clearance from virus. Viral clearance was associated also with presence of Th1 immune response. There was no correlation between viral genotype and disease outcome.

P770

Phylogenetic analysis in a HCV type 5 infected population in the French central district of Clermont-Ferrand

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Objectives: Hepatitis C virus genotype 5 (HCV5) is the predominant genotype in southern Africa but unusual in Europe. In France, it is responsible for less than 2% of HCV infections. In Clermont-Ferrand district (central France), its prevalence is 14.2% representing more than 170 patients since 1990. Epidemiological study and molecular analysis of viral strains were carried out to investigate the high prevalence of HCV5 in a local area.
Methods: A case–control study included 131 patients infected with HCV5 and 393 patients infected by other types. A detailed questionnaire was used to identify potential risk factors for infection. Direct sequencing of NS5B and E1E2 fragments was performed for 97/131 patients. The neighbour-joining algorithm using the Tamura-Nei model was used to obtain phylogenetic trees for NS5B and E1E2 (257 and 715 nt respectively).
Results: No IVDU contaminated patient was found. In multivaried analysis, the following variables were associated with contamination by HCV5: living in the South of the district (ORa = 16.3, $p < 0.0001$); being injected with more than five injections in the South of the district (ORa = 3.5, $p < 0.0001$); being transfused (ORa = 3.3, $p = 0.002$); use of an intrauterine contraceptive device (ORa = 2.2, $p = 0.01$). The mean age of patients with risk factor ''living in the South of district'' is higher than this of controls (61.8 vs 53.2, $p = 0.001$). Genotype 5a was identified in all patients. Comparison of NS5B and E1E2 sequences demonstrated a very high degree of nucleotide similarity between local HCV5 isolates and other homologous sequences (French isolates out of our geographic area or sequences available in Genbank). No cluster was observed within local sequences. However, phylogenetic analysis of E1E2, encompassing the HVR1 region, revealed 12 pairs of tightly closely related sequences supported by bootstrap analysis. Epidemiological data showed that 7/12 pairs were linked by blood transfusion (donor/recipient or recipients of the same donor) between 1978 and 1990, and 3/12 were husband and wife. No sub-grouping within patients living in the South of the district was observed.
Conclusion: The HCV5 infection in this semi-rural settled population was probably due to multiple events between 1960 and 1990 (iatrogenic transmission, transfusion, in-house transmission). Phylogenetic analysis did not evidence a cluster in patients living in the South of the district, but highlighted the existence of in-house transmissions.

P771

Quantification of hepatitis C virus in human plasma samples by using real-time reverse transcriptase PCR

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Objectives: Hepatitis C virus (HCV) is the major causative agent of non-A, non-B Hepatitis. Acute HCV infection is often asymptomatic, and approximately 70% of cases progress to chronic hepatitis. This may lead to progressive liver disease, cirrhosis, liver failure, and hepatocellular carcinoma over 20–30 years. Factors associated with disease progression following HCV infection include the viral genotype, alcohol consumption, and viral load. High viral loads associated with decreased rates of response to interferon therapy, and a decrease in the HCV viral load during the early phase of treatment (2–12 weeks) has been shown to predict effective treatment responses. Because it has been established that viral load is relatively stable in the chronic phase of the infection, of these parameters, viral load seems to be more useful for the tailoring of treatment schedules and the monitoring of HCV replication during therapy. The detection and quantification of HCV RNA in plasma requires highly specific and sensitive assays because HCV circulates in the blood at a low copy number and its genome is extremely heterogeneous, even though the virus titre covers a wide range (from undetectable values to 108 copies/ml).
Methods: The aim of this study was to develop a simple, real-time QRT-PCR assay for the quantification of HCV RNA copies in plasma samples with SYBR Green I detection rather than the use of labelled probes. Real-time QRT-PCR assays were performed with a Light Cycler system. Ninety-four RNA samples from plasma of infected patients were quantified by this method. Comparison of the results with those obtained by other quantification method.
Results: The linearity of this approach was conserved over a wide range of HCV copy numbers. The coefficient of the regression of the standard curve was, on average 0.98. Comparison of the results with those obtained by other quantification method, revealed a significant correlation with all of the results.
Conclusion: Our data demonstrate that Light Cycler is a fast and reliable method for the detection and quantitation of HCV RNA.

P772

PEG-interferon-alpha 2a (40 kDa) in patients on chronic haemodialysis with chronic C hepatitis: Early results

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Objective: The aim of the study was to evaluate the early response to Pegylated Interferon alpha2a (40 kDa) in patients on chronic haemodialysis (HD) with chronic C hepatitis.
Methods: Twenty-two patients were enrolled in this study (12 males and 10 females). The diagnosis was established according to anti-HCV positively, serum HCV-RNA activity with PCR (Cobas Amplicor HCV Monitor; Roche Molecular systems, Branchburg, NJ, US), increased of ALT levels. Liver biopsy was not done in HD patients. We administrated Peg Interferon alpha-2a 135 μg/week for 48 weeks. We used the reduction of two log drop HCV-RNA clearance, or both at week 12 to predict early and response to treatment.
Results: Of 22 HD patients, 12 were male and 10 female, average ages was 35.2 ± 12.1 years old. Three patients were excluded from the study because of lack of compliance. We had to stop the treatment in two patients due to complications (depression, thrombocytopenia and leucopenia). After 12 weeks of treatment with Peg-Interferon alpha 2a (40 kDa) in patients on chronic haemodialysis with chronic C hepatitis, the virological response (HCV-RNA absent by PCR) was obtained in 82.4% (14/17) of the cases. The normal transaminase (biochemical response) was obtained in 70.6% (12/17) of the cases. Even if side-effects occurred in most of the patients (flu-like syndrome, thrombocytopenia or leucopenia) they did not impose the discontinuation of treatment.

Conclusion: The early virological response in our study population was better than those observed in the general population. The sustained response will be established by determining PCR RNA-HCV 6 months after the end of the treatment.

P773

Hepatitis C virus infection in haemodialysis patients

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Objectives: Monitoring the Hepatitis C virus (HCV) infection antibodies in haemodialysis (HD) patients is an important issue of public health. The aim of the study was to analyse the trend of the seroconversion rate and the impact of HCV serologic positive test on mortality.
Methods: The study population consisted of 6412 patients starting HD in Lazio region (Italy) reported to the regional Dialysis Registry between 1/1/1995 and 31/12/2003. The information about HCV serologic status was collected at the beginning of dialysis and then once a year. HCV status was defined using ELISA or RIBA third generation test. To calculate seroconversion rates, we defined as cases those patients, seronegative at the beginning of each period, who became seropositive at the end of the same period. We estimated the cumulative probability of survival using the Kaplan–Meier method; we used the Cox model to estimate mortality hazard ratios (HR) of HCV positive compared to HCV-negative ones. To take into account of those who seroconverted during HD, the HCV serostatus was considered as a time varying covariate.
Results: Mean age at the beginning of dialysis was 63.1 (SD 16.2) years; males were 61.6%. The HCV-seroconversion rates in the period 1/7/1995-31/12/2003 significantly decreased in the first three periods and then remained quite stable at around two cases per 100 person-years (PY). The survival at 5 years was lower both for HCV-seroconverters (45.1%; 95%CI 34.0–55.6) and for those already HCV-positive at HD start (47.6%; 95%CI 42.5–55.6), compared to HCV-negative subjects (55.7%; 95%CI 54.0–57.3) (log-rank test, $p < 0.001$). The estimated crude HR was 1.37 (95%CI 1.23–1.53) for those who were or became HCV-positive compared to those who remained HCV-negative. When adjusting for other variables the HR was 1.29 (95%CI 1.15–1.44).
Conclusions: The HCV-seroconversion rate has significantly decreased in the mid-nineties in HD patients in Lazio region, and has remained stable thereafter. The overall reduction of the HCV-seroconversion rate in the last decade could be related to a lower frequency of transfusions in HD patients; however, the two per 100PY is likely due to nosocomial risk factors. We observed that HCV-positive subject had a higher risk of mortality than negative ones. The monitoring of HCV infection has important public health implications, being the presence of HCV antibodies both at the beginning of dialysis and as seroconversion during HD associated to higher risk of mortality.

P774

Anti-HCV IgG Avidity Index in acute hepatitis C and chronic hepatitis C

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Objective: To evaluate the useful of anti-HCV IgG Avidity Index (HCV AI) to distinguish acute Hepatitis C (AHC) and reactivation of chronic hepatitis C (r-CHC).
Patients and methods: We enrolled 40 consecutive patients with AHC identified by seroconversion to anti-HCV (AHC group) and 37 consecutive patients who had been anti-HCV positive for at least 6 months at the time of reactivation (r-CHC group). For each patient in AHC group we choose an HBsAg negative patient with symptom-free chronic hepatitis C, pair-matched for age (+5 years), sex and risk factor for acquisition of parenteral infection (CHC group). The HCV AI was determined by a commercial immunoenzyme assay evaluating for each serum sample two aliquots of 200 μL diluted 1:10 with guanidine 1 M and phosphate-buffered saline (PBS), respectively; HCV AI has been calculated as the ratio between the OD at 492 nm from the guanidine wells and the OD from the PBS wells.
Results: HCV AI was significantly lower in patients in AHC group (0.50 ± 0.30, range 0.05–1.15) than in those in r-CHC group (0.94 ± 0.25, range 0.28–1.24, $p < 0.0001$) and in those in CHC group (1.06 ± 0.20, range 0.84–1.3, $p < 0.0001$). The analysis of sensitivity and specificity of HCV AI to identify AHC demonstrated that increasing the cut-off of AI the sensitivity increased, but specificity decreased: in fact, considering an cut-off of AI lower than 0.5 as a marker of AHC we found that the sensitivity of the assay was 52.5% and the specificity of 94.6%, whereas a cut-off lower than 0.9 had a high sensitivity (85%) but a low specificity (78.4%). An increase of HCV AI was observed in 60.5% of the 33 patients in AHC group and in none of 24 in r-CHC for whom more than one serum sample collected during the acute stage of the disease, whereas stable HCV AI values were observed more frequently in r-CHC group (91.7%) than AHC (39.5%, $p < 0.005$). Considering the 29 patients in AHC group observed as outpatients for 6–30 months, no difference was found in the HCV AI between the 12 patients who recovered and the 17 who developed a chronic HCV infection.
Conclusion: Our data indicate that the detection of low HCV AI may identify acute hepatitis C, but its senstivity and specificity is not enough high.

P775

Hepatitis B virus genotypes: a Lebanese hospital-based study

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Objectives: Hepatitis B virus (HBV) represents a global health challenge due to its worldwide distribution and serious complications as a result of the chronic viral persistence. The recent interest in HBV genotypes has shown that the various HBV genotypes may have different influence on the prognosis, response to treatment and course of the disease. At present eight HBV genotypes have been identified from A to H with a characteristic geographic distribution. Genotype D is the most prevalent in the Eastern Mediterranean region. However, there are scarce data about HBV genotype distribution in Lebanon where the prevalence of Hepatitis B antigen (HBs Ag) is around 2–5%. Lebanon is situated at a crossroads between Europe, Africa and Asia, where different genotypes have been detected. The aim of the study is to determine the prevalence of HBV genotypes in a Lebanese population sample and to assess the potential correlation between the genotype and viral characteristics such as HBe antigen status and serum HBV DNA viral load.
Materials and methods: This study involves 60 HBs Ag positive individuals with various demographic distribution, of which 30 blood bank donors (HBs Ag positive) and 30 patients with a documented active viral hepatitis B (HBs Ag positive with viremia). Only this latter group was further investigated for viral characteristics. HBV genotype was identified by multiplex-PCR using genotype-specific primers.
Results: Genotype D was the only genotype detected in both groups. Among the active viral hepatitis B patients, 15 out of 30

(50%) have a high viremia (HBV DNA >104 copies/ml) associated with a negative HBe antigen suggesting a precore mutation.
Conclusion: Despite the limited sample size of the study, our data clearly show that genotype D is the major strain in Lebanon, as for the other Eastern Mediterranean countries. It also confirms the previously described correlation between genotype D and HBe antigen negative chronic hepatitis. For further analysis we aim to expand our study and assess the correlation between genotype D, precore mutation, and severity of liver disease.

P776

Evaluation of Bayer Advia Centaur chemiluminescent assays for the analysis of hepatitis B HBs antigen and HBc total antibodies and hepatitis C total antibodies

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Introduction: Screening for hepatitis B and C requires highly automated analytical systems to allow for short turn-around time and high reliability.
Objectives: To compare recently released Bayer Advia Centaur chemiluminescent assays for hepatitis B (HBs antigen, HBc total antibodies) and hepatitis C (total antibodies) with the currently used chemiluminescent assays on Abbott Architect.
Materials and methods: Samples sent to a private routine laboratory were included in the study without prior selection. Samples were analysed on Abbott Architect for HBs antigen and/or HBc total antibodies and/or hepatitis C total antibodies followed by immediate analysis of the correspondig markers on Bayer Advia Centaur to ensure routine conditions.
Results: 2927 sera from 2899 patients were analysed. 1151 sera were tested for HBs antigen only, 599 for HBc antibodies only, and 1001 for both HBs antigen and HBc antibodies. 1756 sera were tested for hepatitis C antibodies. For HBs antigen, at a prevalence of 1.9%, no diagnostically relevant discrepancies were found. 20 sera were found equivocal in Architect and negative in Centaur, whereas two sera were found equivocal in Centaur and negative in Architect. For HBc antibodies, at a prevalence of 10.3%, major discrepancies were found in 24 samples: 20 Architect-reactive sera were found negative in Centaur, whereas four Centaur-reactive sera in were negative in Architect. For HCV antibodies, at a prevalence of 1.5%, one major discrepancy was found: one Architect-negative sample was found reactive in Centaur and was confirmed positive in a recombinant immunoblot.
Conclusions: Good agreement was observed for HBs antigen and for HCV antibodies. For HBc antibodies, Architect may provide somewhat higher sensitivity than Centaur, although low signals in the twenty discrepant sera may point to false reactivity in Architect. From this large-scale comparison of Centaur and Architect under routine conditions we conclude that both systems are equally useful for routine testing for hepatitis B and C infections.

P777

Relationships between luetic infection and acute viral hepatitis

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Background: Acute viral hepatitis (AVH) still represents one of the major problems for the romanian healthcare system. The association between lues and AVH is currently underdiagnosed and represents a problem which should be more focused in future.
Methods: Retrospective clinical study of 469 acute viral hepatitis patients admitted in our hospital in 2004. The diagnosis of coexisting lues was made by an association of positive values of *Treponema* screening tests (i.e. qualitative RPR and VDRL) and of a confirmation test (we used TPHA).
Results: Out of the 469 patients, 330 (70.4%) were diagnosed with acute HAV hepatitis, 116 (24.7%) with acute HBV hepatitis, 8 (1.7%) with acute HDV hepatitis on a chronic HBV infection background, 3 (0.6%) had an HAV+HBV acute coinfection, 2 (0.4%) had an HBV+HDV acute coinfection. The aetiology of the acute hepatitis remained unclear in 10 (2.1%) of the patients, but was presumed viral based on the clinical presentation and evolution and on the associated performed lab tests. TPHA was performed in a total of 62 (13.2%) of the admitted patients, and was positive in 8 (1.7% overall, 12.9% of all TPHA tests performed) cases. All positive TPHA tests were accompanied by positive lues screening tests (qualitative RPR (qRPR) tests (7 positive tests out of 27 performed, overall positivity 1.5% and a positivity of 25.9% with regard to the total qRPR tests performed) and VDRL test [one positive test out of three performed, overall positivity 0.2% and a positivity of 33.3% with regard to the total VDRL tests performed)] which allowed the physicians to diagnose lues in addition to the AVH. The distribution of lues among the eight diagnosed patients with regard to the AVH aetiology was as follows: one case coexisted with HAV AVH, five were associated to HBV AVH, one case was discovered in addition to HBV+HDV AVH on a chronic HCV infection background and one case coexisted with HDV AVH on a chronic HBV infection background.
Conclusions: (1) The association between lues and AVH should be searched for, especially in those patients with parenterally transmitted viral infections. (2) We estimate that by routinely searching for luetic infection in AVH patients, a certain number of currently undiagnosed lues cases may become apparent and may be appropriately treated. (3) We therefore suggest the TPHA testing in documented parenterally transmitted acute viral hepatitis cases, and, if positive, the further testing with any available *Treponema* screening test.

P778

The distribution of HBV genotypes, serotypes and YMDD variants among chronic HBV patients in Kuwait

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Objectives: There is growing evidence that HBV genotypes may play some role in causing different disease profiles in CHB. This relationship between HBV genotypes and severity of liver diseases has not been studied in Kuwait. Therefore, the aim of this study was to elucidate the distribution of different HBV genotypes/serotypes among CHB patients in Kuwait and to investigate their role in correlation with disease severity and therapeutic outcome.
Methods: Sixty-four patients with CHB were enrolled in this study. The age, liver function, HBeAg and anti-HBe status, histology were all analysed for each patient. HBV DNA was detected in all 64 isolates (34 were on treatment, 31 received no treatment). HBV genotype, serotype and YMDD variants were determined for the isolates by using semi-nested PCR and sequencing analysis of the HBV pol gene including the YMDD locus. Retrieved sequences were compared with key nucleotide

positions from genotypically well-defined strains in the GenBank after alignment of the sequences.
Results: Analysis of the 64 CHB patients demonstrated that 86% of the strains were genotype D/ayw followed by 6.25% genotype A/adw. Interestingly, mixed genotypes (D+F, D+A) and C/D hybrid were observed among the local isolates. Male ratio was twice as that for female and the mean ages was fairly uniform. When the correlation between the patient's serological status and the HBV genotype was analysed, the results for genotype D strains were significantly different, in that 68% were anti-HBe positive in comparison with only 4% for genotype A and 2% for mixed genotypes ($P < 0.05$). The ALT levels in samples from patients infected with genotype D were higher than the rest. Furthermore, rtM204 I/V identified in 4/64 HBV strains tested; three patients were on lamivudine for 3–5 years and the remaining didn't receive any treatment.
Discussion: The predominance of genotype D/ayw coincided very accurately with the HBV genotypes expected from the local population. Genotype D-infected patients were found to be anti-HBe positive significantly more often and had higher ALT levels. Therefore, genotype D may lead to more severe liver disease. However, occurrence of heterogeneous populations in clinically local samples indicates that HBV exists as a quasispecies. With regards to disease presentation at the commencement of treatment, YVDD and YIDD types tended to have similar findings with respect to age and histology.

P779

Immunogenicity of recombinant hepatitis B vaccine in treatment-naïve and treatment-experienced chronic hepatitis C patients: The effect of pegylated interferon plus ribavirin treatment

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Objectives: To retrospectively evaluate the vaccination-induced anti-HBs seroconversion rates in treatment-naïve and treatment-experienced chronic hepatitis C (CHC) patients. Also to prospectively evaluate the seroconversion rates in CHC patients during pegylated interferon (PEG) plus ribavirin (RIB) treatment.
Methods: Seventy treatment-naïve CHC patients (group A), 22 sustained virological responders-SVR following interferon (IFN) plus RIB treatment CHC patients (group B) and 121 healthy subjects (group C) had been participated in the same HBV vaccination schedule (20 μg, 0–1–6 months). Seroconversion was considered if anti-HBs levels were above 10 mIU/ml within 3 months following the third dose of the vaccine. Moreover, we prospectively selected 30 non-cirrhotic CHC patients and evaluated them for the efficacy of the same vaccine schedule randomizing them in two groups: Group-1, 15 CHC patients received the first dose of the vaccine in parallel with the initiation of PEG plus RIB treatment and Group-2, 15 patients received the same vaccination schedule without concomitant treatment. Determination of anti-HBs was performed at months 1, 2 and 7. Statistical analysis of data was based on ANOVA student's t-test and chi-square analysis ($p < 0.05$).
Results: Fifty-eight of 70 group A patients (82.85%), 20/22 group B (90.9%) and 112/121 healthy subjects (92.56%) had been seroconverted. The seroconversion rates were significantly higher in the control group than in treatment-naïve CHC patients ($p = 0.04$). The corresponding rates were comparable between group A and group B CHC patients ($p = 0.38$). The vast majority of non-responders (10/14, 71.43%) had been infected by genotype-1 of HCV. The seroconversion rates were comparable between group 1 and 2 CHC patients at month 1 (20% vs 26.7%, $p = 0.67$), month 2 (46.7% vs 60%, $p = 0.46$) and month 7 (86.7% vs 93.3%, $p = 0.54$) of follow-up.
Concusions: The immunogenicity of HBV vaccine seems to be lower in CHC patients as compared to healthy subjects. SVR following IFN plus RIB treatment does not affect the antibody response to HBV vaccine. Infection by genotype-1 seems to negatively influence the seroconversion rates. Vaccination against HBV during PEG plus RIB combination treatment is not beneficial in terms of anti-HBs seroconversion rates.

P780

Serum neopterin levels in patients with replicative and non-replicative HBV carriers

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Objectives: Infection by hepatitis B virus causes complicated biochemical, immunological and histological changes in host immune response against virus can be specific or nonspecific. Nonspecific response occurs via cytokines and other substance. Neopterin is produced by activated macrophages, in response to interferon-gamma derived from activated T cell. Recent attention has focused on neopterin as a marker for the activation of cell mediated immunity The aim of this study is to define the pattern of neopterin levels in replicative and nonreplicative HBV carriers.
Methods: Thirty HBV replicative carriers and 25 nonreplicative HBV carriers and 30 healthy adult patients were included this study. Hepatitis markers were determined by commercial kit based on chemilumminesans assay. HBV DNA was quantified by hybrid capture system. Serum neopterin levels were measured by the method of competitive enzyme-linked immunosorbent assay.
Results: Serum neopterin concentrations were 14.5 ± 10.0 (4.2-41) nmol/L in replicative HBV carriers, 8.9 ± 4.3 (2.1-22) nmol/L in nonreplicative HBV carriers and 7.7 ± 2.3 (4.0-12) nmol/L in the control group. Serum neopterin levels and the rates of abnormal serum neopterin levels in the replicative group were higher than control group ($P < 0.01$ and $P < 0.05$). In the nonreplicative group, serum neopterin levels were not differing from those of control. There was a difference between replicative and nonreplicative group in that respect of neopterin levels.
Conclusion: In the hepatitis B infected carriers, elevated neopterin levels may be an indicator of the presence of replication.

P781

Investigation of the serum neopterin levels in patients with infected hepatitis B

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Objectives: Neopterin is a pyrazino-pyrimidine compound, which originate, from guanosine triphosphate. Its derivatives are produced by human monocyte, macrophages and dentritic cells upon stimulations with interferon-gama. Due to its chemical structure, neopterin belongs to the class of pteridines. Measurement of neopterin concentrations in human body fluids allows insight into a specific aspect of immunity, namely, the cell mediated immune response. The increased concentrations of neopterin in body fluids were used as diagnostic and prognostic criterion for cell mediated immunity in some clinical conditions such as infections, autoimmune diseases, coronary artery dis-

ease, malignencies, allograft rejection, renal and cardiac failure. The aim of study was to evaluate serum neopterin levels in nonreplicative HBV carriers, patients with chronic HBV infection and natural immunized persons against to HBV.
Methods: Twenty patients with nonreplikative HBV carriers (group 1), 20 patients with chronic HBV infections (group 2), 20 persons with natural immunized against to HBV (group 3) and 20 healthy volunteers (group 4) had included to study.
Results: Serum neopterin levels were 7.44 ± 3.44 nmol/l in group 1, 18.58 ± 12.19 nmol/l in group 2, 7.51 ± 10.18 nmol/l in group 3, 5.71 ± 2.92 nmol/l in group 4. The difference between group 1 and group 4, group 3 and group 4 was not statistically significant ($p > 0.05$). The difference among between group 2 and the others groups was statistically significant ($p < 0.05$).
Conclusions: In conclusion; neopterin can be used as a marker in nonreplicative HBV carriers and patients with chronic HBV infection. However, further studies with large series are needed to using routine.

P782

The HBV seroprevalance among high school students in Izmir, Turkey

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Objective: Hepatitis B infection is still a problem that causes morbidity and mortality especially in developing countries in the world. Although neonatales are being immunized against hepatitis B infection routinely by our government since 1998, there are still aspects about its epidemiology that need to be clarified in adolescents and adults. The purpose of this study is to perform a serologic survey among high school students to detect HBV seroprevalance.
Methods: Totally 7237 serum samples from eight different high schools were collected. The HBsAg, AntiHBs, HBeAg, AntiHBe, AntiHBc total, AntiHBc IgM are studied with commercially available ELISA kits.
Results: In this study 7237 serum samples were collected from high school students. Of them 4667 (64.4%) were female and 2570 (35.5%) were male. The mean age was 16.5 (ranged between 15 and 18). HBsAg, AntiHBs were positive in 98(1.35%), 1365(18.8%) of them respectively. Additionally in the HBsAg positive group; HBeAg, AntiHBe, AntiHBc total and AntiHBcIgM were positive in 16(16.33%), 82(83.67%), 98(100%), 0(0%) of the students respectively. In 5774 (79.7%) students both HBsAg and AntiHBs were obtained as negative.
Conclusion: Latest studies show that hepatitis B vaccination prevents both hepatitis B infection and primary hepatocellular carsinoma in especially endemic areas. Although vaccination in neonatal period is being performed for years, adolescent and adult vaccination strategies are not yet well defined throughout the world. As a result of this, hepatitis B infection is still a health care problem. The HBsAg positivity rate is obtained 1.3% in high school students in this study. This result is correlated with the carriage rates in our country. Also both HBsAg and AntiHBs negativity rate was 79.7%. According to this result vaccination in adolescent period is needed. Similar studies should be carried out in different areas in our country in order to detect actual immunity status against hepatitis B. In addition, the immunization procedures are have to be performed not only in neonatale period but also in adolescent and adult ages according to AntiHBs screening results. Our survey is going on and further investigations are being done, also we are evaluating the data about the risk factors affecting the carrier stage and the carrier rate among the students' families, we plan to report them in further meetings.

P783

The epidemiology of hepatitis A in Poland: Prevalence of hepatitis A antibodies in population

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Objective: Hepatitis A is acute infection disease caused by hepatitis virus type a. Severity of disease depends on the age of patient. In Poland a significant decreasing in the incidence of hepatitis A is observed last years. In 2003 and 2004 it was registered respectively only 150 and 95 hepatitis A cases. Morbidity was respectively 0.39/100 000 and 0.25/100 000.
Methods: The presence of anti-HAV antibodies in Warsaw population aged 1–50 years was evaluated. Anonymized residual serum samples were collected from individuals attending selected hospitals and Public Laboratories in 2003 and 2005. About 100 samples were collected from each of age groups: 1–4; 5–9; 10–14; 15–19; 20–24; 15–29; 30–34; 35–39; 40–44; 45+. Serum samples were assayed for hepatitis A-specific IgG using commercially available enzyme-linked immunosorbent assay kit ETI-AB-HAVK PLUS DiaSorin S.p.A, Italy. Samples with titter below 20 mU/ml were regarded as negative.
Results: In presented study the proportion negative samples varied with age. Children at age 1–4 years of life were protected against hepatitis A only in 9.7%, adolescents in about 20%, young adults at age 25–29 years in 33.3% and adults over 45 years were hepatitis A negative below 32%.
Conclusion: A decline in the incidence of hepatitis A in Poland has led to a progressive decrease in circulation of HAV and, as a result, an increase in the proportion of the adult population susceptible to infection. In Poland till now the vaccination against HAV is recommended for children and adolescents and people dealing with food distribution, as well as for travellers to the endemic region of the world or during natural disasters such as for example floods. In 2004 only 31258 persons were vaccinated. Immunisation at a very early age has been proposed as an important mechanism for protecting against HAV infection in adults and adolescent, by interrupting transmission and ultimately eradicating HAV.

P784

Changing epidemiology of acute hepatitis A and hepatitis B in Italy: Further counselling and prophylactic measures are needed for male homo-bisexual subjects?

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Introduction: Small outbreaks and an increased frequency of both hepatitis A and B were recently observed worldwide, with attention on homosexual men.
Methods: An observational survey of all hospitalizations due to acute hepatitis A and B occurred in the Bologna metropolitan area, was performed from 1999 to June, 2004.
Results: One hundred and eighty seven consecutive patients (p) were hospitalized in the examined period. From October 2002,acute hepatitis A largely prevailed over acute HBV, HCV, and HEV hepatitis, and since June 2004 also acute hepatitis B increased its frequency and the last year was characterized by a similar prevalence of the two occurrences. Adult female p and children represented only 27 cumulative p of 187 (14.4%). Among the 126 and 61 p with acute hepatitis A and B respectively, even 108 and 52 (85.6%) were represented by male adults aged 22–49 years, who recognised unprotected

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homo-bisexuals contacts in the 2–4 months before the onset of hepatitis A or B in 119 cases (74.4%). Nobody reported contacts with p with a recently diagnosed hepatitis A or B, and nobody underwent prior anti-HAV/anti-HBV vaccination. Among the 160 adult males with acute HAV or HBV infection, potential STD were found in 45 cases ($p < 0.01$) and included hepatitis C in 21 p, syphilis in 16, and HIV in 14 p. The temporal trend of male adults admitted for hepatitis A showed a significant increase from 1999 to the first 6 months of 2004:a ~300% increase versus 1999 leading to a crude rate of 7.7 per 100.000 residents/y. A more reduced tendency of a frequency increase of acute hepatitis B was also recognized in the same p population (four cases only in 1999, versus 19 cases in the last 18 months of observation; $p < 0.04$).
Conclusions: Despite the availability of anti-HAV and anti-HBV vaccination and information campaigns directed against the spread of STD and HIV, the continued epidemic of hepatitis A and B recognize an increased prevalence of homo-bisexual transmission. Our experience shows a strict link between novel diagnoses of acute HAV and HBV infection and homo-bisexual behaviour, which showed a significant increase of the absolute number of detected cases, especially since the year 2002. A careful epidemiological monitoring, specifically targeted educational campaigns and public health measures (such as an recommendation of active immunoprophylaxis for p at risk) may help contain the outbreak of hepatitis A and B among homo-bisexual men and concurrently reduce the spread of other potential STD.

P785

Intrafamilial transmission of hepatitis E in France

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Introduction: Hepatitis E virus (HEV) infection is an important cause of epidemic and acute sporadic hepatitis in developing countries. HEV is transmitted principally via the faecal-oral route. In some industrialized countries, autochthonous cases have emerged in patients who had never travelled to endemic areas. We report an autochthonous case of acute HEV infection with person-to-person transmission in the same family.
Methods: A 41-year-old man with clinical symptoms of acute hepatitis and increased bilirubin and aminotransferase levels was admitted in our hospital. Anti-hepatitis A, B, C virus antibodies (Ab) (HAV, HBV, HCV) and anti-herpes viruses Ab (CMV, EBV) were negative. Diagnosis of HEV was considered. Samples from the patient and all the household contacts were tested for the presence of serum IgG and IgM anti-HEV (Ab) and HEV RNA.
Results: Anti-HEV IgG and IgM Ab were found in the patient (index case) 14 days after the onset of hepatitis. The presence of serum HEV RNA was detected only 4 months later. In his wife (case contact 1), who presented with asthenia and moderate cytolysis, HEV RNA was detected in sequential samples, before the detection of anti-HEV Ab. Anti-HEV Ab and HEV RNA were also found positive in the second contact case (5 years old child), who had fever and asthenia. The second child was anti-HEV Ab and HEV RNA negative. Clinical interview indicated that the index case and his wife had eaten shellfish and that all the family have had a swim in the Saint-Ferreol lake located in the south west part of France.
Conclusion: Diagnosis of HEV infection have to be considered in case of acute hepatitis when markers of acute HAV, HBV, and HCV infection are negative, even if patients have not recently travelled in endemic areas. In our study, in the first contact case, hepatitis occurred within two weeks after the index case and may not be due to intrafamilial transmission, but to a simultaneous faecal-oral contamination via shellfish consumption or lake swim. In the second case (child), the detection of HEV RNA occurred after 6 weeks from the onset of disease in the index case, suggesting that the infection had been contracted by household contact. This case report and other published data may indicate that HEV is emerging in the south part of France.

P786

Molecular detection and sequence analysis of hepatitis E viruses in humans and animals in Hungary

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Objectives: Hepatitis E virus (HEV) one of the most common cause of hepatitis in endemic areas. However, recently demonstrated that human infection may occur in developed countries without any travel history and that animals may act as a reservoir. The objective of this study was to identify hepatitis E virus both in humans with hepatitis and animals by molecular methods and to determine the viral genetic relationship to each other and to the prototype HEV strains in Hungary.
Methods: Laboratory diagnosis of hepatitis E virus infection was performed in human serum by HEV IgM ELISA and IgM/IgG immunoblots. In addition, HEV-positive human samples were selected and tested together with animal faecal and liver samples by reverse transcription-polymerase chain reaction (RT-PCR) using primers for partial viral capsid region followed by sequence- and phylogenetic analysis.
Results: Twenty-seven patients with acute hepatitis were positive by HEV IgM ELISA and immunoblots treated in Hospital of Szeged, Hungary between year 2001 and 2004. Viral genome was successfully amplified in three human sera and 39 samples from animals (swine, deer and boar) by RT-PCR and confirmed by sequencing in two human and eight animal samples, respectively. All sequences belong to genotype 3 HEV strains. Genetically identical human strains showed 95% nucleotide identity to swine HEV strains (HEV-072swine/HUN and 354/1/02/UK) and having 90% nucleic acid identity to the closest human strain Greece2 (AF110392). In one human case, consumption of home-made slaughtered pork sausage was a potential source of infection.
Conclusions: Hepatitis E virus is present in Hungary. Only genotype 3 strains have been found in both animals and humans, which support the possibility of the endemic infections in developed countries including Hungary and that swine may act as a reservoir of human HEV.

P787

TT virus prevalence and distribution of genotypes and genogroups in Korea

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Objectives: To determine prevalence of TT virus (TTV) and distribution of genotypes and genogroups of TTV among healthy adults and hepatitis B virus (HBV) or hepatitis C virus (HCV) infected individuals in Korea.
Methods: Prevalence of TTV was investigated in serum samples of 69 healthy adults, 59 HBV infected individuals and 34 HCV infected individuals by heminested PCR assays using primers from ORF-1 and UTR regions of viral genome. ORF-1 PCR products were genotyped by sequence analysis. TTV

genogroups were investigated by five different genogroup-specific PCR assays.
Results: TTV DNA was detected in 53/69 (77%) healthy adults, 47/59 (80%) HBV infected individuals and 27/34 (79%) HCV infected individuals. Among the 20 sequenced isolates, 9 (45%) were genotype 2, 8 (40%) were genotype 1, 2 (10%) were genotype 3, and 1 (5%) was genotype 4. TTV genogroup 4 was found most frequently (52/128), followed by genogroup 3 (42/128), genogroup 1 (35/128), genogroup 5 (32/128), and genogroup 2 (1/128). Mixed infections with different genogroups were frequent.
Conclusion: Prevalence of TTV is high in Korea. There was no signigicant difference in TTV prevalence between healthy adults and HBV or HCV infected individuals. TTV genogroup 4 and genogroup 3 are predominant genogroups.

P788

Presence and significance of TT virus infection in voluntary blood donors and patients on maintenance haemodialysis in Tabriz, Iran

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Objectives: Transfusion Transmitted virus (TTV) is a novel DNA virus that is associated with post-transfusion hepatitis. We investigated the prevalence of TTV and its role on liver disease in voluntary blood donors and patients on maintenance haemodialysis.
Methods: This cross-sectional study was conducted in March and April 2005. We tested 407 voluntary blood donors and 324 chronic haemodialysis (HD) patients in the city of Tabriz, northwestern part of Iran, for the presence of TTV. Demographic and clinical data were registered. TTV DNA was detected using semi-nested polymerase chain reaction (PCR). Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also measured. In addition, hepatitis B surface antigen (HbsAg), anti-hepatitis C virus (HCV), hepatitis E virus antibody, and anti-HIV were previously determined in all patients.
Results: The positive rate of TTV was 9.3%(95% CI: 6.1–12.5%) in 324 patients on maintenance haemodialysis and 2.7% (95% CI: 1.1–4.3%) in 407 cases of voluntary blood donors ($P < 0.001$). The prevalence rate of HBV, HCV and HEV infection were 4.6%, 20.4%, and 74%, respectively among HD patients. In voluntary blood donors the prevalence rate of HBV, HCV and HEV infection were 1.2%, 0.5% and 7.6%, respectively. All patients were negative for anti-HIV antibody. No relations were found between TTV infection and elevated levels of ALT or AST. Clinical background including sex, mean duration of HD, history of transplantation, history of blood transfusion, and positive markers for either hepatitis B virus (HBV), hepatitis C virus (HCV) or hepatitis E virus (HEV) did not differ between TTV DNA positive and negative HD patients in both groups. In HD group, the presence of TTV DNA was found to be related to the age of the patients. TTV-positive patients were significantly younger than TTV-negative patients ($P < 0.018$).
Conclusions: TTV infection was more prevalent in Iranian patients on regular HD than voluntary blood donors. No clinical significance of TTV virus could be elicited in both groups; neither it showed any clinical impact as a co-infection with other blood borne infections. Whether blood transfusions increase the risk of TTV infection was not revealed by this study. Our data did not show that when TTV alone is present it induces liver function tests alteration. However, in TTV-positive patients, long-term follow-up is necessary in order to clarify the effects of TTV on liver function.

P789

The prevalence and phylogeny of GBV-C/HGV in eastern Taiwanese indigenes

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Objectives: GB virus C (GBV-C)/hepatitis G virus (HGV) has been detected in eastern Taiwanese indigenes. However, only the prevalence of GBV-C/HGV viral RNA was reported without any information about genotyping and their phylogenetic relationship. We therefore investigated the prevalence, genotypes, and phylogeny of GBV-C/HGV in eastern Taiwanese indigenes to complete their molecular epidemiological data.
Methods: Serum or plasma samples from 140 eastern Taiwanese indigenes were included in this study. Viral RNA was extracted from the 140 samples and cDNA was synthesized with random hexamer primers. A nested PCR was performed in the 5′NCR to part of the E1 gene. PCR products were purified and subjected to direct sequencing. Phylogeny re-construction was performed using the Phylip software package, with the neighbour-jointing method, the Fitch and Wagner parsimony method, and the maximum likelihood method. The robustness of the NJ and pars trees was statistically evaluated by bootstrap analysis with 1000 bootstrap samples.
Results: Among the 140 samples, five were found to be GBV-C/HGV RNA positive (3.5%). A total of 113 GBV-C/HGV sequences from all over the world were included in the phylogenetic analysis. Five major GBV-C/HGV genotypes were identified. Two of the five isolates belonged to the genotype 3 (Asian type) cluster, whereas three were surprisingly clustered within the genotype 1 (Africa type) groups. The tree topology was supported statistically significant by all methods used. A very significant effect (20–5% variance of bootstrap values) of different times of bootstrap replications (100 and 1000 times) was also observed.
Conclusion: About 3.5% prevalence of GBV-C/HGV viral RNA in eastern Taiwanese indigenes was confirmed. Both GBV-C/HGV genotype 1 and 3 were found in this population. The possible sources of the infection with the African type virus remained unclear. A sufficient number of replications needed for accurate estimation of the bootstrap value should be taken into account for phylogenetic studies.

P790

Three years lamivudine therapy in adults with HBeAg-positive chronic hepatitis B

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Introduction: The aim of the study was to assess a three-year administration of lamivudine in adult patients with HBeAg-positive chronic hepatitis B.
Methods: Chronic hepatitis B patients that not responded to interferon-α therapy were included into this study. Lamivudine monotherapy was administrated 100 mg daily for 3 years. In the course of the treatment, every four-week period, ALT activity, haemoglobin levels, leukocyte and thrombocyte counts were determined. Furthermore, in every 12-week periods, prothrombin index and protein fraction were analysed. Serologic markers of hepatitis B virus (HBV) infection and level of HBV-DNA were measured before and after treatment, every 6 months during the treatment and 6 months after the end of treatment. A liver biopsy was performed before the therapy in suitable of the studied patients. (Histological evaluation according to Knodell)

In the assessment of the effectiveness of the Lamivudine therapy was considered according to loss of HBV-DNA in serum and seroconversion in HBeAg/anti-HBe system as well as normalization ALT and AST activity.
Results: Twenty-six patients (22 men, 4 women) aged 30.8 ± 7.5 years old were included into the study. After a twelve-month treatment period, HBV replication and normalization of ALT and AST activity were observed in 15 patients (57.7%). The response of the therapy sustained in 13 (50%) patients at the end of second year. The end of 36 months, biochemical and virological response persisted in nine patients (34.6%). HBeAg/AntiHBe seroconversion was developed in five patients. After sixth month of the end of therapy, permanent response was continued in eight patients (22.6%). Side effects of treatment were not observed in any of the studied patients.
Conclusion: The sustained virological response after 3 years Lamivudine treatment was found 22.6% in chronic HBV infections.

Paediatric infectious diseases

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Antimicrobial susceptibility of *Haemophilus influenzae* ocular isolates collected from outpatients in paediatric hospital

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Introduction: *Haemophilus influenzae* (HI) is responsible for a number of human diseases, ranging from chronic respiratory infection to meningitis, which affect children. Eight biotypes and six serotypes of HI have been identified. Biotyping and serotyping have been used to investigate patterns of colonization of HI, as well as to identify stains of the bacterium that appear to be associated with more severe infections.
Objectives: To determine which subtypes and biotypes of HI are most commonly associated with ocular disease and to monitor the patterns of antimicrobial sensitivity in children with eye infections.
Material and methods: Ocular swabs were collected from the examined population, aged 1 month to 14 years old, who visited our children hospital during the period 2002–2005. Identification and biotyping isolates were performed by classical microbiological methods and by API NH strips (bioMerieux). Capsular subtypes a–f were determined by slide agglutination using commercially available subtype specific antiserum. β-Lactamase production was determined by the chromogenic cephalosporin test with nitrocefin as substrate.
Results: A total of 151 HI isolates were recovered from 755 ocular samples submitted to our microbiological department. The majority of the isolates were serologically non typable. The prevalent biotype of HI isolates was biotype II 48.3% (73/151) followed by biotype III accounted 22.5% (34/151). The remaining biotypes were biotype I 12% (18/151), biotype IV 6.6% (10/151), biotype V 4.6% (7/151) and from each one isolate 2% for biotypes VI–VIII. A significant resistance to cotrimoxazole (25%) and in lower rate to clarithromycin (8%) was observed. All the isolates resistant to ampicillin (8%) were β-lactamase producers and susceptible to cefuroxime, cefotaxime, ciprofloxacin and chloramphenicol.
Conclusions: This study showed that biotypes II and III are the predominant biotypes of HI found in ocular infection. There is a low prevalence of β-lactamase production and resistance of macrolides while all isolates were sensitive to chloramphenicol. Surveillance is necessary to monitor rates of resistance in the community in order to tailor empiric therapeutic recommentators.

P792

Bacterial pathogens isolated from children with bloodstream infection

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During the last two decades, the incidence of the pathogens responsible for Blood Stream Infections (BSI) in children has changed.
Objectives: To determine the frequency of pathogens isolated from children with bacteraemia and to investigate their susceptibility patterns.
Material and Methods: Blood cultures were performed using the Bac T Alert 30 automated system. Vitek 2 automated system (bioMerieux, France) was used for identification and susceptibility testing of pathogens.
Results: A total of 12 301 blood cultures were screened for aerobic, anaerobic and fungi during a five-year period (2001–2005). Bacteraemia occurred in 350 cases (2.8%) of hospitalized children, males: 53%, females: 47%, with a median of 5 years of age. Out of 350 isolated stains, 266 were Gram- positive (76%) and 81 were Gram- negative (24%). Among Gram positive, the most prevalent was Coagulase-Negative *Staphylococci* 70.6% followed by *Streptococcus pneumoniae* 10.9%, *Streptococcus aureus* 8.6%, *Streptococci* 6.0%, Enteroccoci 2.2%. The most frequent of Streptococci were *Streptococcus pyogenes* and *Streptococcus agalactiae*. From the Gram negative the most prevalent were *Escherichia coli* 20.9% followed by *Streptococcus maltophilia* 16% and *Salmonella spp*.12.3%. Other *Enterobacteriaceae*, *Neisseria meningitidis*, H. influenzae and anaerobic bacteria were found in lower rates. *S. pneumoniae*, *E. coli* and *Salmonella spp*. were usually causative agents of BSI in pediatric clinics whereas *Stenotrophomonas*, *Pseudomonas* and *Enterococci* were isolated from Intensive Care Unit (ICU). All Gram-positive isolates were susceptible to glycopeptides. Most of CoNS were resistant to Oxacillin 68%, Clindamycin 40.4%, fluoroquinolones 12.2% and Gentamicin 27.1%. A significant degree of resistance to Penicillin (24.1%) and to macrolides (31%) was reported for *S. pneumoniae*. The majority of *S. aureus* were susceptible to aminoglycosides, fluoroquinolones, and resistant to Oxacillin by 26%. *E. coli* strains produced AmpC-β-lactamases in 29.4% and a low prevalence of ESBL-producing strains was recorded. *Acinetobacter baumannii* and *Stenotrophomonas* strains from ICU, were multiresistant.
Conclusions: 1. CoNS were the leading cause of BSI, followed by *S. pneumoniae*, *S. aureus* and *E. coli*. 2. These four were responsible for 71.5% of all BSI. 3. Significant difference in the microbiology of blood cultures between the paediatric clinics and ICU was noted.

P793

Characterisation of *Haemophilus influenzae* isolates from conjunctivitis (Portugal, 2001–2005)

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Objective: Acute conjunctivitis, caused by nontypable (NT) *Haemophilus influenzae* (HI) strains, is the most common eye disorder, especially in young children. The aim of this work was the phenotypic characterization of HI isolates and the relation with strains previously isolated (1997–2000).
Methods: From January 2001 to June 2005 the Antibiotic Resistance Unit at the National Institute of Health, in Lisbon, received 222 HI strains isolated from conjunctivitis, in 11 collaborating Hospital Laboratories. Most of strains were isolated from children (92%) and from community acquired infections (74%). beta-lactamase production was determined by a nitrocefin assay; ApiNH (BioMérieux) was used to determine the biotype; serotyping was performed by PCR, using primers specific to capsule (a–f); minimum inhibitory concentrations (MIC) of 14 antibiotics were determined by a microdilution assay (Dade Behring).
Results: 12.5% of strains were beta-lactamase producers (all had MIC of ≥4 mg/L to ampicillin); 5.3% of beta-lactamase negative strains had MICs of 2 or 4 mg/L to that beta-lactam and were considered ampicillin resistant non beta-lactamase producers (ARNBLP). Resistance to other antibiotics (considering both resistance and intermediate strains), was as follows: 32% to SXT; 4.6% to cefaclor; 1.4% to rifampicin; and 1% to tetracycline. Biotypes I (23.6%), II (36.4%), III (23.6%), and IV (14%) included most of the strains (98%). As expected, most strains were NT (99%), although two strains were of serotype e, one f and other d. Strains of serotype e are often associated to biotype IV as also found in our study.
Conclusions: In this study we verified an increase of beta-lactamase producing strains, isolated from conjunctivitis (8.5–12.5%), comparing with 64 strains isolated between 1997 and 2000. ARNBLP presented only a slight increase (4.7–5.3%), although we expect an increment of this mechanism of resistance in the future, due to changes on therapy. As ARNBLP strains are difficult to detect with the breakpoints actually proposed by NCCLS (2004), we suggest their revision. Advanced molecular studies would improve ARNBLP detection. HI isolated from conjunctivitis is of high concern since this disease affects very young children and beta-lactam resistance mechanisms are changing. Serotype alterations, due to Hib vaccine should also be monitored.

P794

Antimicrobial susceptibility and serotyping of pneumococci in a Tunisian paediatric hospital

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Streptococcus pneumoniae is one major causative agent of severe infectious diseases. More than 90 pneumococcal serotypes are known, although the majority of invasive and non invasive disease is associated with a much smaller number of serotypes.
Objectives: Antibiotic resistance prevalence and serotype distribution of invasive pneumococci isolates in Tunisia.
Methods: We have studied 131 non-repetitive invasive pneumococcal strains isolated in our laboratory between January 1998 and August 2005. Isolates were identified as *S. pneumoniae* by optochin sensitivity and bile solubility tests. Antimicrobial susceptibility was performed by the disk diffusion method using Mueller–Hinton agar supplemented with 5% defibrinated sheep blood as determined by CA-SFM guidelines. Penicillin susceptibility was determined by oxacillin 5 mcg disk screening test. MICs of penicillin G, amoxicillin and cefotaxime were determined by E-test (AB BIODISK). *S. pneumoniae* ATCC 49619 was used as a control strain. Serotype determination was performed by rapid latex agglutination (Pneumotest Latex) and the capsular reaction test using antisera from Staten Serum Institute, Copenhagen.
Results: Prevalence of penicillin non susceptible pneumococci (PNSP) isolates was 49.6%, the majority of them (43.7%) had a low level of resistance. Amoxicillin resistance concerned 10.8% of isolates with a high level of resistance in 0.8%. All cefotaxime resistant isolates (5%) had a low level of resistance (MIC < 2 mcg/ml). The most prevalent serotypes were 14 (30.5%), 4 (12.5%) and 23F (7.6%). Among PNSP isolates, 35.7% belongs to serotype 14 and 19% to serogroupe 23. The coverage of serotypes included in the conjugate heptavalent vaccine is 62%.
Conclusion: Prevalence of PNSP is high in pneumococci invasive isolates. The most prevalent serotype is 14.

P795

Pneumococcal infection in a children's hospital in 2004, Taoyuan, Taiwan

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Streptococcus pneumoniae is one of the common pathogens related in bacteria infection in children. To study the clinical spectrums, microbiologic characteristics and serotype distribution of pneumococcal infection, 77 *S. pneumoniae* isolates from 77 patients (38 were male) were identified in Chang Gung Children's Hospital in 2004.The isolates were obtained from blood (14), pleural fluid (4), nasal aspirates (12), sputum (25), conjunctival discharge (4), pus from ears (13), parotid gland (1), synovial fluids (2), orbital abscess (1) and scald burn wound (1). The disease spectrum of these patients included bacteremia (9), pneumonia (25), complicated pneumonia (6), suppurative otitis media (14), sinusitis (11), septic shock (2), septic arthritis (2), conjunctivitis (4), suppurative parotidis (1), scald burn wound infection (1), preseptal cellulitis (1), orbital cellulitis (1). The mean age was 3.55 years (range: 4 months to 16.7 years). 31 (40%) patients were younger than 2 years old. Two cases resulted in mortality due to septic shock with the same serotype 23F and both were younger than 2 years old. Patients with complicated pneumonia had the longest mean hospital stay: 17 days. Among 77 isolates, 68 (88%) were not susceptible to penicillin (58% were intermediately susceptible, and 30% were resistant, intermediate: MIC: 0.1–2, resistant: MIC ≥ 4). One strain had MIC 8 μg/mL. No strain was resistant to vancomycin. Most non-susceptible pneumococci were serogroups 23F (29%), 6 (22%), 14 and 19F (19%). The predominant serotypes were 23F (27%), 14 and 19F (18%). Serotypes 19A and 23B were only isolated from sputum. Serotype 14 accounted for 23% of pneumonia cases. Among patients with serotype 14 isolates, 64% were pneumonia cases. All isolates were belonged to or closely related to serotypes covered by the 23-valent vaccine and 7-valent pneumococcal conjugated vaccine.

P796

Repeated isolation of a pneumococcus from a child suffering from IRAK-4 deficiency

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Objectives: The IRAK-4 deficiency is a primary immunodeficiency, mainly associated with pyogenic bacterial infections like those caused by *Streptococcus pneumoniae*. The patients express a

poor inflammatory response and fail to sustain antibody responses. In this case report we describe the repeated isolation of the same invasive pneumococcal strain from an abscess and the blood of a 5-year-old child with this disease, after a 2-year interval.

Methods: The species identity of the strains was confirmed by the optochin sensitivity and by the presence of the *lyt*A gene. Antibiotic susceptibility testing (MIC) was performed by the agar dilution method to 10 drugs. Serotyping was done with the agglutination antisera produced by Mast Diagnostica. PFGE was performed by digestion with ApaI enzyme for 6 h at 37 °C, and the fragments separated using 2s and 30s pulse times, for 22 h at 14 °C. The presence of four macrolide resistance determinants: erm(B), mef(E/A), erm(A) and erm(TR) genes was tested by PCR.

Results: The patient suffered from relapsing joint infections already at early age. The first invasive penumococcal infection (septic hip-joint inflammation) occurred when he was 3 years old, and it was cured with parenteral cefotaxime and non-steroid therapy. A specific anti-polysaccharide antibody deficiency was observed. The second invasive episode occurred 2 years later, when purulent meningitis followed the joint symptoms, and was cured successfully again with parenteral cefotaxime and supportive treatment. The IRAK-4 deficiency was shown at this stage and the patient received intravenous immunoglobulin substitution. The two invasive pneumococcal strains proved to be identical, by both genotyping and serotyping methods and by their antibiotic sensitivity patterns. Both strains carried the erm(B) macrolide resistance gene.

Conclusion: Repeated pneumococcal infections associated with IRAK-4 deficiency have been described before in the USA in 1998. However, to our knowledge, this is the first time that the isolation of the same pneumococcal strain in a patient with IRAK-4 deficiency was proven, and also confirmed by genotyping methods. In the case of pneumococci, serotyping and other phenotypic investigations are not sufficient for the determination of identical strains.

P797

Nasopharyngeal colonisation by *Streptococcus pneumoniae* in children: Impact of amoxicillin treatment

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Introduction: Nasopharynx of children is the main reservoir of *Streptococcus pneumoniae* (SP). The percentage of nasopharyngeal carriers of SP in <2 years-old children ranges from 26% to 62%. The aim of our study was to assess the effect of amoxicillin exposure on the nasopharyngeal colonization (NPC) and on the antibiotic susceptibility of SP in <5-year-old children.

Material and methods: From Dec 2001 to Feb 2004, <5-year-old children with respiratory symptoms and fever seen in the Emergency Department of our hospital and who were prescribed amoxicillin (40–90 mg/kg) were eligible. Three nasopharyngeal swabs were taken: at the time of the initial visit (IV), a second sample within 60 hours after amoxicillin discontinuation (end of treatment visit, ETV), and the third, 4 weeks later (follow-up visit, FUV). SP colonization, serotype distribution and antimicrobial resistance were evaluated over time.

Results: 134 children were included. In the IV 67/134 (50%) were colonized. SP was found in the nasopharynx of 10/17 (58.5%), 15/35 (42.9%) and 42/82 (51%) of <1, 1–2 and >2 years-old children, respectively. Vaccine Serotypes (VS) were identified in 80%, 40% and 55% of <1 year-old, 1–2 years-old and > 2 years-old children, respectively. The proportion of non-susceptible Penicillin SP (NPSP) was 60% in <1 year-old children, 43% in 1–2 years-old children and 40% in > 2 years-old children. 49 out of 134 (36.5%) children completed the three study visits and were the assessable group. 51%, 22.4% and 46.9% were colonized at IV, ETV and FUV, respectively. The percentage of resistant SP was 28%, 45.5% and 8.7% ($p < 0.05$) for penicillin, and 40%, 63.3% and 47.8% (NS) for erythromycin at IV, ETV and FUV, respectively.

Conclusions: In children <1 year of age a higher proportion SP colonization, presence of vaccine serotypes and non-susceptible penicillin SP was found. NPC dropped to a half in ETV and returned to baseline levels 1 month after amoxicillin discontinuation; however non-susceptible penicillin isolates decreased a 68% in FUV with respect to IV due to recolonization by ''de novo'' penicillin susceptible strains.

P798

Prevention of perinatal group B streptococcal infections: evaluation of a new chromogenic medium STREPTO B ID

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Objective: The aim of this prospective study was to compare a method using the new chromogenic agar STREPTO B ID (bioMérieux SA, France), Granada agar (Biomedics) and Columbia blood agar with colistin-nalidixic acid (BA-CNA) (bioMérieux), to detect vaginal colonization by group B streptococci (GBS) in pregnant women.

Method: Specimens for GBS screening received over a 3 month period from pregnant women (683 vaginal swabs and 41 urine), 13 newborn gastric fluid at two hospitals were included and tested during this study. These specimen were cultured in parallel onto the three media by direct inoculation and after enrichment in Todd-Hewitt + antibiotics broth. Each medium were observed after 24 and 48 h incubation.

Results: Overall GBS was detected in 255 cases (35%) on at least one medium after enrichment. Before and after enrichment, the sensitivity was 45.8% and 93.3% for STREPTO B ID, 39.6% and 82.9% for Granada, 45.4% and 92.5% for BA-CNA respectively at 24 h. Prolonged incubation (48 h) enabled to increase the sensitivity: before and after enrichment, the sensitivity was 55.8% and 99.2% for STREPTO B ID, 52.5% and 92.9% for Granada, 52.9% and 96.7% for BA-CNA. Regarding the specificity, the Granada medium method gave 99.8% versus 97.3% for the STREPTO B ID after enrichment and 48 h incubation.

Conclusion: These findings show that the STREPTO B ID medium, the first chromogenic medium to detect GBS, is more sensitive than the Granada medium. However, the Granada medium method is more specific after enrichment and 48 h incubation. The enrichment phase increases significantly the sensitivity of GBS detection especially with STREPTO B ID. This new medium is easy to use due to the incubation under aerobic conditions.

P799

Invasive group a streptococcal infections and incidence of resistance in children's hospital

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Objectives: *Streptococcus* group A (GAS) has been mentioned to cause severe invasive diseases during the last years. Knowledge of resistance towards the most usually used antibiotics is very important, since fast and effective treatment is needed to reduce morbidity and mortality.

Materials-methods: Between 1 January 2001 and 31 October 2005, a total of 51 invasive strains of *Streptococcus pyogenes* (deep tissue infections, blood, synovial, cerebrospinal fluid) were collected from children aged 0–15 years old. All isolates were confirmed as *S. pyogenes* by Lancefield grouping using the Pastorex STREP rapid agglutination kit. Susceptibility testing to antibiotics was performed by disk diffusion method and MIC of penicillin was determined by E-test.
Results: All strains of *S. pyogenes* were penicillin sensitive. Seven isolates (13, 72%) were erythromycin resistant, the main mechanism of which was of M-phenotype. Three isolates (5.88%) were clindamycin resistant. If we compare the results obtained in 2001–2005, we observe a statistically significant increase in erythromycin resistance of *S. pyogenes*. During 2001–2003 there were no clindamycin or erythromycin resistant strains, while during 2004–2005 there were isolated the resistant strains we report.
Conclusions: The prevalence of macrolide resistance among *S. pyogenes* is not uncommon (13, 72%). Efforts should focus on development of guidelines, but also on appropriate use of antibiotics. Penicillin G is still considered to be drug of choice for *S. pyogenes* infections.

P800

Community-acquired methicillin-resistant *Staphylococcus aureus* in Greek children

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Objectives: MRSA has been a major nosocomial pathogen causing severe morbidity and mortality. More recently, serious community-acquired MRSA (CA-MRSA) infections have been reported worldwide. We aimed to define the frequency of MRSA in paediatric patients of a tertiary care hospital and estimate the proportions and drug susceptibilities of MRSA and CA-MRSA in our region.
Methods: All cases of paediatric patients from whom *Staphylococcus aureus* was isolated between Jan 2002 and Dec 2004 were reviewed and compared with the results of a previous study (Jan 1999 to Dec 2001; ECCMID 2003, abstr 1256). CA-*S. aureus* and CA-MRSA were defined as isolates from cultures taken ≤ 2 days after hospital admission from patients not hospitalized during previous 3 months. Fisher's exact test was used for the analysis.
Results: *S. aureus* was isolated from 168 children, and MRSA from 28 (16.7%) as compared to 36.5% in previous study, $p < 0.001$. The frequencies of MRSA and CA-MRSA are shown in Table. Comparing the two periods, the peak frequency of MRSA (42.9%) was in 2000, whilst the lowest frequency (16.9%) in 2004, $p = 0.003$. The highest frequency of CA-MRSA (53.8%) was in 2003, whilst the lowest (33.5%) in 1999, p=ns. From CA-MRSA isolates, 89.3% were susceptible to clindamycin, 82.1% to erythromycin, 100% to co-trimoxazole and 53.8% to tobramycin. One CA-MRSA isolate was resistant to erythromycin and susceptible to clindamycin. The children with MRSA were ranged within 1 d to 17 yr. In general, MRSA tended to be isolated from boys and infants younger than 45 d more frequently than girls and older children, respectively.

Year	*S. aureus*	MRSA	CA-*S. aureus*	CA-MRSA
2002	44	4/44 (9.1%)	39	1/39 (2.6%)
2003	59	13/59 (22%)	44	6/44 (13.6%)
2004	65	11/65 (16.9%)	52	7/52 (13.5%)
Total	168	28/168 (16.7%)	135/168 (80.4%)	14/135 (10.4%)

Conclusion: (1) Approximately 80% of *S. aureus* isolated from pediatric patients in our hospital are community-acquired, and half MRSA's isolated are CA-MRSA's. (2) Compared to early period, frequency of MRSA tends to decrease unlike CA-MRSA, which tends to increase. (3) CA-MRSA isolates are susceptible to alternative antibiotics. (4) Control of MRSA requires continuous surveillance and appropriate use of antibiotics not only in the hospital but also in the community.

P801

Seroprevalence of serogroup specific antibodies against different serogroups of *Neisseria meningitidis* (Serogroup A, C, W 135 and Y) in healthy individuals in different age groups

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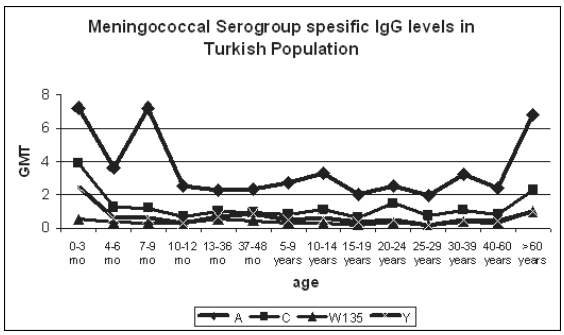
Objective: Meningococcal disease is still a major global health problem causing approximately 1.2 million cases per year and an estimated 135,000 deaths. The predominant serogroups may be different in each region and they can change by the time in the same area. There is no meningococcal vaccine introduced into the routine schedule in Turkey yet. Since the distribution of serogroups is important to determine the antigens that will be included in the vaccine, we aimed to detect the levels of serogroup spesific antibodies from birth to the age after 60 in Turkey.
Materials and methods: From different regions representing the West, East and the North side of the country, a total of 350 serum samples were included in the study. Fourteen different age groups and 25 samples in age group were analysed. Meningococcal serospesific IgG levels were identified by using tetra-plex ELISA in Bioplex Array System, Bio-Rad®.
Results: The pattern of the IgG levels against serogroup A, C, W135 and Y from birth to the age after 60 was dfifferent from each other. Over all age groups, the geometric mean titer for serogroup A was 3.21 μg/ml, serogroup C was 1.22 μg/ml, serogroup W was 0.34 μg/ml, serogroup Y was 0.55 μg/ml. In all serogroups a decline in the IgG levels were detected between the age 6 and 12 months. For serogroup A and C, there was a inreasing trend after the age of 10–14 years. A similar increase was seen in the period of between the ages of 3 and 5 for serogroup W135 and Y.



Conclusions: To determine which serogroups are causing most of the meningococcal infections is important because the disease due to some serogroups can be prevented by active immunization and there are different vaccines including different serogroup antigens. In Turkish population, after the lost of maternal antibodies between the age of 6 and 12 months, the level of serogroup spesific IgG antibodies increases after 10–14 years against serogroup A and C and during 3–5 years of age against serogroup W135 and Y. This pattern of antibody distribution suggests that serogroups A and C were the predominant meningococci 0–14 years ago, however serogroups W135 and Y are much more frequent in early childhood meningococcal infectios currently. This data is parallel to the results of previous and current surveillance studies and shows the importance of age and time spesific seroprevelance investigations.

P802

Increasing nasopharyngeal carriage rate of *Neisseria meningitidis* serogroup W135 in healthy Turkish primary school students after 2000 Hajj epidemic

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Objective: The usual source of meningococcal infections are the asymptomatic human nasopharngeal carriers. Colonising and infecting serogroups differ from country to country and this information is important because the current and developing vaccines are including only the predominant serotypes in that area. Thus our study aimed to determine meningococcal carriage rates among the primary school children and identify the predominant serogroups.
Materials and methods: Throat swabs were cultured from 1500 healthy children randomly selected from primary schools in Ankara, Turkey and recovered *N. meningitidis* were serogrouped by using conventional polymerase chian reaction (PCR). Oligonucleotides in siaD gene (for serogroups B, C, Y and W135) and in orf-2 gene (for serogroup A) were targeted in PCR.
Results: Nasopharyngeal swabs were taken from 1500 pupils attending to schools in the study and the children were aged 6–14. The carriage rate of *N. meningitidis* rate in our region was found to be 7.4% (111 of the 1500 samples). Serogroup W135 was identified in 75 (67.5%), serogroup B in 12 (10.8%), serogroup Y in 4 (3.68%) and serogroup C in 1 (0.9%) of the cultures. Nineteen isolates of *N. meningitidis* could not be serotyped by PCR.
Conclusion: Serogroup C is the most common *N. meningitidis* in the developed countries and vaccination policies are being tailored to prevent the infections caused mainly by this serotype. Since asymptomatic carriers are presumably the major source of pathogenic strains and one of the goals of the vaccination against *N. meningitidis* is to prevent the carrier state the regional rates of the *N. meningitidis* serogroups carried in the nasopharynx is important to know for selecting the vaccine content. Although the dominant meningococcal serogroups were serogroup B and C in carriers and patients with invasive infections up to the recent years, the rate of serogroup W135 infections are increasing after the year of 2001. Our study showed that this serogroup has been intensely colonized in healthy Turkish children. The source of this colonization seems to be the pilgrims in 2000 when international outbreak caused by a previously rare serogroup W135, because approximately 150,000 Turkish pilgrims travel annually to Saudi Arabia for Hajj. Although the vaccine can not protect the persons from becoming asymptomatic carriers, a vaccine including W135 meningococcal antigen has to be used for the vaccination of children to prevent the invasive disease.

P803

Evaluation of IgA secretory antibodies in children with *Helicobacter pylori* infection

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Helicobacter pylori is associated with peptic ulcer disease and represents a risk factor in gastric cancer and maltoma. Secretory IgA, is the predominant gastrointestinal mucosal antibody, with an important effect against different antigens. The objective of this study was to measure the salival specific secretory IgA anti-*H. pylori* value in 30 children with chronic abdominal pain (13 female; 17 male, range 2–15 years old mean 7.3 b 3.8) and 30 children asymptomatic.
Patients and methods: Each child of the symptomatic group underwent clinical evaluation, serum IgG anti- *H. pylori* (Pyloriset-ORION), salival IgAs anti-*H. pylori* (ELISA, developed in our laboratory), gastric biopsy for histology, rapid urea test.
Results: Chronic gastritis was found in 53% (30% positive *H. pylori*, 23% negative *H. pylori*). Chronic active gastritis was detected in 17%, all positive for *H. pylori*. A significant tendency to increase secretory IgA levels, related to the severity of the histological gastric finding was observed ($p = 0.0307$); which was significantly higher in children with positive *H. pylori* and chronic active gastritis than chronic gastritis ($p = 0.0408$). These results probably reflect an important effect of the salival secretory IgA antibody in the gastric immunity against *H. pylori* infection associated with chronicity and activity of gastric infection.
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P804

Resistance pattern of *Staphylococcus aureus* clinical isolates from children

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Staphylococcus aureus is one of the three most common causes of nosocomial infections, while resistance to antimicrobials is gradually rising.
Objectives: To determine antibiotic resistance of *S. aureus* clinical isolates from children.
Methods: We studied retrospectively 124 strains of *S. aureus* isolated during the last 5 years (2000–2004). Twenty-nine strains were isolated from skin lesions, 25 wounds, 15 eye swabs, 14 ear swabs, 13 nose swabs, 16 blood cultures, 7 umbilical swab and 5 urine specimens. Processing and follow-up was based on conventional methods and identification was performed by Api-Staph (Biomerieux). Resistance of staphylococci was tested by Kirby–Bauer method and MICs to 10 antibiotics were determined by the agar dilution method, according to NCCLS guidelines. Detection of inducible resistance to clindamycin was tested with erythromycin-clindamycin 'D-zone' test. Moreover resistance to methicillin was tested with the following methods: (1) incorporation of oxacillin (6 mg/ml) in Muller–Hinton agar enriched with 4% Nacl, (2) Etest method (AB Biodisk,Solna,Sweden), (3) estimation of zone diameter around cefoxitin disc (30ìg) and (4) agglutination with monoclonal antibody against PBP2a protein (Biomerieux).
Results: Resistance of *Staphylococcus aureus* to methicillin (MRSA) with all methods was 12.7%. Resistance rates to antimicrobials was as follow: penicillin (87%), tetracycline (24.4%), erythromycin (10.4%), clindamycin (5.8%), fucidic acid (6.9%) and gentamycin (1.1%). Inducible resistance to clindamycin was detected in 5.8%. The most common resistance phenotype was oxacillin-tetracycline-fucidic acid. All specimens were sensitive to rifampicin, chloramphenicol, glycopeptides, streptogramines and oxazolidinones.
Conclusions: (1) Continuous increase of MRSA, necessitates the use of a combination of laboratory methods for accurate detection of methicillin resistance. (2) 'D-zone' test is a rapid and reliable method for detection of inducible resistance to clindamycin and can be used for discrimination of strains with genetic potential to develop resistance during treatment.

P805

Incidence and antimicrobial resistance of pathogens causing gastroenteritis in childhood

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Objectives: To evaluate the incidence and resistance pattern of enteropathogenic bacteria and viruses in children.
Methods: A total of 6152 stool specimens were tested in our laboratory during the years 1998–2005. All samples were gram stained and plated in the appropriate common and selective culture media. Detection of Rota and Adeno virus antigen was performed with immunochromatography assay. Identification was performed with conventional methods and serological typing with specific antisera in the National Reference Center. Susceptibility to antibiotics was tested by Kirby–Bauer method and MIC determination by the agar dilution method, according to NCCLS guidelines.
Results: Bacterial gastroenteritis was detected in 18.4% of specimens, viral in 14% and co-infection in 5.5%. The most frequent isolate was *Salmonella* spp (54.2%) with *S. enteritidis* being the commonest serotype (48.1%), followed by *Campylobacter jejuni* (30.3%) with HS:2 as the predominant serotype. Third most frequent pathogen was *Shigella* spp (6.8%) with commonest serogroup *Shigella flexneri* (80%) and predominant serotype 2. Enteropathogenic *Escherichia coli* (EPEC) was detected in 5.2% of specimens, Yersinia enterocolitica in 1.4%, with O:3 as the predominant serotype and Aeromonas hydrophila in 1.1%. Antigens of Rota and Adeno virus were detected in 11.5% and 2.5% respectively. Resistance of *Salmonella* spp. to co-trimoxazole was 5.4%, to ampicillin 32.2% and to nalidixic acid 33.5%. Resistance of *S. flexneri* to ampicillin was 61.3% and co-trimoxazole 27.2%, while both pathogens were sensitive to ciprofloxacin. There was no resistance of *Campylobacter* spp. to erythromycin.
Conclusions: 1. In order of frequency *S. enteritidis*, *C. jejuni* and *S. flexneri* were the predominant isolates, while Rota virus is responsible for a high incidence of viral gastroenteritis. 2. High incidence of resistance to ampicillin and co-trimoxazole and susceptibility to ciprofloxacin was observed in *Salmonella* and *Shigella* isolates. 3. *Campylobacter gastroenteritis* is usually self-limited and erythromycin continues to be the drug of choice in cases where treatment is necessary.

P806

Evaluation of bacterial microflora in the colon of children with inflammatory bowel disease

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Objectives: Evaluation of bacterial microflora in the colon of children with IBD and characterisation of quantitative and qualitative changes of the G.I. tract flora in children hospitalised for the first time because of UC (ulcerative colitis) or CD (Crohn's disease).
Methods: The study materials were faecal samples and intestinal biopsies collected from 30 patients 1–18 years of age, hospitalised for the first time because of UC or CD in the Clinic of Paediatrics, Gastroenterology and Nutrition in Cracow. The control group consisted of children without signs of IBD in colonoscopy. The stools were collected in three independent fractions, at the time of preparing patients for colonoscopy: Methods of qualitative and quantitative microbiological diagnostics: phenotyping methods FISH.
Results: In faecal samples of children with confirmed IBD versus control group there were no differences in global populations of bacteria, only quantitative changes within specific bacterial genera, as follows: an increase in the population of bacteria of *Enterobacteriaceae* and *Streptococcus genera*, confirmed both by culture and FISH; a decrease in the population of strictly anaerobic bacteria, mainly of Bifidobacterium and *Bacteroides genera*. Performing analogous examinations of intestinal tissue biopsies, making use only of culture methods, the following dependencies were observed: an increase in the total number of bacteria in IBD patients; the remaining quantitative changes within each of the bacterial genera showed the same tendency as the results obtained from examinations of faecal samples. In case of bacteria from *Lactobacillus genus*, a decrease of bacterial population was noticed in children with IBD (using FISH in faecal sample examinations and culture methods for tissue biopsies).
Conclusions: A decrease in the population of anaerobic bacteria (Bifidobacterium and Bacteroides) in IBD patients may be caused by an inflammatory process which can generate reactive oxygen species (ROS) toxic to anaerobic bacteria. Aerobic bacteria (*Enterobacteriaceae* and *Streptococcus*) are insensitive to oxygen and may grow in its presence; a decrease in the population of Lactobacillus and Bifidobacterium in IBD patients may be one of the reasons of exacerbation of inflammation process.; FISH is more accurate than culture method because hybridisation can detect all bacteria, even dead cells. It is the reason why we observe significantly higher results comparing with culture method.

P807

Molecular epidemiology of *Escherichia coli* diarrhoea in children in Tehran

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Objectives: Diarrhoeal diseases remain a major public health problem in developing countries. Diarrhoeagenic *Escherichia coli* strains include several emerging pathogens of worldwide public health importance. This study investigated the role of four categories of diarrhoeagenic *E. coli* in Tehranian children with acute diarrhoea.
Methods: During a six-month period, stool specimens of children under five years of age with diarrhoea (n = 200) and matched controls without diarrhoea were studied for the presence of enteroaggregative (EAEC), enteropathogenic (EPEC), enterotoxigenic (ETEC) and shiga toxin-producing (STEC) *E. coli*. PCR detection of six different genes of diarrheagenic *E. coli* in primary mixed culture of stool and subsequent colony screening were used for identification purpose. STEC isolates were typed by O157 and H7 antisera.
Results: EAEC was the most prevalent category and was found in 24% children with diarrhoea and 10% of control children without diarrhoea ($p < 0.0001$). ETEC was isolated from 15.5% of children with diarrhoea but was not isolated from control children without diarrhoea ($p < 0.0001$). STEC was isolated from 15% children with diarrhoea and 2% of controls children without diarrhoea ($p < 0.0001$). EPEC was found in 6% of children with diarrhoea and 5% of control children without diarrhoea showing no association with diarrhoea. Of 30 isolates of STEC from children with diarrhoea, seven were O157:H7 while 23 were non-O157:H7.
Conclusion: We concluded that EAEC, ETEC and STEC were significant diarrhoeal pathogens in children with diarrhoea while non-O157:H7 isolates of STEC were more prevalent than O157:H7 isolates in diarrhoeal group.

P808

Antimicrobial susceptibility of urinary pathogens in children from two tertiary care Greek hospitals

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Objectives: Urinary tract infections (UTIs) are the second most common infection after respiratory tract infections in children. The purpose of this study was to determine the current antimicrobial susceptibility patterns of pathogens causing UTIs to pediatric patients from two different tertiary care Greek hospitals.
Methods: Data were collected from patients admitted in the paediatric units of the two hospitals during two-year period (2003–2004). Identification and susceptibility testing were performed using the VITEK 2 automated system (bioMerieux, France). Susceptibility data were interpreted using NCCLS breakpoint criteria. Statistical analysis was performed using the 1-way ANOVA ($p < 0.01$ and $f > 0$ was considered as significant).
Results: During the two-year period 665 isolates from urine cultures in paediatric units of two hospitals were reviewed. *Escherichia coli* (n = 436; 66%) was the predominant pathogen in both hospitals, followed by *Pseudomonas aeruginosa* (n = 53; 8%) and *Proteus mirabilis* (n = 51; 8%). According to ANOVA test there was not statistical differences in the susceptibility rates between the two hospitals. Susceptibility rates to commonly used antimicrobial agents like ampicillin (AMP), amoxicillin/clavulanate (AMC), cefotaxime (CTX), ceftazidime (CAZ), cotrimoxazole (SXT), ciprofloxacin (CIP) for *E. coli* were 57%, 89%, 92%, 92%, 78%, 99% respectively. Among *P. mirabilis* isolates 80% were sensitive to AMC, 96% to CTX, 96% to CAZ, 71% to SXT, 98% to CIP and 80% to AMP. *P. aeruginosa* were only sensitive to ciprofloxacin 96%. Based on the resistance phenotype 8% of *E. coli* and 4% of *P. mirabilis* isolated from urine cultures in paediatrics patients were resistant to broad- spectrum beta- lactam antibiotics.
Conclusions: *E. coli* remain the main causative agent of urinary tract infections of paediatrics patients. The results reinforce the need for continuous local surveillance to show the current antimicrobial susceptibility data which can be used as aid to the empirical treatment of UTIs in children.

P809

Urinary tract infection in neonates, a five-year study (2000–2005)

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Objectives: Urinary tract infection (UTI) is one of the serious bacterial infections in the neonatal period. To determine the epidemiology, aetiology, clinical pattern and antibiotic sensitivity in neonatal UTI a retrospective study was carried out during a 5-year period.
Methods: The medical records of 38 neonates with proven UTI (cultured by suprapubic aspiration) were investigated. Details of gestational age, sex, clinical findings, blood culture, causative organisms and antibiotic sensitivity were reviewed.
Results: During the study period (March 2000–2005), 3623 newborns were admitted in our neonatal unit, of which 38 had UTI which was confirmed by suprapubic aspiration, giving an incidence rate of 1.04% in general. UTI was more prevalent in males than females (ratio 2.4:1). Of the affected newborns 82% were term and 18% preterm.The most frequent symptoms were jaundice (68.4%), lethargy (14%), fever (13%) and poor feeding (5%). Eleven per cent (11%) had positive blood culture simultaneously.*Escherichia coli* (50%) and *Klebsiella* spp. (30%) were the prominent pathogens isolated, both of them were highly sensitive to amikacin and gentamicin respectively.
Conclusion: *E. coli* was the most common isolated microorganism. Jaundice may be the first or the only sign of UTI in newborns, therefore, obtaining urine culture in jaundiced infants is reasonable.

P810

Longitudinal development of tobramycin resistance in *Pseudomonas aeruginosa* isolates from children with cystic fibrosis and the effect on clinical outcome

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Objectives: To evaluate the longitudinal development of Tobramycin resistance in *Pseudomonas aeruginosa* isolated from sputum of children with cystic fibrosis receiving inhaled Tobramycin and to assess the clinical impact of the changes in the Minimum inhibitory concentration (MIC).
Methods: Sputum was collected prospectively from all children with a persistent growth of *P. aeruginosa* receiving Tobramycin Inhaled Solution (TSI). Isolates were collected from Jan 2004 to July 2005. All isolates resistant to Tobramycin (10 mcg/ml) by disk diffusion were tested to evaluate the MIC. The following data was recorded prior to and during treatment with inhaled Tobramycin; anthropometric indices, antibiotic resistance of *P. aeruginosa* strains, use of IV Tobramycin and forced expiratory volume in one second (FEV1).
Results: Twenty-nine patients were treated with TSI during the study period. In twenty-six patients, the *P. aeruginosa* developed resistance to Tobramycin (10 mcg) as evaluated by disk diffusion. In ten of the patients *P. aeruginosa* developed a Tobramycin MIC of >1024 mcg/ml. After one year on TSI, this group showed a greater fall in mean percent predicted FEV1 ((1.7% per year v. +0.32% per year) and mean weight z scores ((0.36 SD over 4 years v. (0.23 SD over 4 years) respectively when compared to the group (19/29) whose Tobramycin MIC was <1024 mcg/ml. This finding was independent of genotype, pancreatic insufficiency or use of other medications. The duration of treatment was similar in those with a MIC of <1024 mcg/ml.
Conclusion: In patients with a MIC >1024 mcg/ml, there was a tendency towards a more rapid fall off in both weight z scores and FEV1. This is a consistent trend and demands further scrutiny and a more detailed clonal analysis of the Pseudomonas isolates.

P811

Predictors of mortality in children with candidaemia

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Objectives: Several studies have documented that survival rates for children with candidaemia are significantly better than survival rates for adults, which has been linked to the higher proportion of infections caused by *Candida parapsilosis* in children. The aim of this study was to present, using multivariate analysis, risk factors for death among paediatric patients with candidaemia.

Methods: This was a retrospective cohort study conducted in a 1200-bed Brazilian teaching hospital during 1995 and 2003. Patients were considered paediatric if their age was ≤13 years-old. Severity of candidaemia was determined by PRISM score and shock requiring inotropic support. Patients who received less than 72 h of antifungal therapy were considered to have received no treatment. This criterion was also applied for patients treated with low doses of antifungals (amphotericin B desoxycholate: <0.6 mg/kg; fluconazole: <6 mg/kg bds; and liposomal amphotericin B: <3 mg/kg). Overall in-hospital mortality was the main outcome studied.
Results: A total of 82 paediatric patients with candidaemia were included in the study, including 19 neonates. Most of these children were female (62.2%) and median age was 0.4 year-old. Major underlying diseases were congenital malformations (41.5%), haematological malignancies (11.0%), and solid tumours (11.0%). Species other than *C. albicans* were the main aetiology of candidaemia (79.3%), mainly *C. parapsilosis* (37.8%), and *Candida tropicalis* (22.0%). *Candida glabrata* occurred in only 1.2%, and *Candida krusei* in 2.4%. Overall mortality was 40.2%. Independent risk factors for death were failure to remove the central venous catheter ($p = 0.012$), time from admission to candidaemia > 45 days ($p = 0.042$), requirement of mechanical ventilation ($p = 0.011$), and heart rate ≥ 175 bpm ($p = 0.023$). No interaction was observed amongst the variables included in the equation.
Conclusions: Our study highlights the importance of central venous catheter removal in paediatric patients with candidaemia. Failure to do that was strongly associated with a poor outcome, independently of severity of infection or receipt of antifungal drugs.

P812

Bacterial isolates during bacteraemia in neonatal units of a medical university hospital in Gdansk

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Objectives: The aim of the study was to determine blood cultures bacterial isolates from the Neonatal Units and their susceptibility.
Methods: We analysed 758 blood cultures in 2003 and 2004. Blood cultures from Neonatal Units patients were performed using the BacT/Alert automated system (BioMerieux). Strains were identified by classical method and VITEK GNI+ cards (BioMerieux). Sensitivity was determined by the agar diffusion method according to NCCLS guidelines.
Result: During this study 150 (20.2%) blood cultures were positive, 605 (79.8%) were negative. A total of 151 bacterial strains were isolated from blood cultures between 2003 and 2004. The coagulase negative and positive Staphylococci: *Staphylococcus epidermidis* (43.7%) and *Staphylococcus aureus* (4.63%) were the most common pathogen recovered from blood, followed by members of *Enterobacteriaceae* (19.86%) including *Klebsiella* spp. (7.28%), *Escherichia coli* (5.29%), *Enetrobacter* spp. (5.29%). Three strains of *Enterobacter aerogenes* were ESBL positive. We found 126 episodes of bacteremia and observed 8 episodes of polymicrobial bacteriemia and 1 persistent bacteraemia.
Conclusion: The coagulase-negative and positive Staphylococci were most frequent casual agents of bacteraemia during study period in neonatal units, however in most cases probably due to contamination of the samples. Gram-negative bacteria especially members of the *Enterobacteriaceae* are the second important causes of bacteraemia in neonatal units. Great percentage of Gram-positive cocci mentioned previously indicates the need of investigation of blood collecting procedures.

P813

Incubation time for clinically significant positive neonatal blood cultures

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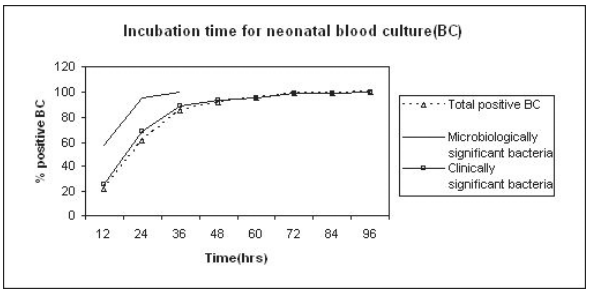
Objectives: To determine the incubation time for clinically significant positive neonatal blood cultures.
Methods: Positive blood cultures from infants on the neonatal intensive care unit (NICU) and post-natal wards of a teaching hospital were prospectively and consecutively identified by a clinical microbiologist. The blood cultures were processed on the BacT/Alert® 3D automated system (bioMérieux, USA). Data was collected between April 2004 and October 2005. A neonatologist made an independent prospective assessment of the clinical significance of the same positive blood cultures.
Results: During the study period there were a total of 229 positive blood cultures. Prospective data on clinical significance was available on 179, from 111 infants. 106 of the infants were on the NICU and five on the post-natal wards. Median gestational age was 27 weeks (range 23–41 weeks) and median birth weight was 950 g (range 385–3955 g). Median age at testing was 10 days (range 0–101 days). Median number of positive blood cultures was 1(range 1–7). All 38 microbiologically significant positive bacterial blood cultures (e.g. Group B *Streptococcus* and *Enterobacteriaceae*) and 8 blood cultures growing fungi were also classified as clinically significant. For the microbiologically significant bacterial isolates, 22(58%) were detected within the first 12 hours, 36(95%) by 24 hours and all by 36 hours. Bacteria of uncertain microbiological significance (e.g. coagulase negative *Staphylococcus*, *Enterococcus* species) were detected in 133 blood cultures, of which 87 were classified clinically significant. The incubation time for clinically significant bacterial blood cultures was longer than for solely microbiologically significant bacteria (Fig. 1): 26% being detected by 12 hours, 69% by 24 hours, 89% by 36 hours, 93% by 48 hours, 95% by 60 hours and 100% by 96 hours.



Conclusions: This prospective study highlights the need to consider a two tier approach with respect to the incubation time for clinically significant neonatal blood cultures; virulent bacteria are detected within 36 hours, whilst bacteria of uncertain microbiological significance can take longer to manifest. We recommend antibiotic therapy can be stopped in clinically asymptomatic infants with a negative blood culture at 36 hours incubation time. However if there are clinical concerns of sepsis, antibiotic therapy should be continued.

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P814

Predictive value of procalcitonin and serum amyloid A protein levels in early detection of pyelonephritis in childhood

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Objectives: The detection of renal involvement during urinary tract infection (UTI) is of great importance for the determination of further investigation and monitoring. The objective of this study was to determine the PCT and SAA values as markers of renal involvement in UTI assessed by a [99Tcm]-dimercaptosuccinic acid (DMSA) scintigraphy.
Methods: Sixty children aged 1 month to 10 years with or without fever, who were admitted in a General Pediatric Hospital with the suspicion of UTI were included in the study. WBC count, CRP, ESR, PCT and SAA levels were measured on admission. Renal involvement was assessed by DMSA scintigraphy within 7 days after treatment initiation.
Results: When comparing the two groups: a. with or b. without renal involvement, in the first group significantly increased levels of PCT (4.38 vs 0.38 μg/L, $p < 0.001$) and increased levels of SAA (483.7 vs 325.3 mg/L, $p < 0.043$) were found; moreover in the first group increased levels of ESR (78.5 vs 44.8 mm/h, $p = 0.012$) and WBC (20.1 vs 15.6×10^3 μL, $p = 0.043$) were found respectively. On the contrary CRP levels were not different between two groups (87.7 vs 53.1 mg/L p = NS). When analysing the groups according to the presence of fever, in the febrile group the levels of PCT, SAA, CRP, ESR and leukocyte counts were significantly increased, in comparison to the afebrile group respectively (PCT: 0.2 vs 2.17 μg/L, $p < 0.001$, SAA: 90.3 vs 467.7 mg/L, $p < 0.001$, WBC: 10.7 vs 18.4×10^3 μL, $p < 0.001$, ANC counts 5.7 vs 10.8×10^3 μL, $p < 0.001$, CRP: 8.5 vs 76.6 mg/L, $p < 0.001$, and ESR: 27.0 vs 66.8 mm/h, $p < 0.001$). PCT was significantly related to the severity of renal lesions (as ranked by DMSA), while SAA and CRP were not.
Conclusion: The serum PCT levels were found significantly increased in children with UTI when renal parenchymal involvement was present. The SAA levels were significantly correlated with the severity of infection while they were not related to the severity of renal lesions. Thus the PCT is a useful marker for the prediction of the risk of severe renal lesions, while the SAA is a marker of infection severity.

P815

Is there a place for moxifloxacin in paediatric practice? A report from a tertiary care paediatric hospital

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Background: Moxifloxacin (moxi) is recommended as a treatment of hospitalised adults with community acquired pneumonia. Its pharmacokinetic allows an oral administration. Unfortunately, there is no data concerning its paediatrics use.
Objective: To assess the indications, the tolerance and the efficacy of moxi use in hospitalised children. To evaluate the costs saved by an earlier discharge.
Methods: All patients who had received moxi in a 170-bed paediatric tertiary care hospital were retrospectively identified by the pharmacy billing data. We reviewed the medical charts and determined the number of outpatient days during which an intravenous (iv) beta lactam therapy would have been required and oral moxi was used. We calculated the cost of these days.
Results: Moxi was used in 23 children from March 2003 to October 2005. The median age was 35 months (from 7 to 172). No patient had received (iv) moxi. The indications were: earlier discharge in 15/23 patients, venous access problems in 5/23, therapeutic failure in 3/23. The main diagnosis was complicated pneumonia associated with empyema (17/23), other diagnosis were mastoiditis (3/23), ethmoiditis (1/23), abscess complicating a knee arthritis (1/23) and a neck cellulites-adenitis (1/23). The oral therapy was begun after a mean of 12.3 days (0 to 43) of iv beta lactam therapy. The children received the following regimens according to their weight: 1×100 mg/day (1/23), 2×100 mg/day (10/23), 2×150 mg/day (1/23), 2×200 mg/day (9/23) and 1×400 mg/day (2/23). The oral moxi was given during hospitalisation for 14/23 patients, whereas it was started at discharge for the others. The children were discharged from hospital after a mean of 15.5 days (7–43), which represents a mean of 3731 euros saving per patient. The hospital stay in these children was statically shorter than the stay with the standard antibiotic regimen for these infections (21 days of iv beta lactams); (t = (3.9, $p = 0.001$). All children had a routine follow-up at 15 days and /or 1 month after discharge and no adverse event was reported.
Conclusion: We report a series of 23 children successfully treated with oral moxifloxacine for severe infections. It was well tolerated and it allowed a significant shortening of the hospital stay. This reduced the cost without compromising the efficacy. However, there is a need for pharmacokinetic and pharmacodynamic studies in children as no appropriate dosage has been determined.

P816

Leptin as an acute-phase reactant in urinary tract infections in children

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Objectives: It has been demonstrated in several experimental animal models that leptin, an adipocyte-derived hormone/cytokine that links nutritional status with neuroendocrine and immune functions, is also an inflammatory mediator that might act as an early acute-phase reactant in some conditions and not in others.
Patients and methods: We studied the leptin response in 40 febrile children (1 month to 9 years of age) with confirmed bacterial urinary tract infection. Leptin levels were measured by an enzymatically amplified ''two-step'' sandwich-type immunoassay (Diagnostic Systems Laboratories).
Results: Leptin levels were significantly increased (mean value: 58.69 ± 5.05 pg/ml) in all 40 patients independently of their BMI, at diagnosis of the infection and returned to normal levels 3 days after initiation of treatment ($p < 0.001$). We also noted that in four of our subjects, leptin levels remained high or even increased. In correlation with the rest of our laboratory data (CRP, WBC and urine cultures) along with the clinical data (fever, anorexia, general condition) these patients had not responded to treatment and their infection was not considered under control.
Conclusions: High leptin levels during inflammation are indicative of normal expression of leptin genes and leptin receptor and this suggests that leptin is also an ''inflammatory marker'' and might be an early indicator of the prognosis of the bacterial infection. Similar results have been described in patients with

sepsis, whereas the non-increase of leptin levels was associated with increased mortality. The secretion of leptin in the first hours of inflammation in children is stimulated by the endotoxins of bacteria and can be induced by other inflammatory mediators such as IL-1 and TNF-alpha, as it has been shown in animal models. Leptin seems to play an important role being part of the cytokine network and modulating the inflammatory-immune response and the host defence mechanisms.

P817

Treatment of acute otitis media in primary paediatric care and the risk factors for antibiotic prescribing

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Backgrounds and objectives: Acute otitis media (AOM) is an important problem mainly in children age. Objective of this work was to analyse and validate antimicrobial treatment at acute otitis media according guidelines. Also the using of laboratory examinations and microbiology diagnostics to assess aetiology of infection and consecutive antibiotic indication were determined. To classify the factors which lead to antibiotic prescription at AOM.
Methods: Multicentric prescription study at the acute respiratory tract infections, four weeks in November 2003 in five Slovak cities. The 66 paediatricians registered patient characteristics (gender, age, weight), diagnosis, assumed aetiology, antibiotic therapy, who prescribed it, risk factors of patients (allergy to penicillin, dysimmunity, chronic respiratory tract disease, etc.) into the protocols according same methodology. We evaluated the using of auxiliary examination (laboratory, microbiology), assumed aetiology and antibiotic prescription for a treatment an acute otitis media. To predict the antibiotic prescription we use a logistic regression (SAS).
Results: Out of 326 patient, to 173 (53.1%) antibiotic treatment was indicated. The most frequent prescribing antibiotic were co-aminopenicillins in 54 patients (31.2%; 95% confidence interval (24.3%; 38.1%)), second were penicillins in 37 cases (21.4%; 95% CI (15.3%; 27.5%)), after that cephalosporins in 35 cases (20.2%; 95% CI 14.2%; 26.2%), aminopenicillins in 24 cases (13.9%, 95% CI (8.7%, 19.0%)), macrolides in 18 cases (10.4%, 95% CI (5.9%; 15.0%)). In six out of ten patients with acute otitis media antimicrobial therapy was according to the guidelines. To settle aetiology of infection paediatricians used laboratory examination and microbiology diagnostics (42.3%; 20.6% respectively). The most frequent risk factor was the attendance of collectively institute (60.1%). The microbiology diagnostics contribute to determine infection pathogen and to avoid antibiotic prescribing ($p = 0.01$). Other statistically significant factors which conduce to antibiotic prescription were aetiology ($p < 0.0001$), city ($p < 0.0001$), dysimmunity ($p = 0.001$) and attendance ($p = 0.005$).
Conclusions: Main reason for antibiotic prescription at AOM is according our results the aetiology which is assumed by GP, risk factors of patient as attendance and dysimmunity and there is also dependence on prescription habits within the city.

P818

The hygiene hypotheses revisited? Recurrent childhood upper respiratory tract infections do not reduce the risk of adult atopic disease

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Background: The role of childhood upper respiratory tract infections (URTI) in the development of atopic disease later in life remains controversial.
Objective: To investigate the association between childhood recurrent upper respiratory tract infections and both subjective and objective measures of atopy and asthma in adulthood.
Methods: A birth cohort of 1055 members was followed prospectively from ages 2 to 21 years. At ages 2–4 years detailed information on upper respiratory tract infections was collected 3-monthly. At age 21 years a standardized questionnaire, lung function, exhaled bronchial Nitric Oxide (FENO), total and specific IgE were measured.
Results: Of the original cohort, 693 (66%) members completed the questionnaire and 404 (60%) of them completed the objective measures at 21 years. Children who experienced recurrent URTI during childhood were not less likely to have asthma at age 21 years than children who did not experience recurrent URTI; RR 1.45 (95% CI 0.95–2.21). Neither were recurrent URTI associated with a decreased risk of allergic rhinitis (RR 0.99 (95% CI 0.79–1.25)), eczema (RR 1.19 (95% CI 0.81–1.75)), lung function parameters, FENO, and IgE at the age of 21 years.
Conclusion: We could not confirm the hygiene hypothesis, i.e. recurrent URTI in childhood did not reduce the risk of atopic disease in young adulthood.

Microbial biofilms

P819

Genetic analysis of the biofilm phenotype in methicillin-resistant *Staphylococcus aureus*

E. O'Neill, H. Humphreys, J.P. O'Gara *(Dublin, IE)*

Objectives: Biofilm development by meticillin resistant *Staphylococcus aureus* (MRSA) represents an additional pathogenic mechanism in its already impressive arsenal of virulence determinants. Moreover the environmental regulation of biofilm formation may reflect an adaptive, pathogenic mechanism employed by staphylococci to enhance colonisation under appropriate clinical conditions. Enzymes encoded by the ica operon synthesise an extracellular polysaccharide adhesin implicated in biofilm formation. However, preliminary research in our laboratory (Fitzpatrick et al., 2005. J. Clin. Microbiol. 43:1973) revealed that glucose-induced biofilm development in MRSA isolates appears to be ica-independent. In this study we investigated the contribution of ica and other biofilm genes associated with biofilm development by clinical isolates of MRSA.
Methods: Bacteriophage transduction with phage 80 was used to generate ica operon deletion mutations in MRSA strains with glucose-dependent biofilm phenotypes. Deletion mutations were also generated in other genes associated with biofilm development namely the staphylococcal accessory regulator (sarA) and the accessory gene regulator (agr).
Results: Ica operon deletion mutations were created in three clinical MRSA strains and the laboratory strain *S. aureus* RN4220. ica operon deletion had no effect on the biofilm phenotype of the clinical MRSA strains but resulted in a biofilm

negative phenotype in *S. aureus* RN4220. Deletion of sarA in one clinical MRSA isolate and in *S. aureus* RN4220 resulted in a biofilm negative phenotype. In contrast, deletion of the agr locus did not affect the biofilm phenotype in four clinical MRSA strains tested or in *S. aureus* RN4220.
Conclusions: These findings support the existence of an ica-independent biofilm phenotype amongst clinical strains of MRSA and further reveals that this MRSA biofilm phenotype is sarA-dependant and agr-independent. Further research is ongoing to investigate the contribution of other genes implicated in biofilm formation in these important hospital pathogens.

P820

Combined exposure to sub-inhibitory levels of vancomycin and therapeutic levels of aspirin enhances biofilm formation by *Staphylococcus epidermidis*

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Introduction: *Staphylococcus epidermidis* is increasingly recognised as a cause of hospital acquired infection, especially associated with implanted medical devices. The main virulence factor in these infections is the ability to form biofilms. Due in part to this fact, but also to the high levels of methicillin resistance in *S. epidermidis*, vancomycin is the drug of choice for treating such infections. Patients receiving vancomycin treatment of prophylaxis may have residual levels of vancomycin in their blood. It has been shown previously that sub-minimum inhibitory concentrations (MIC) of vancomycin may lead to increased biofilm formation. It has also been suggested that acetylsalicylic acid (aspirin) can be combined with vancomycin to inhibit biofilm formation.
Methods: Eleven *S. epidermidis* isolates recovered from cases of prosthetic joint infection were examined for biofilm forming properties in the presence of sub-MIC levels of vancomycin with and without aspirin at a therapeutic level of 300 μg/ml.
Results: Vancomycin at sub-MIC levels significantly enhanced the density of biofilms formed by some of the isolates tested. When combined with aspirin, the findings were similar to those obtained with vancomycin alone.
Conclusions: This study supports the previous suggestions that sub-MIC levels of vancomycin may enhance *S. epidermidis* biofilm formation and highlight the importance of maintaining vancomycin levels above the MIC at sites of medical device implantation. In contrast to some previous reports, we suggest that the use of aspirin at therapeutic levels is of little value in the treatment of device related infection.

P821

Lactobacillus as a competitive bacteria in *Staphylococcus* strains biofilm formation

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Objectives: The commensal microflora of the human body plays a very important protective role, which is associated with the colonisation of skin and mucous membranes, the production of the bacteriocin-like inhibitory substances (BLIS) and fatty acids or with the competition of the nutrients. One of the ''barriers'' against pathogens are the bacteria from *Lactobacillus* genus. In our study we wanted to describe the activity of *Lactobacillus acidophilus* against planktonic and sessile cultures of *Staphylococcus* strains.
Methods: The influence of BLIS produced by ten *Lactobacillus acidophilus* strains on the growth *Staphylococcus aureus* strains (reference MSSA, clinical MRSA and clinical MRSA/hVISA) and *Staphylococcus epidermidis* strains (MRSE) was determined by two-layer plate assay and by dilution assay for bacterial culture supernatants. The activity of BLIS against bacterial adherence and biofilm formation was estimated using spectrophotometric assay (MTT). Colony forming units (CFU) method was used to determine the coaggregation/interference of the bacteria forming dual species biofilms on the plastic carriers.
Results: The obtained results demonstrated variable anti-staphylococcal activity among lactobacilli used in this study. It was observed that the antagonism between selected *L. acidophilus* strains and various staphylococcal strains does not only influence the growth of planktonic staphylococci, but also their adherence and biofilm development. The displacement of *S. aureus* and *S. epidermidis* from the surface by *Lactobacillus* in 50–80% was observed. The BLIS and probably biosurfactant activity of lactobacilli seems to play a very important role in these interactions.
Conclusions: These observations encourage further studies of bacterial interference which can be used for the prevention of biofilm formation. The ability of one strain to exclude others from the same environment may be an alternative for unsuccessful antibiotic therapy.
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P822

Dual species biofilms: do staphylococci like other bacteria?

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Objectives: Different forms of the interdependencies between microorganisms, like tolerance, interference commensalism or synergism, develop during coexistence of bacteria in the same ecological niche. They also play an important role when multispecies biofilm consisting of both physiologic or/and pathogenic microflora is formed. The aim of the study was to determine the interactions between staphylococci and other bacteria and their influence on dual species biofilm formation.
Methods: The interdependencies between *Staphylococcus aureus* or *Staphylococcus epidermidis* clinical strains and selected reference or clinical strains of *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, *Enterococcus faecalis* were estimated in different stages of dual species biofilm formation. The influence of bacteriocin-like inhibitory substances (BLIS) produced by coaggregating strains on the growth of *S. aureus* A3 and *S. epidermidis* A4c was determined by two-layer plate assay and by dilution assay for bacterial supernatants. The activity of BLIS against bacterial adherence and biofilm formation was estimated using spectrophotometric assay (MTT). The quantitative method using the carriers of biofilm was developed to determine the composition of mixed biofilms.
Results: All bacterial strains used for the study were capable of the biofilm formation with *S. aureus* and *S. epidermidis*, however with different intensity. The antagonistic activity of BLIS on the growth, adherence or/and biofilm formation by *S. aureus* or *S. epidermidis* was not always correlated with the blocking of coadhesion. The biggest interference during staphylococcal biofilm formation was observed when *E. coli* and *P. aeruginosa* strains were used as the second element of this structure.
Conclusions: Understanding of the mechanisms of bacterial interference in mixed species biofilms development and the survival of different microbial species living at the close proximity in the same matrix may help in the proceeding on therapeutic strategies.
Acknowledgement: Supported by Grant No. 2 PO5A 149 29 from State Committee for Scientific Research.

P823

Evaluation of the biofilm-forming ability of *Staphylococcus aureus* and *Staphylococcus epidermidis* mastitis isolates

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Objectives: Biofilm-forming ability is increasingly being recognized as an important virulence factor in staphylococci, facilitating their persistence in the host, evading its defences and allowing bacterial survival at high antimicrobial concentrations. *Staphylococcus aureus* remains the major pathogen related to chronic mastitis, but in the last years *Staphylococcus epidermidis* has emerged as an important mastitis agent. The present work aimed at evaluating the biofilm-forming ability of staphylococci field isolates from bovine subclinical mastitis.

Methods: In the present study biofilm production by *S. aureus* (n = 16) and *S. epidermidis* (n = 16) mastitis isolates, was evaluated *in vitro* by three distinct methods: phenotypic expression of colony characteristics in congo red agar (CRA) (Freeman et al., 1989), quantification of biofilm produced by optical density measurement (Cucarella et al., 2002) and direct observation of biofilm occurrence in bacterial suspensions and in artificially contaminated milk samples by fluorescent *in situ* hybridization (FISH). The FISH protocol included fixation with paraformaldehyde, permeabilization of the bacterial membranes by lysostaphin and ethanol, hybridization with two 16S rRNA oligonucleotide probes described by Kempf et al. (2000), stringency washes and observation of the hybridized cells by fluorescence microscopy (Oliveira et al., 2005).

Results: According to the three methods, 37.5% of the *S. aureus* isolates revealed the ability to produce biofilm, being also verified that 37.5% of the *S. epidermidis* isolates present this virulence factor. The positive strains maintained the biofilm-producer ability in the artificially contaminated milk samples, as observed by the FISH analysis.

Conclusion: *Staphylococcus aureus* are well established as clinical and epidemiological pathogens, but our results show that the potential pathogenic role of *S. epidermidis* as a major bovine mastitis agent should not be neglected. Further studies are required to elucidate the relevance of biofilm formation upon antibiotic susceptibility and increased antimicrobial resistance due to horizontal gene transfer.

P824

Comparative activity of linezolid, quinupristin/dalfopristin and vancomycin against biofilm produced by cystic fibrosis isolates of *Staphylococcus aureus* normal and small colony variant phenotypes

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Staphylococcus aureus (SA) is an important pathogen in cystic fibrosis (CF) patients (pts). Persistence of SA in CF has been associated to the formation of biofilm (BF) and the expression of a subpopulation with small colony variant (SCV) phenotype.

Objectives: Determine ability and entity of BF formation of SA and SASCV colonising CF pts attending the Genoa centre; moreover the efficacy of linezolid (L), quinupristin/dalfopristin (QD) and vancomycin (V) to inhibit and disrupt BF produced by both SA phenotypes was assessed.

Methods: Thirty-six SA (15 normal and 21 SCV phenotype) recovered from 21 CF pts were identified by API ID 32 STAPH (BioMerieux) and by nuc gene amplification (BIBLIO), using SA ATCC 29213 as quality control strain. Biofilm quantitative assay, antibiotic inhibition and disruption, were determined in microtitre plates (Christensen et al., 1985) by the spectrophotometrical measure of 595 nm absorbance after crystal violet staining; results were classified according to the categories described by Stepanovic et al. (1999). For BF inhibition the following antibiotic concentrations were used: L (0.25–0.50–1 mcg/ml), QD (0.125–0.250–0.50 mcg/ml) and V (0.125–0.250–0.50 mcg/ml). For BF disruption the following antibiotic concentrations were used: L (2–4–8 mcg/ml), QD (4–8–16 mcg/ml) and V (4–8–16 mcg/ml).

Results: SA BF formation revealed 9/15 low and mid-low (mean OD: 0.1552) 2/15 mid (mean OD: 0.2800), 4/15 mid-high and high (mean OD: 0.9550) producers. SASCV BF formation revealed 8/21 SASCV mid-low (mean OD: 0.1676), 7/21 mid (mean OD: 0.2469) and 6/21 mid-high and high producers (mean OD: 0.2932). Antibiotic activity on BF inhibition and disruption is shown in Table 1.

		Inhibition (%)				Disruption (%)			
	mcg/ml	0.125	0.25	0.5	1	2	4	8	16
SASCV	Linezolid		55.4	65.0	86.5	46.3	49.0	58.0	
	Quinupristin/ Dalfopristin	88.2	96.7	99.8			42.0	53.2	61.8
	Vancomycin	40.4	55.0	73.8			43.3	52.2	57.7
SA	Linezolid		30.4	49.7	70.0	8.0	16.0	23.0	
	Quinupristin/ Dalfopristin	97.0	97.8	99.8			10.5	25.4	33.3
	Vancomycin	95.0	97.8	99.7			7.7	21.1	32.8

Conclusions: In our study SASCV isolates showed higher BF production compared to SA phenotypes. For BF inhibition, QD and V showed a better activity on SA than on SASCV, while L was more active on SASCV. For BF disruption all the three antibiotics seemed more active on SASCV.

P825

Low prevalence of BAP gene (coding for biofilm-associated protein) in French isolates of *Staphylococcus aureus* recovered from human and animals species

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Objectives: *Staphylococcus aureus* is a common cause of mastitis in dairy farm animals (cows, sheep and goats). The implication of biofilm in chronic infection in all species have triggered an increasing interest in the characterization of genes involved in biofilm formation. Cucarella et al. (2001) identified a new gene (6,831 nt) involved in biofilm formation (bap coding for a biofilm-associated protein, Bap) in a small proportion of *S. aureus* strains from mastitis in different species. The purpose of this study was to investigate the presence of bap in various isolates from human and animals species.

Methods: One hundred and fifty *S. aureus* isolates, associated to different pathologies, were recovered from various locations in France and different species (cows, sheep, goats, pigs, rabbits, poultry, horses and human). DNA extraction was performed using an commercial kit according to the manufacturer's instruction with slight modifications. PCR were performed in duplicate, using a primer pair (sasp-6m and sasp-7c) as described by Cucarella et al. (2004) to detected the bap gene and (coag2 and coag4) as described by Goh et al. (1992) to control the negative results in the PCR.

Results: Although, the bap gene was detected in the bap positive control strain (kindly provided by J.R. Penadés, Spain), all *S. aureus* tested so far, including biofilm producing isolates (data not shown), do not seem to carry the bap gene. The presence of another gene (coa) was detected which indicated that DNA degradation did not occur thus eliminating the risk of false negative.

Conclusion: The bap gene, also present in other pathogenic *Staphylococcus* species, is not widely distributed in *S. aureus* isolates. As shown by Tormo et al. (2005), it was found in a small proportion among mastitis isolates of different species. This gene is normally carried by SaPIbov2, a bovine *S. aureus* pathogenicity island (Ubeda et al., 2003), and should be associated with well-known genes [icaADBC operon (Cramton et al., 1999), hla gene (Caiazza and O'Toole, 2003), rbf gene (Lim et al., 2004)] to explain biofilm production capacity. *S. aureus* is fully capable of forming biofilm in the absence of the bap gene.

P826

Contribution of biofilm phenotype to the pathogenesis of *Staphylococcus epidermidis* neurosurgical device-related meningitis

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Objectives: The coagulase-negative staphylococci (CoNS), in particular *Staphylococcus epidermidis*, have been identified as the most common causative agents of neurosurgical device-related meningitis. A common pathogenic feature amongst these bacteria is the ability to form biofilm, and the intercellular adhesin (ica) operon of *S. epidermidis* has an important function in adherence and biofilm development. The objective of this study was to determine the role of icaADBC, which results in the production of polysaccharide intercellular adhesin (PIA), in the pathogenesis of neurosurgical device-related meningitis caused by *S. epidermidis* isolates.
Methods: Eleven clinically significant *S. epidermidis* isolates were collected from neurosurgical patients with meningitis and with an extra-ventricular drain *in situ*. The ability to form biofilm under laboratory conditions was determined using a biofilm assay. Each isolate was grown in brain heart infusion broth (BHI) or BHI supplemented with 4% ethanol, 4% NaCl or 1% glucose. The PIA phenotype, as assessed by growth on congo red agar (CRA), was determined for each isolate. PCR, using primers specifically designed to amplify the entire ica operon, and Southern blot analysis were used to determine the ica genotype of each isolate. Reverse-transcription PCR (RT-PCR) was used to determine icaR and icaA expression in the same environmental conditions as above.
Results: PCR and Southern blot analysis confirmed that 7 (64%) of the isolates had an ica+ genotype. Under standard growth conditions only one (9%) isolate had a strong biofilm positive phenotype, while biofilm growth could be enhanced in a further two (18%) isolates by the supplementation of the media with 4% ethanol or 4% NaCl. RT-PCR analysis of icaA expression was consistent with this. Interestingly, although ica transcription was detected in the four remaining ica+ isolates, they were not biofilm-positive under any growth condition. There was also a good correlation between the biofilm phenotype and the PIA phenotype, as determined by CRA colony morphology analysis.
Conclusion: This initial data suggests that icaA expression alone is not sufficient for biofilm formation. Other genetic factors, along with local conditions within the neurosurgical device, may be important in this complex pathological process.

P827

Biofilm formation of invasive GAS isolates collected in immunocompromised and immunocompetent patients

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Objective: The ability of Streptococcus pyogenes (GAS) to form biofilm-like bacterial communities during infection suggests that the production of biofilm may be important for pathogenesis of invasive streptococcal infections. To examine a possible relationship between biofilm-forming and underlying disease we examined 35 isolates of GAS from blood cultures collected between 1999 and 2005 (35 patients with soft tissue infections).
Methods: To test the ability of 35 blood stream isolates to form biofilms, the isolates were grown in microtitre plates in tryptone soya broth for 24 or 48 hours. To examine the morphology of the biofilm formation the isolates were either examined unfixed or fixed with 2% glutaraldehyde using electron microscope scanning (Philips XL30 ESEM). For quantification they were fixed with 2% glutaraldehyde and dyed with 1% crystal violet to measure the mean optical density (OD using a routine microtitre-plate-reader at 550 nm wavelength).
Results: Nineteen isolates (54.3%) were biofilm forming and 16 (45.7%) of them showed no biofilm. All isolates were sensitive to penicillin, vancomycin and tigecyclin. Four isolates showed a resistance to erythromycin and six isolates were resistant to clindamycin. Twenty GAS were isolated from patients with no immunosuppression and 15 GAS were found in blood cultures from immunocompromised patients (six patients with HIV infection; nine patients with long-term treatment with methotrexate or steroids). From the 20 isolates of immunocompetent patients 11 (55%) isolates were biofilm forming and 9 (45%) isolates had no capabilities of biofilm forming. Eleven GAS isolates of 15 (73.4%) immunocompromised patients were biofilm forming and 4 (26.6%) strains showed no biofilm. In the six patients which were HIV positive the distribution between biofilm positive and negative were equal. Eight (88.9%) isolates from patients receiving long-term methotrexate or high-dose steroid treatment produced biofilm.
Conclusion: These data suggest that biofilm formation is not pivotal for the pathogenesis of invasive GAS infection, however hosts being immunocompromised because of long term methotrexate or steroids treatment may be more susceptible to invasive infection due to biofilm forming GAS. Penicillin G is still the therapy of choice. Resistance of clindamycin in 17.1% warrants cautiousness towards the treatment with clindamycin.

P828

Biofilm formation by *E. faecalis* in the microtitre-plate assay does not correspond to biofilm formation on clinically relevant materials

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Objective: To compare an often used screening method for biofilm formation, i.e., the microtitre-plate assay with 24-hour biofilm formation of *E. faecalis* on catheters and laboratory dialysis membranes.
Methods: Four clinical *E. faecalis* isolates and a laboratory strain (OG1RF) were used. The microtitre-plate assay was performed as previously described with some modifications, using TSB without glucose as growth medium and 0.1% saffranin or 0.5% crystal violet as dyes. Each test was performed in quadruplicate and performed at least twice on different days. For biofilm formation on clinically relevant materials, sterilely cut pieces of lubricant coated latex urinary catheters, polyurethane central venous catheters (CVCs) or cellulose membranes were placed in 5 mL of TSB without glucose, inocculated with 50 μL of an overnight culture of respective isolate and incubated in 37 °C for 24 h with gentle shaking. The pieces were washed three times and subsequently sonicated and vortexed (10 s + 10 s) twice in 1 mL PBS. An aliquot of the bacterial suspension was then serially diluted and plated for viable count. All tests were performed in duplicate and performed at least twice on different days.

Results: With the microtitre-plate using crystal-violet two isolates were considered non-biofilm forming and three isolates strong biofilm forming, according to the criteria proposed by Baldasarri et al. Using saffranin as stain gave similar results with all the strong biofilm forming isolates having comparable A490 values. However, on urinary catheters one strain (1128) formed 4–5 times more biofilm (~107 cfu/cm^2) than other isolates (including OG1RF) with comparable values of absorbance in the microtitre-plate assay. Similar results were seen when cellulose membranes were used. The results the least divergent from the microtitre-plate assay were when CVCs were used. The two isolates considered non-biofilm forming isolates, formed less biofilm than the others but still showed biofilms with ~105 cfu/cm^2 on catheters, CVCs and cellulose membranes.
Conclusion: Use of the microtitre-plate assay for screening isolates for biofilm formation might not give accurate information concerning biofilm formation on clinically relevant surfaces.

P829

Cell surface hydrophobicity, motility, and biofilm formation of *Stenotrophomonas maltophilia* clinical isolates

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Introduction: *Stenotrophomonas maltophilia* is an opportunistic pathogen colonizing patients in intensive care settings, especially those with underlying debilitating conditions such as immunosuppression, malignancies, and implantation of foreign devices. *S. maltophilia* has been reported to adhere to abiotic and living surfaces and to organize itself in biofilm on polystyrene surfaces. The formation of biofilms is a multistep process that requires participation of structural appendages, such as flagella and type IV pili in *P. aeruginosa*. Further, the hydrophobicity of bacterial surface is an important determinant in the adherence of bacteria to both living and non-living surfaces, and it may facilitate *in vivo* bacterial colonization of epithelial mucous tissues and adherence to medical devices.
Objective: To investigate the relationship between motility, cell surface hydrophobicity and biofilm formation in *S. maltophilia*.
Methods: Forty *S. maltophilia* strains isolated from blood of neutropenic patients were assayed for motility (swimming, swarming, and twitching). Cell surface hydrophobicity was assayed by microbial adherence to n-hexadecane test (MATH). Strains were considered as hydrophilic when MATH < 20%. Biofilm formation on polystyrene microtitres was quantified by absorbance (OD492) following staining by crystal violet dye (CV). Pearson's correlation index was calculated to determine the relationship between CV, MATH, and motility. All tests were performed in triplicate and repeated twice.
Results: Thirty-seven out of 40 (92.5%) strains were able to form biofilm (OD492 range: 0.119–0.345; mean OD492: 0.201). All isolates showed swimming. On the contrary, swarming an twitching were not visible in all samples. Eleven out of 40 (27.5%) strains resulted hydrophobic by MATH. Positive correlation ($r^2 = 0.510$; $P < 0.05$) was observed between CV and MATH. Two strains with the biggest MATHs (50 and 47.5%, respectively) were also the biggest biofilm producers (0.345 and 0.323, respectively). There was no significant correlation between swimming and CV or MATH.
Conclusions: Our results suggested, for the first time, that hydrophobicity is a major determinant of biofilm formation in *S. maltophilia*. While the relative contributions of hydrophobicity to the different steps of biofilm formation in *S. maltophilia* are not known, our results showed that hydrophobycity is not related to the presence of flagella.

P830

Molecular epidemiology and phenotypic characteristics of chronic *Pseudomonas aeruginosa* populations in cystic fibrosis lungs

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Objectives: Chronic *P. aeruginosa* infections are associated with poor prognosis for cystic fibrosis (CF) patients. We have investigated the geno- and phenotypes of isolates of *P. aeruginosa* from seven chronically infected CF patients. From each patient we have characterised clones isolated over periods of 1–28 years.
Methods: In all cases the genotypes have been identified by the use of a DNA dot-blot array. Using quantitative 16S rRNA *in situ* hybridization (FISH) we have determined the apparent growth rate distribution of *P. aeruginosa* in the CF lung.
Results: Our data show that most of the investigated patients carry one or very few different strains, that some of these belong to genotype clusters also found in other patients belonging to the Danish CF Centre in Copenhagen whereas others have unique clones. Clonal substitutions have taken place at some point in time in most of the patients.In a search for general phenotypic traits shared by most or all of the CF isolates of *P. aeruginosa* we have focused on growth rates and related phenotypes. All the isolates we have investigated so far display slow growth in comparison with reference laboratory strains and environmental isolates. In contrast, strains isolated from newly infected CF children grow significantly faster, suggesting that there is a selection for slow growth in the CF lung. Quantitative FISH data suggest that a large majority of the bacterial cells in the CF lung grow with rates comparable to those observed for the same cells growing in lab media. The adaptation of *P. aeruginosa* in the CF lung is often associated with biofilm-like growth and over-production of alginate. From direct *in situ* imaging of sputum samples we have identified several modes of multi-cellular organisation. Some of these may be comparable to biofilm structures also found *in vitro* and others, which share only little or no homology with the commonly observed structures described for *in vitro* biofilms.
Conclusions: Strain substitutions in chronically infected CF patients take place frequently. Growth rates of CF isolates were found to be low. Estimates of bacterial growth rates in the CF lung show that the majority of the cells grow actively. Bacterial aggregates were observed in the sputa from the majority of the patients.

P831

Morphological changes induced by subinhibitory concentrations of imipenem and piperacillin/tazobactam on surface properties and adhesion abilities of *Pseudomonas aeruginosa*

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Objectives: Various antimicrobial agents are known to retain some activity at subinhibitory concentrations (sub-MICs), causing bacteria to undergo ultrastructural changes. In general, carbapenems such as imipenem (IMP), have a high affinity for penicillin binding proteins (PBP)-2, and cause the bacilli to form round cells, whereas piperacillin/tazobactam (P/T), a β-lactam, has a high selective affinity for PBP-3, and induces the formation of long filaments (1), which are associated to possible decrease in protein adhesins. The aim of this study was to evaluate the influence of the morphological changes induced by sub-MICs of

IMP and P/T on surface properties and adhesion abilities of *P. aeruginosa* strains.
Methods: *In vitro* antimicrobial activities were evaluated by microdilution method (NCCLS) against three reference strains (ATCC 27853, PAO1, AK1), three defined PAO1 mutants with deviating surface characteristics (MT1562, PT623, PAO1 algC) and five *P. aeruginosa* clinical isolates. The strains were grown in LB in the presence and absence of 0.5 times MIC of IMP or P/T. The effects of different antibiotics on bacterial adhesion (1 h) were studied using a modified microtitre-plate assay. The changes on microbial adhesion to solvents (MATS) were estimated by using a biphasic method, which calculates the percentage of cells adhering to hexadecane (CSH) (apolar solvent), chlorophorm (acidic solvent) and ethyl acetate (basic solvent). The changes in cell morphology were assessed by light microscopy, after a gram stain of all the strains.
Results: There were no differences between adhesion in strains grown with IMP (round cells) or without in the majority of the strains tested. A significant decrease in adhesion and CSH was found with P/T (filamentous cells) (1). In almost all the strains there were no significant differences between the presence and absence of antibiotics in adhesion abilities to chloroform and to ethyl acetate.
Conclusion: The formation of round cells is associated with no modification in adhesion and CSH comparing to a significantly decrease in the filamentous forms (1). In both morphologies there was no modification of the electron donor/basic and electron acceptor/acidic characteristics of the bacterial strains which allow them to maintain their bipolar character.(1) Fonseca AP et al. Effect of subinhibitory concentration of piperacillin/tazobactam on Pseudomonas aeruginosa. J Med Microbiol 2004; 53: 903–910.

P832

Influence of the salt concentration on biofilm formation by *Salmonella* spp.

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Salmonella spp. are one of the most important foodborne pathogens. More than 95% of cases of infections caused by these bacteria are foodborne and these infections account for about 30% of deaths resulting from foodborne illnesses. The objective of the present study was to investigate the influence of concentration of NaCl on biofilm production by *Salmonella* spp. The quantification of biofilm formation by 30 *Salmonella* spp. strains was performed by the modified microtitre-plate test. After overnight incubation in Trypcase-soy broth, supplemented with 0.5%, 2.5%, 5%, 7.5% and 10% of NaCl, poured in 96-well flat-bottomed plastic microplates, bacterial biofilms were fixed with methanol and stained with crystal violet. The bound dye was released with 33% glacial acetic acid, and optical density was measured at 570 nm by using an automated microtitre-plate reader. Upon the optical densities of bacterial biofilms, all strains were classified into the following categories: no biofilm producers, weak, moderate or strong biofilm producers. Differences in the quantity of produced biofilm were examined by the Friedman test, followed by the Wilcoxon signed ranks test. P values of <0.05 were considered significant. The number of biofilm producing strains was highest in broth with 0.5% of NaCl (23, 76.7%), while 18 strains (60%) produced biofilm in broth supplemented with 2.5% NaCl and only one strain (3.3%) in broths supplemented with 5%, 7.5% and 10% of NaCl. The highest number of strains were moderate biofilm producers (30%) in broth with 0.5% of NaCl and weak biofilm producers (36.7%) in broth with 2.5% of NaCl. The quantities of biofilm produced by tested *Salmonella* spp. strains were significantly higher in medium supplemented with 0.5% and 2.5% of NaCl than in medium with 5%, 7.5% or 10% of NaCl. The obtained results showed that high salt concentration reduces the ability of *Salmonella* spp. to produce biofilm. The shown decrease capacity for biofilm production to increase salt concentration by *Salmonella* spp. is also a result of possible interest in food industry.

P833

Effect of the acquisition of quinolone resistance in biofilm formation by *Acinetobacter baumannii* clinical isolates

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Objectives: The ability of *Acinetobacter baumannii* to form biofilm could, in part, explain the long survival of this microorganism in hospitals. The objective of this work was to analyse the potential relationship between the acquisition of quinolone resistance and biofilm production in *A. baumannii*.
Methods: Biofilm formation was determined in 88 epidemiologically unrelated *A. baumannii* strains. Two quinolone-susceptible and biofilm-producing *A. baumannii* strains (A15-43 and 77) were chosen to select for quinolone-resistant mutants with clinafloxacin and ciprofloxacin, respectively. Biofilm: An overnight culture was diluted 1:100 in LB broth and incubated stagnant at 37 °C for 24 and 48 h. The biofilm was stained with 1% crystal violet and quantified at 570 nm after solubilization with ethanol–acetone. Two-dimensional gel electrophoresis: cell envelope proteins were purified and analysed by two-dimensional gel electrophoresis, followed by protein identification using MALDI-TOF/TOF mass spectrometry.
Results: Thirty-eight percent of the strains were ciprofloxacin-susceptible and biofilm-positive while 20.5% of the strains were ciprofloxacin-resistant and biofilm-positive. The relationship between biofilm formation and ciprofloxacin susceptibility was statistically significant ($p = 0.0014$). The quinolone-resistant mutants with a MIC for ciprofloxacin of 64 mg/L lost the ability to form biofilm. The comparative 2D gel electrophoresis between the wild-type and mutant strains showed differences in the expression of several proteins. One had a high homology with CsuA/B, a protein involved in type 1 pili formation. This protein appeared in the gel of the wild-type strains but it did not appear in the gel of their quinolone-resistant mutants.
Conclusion: Quinolone-resistant *A. baumannii* strains are less prone to produce biofilm than their susceptible counterparts. This association is linked to a decreased expression of type 1 fimbrae, the first step in biofilm formation. Therefore, the results obtained suggest that there is a relationship between biofilm formation and resistance to quinolones.

P834

Virulence factors of *Proteus mirabilis* involved in encrustation of urinary catheters

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Objectives: Catheter blockage by crystalline *Proteus mirabilis* biofilm is a major complication in long-term catheterized patients. These bacteria adhere to catheter surface and form biofilm communities embedded in a polysaccharide matrix. The bacterial urease generates ammonia from urea and elevates the pH of the urine and biofilm. Under this conditions calcium and magnesium phosphates crystallize and became trapped in the matrix. It blocks urinary catheter, causes obstruction of urine flow which can induce pyelonephritis, septicemia and urinary

calculi. The aim of this study was to establish a role of *P. mirabilis* major virulence factors in encrustation of catheter surface.
Methods: *P. mirabilis* strains were recovered from Foley catheters of long-term catheterized patients. The *in vitro* model was used to analyse formation and crystallization of *P. mirabilis* biofilm. Catheter fragment was sonicated and the fluid was diluted and plated to establish the number of bacteria. Crystallization was determined as the amount of calcium and magnesium ions by atomic absorption spectroscopy. Activity of urease was tested using method of Weatherburn. Colorimetric method with Alcian Blue dye and enzyme-linked lectinsorbent assay were used to study the ability of these bacteria to form exopolysaccharides.
Results: It was found that all of tested *P. mirabilis* strains show urease activity, form biofilm and cause encrustation of catheter surface. It has been shown some differences in intensity of crystallization between the strains which may depend on capacity to colonization of catheter surface and the presence of bacterial polysaccharides. These strains produced polysaccharides, but only three of them in significant amount. *P. mirabilis* strains producing polysaccharides had the strongest ability to catheter encrustation, although urease activity of these strains was low. It was also shown no correlation between adhesion ability, urease activity of *P. mirabilis* and intensity of crystallization.
Conclusion: Our observations suggest that in encrustation of catheter by *P. mirabilis* macromolecules, e.g., polysaccharides on bacterial surface may play an important role in enhancement of crystallization initiated by urease activity.

P835

In vitro biofilm formation by *Candida* spp. on the surface of polyurethane and PVC catheters

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Objective: Catheterized patients have a significantly increased risk of infection. There are many associated risk factors such as the frequency of lines changes, the duration of catheterization and the type of catheter used. The aim of this work is to study the biofilm formation by different species of *Candida* on the surface of two catheter materials such as polyurethane and Polyvinyl chloride (PVC).
Material and methods: Seventy-two clinical isolates of *Candida* and five from ATCC collection were used in this study (22 *C. albicans*, 22 *C. parapsilosis*, 16 *C. tropicalis* and 17 *C. glabrat*). All yeasts were grown in Sabouraud dextrose broth medium (SDB) and incubated at 35 °C in an orbital shaker at 60 rpm. Cells were harvested after 18 h of incubation, washed twice with PBS pH 7.2 and cell suspensions were adjusted to an optical density of 1.0 at 540 nm. Catheters were cut into 1 cm segments and sterilized with ethylene oxide. Standardized cell suspension 1 ml was applied to each segment and incubated for 90′ at 35 °C for adhesion period. Segments were then incubated for up to 72 h at 35 °C, in 1 ml of SDB with 500 mM galactose. After biofilm formation, catheter segments were removed and washed with PBS to remove nonbiofilm cells. To determine the number of viable cells attached, segments of catheter were washed, placed in 2 ml of PBS and vortexed for 2 min. After dilution with PBS, yeast suspensions were plated onto Sabouraud agar plates, incubated at 35 °C for 24 h.
Results: Results are expressed as logarithm of viable cells count for ml (CFU/ml) for each species. For polyurethane the mean CFU/ml for each specie (as expressed in log) were 12.329, 12.559, 12.760 and 11.768 for *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata,* respectively. For PVC the results were 12.969, 13.258. 12.626 and 12.516 for the same species. Evaluation of two catheter materials with analysis of variance showed that biofilm formation by *Candida* species was slightly increased on PVC, compared with polyurethane. The differences between materials were significatives ($p < 0.05$) for *C. parapsilosis* and *C. glabrata*. Biofilm formation by *C. glabrata* gave less biofilm growth than the other *Candida* species. *C. parapsilosis* showed the greater biofilm formation in PVC and *C. tropicalis* in polyurethane. Scanning electron microscopy showed that after 72 h *Candida* spp biofilms consisted of a dense network of yeast, pseudohyphae, and hyphae. Slime production was visible on the surface of two materials.

P836

Biofilm production in well characterised *Candida* spp. isolated in Italy during the period 2002–2005 from paediatric and adult patients

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Objectives: To assess the rate of biofilm production in a large number of previously well characterised *Candida* spp. isolated from clinical specimens in adult and paediatric patients. To establish whether a correlation exists between biofilm production, resistance, site of infection and age of patients.
Methods: Five hundred and thirty-five *Candida* spp., including 418 *C. albicans*, 108 *C. glabrata*, 45 *C. tropicalis*, 43 *C. parapsilosis* and 11 *C. krusei*, isolated from blood, urine, vaginal swabs and respiratory samples have been studied. Two biofilm producing *C. albicans*, kindly provided by Jin et al. (2003), were used as positive controls. Biofilm production was quantified spectrophotometrically.
Results: Production of biofilm was more common among non-albicans species (72.9%) than among *C. albicans* (46.4%) ($p < 0.001$). In particular *C. tropicalis* and *C. parapsilosis* produced more biofilm than *C. albicans* and *C. glabrata* ($p < 0.001$). No significant differences in biofilm production were observed among *C. albicans* isolates from different specimens with the exception of *C. albicans* strains from respiratory samples vs urinary tract isolates or after grouping the strains according to the patient's age. Similar results were obtained for the other species studied. *C. tropicalis* isolates resistant to one or more antifungal drugs (fluconazole, ketokonazole, flucytosine and amphotericinB) were found to be more frequently biofilm producers than susceptible strains ($p < 0.001$). This correlation was not observed for the other species.
Conclusion: Biofilm production was more related to the species of *Candida* than to the site of infection. There were no important differences in biofilm production when grouping the strains according to the patient's age, site of infection and antifungal susceptibility patterns, with the exception of antifungal resistant *C. tropicalis* strains that produced biofilm more frequently than susceptible strains.

P837

Biofilm communities on different construction materials

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Introduction: Biofilm forms when bacteria adhere to surfaces in aqueous environments and begin to excrete a slimy, glue-like substance that can anchor them to surfaces. The formation and presence of biofilms in water systems have been reported

frequently. The aim of this study is to determine the material dependence of biofilm and select the appropriate manufacturing material for water systems.
Methods: The study was performed using polypropylene re-circulating model system under constant hydraulic conditions, which was fed up with tap water. Coupons of each material were removed monthly from the system. Biofilms on surfaces were scraped by sterile swab and suspended in phosphate buffer and vortexed for 60 s. Faecal and total coliforms (FC,TC), aerobic mesophilic heterotrophic bacteria (AMHB) (22–37 °C), Pseudomonas, amoeba adhered to surface on different materials [polyvinyl chloride (PVC), polyethylene (PE), polypropilene (PP), galvanized steel (GSS), stainless steel (SS), copper (Cu), glass (G), ceramic] in model system have been examined during 6 months period. On the other hand, the same microorganisms have also been examined in the water at same model system. Microorganism counts in the water were determined with membrane filtration method. Media used were m.FC-NKS for FC, Endo-NKS for TC, R2A agar for AMHB, Cetrimide Agar for Pseudomonas and NNA for amoeba. Cultures were incubated at suitable temperatures and periods. After incubation, suspicious bacterial colonies were counted by a Colony Counter, cultures of amoeba were examined microscopically (10×).
Results: Neither FC nor Pseudomonas have determined on all the material during 6 months periods. In this period, it has determined that TC have increased gradually, have arrived at maximum level at the fourth month, and have possessed on Cu material at high level. It was found that AMHB counts have reached maximum level at the second month, decreased later, and have accumulated on GSS at high levels. Although amoeba found on all materials during 4 months, they have not been found on PVC, PP, PE at fifth and sixth month. It has revealed that GSS generally enhance to biofilm formation significantly. On the other hand, plastic polymers, especially PVC were supported the lowest bacterial numbers.
Conclusion: GSS surfaces support more microorganisms than do other materials. We think that PVC can be used in the water systems reliably because it supports less biofilm.

P838

Biofilm-positive microbes isolated from the environment of life-boxes for allogenic transplantations and from immunocompromised patients

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The patient after an allogenic transplantation is immunosuppressed and whatever microbe which is normally considered as non-pathogenic, can cause an infection. The life-boxes prevent their contact with potential pathogens to avoid risk of infection. We focused on the biofilm-forming microbes in life-boxes for allogenic transplantations, because the biofilm formation is an important factor of pathogenicity in all microorganisms. Biofilm protects bacteria against actions of antibiotics and disinfectants and therefore bacteria can survive concentrations of antibiotics and disinfectants even 1000× higher than planktonic forms of the same bacteria. The aim of the study was to compare microbial contamination of the environment of the life-boxes for allogenic transplantations with standard hospital wards of the same clinic. From October 2004 to January 2005 we collected 60 samples from standard hospital unit and from life-boxes for allogenic transplantations of the Clinic of Internal Medicine—Haematology and Oncology. After isolation of individual strains, the microbes were determined and tested for the ability to form biofilm by the modified Christensen's method. From 60 samples we isolated 116 different strains of microbes. In 49 (81.7%) samples bacteria were present, only 11 samples were sterile (18.3%). The isolated strains were mostly Gram-negatives (*P. aeruginosa*, *S. maltophilia*, *K. pneumoniae*, *S. marcescens*, *E. aerogenes*, *P. mirabilis*, *Acinetobacter* sp.), of Gram-positive bacteria, *S. epidermidis*, *M. luteus* and *Bacillus* sp. were isolated. The most frequently isolated species were *P. aeruginosa* and *S. maltophilia*. The ability to form biofilm was demonstrated in 71.2% of strains isolated from life-boxes and in 34.5% of strains isolated from standard wards. The different number of isolated strains on both departments may be caused by different sanitation modes. The higher sanitation standards in life-boxes might cause higher proportion of resistant biofilm-positive bacteria, although the absolute number of bacteria is lower in comparison with standard hospital wards. Although all microbes which we isolated from the hospital environment are generally considered as low pathogenic or non-pathogenic, the presence of virulence factors increases their clinical importance and these strains represent risk of infections and colonization of indwelling devices, particularly for immunocompromised patients.
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P839

The effectiviness of impregnation of graft with cefazolin in foreign body infection

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Objective: Foreign body infection (FBI) is a real problem in clinical situations. In the present study, *in vitro* and *in vivo* efficiacy of impraganation of mesh with cefazolin in prevention of FBI.
Materials and methods: Strain: The microorganism was slime positive, methicillin-resistant *S. epidermidis* (MRSE). Impregnation: Cefazolin impregnated grafts were prepared by dipping method. Naive meshes, in size of 5 × 10 mm were immersed into the polylactic acid (PLA) solution in dichlorometahane including cefazolin with different concentrations (i.e., 0.025, 0.05 and 0.3 g sefazolin/5 ml and 2% w/v of PLA solution). Cefazolin impregnated and naive grafts with different concentration (Group 1 = 0.025 g, Group 2 = 0.05 g, and Group 3 = 0.3 g) were incubated with 108 cfu/ml slime positive MRSE. *In vitro*: After 24 and 48 hours of incubation, the numbers of colonies were counted in an aliquot and adhered to catheter. *In vivo*: Contaminated naïve and cefazolin-impregnated grafts (n = 10 in each groups) were implanted subcutaneously in the back of Swiss albino mouse. Grafts were explanted at 7 days following implantation. Microbiologic assessments and electron microscopic (JOEL JSM-5600 Japan) evaluation of catheter segments were performed.

	Comparisons of Groups	
Adherent bacterial counts at 24th hours	Gp 1 vs Gp3	P=0.019
	Gp 2 vs Gp 3	P=0.017
Adherent bacterial counts at 48th hours	Gp 1 vs Gp 3	P=0.020
	Gp 2 vs Gp 3	P=0.623
Count of bacteria in aliquot at 24 hours	Gp 1 vs Gp 3	P=0.019
	Gp 2 vs Gp 3	P=0.019
Count of bacteria in aliquot at 48 hours	Gp 1 vs Gp 3	P=0.027
	Gp 2 vs Gp 3	P=0.037

Results: Impregnation of cefazolin decreased the numbers of adherent bacteria to the grafts and the number of free bacteria within the liquid medium significantly in all groups. In all comparisons; the decrease of bacterial counts were statistically significant, except the values at 48 hours of group 2 vs group 3. Cfu counts in explanted grafts were significantly less in treatment than in control group and wound infection rates also decreased, accordingly.
Conclusions: Impregnation of cefazolin to grafts by dipping method may protect against FBI with MRSE during peri-operative period.

P840

Biofilm production by different strains of *Salmonella typhimurium*: genetics, morphology and role of surface and nutrient content of medium

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Objectives: To investigate the ability of biofilm production by 14 different strains of *Salmonella typhimurium* in depending on genotype and culture conditions in artificial systems: in 96-well plastic microtitre plates, plastic and glass tubes, plastic Petri dishes and on microscope glasses.
Methods: Quantitative biofilm growth was monitored by using an assay based on crystal violet staining, while planctonic growth in the same cultures was monitored by absorbance in iEMS Reader MF, and qualitative biofilm growth—by digital photo and visually. Morphology of the planktonic cells and cells in the biofilm was investigated by methods of transmission microscopy of ultra-thin cessions.
Results and conclusions: Optimal rate between growth and biofilm indications for all strains was determined at initial cell concentration 106-7 KOE/ml and T°= 28 °C culture incubation. The nutrient content of the medium significantly influenced the quantity of produced biofilm. The nutrient broth LB without NACl was the most effective in promoting biofilm formation, than LB itself. The least quantity of biofilm was formed in water. The chemical content of plastic and glass also influenced biofilm formation. The ability to induce biofilm on the walls of plastic tubes is very suitable for visual screening of a number of strains. We used this property for the testing of influence of some proteins on the biofilm production intensity. Plastic tubes were treated before incubation by 1% BSA or milk proteins and sterilized by ethanol. It seems to be, that protein pretreatment of plastic surfaces increased adhesion and biofilm production. It is possible to conclude the more effective biofilm production on surfaces, contacting to blood proteins or food products. The genotype of the strains also critically influenced the quantity of produced biofilm. Nonmotile mutants cells reduced ability to form biofilm. RpoS mutant cells produced significantly less biofilm as compared with cells of isogenic parent strains. The morphology of *S. typhimurium* grown as biofilm or as planctonic cells was also different.

P841

Biofilm-associated anaerobic sulphate reducing bacteria on galvanized steel surfaces

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Objectives: Sulphate reducing bacteria (SRB) are found in marine and freshwater sediments and even in human intestine. Anaerobic SRB conduct dissimilatory sulphate reduction to obtain energy, resulting in the release of a great quantity of sulphide (H_2S). H_2S is toxic to human cells. While H2S causes ulcerative colitis (UC) in human, SRB trigger liver abscess and septicaemia. In addition to the effect of H_2S, extracellular polymeric substances produced by SRB may contain highly immunogenic O-antigen. In addition to the effect of H_2S, extracellular polymeric substances produced by SRB may contain highly immunogenic O-antigen. Such antigens may cause an immune response in genetically predisposed individuals and initiate to the inflammatory process characteristic of UC. Also it has been reported that SRB cause corrosion of various metals including carbon steels, stainless steels and copper alloys. Galvanized steel (GS) is frequently used in the construction of water containers and pipes, etc. owing to its good resistance to corrosion and biofouling. In this study, survival and enumeration of the SRB and heterotrophic bacteria were investigated on GS coupons in biofilm reactor during 180 days. Also total carbohydrate amount in biofilm was detected.
Methods: Biofilms were allowed to develop for 180 days on galvanized steel coupons within annular biofilm reactor. For the enumeration of heterotrophic bacterial counts, samples are plated on R2A agar for 10 days at 27 °C. Postgate medium B was used for SRB cultures. Numbers of cultivable SRB were determined by the most-probable-number technique. The amount of carbohydrate on coupons was quantitated colorimetrically using the phenol-sulphuric acid method.
Results: There was a gradual increase in growth of SRB with the time, similarly to heterotrophic bacteria. The cell concentrations of SRB increased to a maximum of 140,000 SRB/cm^2 after 180 days. The carbohydrate contents on coupons were correlated with heterotrophic counts ($R = 0.91$, $P < 0.05$). After 90-day period sessile heterotrophic counts and carbohydrate quantity have reached to plateau stage.
Conclusion: GS is frequently used in construction of water containers which provide ideal conditions for growing of microorganisms especially SRB. Our findings suggest that SRB cells attached and located in biofilm on the GS surfaces even though the toxic effect of zinc. This study points out that because of SRB could be colonize on GS easily, they form a risk for human health.

P842

Mechanism and effects of biofilm formation on fat-based materials

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Objectives: Non-petroleum based polymers are attracting more attention nowadays. For this group of polymers, especially fat based polymers, find a great application areas. After literature search no related work was found about biofilm formation on fat based polymers. Therefore, the adhesion affinities of different candidate materials were tested regarding bacterial accumulation using modified robbins device (MRD). Fat based polymers that have different functional groups were compared against changes on their surfaces by the aid of multiple internal reflectance (MIR) spectroscopy before and after the experiments.
Methods: The biofilm was established under standard hydraulic conditions and tubular pipe flow using a MRD. Discs of different material are immobilized on MRD plugs and the experimental setup was run 60-day period. The device is connected to the distributed drinking water network. Twelve different materials [acrylated-ESO (epoxidized soybean oil), acrylated-ESO co-styrene, acrylated-ESO co-acrylic acid, ESO-phtalic anhydrite, PEG-400 modified ESO co-styrene, ESO-malonic acid, ESO-triethylenetetraamine, ESO-hekzamethylen-ediamine, ESO-bis-phenol, polyvinylchloride, polyurethane,

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stainless steel] were tested in this study. The bacterial numbers were determined by heterotrophic plate counting (HPC) after 30- and 60-day periods. Extracellular polymeric carbohydrates on surfaces were analysed by phenol-sulphuric acid method. The polymeric materials were analysed by MIR to determine whether the chemical modifications were present or not on the surfaces.
Results: We found the amount of bacterial accumulation on the surfaces is largely depends on the functional groups at the surfaces and some chemical modifications on the surfaces took place. Significantly high count of bacterial accumulation was found on PEG 400 ESO after two periods. On ESO acrylic acid and bis-phenol, significant differences were observed between 30- and 60-day periods.
Conclusion: Bacterial accumulation was higher on the functionalized surface than neutral surfaces. Amine functionalized surfaces show the highest accumulation. The positive charge, which causes from the ionization of amine groups, on the surfaces would attract negatively charged bacteria. When bacteria invade the surface they would use the fatty parts of polymers as a nutrient.

P843

Impact of daptomycin compared to linezolid, vancomycin, gentamicin, rifampin and ceftriaxone on resolution of staphylococcal adherence from surface of *in vitro* Silastic Antibiotic Lock and Prosthetic Vascular Graft biomedical devices

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Introduction: Infection of intravascular or implanted biomedical devices often involves biofilm-forming staphylococci, recalcitrant to antimicrobial therapy.
Objective: The present investigation compares the antimicrobial activity of Daptomycin to five selected anti-infectives in resolving staphylococcal adherence from surface of biomedical devices.
Methods: Five staphylococcal (four *S. epidermidis* and one *S. aureus*) strains were selected for in-vitro adherence studies: (a) ALM (silastic catheter) and (b) VGM (Dacron and polytetrafluoroethylene). RP62A, M187sp11 and Sef141-98 are strongly adherent strains, producing a copious biofilm, while Sef141-98 and *S. aureus* ATCC 25923 are both negative for biofilm formation. Test strains were inoculated for 30 min (4.5 log10/cfu) to ALM and VGM, rinsed 3×, incubated in TPN (Ca^{2+} = 3 mEq/L) solution (ALM) or phosphate-buffered saline (VGM) with dextrose (0.75%) for 12 hours (35 °C) to stabilize microbial (biofilm) adherence. Following stabilization, anti-infectives were added at concentrations 20–40 × MIC. Antibiotic solutions were replaced daily and antimicrobial impact on bacterial adherence assessed at 0, 2, 4, 7 and 10 days postinoculation. Ten specimens were evaluated at each time interval. Selected samples were prepared for SEM.
Results: (a) ALM: Daptomycin = 100% reduction in bacterial adherence by day-4; Vancomycin = 90% reduction by day-10; Ceftriaxone = 85% reduction by day-10; Rifampin = 100% reduction by day-4; Gentamicin = 100% reduction by day-7; and Linezolid = 100% reduction by day-7. (b) VGM: Dacron—Daptomycin = 100% reduction in bacterial adherence by day-4; Vancomycin = 90% reduction by day-10; Ceftriaxone = 78% reduction by day-10; Rifampin = 100% reduction by day-4; Gentamicin = 95% reduction by day-10; and Linezolid = 100% reduction by day-7. PTFE—Daptomycin = 100% reduction in bacterial adherence by day-2; Vancomycin = 100% reduction by day-7; Ceftriaxone = 90% reduction by day-10, Rifampin = 100% reduction by day-4, Gentamicin = 100% reduction by day-7; and Linezolid = 100% reduction by day-2.
Conclusion: Daptomycin, rifampin and linezolid demonstrated greater efficacy in reducing microbial adherence of both biofilm negative and positive strains on surface of selected biomedical substrates compared to vancomycin, gentamicin or ceftriaxone ($p < 0.01$). Further studies are warranted, validating the clinical efficacy of Daptomycin in the treatment of biomedical device-associated infections.

P844

The development of an *Aspergillus fumigatus* biofilm model to determine the effectiveness of antifungal treatments *in vivo*

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Objectives: To devise an *in vitro* biofilm model of *Aspergillus fumigatus* to allow the examination of the ability of antifungal agents to inhibit/kill these biofilms.
Methods: Polystyrene microtitre plates were selected to grow biofilms upon, based on previous studies for high throughput analysis of biofilms. Spores were collected from NCPF 7367 and standardized at different densities in RPMI. Biofilm growth kinetics were then observed microscopically over 48 h (1, 2, 4, 6, 24 and 48 h) using a metabolic (XTT) and a biomass assay (crystal violet). Standardized biofilms formed from NCPF 7367 and several clinical isolates were then treated with antifungal agents for 48 h and assayed for viability and biomass. We also examined the effects of biofilm development when antifungals were added at 0, 1, 2 and 4 h postspore inoculation. Following 24 h incubation, the antifungal was removed and replaced with fresh RPMI, and biofilm development reassessed.
Results: Spore concentrations of 1×10^6/ml produced the optimal confluent biofilm after 24 h. Biofilm development, assessed by both metabolism and biomass, indicated a slow increase from 0 to 6 h, exponential growth to 24 h, followed by a plateaus. Treatment of mature biofilm structures with amphotericinB, voriconazole and caspofungin was ineffective at clinically achievable concentrations; even at high dosages both metabolism and biomass only decreased minimally. When voriconazole was added during the early stages of adhesion, a dose-dependant effect was observed, and appeared effective at therapeutic concentrations. Replenishment with fresh media allowed biofilm growth in at all concentrations.
Conclusions: We have developed a reproducible and robust assay to analyse *A. fumigatus* biofilms. The results suggest that treatment of mature *A. fumigatus* biofilms with antifungals is futile. Nevertheless, voriconazole could be used as a prophylactic. The use of this antifungal on early exposure to spores prevented filamentation and subsequent biofilm formation. The continued use of voriconazole would be advocated, as removal of the drug permits biofilm regrowth. Overall, voriconazole appears to offer excellent prophylactic properties against invasive *Aspergillosis*.

P845

Influence of subinhibitory vancomycin concentrations on biofilm formation in coagulase negative *Staphylococci*

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Objectives: Detection of vancomycin MICs changes in coagulase negative *Staphylococci* (CoNS) growing in biofilm and in

planktonic cultures. Influence of pretreatment with vancomycin subinhibitory concentrations on biofilm formation with respect to use of vancomycin in prophylaxis.
Methods: Eighty-two CoNS strains isolated from joint prostheses infections of patients from first orthopaedics clinic were identified by standard microbiological methods. Antimicrobial susceptibility was tested according to NCCLS. MICs to vancomycin (VAN) were established by broth dilution according to NCCLS, as well as by the E-test (AB Biodisk, Sweden). Biofilm production was detected by use of congo red agar plates, Christensen method and microtitre plate biofilm assay. Effect of VAN on CoNS growing in biofilm was detected by modified microtitre plate biofilm assay: biofilm was formed in 96-well microtitre plates, and than VAN was added in different concentrations. Results were expressed as minimal biofilm inhibitory concentration (MBIC), and minimal biofilm eradication concentration (MBEC). Influence of pretreatment with VAN subinhibitory concentrations was detected in all strains: biofilm formation was detected by microtitre plate assay, which followed after overnight cultivation of tested strains on solid medium with VAN in subinhibitory concentrations.
Results: Biofilm production was detected by three *in vitro* methods. There were differences in amounts of produced biofilm when tested by different methods. The microtiter plate assay, which detected biofilm production in all of the tested strains, seemed to be the most susceptible one. Detected MBICs were higher than MICs in most of the strains. The greatest differences were observed in strains with most extensive biofilm production. MBECs were 2–32 times higher than MBICs. In 50 (61%) strains, pretreatment with VAN in subinhibitory concentrations considerably decreased the amounts of produced biofilm.
Conclusions: The most susceptible method for biofilm detection was the microtiter plate assay. According to our results, for therapy of endoplastitis caused by CoNS is not advisable to use therapeutic guidelines based on MICs obtained in planktonic cultures. Individual testing of MBEC should be necessary for successful therapy of endoplastitis. Subinhibitory concentrations of VAN decrease biofilm production by CoNS; prophylaxis by VAN during arthroplasty may have protective effect.

Brucella and Lyme borreliosis

P846

Brucellosis: retrospective evaluation of 133 hospitalized cases

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Objectives: In this study, we aimed to evaluate the clinical, laboratory and treatment data of our cases with brucellosis and to compare them with the data of other centres.
Methods: Medical records of 133 patients hospitalized between January 2003 and May 2005 with the diagnosis of brucellosis were reviewed. One of the two diagnostic criteria was required along with the clinical findings; one being a SAT result of 1/160 or higher and the other is *Brucella* spp. isolation in automated system blood culture bottles. Complete blood count, erythrocyte sedimentation rate, CRP value, liver function test results on admission for each patient were recorded. Cases presenting with duration of symptoms less than 8 weeks were considered to be acute, duration of 8 weeks to 1 year subacute and the duration of more than 1 year was considered to be chronic.
Results: Eighty-one (61%) of the cases were female. Mean age was 44.7 years. Eighty-four (63%) of the cases were diagnosed as acute, 35 (26%) were subacute and 14 (11%) were chronic brucellosis. History of fresh cheese or diary product consumption was positive in 93 (69%) of the cases. Seventeen (13%) of the patients were cattle-dealer and four of the patients had occupational risks (two veterinary surgeons and two laboratory technicians). The most common symptom was fever (68%), others were night sweats (64%), joint pain (52%), low back pain (52%) and malaise (42%). The most common complications were related to the haematopoietic system (38%). Anaemia was seen in 41 cases, leukopenia in eight cases, and thrombocytopenia in two cases. Elevation of erythrocyte sedimentation rate and CRP were noticed in 61 (46%) and 46 (35%) of the cases, respectively. Blood cultures grew *Brucella* spp. in 53 (40%) of the cases. Second most commonly involved site was osteoarticular system with 34 cases (26%). Nineteen cases had spondylodiskitis, seven sacroileitis, three peripheric arthritis, four spondylodiskitis and neurobrucellosis, one spondylodiscitis and sacroileitis. Eight (6%) patients had neurological involvement and five (4%) had genitourinary system involvement. The most commonly prescribed regimen was doxycyclin plus rifampicin and streptomycin was added in most of the complicated cases. Relaps was seen in 14 (11%) of the cases. The most common side effect was gastrointestinal intolerance.
Conclusion: Brucellosis can present with various clinical forms in endemic areas and mimics several diseases.

P847

Brucellar epididymo-orchitis in South-eastern Anatolia, Turkey

M.K. Celen, M.F. Geyik, C. Ayaz, S. Hosoglu, M. Ulug *(Diyarbakir, TR)*

Objective: The different clinical and laboratory features and response to treatment of patients with acute brucellar epididymo-orchitis reporting to a reference hospital in South-eastern Anatolia of Turkey.
Patients and methods: In this study, 22 of 191 adult patients with brucellosis, who presented with epididymitis or epididymo-orchitis at a university hospital in Diyarbakir from 1998 to 2004, were included. Positive blood culture or high agglutination titres of 1:160 and positive clinical manifestations of brucellosis were the main criteria for diagnosing brucellosis.
Results: Epididymo-orchitis occurred in 22 patients (11.5%) of 191 with brucellosis. Most (68.2%) were 18–34 years old. All patients complained of swollen painful testicles. Other presenting symptoms included undulant fever (90.9%), sweating (63.6%) and arthralgia (13.6%). Sixteen patients gave a positive history of ingestion of raw milk and milk products. Ten patients had unilateral epididymo-orchitis; the remaining 12 had only orchitis (bilateral in two, right in seven and left in three). Leukocytosis was present in two patients; 18 had initial agglutination titres of 1:160 and the remaining patient had a positive blood culture. All patients received combined therapy with streptomycin for the first 21 days (or oral rifampicin for

6–8 weeks) with doxycycline or tetracycline for 6–8 weeks. All showed improvement, fever subsided in 2–4 days and the scrotal enlargement and tenderness regressed. Only one patient had a relapse within 1 year.
Conclusion: In brucellosis-endemic areas, clinicians encountering epididymo-orchitis should consider the likelihood of brucellosis. Conclusively, brucellosis must be considered as a cause of orchitis in especially endemic regions like Turkey. The incidence of epididymo-orchitis was determined 11.5% in our region. Most of the cases (90.9%) were unilateral. All patients respond to medical management very well. Conservative management with combination antibiotic therapy is adequate for managing brucellar epididymo-orchitis.

P848

Neurobrucellosis: experience with eight cases

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(Diyarbakir, TR)

Objectives: Brucellosis is a common infection in Turkey and a serious medical problem. Neurobrucellosis is a rare manifestation of the diseases. The aim of this study is the presentation of the cases with neurobrucellosis in our clinic.
Methods: Eight patients with neurobrucellosis, which were followed during the period from 1998 to 2005 in our hospital, were investigated retrospectively.
Results: Five of all patients were female and, three patients were male. Mean age of patients was 34.7 ± 11.4 (range, 24–54) years old. Touch on an animal was present all of patients and eating of fresh cheese story was determined in six patients. All of patients had complaints and clinical findings of brucella infection. All of them applied to us with symptoms of meningitis. In seven cases meningitis and one of them meningoencephalitis, were determined. Cerebrospinal fluid (CSF) findings were abnormal in all of them. When the CSF vas investigated 40–230 leucocyte/mm^3 and erythrocyte 0–60/mm^3 in CSF, increasing of CSF glucose (range, 87–423 mg/dl), decreasing of CSF/plasma glucose ratio (range, 15–38%), were determined. While serum brucella antibody was positive in all of them (standard agglutination ≥1/160), brucella antibody was positive too in CSF at three patients (standard agglutination ≥1/40). Trio combinations of antibiotherapy (rifampicin, doxocycline and cotrimoxazole/streptomycin/ceftriaxone) were given for 3 or 6 months to all patients. Prednisolone 60 mg/day was given to a patient which myelitis. The response of the therapy was very well in all patients. The therapy was completed and CSF findings were improved in all patients except one. Motor dysfunction and losing of muscle force was developed in lower army in this patient which was meningoencephalitis.
Conclusion: Neurobrucellosis is a rare but serious complication of brucellosis and a diagnostic delay can enhance residual disturbances.

P849

The effects of oxidative stress in patients with infection of *Brucella melitensis*

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Objectives: In Turkey, brucellosis, mainly produced by *Brucella melitensis* (*B. melitensis*), is one of the most important zoonoses causing severe morbidity in humans. The infection in humans is commonly acquired by drinking unpasteurized milk or eating milk by-products derived from infected goats or cows. Oxidative stress has been suggested to play a role in some physiological conditions and infectious diseases. The present study aimed to determine the effect of infections with *B. melitensis* on antioxidant enzymes and malondialdehyde (MDA) in the serum samples of patients.
Methods: The blood samples were inoculated at a volume of 10 ml into BACT/Alert Plus+Aerobic/F blood culture bottles. When growth was detected, identification of brucellae was performed by H_2S, urease production, and dye tests. Superoxide dismutase (SOD) activity was measured according to the method described by Fridovich. Catalase (CAT) activities were determined by measuring the decrease in hydrogen peroxide concentration at 230 nm by the method of Beutler. The protein concentration of the tissue was measured in digital Spectronic-20 spectrophotometer by the method of Lowry.
Results: Patient group consists of 14 (60.8%) male and 9 (39.1%) female, control group were consist of 11 (55.0%) male and 9 (45.0%) female. There was no difference with respect to age, sex, smoking status and body mass index among the patients and healthy subjects. This parameters were taken out of evaluation which can be thought to have effects on oxidative stress. As shown in Table 1, the antioxidant enzyme activities and MDA levels were higher in serum samples from patients with *B. melitensis* compared to that from healthy subjects ($p < 0.05$).

Tablo 1: Activities of catalase and superoxide dismutase and malondialdehyde levels in both group

	n	CAT	SOD	MDA
Patients	23	*5,96±0,13	*7,27±0,24	*4,65±0,73
Control	20	2,33±0,50	3,15±0,53	2,20±0,28

*p<0.05

Conclusions: Cells and biological fluids have an array of protective antioxidant mechanisms such as glucose-6-phosphate dehydrogenase, SOD, CAT and also reduced glutathione both for preventing the production of free radicals and for repairing oxidative damage.

P850

Doxycycline plus streptomycin versus ciprofloxacin plus rifampicin in spinal brucellosis

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Objectives: The optimal treatment regimen and duration of the therapy is still controversial in spinal brucellosis. The aim of this study is to compare the efficacy, adverse drug reactions, complications and cost of ciprofloxacin plus rifampicin versus doxycycline plus streptomycin in the treatment of spinal brucellosis.
Methods: The patients diagnosed as spinal brucellosis between January 2002 and December 2004 were enrolled into the study. Patients were enrolled into the two antimicrobial therapy groups (doxycycline plus streptomycin vs. ciprofloxacin plus rifampicin) consecutively. All the patients had at least 12 weeks antibiotic therapy in the both group. The antibiotic therapy was prolonged according to clinical improvement and the resolution of magnetic resonance imaging findings. Furthermore, the patients were followed up until 12 months after cessation of

the therapy for the evaluation of sequelae and relapse. Only the cost of antibiotic therapy was analysed for each patient.
Results: During the study period, 31 patients with spinal brucellosis were enrolled into the two antimicrobial therapy groups. Fifteen patients were included in doxycycline plus streptomycin group and 16 patients were included in ciprofloxacin plus rifampicin group. Forty-two levels of spinal column were involved in 31 patients. The most common affected site was lumbar vertebra (n = 32, 76%) and involvement level was not different in two groups. The median duration of antibiotic therapy was 12 weeks (range 12–24 weeks) in all patients. Eight (25.8%) patients underwent surgical intervention. Despite the disadvantages (older age, more prevalent operation and abscess formation before the therapy) of the patients in the ciprofloxacin plus rifampicin group, the duration of the therapy (median 12 weeks in both groups) and clinical response were not different from the DS. The cost of ciprofloxacin plus rifampicin therapy was 1.2 fold higher than the cost of doxycycline plus streptomycin therapy.
Conclusion: Classical regimen (doxycycline plus streptomycin), with the appropriate duration (at least 12 weeks), is still the first line antibiotics and alternative therapies should be considered when adverse drug reactions were observed.

P851

Epidemiological features and clinical manifestations of adult brucellosis in Turkey

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Objectives: Brucellosis is one of the prevalent diseases in Turkey. The object of this study is to determine the epidemiological features and clinical manifestations of adult cases of brucellosis admitted to our Department of Infectious Disease and Clinical Microbiology.
Methods: A total of 324 patients diagnosed as brucellosis in our clinic between 1999 and 2005 were reviewed. The diagnosis of the cases was established by seropositivity and/or blood culture positivity. Duration of symptoms, clinical symptoms and signs, laboratory test results, clinical type of the illness (acute and subacute/chronic) and coexistence of complications were recorded. The chi-square, Fisher's exact tests and Student's *t*-test were used for statistically analysis.
Results: The mean age of patients was 44.02, b 18.31 (range 15–83 years). The male and female percentages were 56% and 44%, respectively. Animal husbandry (59%), consumption of raw milk and/or fresh cheese (25%), were the main risk factors for brucellosis while there was no risk factors in remaining 16% of patients. The percentage of patients who have/has brucellosis in her/his family member or surrounding area was 36.7%. Fever, sweating, fatigue and myalgia were the most frequent clinical symptoms. Fever was the most frequent physical finding. The percentages of acute, subacute and chronic cases were 61%, 24% and 15%, respectively. High fever was detected more frequently in the acute group of patients ($p < 0.05$). Complications were detected in 163 of 324 (51.5%) cases. Osteoarticuler involvement (25.2%), neurological involvement (6.2%), and genitourinary involvement (6.2%) were the most common complications. In 9.5% of patients, elevated ALT levels due to brucellosis were detected. Blood/bone marrow culture positivity rate was 45% (123/273 patients). Culture positivity was 56% and 27% in acute and subacute/chronic group of patients, respectively ($p < 0.0001$). The complication rate was higher in acute cases compared with subacute/chronic cases ($p = 0.0.3$). The male patients had more complications ($p < 0.01$). Age was not found as a risk factor for any complication ($p > 0.05$).
Conclusion: Most patients with brucellosis did have known risk factors. The main risk factors were animal husbandry and/or consumption of raw milk and/or fresh cheese. The complication rate is also quite high due to high prevelance of brucellosis in our country. It is important to determine the epidemiological features of the illness to control of the infection.

P852

Analysis of the risk factors for brucellosis in an endemic region

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Objectives: Early diagnosis and treatment of the acute brucellosis cases were targeted by screening the household members of the index cases. It was also aimed to describe the causal relations of acute brucellosis in an endemic region.
Methods: The study was performed in a brucellosis endemic country. The household members of the index cases were screened by agglutination test. The bacteria were isolated by blood culture. After the household members were screened, the risk factors for seropositive and index cases were studied by multivariate analysis. Independent variables were gender, consuming fresh cheese, blood groups, dealing with husbandry, contact with the placenta of the infected animals were included to the model. Backward and forward selection were performed.
Results: After admission of 30 index cases to the clinic, 112 household members of these cases were screened. Eighteen of 112 (16%) screened individuals had agglutination of >1/160. The mean age was 31 (SD 19), and 52% of the subjects were female. In multivariate analysis, consuming fresh cheese (OR: 3.2, CI: 1.01–10.4, $p = 0.047$), blood group A (OR: 2.4, CI: 1.06–5.4, $p = 0.035$), dealing with husbandry, and contact with the placenta of the infected animals (OR: 3.6, CI: 1.4–9.3, $p = 0.009$ were found to be associated with brucellosis. On the other hand, in univariate analysis, the individuals with blood group B were found to be protected from brucella infection ($p = 0.015$).
Conclusion: (1) Screening of the people in brucellosis endemic area should be considered, because of the opportunities for early diagnosis and treatment. (2) The people in endemic areas should be educated for not to eat fresh cheese and protect themselves from the infected animals (3) To our knowledge, the different blood groups were studied firstly by this study, and higher prevalence of brucellosis among the individuals with blood group A, and less prevalence among the individuals with blood group B should be considered for further studies on pathogenesis mechanisms.

P853

Neurologic involvement among brucellosis cases

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Objectives: To determine the neurological involvement among the brucellosis cases and describe the risk factors for the development of neurobrucellosis.
Methods: In patient brucellosis cases were followed up prospectively between 2002 and 2005 in an endemic region. The patients with serum culture positivity or Standard tube agglutination with Coombs (STA) >160 or fourfold increase of STA were included to the study. Neurologic involvement was defined

as (i) isolation of *Brucella* spp. from CSF, or (ii) demonstration of antibodies to Brucella >1/4 in the CSF and the presence of lymphocytosis, increased protein and decreased glucose levels in the CSF, or (iii) neurological findings not related to any other neurological disease. Risk factors for neurological involvement was analysed by logistic regression. Multivariate analysis was performed to determine the predictors of neurobrucellosis. Age, gender, numerous visits to physicians, brucellosis history, duration of symptoms were included to the model.

Results: Two hundred brucellosis patients were included. The mean age was 43, and 43% of the patients was female. The number of neurobrucellosis cases was 68 (34%). *Brucella* spp. was isolated in CSF of seven patients, STA in CSF was positive among 62 cases, and abnormalities in CSF findings were detected among 48 patients. In 12 cases, magnetic resonance and/or computerized tomography revealed useful findings. Headache was significantly more common among neurobrucellosis cases ($p = <0.001$). The most common neurological findings were meningeal irritation signs, unconsciousness, disorientation, confusion, paraparesia, incontinence, amenorrhea, diplopia, dysarthria, papilledema, hipo-hyperreflexia, areflexia, loss of hearing, cerebellar ataxia.The level of the aspartate aminotransferase was higher among neurobrucellosis cases ($p = 0.014$). Six patients with positive STA Coombs in CSF had isolated serious headache as the neurologic symptom. Multivariate analysis revealed that neurobrucellosis cases visited numerous physicians (OR: 3, CI: 1.17–7.5, $p = 0.021$), particularly psychiatrists. One out of 68 neurobrucellosis cases was died, three had neurological sequela.

Conclusions: (1) Magnetic resonance imaging and computerized tomography support the diagnosis of neurobrucellosis. (2) The patients with serious headache should be considered for neurobrucellosis in endemic regions. (3) STA in CSF could be positive without any neurologic signs in patients with systemic brucellosis.

P854

Clinical manifestations of Lyme borreliosis in Bulgaria and identification of *Borrelia* species in ticks

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Objectives: Data on disease expression and epidemiological characteristics of Lyme borreliosis in south-eastern Europe are scarce.

Methods: To reveal features of Lyme borreliosis in Bulgaria, clinical data and epidemiological characteristics of over 1200 patients were analysed. In addition, 300 Ixodes ricinus ticks collected in two consecutive years by flagging vegetation were examined by polymerase chain reaction and reverse line blot hybridization for the presence and identity of *Borrelia burgdorferi sensu lato* species.

Results: Among patients, the most affected age group was 5–9 years, followed by 45–49, 50–54, and 10–14 years. Lyme borreliosis cases occurred throughout the year with two peaks—one in June and second smaller one in September. The most common clinical manifestation was erythema migrans (EM), diagnosed in 69% of the patients. Multiple EM was detected in 4.3% of the EM cases. Neuroborreliosis was the second most common presentation of Lyme borreliosis, diagnosed in 19% of the patients. Lyme arthritis was found in only 8% of the patients. Heart and ocular manifestations were very rare. Analysis of *Borrelia* prevalence revealed that ticks collected from the same location and in the same month, but in two consecutive years, had different prevalences of *Borrelia* infection. *B. afzelii* was the predominant species, representing over 50% of all *Borrelia*-positive adult ticks. The second most frequently found species was *B. burgdorferi sensu stricto*. The prevalence of *B. garinii*, *B. valaisiana* and *B. lusitaniae* was about 5%. Approximately 6% of the adult ticks carried more than one *Borrelia* species simultaneously.

Conclusions: The high prevalence of *B. afzelii* is remarkable, since neuroborreliosis, most frequently associated with *B. garinii*, is the most common clinical manifestation of disseminated Lyme borreliosis in Bulgaria. It could be speculated that, in many cases, infections with *B. afzelii* are self-limiting, whereas *B. garinii* has a greater capacity to survive in the host, leading to progression of the disease.

P855

Seasonal patterns of the activity of host-seeking *Ixodes ricinus* ticks in a tick-borne encephalitis and Lyme borreliosis natural focus (Czech Republic)

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Objectives: The aim of our research is focused on the short-term risk assessment and prediction of day-to-day variations of *I. ricinus* questing patterns informing about the actual changes in the level of risk in tick-borne encephalitis and Lyme borreliosis natural foci due to the daily weather conditions. Field studies include also an examination of the closeness of relationship between the so-called macro-scale weather as presented by standard meteorological stations, and the authentic microclimate of a typical forest ecosystem containing *I. ricinus* ticks and tick-borne diseases pathogens.

Methods: Field observations were realized in the south-eastern periphery of Prague where the experimental plots for tick monitoring were established in relevant type of forest growth (Querceto- carpinetum).The occurrence of tick-borne encephalitis virus in *I. ricinus* ticks and human infection of Lyme borreliosis (caused by *Borrelia afzelii*) were reported from this area. *I. ricinus* activity was investigated by flagging method on three plots (200 m^2 each) in weekly intervals (March–November) during 2001–2005. The instruments for micrometeorological observations were installed between the experimental plots. Macrometeorological data were used from the nearby Czech Hydrometeorological Institute observatory. Simple and multiple linear regression and quadratic regression were used to test the relation between the weather and *I. ricinus* activity.

Results: Eight models of the relationships were constructed and tested, of which four were single-parametric and four were two-parametric. Double quadratic regression provided far the best results. The relationship between daily *I. ricinus* activity and weather variation can be described using two- to three-parameter models. Three following models were found as the best: (a) activity is quadratic function of temperature and soil moisture; (b) activity is quadratic function of temperature and relative humidity; and (c) activity is quadratic function of temperature and precipitation.

Conclusion: The relationship between weather and tick behaviour is solid enough to be used for the prediction of tick host-seeking activity and thus for the prediction of tick-borne diseases infection risk using macrometeorological data as a predictor.

Diagnositic and laboratory methods in parasites and fungi

P856

Diagnosis of *Giardia lamblia* with microscopy, striptest, ELISA and real-time PCR

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Objectives: *Giardia lamblia* is the most frequently diagnosed pathogenic intestinal parasite in the Netherlands. We compared four different diagnostic methods for the detection of *G. lamblia* in faeces in both acute and chronic diarrhoea.
Methods: Microscopic examination was carried out on stained samples collected with triple faeces test (TFT). ELISA (Novitec and Novatec Giardia lamblia ELISA), Giardia-strip (Coris Bioconcept), and real time PCR for the detection of *G. lamblia* were performed on corresponding fresh stool samples.
Results: Five hundred and fifteen faeces were included. 154 fresh watery specimens from acute diarrhoea were sent for bacteriological examination and 361 TFT-samples, representing a more chronic form of diarrhoea, were sent to the parasitology department. Using real time PCR as the gold standard, the positive predictive values of microscopy, ELISA and Giardia-strip were 100%, 99% and 50%, respectively. The sensitivity of microscopic detection was 71% while that for Giardia-strip was only 5%. Novitec ELISA was more sensitive (67%) than Novatec ELISA (51%). Specificity of all methods was never lower than 97%.
Conclusion: Microscopy with triple faeces test is a very specific method for the laboratory detection of *G. lamblia* in faeces with good sensitivity. Both ELISA's also have good sensitivity and can be used as acceptable alternatives for microscopy. Giardia-strip can not be used as an alternative, because of its very low sensitivity. Real time PCR is a very sensitive and specific method for the detection of *G. lamblia*.

P857

Diagnosis of *Cryptosporidium parvum* with microscopy, striptest, ELISA and real-time PCR

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Objectives: *Cryptosporidium parvum* remains largely under diagnosed in current routine diagnostic procedures in microbiology laboratories. We compared four different diagnostic methods for the detection of *C. parvum* in faeces in both acute and chronic diarrhoea.
Methods: Microscopic examination (Auramin stain confirmed by Kinyoun stain), Crypto-strip (Coris Bioconcept), ELISA (Novitec Cryptosporidium ELISA) and real time PCR for the detection of *C. parvum* were compared.
Results: Five hundred and fifteen faeces were included. One hundred and fifty-four watery specimens from acute diarrhoea were sent for bacteriological examination and 361 triple faeces test (TFT)-samples, representing a more chronic form of diarrhoea, were sent to the parasitology department. Using real time PCR as the gold standard, the positive predictive values of microscopy, Crypto-strip and ELISA were 100%, 85%and 99%, respectively. The sensitivities of microscopic detection, Crypto-strip and ELISA were 37%, 78% and 71%, respectively, while the specificities of the three methods were never lower than 98%. Remarkably, the majority of the positive *Cryptosporidium* samples were not found in watery stools, as described in all textbooks, but rather in loose to mushy stools (57%). Furthermore, the majority of the positive watery samples were not sent for parasitological examination but only for bacterial culture.
Conclusion: The widely used microscopy is a very specific but less sensitive method for the laboratory detection of *C. parvum* in faeces. Both ELISA and Crypto-strip have good sensitivity and both positive and negative predictive values. Real time PCR is a very sensitive and specific method for the detection of *C. parvum*. The majority of positive *Cryptosporidium* samples were found in mushy stools from children younger than 10 years old. Examination of watery stools sent only for bacteriological examination, for the presence of *C. parvum* yields additional positive samples which would otherwise not have been detected.

P858

New diagnostic method for pneumocystis using flow cytometry

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Objective: Pneumocystis jiroveci is an opportunistic fungal agent infecting immunocompromised hosts like AIDS and those receiving immunotherapy. Diagnosis of pneumonia due to Pneumocystis requires an accurate method. The laboratory diagnosis is based upon the detection of the agent using fluorescent monoclonal antibodies on respiratory samples using commercial kits. The organism does not grow in culture and nucleic acid amplification is still performed only in research setting. Flow cytometry has been used as a valuable tool on microbiology showing several advantages.
Materials and methods: Two hundred and twenty respiratory samples (188 bronchial or bronchoalveolar samples and 32 traqueal aspirates secretions) were evaluated. The last were treated with *N*-acetil-cysteine prior to analysis. The samples were centrifuged and the sediment evaluated according two methods. For fluorescence microscopy the samples were smeared on a slide and stained with 25 μl of the monoclonal antibody of MerifluorR- Pneumocystis. To optimize the staining serial concentrations of the specific monoclonal (5, 10, 15, 20 and 25 μl), were used to stain positive samples. Thereafter, that all the samples were stained with 5 μl, centrifuged (10 min at 3000 rpm) and resuspended on deionized water for flow cytometry analysis (FC). FC acquisition protocol was optimized in order to define a scattergram and to determine the intensity of green fluorescence, FL1. A threshold of detection was evaluated after diluting a positive sample and performing both analysis, that is microscopy and FC analysis. Negative samples were contaminated with 25 μl of suspensions (0.5 McFarland) of rods, cocci and yeasts.
Results: All the positives samples by microscopy were positive by flow cytometry. FC detected eight positive samples that were negative by microscopy. Such cases correspond to AIDS patients with CD4 count below 200/mm^3, presenting fever and respiratory infection; they were treated as positive and seven improved. Samples contaminated with bacteria or fungi did not show increased fluorescence that is, no unspecific staining. FC using a specific fluorescent antibody, proved to be sensitive and useful to detect Pneumocystis.

P859

Rapid identification and separation of the yeast according to their isoelectric point

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Objectives: The yeasts of the *Candida* genus are considered as important aetiological agents of nosocomial infections. The frequent use of indwelling medical devices in the last decades

is the cause of increasing incidence of non-albicans yeast infections. The rapid detection and identification of these aetiological agents can help to choose appropriate therapy. The yeasts as amphoteric particles are characterized by their isoelectric points (pI). This value is determined by the balance between the positive and negative surface charges, which are determined by the surface composition of the bacterial cell. Using one of the electromigration techniques, capillary isoelectric focusing (CIEF), the bacteria are focused and separated according to their pI. One of the advantages of this method is the rapidity of the examination. Additionally, it enables to work with small volumes of sample and to measure the results more accurately.

Methods: The aim of the work was to evaluate the pI as a tool for the detection, differentiation and identification of clinically important yeasts. The strains of *Candida albicans, C. krusei, C. parapsilosis, C. glabrata, C. kefyr, C. lusitaniae, Yarrowia lipolytica, Saccharomyces cerevisiae, Trichosporon asahii* and *Geotrichum candidum* were examined by means of the CIEF with on-column UV detection. The isoelectric points of examined yeasts were calculated via the comparison of the migration times of these yeasts with the migration times of pI markers.

Results and conclusions: All examined yeast species were focused according to their surface characteristics in distinct zones. The independence of pI value on the reaction conditions enables the standardization of the results. Assessment of the isoelectric points of yeast strains isolated from serious infections by means of CIEF represents the possible way for the detection and identification of these pathogens. This method is able to reveal the simultaneous presence of different yeast species in the sample. Moreover, the quantification of bacteria is possible according to the peak area in the electrophoreogram.

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P860

Evaluation of a rapid colorimetric test based on trehalose use for identification of *Candida glabrata*

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Objective: Non-*Candida albicans Candida* species have increasingly become important cause of morbidity and mortality in immunocompromised patients. *Candida albicans* remains the most frequent cause of candidiasis, and *Candida glabrata* is the second most frequent agent isolated from clinical infections. Due to developing resistance to many azole antifungal agents, including fluconazole, the rapid identification of *C. glabrata* is essential for appropriate antifungal therapy. Many tests have been developed for identification of *C. glabrata* based on their trehalose use, which is characteristic property of this species. The aim of this study was to determine the ability of a rapid trehalase test to identify *C. glabrata* isolates.

Methods: One hundred and fifty-four isolates from several clinical specimens were tested by a method developed by Peltroche-Llacsahuanga et al. to evaluate its ability to discriminate *C. glabrata* strains. *Candida* strains were identified by germ tube test, their growth on corn meal Tween 80 agar, colonies appeared on Mast ID-CHROMagar Candida medium (Mast Diagnostics, Merseyside, UK), and if required by API 20C AUX (bioMérieux, France) commercial kit. All isolates were taken from Sabouraud dextrose agar containing 4% glucose incubated at 37 °C for 24 hours. The reference strains (*C. glabrata* standard laboratory strain, *C. albicans* ATCC 90028, *C. tropicalis* ATCC 750, *C. krusei* ATCC 6258) were used as control. One yeast colony emulsified in 50 μl of citrate buffer (0.1 M, pH: 5.0) containing 4% (wt/vol) trehalose for 3 hours at 37 °C. Presence of glucose was detected by spotting 10 μl of this suspension onto a dipstick. Glucose, generated by cleavage due to cell-bound trehalase enzyme, was detected a commercial dipstick.

Results: Among 151 strains tested, 76 were *C. glabrata*, 47 were *C. albicans*, 17 were *C. krusei*, five were *C. parapsilosis*, four were *C. tropicalis*, three were *C. keyfr*, and one was *C. utilis*. Among 76 *C. glabrata* strains tested all were found positive by trehalase test. Non-*C. glabrata* isolates were found negative by rapid trehalase test. The trehalase tests allowed identification of *C. glabrata* in 3 hours with 100% sensitivity and 100% specificity.

Conclusion: Our study showed that the trehalose assimilation test is rapid, cost-effective, and simple to use. This test may be helpful as a rapid identification method of *C. glabrata* in routine medical mycology laboratory.

P861

Immunodiagnosis of invasive candidiasis: prospective serological evaluation of at-risk patients in the French university hospital of Grenoble

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The diagnosis of invasive candidiasis (IC) remains difficult to assess before positive blood cultures or positive biopsies. To evaluate the interest of immunodiagnosis in hospitalized at risk patients for the management of this fungal infection, we evaluated the performance of serological methods routinely used in the laboratory for *Candida* infections from January 2004 to July 2005. *Candida* antibodies detection was performed by indirect immunofluorescence (IFI) and immuno electrophoresis (IEP) on 1598 sera of 690 patients (mainly from haematology and IUC: 54% and surgical patients: 30%). The latex agglutination test Cand-tec (Ramco, USA) was used for antigen detection in 1450 sera of 495 patients. IFI slides have been prepared with a *Candida albicans* strain (VW 32 Pasteur strain, Lille) and for IEP two commercial somatic antigens of *Candida albicans* were used (Bio Rad, USA and FSK2 from Microgen Bio products, USA). The efficiency of the immunodiagnosis was evaluated in the group of patients with proven IC according the criteria of OERTC. Among the 39 patients with candidemia, antibody detection was performed only on 17 patients and five of them showed high antibodies level (sensitivity 30%). Fifteen other patients, mainly from surgical departments, developed an invasive candidosis (positive cultures from sterile fluid, blood excluded, or positive culture from organ biopsies). In these cases, the serological tests were positive in sera of 12 patients (sensitivity 80%). The overall sensitivity of the antibody detection was of 47%, the specificity, the PPV and the NPV were respectively of 91%, 20% and 97%. High antibody levels in patients without diagnosis of IC were associated with abnormal *Candida* colonization. A particular arc in IEP was more often present in sera of patients with deep candidosis (PPV 38%). The sensitivity of the Cand-Tec was disappointing; the sensitivity was of 5% in patients with candidemia and only two patients with deep *Candida* infections out of 15 showed positive results. The specificity, PVP and NPV were, respectively, of 91%, 15%, and 93%. The antibody detection in our survey showed higher performance than antigen detection by the Cand-Tec method but the combination of both enhanced the efficiency to diagnose IC. Despite the weak sensitivity, especially in patients with haematological diseases, high antibody level and the presence of specific precipitin must lead to highly suspect *Candida* dissemination.

P862

Influence of culture medium on performance of a yeast identification system (MICRONAUT Candida®)

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Objectives: Recently several novel antimycotic agents had been introduced, e.g., caspofungin, voriconazole, and posaconazole. Due to different susceptibility of yeast species recovered from human specimens (e.g., low susceptibility of basidiomycetal yeasts in the case of caspofungin) reliable species identification is of growing importance in order to select them for appropriate resistance testing. The influence of the type of culture media used for preparation of the inoculum for biochemical based identification kits had not been studied in depths. We have tested the influence of three culture media on the performance of a microtiter based identification system (MICRONAUT Candida [M-C], Merlin, Germany).
Methods: We tested 99 isolates compromising 21 different yeast species. Isolates used were reference strains or had been identified by sequence analysis (5′ end 28S rDNA). Identification achieved by M-C was compared to the respective results of the ID 32C [ID] identification kit (bioMérieux, Nürtingen, Germany). Inoculum was prepared from three different commonly used primary platting media (CHROMagarTM Candida, MAST, Reinfeld, Germany, Sabouraud Dextrose Agar, Sabouraud Dextrose Agar [SD] with 0.5% chloramphenicol [SDC], BD, Heidelberg, Germany) in case of M-C. Microtitre plates were photometrically evaluated after 24 h of incubation and identification was achieved by application of the respective software. Inoculum for the ID was prepared from SDC and evaluated (24 and 48 h) by using the miniAPI reader.
Results: Overall false identification was observed in case of *C. dubliniensis* (M-C/ID; 1/2), *C. guilliermondii* (M-C/ID; 2/1), *C. lusitaniae* (M-C/ID; 1/1), *C. tropicalis* (M-C/ID; 1/4), *C. neoformans* (M-C/ID; 4/1), and *S. cerevisiae* (M-C/ID; 1/1). Failure of identification due to lack of the species in the respective data base was observed in case of *C. africana* (M-C/ID; 4/4), and *C. viswanathii* (M-C/ID; 1/1). Type of culture medium used had only a minor influence on the identification score. Singular or dual misidentification was observed in 4.4% of all M-C identification mostly seen when using SD (n = 7). Results of this study are currently used for improvement of the M-C identification algorithm.
Conclusion: Type of culture media used for preparation of the inoculum seems to have only a minor impact on the outcome of biochemical tests. Verification of performance of an identification system might only be necessary when changing the culture media.

P863

Function of *Candida albicans* ALS5 and ALS7 genes on adhesion to FEP (polymer of tetrafluoroethylene and hexafloropropylene) catheter and polyurethane catheter

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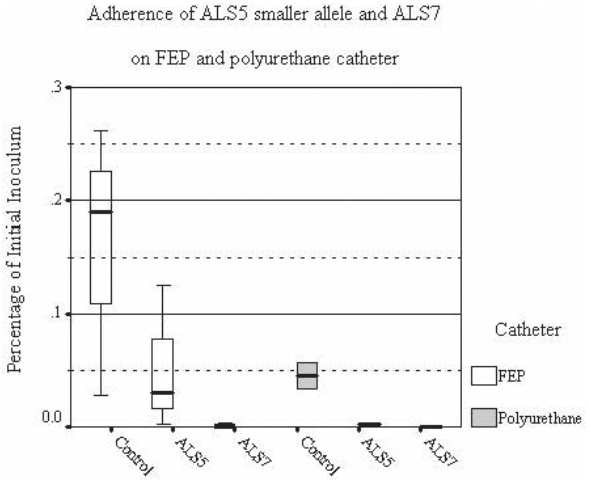
Objectives: To investigate the adherence of *Candida albicans* ALS5 and ALS7 genes on FEP catheter and polyurethane catheter.
Methods: ALS5 smaller allele and ALS7 from *Candida albicans* strain SC5314 were cloned into pYES6CT plasmid and under a GAL promoter control. Plasmids were transformed into *Saccharomyces cerevisiae*. The transformed *S. cerevisiae* was cultured in SC selective medium with galactose for expression of Als5p or Als7p. About 0.5 cm fragment of FEP catheter and polyurethane catheter were incubated in 1 ml of the *S. cerevisiae* culture for 30 min. Catheters were washed and sonicated. One hundred microlitre of the sonication fluid was plated on sabouraud dextrose agar and was incubated at 30 °C for 2 days. Adherence was expressed as a percentage of the number of colony forming unit of the initial inoculum. The experiment on FEP catheter was conducted in triplicate and the experiment on polyurethane catheter was conducted in duplicate. Two independent experiments were performed.
Results: The percentage of initial inoculum, representing the adherence of the *S. cerevisiae* transformed with pYES6CT (control), ALS5 smaller allele or ALS7 on both type of catheter is low and nearly zero. There is no significant difference in the percentage of initial inoculum between the three groups for both type of catheter ($p = 0.058$ for FEP; $p = 0.104$ for polyurethane). The result is summarized in the figure followed.



Conclusion: Transformation of ALS5 smaller allele or ALS7 of *Candida albicans* strain SC5314 into *S. cerevisiae* does not confer adhesion properties on FEP catheter and polyurethane catheter.

P864

Comparative evaluation of *Candida* DNA, mannan, anti-mannan antibodies and (1-3)-*β*-D-glucan in the diagnosis of candidaemia

Z.U. Khan, F. Alam, A.S. Mustafa *(Kuwait, KW)*

Background and objective: A delayed diagnosis of candidaemia is associated with high mortality, and therefore, early diagnosis and institution of appropriate therapy is essential to improve prognosis. The aim of this study was to evaluate sensitivity of four currently available assays, namely Platelia *Candida* Ag for the detection of mannan, Platelia *Candida* Ab for the detection of anti-mannan antibodies, snPCR for the detection of DNA, and Fungitell for detection of (1–3)-*β*-D-glucan in the diagnosis of candidaemia.
Methods: Thirty-two sera samples from 27 culture confirmed cases of candidaemia, 10 sera from Candida vaginitis patients, and 30 sera from healthy controls were included in the study. The ELISA-based tests were performed as per the instructions provided by the manufacturers. The cut-off values for each test were determined by determining mean + 3 standard deviation of the control sera. DNA was extracted from the sera using standard techniques and PCR was performed with generic as

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well as species-specific primers for *C. albicans*, *C. parapsilosis*, *C. glabrata*, and *C. tropicalis*.
Results: In candidaemia patients, the sensitivity and negative predictive value for the assys were as follows: Platelia mannan 84%, and 86%, anti-mannan antibody 56% and 68%, (1–3)-β-D-glucan 75% and 67%, and *Candida* snPCR 88% and 88%, respectively. When the tests were combined, the sensitivity for mannan and anti-mannan improved to 94% and mannan and (1–3)-β-D-glucan to 97%.
Conclusions: The results of the study suggest that the combined detection of mannan and anti-mannan antibodies or mannan and (1–3)-β-D-glucan may contribute to the early and sensitive diagnosis of candidaemia or invasive candidiasis.. However, performance of snPCR offered an advantage over mannan and glucan tests since it was species-specific and detected 4 (12%) patients who were infected with more than one species. This observation has therapeutic implications, since *Candida* species have different anti-fungal susceptibility profiles.
Acknowledgement: Supported by College of Graduate Studies, Kuwait University, Kuwait.

P865

Evaluation of tobacco agar for differentiation of *Cryptococcus neoformans*

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Background and objective: *Cryptococcus neoformans* is the etiologic agent of cryptococcosis, a systemic mycosis of humans and animals with a world-wide distribution. The aim of the study was to evaluate the efficacy of tobacco agar as a differential medium for *C. neoformans* and its serotypes.
Methods: One hundred and sixty-eight isolates of *C. neoformans* originating from clinical specimens (n = 8), and environmental sources (n = 160) were evaluated for their ability to produce brown melanin-like pigment on tobacco agar. In addition, reference strains or clinical isolates of *C. neoformans* (n = 10), *C. laurentii* (n = 1), *C humicola* (n = 1), *Candida albicans* (n = 55), *C. parapsilosis* (n = 9) , *C. tropicalis* (n = 8), *C. krusei* (n = 5) and *C. glabrata* (n = 7) were also tested. Twenty-five gram of tobacco obtained from commercially available cigarette brand (Marlboro; tar 8 mg, nicotine 0.6 mg; Philip Morris Products SA, Richmond, VA, USA) was mixed with 1 l of distilled water. The mixture was boiled for 30 min and then filtered through several layers of gauze. To this filtrate 20 g of agar was added and the volume was made up to 1 l. The pH of the medium at this point was 5.4. It was autoclaved at 121 °C for 15 min. Twenty millilitres medium was poured into each plate. The test cultures were streaked on the medium and plates were incubated at 280 °C and examined for development of brown-coloured colonies up to 96 h.
Results: All the isolates of *C. neoformans* developed brown-coloured colonies within 48 h. Induction of brown pigmentation was discernible as early as12 h. Isolates belonging to serotypes B (n = 62) and C (n = 1, reference strain) produced more intense pigmentation (cherry brown) as compared to serotypes A (n = 106) and D (n = 1, reference strain).
Conclusion: The results of the study suggest that tobacco agar can be used as a differential medium for presumptive identification of *C. neoformans* from other *Cryptococcus* species and *Candida* species. While isolates of serotype B produced more intense brown pigmentation as compared to serotype A, this phenomenon needs to be evaluated more extensively with respect to serotypes C and D.
Acknowledgements: The authors are thankful to Prof. H. S. Randhawa and Dr. Anuradha Chowdhary for providing environmental isolates and some reference strains of C. neoformans.

P866

Comparison of *Aspergillus fumigatus* DNA and galactomannan in serum and bronchoalveolar lavage specimens of experimentally infected rats

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Background and objective: Invasive aspergillosis is an important opportunistic mycosis associated with high mortality. Its diagnosis is difficult due to non-specific signs and symptoms and low culture positivity. The aim of this study was to detect *Aspergillus fumigatus*-specific DNA by recently developed nested PCR (nPCR) in serum and bronchoalveolar lavage specimens of experimentally infected rats and compare the results with galactomannan detection.
Methods: Thirty Wistar rats, weighing between 150 and 170 g, immunosuppressed with intraperitoneal injection of cyclophosphamide were infected intravenously with 1×10^6 *A. fumigatus* conidia. The rats were sacrificed on day 1, 3, 5, 7, and 9 post-infection in groups of six each and their bronchoalveolar lavage (BAL), blood and lung tissues were cultured. The serum and BAL specimens were collected for galactomannan and nPCR tests. Sera and BAL specimens of six normal rats were obtained to standardize base line values. The DNA from serum and BAL specimens was extracted using standard procedure. Galactomannan was detected by Platelia Aspergillus kit (Bio-Rad, France) and *A. fumigatus*-specific DNA by nested PCR using primers derived from the internally transcribed spacer (ITS) regions 1 and 2.
Results: Sera and BAL specimens of six normal rats were negative for galactomannan and *A. fumigatus* DNA. The per cent of positive tests for galactomannan (cut-off value 0.5 ng/ml) and nPCR in serum samples on day 1, 3, 5, 7, and 9 post-infection in each group of animals was 83% and 50%, 100% and 66%, 66% and 66%, 100% and 50% and 33% and 16%, respectively. The overall positivity for galactomannan and nPCR was 76% and 50%, respectively. In BAL specimens, the per cent of positive tests for galactomannan and nPCR in each group on day 1, 3, 5, 7, and 9 post-infection was 100% and 83%, 66% and 83%, 66% and 66%, 100% and 66%, and 33% and 33%, respectively. The overall percent positivity for galactomannan and nPCR was 73% and 66%, respectively.
Conclusion: The results suggest that while the culture of the lung tissues was positive for all the animals infected with *A. fumigatus*, the overall positivity of galactomannan was slightly higher than nPCR in both serum and BAL specimens in the 9 days post-infection follow-up.
Acknowledgements: Supported by Kuwait University Research Admninistration Grant MI04/02.

P867

Candida spp. colonization and serum anticandidal antibody levels in patients with chronic urticaria

M.C. Ergon, T. Ilknur, M. Yucesoy, S. Ozkan *(Izmir, TR)*

Objectives: Bacterial and viral infections, parasites, fungi, food and food additives are some of the aetiological factors hold responsible for the pathogenesis of chronic idiopathic urticaria. In this study, we aimed to investigate the triggering role of *Candida* spp. colonization and infection in patients with chronic idiopathic urticaria.
Methods: Thirty-eight patients with chronic idiopathic urticaria who applied to Dermatology Clinic of Dokuz Eylul University, Faculty of Medicine, and a control group consisted of 42 healthy

individuals were included in the study. Stool and oral specimens of two groups were cultured quantitatively on Sabouraud dextrose agar (SDA) with chloramphenicol and gentamicin. Yeast growth after 48 hours of incubation at 37 °C on SDA was evaluated as CFU/gram for stool specimens and CFU/ml for oral specimens. *C. albicans* ELISA IgG/IgM/IgA test kits were used for the detection of these antibodies against *C. albicans* in sera of the patient and control group individuals.

Results: Yeasts were isolated from the stools of 60.5% of the patient group and 50.0% of the control group and these rates were 47.4% and 42.9% for oral specimens. When the results of two groups were compared with *t*-test, no statistically significant discrepancies were detected between the groups for stool ($t = 0.28$, $p = 0.78$) and oral ($t = 0.19$, $p = 0.85$) cultures. If the colony counts of the cultures were compared with Mann–Whitney *U* test, there were no significant differences between two groups for stool ($z = 0$, $U = 241.5$, $p = 1$) and oral ($z = 0$, $U = 162$, $p = 1$) specimens. IgG, IgM and IgA antibodies were positive in 36.8%, 23.8 and 5.3% of the patient group and in 42.9%, 19.1% and 4.8% of the control group. When the quantities of IgG, IgM and IgA antibodies were compared by using paired *t*-test, no statistical difference was detected between the two groups ($p = 0.93$, 0.56 and 0.67, respectively). Chi-square test was applied for the comparison of the qualitative values of IgG, IgM and IgA antibodies of two groups and there was no statistical difference between the groups ($^2 = 1.34$, $p = 0.26$; $^2 = 0.27$, $p = 0.88$ and $p = 0.70$, respectively).

Conclusion: It is concluded that intestinal and oral colonization of *Candida* spp. and previous *Candida* infection do not play important triggering role in the aetiology of patients with chronic idiopathic urticaria.

P868

Performance of three differential media for the presumptive identification of yeasts

M. Yucesoy, M.C. Ergon, S. Ozer *(Izmir, TR)*

Objectives: In order to facilitate yeast identification process, several chromogenic isolation media for species identification of clinical yeasts have been developed as rapid tests. This study was performed to evaluate three chromogenic media, CHROMagar Candida (CAC) (CHROMagar, France), Albicans ID2 agar (AID) (bioMérieux, France) Bromcresol Green Agar (BCG) (Difco, France) for the presumptive identification of yeasts.

Methods: A total number of 203 yeast strains including 128 *Candida albicans*, 21 *C. tropicalis*, 18 *C. glabrata*, 17 *C. parapsilosis*, 7 *C. krusei*, 6 *Trichosporon* spp., 4 *C. kefyr*, 1 *C. guilliermondii*, 1 *Geotrichum candidum* which were isolated from various clinical specimens were included. The isolates were first identified by germ tube test, morphological characteristics on corn meal Tween 80 agar and API 20 C AUX systems. The strains were saved as stock cultures. Then they were inoculated into Sabouraud dextrose agar and after purity check, they were streaked onto CAC, AID and BCG plates. The results were read by three different people and interpreted according to the colour, texture of the colonies, colour of the plate and the existence of halo around the colony after 24, 48 and 72 hours of incubation at 37 °C in the dark.

Results: All of the isolates grew well on the three media tested. The sensitivity and specificity values for *C. albicans* were found to be 100% and 100% for CAC; 97.7–100% and 94.7–97.3% for AID; 93.0–99.2% and 24.0–68.0% for BCG at different incubation periods, respectively. These values for *C. tropicalis* were 95.2–100% and 100% for CAC; 14.3–95.2% and 9.3–23.1% for BCG at various incubation periods, respectively. CAC was found to be 11.1–88.9% sensitive and 100.0% specific for *C. glabrata*; 100% and 99.5% sensitive and specific for *C. krusei*.

Conclusion: CAC and AID can be recommended as reliable methods for the presumptive identification of *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. albicans*, respectively. The sensitivity and specificity values obtained for the most isolated *Candida* species for BCG are very low to be used for the identification process however; its usefulness as a differential medium for primary isolation and detection of *Candida* species from clinical specimens should be tested.

P869

Detection of *Aspergillus galactomannan* antigen levels for antimicrobial agents by ELISA

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Objectives: False positive results in *Aspergillus galactomannan* antigen detection by ELISA method from patients receiving piperacillin-tazobactam have been reported. In this study, we aimed to investigate *A. galactomannan* antigen levels for piperacillin-tazobactam and other various antimicrobial agents that are often used for the treatment of infections in immunocompromised patients.

Methods: The level of galactomannan antigen was investigated for piperacillin-tazobactam, ampicillin-sulbactam, ampicillin, penicillin G, ceftriaxone, cefepime, imipenem, clarithromycin, ciprofloxacin, vancomycin, gentamicin, trimethoprim-sulfamethoxazole, ornidazole, fluconazole and amphotericin B. Antigen levels were determined by using the Platelia Aspergillus ELISA (Bio-Rad, France) according to the manufacturer's instructions. Samples were run in triplicate and the entire experiment was repeated.

Results: Among the 15 antibiotics, ampicillin expressed highest level of galactomannan with a galactomannan index (GI) of 0.540. Although this index value is not a positive result for the presence of galactomannan according to the manufacturer's instructions, it can be accepted as positive according to the test products with FDA approval. Piperacillin-tazobactam expressed the second high level of galactomannan (GI = 0.235), however this value is below the both cutoff limits. Galactomannan levels for the other antibiotics ranged from 0.011 to 0.188.

Conclusion: It can be concluded that among the antibiotics that was investigated in this study, ampicillin might cause a false positive result in galactomannan antigen test by ELISA because it showed significant level of galactomannan antigen and piperacillin-tazobactam can also cause cross reactivity in the sera of patients due to its relatively high galactomannan level in the test.

P870

Mycological evaluation and suitability for detecting dermatophytes on sabouraud-gentamicin-chloramphenicol-2-agar

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Sabouraud-Gentamicin-Chloramphenicol-2-agar (SGC2; 43651-bioMérieux) is designated for the isolation of yeasts and moulds from clinical specimens. Under the ''Limitations'' section in the package insert, the description of the culture medium states: ''This medium is not recommended for the detection of dermatophytes''.

Objectives: The purpose of this study was to evaluate the suitability of SGC2 for the detection of dermatophytes in clinical specimens and with clinical isolates. Performance of SGC2 was

compared in terms of growth rate, colony morphology, pigmentation and formation of conidia with the following media: Sabouraud dextrose agars from Merck and from Oxoid and dermatophyte selective media (Selektiv-Agar für Pathogene Pilze from Merck, Dermasel agar from Oxoid).
Methods: One hundred clinical specimens (nail cuttings, skin scrapings and hair stubs) were inoculated on SGC2 and incubated at 25 °C up to 21 days. The presence of a positive dermatophyte culture had already been confirmed via prior culturing all materials on dermatophyte-selective media. Moreover, four hundred clinical isolates including 312 strains of *Trichophyton* spp., 76 strains of *Microsporum* spp. and 12 strains of *Epidermophyton* spp. were examined on SGC 2, in comparison to the media specified above. Growth rate, morphology and pigmentation of the colonies and the microscopic structures were studied simultaneously for all media after incubation at 5, 7, 10,14 and 21 days.
Results: Ninety-nine of one hundred clinical specimens were detected positive for dermatophytes on SGC2. All dermatophytes exhibited good growth on SGC2 and growth rates typical for the particular species, as well as typical colony morphology, pigment formation and microscopic characteristics. Performance was comparable to that observed for prepared agar plates from other manufacturers and occasionally somewhat better.
Conclusion: Due to these results, SGC2 medium permits the isolation of dermatophytes in spite of the current restrictive wording in the limitation section of the package insert. Thus, the SGC2 medium is an universal mycological medium that enables detection of dermatophytes in addition to yeasts and moulds.

Toxoplasmosis

P871

Pregnant women and toxoplasmosis

I. Machado, I. Sousa, H. Angelo *(Porto, Lisbon, PT)*

Objectives: Evaluation of the knowledge of women on toxoplasmosis in Portugal.
Methods: A survey on the self-perceptive knowledge was carried out, in parturient women of Portugal. Results were obtained from 48 out of 50 hospitals, in total 7362 valid surveys.
Results: 77.3% of the women had already heard about toxoplasmosis; among these 85.7% had declared to know what this infection is. Age was a very important factor to knowledge, as about 50% of women <20 years had never heard of toxoplasmosis before. Compared to women with 30–39 years, the adjusted odds ratio (OR) for women <20 years was 3.6 (95% CI: 2.72, 4.77). Education is the main factor that influences the knowledge of parturient women on toxoplasmosis, being deeply related to the profession. Compared to graduate women, the OR for women with only the primary school was 43.6 (95% CI: 22.58, 84.08). The knowledge of this infection also varies with the place of information, but mostly with the region of the country. It is in the North of the country that the knowledge is worse—OR 2.4 (95% CI: 1.94, 2.85), compared to Lisbon and Tejo Valley. Women's most common source of information is the doctor (77%). The information places are mainly ''other places/private consultation'' (38.5%) and the ''local healthcare center'' (35.8%), even though the percentages vary depending on the district surveyed.
Conclusions: As health professionals do not always transmit their patients the information in a way that allows its exact perception, education influences the most the knowledge parturient women have on toxoplasmosis. It is essential that health professionals adapt the way they communicate with the women they assist.

P872

Congenital toxoplasmosis prevention in Portugal

I. Machado, I. Sousa, H. Angelo *(Porto, Lisbon, PT)*

Objectives: To evaluate the prevention done in Portugal on congenital toxoplasmosis, concerning primary and secondary prevention.
Methods: A survey on the self-perceptive knowledge was carried out, in parturient women of Portugal. Results were obtained from 48 out of 50 hospitals, in total 7362 valid surveys.
Results: Primary prevention precautions that are most commonly known among parturient women are ''eating well-cooked meat'', ''avoiding contact with cats'' and ''eating well-washed salads''. It was detected some confusion related to the choice of certain correct and incorrect primary prevention steps, such as between ''avoiding contact with cats'' and ''avoiding contact with dogs'' or between ''eating well-cooked meat'' and ''eating well-cooked fish''. 67.1% of the women had declared to have done prevention during the pregnancy to be protected from toxoplasmosis, even though the prevention was not always the most correct. Thus, 62.1% of the pregnant women with positive serology for *Toxoplasma gondii* had taken unnecessary precautions, but 18% of the pregnant women with negative serology did not take the necessary precautions. It was also verified that, due to incorrect procedures in secondary prevention, there are useless expenses: between women with positive serology for *T. gondii*, 98.8% of primiparous women repeated useless analysis for toxoplasmosis, as well as 57.9% of the multiparous women. Still in the group of women with positive serology to *T. gondii*, 10.7% had done monthly analysis during the pregnancy, and 49.5% had done quarterly analysis.
Conclusions: Gestations are frequently badly planned, leading to an incorrect implementation of the prevention. The prevention for congenital toxoplasmosis in Portugal is frequently being incorrectly made, not only as far as the primary prevention is concerned, but also regarding the secondary one. In this area specialized training should be carried out for health professionals.

P873

Screening for *Toxoplasma gondii*, Rubella virus and cytomegalovirus in pregnant women

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Objectives: *Toxoplasma gondii*, rubella virus and cytomegalovirus (CMV) are responsible for some of the most common infections associated with congenital anomalies. These infections also cause mild maternal morbidity. Nevertheless, recognition of maternal disease is very important for the clinician. In order to determine the immune status of the organism against those microorganisms, seroprevalence of IgM and IgG antibodies proved to be very helpful tests. Ideally, these tests should be performed before the pregnancy is diagnosed, but usually, they are requested in the first trimester of pregnancy. We conducted this study in order to detect the seroprevalence of IgM and IgG antibodies to *Toxoplasma gondii*, rubella virus and CMV.

Methods: We analysed antibodies against *T. gondii*, rubella virus and CMV, performed as a routine check in the first trimester of pregnancy in women attending our hospital. IgM and IgG were determined using enzyme immunoassays, EIA WELL, *Toxoplasma* IgM and IgG, Rubella IgM and IgG, and Citomegalovirus IgM and IgG (RADIM, Italy), respectively.
Results: A total of 1466 patients were tested for the presence of antibodies. In agreement to our findings, we divided our study population as having: acute infection (when only IgM were positive), recent infection (both IgM and IgG positive) or past infection (only IgG positive). Out of the 1466 pregnant women we determined one acute *Toxoplasma* infection, whereas recent infection in 1.2% and past infection in 20.1%. For rubella and CMV acute infection was detected in 0.2% and 0.3%, recent infection in 1.0% and 1.8%, while past infection in 81.2% and 69.2%, respectively.
Conclusion: Our data demonstrate that even a low prevalence of primary infections during pregnancy or a high prevalence of seronegative women supports the idea that routine prenatal screening is justified. Clinicians must appropriately advise women on preventive measures to avoid these infections during their pregnancy.

P874

Screening for acute toxoplasma infection during pregnancy: compliance to the French programme of 41,086 pregnancies in the Rhône-Alpes region

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A national programme aimed at preventing congenital toxoplasmosis has been set up in France which is legally mandatory and requires a serology during the first three months of pregnancy for women for whom there is no certitude about a previous immunity against toxoplasmosis, and for all women identified as seronegative, a monthly serology throughout their pregnancy including a final serology at delivery. However, neither the compliance with the program nor its efficacy have been evaluated.
Objectives: To evaluate the compliance with the mandatory French prenatal screening program for toxoplasmosis in pregnant women.
Methods: Descriptive, transversal study, using the data bases of the Rhone-Alpes public health insurance system. Compliance with the recommended screening schedule of 37,353 pregnant women who delivered between July 1st, 2002 and June 30th, 2003 and had at least one serology for toxoplasmosis was analysed. Our criteria for satisfying compliance were: (1) first test before 13 weeks after conception, (2) no between-test interval greater than 35 days, (3) last test within 28 days of delivery, and (4) a global assessment based on the combination of the three individual criteria. Patients characteristics associated with a poor compliance were studied for each criteria.
Results: Mean age of pregnant women was 29 years (SD 5) and mean gestation duration was 263 days (SD 13), 37.6 weeks. The first diagnostic test was done before 13 weeks of gestation in 73% of women and after the 14th week in 9.53%; 20% of women had all subsequent tests done within 35-day intervals; 60% had their last test done within 28 days of delivery and 7% only met all three criteria. Multivariate analysis identified factors (age, social and employment status of women, profile of physicians who prescribed the tests, delivery in a public or private hospital) which were associated with satisfying compliance with one or more criteria. Their exact impact will be discussed in an attempt to assess the responsibility attributable to women, physicians, biologists for this poor application of legal recommendations.
Conclusion: Identification of likely explanations for the non respect of the French legal recommendations regarding prevention of congenital toxoplasmosis will be followed by specific measures which effectiveness in the long term will have to be assessed.

P875

High prevalence of IgM antibodies against *Toxoplasma gondii* in Mexican pregnant women

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Objective: To know the prevalence of anti-*Toxoplasma gondii* IgM antibodies in pregnant women that are attended at a Third level Hospital of Perinatal Medicine in México.
Methods: At the National Institute of Perinatology (INPer) (México, City), a Third level Hospital of Perinatal Medicine, where high risk pregnancies are attended, a screening study of anti-*Toxoplasma* IgM antibodies was performed, using blood spots and serum samples taken from pregnant women. The study period was from February 25th to July 7th, 2005. The patients were captured at the prenatal control consult, and signed a consent to be included in the study; afterwards 6–10 blood drops that were spotted onto standard filter papers normally used for screening purposes. The screening serologic test was a specific IgM-capture ELISA. From positive patients, we obtained a second venous blood sample that was used for confirmatory IgM-western blot and IgG-ELISA.
Results: Screening was performed on 437 pregnant women within a gestation period between 14 and 28 weeks. The mean age of the patients was 26.3 + 6.5 years. There were 38 patients positive to IgM anti-*Toxoplasma* antibodies, giving a prevalence of 8.7 per 100 pregnant women screened. Nine positive patients were younger than 20 years (23.7%), 16 (42.1%) were between 20 and 30 years and 13 (34.2%) were older that 30 years. The prevalence of specific IgM according the age group was: 13 per 100 in pregnant women below 20 years of age, 9 per 100 in those pregnant women between 20 and 30 years and 6 per 100 in the group older than 30 years.
Conclusion: At the INPer the prevalence of *Toxoplasma* infection is high. The youngest pregnant women presented a higher incidence. The high incidence was expected because of the type of patients attended, many of them with history miscarriages. *Toxoplasma* infection screening during pregnancy is the principal action to prevent foetal infection and development of the congenital toxoplasmosis syndrome, our data support the specific screening of this infection in the high risk pregnancy group.

P876

Impact of health education for the primary prevention of *Toxoplasma* infection in pregnancy: lessons from the ERIS study

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Objectives: Our study was designed to assess the ability of health education to improve knowledge and preventive behaviour of susceptible pregnant women regarding toxoplasmosis.
Methods: An intervention trial was initiated in the French Rhone Alpes region in 1993. Pregnant women seronegative for *Toxoplasma gondii* were enrolled in the first trimester of gestation by physicians randomly allocated to two groups according to their city of practice. Physicians in the intervention cities (group

A) were asked to give their patients a booklet and an audiotape including advices on how to avoid toxoplasmosis mixed with global information regarding pregnancy while physicians in the control cities (group B) were not instructed to change anything from their usual practice. Physicians and participants were blinded to the study question. Knowledge and risk behaviour for toxoplasmosis and other topics related to pregnancy were measured through two questionnaires completed at inclusion and at delivery. Analysis was limited to 2790 women (56%) for whom both questionnaires were available.
Results: No difference was found between arms regarding socio-demographic indices. At baseline no significant difference was found between group A and B regarding (a) knowledge or (b) behaviour: (a) 64% and 66% respectively knew all answers regarding consumption of raw meat or unwashed salad as risk factors; 44% and 46%, respectively, correctly answered all questions on the preventive effect of hand washing before eating or after handling raw meat (b) 88% and 89%, respectively, reported washing vegetables eaten raw; 26% and 27% reported always washing hands before eating or after handling potential sources of contamination; among the 97% who ate meat in the 60 days preceding inclusion, 44–45% did not eat undercooked meat. Comparison between delivery and baseline showed a moderate gain in knowledge that was significantly associated with assignment to group A. Better habits regarding meat consumption and hand washing were also reported at delivery, significantly associated with good baseline knowledge and behaviour but not with assignment to group A.
Conclusion: Education can improve knowledge of risk factors for *Toxoplasma* infection but no evidence was found regarding changes in behaviour. Additional anthropological studies are needed to identify effective ways to reduce risk behaviours in an attempt to prevent acute maternal infections in pregnancy and consequently congenital toxoplasmosis.

P877

Multicentre proficiency testing programme for molecular detection of *Toxoplasma gondii* in amniotic fluid

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Diagnosis of *Toxoplasmosis* during pregnancy is hampered by the lack of reliable method and by lab to lab discrepancies. PCR is widely used with various predictive values. In order to assess the performance of nucleic acid amplification technologies (NATs) for the detection of *Toxoplasma gondii*, a pilot proficiency panel was designed. The proficiency panel consisted of five lyophilised coded samples with various concentrations of parasites diluted in amniotic fluid and a negative control. The positive samples included *T. gondii* in a range of concentration between 5 and 1000 parasites per ml. Five reference laboratories evaluated the production process. The performance was analysed in combination with a questionnaire on the applied methods. Thirty-three laboratories in 17 countries participated with a total of 38 data sets. An extensive heterogeneity in the pre analytic and analytic procedures was observed. The percentage of data sets achieving correct results on all panel samples was 42.1%, two or more incorrect or equivocal results were reported in 14 (36.8%) data sets. The lowest concentration corresponding to five parasites per ml was not identified correctly in 15 (39.5%) data sets. False-positive was reported by two laboratories witch were not checking for contamination. In 32 (84.2%) data sets an ''in-house'' methods was used, and in 16 (15.8%) sets a commercial assay was tested. Overall, the results of this study demonstrated the need for improvement in the molecular detection of *T. gondii* and for procedure standardization in the diagnosis by nucleic acid amplification.

P878

Using the new IMMULITE 2000 toxoplasmosis IgM (μ-capture) kit for the diagnosis of human toxoplasmosis

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Objectives: In many countries, the prevention of congenital toxoplasmosis calls for regular serological monitoring of nonimmunized pregnant women throughout pregnancy. Diagnosing toxoplasmosis is difficult because of the lack of specific clinical signs. Diagnosis therefore relies heavily on the serological detection of IgM and IgG antibodies directed against *Toxoplasma gondii*. Identification and quantification of IgG antibodies rarely poses any difficulties as most standard tests have a high level of sensitivity and specificity. However, detecting IgM antibodies is often more problematic given the poorer specificity of the available methods. In contrast with methods using an indirect sandwich format, it has been demonstrated that a μ-capture methodology can be more specific and sensitive. This is the context in which we assessed the new IMMULITE 2000 *Toxoplasma* IgM test (μ-capture) from Diagnostic Products Corporation (Los Angeles, USA).
Methods: In May and June 2005, we tested 151 samples, 23 of which were frozen, using four different systems for the detection of IgM antibodies against *T. gondii*: the DPC IMMULITE 2000 μ-capture, bioMerieux VIDAS, Abbott AxSYM and DiaSorin LIAISON assay. VIDAS Toxo IgG Avidity was also used to measured the avidity of anti-*Toxoplasma* IgG in samples established as either discordant or positive by the four investigated diagnostic methods.
Results: Of the 151 samples tested, 13 were positive and 120 proved to be negative according to all four methods, while 18 showed discordance by at least one of the techniques used. Seven samples showed discrepant values on the LIAISON platform as compared to the other three systems, five on the AxSYM, two on the VIDAS and on the IMMULITE 2000 only one. With the IgG Avidity method we were able to determine for each method the relevance of the IgM antibodies compared to the stage of infection.
Conclusion: The result of our work should be taken into account when interpreting a positive result using one of the four methods examined. The VIDAS and AxSYM methods have been available for some time and have been discussed in numerous publications. Moreover, it has been demonstrated that VIDAS offers better specificity than AxSYM. We can state that the IMMULITE *Toxoplasma* IgM (μ-capture) assay have shown very good sensitivity and specificity as well as excellent discrimination between serum samples obtained in the early and late stages of *Toxoplasma* infection.

P879

Development of novel cytomegalovirus and toxoplasma IgG avidity assays using an antigen competitive format ''AVIcomp'' on the Abbott ARCHITECT Instrument

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Objectives: Development of novel Cytomegalovirus (CMV) and Toxoplasma (Toxo) IgG avidity assays using an antigen

competitive ''AVIcomp'' format on the automated ARCHITECT instrument.
Methods: Conventional IgG avidity assays for a variety of infectious diseases employ chaotropic reagents, for example, urea or diethylamine, to distinguish between antibodies of low and high avidity. The AVIcomp competitive assay format does not use chaotropic reagents but relies on mass action using aqueous antigen to discriminate between low and high avidity antibodies. Two IgG assays were performed in the AVIcomp format to determine the Avidity Index (AI) on the ARCHITECT instrument as follows. In Assay No. 1, the patient sample was pretreated with buffer and in Assay No. 2 the patient sample was pretreated with aqueous CMV (viral lysate) or Toxo (purified MBP-P30 (SAG1)) antigen. Pretreatment of the patient sample with aqueous antigen blocks binding of high avidity IgG antibodies to the solid phase in the subsequent assay step. Microparticles coated with CMV or Toxo antigens were then added to each assay, washed, and anti-human IgG conjugate was then added and signal generated. The ratio of the signal in Assay No. 2 over the signal in assay No. 1 was proportional to the amount of low avidity anti-CMV or anti-Toxo IgG present in the patient sample. Since the Avidity Index for an avidity assay is defined as the proportion of high avidity antibodies present in the patient sample, the results of the AVIcomp assay were transformed mathematically as follows: AI(%) = [1-(Signal Assay No. 2/Signal Assay No. 1)] × 100.
Results: The preliminary results of the ARCHITECT CMV IgG avidity assay and the ARCHITECT Toxo IgG avidity assay were compared to the Radim CMV IgG and Vidas Toxo IgG avidity assays, respectively, by testing seroconversion panels, pregnant women and random blood donor patient populations. The relative agreement between the ARCHITECT and Radim CMV IgG assays was 98.7% with a correlation coefficient r = 0.88. The relative agreement between the ARCHITECT and Vidas Toxo IgG assays was 100% with a correlation coefficient r = 0.97.
Conclusion: These preliminary results suggest that the performance of the novel CMV and Toxo IgG avidity assays employing AVIcomp technology was equivalent to conventional avidity assays using chaotropic reagents.

P880

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

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Objectives: Preliminary evaluation of a panel of human anti-Toxoplasma (Toxo) immunoassays on the automated ARCHITECT instrument.
Methods: The three Toxo assays for the ARCHITECT instrument are two-step immunoassays utilizing recombinant antigen coated paramagnetic microparticles for the capture of human anti-Toxo antibodies and an acridinium-labeled anti-human IgG or IgM monoclonal antibody for human anti-Toxo antibody detection. Recombinant MBP-P30 (SAG1) coated microparticles were used in the ARCHITECT Toxo IgM and IgG avidity assays whereas MBP-P30 (SAG1) and CKS-P35 (GRA8) coated microparticles were used in the ARCHITECT Toxo IgG assay. Samples from pregnant women, blood donors, hospital patients, selected RF and characterized Toxo IgM positive samples, and seroconversion panels were tested on the new ARCHITECT Toxo IgG, IgM, IgG avidity assays in comparison to the Abbott AxSYM Toxo IgG, IgM, or Vidas Toxo IgG avidity assays. The performance of the ARCHITECT Toxo IgG avidity assay was also evaluated by comparison to clinical information.
Results: The ARCHITECT Toxo IgG assay has a relative sensitivity of 99.4% and a relative specificity of 99.4% when compared to AxSYM Toxo IgG assay. The ARCHITECT Toxo IgM assay has a relative sensitivity of 98.7% and a relative specificity of 94.6% when compared to AxSYM Toxo IgM assay. The seroconversion sensitivity of the ARCHITECT Toxo IgG and IgM assays was comparable to the AxSYM assays. The ARCHITECT Toxo IgG avidity assay using ''AVIcomp'' technology has a clinical sensitivity and specificity of 100% and 99.3%, respectively. The relative agreement between the ARCHITECT and Vidas Toxo IgG avidity assays was 100%.
Conclusion: The performance of the three new ARCHITECT Toxo immunoassays was comparable to the reference assays.

P881

Performance evaluation of the VIDIA toxoplasmosis IGG and IGM assays

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Objective: Compare the clinical performance of the new VIDIA Toxoplasmosis IgG and IgM assays (bioMérieux, France) with in-house serologic tests using clinical specimens.
Material: A total of 1148 frozen and fresh serum samples from pregnant women, male patients, infants and immuno-compromised patients was used for testing. Sensitivity and specificity of the VIDIA TOXO IgG were determined on all sera in comparison with the Dye test. Sensitivity and specificity of the VIDIA TOXO IgM were determined on 628 sera in comparison with the in-house IgM-ISAGA and patient clinical data. A correlation study was performed by testing 110 sera for IgG and 115 for IgM in comparison with the VIDAS Toxo IgG II and VIDAS Toxo IgM (bioMérieux, France) respectively. Seroconversion panels (n = 10) were tested on both VIDIA and VIDAS systems. In case of discrepancies, complementary testing was performed with the in-house high-sensitivity (HS) agglutination test for IgG.
Results: For clinical samples, sensitivity was found 99.7% for the VIDIA Toxo IgG and 100% for the VIDIA Toxo IgM. The specificity was 99.5% for the VIDIA Toxo IgG when compared to the Dye test and the specificity of the VIDIA Toxo IgM was 98.9% when compared to the ISAGA assay. The correlation coefficient of IgG titers was 0.91. IgM detection agreement was found 100% between VIDIA and VIDAS. The same number of seroconversion panels (n = 10) was detected with the VIDIA and VIDAS systems.
Conclusion: The VIDIA Toxo IgG and VIDA Toxo IgM assays show an excellent sensitivity and specificity. Early seroconversion samples show the same delayed detection of IgG with the VIDIA as the VIDAS when compared with the Dye and HS tests.

P882

Toxoplasma gondii antibodies in 72 patients who attended emergency wards in three hospitals in Stockholm due to infective cat bites

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Objectives: *Toxoplasma gondii* causes severe infections in immunocompromised patients and may also infect the foetus of the pregnant women. The seroprevalence of toxoplasmosis in fertile women in Stockholm has in previous studies been reported to be 14%.
Methods: Patients who attended Emergency Wards in three hospitals in Stockholm due to infected cat bites were investigated by antibodies to *Toxoplasma gondii*, IgM and IgG (ELISA,

commercial methods). The patients were offered a follow-up at the Outpatients. Department and an additional sample of antibodies was performed.
Results: In 72 patients (51 women and 21 men, median age 53 years) antibodies to *T. gondii* were analysed in samples from the first visit at hospital, IgG antibodies were found in 20 patients (15 women and 5 men), in addition both IgM and IgG antibodies were found in one (female) patient. At follow-up at the Outpatients Department *Toxoplasma gondii* antibodies from 34 patients were analysed, no case of seroconversion was found between the visits.
Conclusion: Seropositivity to *T. gondii* was found in 28% of the patients with infected cat bites that is higher than previous studies from fertile women, however in this study the median age was higher.

Staphylococci and surgical infections

P883

Survey of the epidemiology of methicillin-resistant *Staphylococcus aureus* infections in Greece

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most frequent causes of community- and hospital-acquired infections (CA-MRSA, HA-MRSA). The aim of the present study was to characterize the MRSA clones in relation to clinical specimens and to investigate their spread in the hospital environment and the community during a four-year period.
Methods: We collected the MRSA from clinical specimens of different patients admitted at the University Hospital of Patras during 2001–2004. The MIC of oxacillin was determined by the agar dilution method in Mueller-Hinton supplemented with 2% NaCl, according to the guidelines of NCCLS. PBP2a production was investigated by a Latex agglutination test (bioMerieux) in all *S. aureus*. PCR amplification with specific primers was applied for the detection of mecA gene and 338 MRSA were identified. Clonal types were determined by PFGE of chromosomal DNA SmaI digests.
Results: An increase of MRSA infections was observed: 23% in 2001, 28% in 2002, 28% in 2003 and 44% in 2004. The majority of MRSA were isolated from the Department of Surgery (DS) (range 34–46%); while in 2004 there was a significant increase in the Intensive Care Unit (ICU) (17%) and the community (40%). PFGE classified the 338 isolates into six clonal types with distinct resistant phenotypes. Type A included 28 strains (8%) resistant only to b-lactams. Type B 70 (21%) and type E 21 (6%) strains exhibiting a multiresistant phenotype were isolated mainly from abscesses and pulmonary tract, respectively. Two hundred and twelve strains (63%) classified to type C were resistant to b-lactams and fusidic acid, associated with wounds and soft tissue infections. Three (0.8%) strains were characterized as type F resistant to erythromycin, while the newly described type G included 4 strains (1.2%) resistant to gentamicin, tobramycin and kanamycin. Among the CA-MRSA predominated PFGE type C, while in the ICU were spread strains of type E.
Conclusions: An increase of infections caused by MRSA was observed during the study period due to the spread of clone C in the community and the hospital environment.

P884

Nasal carriage rates of *Staphylococcus aureus* which are resistant and sensitive to oxacillin in hospital staff and hospitalised patients

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Aim: The aim of this study is to determine the carriage rate of methicillin resistant and sensitive *Staphylococcus aureus* (*S. aureus*) in hospital staff (physician, nurse and auxiliary personnel) of the Ankara Training and Education Hospital) and patients hospitalized in intensive care unit, and surgery and internal medicine wards and risk factors related to carrier status.
Material and method: Overall 1000 people (500 hospital staff and 500 hospitalized patients) were included in the study. Samples of nasal swab were inoculated into mannitol-salt agar, oxacillin resistance screening agar base (ORSAB) and chromogenic MRSA agar media, respectively. Coagulase positive colonies that yielded yellow colour in mannitol-salt agar were considered as *S. aureus*. Colonies that yielded blue colour in ORSAB medium and/or those that yielded pink or lilac colour in chromogenic MRSA agar medium were considered as methicillin resistant *S. aureus* (MRSA). Coagulase positive colonies that were grown only in mannitol-salt agar were accepted as methicilline sensitive *S. aureus* (MSSA). The confirmation of methicillin resistance in *S. aureus* strains were made in accordance with CLSI criteria using oxacillin, methicillin and cefoxitin discs in Mueller-Hinton agar medium.
Results: Nasal carriage rates were found to be 2% (10/500) for MRSA and 9.2% (46/500) for MSSA in hospital staff. In hospitalized patients, MRSA carriage rate was 6.4% (32/500) and MSSA carriage rate 14.4% (72/500). The majority of the staff who were nasal carriers were auxiliary health personnel (attendant, cleaning personnel) and most of them worked in intensive care units and surgical clinics.
Conclusion: The training of auxiliary health personnel on hospital infections, routes of transmission and preventive measures, screening of health staff working in clinics where hospital infections and colonization are prevalent and the patients hospitalized in these clinics for colonization at periodical intervals and treatment of colonization if necessary are important for the prevention of MRSA infections. This project was supported by Scientific and Technical Research Council of the Turkish Republic (TUBITAK) with project number of SBAG-AYD 493.

P885

Nasal carriage of *Staphylococcus aureus* among patients and personnel of a haemodialysis unit

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Nasal carriage of *Staphylococcus aureus* portends an increased risk of peritonitis and other serious infections for haemodialysis patients, thus increasing morbidity and mortality.
Objectives: The aim of our study was to determine the incidence of *S. aureus* nasal carriage among patients and personnel of the Haemodialysis Unit of a Secondary General Hospital (120 beds) and to investigate the study of the genotypical and phenotypical characters of the strains yielded.
Methods: A total of 35 individuals were examined during autumn 2005. Samples taken from the anterior snares of the

twenty-five haemodialysed patients and the ten personnel of the Haemodialysis Unit of our Hospital were cultured. The strains' identification was performed by catalase production, 24-hour coagulase test, and the API Staph system (Biomerieux, Marcy L' toile, France). All the strains were tested for the production of PBP2a (Slidex MRSA, Biomerieux, Marcy L' toile, France). Their susceptibility to antibiotics was performed by the disk diffusion technique and the results were interpreted according to the criteria of the N.C.C.L.S. Moreover, molecular analysis for the mecA gene and the Panton-Valentine leucocidin (PVL)-encoding genes, lukF and lukS, was performed by PCR.

Results: The prevalence of *S. aureus* nasal carriage among patients was 48% (12 out of 25) and 20% among personnel (2 out of 10). The strains isolated from the patients were all MSSA and did not carry the PVL-encoding genes. All the MSSA strains were susceptible to glycopeptides, linezolid, quinopristin/dalfopristin, cotrimoxazole, quinolones, rifampicin, amikacin, gentamicin, and netilmicin, whereas their susceptibility to fucidic acid, erythromycin and clindamycin was 92%, to tetracycline 85% and to penicillin 23% respectively. Only one strain carrying the mecA gene was recovered. This MRSA strain was isolated from a nurse and proved to be multisusceptible.

Conclusions: The haemodialysis unit of our hospital was found to have higher incidence of *S. aureus* nasal carriage among patients and personnel compared to other larger haemodialysis units of tertiary hospitals in Greece. However, MRSA carriage was rare and the most of the *S. aureus* strains isolated were multisusceptible.

P886

MRSA carriage in children undergoing cardiac surgery in Georgia

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Objectives: *Staphylococcus aureus* has been and continues to be a significant human pathogen. Although asymptomatic colonization with *S. aureus* is common, it appears to be an important factor in the development of most infections due to this organism. In the past two decades, methicillin-resistant *S. aureus* (MRSA) has increased in incidence in many parts of the world as agent of nosocomial infection. The spread of MRSA has become an alarming problem throughout the world. We studied the problems of the carriage of *S. aureus* in children, prevalence of MRSA, the correlation of the *staphylococci* carriage with the cardiac surgery intervention in the past.

Methods: At the Tbilisi Children's Cardiac Surgery Hospital it has been studied 294 patients, who underwent cardiac surgery from January 2003 to December 2004. The age of patients varied from 1 day to 18 years. From 294 operations 246 were primary ones, and 48 cases were reoperations. The average time period between the primary and reoperations was about 1.5 years. Prior every operation the smears from the throats of the patients were investigated.

Results: From 294 patients, prior the operation *Staphylococci* carriage was discovered in 121 cases (41.2%). Prior 171 primary operations – in 72 cases (41,9%), and prior 48 reoperations – in 18 cases (37.5%). 121 isolated strains of *S. aureus* the Oxacillin resistance was registered in 17 ones. Thus, 5.8% of 294 patients examined were the MRSA carriers. Prior the primary operation 10 patients (4.1%) were MRSA carriers, and in the case of reoperations 7 patients (14.6%) were MRSA carriers.

Conclusion: Prior the primary and reoperations a significant difference between the numbers of *S. aureus* carriage was not discovered. A substantial difference in the numbers of MRSA carriage was discovered prior primary (4.1%) and reoperations (14.6%). Thus, according to the studied cases, the operation did not affect the percentage of the *S. aureus* carriage, but increases the number of the carriage of the MRSA strains.

P887

Coagulase-negative staphylococci in a surgical hospital

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Objectives: The growing interest in coagulase-negative *Staphylococci* (CoNS), particularly, *S. epidermidis* and *S. haemolyticus*, as the etiological factors of nosocomial infections, is connected with the increasing number of implanted medical device or catheter related infections and bacteraemia. The aim of this study was to detect the prevalence of CoNS infections in a surgical hospital, and to characterize the species spectrum and methicillin resistance (MR).

Methods: The study was conducted during 2003–2004 at the Hospital of Traumatology and Orthopaedics, Riga, Latvia. CoNS isolated from clinical specimens were identified up to a species level. The antimicrobial susceptibility to a panel of antimicrobials was tested by the standard agar disk diffusion test (BBL) according to NCCLS standards and the E-test. Methicillin resistance was confirmed by genotypic profiles, defined by hybridization with the mecA gene in PCR and the latex agglutination test with a Slidex MRSA detection kit.

Results: The incidence rate of the isolated MR CoNS was significantly increased from 1.15% in 1998 to 18.5% in 2003 and 33.3% in 2004. During 2003, 143 CoNS cultures were isolated and identified. 89 of them were *S. epidermidis*, 16 – *S. haemolyticus*. A few cultures of *S. hominis*, *S. capitis*, *S. warneri*, *S. saprophyticus*, *S. simulans* were documented. During 2004, 54 and 11 cultures of *S. epidermidis* and S. *haemolyticus* were isolated. Methicillin resistance among *S. epidermidis* was 17.3% in 2003, and 37.2% in 2004. In *S. haemolyticus*, it was high – 52.9% and 100%. 17 cultures of *S. haemolyticus* were analysed for the presence of the mecA gene. According to the PCR results, all showed the presence of the 310-bp fragment of the mecA gene. The Slidex MRSA test was shown as very accurate for detection of the methicillin-resistant *S. haemolyticus* – the latex agglutination was clearly observed in all 17 cultures. 11 cultures were tested for MIC. According to the E-test results, only 2 cultures exhibited 8 mg/ml. In others, MIC was 256 mg/ml. For antibiogram typing, 46 cultures of MR *S. haemolyticus* were used. At least five different profiles were observed.

Conclusion: The results suggest that the methicillin resistance among CoNS has markedly increased in our hospital within recent years. The mecA gene detection and Slidex MRSA latex agglutination tests provide a reliable identification of MR *S. haemolyticus*.

P888

Prevalence of methicillin-resistant *Staphylococcus aureus* infection among inpatients colonised with MRSA

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St Vincent's University Hospital is a 492-bed tertiary referral teaching hospital. Like many Irish hospitals, methicillin-resistant *Staphylococcus aureus* (MRSA) is endemic. While information on both numbers of patients colonized with MRSA and rates of MRSA bloodstream infections is routinely collected, data on the proportion of MRSA-colonized patients who had infection attributable to MRSA was not available. We carried out a point prevalence study on a single day, which included all inpatients colonized with MRSA to determine the rate of MRSA

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infection in our hospital. All patients known to be colonized with MRSA were assessed to determine whether or not they were infected with MRSA on the day of study. Routine demographic data was collected along with information relating to duration of MRSA colonization, source of acquisition, length of stay, risk factors for MRSA infection, antimicrobial treatment and site of infection. Patients were divided into 2 groups: those who were colonized only and those who were being treated for MRSA infection. The patients in the second group were further subdivided according to whether or not they had infection according to CDC criteria. Forty-one inpatients were colonized with MRSA (9% of all inpatients) on the day of study – 22 (54%) were male, 19 (46%) female and the average age was 68.4 years. Thirteen patients (32%) were being treated for MRSA infection – of which seven (17% of MRSA-colonized inpatients and 1.5% of all inpatients) had definite infection according to CDC criteria. Of those, five infections were acquired in our hospital and two were healthcare-associated. One patient had probable infection; another was receiving appropriate empirical antimicrobial therapy. The remaining four were being treated inappropriately. We now have an indication of the rate of definite MRSA infection and the number of cases treated as MRSA infections in our institution. Although an incidence study would be ideal, there are simply not the resources to put this in place at present. We plan to repeat the point prevalence study monthly to allow us to monitor rates of MRSA infection and identify risk factors for infection.

P889

The role of screening and antibiotic prophylaxis in the prevention of percutaneous gastrostomy site infection caused by methicillin-resistant *Staphylococcus aureus*

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Introduction: Peristomal wound infection is a common complication of percutaneous endoscopic gastrostomy (PEG) especially in hospitals where methicillin-resistant *Staphylococcus aureus* (MRSA) is endemic. Recent evidence suggests antibiotic prophylaxis at the time of the PEG insertion may reduce post-procedure infections. We have examined rates of PEG associated MRSA infection before and after the introduction of an MRSA screening, decontamination and antibiotic prophylaxis protocol.
Patients and methods: Retrospective case detection ascertained new MRSA associated PEG site infection (isolated 1 month post procedure), over a 33-month period (January 2002 to September 2004). Prospectively from October 2004, patients requiring PEG insertion and found to be MRSA positive underwent nose (Mupirocin ointment, tds) and skin (Aquasept shampoo 2% triclosan) decontamination for 5 days prior to PEG insertion and received prophylactic teicoplanin 400 mg IV 30 minutes before the procedure. MRSA negative patients were given co-amoxiclav 1.2–1.8 gm IV. Peristomal wound sites were monitored by hospital infection control nurses for 1 month post PEG insertion for inflammation and purulent discharge and infected looking wound sites were swabbed. Rates of peristomal MRSA infection in patients pre- and post-screening/prophylaxis were compared.
Results: Peristomal MRSA infection was identified in 5 of 41 (12%) PEG insertions in 2002, 7 of 35 (20%) in 2003 and 7 in 24 (29%) in 9 months of 2004: Overall infection rate of 19%. Of 25 patients undergoing new PEG insertions from Oct 2004 (4 known and 5 identified by the screening as MRSA positive) only 1 (not previously MRSA positive) developed MRSA PEG site infection, but only 14 days post procedure (4%) (^{2} $p < 0.05$ for 2004 comparison, $p > 0.05$ for 2002, 2003 and pooled).
Conclusions: Screening for MRSA before a PEG procedure, followed by treatment of positive patients and appropriate antibiotic prophylaxis, can reduce PEG associated MRSA wound infections. Topical nasal mupirocin, combined with an anti-staphylococcal skin agent and followed by intravenous teicoplanin prophylaxis at the time of the procedure appears to be an effective regimen. The protocol and treatment used here was well tolerated by all patients and was easy to administer for nursing/medical staff. In MRSA endemic clinical areas, the risk of developing wound site infection may remain for some time post procedure unless high standard wound care is maintained.

P890

Surgical site infection and delayed sternal closure after open-heart surgery

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Introduction: Delayed sternal closure is a surgical technique aimed to avoid cardiac compression in some open heart surgery patients. Little is not known about the risk of surgical site infection related to this procedure.
Methods: Retrospective study of sternotomies left open after cardiac surgery and delayed sternal closure between 1994 and 2000. Characteristics of patients and surgery, as well as postoperative complications were recorded.
Results: 51 patients were included. Thirty (58.8%) were men and 21 (41.2%) women. Mean age was 68.7 (9.7) years. 30 (59%) patients had a comorbidity Charlson index > 2. Nine (17.6%) patients were diabetic. Indications for cardiac surgery were: valve replacement 18 (35.3%), myocardial revascularization 14 (27.5%), both procedures 11 (21.6%) and other 8 (15.6%). Mean (SD) surgery duration was 302 (135) minutes. Fifty (98%) patients received antibiotic prophylaxis, 17 (34%) of them during more than 1 day. Indications for DSC were: incontrollable haemorrhage 25 (49%), cardiac compression 23 (45%) and arrhythmia 3 (6%). Sternotomy remained opened for 1.8 (1.2) days. Among patients with delayed sternal closure (51), there were 0 deep surgical site infection (mediastinitis) and 2 (5.7%) superficial surgical site infection due to *Citrobacter freundii*, *Corynebacterium striatum*, and *Staphylococcus epidermidis*. Other infections recorded were: 20 (39.2%) respiratory tract infections, 4 (7.8%) catheter-related bacteremia, 2 (3.9%) urinary tract infections, and 1 (1.9%) other. Mean days of hospitalization was 36.5 (39) days. Global in-hospital mortality was 22 (43.1%). Death occurred in a mean (SD) time of 20 (36) days. 8 (36%) patients died during the period with sternotomy opened. Causes of death were: cardiogenic shock 15 (68%), septic shock 4 (18%), hypovolemic shock 1 (4.5%) and anoxic encephalopathy 2 (3.9%).
Conclusions: In patients with delayed sternal closure the risk of surgical site infection is low. However, mortality is very high, mainly due to non-infectious complications.

P891

The importance of risk indexes for stratifying surgical site infection rates

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Objectives: To determine compliance in completion of the three fields required to determine the NNIS risk index score and to identify categories of surgery that are not stratified well by the NNIS risk index.
Methods: From 1st January 2004 to 31st December 2004, data on 14660 surgical procedures were collected as part of the mandatory surgical site infection (SSI) surveillance programme in

Scotland. The percentage of not recorded values for the three fields required to calculate the NNIS risk index score; ASA score, wound class and operation duration were calculated for each category of surgery. The percentage of records where the NNIS risk index score was unable to be determined due to not recorded data was calculated for each category of surgery. The Scottish surgical category specific infection rates were stratified by NNIS risk index and plotted in a graph alongside the corresponding NNIS category specific infection rates.
Results: Completion of the fields used to calculate the NNIS risk index varied depending on the risk factor and surgical procedure category. The percentage of records where the NNIS risk index could not be determined due to missing risk factor data ranged from 12.8% for operations for fractured neck of femur to 42.3% for caesarean section surgery. The infection rates for all surgical procedures were not stratified well by the NNIS risk index. Additionally, the procedure specific infection rates did not follow the expected pattern when stratified by the NNIS risk index score.
Conclusion: Compliance in completion of fields used for risk stratification should be improved to allow adjustment for risk factors. In addition, the currently used NNIS risk index does not adequately stratify the Scottish SSI infection rates and further modelling work on risk factors is required to develop procedure category specific risk indexes for use in the Scottish setting.

P892

MRSA hand colonisation among health care workers

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Objectives: In this study, it is intended to find out the colonisation of methicillin-resistant *Staphylococcus aureus* (MRSA) on hands of doctors and other health care workers (nurses, attendants, etc) during their daily work. The study was held in Ministry of Heath Ankara Research & Training Hospital.
Methods: Totally 100 persons including doctors, nurses and other health care workers who work in intensive care units, general medicine and surgery departments and have direct contact with patients' open wounds were included to this study. Screening was performed during routine daily work regardless of hand washing. Samples were inoculated directly as smears from both hands to Baird Parker agar (RTA laboratories, Kocaeli, Turkey). After 48 hours of incubation, black colour forming colonies which grow 103 cfu or more were accepted for evaluation of *Staphylococcus aureus* (*S. aureus*). The colonies which are Gram positive, catalase positive, coagulase positive and shows yellow pigmentation in mannitol agar were identified as *S. aureus*. The isolated strains were inoculated to ''oxacillin resistance screening agar '' and chromogenic MRSA agar medias to investigate metisillin resistance. Then the methicillin resistances of the colonies which grow in these medias were confirmed with disc diffusion method using oxacillin disc in Mueller Hinton agar.
Results: There were 103 or above *S. aureus* growth in 68% of hands of medical staff while remaining 32% show no growth or less than 103 so not included to study. MRSA hand colonisation was found in 12 staff of 68 (17.6%).
Conclusion: The high rate of MRSA hand colonisation shows that the medical staff do not obey the hand washing rules during their daily work. But it is not possible to determine if this colonisation is persistent or temporary because the study was held regardless of hand-washing. Because of that our study which we will also compare the colonisation rates before and after handwashing is continuing.

P893

Susceptibility of MRSA to octenidine dihydrochloride

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Methicillin-resistant *S. aureus* (MRSA) strains are a major cause of sepsis in hospitals and they can lead to skin infections, septicaemia and death. Antiseptics, such as octenidine dihydrochloride, are used for the treatment of MRSA infected patients. The ability of bacteria and especially MRSA to develop resistance to antimicrobials, especially antibiotics is well documented and resistance to biocides has also been reported. It is important that products should be tested for any resistance arising from repeated and continued exposure. 76 MRSA (including several clonal variants of the two dominant UK MRSA: EMRSA-15 and EMRSA-16) and 24 MSSA from Scottish hospitals were tested for MIC to octenidine dihydrochloride (OCT) according to NCCLS methodology. Adaptation/Tolerance studies were performed on representatives of the five major international MRSA clones (CC5, CC8, CC22, CC30 and CC45). Isolates were grown in BHI broth in the presence of increasing concentrations of OCT for a period of up to 3 months. Octenidine dihydrochloride MIC of the parent and adapted isolates were determined. The MIC50 for the 76 MRSA and 24 MSSA was 4 μg/ml and 2 μg/ml, respectively and MIC90 for both was 4 μg/ml (range 24 μg/ml). The parent isolates of all five clones tested in the adaptation studies had an MIC of 4 μg/ml to OCT. Following continuous exposure to increasing concentrations of OCT over a three months period some differences were observed between clones in their ability to grow at increasing concentrations. The highest OCT concentrations allowing growth in broth were as follows: CC5 (8 μg/ml), CC8 (4 μg/ml), CC22 (6 μg/ml), CC30 (7 μg/ml), and CC45 (8 μg/ml). Although growth occurred at concentrations higher than the MIC of the ''parent'' strain the MIC of the adapted isolates was identical to that of the parent (4 μg/ml). In conclusion, the data indicates that, under these experimental conditions, the five epidemic MRSA clones tested failed to acquire resistance/reduced susceptibility following continuous exposure to increasing concentrations of octenidine dihydrochloride.

P894

Study on bacterial contamination of surgical rooms and delivery centres of Hamadan hospitals and health care centres

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Objectives: In order to identify bacterial agents that causing nosocomial infection, a cross-sectional study was carried out in 24 health care centres and hospitals in Hamedan, western Iran.
Materials and methods: In this study 554 samples were collected from different parts of surgical rooms and delivery centres from 24 health care centres, during 1 year. The samples were collected from floor, air, suction device, cuters, tables, surgical beds, walls, electric shock devices, sialectic light, eye microscope manometer, incubator, infant scales. The samples were culture on Blood agar and EMB agar by sterile cotton swabs. A smear was also prepared for Gram staining. The species were then identified by serotyping and biochemical tests.
Results: The mean frequency of contamination was 66.9% in surgical rooms (66.5%) and delivery centres (69.3%), the distribution of Gram-positive bacteria was 52.8% and Gram-negative

bacteria was also 47.2%. The most important Gram-positive bacteria were: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis* and *Bacillus subtilis*. The most important Gram-negative bacteria were: *Klebsiella pneumoniae*, *Enterobacter* spp., *E. coli*, *Pseudomonas aeroginosa* and *Acinetobacter baumanii*.
Conclusion: Our results showed that Gram-positive bacteria were most common bacterial agents that isolated from surgical rooms and delivery centres in Hamedan, western Iran. Our results also indicated that contamination was relatively high in studied places. It is suggested that operative rooms and delivery centres should be controlled regularly by health authorities.

P895

Reduction in the incidence of staphylococcal infections in hospital environment after the starting of the guideline of perioperative antibiotic prophylaxis

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Nosocomial infections caused by MRSA are increasing; moreover only few studies concerning the trend of MRSA after the application of perioperative antibiotic prophylaxis (PAP) guideline have been conducted.
Objectives: Comparing the general incidence of staphylococcal and MRSA infections in the Hospital of Mantua versus the incidence of staphylococcal and MRSA in the surgical wards after the introduction of PAP's guideline.
Methods: The hospital is a 530-bed tertiary care teaching hospital in Mantua with 30,000 patients admission per year. Two periods have been compared: 2nd Six Months (S) of 2002-1st S of 2003 (period A) and 2nd S of 2003-1st S of 2004 (period B), respectively before and after the introduction of the guideline of PAP. Statistical analysis has been performed using Mercurio (DIANOEMA) software; the rates of incidence of Staphylococcal and MRSA infections has been compared using the Fisher test.
Results: In the period A 10407 microbiological examinations have been executed; 637/10407 cases (6.1%) were positive for *S. aureus* and 119/637 (18.6%) were MRSA. In the period B 8421 microbiological exams have been performed: 315/8421 (3.7%) *S. aureus* and 81/315 (25.7%) MRSA. In the period A a significant increase of MRSA was found in the surgical wards comparing to the other wards: 58.6% vs 22.9% ($p = 0.001$), whereas in the period B was checked a relevant decrease of MRSA in the surgical wards compared to the general data (25.0% vs 25.7%) (p = ns). In the second period the data of MRSA in the surgical wards were found significantly reduced (58.6% vs 25.0%) ($p = 0.001$). Moreover in the period B the global antibiotic consumption was reduced with an increment of the cephalosporins of 1st generation (+11.5% of cephazolin) used correctly in surgical prophylaxis, while a remarkable decrease of consumption of cephalosporin of 3rd and 4th generation (respectively: −59% and −12.7%).
Conclusions: 12 months after the introduction of PAP a significant reduction of incidence of MRSA infections was found in the surgical wards; moreover such result can be ascribed to a greater adhesion to norms of ''good clinical practice''.

Streptococci - skin and soft tissue infections

P896

Characterisation of a clone of methicillin-resistant *Staphylococcus aureus* responsible for community-acquired staphylococcal toxic shock syndromes

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Objectives: To characterize *Staphylococcus aureus* isolates producing TSST-1 resistant to methicillin and fusidic acid but susceptible to ciprofloxacin, recovered from community-acquired as well as hospital-acquired infections.
Background: *Staphylococcus aureus* can produce many virulence factors and some of them, like the Panton-Valentine leucocidin (PVL) or the toxic shock syndrome toxin (TSST-1), are coded by accessory genes which have been acquired by some strains. Until recently, community-acquired staphylococcal infections were caused by methicillin-susceptible strains, but since a few years, epidemic infections due to methicillin-resistant *S. aureus* (MRSA) have been reported worldwide. Many community-acquired MRSA from European countries produce PVL, but to our knowledge only few strains have been described to produce TSST-1. We report here two cases of staphylococcal toxic shock syndrome (TSS) due to methicillin-resistant *S. aureus* (MRSA). If one of them was clearly community-acquired, acquisition of the other one was perhaps related to childbirth at hospital maternity although the onset occurred at home with colonization of all members of family.
Methods: MRSA responsible for TSS have been characterized by MLST, SCCmec typing, spa typing and PFGE. Furthermore we have looked for TSST-1 production by isolates with the same antibiotic resistance pattern (susceptible to ciprofloxacin and resistant to oxacillin, tobramycin and fusidic acid) isolated between May 2004 and August 2005. Consequently nine additional isolates have been characterized by PFGE, SCCmec typing, and spa typing.
Results: MRSA strains producing TSST-1 have been recovered from 11 infections or colonization but TSS have been diagnosed for only 2 patients. Isolates from TSS cases were identified as MRSA ST5-IV. By PFGE, among the nine additional strains seven were found closely related to each other and to the previous one. All these 9 isolates have a SCCmec type IV and a t002 or t002-related spa type.
Conclusion: Although some isolates have been recovered from hospitalized patients our findings suggest that strains belonging to the clone ST5-IV TSST-1+ were community-acquired. Among 9 patients infected or colonized, only 2 have developed a TSS.

P897

Antibiotic resistance of Gram-positive bacteria that cause severe skin and soft tissue infections in a Turkish university hospital

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Background: Since assessing the extend and severity of skin and soft tissue infections is difficult, therapy is often started before definitive microbiological and diagnostic data are available. The majority of SSTIs are caused by *Staphylococcus aureus* and B-hemolytic *Streptococci*. B-lactam/B-lactamase inhibitor

therapy have been using in empirical therapy for SSTIs in our hospital. The purpose of this study is to determine the research-based evidence and to re-evaluate our antibiotic regime used.
Methods: Eligible patients were hospitalized adults, between June–November 2005, with a diagnosis of a SSTIs, proven to be caused by a *Staphylococcus* or *Streptococcus* strain. The presence of at least 2 of the following characteristics was required: local heat; purulent drainage from a wound; erythema; body temperature 37.8 ° C; stage 1, 2, 3 ulcer severity on Wagner scale; and WBC count 10,000/mm^3.Cases of osteomyelitis, decubitis ulcers and burns were excluded. Resistance against nine-teen known or novel antibiotic were investigated with VITEC 2 system.
Results: Ninety-six gram-positive bacteria were isolated from 73 patients. Of these bacteria, 60.2% were *S. aureus* (60.9% were methicillin resistant), 16.1% *S. epidermidis*, 5.8% *S. haemolyticus*, 5.8% *Streptococcus viridans*, 4.4% *S. pyogenes*, 4.4% *S. agalactia*, and 2.9% were *S. hominis*. The highest resistance ratios were belong to ampicillin (85.2%), tetracycline (44.8%), and erythromycin (33.8%) groups. Second or 3rd generation cephalosporin resistance (cefaclor 30.8%-cefotaxim 32.3%) was not different statistically from amoxicillin/CA 29.4%,ampicillin/SB 32.3% or imipenem 30.8%. Gentamycin resistance was 19.1%, rifampin resistance was 17.6%, clindamycin resistance was 10.2%. Ciprofloxacin resistance was higher (17.6%) than levofloxacin (10.2%). No resistance found against glycopeptides (vancomycin, teicoplanin), Zyvoxid, quinupristin-dalfopristin and moxifloxacin.
Conclusion: Our recent surveillence datas support that 8090% of these pathogens remain susceptible to clindamycin, rifampin, and quinolones. But, we have an increasing resistance against cephalosporin, antistaphylococcal penicillin, or other β-lactam group antibiotics. These drugs with newer quinolones, and zyvoxid may be an effective choice both in outpatient clinics and in hospitalized patients.

P898

Contrasting features of Group A streptococci from asymptomatic carriage and diverse infection sites

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Objectives: To determine whether particular clones of Group A streptococci (GAS) were related to a particular tissue site of isolation or disease and the role of carriers as reservoirs of virulent and macrolide-resistant clones.
Methods: 854 GAS collected during 19992003, in Lisbon area, from four origins: asymptomatic oropharyngeal carriage (CA, n = 592), tonsillitis/pharyngitis (TS, n = 180), skin and soft-tissue infection (SI, n = 47) and invasive sites (IV, n = 35), were tested for macrolide resistance frequency and phenotypes (M or MLSB) by disk diffusion. All drug-resistant isolates (n = 224) and a fraction of the drug-susceptible isolates from all origins (n = 133) were characterized by T-typing and pulsed-field gel electrophoresis (PFGE), which was used to estimate a clonality index (no. of PFGE patterns/ no. of isolates per origin) and select major clones for sequencing (emm typing and MLST). Macrolide resistance genes - mef(A), erm(B), erm(A), and virulence genes - speA, speC, ssa, prtF1, were detected by PCR.
Results: Macrolide resistance was much higher in TS (30%), SI (25.5%) and CA (17.4%) than in IV (5.7%) isolates. Among the macrolide-resistant isolates, the M phenotype (mefA+) was predominant in CA (75%) and TS (60%) while the MLSB phenotype (ermB+/ermA-) was found in 50% of the SI isolates. The most common T-types in relation to clinical origin were: T12/T3.13.B3264/T4/T1 (62.5%) in CA, T8.25.Imp19/ T12/T3 (47.6%) in TS, T28/T12 (36.4%) in SI and T1/T3.13.B3264 (28.6%) in IV. The most predominant virulence gene in each origin was: speC in SI (57.1%), speA in CA (34.1%), ssa and prtF1 in TS (35.4% and 51%, respectively). In IV isolates, speC and prtF1 were more frequent (49% and 43%, respectively) than speA (26%) and ssa (3%). Clonality was higher in CA isolates (0.1) than among infection GAS populations (0.6). Clonality of M phenotype isolates was high in CA (0.1) and TS (0.2) and three major M phenotype clones comprised the majority of the throat isolates (CA and TS). One of these clones (T1; emm1;MLST-ST28) also included GAS from SI and IV infections.
Conclusion: The abundance of speA and speC exotoxins in asymptomatic pharyngeal GAS was impressive stressing the role of carriers as reservoirs of highly virulent strains, which seem to be associated mainly with throat infections rather than with skin or severe invasive infections. Carriers may also be the reservoirs of macrolide resistant M phenotype GAS strains with no apparent tissue site preference.

P899

Description of the epidemiology of multiple clusters of *Streptococcus pyogenes* infection in a rehabilitation hospital

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Objective: Description of the epidemiology and management of multiple clusters of *Streptococcus pyogenes* in a rehabilitation hospital
Method: Multiple clusters of infected and colonised patients with *Streptococcus pyogenes* including one staff were identified on two wards over six months. In February a cluster of five patients and a member of staff were identified with *Streptococcus pyogenes* isolated from hand lesion, ear and eye swabs and wounds. The ward was closed, deep cleaned, appropriate antibiotic treatment administered and symptomatic staff were screened. Subsequently two further patients with the organism were identified on the same ward despite implementing an education programme and reinforcing standard infection control measures. A further cluster of three patients was identified on another ward in May. One of these patients continued to be positive for the organism when a third cluster of five patients was identified two months later.
Results: The wards were closed, patients isolated or cohorted, screening throat and skin lesion swabs were obtained from staff and patients, environmental cleaning implemented and standard infection control precautions and education was reinforced. The strains were typed and the three major types detected were emm1, emm87 and emm28. These also reflected the major types found in the local community. The strains isolated from patients differed from the staff member. All strains from one ward had the emm28 type whilst the other ward had emm87 type, suggesting spread within the same ward but not between the two wards. In two patients, the organism was isolated despite at least 48 hours of previous penicillin treatment.
Conclusion: Control of the incident required communication of all stakeholders including infection control, public health, ward staff and managers. Characterisation of the strains confirmed the epidemiology of these infections. Azithromycin was successful in eradicating *Streptococcus pyogenes* especially in the patients with persistent infection despite penicillin therapy.

P900

The EPISA study: a recent epidemiological study in outpatients with skin and soft tissue infection in France

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The EPISA study was instigated to monitor the susceptibility of *Staphylococcus aureus* causing primary or secondary skin and soft tissue infections in the community in various regions of France. General Practitioners in 60 centres, located in 7 different geographic areas (Reims, Evreux, Cholet, Niort, Toulouse, Montpellier and Marseille) participated in the study. Between December 2003 and August 2004, 480 patients were included of whom 477 provided laboratory data and from whom 205 *S. aureus* isolates were grown from skin or soft tissue swabs (197 patients, 8 with 2 distinct strains). Of the 197 patients with *S. aureus*, 104 (53%) had a primary and 93 (47%) had a secondary skin and soft tissue infection. Swabs from patients were analysed by a central laboratory (GR Micro, London) and susceptibility to penicillin, oxacillin, erythromycin, clindamycin, tetracycline, gentamicin, fusidic acid, rifampicin, ciprofloxacin, vancomycin and mupirocin was tested by microbroth dilution. All but fourteen of the 205 *S. aureus* isolates were resistant to penicillin (86%) and 12 (5.8%) were resistant to oxacillin. Rates of resistance to other antibiotics were as follows: erythromycin 32%, clindamycin 3.4%, tetracycline 5.8%, gentamicin 0.5%, ciprofloxacin 9.3%, fusidic acid 4.4%, rifampicin 0%, vancomycin 0% and mupirocin 1%. Fourteen (6.8%) isolates were susceptible to all of the 11 tested antibiotics, 112 (54.7%) were resistant to one antibiotic, 58 (28.3%) to two antibiotics, 12 (5.9%) to three antibiotics, 4 (2.0%) to four antibiotics and 5 (2.5%) to five antibiotics. Results from the French EPISA study indicate that multiple drug resistance is nowadays common among *S. aureus* isolates from skin and soft tissue infection in the community in France, since 10.4% of the isolates were resistant to at least three antibiotics. Whether those multiple resistant strains spread from the hospitals or are related to clones prevalent in community remains to be investigated.

P901

Serotypes and antimicrobial susceptibilities of pneumococci isolated from children with acute otitis media during 2003–2004

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Objectives: To determine the serotypes and antibiotic resistance of pneumococci isolated from children of our geographical area suffering from acute otitis media (AOM) during a two year period (20032004).
Methods: A prospective study was conducted in three major Paediatric Hospitals of major area of Athens to study pneumococcal AOM in children up to 14 years, from January 2003 to December 2004. In children with clinical evidence of AOM, middle ear fluid was taken by tympanocentesis or in those presenting with otorrhea sample was taken by calginate swabs. The serotyping was carried out by the Quellung reaction. The antimicrobial testing was performed by the disc diffusion method according to the current NCCLS guidelines. The MICs of penicillin, amoxicillin and cefotaxime in non-susceptible to penicillin strains (PNSP) were determined by the Etest method. The erythromycin resistant determinants were characterized by the double-disc induction test.
Results: Overall, 215 of *Streptococcus pneumoniae* isolates were obtained from children with AOM. The major serotypes identified including 19F (53.0%), 14 (9.8%), 9V (7.9%), 6B (7.0%), 3 (6.5%), and 23F (4.6%), all together comprising 88.8% of isolates. The potential coverage rates by the 7-valent conjugate vaccine formulation was 84.6%. The resistance rates of the isolates were as follows: penicillin; P (62.3%; intermediately susceptible 42.3% and resistant 20.0%) cefotaxime (1.9%; intermediately susceptible 1.4% and resistant 0.5%), amoxicillin (2.3%), erythromycin; E (60.0%), clindamycin (18.6%), cotrimoxazole; Co (54.9%), tetracycline; T (48.4%), chloramphenicol (4.2%). All pneumococci were susceptible to rifampin and vancomycin. The majority of the erythromycin-resistant pneumococci exhibited the M-phenotype (65.1%), followed by the constitutive (31.0%) and inducible (3.9%) MLSB phenotype. Multi-drug resistant isolates (resistant to three or more classes of antimicrobials) were detected in 54.4% of the middle ear sample with the multi-drug resistant pattern PECoT more frequently isolated (53.8%).
Conclusions: Our data indicate that: a) The 7-valent conjugate pneumococcal vaccine could reduce morbidity in children. b) Despite the high rates of resistance to multiple antimicrobials, a spectrum of other antibiotics, including amoxicillin, remains highly active against the penicillin non-susceptible pneumococci.

P902

Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* causing invasive disease in childhood (2001–2004); microbiological observations from Central Greece prior to the systemic pneumococcal vaccination era

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Objectives: To determine the serotypes and antibiotic resistance of pneumococci isolated from children with invasive disease during 20012004, just before the systematic application of the 7-alent conjugate vaccine in Greece.
Methods: Concecutive pneumococcal isolates from children with suspected bacteremia/sepsis seen at three major Paediatric Hospitals of Athens from January 2001 to December 2004, were included in the study. The antimicrobial testing was performed by the disc diffusion method. The MICs of penicillin, amoxicillin, cefotaxime and ofloxacin in non-susceptible to penicillin strains (PNSP) were determined by the Etest method. The erythromycin resistant determinants were characterized by the double-disc induction test. The serotyping was carried out by the Quellung reaction.
Results: A total of 186 invasive *Streptococcus pneumoniae* isolates (IPD) were obtained from children with bacteremia/sepsis. Of these, 161 were recovered only from blood, and 25 from an additional source; 20 from CSF, and 5 from other material (joint fluid, ascitic fluid, and bone aspirations). The age of bacteremic patients ranged from 22 days to 14 years. Most of pneumococci (55.4%) were from children aged <2 years. The IPD isolates were distributed into 19 serotypes of which serotype 14 was prevalent (44.6%), followed by 6B (11.3%), 9V (7.5%), and 19F (6.5%). The potential coverage rates by the 7-valent conjugate vaccine formulation was 75.3%, and encountered more often among isolates derived from children <2 years of age than older ones (87.4% coverage vs. 60.2%). Among the IPD isolates 17.2% were PNSP and 1.2% intermediately susceptible to cefotaxime. Only a small number of PNSP (0.5%) exhibited MICs of penicillin >1 mg/l. High rates of resistance were observed to erythromycin and cotrimoxazole (30.1% and 23.7%, respectively). All

pneumococci were susceptible to amoxicillin, ofloxacin, and vancomycin. The majority of the erythromycin-resistant pneumococci (85.7%) exhibited the M-phenotype. Multi-resistant isolates accounted for 11.3% of the IPD sample and were more often isolated from younger (<2 years) than older children (71.4 vs. 28.6%).
Conclusions: These data indicate that: a) The 7-valent conjugate pneumococcal vaccine could potentially prevent a substantial proportion of episodes of bacteremic disease in Greek paediatric population. b) The β-lactams remain suitable antimicrobials for empiric therapy of invasive disease in this setting.

P903

Clinical characteristics and outcome of 22 patients with invasive infection due to vancomycin-resistant *E. faecium*

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Objective: Until recently, vancomycin-resistent *Enterococci* (VRE) have not been a major clinical problem in many European countries, including Germany. Here we report the clinical and microbiologic features of a cohort of patients with invasive VRE infections in a German tertiary care hospital.
Methods: During a prolonged outbreak that started in 2004, a total number of 167 adult patients were identified to be colonized and/or infected by VRE of whom 22 (13%) developed invasive infection [20 bloodstream infection (BSI), 2 peritonitis]. Clinical characteristics and outcomes of these patients were assessed after structured retrospective chart review.
Results: All VRE were identified as *E. faecium*, and all infections were hospital-acquired. The mean age was 53 years. 20 (91%) patients with invasive VRE infection had cancer (16 haematologic malignancies, 4 solid tumours), and two patients had liver cirrhosis. Of the cancer patients, 17 (85%) were not in remission. Nine had recently undergone allogeneic haematopoietic stem cell transplantation. 12 (60%) had neutropenia at the time of the BSI. A significant co-pathogen was found in 10 (45%) of patients. 36% of patients had received vancomycin, 23% metronidazol and 45% a 2./3. generation cephalosporin within 7 days prior to VRE bacteraemia. The 4-week crude mortality rate in our cohort was 59%. The mean duration of bacteraemia was 2, 7 days. In patients dying with VRE bacteraemia the duration of bacteraemia was significantly longer than in those surviving (4, 3 vs. 1, 1 days, $p = 0.04$). 4 deaths (33%) were thought to be attributable to VRE bacteraemia by clinical judgement. 81% of patients received an antimicrobial agent effective against VRE (15 linezolid, 1 quinupristin/dalfopristin, 2 a combination of both).
Conclusion: In our study population, VRE bacteraemia was largely confined to high-risk patients and especially those with uncontrolled haematologic malignancy. In a significant number of patients, additional significant co-pathogens were found. The overall and attributable mortality of VRE bacteraemia in this cohort was high, despite utilisation of antimicrobials active against VRE.

P904

Molecular characterisation of group B *Streptococcus* serotype III subtypes 1–4 in Hong Kong

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Objectives: To characterize Group B *Streptococcus* (GBS) Serotype III Subtypes I–IV by PFGE, surface protein genes, mobile genetic elements and multi-locus sequence typing.
Methods: 136 non-duplicate GBS serotype III isolates from patients admitted to Prince of Wales Hospital, Hong Kong, during 1993–2003, were examined. These included 73 isolates from bacteraemia and meningitis cases, 15 from wound/pus and 48 from swabs of newborns or from genital tracts of pregnant mothers. The isolates were differentiated into four subtypes, III-1, III-2, III-3, and III-4, by sequencing of the capsular polysaccharide genes, cpsE to cpsG and the isolates were typed by PFGE, gene specific PCRs on surface proteins (Rib or C alpha and beta, Alp2 and Alp3 protein genes) and mobile genetic elements including IS1548, IS861, IS1381, ISSa4 and GBSil. Multi-locus sequence types of the representative strains for each subtype were also identified by PCR and sequencing.
Results: 52.2% (71) of isolates belonged to subtype III-1, 25.0% (34) were subtype III-2, 5.1% (7) were III-3 and 17.7% (24) were III-4. Subtype III-1 was characterized by possessing the IS1548 and Rib protein gene, representatives belong to ST19 by MLST. Subtype III-2 also possessed the Rib protein gene, and GBSil, and belong to ST17. Subtype III-3 are heterogenous on PFGE, possessed Alp2 protein gene, and representatives belonged to ST23. Subtype III-4 constituted mainly invasive isolates from non-pregnant adults, and these strains have indistinguishable PFGE pattern, possessed C alpha protein and IS1381, and have unique ST by MLST (allelic profile 9-5-7-1-3-3-2) The distribution of strains from invasive disease versus colonizing strains for each subtype was calculated by Chi-squared test. Isolates of subtype III-4 predominantly were invasive strains ($p \leq 0.01$) whilst non-invasive strains were significantly distributed in subtype III-1 ($p \leq 0.05$) and no statistical significance for subtype III-2 or 3. Subtype III-4 constituted invasive isolates from non-pregnant adults, and was not isolated in neonates or genital tract of pregnant mothers.
Conclusion: GBS Serotype III subtypes-1 and -2 are known to cause invasive neonatal disease. An increase in invasive disease in healthy non-pregnant adults recently in Hong Kong may be due to the presence of subtype III-4 which was not seen in neonates.

P905

Role of isolation of *Corynebacterium* spp. organisms in different clinical settings, including intensive care

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Background: *Corynebacterium* spp organisms are commonly isolated, by their pathogenic role is still poorly known.
Methods: A bacteriological-clinical survey was conducted on all inpatients of our teaching Hospital, in order to identify all *Corynebacterium* isolates, and to assess them according to involved species, clinical materials, and hospital setting, with special attention on intensive care units (ICU).
Results: Of 161 *Corynebacterium* isolates of year 2004, 17 *C. glucuronolyticum* strains came from semen assessed during FIVET screening procedures, and were not further evaluated. *Corynebacterium* spp represented the most frequent isolate (78 episodes, with 4-11 repeated isolation in 2 patients), followed by *C. striatum* (33 strains), *C. jeikeium* (15), *C. urealyticum* (6), *C. propinquum* (5), *C. afermentans* (3), *C. macgihley* and *Corynebacterium* group G-1 (2 cases each). The most frequent clinical specimens were blood cultures (41), blood/CVC (14), bronchial aspirates/BAL (25), ascites/pleural effusion (14), surgical wound/ulcers (13), urine (12), and drainages (11). Interestingly, while all *C. jeikeium* and *C. propinquum* isolates came from blood

and catheters, *Corynebacterium* spp prevailed (>80%) in blood and lower respiratory tract cultures, C. striatum predominated in wound infections, ulcers, and drainages (>60%). When making a four-year survey of *Corynebacterium* strains isolated from all ICU since year 2001, 24 disease episodes were confirmed in 21 patients (15 males and 6 females, aged 32–90 years). *Corynebacterium* spp accounted of 11 isolates, followed by *C. striatum* (6), *C. jeikeium* (4), C. group G-1 (2), and *C. macginley* (1), while among clinical specimens bronchial aspirate/BAL prevailed (11 cases), over blood (6), peritoneal fluid/abdominal drainage (5), and surgical wounds (2).
Conclusion: *Corynebacterium* spp. are assessed with increasing interest, but their clinical role remains unclear. Based on their unpredictable sensitivity profile, further epidemiologicaò, clinical, and therapeutic studies are strongly needed.

P906

Skin colonisation of *Corynebacterium jeikeium*

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Objective: *Corynebacterium jeikeium* is an opportunistic pathogen particularly in immunosuppressed and neutropenic patients. The multiresistance of *C. jeikeium* clinical isolates is a common feature and often responsible for failure of antibiotic therapy. The aim of this study was to evaluate occurrence of these microorganisms in skin flora of different patient populations and to demonstrate antibiotic resistance profiles.
Methods: Fifty hospitalized patients from intensive care unit, 50 patients with different dermatological disorders from policlinic and 20 volunteers as the control group were included to the study. Skin smears were taken from axillar region of patients. Specimens were cultivated in blood agar + tween 80 medium for 48 hours – 7 days, at 37°C. Identifications were based on conventional methods and Api Coryne test. Antibiotic sensitivity tests were performed by disc diffusion and agar dilution methods according to NCCLS standards.
Results: *C. jeikeium* were isolated from 38% of hospitalized patients, 6% of patients with dermatological problems and 2% of control group. Multidrug resistance was established from 77% of isolates.
Conclusion: This study showed us the colonization rates of *C. jeikeium* were higher in hospitalized patients and dermatological disorders did not affect these rates. Opportunistic infections are usually caused by endogenic flora so the high colonization rate may be a problem for hospital acquired infections with these multidrug resistant organisms.

P907

Serotype distribution of *Streptococcus pneumoniae*-resistant strains isolated in Western Pomeranian Region of Poland in 2001-2003

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Objectives: *S. pneumoniae* is a main causative agent of upper respiratory tract infections and severe systemic diseases. In face of abrupt antimicrobial resistance increase and clonal spread of multiresistant strains, vaccine prophylaxis seems to be an important way of antipneumococcal defence. The aim of this study was to analyse serotype distribution of *S. pneumoniae* resistant strains isolated in our region during three years (2001–2003) and determination of pneumococcal vaccines effectiveness.
Methods: Using E-test method and the NCCLS criteria for benzylpenicillin, erythromycin, clindamycin, tetracycline, cotrimoxazole, ceftriaxone, chloramphenicol, vancomycin, imipenem, 80 resistant strains were obtained. Serotyping was performed using reference method of capsular swelling with pneumococcal diagnostic antisera kit of Statens Serum Insitut (Copenhagen).
Results: Resistance to 8 out of 9 determined antibiotics (except vancomycin) was described, with 63% of MDR strains. Serotype was determined for 73 strains, 2 were described as non-vaccine related, 5 were untypeable. We observed 9 different serotypes in following percentages: 6B—27.5%, 19F—21.3%, 23F—12.5%, 9V—12.5%, 14—8.8%, 18C–2.5%, 11A—1.3%, 33F—1.3%, 6A—3.8%. Eight of them can be found in 23-valent vaccines (except 6A type, which shows cross immunogenicity in serogrup 6), including 93.3% (and with 6A—97.3%) of serotyped resistant strains. In case of 7-, 9-, 11-valent vaccines this percentage is similar and concern 90.7% strains (with 6A – 94.7%). Serotype 23F was the most penicillin nonsusceptible with 80% of such strains and 4 of all 5 high penicillin resistant strains observed in the study. The highest number of MDR strains (41%) belonged to serotype 6B and all strains of this serotype were MDR. Other serotypes consisted of following percentages of MDR strains: 23F-80%, 14-71%, 19F-58.8%, 9V-40%, 18C, 11A, 33F-0%.
Conclusions: The majority of strains in the study are of serotypes present in pneumococcal vaccines. New generation 7-, 9-, 11-valent conjugated vaccines despite smaller serotypes spectrum, cover not much lower percentage of strains observed in our region, and because of their immunogenicity in children under two year of live, can be more effective in antipneumococcal defence. Such a low amount of non vaccine related resistant strains in study could be a result of low antipneumococcal vaccination in Poland. The higest multiresistance degree show serotype 6B, and serotype 23F is the most nonsusceptible.

ESBL and carbapemenase producing Gram-negative organisms

P908

First report of a metallo beta-lactamase produced by a *Klebsiella pneumoniae* clinical isolate in the United Kingdom

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Background: Class B carbapenem-hydrolysing beta-lactamases are metallo-beta-lactamases. Such enzymes are of great clinical significance as they degrade virtually all beta-lactams; so there may be very few therapeutic options remaining. They have been reported worldwide almost exclusively in *Pseudomonas aeruginosa* and other non fermenting Gram-negative bacilli.
Methods: The *K. pneumoniae* isolate was recovered from the urine of a 4-year old febrile neutropenic child who had received previous beta-lactam agents including meropenem. The isolate had reduced susceptibility to meropenem (MER) and imipenem (IMP) by disk diffusion testing and was only susceptible to ciprofloxacin.
Results: The isolate was identified using API 20E (Biomerieux, France). The Minimum Inhibitory Concentration (MIC) of IMP and MER as measured by E-test (AB Biodisk, Sweden) were

16 mg/l and 8 mg/l respectively. PCR amplification using specific primers for bla IMP and bla VIM was performed and an 830 bp product was amplified when using primers specific for bla IMP. Nucelotide sequence analysis of the PCR product identified the gene as bla IMP-9. Further molecular screening showed the bla IMP-9 to be located on a 200 kb plasmid and associated with a Class 1 integron.
Conclusions: Metallo-beta-lactamases producing *P. aeruginosa* have been increasingly reported from different parts of the world including from the UK. This important class of enzymes is also emerging in *Enterobacteriaceae*. This is the first report of an isolate of the *Enterobacteriaceae* family expressing a metallo-carbapenemase from the UK. The spread of new resistance determinants among nosocomial pathogens is of concern. This report highlights the continuing global emergence of these resistance determinants.

P909

Pandrug-resistant *Providencia rettgeri* producing PER-1 extended-spectrum beta-lactamase and VIM-2 metallo-beta-lactamase

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Objectives: The PER-1 enzyme, an Ambler class-A extended-spectrum beta-lactamase (ESBL), can hydrolyse most extended-spectrum beta-lactams except carbapenems and the VIM-2 type metallo-beta-lactamase (MBL) can hydrolyze all beta-lactams except monobactams. These *bla* genes are identified mostly in *Pseudomonas* spp. and *Acinetobacter* spp. During May to July, 2004, three strains of *Providencia rettgeri* with pandrug-resistance were isolated from urinary specimen of three patients hospitalized with a same hospital room and they were examined for the phenotypic and genotypic characteristics.
Methods: The minimal inhibitory concentration [MIC] was determined by broth microdilution method. The double disk synergy test and EDTA-disk synergy test were carried out for the screening of ESBL and MBL production, respectively. The PCR and sequencing analysis were performed for *bla*PER-1, blaIMP-1, *bla*VIM-1, *bla*VIM-2, and class I integron. The DNA fingerprinting of genomic DNAs was performed by a random amplified polymorphic DNA [RAPD] analysis.
Results: *In vitro* antimicrobial susceptibility test, these strains were resistant to all tested antibiotics, including ampicillin, piperacillin, cefazolin, cefotaxime, ceftazidime, cefepime, aztreonam (MICs, >256 μg/mL), cefoxitin (MIC, 128 μg/mL), imipenem (MIC, 16 μg/mL), meropenem (MIC, 8 μg/mL), amikacin (MIC, 128 or >128 μg/mL), tobramycin (MIC, 128 or >128 μg/mL), gentamicin (MIC, 64, 128 or >128 μg/mL), and ciprofloxacin (MIC, >64 μg/mL). All three strains were positive for the double disk synergy test and EDTA-disk synergy test indicating co-production of ESBL and MBL both and blaPER-1 and blaVIM-2 were detected in all three strains by a subsequent PCR analysis. PCR amplification for class 1 integron yielded a ca. 3,700 bp fragment, which was subsequently sequenced, revealing that the detected *bla*VIM-2 gene was part of a class 1 integron. RAPD analysis showed the same pattern of three strains indicating a single clonal origin.
Conclusions: Pandrug-resistant *Providencia rettgeri* strains were isolated from urinary specimen and they carried *bla*PER-1 and *bla*VIM-2 both in a single strain. The emergence of the strains which co-produce ESBL and MBL both in a single strain deserve great attention due to the limitation of antibiotics for the treatment of infection caused by these bacteria.

P910

An outbreak of VIM-4 producing multidrug-resistant *Pseudomonas aeruginosa* isolates from three different towns in Hungary

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Objectives: We have recently started monitoring metallo-beta-lactamase (MBL) producing clinical isolates at the National Center for Epidemiology, Hungary. In 2005 a cluster of MBL producing *P. aeruginosa* infections were observed in North-West Hungary. Our aim was to characterise these isolates by molecular and phenotypic tools and to establish their epidemiological relationship.
Methods: Antibiotic resistance was determined according to the CLSI recommendations. The MBL Etest and IPM-EDTA disk test were used for screening multidrug-resistant *P. aeruginosa* isolates. PFGE was carried out using the *Spe*I enzyme and analysed by computational methods. Integrons and *bla*VIM genes were amplified by PCR and sequenced with an ABI Prism 3700 Sequencer. Serotyping was carried out by monovalent O11 and O12 antisera.
Results: To date we have characterised 31 VIM-positive *P. aeruginosa* isolates from three intensive care units in three different towns in North-West Hungary. The isolates were cultured from clinical samples of thirteen patients, from the hospital environment and from faecal samples. Among the clinical samples trachea and drain samples were most prevalent. One patient with no previous related clinical history was identified as a carrier on admission, suggesting the presence of VIM-positive strains in the community. We have also identified carrier patients who were transferred between the concerned hospitals thus providing an epidemiological link between them. Several isolates were only sensitive to polymyxin. Macrorestriction profiling revealed that the strains selected for PFGE analysis from three different towns were clonally related and were also of serotype O11. One of these isolates was analysed for integron content and structure. These studies revealed the presence of three different integrons within this strain, with the largest one of more than 3 kb. This class 1 integron was sequenced and carried a *bla*VIM-4 gene cassette in its first position.
Conclusions: This is the first reported outbreak of MBL producing clinical pathogens from Hungary. Our observations together with several other VIM-producing isolates from different regions of the country suggests that MBL producing *P. aeruginosa* should be regarded as an important emerging multidrug-resistant pathogen in Hungary.

P911

Molecular epidemiology of VIM-producing *P. aeruginosa* isolated in four European countries

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Objectives: *P. aeruginosa* producing VIM-type metallo-b-lactamases have originally been detected in Mediterranean countries and, more recently, also in Poland, Sweden and Hungary. In the last two cases strain import from Greece was suggested. Our study was designed to explore relatedness between *P. aeruginosa* isolates producing enzymes of the VIM-1 lineage from Mediterranean countries and those emerging in Northern and Eastern Europe.

Methods: Twelve clinical isolates of *P. aeruginosa* producing VIM-1-like enzymes from Italy (n = 7), Hungary (n = 2), Sweden (n = 2) and Greece (n = 1) were studied. The genetic typing methods MLST, PFGE, RAPD and *fli*C sequence analysis were applied, as well as comparison of integron structure and serotype.
Results: Based on the results of the typing methods isolates were classified into four major groups. Group 1 was formed by 6 serotype O11 isolates from Italy with similar PFGE profile, identical MLST profile and *fli*C type a. Five different integron structures were observed in this group, all of them with the *bla*VIM-1 cassette in the first position. Group 2 contained a single non-serotypable Italian isolate with clearly distinct MLST and PFGE patterns. Three serotype O12 isolates constituted Group 3: two from Hungary [PA396, PA555] and one from Sweden [AK5493]. These three strains clustered together by RAPD, had an identical ST, and harboured *fli*C type b. The two Hungarian isolates were potentially related by PFGE, while the Swedish isolate was not. Both PA396 and AK5493 were isolated from Greek national patients. The Hungarian isolates had similar integron structures, featuring blaVIM-4 in the last position, while the Swedish isolate had a different structure. The fourth group comprised two serotype O11 isolates, one from another Greek patient in Sweden, and the other [Ps100] from Heraklion, Greece. The two strains had identical STs and *fli*C type a, but different PFGE patterns. In both isolates blaVIM-4 was found in the first position of the integron, but the other cassettes differed. Interestingly, Ps100 was found to be closely related to group 1 isolates by RAPD, although their MLST profiles were different.
Conclusions: Our results indicate a clonal diversity of VIM-1-like-positive *P. aeruginosa* spreading in Europe, but also underscore the role of human carriage in the international spread of MBL producing *P. aeruginosa*. MLST can be useful to establish epidemiological relationships between isolates that are discriminated by PFGE.

P912

Metallo-beta-lactamase-producing *Pseudomonas* spp. and *Acinetobacter* spp. in Korean hospitals: emergence of SIM-1-producing *Acinetobacter baumannii*

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Objectives: Acquired metallo-beta-lactamases [MBLs] have been increasingly reported in clinical isolates of gram-negative bacilli worldwide. The 5th MBL type, *bla*SIM-1 was first detected in *A. baumannii* clinical isolates at a Korean tertiary care hospital. The aim of this study was to determine any change of the types of MBL and prevalence of MBL-producing isolates among *Pseudomonas* spp. and *Acinetobacter* spp. collected from Korean Nationwide Surveillance of Antimicrobial Resistance (KONSAR) program-participating hospitals in 2005.
Methods: Non-duplicate, imipenem-resistant isolates of *Pseudomonas* spp. and *Acinetobacter* spp. were collected in 2005 from 23 KONSAR hospitals. MBL production was screened by the imipenem disk Hodge test and imipenem-EDTA + SMA double disk synergy test. blaIMP-1, blaVIM-2 and blaSIM-1 alleles were detected by PCR using heat-extracted DNA template.
Results: Among the imipenem-resistant isolates tested, 42 of 397 (10.6%) *P. aeruginosa*, 8 of 12 (66.7%) *Pseudomonas* spp. and 27 of 177 [15.3%] *Acinetobacter* spp. were MBL producers. Among the MBL producers, 37 and 13 isolates of *Pseudomonas* spp. had *bla*VIM-2 and *bla*IMP-1 alleles, respectively, and 10, 9 and 8 isolates of *Acinetobacter* spp. had *bla*IMP-1, blaSIM-1 and blaVIM-2 alleles, respectively. MBL-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. were detected in 15 of 23 (65%) and 9 of 23 (39%) hospitals, respectively. MBL-producing isolates existed in 9 of 10 cities/provinces in Korea.
Conclusion: VIM-2 type MBL continued to be dominant type in *Pseudomonas* spp., but in *Acinetobacter* spp., SIM-1-producing isolates emerged and prevalence of IMP-1-, SIM-1- and VIM-2-producing isolates were similar. It is a concern that *Acinetobacter* isolates producing the new MBL, SIM-1, are detected in two hospitals.

P913

Outbreak of multidrug-resistant *Acinetobacter baumannii* producing the carbapenem hydrolysing beta-lactamase OXA-58 in a general intensive care unit in Southern Italy

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Objectives: The oxacillin-hydrolyzing beta-lactamases belonging to Ambler class D are emerging resistance determinants in Gram-negative nosocomial pathogens. Since the first description of a carbapenem-hydrolyzing oxacillinase in 1993, several oxacillinases with a carbapenem-hydrolysing activity have been reported in MDR isolates of *A. baumannii*, but their epidemiology remains largely unknown. In this work we describe an outbreak due to *A. baumannii* producing the OXA-58 class *D. carbapenemase* in a general Intensive Care Unit of an Italian Hospital.
Methods: Sixteen nonreplicate carbapenem-resistant isolates of *A. baumannii* were collected from 16 inpatients at the Intensive Care Unit of the S. Giovanni Rotondo Hospital (Southern Italy) during the period November 2003 February 2004. Most isolates (14/16) were from the lower respiratory tract. MIC of imipenem (IPM) was determined by a microdilution test as recommended by CLSI. Double-disk test in MH agar plus cloxacillin (250 mg/L), was used for phenotypic detection of oxacillinases producers. IEF coupled with a bioassay, PCR amplification with primers specific for blaOXA-23, *bla*OXA-40 and *bla*OXA-58 were carried out to identify the OXA-type determinants. The clonal relationships between the isolates were evaluated by PFGE and Rep-PCR.
Results: All carbapenem-resistant *A. baumannii* isolates (MICs range 16–64 mg/L) were shown to produce a beta-lactamase (pI 7.2) active on oxacillin and IPM by the bioassay. In all cases the oxacillin hydrolysing beta-lactamase was identified as OXA-58 by molecular methods. The OXA-58 producers were also resistant to all beta-lactams, all aminoglycosides but amikacin and to fluoroquinolones. Genotyping showed that all the OXA-58 producers were clonally related suggesting a clonal spread within the ward.
Conclusion: This is the first report of a nosocomial outbreak caused by *A. baumannii* producing OXA-58 in Italy. The spreading of carbapenem-hydrolysing oxacillinases is of considerable concern for antimicrobial chemotherapy.

P914

Carbapenem-hydrolysing oxacillinase OXA-40 in an *Acinetobacter haemolyticus* clinical isolate

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Objectives: *A. carbapenem*-resistant *A. haemolyticus* strain was isolated, in 2002, from an university hospital where an endemic

multi-drug resistant (MDR) *A. baumannii* is currently been observed. The aim of this study was to determine the molecular basis of carbapenem resistance in this isolate.

Methods: *A. carbapenem*-resistant *A. haemolyticus* strain was isolated from a urine sample of a medicine ward attending patient in a Porto university hospital. Identification was performed using the API32GN system and by sequencing the 16S rRNA genes. MICs of beta-lactams and colistin were determined by Etest and agar dilution method, respectively. Susceptibility to non-beta-lactam antibiotics was performed by the disk diffusion method. Genes were sought by PCR with *bla*IMP, *bla*VIM and *bla*OXA-24-like specific primers. Obtained products were sequenced.

Results: The isolate was identified as *A. haemolyticus*, based on the results of 16S rRNA genes sequencing. It presented resistance to imipenem, meropenem (MIC of ≥ 32 mg/L), to amoxicillin and its association with clavulanic acid, ureidopenicillins and their associations (MIC of ≥ 256 mg/L), and was susceptible to cefepime and cefpirome (MIC of 8 mg/L), ceftazidime and aztreonam (MIC of 4 mg/L). It showed resistance also to colistin (MIC of 4 mg/L), to ciprofloxacin and variable behaviour to aminoglycosides. No PCR evidence suggested the presence of *bla*IMP or *bla*VIM type genes but a PCR product with *bla*OXA-24-like specific primers was obtained. Sequencing showed the presence of a OXA-40 enzyme.

Conclusion: In this study we describe, for the first time, the presence of OXA-40 enzyme in a *A. haemolyticus* clinical isolate. Although the spreading of OXA-40, both in the Iberian Peninsula and France, has been correlated with the progressive dissemination of a single *A. baumannii* clone, the observation of this enzyme in a different, previously unreported, genomic species, *A. haemolyticus*, poses new questions on OXA-40 dissemination.

P915

Influence of carbapemen resistance on outcome in *Acinetobacter baumannii* bacteraemia in an intensive care unit

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Objectives: The aims of this study were to determine the prevalence and clinical features of *A. baumannii* bacteremia (ABB) in an Intensive Care Unit, to identify the clinical differences between Carbapenem resistant and carbapenem-susceptible episodes, to know their prognosis, and finally to define if carpapenem resistance is a factor independently associated to hospitality and related mortality in ICU patients with ABB.

Methods: From 1996 to 2005, 322 patients with a clinically significant bacteremia were prospectively evaluated in ICU of a teaching hospital. Carbapenem resistant *A. baumannii* (CR) was defined as the laboratory documentation of strains of *Acinetobacter* which were not susceptible to Imipemen and Meropenem. Clinical and microbiological variables were studied. A multivariate analysis was performed to determine the factors independently associated to hospitality and related to bacteremia mortality in ICU patients with ABB.

Results: Seventy-nine (29%) of 322 ICU bacteremias were due to ABB. A sixty- eight percent of them were CR episodes. The mean age of patients with ABB was 60.6 years (SD 13.3) and the relation between men/women was 3.1. APACHE II score was 19.3 (SD 7.8). The incidence of inadequate empirical antibiotic treatment was 51.8% in ABB patients. The principal origins of ABB were: respiratory (49.3%), unknown (20.2%) and catheter (15.1%). Severe sepsis or septic shock was present in 56.9%. The global and related mortality rate for ABB was 56.9% and 25.3 There were no differences in APACHE II or SOFA score, presence of septic shock, foci of infection between CR and susceptible episodes. Only the incidence of inadequate empirical antimicrobial treatment was statistically higher in CR group (59.2% vs 25%; p = 0.009). No differences were also found in Global (62.9% vs 55%) and related mortality (27.7 vs 15%) rates between two groups. In a multivariate analysis CR was not independently associated with global or related mortality in ABB patients. Only the presence of severe sepsis or septic shock (OR 6.3; 95% CI 2.1-18.9; p = 0.001) was an independent predictor of hospitality mortality in critically ill patients with ABB.

Conclusions: The prevalence of ABB is very high among critically ill patients, and majority of them are CR. Although CR implies a higher rate of inappropriate empirical antimicrobial treatment, CR is nor associated with an increased global or related mortality in critically ill patients with ABB.

P916

Risk factors for nosocomial imipenem-resistant *Acinetobacter baumannii* infections

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Objectives: To identify the risk factors for nosocomial imipenem-resistant *A. baumannii* (IRAB) infections.

Methods: The study was conducted prospectively between January to December 2004 at a tertiary care hospital in Ankara. The patients who had nosocomial *A. baumannii* infections were enrolled to the study. The characteristics of the patients who had IRAB infections and imipenem-sensitive *A. baumannii* (ISAB) infections were compared. Only the first isolation of *A. baumannii* was considered. Nosocomial infections were defined according to CDC criteria.

Results: IRAB was isolated from 66 (53.6%) patients, and ISAB was isolated from 57 (46.3%) patients during the study period. IRAB were most frequently isolated from tracheal aspirate (28.7%) and blood (21.2%) cultures whereas, ISAB were most frequently isolated from wound (33.3%) and blood (31.5%) cultures. Mean duration of hospital stay until *A. baumannii* isolation was 20.8 ± 13.6 days in IRAB infections whereas 15.4 ± 9.4 days in ISAB infections. 65.1% of the patients with IRAB infection and 40.3% of patients with ISAB infection were followed at intensive care unit (ICU). Previous carbapenem use was present in 43.9% of the patients with IRAB and 12.2% of the patients with ISAB infection. According to the univariate analysis female sex, ICU stay, emergent surgical operation, total parenteral nutrition, having central venous catheter, endotracheal tube, urinary catheter, previous antibiotic use and previous administration of carbapenems were significant risk factors for IRAB infections ($p < 0.05$). In multivariate analysis, longer duration of hospital stay until *A. baumannii* isolation [odds ratio (OR), 1.043; 95% confidence interval (CI), 1.003 -1.084, p = 0.032], previous antibiotic use (OR, 5.051; 95% CI, 1.004-25.396, p = 0.049) and ICU stay (OR, 3.1; 95% CI, 1.398-6.873, p = 0.005) were independently associated with imipenem resistance.

Conclusions: Our results suggest that the nosocomial occurrence of IRAB is strongly related to an ICU stay, duration of hospital stay and IRAB occurrence may be favoured by the selection pressure of previously used antibiotics.

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P917

Dissemination of extended-spectrum beta-lactamase producers in natural environments in Northern Portugal

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Objectives: The aim of this study was to detect ESBL producers, in natural water streams reaching the sea and compare with those isolated from sea water samples. Our previous work, showed contamination of marine coastal water with antimicrobial resistant bacteria, namely ESBL (extended-spectrum β-lactamases) producers. This question alerted us to the origin of this contamination. In that way, it was our purpose to look for possible contamination sources, in water streams reaching the beach.
Methods: Natural water streams (probably including raining water streams) reaching the sea, were collected in July 2004 and 2005 (beach season, in Portugal), from 3 beaches of the Porto area. Isolates were selected by membrane filtration technique and the filters were placed on Mac Conkey agar and Mac Conkey agar with ceftazidime (2°mg/l) or cefotaxime (2°mg/l). Colonies of lactose fermenters were randomly selected and screened for ESBL production by the double disc synergy test. Identification of the selected strains was achieved by classic biochemical tools and ID 32 GN. Susceptibility to antimicrobial agents was determined according to the CLSI guidelines. β-lactamases were characterized by isoelectric focusing.
Results: The natural water streams accessed, in this work, seem to be impacted by faecal contamination of unknown origin, with antimicrobial resistant strains, namely ESBL producers. At least 4 water streams isolates, were able to transfer the ESBL gene, by conjugation.
Conclusion: Our tries to understand coastal sea water contamination with ESBL producers, showed that natural water streams reaching the seashore, are, in at least some part, responsible for seawater contamination with ESBL producers. Future work intends to find the origin of contamination of these natural environments. This situation seems relevant in terms of public health and environmental protection, once these are some of the beaches used by the Porto population. The incoming of ESBL producers to natural environments and the transferability of the ESBL genes by conjugation, might provide a track for environmental dissemination of resistant bacteria and genes, that may create a source of transferable traits for environmental bacteria, influencing natural reservoirs of resistance.

P918

The presence of extended-spectrum β-lactamase-producing *Escherichia coli* is a prognostic factor for patients with *E. coli* bacteraemia

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Objectives: *Escherichia coli* is one of the first causal agents of bacteraemia. However the published information about their prognostic factors is scarce, and not recent, so we decided to analyse this aspect in our patients with *Escherichia coli* bacteraemia.
Methods: Cases of Escherichia coli bacteraemia were identified by using the microbiology laboratory database. The charts of all patients with Escherichia coli bacteraemia attended at our hospital between January 2001 and December 2004 were reviewed with a questionnaire. Blood cultures had been incubated in the BacT/ALERT system (BioMerieux). In statistical analyses, Student's *t*-test was used for the comparison of mean values and chi square test and Fisher's exact test for the comparison of categorical data (two tailed). A stepwise logistic regression was performed for multivariate analysis.
Results: During the period of the study blood cultures were performed in 10132 patients, 372 of them (3.7%) with *Escherichia coli* isolation. The origin of the bacteraemia was a urinary infection in 219 (80.5%), unknown in 61 (16.4%), biliar in 57 (15.3%) and other origins in 35 (9.4%). Twenty three patients (6.2%) died during the admission. Patients who died were older (76.8°±°10.5 years vs 70.4°±°16.1 years), had a higher Charlson index (2.1°±°2 vs 1.1°±°1.6), more frequently had an antecedent of dementia (17.4% vs 3.6%), a non-urinary origin of the bacteraemia (59.6% vs 37.6%), a severe sepsis or shock (56.5% vs 8.3%), had a lower albumin plasmatic concentration (2.2°±°0.6°mg/dL vs 2.7°±°0.5°mg/dL) and more frequently had a bacteraemia cause by Extended-Spectrum β-Lactamase producing *Escherichia coli* (21.7% vs 2.7%). In the multivariate analysis only a non urinary origin of the bacteremia (OR: 3.36; 95% CI, 1.2–9.38), the presence of severe sepsis or shock (OR: 9.14; 95% CI, 3.47–24.07) and the presence of extended-spectrum β-lactamase producing *Escherichia coli* in the blood cultures (OR: 5.78; 95% CI, 1.38–24.47) were associated with the prognosis.
Conclusions: *Escherichia coli* bacteraemia, had a relatively low mortality among our patients. The existence of severe sepsis or shock, a non-urinary origin of the bacteraemia, and the presence of extended-spectrum β-lactamase producing *Escherichia coli* in the blood cultures were prognostic factors.

P919

Extended-spectrum β-lactamase producing *Enterobacteriaceae* in Lebanese ICU patients: epidemiology and patterns of resistance

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Introduction: The objective of this study is to assess the faecal carriage of ESBL producing bacteria in patients and health workers of intensive care unit of five Lebanese hospitals over a three-month period.
Methods: Faecal samples were collected in a period of 4 months from 378 patients that were admitted to the ICU in addition to 58 health workers of the same units. ESBL production was detected by double disk synergy as described by Jarlier et al. Then antibiotic susceptibility of ESBL-producing strains was determined by disk diffusion method and an enhancement of the zone of inhibition zone around ceftazidime, cefepime, aztreonam, and cefotaxime towards the clavunate-containing disk indicated the presence of ESBL's. Antibiotic susceptibility and MIC were determined by E-test.
Results: In total, 1442 faecal samples were collected during the whole study period from 278 ICU patients of the participating 5 hospitals. 118 strains isolated from 72 subjects were identified as ESBL-PS including 95 (80.5%) *E. coli*, 16 (13.6%) *Klebsiella pneumoniae* and 7 (5.6%) *Enterobacter cloacae*. The general characteristics of patients are represented in the table 1. Fourty one new patients, for whom a conversion from negative carriage at ICU admission to positive carriage after admission was noted, in addition to 18 patients who were previously colonized (at admission) then recolonized after at least 48 hours of ESBL producing strain eradication, were considered as acquisition cases (59 patients and 86 isolates). A higher rate of multiple carriages was detected among these acquisition cases (21double carriages and 3 triple carriages of ESBL-PS).

The figure shows the distribution of carriage of ESBL-PS over the different phases of ICU stay. The best susceptibility of ESBL-PS was associated to amikacin with values raging between 71.4%–92%. Ciprofloxacin followed by Trimethoprim-sulfamethoxazole seem to be associated with the lowest susceptibility (0–10% for ciprofloxacin and 9.5%–50% for TMX) excluding NN hospital in both cases.

Table 2: Antimicrobial activities (MIC_{50}, MIC_{90}, and percentages of susceptibility) of Imipenem, Cefoxitin, Ceftazidim, Cefotaxime, Ceftriaxone, Cefepime, Gentamycin, Amikacin, Amoxicillin/ Clavulanic acid[c], Piperacilline/Tazobactam[¶], Aztreonam, and Ciprofloxacin on ESBL-producing *E.coli, Klebsiella spp., and Enterobacter spp.*

	E. coli (N= 95)				*Klebsiella* spp. (N= 16)				*Enterobacter* spp. (N=7)			
	MIC_{50}	MIC_{90}	MIC range	% S	MIC_{50}	MIC_{90}	MIC range	% S	MIC_{50}	MIC_{90}	MIC range	% S
Imipenem	0.19	0.25	0.002- 0.5	100.0	0.12	0.25	0.016-0.19	100.0	0.12	0.5	0.094-0.75	100.0
Cefoxitin	1.5	16	0.5-32	82.1	6	20	1.5-16	50.0	32	64	16-64	26.8
Ceftazidime	128	>256	4- >256	10.5	96	>256	16-256	6.3	96	>256	48->256	0.0
Cefotaxime	32	96	2- >256	34.7	16	>256	2- >256	12.5	48	>256	3- >256	14.3
Ceftriaxone	>256	6	>256 48- >256	--	12	>256	4- >256	--	>256	6	>256 4-300	--
Cefepime	16	96	1- 256	--	2	64	0.75-64	--	8	96	1- 84	--
Gentamycin	4	64	0.25- 64	60.0	28	64	0.5-64	31.3	16	48	1- 48	42.9
Amikacin	2	4	0.125-16	83.2	4	8	0.25-16	75.0	2	8	0.25-8	100.0
Amx/ Clv[c]	16	32	1- >256	--	32	128	24-256	--	32	128	32-48	--
Pip/ Tazo[¶]	4	16	0.016- >256	66.3	8	32	4- >256	25.0	8	64	2- >256	0.0
Aztreonam	24	196	2- >256	--	64	>256	32- >256	--	128	>256	64- >256	--
Ciprofloxacin	64	64	16- 64	7.4	64	64	48-64	18.8	64	64	32- 64	28.6

Conclusion: Our results highlighted the increasing occurrence of ESBL-PS in the ICU environment of Lebanese hospitals suggesting the serious endemicity of this issue. On the other hand, it shows clearly that limited therapeutical alternatives are available for the treatment of infections produced by ESBL-PS. This is alarming for Infection Control and in the Lebanese hospitals and medical centres.

P920

Susceptibility pattern and prevalence trend over four years of extended-spectrum β-lactamase producing *Klebsiella* spp. from intensive care units: MYSTIC Program Brazil

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Objective: To evaluate the susceptibility pattern and prevalence trend over four years of ESBL-producing *Klebsiella* spp responsible for nosocomial infections in intensive care units (ICUs) participating in 2001, 2002, 2003, and 2004 editions of the MYSTIC Program in Brazil.

Methods: Two hundred and fifteen *Klebsiella* spp clinical isolates were collected by the five ICUs participating in each of the four yearly editions of the MYSTIC Program in Brazil, as follows: n = 50 (2001), n = 43 (2002), n = 66 (2003), and n = 56 (2004). Minimal inhibitory concentrations (MICs) were determined by E-test methodology to cefotaxime (CTX), ceftazidime (CAZ), cefepime, piperacillin/tazobactam, imipenem, meropenem, tobramycin, gentamicin, and ciprofloxacin. Isolates of *Klebsiella* spp with increased MICs (≥1.5 mcg/ml) for CAZ and/or CTX were considered as possible ESBL-producing phenotypes according to NCCLS criteria. Interpretations followed the respective year NCCLS documents. A chi-square for trend test (Altman, 1999) was applied to identify ordered differences in the ESBL rates along the four years studied. A chi-square test was also applied to compare the aggregated ESBL production rates of 2001–2002 and 2003–2004. P values below 0.05 were considered significant.

Results: The table presents susceptibility and ESBL producing rates of all *Klebsiella* spp isolates collected from the ICUs according to the year of isolation. Both carbapenems presented virtually full and stable susceptibility rates at all years. However, the remaining drugs showed decreasing susceptibility rates from 2001–2004, except for 2002. The chi-square for trend test yielded a p value of 0.02 for the ESBL rate among all four years. The aggregated 2001–2002 ESBL production rate was 53.7% (n = 50/93) and the 2003–2004 was 69.6% (n = 85/122). The chi-square test to compare the aggregated ESBL production rates among 2001–2002 and 2003–2004 yielded a p value of 0.01.

Susceptibility and ESBL producing rates of *Klebsiella* spp isolates from ICUs according to the year of isolation: MYSTIC Program Brazil

	%Susceptibility / Year			
	2001	**2002**	**2003**	**2004**
	n=50	n=43	n=66	n=56
Imipenem	100.0	100.0	98.5	100.0
Meropenem	100.0	100.0	98.5	98.2
Ciprofloxacin	74.0	76.7	68.2	35.7
Piperacillin/tazo	86.0	81.4	80.3	58.9
Cefepime	42.0	51.2	34.8	25.0
Ceftazidime	42.0	51.2	34.8	25.0
Cefotaxime	42.0	51.2	34.8	25.0
Gentamicin	52.0	67.4	40.9	26.8
Tobramycin	52.0	60.5	48.5	33.9
ESBL	**58.0**	**48.8**	**65.2**	**75.0**

Conclusions: The prevalence of isolation of ESBL-producing *Klebsiella* spp has increased significantly over four years in the units evaluated. Thus, decreasing susceptibility rates were observed among all antimicrobials evaluated in each year against these pathogens, with only the carbapenems presenting virtually full and stable activity. However, a biased sample cannot be ruled out, due to either possible clonal spread or overvalued resistant clinical isolates.

P921

Nosocomial outbreak due to an extended-spectrum betalactamase producer *Enterobacter cloacae*

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Enterobacter spp. have been associated with nosocomial outbreaks, generally involving strains that overproduce their chromosomal β-lactamase. However the presence of extended-spectrum β-lactamases (ESBL) have been rarely reported in these strains. Only sporadic cases of ESBL-*E. cloacae* (ESBL-EC) have been recognised in our hospital for the last years (5 unrelated cases in 2004).

Objective: To describe the clinical and molecular epidemiology of an outbreak caused by an ESBL-EC strain, in a Cardio-Thoracic Intensive Care Unit (CT-ICU).

Methods: Prospective surveillance of patients with infection/colonisation caused by ESBL-EC and weekly screening for faecal carriage among all patients admitted to the CT-ICU during the outbreak period (Jul–Sep 05). Production of ESBL was suspected by decreased susceptibility to expanded-spectrum cephalosporins and confirmed by a positive sinergy test with clavulanic acid. Clonal relatedness was determined by pulsed-field gel electrophoresis (PFGE).

Results: From July to August 2005, 7 patients were identified in the CT-ICU as having ESBL-EC, 4 males, mean age 67 + 10 years. Six patients had clinical isolates and 1 was identified by screening samples. Prior ICUs stay was 13.9 + 5.8 days and 6 patients had cardiac surgery. All were exposed to central venous and urinary catheters and mechanical

ventilation. Four patients developed infection: 3 pt. primary bacteraemia, 1 pt. Ventilator-associated pneumonia. Three patients were treated with carbapenems and 1 pt. with catheter withdrawal. The observation of resistance to expanded-spectrum penicillins and cephalosporins including cefepime, along with quinolones, gentamycin and tobramycin, allowed us to suspect the presence of an ESBL in these strains. PFGE revealed a clonal spread. The review of antibiotic consumption showed a huge increase of cefepime use during June and July 05. The outbreak was stopped with implementation of barrier measures and cephalosporins restriction.
Conclusion: The accurate microbiological surveillance identified an outbreak of ESBL-EC in an CT-ICU, related to cefepime consumption. The ESBL production could be an increasing problem in common nosocomial pathogens other than *E. coli* or *K. pneumoniae*.

P922

Clinical and epidemiologic features of extended-spectrum β-lactamases strains for a two-year period in a third level hospital

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Objectives: For the last years we are being witness of an increase of ESBLs productive strain isolations, making more difficult managing carrier patients. Our aim is to get to know the clinical and epidemiologic features of the ESBLs productive strains in the population attending our hospital.
Methods: A retrospective study was carried out from January 2003 to December 2004, where a total number of 6795 strains were isolated out of which 5847 were *E. coli* and 948 were *K. pneumoniae*. Antimicrobial suceptibilities were carried out by the MicroScan Walkaway automized system and by disk difussion test and E-test. Double-disk synergy test and E-test were used for screening ESBLs. The results interpretation were according NCCLS guidelines.
Results: Out of the total number of isolations obtained, the percentage of ESBLs strains was 3.3% (224) of which 87 (38.8%) were isolated during the year 2003 and 137 (61.1%) during 2004. Most of the strains were *E. coli* isolations 212 (94.6%) and only 12 (5.3%) of *K. pneumoniae*. In 59.37% of the cases the origin was extrahospitalarian being *E. coli* the species isolated more frequently, while in the intrahospitalarian 58.3% were *K. pneumonia*. Urine was the sample where it was isolated more frequently. The 224 ESBL strains belonged to 150 patients 92 (61.3%) women and 58 (38.6%) men with an average age of 60 years old (range: 1–98). Hospitalized cases were 37.33% (56) and most of them in the internal medicine department (30.3%), followed by the Intensive Care Units. Progress of the hospitalized patients went to resolution in 71.4% of the cases being exitus in 28.5% of them. In reference to sensitivity, co-resistance was detected with quinolones, fosfomycin, trimethoprim-sulfamethoxazole (TSX) and aminoglycosides, observing that the co-resistance more frequently found was with quinolones 68.1% followed by TSX 9.2%, aminoglycosides 28.3% and fosfomycin 4.4%. Only 37 (18.4%) did not show co-resistance with other groups of antibiotics. The co-resistance combination more frequently found was ESBLs more resistant to quinolones and TSX in 30.3% of the cases.
Conclusions: An important increase of ESBLs isolations have been proven from the year 2003 to the year 2004. Most of the strains were *E. coli* and of extrahospitalarian origin, being *K. pneumonia* more frequently isolated at intrahospitalarian level. A high level of co-resistance has been detected among ESBLs strains, where quinolones are the antibiotic family mostly affected.

P923

Risk factors for cephalosporin-resistant (ESBL and AMPc producing) Gram-negative enteric bacilli infection in renal and kidney-pancreas transplant patients

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Objectives: In our setting, cephalosporin-resistant gram-negative infections in renal and kidney-pancreas (RRPT) patients have been increasing over time. For this reason, we decided to investigate the risk factors for infection with cephalosporin resistant gram-negative bacilli (CRGN) in this group of patients.
Methods: From January to July 2005 all patients undergoing RRPT were prospectly evaluated. Pre-transplant detection of ESBL-producing gram-negative bacilli colonization was performed by culture of a rectal swab in MacConkey agar supplemented with cefpodoxime. The variables evaluated were: gender, age, underlying diseases (mellitus diabetes, chronic liver and heart diseases), immunosuppression, acute rejection episodes, surgical reinterventions, need for nephrostomy, use of antibiotics and post-transplant infections.
Results: We included 58 patients, 37 of whom were men (64%), with a mean age of 46 years (SD: 14 years). Forty-eight patients underwent kidney and 10 kidney-pancreas transplantation. The incidence of pre-transplant ESBL-producing gram-negative bacilli bowel colonization was 14% (in all cases *Escherichia coli*). The incidence of CRGN infection was 15.5% (9 patients), of which 6 were urinary tract infections (3 ESBL *E. coli*, 2 AMPc *C. freundii* and 1 ESBL *Klebsiella* spp) and 3 surgical wound infections (1 ESBL *E. coli*, 1 AMPc *C. freundii* and 1 ESBL *Klebsiella* spp). Three cases developed bacteremia (3 cases *E. coli*). Underlying diseases, immunosuppressive therapy, prior bacterial infection, use of antibiotics and prior acute rejection were not associated with increased risk of CRGN infection. Previous use of carbapenems did not prevent CRGN infection ($p = 1.0$). A trend towards the association of ESBL-producing *E. coli* colonization with ESBL-producing *E. coli* infection was observed (25 vs 4%;OR 8.0; 95% CI 0.95–67.7; $p = 0.09$). The need for nephrostomy (OR 11.25; 95% CI 1.6–81.6; $p = 0.025$) and surgical reintervention (OR 5.6; 95% CI 1.2–24.9; $p = 0.03$) were associated with higher risk of CRGN infection.
Conclusion: In our setting, the incidence of infection with CRGN and the prevalence of stool colonization with ESBL-producing gram-negative bacilli in RRPT patients was high. Patients requiring major surgical reintervention or those who need nephrostomy are at higher risk of developing CRGN infections. Patients with rectal carriage of ESBL-producing gram-negative bacilli may be at risk for ESBL infections, but a larger study is needed to confirm this results.

P924

Distribution of class 1 and class 2 integron types among ESBL-producing *Enterobacteriaceae* recovered from Portuguese hospitals

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Objectives: To analyse the overall prevalence and distribution of class 1 and class 2 integron types among ESBL-producing *Enterobacteriaceae* from different Portuguese nosocomial environments.

Methods: We studied 105 ESBL-producing *Enterobacteriaceae* recovered from patients attending at Portuguese hospitals (Oct 2002–May 2004): 41 *Klebsiella pneumoniae* (KP), 6 *Klebsiella oxytoca*, 37 *Escherichia coli* (EC), 10 *Enterobacter aerogenes* (EA), 3 *Enterobacter cloacae*, and 8 *Proteus mirabilis* (PM). Bacterial identification was performed by the Vitek System. Antibiotic susceptibility was performed by the standard disk diffusion method. ESBL expression was searched by the double disk synergy test. Characterization of ESBL was performed by IEF, PCR for blaTEM, blaSHV, blaCTX-M, and sequencing. Class 1 and 2 integrons were searched by PCR, typed by RFLP using *Alu*I and *Taq*I as restriction enzymes, respectively, and identified by sequencing (one per RFLP type).
Results: ESBL among the isolates studied were identified as 53 TEM, 28 SHV, and 25 CTX-M. Class 1 integrons were more prevalent than class 2 integrons (70% vs 5%). Presence of class 1 integrons was higher among TEM (46/53, 87%) than among the other ESBL—types-producing isolates (19/28, 68% for SHV; 7/25, 28% for CTX-M), and among KP, EA, and PM isolates (85%, 100%, and 100%, respectively). Class 2 was only detected among TEM-producing EC and PM (5/53, 10%). Eight different class 1 integrons were found, each containing different gene arrangements: A) aacA4, B) aadA1, C) aadA2, D) dfrA10-aadA1, E) dfrA17-aadA5, F) drfA12-orfF-aadA2, G) aac3Ia''-orfX- -aadA1, and H) aac6'Iic-orf. Type A was the most frequently found and observed in all TEM-24 producing isolates. Type B was associated to isolates producing SHV-55. For CTX-M-producing isolates we only detected the presence of Type E (3 CTX-M-14), and F (1 CTX-M-15). Types C, D, G, and H were only detected in isolates harbouring SHV-2, TEM-52, TEM-110 and SHV-12, respectively. Only one class 2 integron type was found (dfrA1-sat1-aadA1). Ten isolates harboured two class 1 integrons of different size and in four strains was observed simultaneous presence of class 1 and 2 integrons.
Conclusion: Class 1 integrons are widely distributed among ESBL-producing *Enterobacteriaceae* from Portuguese hospitals. A low diversity of integron types was observed, some of them being detected only in strains producing a specific ESBL which suggests their co-transference in mobile elements.

Metallo β-lactamases and integrons

P925

Interactions of ceftobiprole with serine *carbapenemases*

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Objectives: Ceftobiprole is a cephalosporin with broad-spectrum antimicrobial activity against methicillin-resistant *staphylococci*, penicillin-resistant *streptococci*, and most gram-negative pathogens. Ceftobiprole is very stable to hydrolysis by the *staphylococcal* β-lactamase PC1 and the AmpC β-lactamases from gram-negative bacteria, with hydrolysis rates of less than 1% those for cephaloridine. The class A *carbapenemases* that are occurring more frequently among the *Enterobacteriaceae* have not been studied with ceftobiprole. Therefore, the stability of ceftobiprole and the comparator cephalosporins, ceftazidime and cefepime, was determined in the presence of the Class A serine *carbapenemases* found in *K. pneumoniae* (KPC-2) and *S. marcescens* (SME-3).
Methods: β-lactamases were partially purified by gel filtration and ion exchange chromatography. Initial hydrolysis rates were determined spectrophotometrically in 50 mM phosphate buffer pH 7.0. KM and Vmax were determined using the Hanes plot. Hydrolysis rates were expressed relative to hydrolysis of cephaloridine. Imipenem was used as a positive control for hydrolysis.
Results: The SME-3 *carbapenemase* hydrolyzed ceftazidime, cefepime and ceftobiprole at less than 1% the rate for cephaloridine (0.01, 0.17 and 0.49%, respectively). The KM for ceftazidime was 170 mM, and ceftobiprole and cefepime had similar KM values of 270 and 280 mM. The KPC-2 *carbapenemase* hydrolyzed ceftazidime, cefepime and ceftobiprole at ≤11% the rate for cephaloridine (0.06, 2.4 and 10.6%, respectively). The KM values for these three cephalosporins were similar, at 231, 306 and 213 mM, respectively. The rates of hydrolysis for the cephalosporins were generally less than for imipenem.
Conclusion: Ceftazidime, cefepime and ceftobiprole were only slowly hydrolysed by both *carbapenemases* tested, and had sufficiently high KM values such that the enzymes would not be saturated under physiological conditions and Vmax would never be attained. The KPC-2 β-lactamase displayed higher relative hydrolysis rates for the *cephalosporins* than the SME-3 β-lactamase. Ceftobiprole stability was more similar to cefepime than to ceftazidime.

P926

Post-genomic detection of CAR-1, a new subclass B3 metallo-beta-lactamase from the important plant pathogen *Erwinia carotovora*

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Objectives: Metallo-beta-lactamases (MBLs) are zinc-dependent enzymes which commonly hydrolyse most beta-lactams. Apart from the clinically important enzymes (IMP, VIM, SPM, GIM and SIM), most MBLs are resident enzymes found in bacterial species of minor clinical interest (e.g. *Flavobacteria*, *Caulobacter crescentus*). The recent progress of genome sequencing revealed a wide distribution of genes encoding MBL-like proteins in both prokaryotes and eukaryotes. Although the presence of genes encoding functional MBL in these organisms and their relationship with acquired enzymes remain unclear, such enzymes represent interesting models for structure – function relationship studies. Here, we report the functional properties of CAR-1, a new MBL from *Erwinia carotovora*.
Methods: The blaCAR-1 ORF was detected by screening the available genome databases for the protein sequences sharing high sequence identity with known MBLs and conserved zinc-binding motifs. The ECA2849 (blaCAR-1) ORF from *E. carotovora* subsp. *atroseptica* SCRI1043 was cloned in vector pBC-SK to obtain recombinant plasmid pBC-CAR where the beta-lactamase gene was expressed under the Plac promoter in *Escherichia coli* DH5alpha. The *in vitro* antimicrobial susceptibility profile was determined using the microdilution method as recommended by the CLSI and a inoculum of 105 CFU/well. Beta-lactamase activity was measured spectrophotometrically with different beta-lactam compounds.
Results: In the genome of *E. carotovora*, an important plant pathogen, an ORF was detected (ECA2849) that encodes a 342-aa

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protein exhibiting 23% and 27% sequence identity with CAU-1 and JAP-1 MBLs, respectively, and a notably longer N-terminal domain (41 additional residues in comparison with L1). blaCAR-1 was expressed in *E. coli*, and beta-lactamase activity was detected (Sp. act., 3600 nmol/min·mg protein using cefuroxime as substrate), that was inhibited >95% after addition of 5 mM EDTA. In kinetic assays, the enzyme exhibited a strong preference for cephalothin (KF), cefuroxime (CXM) and cefotaxime (CTX). These data were in agreement with the susceptibility profile observed in *E. coli*, which showed resistance to KF, CXM and CTX (MICs: 64, 256 and >16 mg/l, respectively) but only decreased susceptibility to other agents, except cefepime and aztreonam.
Conclusion: A new functional subclass B3 MBL was identified in the genome of the important plant pathogen *E. carotovora*.
Acknowledgement: Supported by EU grant no. HPRN-CT-02-00264.

P927

Co-existence of VIM-type metallo-beta-lactamase and PER-1 type extended-spectrum beta-lactamase in clinical isolates of *Pseudomonas aeruginosa*

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Objective: Beta-lactamases are one of the major resistance determinants described for *Pseudomonas aeruginosa* strains. The PER-1 type extended-spectrum beta-lactamase (ESBL) has been first reported in some *P. aeruginosa* isolates in Turkey and widespread across the country. The VIM-type metallo-beta-lactamase (MBL) is another enzyme acting as carbapenamases and 11 variants have been identified. VIM-5 has recently been reported in a *P. aeruginosa* isolate in Turkey. We have conducted a study aimed to determine the probability of co-existence of those different types of beta-lactamases in the clinical isolates of multi-drug resistant *P. aeruginosa* in Hacettepe University Adult Hospital.
Method: Sixty-seven clinical isolates of *P. aeruginosa* recovered from patients with nosocomial infections between January 2002 and December 2004 are included in the study. The isolates are identified by Sceptor (Beckton–Dickinson, USA) and stored at -80°C until study time. PCR is performed for detection of blaVIM and blaPER-1 genes.
Results: According to the results of PCR experiments, 15 isolates are positive for PER-1 and 4 isolates are VIM positive. Interestingly, 3 of the PER-1 positive isolates are also positive for VIM enzyme. Two of the VIM and PER-1 positive isolates are recovered in 2002 and the other in 2004 from different units of the hospital.
Conclusion: Carbapenems are the first drugs of choice for the treatment of infections due to ESBL producing Gram negative bacilli. With the exception of aztreonam, VIM type MBLs hydrolyze all beta-lactamases, including carbapenems. The co-existence of MBLs and ESBLs in the same strain complicates the situation, resulting in a multidrug resistant strain of *P. aeruginosa*. This situation has been rarely reported as case reports in the English literature and to the best of our knowledge, this limited study is the first epidemiological analysis to identify a clinical isolate of *P. aeruginosa* producing both PER-1 type ESBL and VIM type MBL. These findings highlight the need for further systematic surveillance to allow for early recognition of threatening combinations of resistance determinants among nosocomial pathogens.

P928

VIM-2 metallo-beta-lactamase in *Pseudomonas aeruginosa* strains from Zagreb, Croatia

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Objectives: The worldwide spread of acquired metallo-beta-lacctamases (MBLs) in gram-negative bacilli has become a great concern. MBLs possess a broad hydrolysis profile that includes carbapenems and almost all extended-spectrum beta-lactams. The aim of this investigation was to characterize metallo-beta-lactamases (MBLs) in *P. aeruginosa* isolates from Zagreb, Croatia.
Materials and methods: Hundred *P. aeruginosa* isolates with reduced susceptibility to either imipenem or meropenem were tested for the production of MBLs by E test MBL. The strains were isolated during 2002 to 2005 at the Clinical Hospital Center Zagreb and University Hospital Merkur in Zagreb. The susceptibility to a wide range of antibiotics was determined by broth microdilution method. The strains which gave a positive results in the E test were chosen for the further investigation. The presence of blaVIM and blaIMP genes was tested by PCR. The amplicons were sequenced from both sides. Hydrolysis of 0.1 mM imipenem by crude enzyme preparations of beta-lactamases was monitored by UV spectrophotometer at 298 nm. Inhibition of enzyme activity was determined by measuring the residual carbapenemase activity after incubation of the crude extracts with 2 mM EDTA. Outer membrane proteins were prepared and analysed by SDS-PAGE. Pulsed field gel-electrophoresis (PFGE) was performed to determine if the strains were clonally related.
Results: Eight out of 100 isolates were positive for MBLs by E test. Six strains were resistant to imipenem and meropenem. Resistance to ciprofloxacin was detected in 4 and to aztreonam in 3 of these strains. Six strains were identified as VIM MBLs producers by PCR. Sequencing of blaVIM genes revealed the production of VIM-2 beta-lactamase in all six strains. No IMP MBLs producers were detected by PCR. All VIM beta-lactamase producing isolates hydrolyzed imipenem. The enzyme activity ranged from 1.2 to 42 nmol/imipenem/min/mg of protein. Carbapenemase activity was almost completely inhibited by 2 mM EDTA. Loss of OprD2 protein was found in 4 strains. The strains were not clonally related by PFGE.
Conclusions: This investigation proved the occurrence of VIM-2 beta-lactamase among *P. aeruginosa* strains from Zagreb, Croatia. The strains harbouring VIM-2 beta-lactamase were resistant to all beta-lactam antibiotics, aminoglycosides and fluoroquinolones and pose a serious therapeutic problem in our hospitals.

P929

First detection of blaVIM-2 and blaVIM-4 metallo-beta-lactamase genes in *Pseudomonas putida* isolates in Belgium

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Objectives: Multi-drug resistant *Pseudomonas aeruginosa* (Pa) are emerging nosocomial pathogens. Metallo-beta-lactamase (MBL)-producing Pa resistant to carbapenems have escalated worldwide since 2000, limiting therapeutic options and causing major concern. MBL have also been reported occasionally in non-*aeruginosa Pseudomonas* (*P. putida*, *P. fluorescens*). We describe the first identification of VIM-2 and VIM-4 producing *Pseudomonas putida* in Belgium.

Methods: During systematic screening of multi-drug resistant *Pseudomonas* species isolates in two Belgian university hospitals, we collected two clinical isolates of *Pseudomonas putida* with MBL-producing compatible susceptibility profile. The two strains were further analysed for determining resistance mechanism and were characterised using PCR, sequencing of the integron and PFGE genotyping.
Results: Two clinical isolates of *Pseudomonas putida* (Pp) originating from two different Belgian hospitals located in Brussels were highly resistant to meropenem and imipenem (CMI > 32 mg/L). Synergy imipenem/EDTA was observed by two different double disk tests (diam. imipenem/EDTA vs diam. imipenem >6) and by the E-test® MBL strip (ratio MIC imipenem/MIC imipenem-EDTA ≥8). Specific PCR targeting blaIMP gene was negative but PCR targeting blaVIM and integrase 1 genes were positive for both strains. Sequencing the integron between 5′-conserved sequence and 3′-conserved sequence revealed two different gene organisations inside the class 1 integron. One strain revealed blaVIM-2 gene next to an unidentified ORF. The other strain contains a blaVIM-4 gene next to an aacA4 gene that share common structural features with class 1 integron previously reported in Poland *Pseudomonas aeruginosa* isolates [JAC (2004) 53, 451–456]. PFGE genotyping confirms that the two Belgian isolates were sporadic.
Conclusion: To our knowledge, this is the first report of a VIM-2/VIM-4 MBL-producing *Pseudomonas putida* strains identified in Belgium in a clinical setting. The different and original structure of their integrons and distinct genomic backgrounds revealed that these strains were not epidemic and were unrelated to each other. These findings illustrate the horizontal spread of MBL genes among species of pseudomonads other than *P. aeruginosa*.

P930

Genetic structure of a VIM-2-encoding integron from a *Pseudomonas aeruginosa* clinical isolate from Belgium

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Objectives: Acquired metallo-beta-lactamases (MBLs), due to their extremely broad substrate profile and their potential for diffusion via horizontal transfer of mobile genetic elements, represent a worrisome evolution in the antimicrobial resistance landscape. VIM-type MBLs, first detected in Italy, are now widespread in Europe, Asia and the Americas, and have been detected in clinical isolates of *Pseudomonas* spp., *Acinetobacter* spp., *Enterobacteriaceae* and other nonfastidious gram-negative nonfermenters.
Methods: Bacterial identification and susceptibility profiles were determined using an automated Vitek II platform. Carbapenem-resistant clinical isolates obtained in the period Oct–Dec 2003 from different wards of the University Teaching Hospital in Liège (Belgium) were assayed for imipenem-hydrolysing activity using a spectrophotometric test and were screened for the presence of IMP- or VIM-type MBLs by RFLP–PCR. The structure of the MBL-containing class 1 integron was determined by PCR mapping and direct DNA sequencing of amplification products.
Results: Among 10 carbapenem-resistant *P. aeruginosa* isolates, only one produced an EDTA-inhibited imipenem-hydrolysing activity. A PCR analysis revealed the presence of a blaVIM gene. This isolate (3163608), isolated from a decubitus ulcer of a 73-year-old male inpatient, was resistant to most beta-lactam antibiotics (including carbapenems, expanded-spectrum cephalosporins and beta-lactam/beta-lactamase inactivator combinations), aminoglycosides and fluoroquinolones while it was intermediate to aztreonam (MIC, 16 mg/l), gentamicin (8 mg/l) and susceptible to colistin (2 mg/l). DNA sequencing revealed an original integron structure containing two gene cassettes encoding a VIM-2 MBL and an AADA5 aminoglycoside adenylyltransferase, respectively.
Conclusion: Although carbapenem-resistant *Pseudomonas aeruginosa* isolates are relatively unfrequent at our institution, one VIM-2-producing isolate was identified. The original structure of the VIM-2-encoding class 1 integron found in this isolate underlines the important potential of diffusion of such genes in different genetic contexts.
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P931

Spread of In58 containing blaVIM-2 among *Pseudomonas aeruginosa*

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In recent years, a large proportion of resistance to carbapenems in *Pseudomonas aeruginosa* can be attributed to metallo-beta-lactamases (MBL) genes presented in class 1 integrons. Six imipenem resistant *P. aeruginosa* were isolated in 2004 (2 isolates) at Santa Maria Hospital (HSM) and in 2005 (four isolates) at Santo António Capuchos Hospital (HC), located in Lisboa. The clinical isolates were screened for MBL production by EDTA disk synergy test. PCR experiments with specific primers were used to detect MBL genes (blaIMP and blaVIM) as well as to characterise the integrons containing these genes. The blaVIM-2 gene was found in all isolates and was located in integron containing more three gene cassettes: aacA7, aacC1 and aacA4. This integron was identical to the integron In58 previously found in *P. aeruginosa* identified in 1998 in Paris. In Portugal the blaVIM-2 gene cassette has already been identified in a different class 1 integron from *Klebsiella pneumoniae* clinical isolate. To assess the clonal relatedness between the isolates, molecular characterisation was performed using M13 fingerprinting. The two isolates from HSM had the same M13 pattern as two others isolates recovered from HC, suggesting a clonal spread between the two hospitals. The remaining isolates from HC were epidemiological unrelated. Our finding contributes to the observation that blaVIM-2 genes are disseminated in Portugal and underline the need for intensified epidemiological surveillance.

P932

Metallo-beta-lactamase gene blaSPM-1: evaluation of its vicinities in unrelated *Pseudomonas aeruginosa* strains isolated from distinct Brazilian hospitals

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Objective: To reveal the genetic environment around blaSPM-1 in unrelated *Pseudomonas aeruginosa* (PSA) strains and the possible role of the common region 4 (CR4) in the blaSPM-1 mobilization. Although common regions (CR) have been located near resistance genes, the role and function of these genetic

elements has not been well established. A new allele of CR element, CR4, was recently described upstream of blaSPM-1. CR4 was located downstream of groEL, a chaperonin encoding gene.

Methods: 25 clonally unrelated PSA strains (distinct ribotype and PFGE patterns) harbouring blaSPM-1 isolated from 7 Brazilian cities were evaluated. Primers designed for detection of blaSPM-1 were used with primers targeting the CR and groEL to determine the presence of these elements in the vicinity of blaSPM-1. In addition, degenerated primers were designed against CR elements and used to amplify target strains. The amplicons had been sequenced in both strands and the DNA sequencing results analysed.

Results: Amplicons of expected size (800 pb) with CR primers were detected in all 25 isolates. PCR performed anchoring CR primers to blaSPM-1 produced amplicons of 1.5 kb. Sequencing showed that CR4 was located straight upstream of blaSPM-1 in all evaluated isolates. The presence of groEL was also detected in the 25 isolates. DNA sequencing results demonstrated the same features in all 2 kb amplicons obtained by PCR using groEL primers.

Conclusion: groEL followed by CR4 were found upstream of blaSPM-1 in all unrelated PSA isolated from distinct Brazilian geographic regions. The same arrangement of these genes, without any insertions or deletions, was recovered from all 25 PSA isolates, showing a very conserved structure. These findings indicate that CR4 and groEL have been mobilized along with blaSPM-1 and that CR4 may not be responsible for blaSPM-1 dissemination among SPM-producing PSA isolated in Brazil. Although the mobilization of the plasmid carrying blaSPM-1 is difficult due to its size (around 100 kb), based on our results, it seems more likely that this element may be responsible for the mobilization of blaSPM-1.

P933

Discrepancy in the metallo-beta-lactamase phenotypic tests results associated with diversity in the promoter region of class 1 integrons

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Objective: The expression of metallo-beta-lactamase (MBL) genes was studied in MBL-producing *Enterobacter cloacae* and *Klebsiella pneumoniae* isolates due to discordant phenotypic test results for detection of MBL production.

Methods: Four *E. cloacae* and two *K. pneumoniae* isolates that possess blaVIM and blaIMP (see table below) isolated between 2001 and 2005 were evaluated. All isolates were submitted to MBL phenotypic detection: disk approximation method with a beta-lactam substrate (ceftazidime or imipenem) associated with a MBL inhibitor (EDTA and mercaptopropionic acid) and hydrolysis assay against imipenem. Search for the MBL genes was carried out by PCR followed by DNA sequencing. Class 1 integron promoter regions were also sequenced.

Results: The results are summarized in the table shown below. The disk approximation test detected all isolates as MBL producers. In contrast, only 3 of 6 isolates demonstrated positive hydrolysis results against imipenem. The isolates showing imipenem hydrolysis also had a significant inhibition of imipenem activity when previously treated with 25 mM EDTA. All MBL genes were located in the first position of class 1 integrons and just one promoter, Pant, was present in the MBL carrying integrons. However, the DNA sequence of the promoter region was different among the MBL carrying integrons.

Isolate	Organism	MBL	Hydrolysis Assay ΔAbs/min/mg of protein (EDTA inhibition)	Disk Approximation	promoter (P_{aac}) strength
KPN1	*K. pneumoniae*	IMP-1	- 0.416 (61%) – positive hydrolysis	positive	strong (-35 TTGACA 17bp -10 TAAACT)
KPN2	*K. pneumoniae*	IMP-1	- 0.289 (65%) – positive hydrolysis	positive	strong (-35 TTGACA 17bp -10 TAAACT)
ECL1	*E. cloacae*	VIM-1	+ 0.022 – negative hydrolysis	positive	intermediate (-35 T**G**GACA 17bp -10 TAA**G**CT)
ECL2	*E. cloacae*	IMP-like	+ 0.016 – negative hydrolysis	positive	intermediate (-35 T**G**GACA 17bp -10 TAAACT)
ECL3	*E. cloacae*	VIM-like	+ 0.025 – negative hydrolysis	positive	intermediate (-35 TTGACA 17bp -10 TAA**G**CT)
ECL4	*E. cloacae*	VIM-like	- 0.062 (80%) – positive hydrolysis	positive	intermediate (-35 TTGACA 17bp -10 TAA**G**CT)

Conclusions: Hydrolysis assays against imipenem and meropenem have been considered an important tool for MBL detection; however, in the current study the disk approximation method demonstrated better performance than the imipenem hydrolysis assay for detection of MBL production. Our findings suggest that differences in the promoter DNA sequence directly influenced the expression of MBL genes, and consequently the proper phenotypic method to detect them. This is the first report to correlate the diversity of the promoter strength with MBL expression in *Enterobacteriaceae* isolates.

P934

Biochemical characterisation of IND-NF16 metallo-beta-lactamase

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Objectives: Metallo-β-lactamases (MBLs) constitute an heterogenous family of enzymes that are most prevalent in nonfermenting Gram-negative bacteria and more recently in *Enterobacteriaceae*. The spreading of new metallo-β-lactamases resistance determinants among nosocomial pathogens could represents a world wide problem. Actually several type of MBLs were characterized: VIM-, IMP-, GOB-, SPM-, IND-types belonging to three subclasses.

Methods: The nature of gene was determined by PCR and direct sequencing. Sequence was determined on two independent amplification products for each isolate. The amplicons corresponding to blaIND-NF16 was cloned in pET-9a vector and transformation of recombinants plasmids was performed using *Escherichia coli* BL21(DE3) competent cells. IND-NF16 metallo-β-lactamase was purified from *E. coli* BL21(DE3) (pET-9a-INDNF16), by two chromatographic steps using Mono Q and Mono P chromatographic column. Steady-state kinetic parameters (K_m and k_{cat}) were determined by measuring substrate hydrolysis under initial rate conditions and by using Hanes linearization of the Michaelis–Menten equation. Inhibition by chelating agents was studied in 30 mM sodium cacodilate buffer pH 6.5 at 30 °C, using 100 μM nitrocefin as the reporter substrate, in the presence of different concentration of EDTA, 1,10-*o*-phenantroline, and pyridine-2,6-dicarboxylic (dipicolinic) acid.

Results: Compared to IND-1, the IND-NF16 enzyme showed 17 amino acid residues mutated. The IND-NF16 metallo-β-lactamase was purified by two chromatographic steps which yielded the enzyme as more than 95% pure. Under our experimental conditions, IND-NF-16 metallo-β-lactamase was able to hydrolyze several β-lactams except ceftazidime, cefepime, aztreonam and moxalactam. Concerning the interactions with chelating agents, such as EDTA, dipicolinic acid and 1,10-*o*-phenantroline, IND-NF16 showed a K_i value of 815 μM, 3.0 μM, 7.3 μM, respectively. The enzyme was shown to have $t/2$ of stability of 90′ (40°C), 25′ (45°C) and <1 min (55°C).

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 an efficient mechanism of carbapenem resistance in these isolates.

P935

Phenotypic detection of metallo-beta-lactamases in *Acinetobacter baumannii* strains intermediately resistant to imipenem and susceptible to meropenem

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Objective: The purpose of our study was to determinate the proportion of metallo-beta-lactamases producing *Acinetobacter baumanii* strains that were multidrug resistant and showed intermediate resistance to imipenem and susceptibility to meropenem.
Methods: We studied 57 non-repetitive *Acinetobacter baumanii* strains collected from hospital infection samples over a 3-year period (from 2003 to 2005). Identification and susceptibility testing were performed using the Vitek 2 Automated System (Biomerieux). The examined strains were intermediately resistant to imipenem and susceptible to meropenem. MIC values of imipenem and meropenem were determined by the Vitek 2 system and confirmed by E-test (AB Biodisk, Solna, Sweden), according to NCCLS guidelines. These strains were also tested by E-test MBL [Imipenem (IP)/Imipenem plus EDTA (IPI)] for a possible metallo-beta-lactamases production.
Results: The imipenem MIC values of the examined strains were greater or equal to 6 mg/L and less than 16 mg/L. The meropenem MIC values were lower than 4 mg/L. We found 14 (24.5%) positive results, that showed a reduction in the imipenem in the presence of EDTA (IPI) of greater or equal to 8-fold (IP/IPI > 8) and they were interpreted as probably metalo-beta-lactamases producers, 9 (15.8%) were negative and 34 (59.7%) undetermined results. 29 out of 34 strains with undetermined results had imipenem-MIC value of 6 mg/L.
Conclusions: The phenotypic detection of metallo-beta-lactamases was easy to perform but the interpretation of the results was rather complicated. Nevertheless, the laboratory detection of these enzymes is important because our results showed a notably high proportion of metallo-beta-lactamases producing intermediately resistant to imipenem and susceptible to meropenem *Acinetobacter baumanii* strains. These strains should not be underestimated since they are isolated mainly from samples of ICU patients, are multidrug resistant and can cause therapeutic failure.

P936

Metallo-beta-lactamase production in Gram-negative bacteria detected by double disk synergy and combined disk tests in Turkey

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Objective: Anti-bacterial resistance due to metallo -lactamases (MBL) has been reported in Gram negative bacilli such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Acinetobacter baumanii* and *Stenotrophomonas maltophilia* which are among the most common bacterial agents in nasocomial and neutropenic infections. This class of enzymes hydrolyse carbapenems as well as broad spectrum cephalosporins such as ceftazidime. Although antibacterial resistance rates are quite high against both of these drugs, it is not known how much of this resistance is related to MBLs in this area, we aimed to investigate the rate of MBLs positivity in Gram negative isolates resistant to carbapenems, ceftazidime or both.
Materials and methods: During the period from May 2004 to July 2005, a total of 92 isolates found to be resistant to carbapenems, ceftazdime or both were included in the study. To determine the MBL activity, double-disk synergy test using a ceftazidime disk and 2-mercaptopropionic acid (2MPA) disk and IMP-EDTA disk diffusion method were used.
Results: Out of 92 isolates, 34 (36.9%) were carbapenem resistant, 81 (88.0%) were ceftazidime resistant and 21 (22.8%) were resistant to both antibiotics. The rate of MBL positivity was 11 in 26 (42.3%) *P. aeruginosa*, 2 in 46 (4.3%) *K. pneumonia*, 7 in 8 (87.5%)*S. maltophilia*, 3 in 5 (60.0%) *A. baumanii*. None of the *K. oxytoca* was MBL positive. Eight of 24 MBL-positive isolates were resistant to one of the study drugs (3 to ceftazidime and 5 to carbapenems) and remaining 16 isolates were resistant to both antibiotics. This means that the rate of the MBL causing resistance to both carbapenem and ceftazidime is 66.6% in MBL positive isolates and 17.4% (16/92) in carbapenem and/or ceftazidime resistant Gram negative bacilli.
Conclusions: The present study has shown that MBLs are among the most important reasons for the antimicrobial resistance to carbapenems and ceftazidime in Gram negative bacilli in our region and most of them cause resistance to both carbapenems and ceftazidime. Since these enzymes are transferable, infection control and antibiotic control measures are important to prevent the spread of MBLs.

P937

Real-time PCR detection of free circular intermediates of gene cassettes inserted in resistance integrons

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Objectives: Integrons provide a major contribution to the spread of antimicrobial resistance, by promoting the dissemination and expression of functional genetic units, the gene cassettes, in bacterial genomes. The aim of this study was to set up a real-time PCR assay to investigate the mobilisation of resistance determinants carried by gene cassettes via circular intermediates.
Methods: Real-time PCR experiments were performed on DNA samples obtained from a *Pseudomonas aeruginosa* clinical isolate harbouring a chromosomal resistance integron (RI) that carries clinically-relevant resistance gene cassettes (blaVIM-1, aacA4, aphA15) and from laboratory strains of *P. aeruginosa* and *Escherichia coli* carrying the same RI on a non-conjugative plasmid. Circular intermediates of gene cassettes were detected by real-time PCR using primers designed in divergent orientation on the resistance genes. An *E. coli* strain carrying the RI and over-expressing the intI1 integrase gene, known to enhance the frequency of cassettes excision and representing a widely used model, was used as control.
Results: Real-time PCR protocols were established to detect and quantitate circular intermediates of blaVIM-1, aacA4 and aphA15 gene cassettes. The size and nucleotide sequence of the obtained amplicons were consistent with the expected excision product. Contribution of duplicated gene cassettes in the detection of circular intermediates was also investigated. Notable diversity of generation of circular intermediates of the various gene cassettes was observed, depending on the nature of the cassette and its position inside the cassette array, and on the bacterial host.

Conclusions: A real-time PCR assay was set up to detect and quantitate circular intermediates of RI gene cassettes, that could be useful to investigate the mobilization rate of resistance determinants in clinical isolates and to compare their relative rates, under different growth conditions and in different bacterial hosts.

P938

ISAba1 transposition induced by ciprofloxacin produces hyperexpression of the AmpC cephalosporinase of *Acinetobacter baumannii*

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Objective: The purpose of this study was to analyse the prevalence of ISAba1 (IS) in 76 epidemiologically-unrelated *A. baumannii* clinical isolates and to investigate the induction of ISAba1 transposition by ciprofloxacin (CI).
Materials and Methods: 221 *A. baumannii* strains were isolated from 28 different hospitals in Spain in a multicentre study. MICs of ceftazidime (TZ) and CI were determined by a microdilution system and the E-test. Genotyping was performed using PFGE and REP-PCR. The ampC gene, the IS element and the location of IS upstream from the ampC gene were detected by PCR. Two strains were chosen to analyse the induction of IS transposition by CI since both were susceptible to CI (MIC of 0.5 μg/ml) and TZ (MIC of 4 μg/ml), had the IS element located outside the promoter region of the ampC gene. These strains were also incubated overnight with LB broth containing 0.25 μg/ml with and without CI. The next day they were spread onto Mueller-Hinton plates containing 32 μg/ml of TZ. Analysis of the mRNA of these strains was performed by RT-PCR.
Results: In 74 (97.3%) out of the 76 strains analysed, the ampC gene was detected and 51 (69%) strains were positive for the IS element. Among the *A. baumannii* strains containing the IS element in 40 (78.4%) this element was located in the promoter of the ampC gene. All strains with the IS element located in the promoter region of the ampC gene were resistant to TZ, whereas only 27.3% of the *A. baumannii* strains without it were resistant to this antibiotic. All the strains carrying this construct were resistant to CI. The average ratio of IS transposition induced by CI was $\sim 2.5 \times 10^{-4}$.
Conclusion: Quinolones can induce the transposition of the IS element to the promoter region of the ampC gene generating overexpression of ampC and transforming the susceptible strain into one that is resistant to TZ.

P939

Prevalence of class 1 integrons and MIC values in indigenous and pathogenic *E. coli* strains in different age groups

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E. coli belongs to indigenous intestinal microflora yet causing several intestinal and extraintestinal infections. The antibacterial treatment can facilitate the transmission of resistance gene cassettes, e.g. integrons between indigenous and infectious strains. However, there are few data on the prevalence of Class I integron and antibiotic resistance pattern of *E. coli* from different origin.
Objective: We aimed to compare the occurrence of Int1 gene and its associations with MIC values in indigenous, uropathogenic and sepsis-causing strains of *E. coli* in children and in adults.
Methods: A total of 30 indigenous *E. coli* strains were isolated from stool of 10 antibiotic naïve children (age <1 year, born in 1998) and another 30 strains from stool of 9 people aged >65 years. The pathogenic *E. coli* strains were recovered from urine (n = 32) of 13 children (aged 2 mo–8 years, born in 1997–98) with recurrent urinary tract infection and from blood of 27 septic patients (age 45–84 years). The MIC values to ampicillin, cefuroxime, cefotaxime, meropenem, gentamicin, ciprofloxacin and sulfamethoxazole were measured by E-test. Microbial DNA was extracted using QIAamp DNA Mini Kit. Int1 was detected by PCR with primers IntI-F and IntI-R.
Results: The prevalence of IntI gene in indigenous and pathogenic *E. coli* strains was 28% and 29%, respectively. The prevalence of the gene was higher in children than in adults (28 vs 6; $p < 0.001$) but it did not depend on the origin of the strain nor on the underlying illness or previous antibacterial treatment. The presence of IntI gene was positively correlated with MIC values to ampicillin ($r = 0.246$; $p = 0.009$), cefuroxime ($r = 0.29$; $p = 0.002$), cefotaxime ($r = 0.206$; $p = 0.03$), sulfamethoxazole ($r = 0.280$; $p = 0.003$) and negatively to gentamicin ($r = -0.323$; $p < 0.001$).
Conclusions: The presence of IntI gene in *E. coli* is associated with higher MIC values to beta-lactam antibiotics and sulfamethoxazole but not to gentamicin. The prevalence of IntI gene does not depend on the origin of the strain (indigenous or pathogenic) but is associated with the age: the presence of IntI genes in *E. coli* was higher in children.

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New integron-independent trimethoprim resistance gene dfr24, detected in urinary isolate of *Escherichia coli*

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Objectives: In a material of 69 trimethoprim resistant Gram-negative urinary isolates, as many as 11 resulted negative with PCR for all known classes of integrons and four of the five known dfr-genes not carried by integrons. The mechanisms for trimethoprim resistance in these 11 isolates are the subject for further investigations. Here we report the finding of a previously unknown trimethoprim resistance gene in one of the 11 selected isolates.
Methods: A shotgun cloning approach has been used to identify DNA fragments containing the trimethoprim resistance mechanism. The entire DNA from the isolate was digested with four restriction endonucleases separately and randomly ligated into pUC18 vectors. The ligation reactions were transformed into TOP10 *E. coli* recipient cells without any further purification. The transformed *E. coli* were grown on antibiotic plates containing trimethoprim and ampicillin to select for vectors with inserted fragments containing the desired resistance mechanism. The fragments were subjected to sequence analysis from primers directed to the flanking regions of the polylinker in the vector.
Results: So far, one isolate not yet characterized with respect to molecular cause of trimethoprim resistance was showed to carry a previously unknown dfr-gene. This gene, which has been named dfr24, is in contrast to most known dfr-genes not carried as a gene cassette in an integron. The gene product, the dihydrofolate reductase DHFR24, is only 38% identical to DHFR8 and 32% identical to DHFR9 which are also encoded by genes not inserted as gene cassettes in integrons. The protein seems however somewhat more related to housekeeping

dihydrofolate reductase genes of various organisms. The sequence of the new dfr24 gene has been published at EMBL with accession number AJ972619.

Conclusions: The total number of trimethoprim resistance dfr-genes is probably far from known yet. Of the 25 dfr-genes known at present, 12 were published the last 5 years and the majority of them were identified in sequence analysis of integrons. The new gene detected in this study, dfr24, is only distantly related to integron-borne dfr-gene cassettes, and with this approach of shotgun cloning probably more integron-independent dfr-genes can be identified. Furthermore, the sub-selection of integron-negative trimethoprim-resistant isolates this new gene origins from has a potential for more such discoveries.

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