

Mechanism of antifungal action and resistance to antifungals in fungal disease

P1198

Management of semi-invasive aspergillosis and *Aspergillus* empyema with combination of systemic antifungals and intrapleural amphotericin B in an immunocompetent patient

Y. Tezer, O. Memikoglu, H. Kur (Ankara, TR)

The spectrum of pulmonary aspergillosis varies, ranging from allergic bronchopulmonary aspergillosis to invasive diseases depending on the immune status of the patient and the underlying lung condition. Pleural invasion by *Aspergillus* species occurs commonly as a late complication of thoracoplasty with bronchopleural fistula. Rarely the rupture of cavitary aspergillosis into the pleural space can produce *Aspergillus* empyema.

Case report: A 39-year old man referred to tertiary hospital, with a history of destroyed left lung and *Aspergillus* fungus ball for a thoracotomy in thorax surgery department. He had no known medical problems or smoking history. He suffered from cough, hemoptysis and weight loss for a month. He had been operated for fungus ball. But after a week fever and leukocytosis were predominant. Chest tube continually drained purulent fluid and air, indicative of bronchopleural fistula. Control thorax computerized tomography had been confirmed that. Pleural fluid specimens examined biochemically, microscopically and cultured for bacteria, mycobacteria and fungi. In exudative fluid *Aspergillus fumigatus* was grown. Then caspofungin therapy was started. After 14 days, due to culture positivity and allergic reactions we changed to the liposomal Amphotericin B. After 14 days, purulent fluid discharge were continue. On cultures *Aspergillus fumigatus* were grown. Then we combined systemic amphotericin B with caspofungin. But purulent fluid discharge had not been diminished. So we decided to attempt intrapleural antifungal treatment and systemic antifungals together. As described in literature, liposomal amphotericin B was given intrapleurally. During therapy there were no complications occur. After 3 weeks his fever and leukocyte were in normal range and his galactomannan and CRP levels were dropped. His chest tube drainage was diminished but not stopped in 66th day. So he required radical surgery with thoracostomy. Then we continued the treatment with oral voriconazole up to 6 months.

Result: After 1 year of follow-up, the patient is well. In our experience we were able to control clinic progress of disease when considered fever and status of the patient by using intrapleural amphotericin B and combined systemic antifungals. But we were unable to control aspergillosis totally.

Conclusion: A successful management with remarkable clinical improvement was achieved by pleural drainage, combined antifungal treatment and surgical approach.

P1199

Clinical presentation and epidemiology of Mucorales infections in Geneva university hospitals

I. Uçkay, M. Djordjevic, J. Garbino, C. Garzoni, Y. Chalandon, V. Jacomo, P. Rohner, C. Van Delden (Geneva, CH; Nis, CS)

Objective: Fungal infections by Mucorales are rare, even in highly immunocompromized patients. Unless diagnosis can be

made early, local invasive disease tends to progress with a poor prognosis. Initial symptoms are unspecific and an early high index of suspicion is crucial. We are interested in the epidemiology, microbiology, therapy and outcome of mucormycosis in our institution.

Methods: University of Geneva Hospitals is a tertiary hospital with 1600 acute care beds and 40,000 admissions each year, containing all disciplines including several intensive care units and a transplantation centre. Retrospective analysis of all electronical and clinical databases from 1989 till 2004. All results containing Mucorales were analysed.

Results: We identified 7 cases of infection: 2 bone marrow transplant patients, 1 liver transplantation patient, 1 renal transplantation patient, 1 patient with AIDS and 2 patients receiving high doses of steroids. *Rhizopus* and *Absidia* sp were the causative fungus agent in 3 cases each. Survival (3 of 7 patients) could only be achieved during local disease such as the involvement of the orbita, associated with early diagnosis, early and aggressive surgical therapy and specific antifungal treatment. Table 1 resumes the characteristics of the infection cases. In 35 cases the presence of mucormycosis were considered as colonisation since there was no invasive disease, no secondary prophylaxis, no specific surgical or antifungal treatment and no clinical importance reported in the records. The most prevalent body sites for colonisation were nasal sinuses and tracheal aspirates.

Table 1. Characteristics of the seven cases.

	Immunosuppression	Site	Fungus	Surgical interventions	Antifungal agent	Outcome
Case 1	Bone marrow transplantation, Graft versus Host Disease, Deferoxamine	Lung, Heart, Aorta, Brain, Abdomen	Rhizomucor	None	None	Exitus
Case 2	Liver transplantation	Liver, Abdominal wall	Rhizopus	Four	Amphotericin B desoxycholate (systemic and local)	Survival
Case 3	Bone marrow transplantation, Graft vs. Host Disease	Maxillary sinus, Lunge?	Rhizopus	None	Amphotericin B desoxycholate	Exitus
Case 4	Facial trauma, High dose steroids	Orbita, Temporal region	Absidia	Numerous	- Amphotericin B desoxycholate - Liposomal amphotericin B - Posaconazole	Survival
Case 5	Diabetes mellitus, Dexamethasone	Orbita, Ethmoidal sinus	Rhizopus	Two	- Amphotericin B lipid complex - Amphotericin B desoxycholate	Survival
Case 6	AIDS, CD 4 count: 7	Kidney	Absidia	None	Amphotericin B desoxycholate	Exitus
Case 7	Renal transplantation, Anti-rejection treatment	Lunge	Absidia	None	Amphotericin B desoxycholate	Exitus

Conclusion: Mucormycosis is a rare disease even in a tertiary hospital. Disseminated fungal infections have a poor prognosis and may evolve into multiorgan failure within a short period of time, whereas early recognized local infections can be successfully treated. In the presence of a severely immunocompromized host, a high index of suspicion remains mandatory. Physicians should be aware of the possibility of this fungal infection in order to begin a rapid diagnostic workup including aggressive surgical and antimycotic therapy with amphotericin B or posaconazole.

Abstracts

P1200

Caspofungin therapy in documented fungal infections: Spanish experience before licencing of the drug

C. Sanz-Rodriguez, J.M. Cisneros, J.M. Aguado, R. Martino *on behalf of the Spanish Caspofungin Clinical Study Group*

Objective: Retrospective evaluation of clinical effectiveness and safety of caspofungin in the compassionate treatment of invasive or superficial fungal infections before marketing of the drug in Spain in August 2002.

Methods: Eighty-two patients received compassionate treatment with caspofungin. All patients were considered evaluable for safety, yet only those treated for ≥ 7 days for mould infections or ≥ 5 days for yeast infections were considered evaluable for clinical effectiveness. Response was graded as favourable (complete or partial resolution of clinical, microbiological and/or radiographic evidence of fungal disease) or unfavourable (stable or progressive disease).

Results: The main underlying disorders were haematological neoplasias (48 patients; 59%), COPD (9; 11%), and AIDS (8; 10%). Seventeen patients (21%) underwent an allogeneic stem cell transplant, while another 6 (7%) were solid organ transplant recipients. Sixty-three patients (77%) were diagnosed of invasive mould infection (16 proven, 24 probable, 23 possible per MSG/EORTC criteria), 10 (12%) invasive yeast infection, 7 (9%) esophageal candidiasis, and 2 (2%) tracheobronchial aspergillosis. The median length of therapy was 15 days (range, 2–154), typically a 70-mg loading dose followed by 50 mg daily. Sixty-six (80%) patients were evaluable for clinical effectiveness. Of them, 63 patients (95%) were refractory to other antifungals, including 35 (53%) treated with ≥ 2 drugs. Most patients (63/66; 95%) progressed to ≥ 1 amphotericin B (amphB) formulations (median total dose 3,778 mg, range 60–31,920). Favourable response rates were 66% (21/32) for proven or probable invasive aspergillosis (caspofungin monotherapy, 62% [8/13] vs combination therapy, 68% [13/19]), 69% (11/16) for possible invasive mould infections, 67% (6/9) for invasive candidiasis, and 100% (7/7) for oesophageal candidiasis. One patient with disseminated fusariosis did not respond to caspofungin + liposomal amphB, while another patient with an invasive yeast infection (species unknown) responded fully to caspofungin + amphB lipid complex. Overall, the safety and tolerability profile was favorable; no treatment was discontinued due to caspofungin-related adverse events.

Conclusion: Caspofungin was efficacious and generally well tolerated in the treatment of invasive and superficial *Aspergillus* and *Candida* infections, and provided an alternative for those who failed to respond or became intolerant to other available antifungals.

P1201

Zygomycosis in a general hospital during a 17-year period. Is there an increase in incidence after introducing voriconazole?

M. Torres-Narbona, J. Guinea, J. Martínez-Alarcón, T. Peláez, P. Muñoz, E. Bouza (*Madrid, ES*)

Objectives: The question of an increase in the number of cases of zygomycosis (ZM) has been raised in American and European institutions. The use of voriconazole (Vori) as antifungal prophylaxis may correlate with this increase but that may not be the case in institutions in which Vori is not used prophylactically. We reviewed the evolution of ZM, before and after the introduction of Vori in our hospital.

Methods: We retrospectively evaluated all cases of ZM in Gregorio Marañón Hospital from 1987 to 2005. Proven or probable diagnosis of ZM was performed according to established criteria (Ascioglu, CID 2002). Vori was introduced for therapeutic use in our hospital in 2002.

Results: During the study period, 10 patients had proven or probable ZM (0.6 cases/year). The clinical forms and underlying conditions of the patients were 1 rhino-cerebral (kidney transplant), 2 sinuses (1 diabetes mellitus, 1 bone marrow aplasia), 3 cutaneous (1 diabetes mellitus under corticosteroid treatment, 1 heart transplant, 1 lymphoma), 3 pulmonary (1 COPD under corticosteroid treatment, 1 heart transplant, 1 leukaemia) and 1 cerebral (HIV infection). The cases had a sporadic distribution. Only one case of ZM occurred after the introduction of Vori. The consumption of Vori in 2004 was 1110 DDDs. Mean consumption per month was 88.6 DDDs (Standard deviation of 48.91 DDDs).

Conclusions: Since 2002, therapy with voriconazole in our institution has not been associated with changes in the incidence of ZM.

P1202

Candida colonization, colonization index and invasive candidiasis in patients at a multidisciplinary intensive care unit, Sweden

C. Agvald-Ohman, L. Klingspor, H. Hjelmqvist, C. Edlund (*Huddinge, SE*)

Objectives: Intensive care units (ICU) have emerged as epicentres for fungal infections such as candidaemia. The aim of the study was to investigate *Candida* colonization, colonization index (CI) and its relation to invasive candidiasis in patients with a length of ICU stay (LOS) ≥ 7 days.

Material and methods: ICU patients with a LOS ≥ 7 days were consecutively included for sampling during Mars 2004–July 2005. The study was approved by the Ethical Committee in Stockholm. A total of 59 patients, 38 men and 21 women, mean age 59 years (range 19–81) were included. Mean LOS was 19.8 days (range 7–77) and mean days of ventilation was 16 (range 0–74). A majority of the 59 patients were exposed to antimycotic drugs during their ICU stay, 35 received fluconazole, 16 liposomal amphotericin 3 voriconazole and 3 caspofungin. Samples were collected at day 7 and then weekly as long as the patient was admitted to the ICU. Sampling sites were oral cavity, lower airways, urine, blood and rectum from all included patients and from drainage and wounds when this was an option.

Results: Fourteen patients were not colonized by *Candida* (CI = 0). *C. albicans* was isolated from 35 patients, *C. glabrata* from 10 and 12 had other non-*albicans* species. Seven patients were colonized by ≥ 2 species of whom two by ≥ 3 species. At the first sampling occasion 42% of the patients (25/59) had a CI ≥ 0.5 , eight had CI 1.0, while 26% (8/31) had an index ≥ 0.5 and two had CI 1.0 at day 14. Only 10 patients or fewer were sampled at days 21, 28, 35, 42 and 49, range of mean CI were 0.4–0.7. Ten patients developed invasive candidiasis, of whom six had candidaemia. Mean CI for these 10 patients were 0.8 and all invasive species were also colonizing species. Infections were caused by *C. albicans* (6), *C. glabrata* (3), *C. tropicalis* (1) and *C. dubliniensis* (1). Nine patients were treated with at least one antimycotic drug, the majority with liposomal amphotericin. The three months mortality among these patients was 70% compared to 49% (29/59) among all included patients.

Conclusion: The ICU in the present study is a tertiary unit at a university hospital and 66% of the patients were treated

with immunosuppressive drugs (17/59) and/or cortisone (39/59). Despite the fact that all patients were treated with antimycotic drugs as soon as CI exceeded 0.5, invasive candidiasis was diagnosed during the ICU stay in 17% of included patients. A high CI was correlated to invasive candidiasis and high mortality.

P1203

Treatment of invasive *Pseudallescheria boydii* infections with voriconazole or voriconazole plus caspofungin

A. Pan, A. Soresina, C. Cattaneo, S. Lorenzotti, S. Magri, N. Manca, A. Plebani, G. Pinsi, G. Rossi, L. Soavi, L. Signorini, R. Stellini, G. Carosi (Brescia, IT)

Objective: To report the efficacy of new antifungal drugs in the treatment of *Pseudallescheria boydii* infections, to date associated with a very high mortality.

Methods: We report 4 cases of *P. boydii* infections successfully treated with voriconazole at our Institution.

Results:

Case 1: A 26-year old male with chronic granulomatous disease (CGD) was admitted to the Infectious Disease Department (IDD) and pulmonary and mediastinic lesions identified on thoracic CT scan. Hyphae were identified on histology and biopsy yielded *P. boydii*. Oral voriconazole was started and an initial reduction of the lesion observed on CT scan. Repeated CT scans performed thereafter monthly showed mild further improvement. The patient is now in fair conditions and still on oral voriconazole.

Case 2: A 54-year old male, woodcrafter, in general good conditions, was admitted to neurosurgery because of identification of cerebral mass on a brain CT scan. He was operated and the surgical material showed the presence of hyphae and yielded *P. boydii*. Intravenous (IV) voriconazole was started and reduction of the lesion observed on CT scan. Voriconazole was switched to oral formulation after 1 month and stopped after 3 months of therapy. One month after stopping voriconazole he still is in fine conditions.

Case 3: A 70-year old male affected by multiple myeloma, on chronic corticosteroid therapy was admitted to the haematology because of respiratory symptoms. No biopsy was performed. The sputum culture yielded *P. boydii*. The patient was started on oral voriconazole and an improvement of the pulmonary lesions observed over the following months. Treatment with voriconazole was stopped after 3 months.

Case 4: A 28-year old male with CGD was admitted to the IDD because of worsening of pulmonary and mediastinic lesions on thoracic CT scan while on voriconazole therapy, observed over a 6 months period. After *P. boydii* was isolated from a biopsy and hyphae identified on histology, a surgical de-bulking was performed. Caspofungin was associated to voriconazole and improvement of the general conditions observed. Combined IV therapy was continued for 4 months with progressive reduction of the mycosis and improvement of his general conditions.

Conclusions: Voriconazole is an effective and safe therapy for invasive *P. boydii* infections either alone or combined with surgery depending on the severity of the disease. Patients who fail voriconazole may respond to salvage treatment with caspofungin.

P1204

The emergence of caspofungin resistance during treatment of recurrent *Candida glabrata* candidaemia

N. Daneman, A.K. Chan, S.M. Poutanen, R. Rennie, C. Sand, S. Porter (Toronto, Edmonton, Vancouver, CA)

Objective: The development of caspofungin resistance during treatment has not been frequently described. This report documents caspofungin resistance emerging during treatment of recurrent *Candida glabrata* candidemia.

Methods: The patient's clinical chart was reviewed. Susceptibility testing of all saved *C. glabrata* isolates was completed following the NCCLS M27-A2 standards. Electrokaryotyping and restriction enzyme pulsed field gel electrophoresis (RE-PFGE) were performed to assess the relatedness of the isolates.

Results: A 45 yo female with Crohn's disease with fistulae and dependent on TPN was admitted in April 2004 with *C. glabrata* candidemia. Her central venous catheter was changed and she was treated with 3 weeks of caspofungin at 70 mg od x 1, then 50 mg od with clinical response. Blood cultures and line tip were negative. In January 2005, her *C. glabrata* candidemia recurred. Her central line was changed and caspofungin at the same dose was started. Follow-up blood cultures were negative. Ophthalmologic examination and ECG were normal. Line tip cultures were not completed. Her course was complicated by septic shock. Abdominal CT scan revealed a small bowel fistula and intra-abdominal abscess. Broad-spectrum antibacterial therapy was added with clinical response. Caspofungin therapy was discontinued after 2.5 weeks. Nine days later in February 2005, the patient developed recurrent *C. glabrata* candidemia. The central catheter was changed and caspofungin was re-started at the same doses. The line tip grew *Candida* spp, not *albicans*. Repeat blood cultures were negative. Caspofungin was continued for a total of 1 month. Follow-up blood cultures off caspofungin were negative. The patient was lost to follow up until she returned to hospital in October 2005 with a lower gastrointestinal bleed. She died 3 days after admission. Antemortem blood cultures grew *C. glabrata*. Multiple *C. glabrata* blood culture isolates from April 2004, January 2005, and February 2005 were available for testing. Caspofungin MICs were 0.5 mg/L, 8 mg/L, and >16 mg/L respectively. Molecular testing indicated that the multiple isolates were probably related.

Conclusion: This case demonstrates that caspofungin resistance may emerge during treatment. An undetected nidus of infection may contribute to fungal persistence with development of antifungal resistance despite apparent adequate therapy and documentation of negative blood cultures after treatment.

P1205

An open-label study of anidulafungin in invasive candidiasis and candidaemia

R. Betts, D. Kett, K. Clark, H.T. Schlamme, D.S. Krause (Rochester, Miami, New York, US)

Objectives: *Candida* species (spp.) are the leading cause of invasive fungal infections in hospitalised patients (pts), and are associated with excess morbidity and mortality, despite the availability of several new agents. Anidulafungin (ANID) is a novel echinocandin with potent *in vitro* and *in vivo* fungicidal activity against *Candida* spp. ANID was superior to fluconazole (FLU) in a Phase 3 efficacy study of invasive candidiasis and candidaemia (IC/C) previously reported.

Abstracts

Methods: We conducted an open-label trial of the efficacy of ANID. In contrast to the Phase 3 trial, pts on prior FLU were allowed. Adult pts with IC/C received a loading dose of ANID on Day 1 of 200 mg, then 100 mg IV daily for a total of 14–42 days. Pts could be switched to oral FLU after at least 10 days of IV ANID. Pts were assessed at the end of IV therapy (EIV), and at 2 & 6 week follow-up (FU) after the end of all therapy. A successful global response required clinical improvement and microbiological eradication. The EIV was the primary time point of interest. Safety evaluations included haematology and biochemistry labs and recording of all adverse events (AEs), regardless of attribution.

Results: Thirty three (33) pts were enrolled; 22 completed through the 6-week FU visit. The mean APACHE II score was 14 (range 2–27); 20% had scores > 20. Presence of a central venous catheter was the most common (84%) reported risk factor. Most (91%) received at least one prior dose of FLU. Candidaemia was present in 90% of pts; most had multiple positive cultures at baseline. *C. albicans* and *C. glabrata* were isolated from 42% and 38% of pts, respectively. The median exposure to ANID was 14 doses. Most pts (26/33) did not switch to oral FLU. A successful global response was observed in 21/31 (67.7%) culture positive pts. Three pts (9.7%) had persistent infections. On a pathogen level, success was similar for albicans and non-albicans isolates. AEs were reported by all pts; 11 pts had AEs that were related per investigator attribution. Only 2 pts experienced AEs that led to discontinuation of study drug. At the 6 week FU 79% of pts survived. Few pts experienced infusion reactions. Clinical laboratory results did not suggest drug toxicity.

Conclusions: ANID was efficacious and well tolerated. These results confirm and extend the phase 3 data previously reported.

P1206

Systemic fungal infection due to *Trichosporon beigelii* in immunocompromized patient

I. Radonjic, S. Mitrovic, V. Arsic Arsenijevic, I. Kranjic Zec, A. Dzamic (Belgrade, CS)

Objectives: Invasive trichosporonosis caused by yeast *Trichosporon beigelii* often results in rapid, widespread dissemination and high mortality because diagnostic and therapeutic means have not been established well. The authors report a case of fatal *T. beigelii* fungemia in a 28-year old woman with acute leukemia and neutropenia. The patient was not on antifungal therapy before this episode of fungemia.

Methods: During five days seven blood samples were collected. All samples were inoculated on Sabouraud dextrose broth and agar and incubated at 37°C for 10 days. Yeasts were identified by morphologic criteria, germ tube test, chlamydospore formation test and assimilation test API 20 C AUX. Antifungal susceptibility was evaluated by disk agar diffusion method to amphotericin B, miconazole, ketoconazole, fluconazole, itraconazole and flucytosine (1 µg and 10 µg) and by broth macrodilution method for amphotericin B (inoculum 104 CFU/ml, YNB medium, incubation at 37°C, 24 h and 48 h).

Results: All blood samples were positive and isolates identified as *T. beigelii*. All isolates had the same pattern of susceptibility *in vitro*. They were sensitive to miconazole, ketoconazole, fluconazole, itraconazole and flucytosine, but resistant to amphotericin B. The resistance to amphotericin B was confirmed by broth macrodilution test with MIC = 12.8 µg/ml.

Conclusion: We observed that the strain of *T. beigelii* was primary resistant to amphotericin B. Amphotericin B is the most important agent in therapy of disseminated fungal infections but most of *T. beigelii* strains are resistant to this antifungal.

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Therefore it is important to test susceptibility to antifungal agents *in vitro* for any isolate from blood samples, especially in immunocompromized patients.

P1207

Epidemiology of dermatophytosis in Khouzestan province, southwest Iran, from 2002 to 2005

A. Rafiei, M. Omidian, M. Radmanesh, M. Mapar, R. Yaghoobi, R. Rasaei, R. Taddaion (Ahwaz, IR)

Objectives: Cutaneous fungal infection especially dermatophytosis are common in different parts of Iran. Dermatophyte organisms are in constant competition for their particular environmental niche. These data can be used for ascertain past and present trends in incidence and the emergence of more predominant dermatophytes. The aims of this study were to identify the epidemiologic trends and causative organisms of dermatophytosis in Khuzestan province.

Methods: A total of 2953 specimen were collected from patients clinically suspected to have fungal infection from 2002 through 2005. Materials collected from hair, nail and skin and were investigated by direct examination and cultured in Sabouraud dextrose agar and mycobiotic Agar (Difco). Fungal colonies were identified by macroscopic and microscopic examination and supplementary tests.

Results: The prevalence of dermatophytosis among the suspected patients was 24.1%. The most predominant types of infection of dermatophytosis were as follows: *Tinea cruris* (40.1%), *Tinea corporis* (14.5%), *Tinea pedis* (14.5%), and *Tinea capitis* (10%) *Tinea pedis* (6.7%), *Tinea faciei* (6.5%), *Tinea unguium* (6.0%) and *Tinea barbae* (1.2%). The most frequent dermatophyte organisms were: *Epidermophyton floccosum*, *Trichophyton Verrucosum*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Trichophyton Violaceum*, *Microsporum canis* and *Trichophyton schoenleinii*. The frequency rate of dermatophytosis was higher in males than females. The duration of infection had a wide range from 70 days to 20 years. Age patients were from 4 months to 85 years old, but 21–40 years age group was found to be the most common infected group (45.2%).

Conclusion: Dermatophytosis is still one of the most infectious diseases in Khozestan and probably in Iran. As the causal agent, the anthropophilic (*E. floccosum*) and zoophilic (*T. verrucosum*) species were the most common dermatophyte of tinea in khozestan province, Iran.

P1208

Cutaneous infection by *Absidia corymbifera* and *Syncephalastrum racemosum* treated with amphotericin B and surgical debridement

R. Bandettini, E. Mantero, A.M. Tamisani, P. Tuo, R. Ceccarelli, L. Pescetto, L. Ricagni, G. Melioli (Genoa, IT)

Objective: The infections by *Absidia* spp. and *Syncephalastrum* spp. are opportunistic invasive diseases in immunocompromized hosts, in patients with underlying disease or with skin lesions. A high mortality is described even when aggressive invasive antifungal therapy and surgical debridement are used. We describe a clinical case of a paediatric patient with cutaneous infection by *Absidia corymbifera* and *Syncephalastrum racemosum*.

Materials and Methods: A 1-year-old child was hospitalized in February 2005 for *Streptococcus pneumoniae* septicemia and *purpura fulminans* followed by severe reliquates like cutaneous necrosis (hands, feet, legs and nose). For this reason in April the patient was treated with multiple escaectomy and multiple

grafts of skin and autologous keratinocytes but only the 50% of the treated surface improved. In the half of May some pieces of debrided tissue submitted for microbiological examination resulted positive for *Absidia corymbifera* and *Syncephalastrum racemosum*. In this period the patient presented an intermittent fever >38.5°C. The laboratory findings showed leukocytosis with neutrophilia (WBC: 18,000/mm³ and neutrophils 79%), CRP > 10 mg/dl and negative blood cultures. In May 26 an antifungal therapy with liposomal amphotericin B (10 mg/kg/day) was started and the debridement of cutaneous infected sites was performed. The antifungal treatment was completed with topical amphotericin B and continued until June 13, 2005.

Results: In May 30 the microbiological examinations of the skin biopsies were negative, the fever disappeared and haematochemical tests turned to normal values. The side effects of antifungal therapy were not observed. The cutaneous lesions recovered and from July the patient had not signs and symptoms of fungal infection.

Conclusions: Zygomycosis are very severe infections with high mortality despite the use of *in vitro* effective antifungal therapy. We present a clinical case of successful treatment of cutaneous infection by *Absidia corymbifera* and *Syncephalastrum racemosum* with liposomal amphotericin B and topic amphotericin B, associated with surgical debridement. The therapy was well tolerated and the patient recovered without any diffusion of the infection to other sites. We think that the combination of two different approaches, medical and surgical, offers the best chance for survival.

P1209

The Janus head of histoplasma

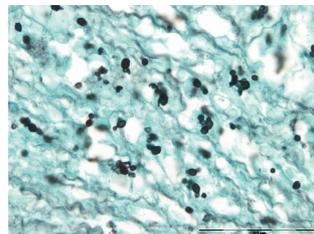
E. Vlieghe, M. Ieven, H. Jansens, B. Hendriks, K. Van de Vyvere, P. Fonteyne, E. Van den Eenden, R. Colebunders (Antwerp, Brussels, BE)

Objectives: To describe an unusual case of invasive infection with *H. capsulatum*

Methods: A description of the clinical, pathological and microbiological findings.

Case study: A 33-year old African man was admitted to the hospital with fever, night sweats and a tender mass in the left fossa iliaca. He was recently diagnosed HIV-positive, with CD4-count 2 μ l (0.6%) and viral load 649,000 copies/ml. He appeared in good clinical condition besides a generalised lymphadenopathy, a splenomegaly and a tender mass (7 × 5 cm) in the left iliac fossa. The relevant lab analyses were: ESR 53 mm/h, CRP 5 mg/dl, Hb 12.5 g/dl, thrombocytes 172 × 10⁹/l, WBC 2.5 × 10⁹/l, LDH 870 U/l. Serology for syphilis and toxoplasmosis was negative and showed immunity for CMV and EBV. Cryptococcus Ag and Histoplasma Ag were negative. Retinal fundoscopy showed no abnormalities. Cultures of sputum and blood remained initially sterile. CT scan of the thorax and abdomen showed enlarged lymph nodes in the upper mediastinum, retroperitoneum, peripancreatic, para-aortic and along the left a. iliaca externa and femoralis; the latter nodes appearing with a necrotic centre. A CT-guided fine-needle biopsy was taken from the iliacal mass. Histology of the biopsy showed yeast forms of *Histoplasma capsulatum*. The patient was started on IV liposomal amphotericin-B 3 mg/kg and switched after 10 days to PO itraconazole 2 × 200 mg for 3 months. The symptoms and the mass subsided soon thereafter. After four weeks, growth of a filamentous fungus was observed in one of the mycobacterial blood culture bottles. It was identified as *H. capsulatum* through PCR carried out on a subculture on blood agar at 37°C. The test was positive after 2 different extractions

and with 2 different PCR-protocols. DNA sequencing of the ITS locus, our reference method for molecular identification confirmed these results. Twelve weeks later, the patient was doing well and itraconazole was reduced tot 1 × 200 mg/d for secondary prophylaxis.



Conclusion: Invasive histoplasmosis is a well-known opportunistic infection in AIDS-patients. The growth of a filamentous fungus in the blood of this patient was surprising, since this dimorphous organism normally converts to the yeast phase at 37°C. Apparently this conversion did not take place, possibly due to the culture medium or the environment.

P1210

Successful therapy of zygomycosis with posaconazole in a patient intolerant of liposomal amphotericin B

S. Savvanis, A. Papadopoulos, D. Kavvatha, H. Giamarellou (Athens, GR)

Introduction: Zygomycosis is a rare invasive fungal infection, but incidence is increasing. Infection typically occurs in patients with immunodeficiency or diabetes mellitus and is associated with high mortality rates. Treatment includes surgical debridement and the use of antifungal agents, such as liposomal amphotericin B and new and investigational azoles (eg, posaconazole).

Case presentation: A 33-year-old woman with an unremarkable medical history was admitted to the hospital with signs of diabetic ketoacidosis, intense pain in her left shoulder extending to the left upper limb in an ulnar nerve distribution, and Claude Bernard-Horner syndrome. A computed tomography scan of the chest revealed a mass in the upper lobe of the left lung. The patient underwent surgery, and a mass infiltrating the cervical sympathetic chain, the stellate ganglion, the left subclavian artery, and the brachial plexus was partially excised. Biopsy specimens revealed aseptate hyphae with right-angle branching compatible with zygomycosis. Liposomal amphotericin B was initiated at a dose of 8–12 mg/kg/day. A postoperative magnetic resonance image (MRI) revealed reoccurrence of the mass infiltrating the left lung apex and the stellate ganglion and extending to the C7-Th1 intervertebral foramen and the left subclavian artery, causing focal occlusion, and an embolized mycotic aneurysm. New lesions appeared in the glottis and the right upper lobe. The patient received 48 g of liposomal amphotericin B in a 5-month period; this therapy was stopped owing to nephrotoxicity. The patient subsequently received posaconazole 400 mg twice daily as an oral solution for 11 months. Posaconazole therapy resulted in almost complete resolution of the pathologic findings and was well tolerated. Three months after the end of the treatment, the patient remains in excellent clinical condition, and the latest MRI shows no evidence of relapse.

Conclusion: Radical surgery combined with control of immunodeficiency or diabetes mellitus represents the gold standard of treatment for zygomycosis. Antifungal agents may resolve

Abstracts

remnant lesions as well as prevent relapse. Liposomal amphotericin B may be given over the long term with strict surveillance of toxicity. The investigational antifungal agent posaconazole has shown high antifungal activity against *Zygomycetes* with low toxicity, and may be administered orally.

P1211

Carbon assimilation profiles as a tool for *Zygomycetes* species identification

P. Schwarz, O. Lortholary, F. Dromer, E. Dannaoui (Paris, FR)

Objectives: Identification of *Zygomycetes* to the genus and/or species level remains difficult by standard mycological procedures. The aim of this study was to evaluate carbon assimilation profiles as a tool for identification of the most common *Zygomycetes* responsible for infections in humans.

Methods: ID32C yeast identification system was used. Strips were inoculated by spore suspensions of 47 well-characterized isolates belonging to 8 *Zygomycetes* species (15 *Rhizopus oryzae*, 8 *Absidia corymbifera*, 7 *Mucor circinelloides*, 7 *Rhizomucor pusillus*, 4 *Rhizopus microsporus*, 3 *Syncephalastrum racemosum*, 2 *Mucor indicus* and 1 *Cunninghamella bertholletiae*). Strips were incubated at 28°C and were read visually after 72 hours of incubation.

Results: Overall seventeen carbon sources were useful for discriminating among the examined *Zygomycetes* species. The assimilation of the majority of carbon sources was homogeneous within a given species and heterogeneous between species. Each species showed a specific carbon assimilation profile allowing accurate identification except for *M. circinelloides* and *M. indicus* that shared similar profiles. Table 1. Percentage of positive assimilation results for the most useful carbon sources

Carbon source	Percentage of positive assimilation					
	R. oryzae*	R. micr	A. cory	M. circ	Rh. pus	S. race
cycloheximide	53	0	100	100	0	100
arabinose	100	0	100	100	100	100
raffinose	27	0	100	0	100	100
ribose	100	100	13	100	14	100
glycerol	100	25	13	0	0	0
palatinose	0	0	0	71	0	100
melibiose	0	0	100	0	100	100
melezitose	20	0	0	86	0	0
K. gluconate	0	75	0	86	0	0
lactose	0	0	100	0	100	100
glucosamine	100	50	0	100	71	0

* R. oryzae, R. oryzae; R. micr, R. microsporus; A. cory, A. corymbifera; Rh. pus, Rh. pusillus; S. race, S. racemosum

Conclusions: These results demonstrate that diagnosis of the main pathogenic *Zygomycetes* to the genus and species level is possible based on their carbon assimilation profiles. This technique using a commercially available system provides a fast and easy tool for identification of the most common *Zygomycetes* species.

P1212

Is posaconazole a new treatment option for brain abscess?

L. Matkham, C. Garzoni, B. Hirschel, S. Harbarth, K. Bouchuigui-Waf, J. Garbino (Geneva, CH)

Introduction: *Cladophialophora bantiana* is a dematiaceous fungus, a rare cause of cerebral abscess. Its high mortality is

related to its poor response to currently available anti-fungal therapy.

Case report: A 60-year old Egyptian woman with a systemic sclerosis and long term corticoid therapy, pulmonary fibrosis chronic hepatitis C with cirrhosis, chronic thrombocytopenia and arterial hypertension. The patient was hospitalised for frequent falls and confusion. On admission, the patient was febrile, sleepy and confused. A CT-scan showed an expansive lesion compressing the left lateral ventricle. The magnetic resonance image (MRI) suggested an image of glioblastoma and a chemotherapy was started. The patient developed a progressive right hemiparesis, aphasia and worsened in sensorium. Cerebral biopsy was performed on hospital day 12. Due to its location resection was not possible and aspiration was done. The microbiological cultures yielded a *Cladophialophora bantiana*. A treatment was initiated with voriconazole 400 mg po/12 h and liposomal amphotericin B 7 mg/kg/d. The MRI 5 days after showed increased size lesion. Increased in liver tests suspected hepatic toxicity and voriconazole was replaced by posaconazole 400 mg/12 h. A MRI done after 16 days of posaconazole treatment showed a similar size of the lesion, but without peri-lesional oedema. In addition to her neurological problems the patient developed pneumonia with severe sepsis and continued to deteriorate and died.

Discussion: Phaeohyphomycosis is the infection caused by dematiaceous moulds which are unique owing to presence of melanin pigments in their cell walls and spores. Melanin may be a virulence factor in these fungi. Dematiaceous fungi are widespread in the environment especially in the soil and decaying vegetation. One of them *C. bantiana* has a predilection for brain tissue. Recommendation for therapy is based upon the experience of published cases. Long-term survival is reported only when complete surgical resection of the lesion is possible accompanied by an anti-fungal treatment. The recommended treatment is high dose of amphotericin B in combination with a new triazole and if it is possible 5 flucytosine. Posaconazole could be a new drug option for its better tolerability and less drug-drug interactions.

P1213

Clinical and epidemiological analysis of candida bloodstream infection in a university hospital

M. Cuervo, M.A. Miguel, M. Lecuona, S. Campos, M.I. Montesinos, A. Torres, T. Delgado, A. Sierra (La Laguna Sta Cruz Tenerife, ES)

Objectives: *Candida* spp is emerging as an important pathogen or coloniser in hospital settings. The aim of this study was to determine demographic, clinical and microbiologic features in *Candida* bloodstream infection in our hospital.

Methods: We have revised prospectively demographic, clinical and microbiologic data from 125 patients with candidemia in a period between 1/06/2002 to 1/06/2005. All the specimens were performed with Bact/ALERT 3D (bioMérieux) initially during five days either 30 days in special cases. The yeast identification was performed with ID-YST Vitek2 and/or api 20 C AUX (bioMérieux).

Results: Of 33043 blood cultures processed in our laboratory, 9500 shown any kind of grown and 355 isolates (3.73%) proved to be yeast strains which belong to 125 patients. The infection rate of males and females was 56.8% and 43.2% respectively. The highest rate was seen in persons >60 years old (47.25%) followed by the group <10 years old (16.8%), most frequently related to neonatal period, especially in premature newborn. Most strains were recovered from patients attended to internal medicine

departments (44%), 31.2% belong to surgical departments and 24.8% were in intensive care units. Of a total of 125 candidemias, the most common origins were unknown (52.58% cases) and 37.11% were secondary to central venous catheter. The most common species isolated was *C. albicans* (51.2%) followed by *C. parapsilosis* (21.6%), *C. tropicalis* (14.4%) and *C. glabrata* (7.2%).

	Creatininemia ($\mu\text{mol/L}$) Median (range)		Creatinine Clearance (ml/min) Median (range)	
	Baseline	End of therapy	Baseline	End of therapy
All patients (n= 88)	84,0 [38,0 ; 465,0]	111,5 [31,0 ; 363,0]	89,5 [17,2 ; 172,2]	60,8 [22,1 ; 207,0]
Abelcet (n= 68)	81,3 [38,0 ; 465,0]	115 [45,0 ; 241,6]	94,6 [17,2 ; 172,2]	60,4 [22,1 ; 183,0]
Ambisome (n= 20)	88,8 [42,0 ; 356,0]	106 [31,0 ; 363,0]	85,2 [21,5 ; 145,6]	71,6 [24,0 ; 207,0]

Conclusions: Comparing the distribution of the demographic data to other Spanish hospitals similar results were found. In our hospital, *C. albicans* was the yeasts most frequently isolated followed by *C. parapsilosis*, *C. tropicalis* and *C. glabrata*. Most candidemias were primary or secondary to intravascular catheter related infections. It should be necessarily to remove the catheter when it is no longer essential for medical management.

P1214

A prospective French national survey to evaluate renal function in patients treated with amphotericin B lipid formulations

S. Faouzi (Villejuif, FR)

Objective: To evaluate renal function in patients treated with Amphotericin B lipid formulations (recommended for treating patients with renal failure).

Methods: A prospective multicentre national survey to evaluate the renal function in adult patients (pts) treated for fungal infections with the two available lipid formulations Abelcet and Ambisome. From April 2003 to December 2004, 99 pts were treated with lipid formulations, 88 pts (43F; 45M) with mean age of 49 + 13 years were evaluable from renal safety. 44% of pts had neutropenia $<500/\text{mm}^3$.

Results: 28 pts (32%) were treated for invasive candidosis, 29 pts (33%) for aspergillosis and 8 pts (9%) for rare fungal infections. 23 pts (26%) had empirical treatment for febrile neutropenia. 68% of the pts received 2 or more nephrotoxic drugs (72% for Abelcet; 61% for Ambisome). 60 pts were treated with Abelcet (median dose: 4.8 g/kg/day) and 38 pts with Ambisome (median dose: 3.3 g/kg/day). The mean duration of treatment was 13.5 \pm 8 days for Abelcet and 15 \pm 11 days for Ambisome. In the group of 26 pts with renal failure, no significant changement occurred between mean serum creatinine level and creatinine clearance at baseline (188 + 98 $\mu\text{mol/l}$; 47 + 24 ml/min) and end of the therapy (173 + 70 $\mu\text{mol/l}$; 45 + 20 ml/min).

Conclusion: Amphotericin B lipid formulations have a good renal safety profile in patients with either altered or normal renal function combined with nephrotoxic drugs.

P1215

Fungal peritonitis in continuous ambulatory peritoneal dialysis patients

B. Arda, O. Sipahi, G. Asci, M. Ozkahya, S. Ulusoy (Izmir, TR)

Objective: The aim of this study was to evaluate the continuous ambulatory peritoneal dialysis (CAPD) patients with culture proven peritonitis between 1992–2004 in our center.

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Method: We analysed the hospital charts of all patients that received diagnosis of fungal peritonitis between 1992–2004, retrospectively. Patients with either cloudy peritoneal effluent containing more than 100 white cells/ mm^3 or signs compatible with peritonitis together with one or more isolations of fungal pathogens were considered to have fungal peritonitis.

Results: During the study period a total of 9 (5 males, 4 females, aged 49.0 \pm 12.2) of 342 patients (overall fungal peritonitis rate 2.6%), who were started CAPD had fungal peritonitis. Four patients had diabetes mellitus. The infecting pathogen was *Aspergillus* spp. in three, *Candida parapsilosis* in two, *Candida tropicalis* in two and *Candida albicans* in two patients. All but two patients had earlier bacterial peritonitis attacks (mean bacterial peritonitis attack 2.2 \pm 1.6, range 0–6). Leucocyte number in peritoneal fluid was 791 \pm 504/ mm^3 (Range 300–1400/ mm^3). Overall mortality rate was 22.2% (one with *Aspergillus* spp. one with *C. parapsilosis*). CAPD catheter was removed in all patients. Mean duration of treatment was 27 \pm 19 days, range 14–70 days). Three patients were treated with amphotericin b deoxycholate, five with fluconazole and one with amphotericin b cholesterol dispersion. All patients received conventional antibiotic treatment before the diagnosis of fungal peritonitis.

Conclusion: Fungal peritonitis is a mortal and morbid complication of CAPD: Fungi especially non-*albicans* *Candida* spp. should be taken into consideration in peritonitis patients not responsive to conventional antibiotics.

P1216

Intra-abdominal fungal infection in surgical patients

I. Gutiérrez, N. Batista, A. Varona, O. Díez (Santa Cruz de Tenerife, ES)

Objective: Several surgical procedures into abdominal cavity are associated with a high incidence of fungal infections. The aim of the study is to analyse the clinical and microbiological aspects of patients with abdominal fungal infections after surgical procedures.

Material and methods: We investigated retrospectively forty-five patients, admitted to the Surgical Digestive Service for urgent selective surgery. Clinical investigation included: host and risk factors, history of the disease, surgical procedures, postoperative complications, intraocular findings. Microbiological criterion included: positive cultures from intra-abdominal liquids and tissues, intraocular and blood samples. Bacteriological and mycological tests included cultures for aerobic and anaerobic, yeast and fungi. Fungal isolates were identified using standard mycologic laboratory methods and tested for susceptibility to fluconazol (FCZ), amphotericin B (AMB), itraconazol (IT), 5-fluorocytosine (5FC) and voriconazol (VOR).

Results: The most commonly identified yeast species were *Candida albicans* (64%), followed by *Candida glabrata* (21%), *Candida tropicalis* (6%), *Candida parapsilosis* (3%), *Candida krusei* (2%), other species (4%). In 67% patients coexisted yeast and bacteria, and in 33% pure yeast culture was found. The bacteria most frequently associated were enterobacteria (26%), enterococcus (25%), non fermenting gram negative rods (18%), gram positive cocci (20%), other (2%). The predominant fungal association was *C. albicans/C. glabrata* (64%), *C. albicans/C. tropicalis* (24%). All the yeast isolated showed susceptibility to fluconazol except 50% isolates of *C. glabrata* and one *C. krusei* isolate. From the forty-five patients 45% showed post-surgical complications, 33% perforation of hollow viscus, 11% pancreatitis, and 11% biliary pathology. Five patients showed invasive fungal infection, one presented mediastinitis and three died.

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Conclusions: High percentage of *C. glabrata* isolated in intra-abdominal infections precludes the election of fluconazol as empiric antifungal treatment in most severe cases.

P1217

Mucormycoses: a report of eleven cases

T. Turunc, Y.Z. Demiroglu, H. Uncu, S. Colakoglu, H. Arslan (*Baskent, TR*)

Objectives: Mucormycoses are infections caused by fungi of the order Mucorales. We reviewed the clinical features of 11 patients who developed mucormycosis. The aim of this study is to evaluate the clinical spectrum, diagnosis and treatment of Mucormycosis.

Material-methods: This was a retrospective study conducted over a 2-year period (2003–2005). The study included 10 patients with hematologic malignancies, diabetes mellitus (DM), chronic renal failure, an one patient was immunocompetent with proven or probable mucormycosis.

Results: The research data base includes 8 men and 3 women (age range: 30–80 years; median age: 53.5 years). Predisposing conditions in decreasing order of frequency were diabetes mellitus (DM) (4), DM and chronic renal failure (3), DM and chronic myeloid leukemia (2), acute lymphoblastic leukemia (1), and immunocompetent (1). Six patients presented with the rhinocerebral form of the disease and five patients presented with central nervous system involvement. The *Mucor* spp. was isolated from cultures in four cases while it was diagnosed by histopathological examination along with clinical and radiological findings in other cases. Seven patients underwent surgical treatment. Liposomal amphotericin B (3 mg/kg/day) was administered in the treatment of 11 patients. Five of the patients responded well to the therapy. Six patients died within 1 month of the diagnosis of fungal infection. Mucormycosis was the cause of death in all of them.

Conclusions: Mucormycosis occurs most frequently in neutropenic patients with haematological diseases. In our study, DM and chronic renal failure were found to be more significantly related with mucormycosis than the hematological malignities. These results suggest that mucormycosis should be considered in the differential diagnosis of orbital cellulitis not only in the patients with hematologic malignities but also in the patients with DM and/or chronic renal failure.

P1218

***Bipolaris spicifera* isolation from the nasal polyps of an allergic boy: a case report**

A. Sergounioti, H. Kirikou, A. Velegraki, K. Chryssaki, M. Choulakis, K. Stefanaki, A. Pangalis (*Athens, GR*)

Allergic fungal sinusitis (AFS) is a non-invasive form of paranasal sinus mycosis caused by the dematiaceous fungi, usually *Bipolaris*, *Curvularia*, *Exserohilum*, *Alternaria*, *Drechslera*, and less often by *Aspergillus*. We present a case of a boy with a massive paranasal polyp, from which *Bipolaris spicifera* was isolated.

Case report: A 10-year-old immunocompetent boy, with a history of allergic rhinitis, nasal congestion, postnasal purulent discharge and restricted nasal breathing during last year, was admitted to our hospital for investigation of his symptoms. The clinical examination revealed the presence of a massive polyp which obstructed the left nostril. Chest radiography was normal. The eosinophils of the peripheral blood were not elevated. The

C/T showed an extended polyp which occupied the left maxillary sinus and protruded from the maxillary sinus ostium in the left nostril. Bony erosion of the sinus walls was also apparent. Levels of fungal-specific IgE(RAST)for *Cladosporium herbarum* and *Alternaria alternata* were elevated. The polyp was surgically removed and full histological and microbiological investigation followed. The histologic findings included inflammation of a chronic inflammatory process, presence of allergic mucin, eosinophils and lymphocytes infiltration of the mucosa, whereas Grocott's and PAS staining rendered fungal hyphae. After 72 hours' incubation, the histological specimen culture yielded a dark olive to black fungus identified as *Bipolaris spicifera*. Conventional identification was confirmed by sequencing (Marcogen, Seoul, Korea) of the amplified ITS1 and ITS2 regions showing 99% homology (Bioedit) with the published rRNA sequences (GenBank Acc. No AY253918). The patient, after the surgical removal of the polyp, received therapy with budesonide and desloratadine and was regularly followed up by the otolaryngology department of our hospital. Antifungal therapy was not administered Eight months after his admission to the hospital, recurrence of the disease was observed.

Conclusions: Recent evidence supports the theory that AFS represents an immunologic, rather than infectious, disease process, similar to that of allergic bronchopulmonary fungal disease. Although important, surgery alone does not lead to a long-term disease-free state and plan incorporating medical, surgical and immunologic care remains the most likely means of providing long-term control of disease.

P1219

Mucormycosis: still a problem (a report of 4 cases)

L. Ammari, B. Kilani, H. Tiouiri ben Aissa, F. Kanoun, E. Chaker, A. Goubontini, T. Ben Chaabane (*Tunis, TN*)

Mucormycosis is a rare and invasive fungal infection which is frequently lethal. It affects commonly on immunocompromised patients, especially diabetics. The rhinocerebral form is the most frequent complication, but other localizations may occur.

Objective: The aim of this study is to analyse demographic characteristics, clinical presentation, radiological findings, management and outcome of mucormycosis.

Methods: We conducted a retrospective study of patients who developed a mucormycosis between January 1988 and December 2004 and admitted in the department of infectious diseases of Rabta hospital in Tunis. Diagnosis of mucormycosis was confirmed by mycological and/or histological findings.

Results: 4 patients with mucormycosis were included in the study, 3 male and one female. The mean age is 53 years. All patients were diabetics, three of them had diabetic ketoacidosis at the time of diagnosis. The sites of infection were: sinonasal (2 cases), rhinocerebral infection (2 cases). One patient with rhinocerebral involvement had carotid artery thrombosis on RMI. The direct detection of fungus in tissues and samples were noted in all cases and *Rhizopus oryzae* grew in 3 cases. Our patients were treated with amphotericin B associated to surgical debridement of devitalized tissue in 3 cases. A fatal outcome was observed in two patients, whereas two patients are still alive with sequelae: facial paralysis and numbness (1 case) and facial necrosis (1 case).

Conclusion: Mucormycosis remains a severe infection disease in diabetics patients. Early diagnosis and treatment are mandatory to improve survival and prevent brain dissemination.

P1220

Candida deep sternal wound infections: an emerging problem?

T. Ejlersten, I. Modrau, L. Lemming, B.S. Rasmussen (Aalborg, DK)

Objectives: Deep sternal wound infection (DSWI) imposes serious consequences to the patient after open heart surgery in terms of significantly increased morbidity and mortality including prolonged hospital stay. *Candida* as a causative organism for DSWI has only been reported in few cases in relation to nosocomial outbreaks. The increasing number of DSWI with *Candida* at our institution required systematic investigation.

Methods: The study was conducted for all open heart surgery performed at the department of Cardiothoracic Surgery Aalborg Hospital, Aarhus University Hospital, in the period of January 2001 through December 2004. All cases of DSWI were investigated retrospectively. DSWI was defined as clinical signs of infection requiring open exploration of the sternum with findings of bacteria or yeast from bone biopsy, deep tissue biopsy, or aspiration of pericardiac fluid.

Results: From year 2001 to 2004, a total of 2138 patients underwent an operation involving median sternotomy. DSWI was identified in 39 patients, 29 cases were early onset (0–30 days), (incidence 1.4%); 10 cases were late onset (32–165 days). Twelve patients experienced more than one episode of infection (a total of 55 episodes). *Candida* was a frequent agent (12 episodes), only second to coagulase-negative staphylococci (36 episodes). Re-operation, prolonged stay in ICU, and colonisation with *Candida* prior to infection were risk-factors for *Candida* infections. Longterm mortality (0–4 years) of patients with *Candida* DSWI was 45%, whereas mortality of patients with DSWI of other aetiology was 18%.

Conclusions: *Candida* infections in DSWI following median sternotomy have become a problem of major concern in our hospital. Spread over time together with presence of several risk factors contradicted a clonal outbreak. *Candida* infections require a prolonged course of antimycotic chemotherapy and have a poor clinical outcome. Findings of *Candida* species from sternal wound should prompt further investigations including bone or tissue biopsies to verify a DSWI. Antimycotic chemotherapy should be initiated as soon as a diagnosis of *Candida* infection has been made.

P1221

Survey on dermatophytosis in patients referred to a dermatology hospital in Ghazvin, Iran

M.R. Aghamirian, D. Keshavarz, J.H. Hashemi (Ghazvin, IR)

Dermatophytosis has been considered to be a major public health problem in many parts of the world. The aim of this study was to identify aetiological agents of patients with dermatophytosis infections in referred to the dermatology Hospital Boali Sina, Ghazvin, Iran. A total of 341 patients suspected to have dermatophytosis lesions were examined over a period of one year (2004–2005). Material collected from skin, hair and nails was submitted to direct microscopic examination using KOH, cultured in Sabouraud dextrose agar and microscopically examined for colony morphology, in order to identify 116 dermatophytes isolated. *Epidermophyton floccosum* was the most frequent dermatophyte isolated (32.8%) followed by *Trichophyton mentagrophytes* (22.4%), *T. rubrum* (18.1%), *T. verrucosum* (17.2%), *T. violaceum* (0.86%). *Tinea cruris* (31.9%) was the most common type of infection, followed by *Tinea corporis* (20.7%), *Tinea pedis* (19%), *Tinea unguium* (11.2%), *Tinea faciei* (7.7%), *Tinea*

manuum (5.2%), *Tinea capitis* (4.3%). The frequency rate of *Tinea* was higher in males than in females. The anthropophilic species *E. floccosum* was the most common dermatophyte causative agent of tinea.

P1222

Caspofungin and G-CSF as first-line therapy of pulmonary invasive fungal infections in 32 neutropenic patients with haematologic malignancies

A. Candoni (Udine, IT)

Caspofungin is a large lipopeptide molecule able to inhibit the enzyme complex 1,3-D-glucan synthetase; this action specifically damages the fungal cell wall. Caspofungin (CAS) is active, *in vitro* and *in vivo*, against most *Candida* species and *Aspergillus* species. Herein we report our experience with this drug as a first-line therapy for pulmonary proven or probable IFI in neutropenic patients with hematologic malignancies. Thirty-two consecutive patients (pts) have been treated with CAS (27 acute leukemias, 3 lymphomas, 1 chronic leukaemias and 1 myeloma): 20 males and 12 females with a median age of 52 yrs (range 22–72). 16/32 (50%) pts had a relapsed or resistant haematologic disease, while 12 pts were in complete remission and 4 pts were at onset of disease; 8/32 (25%) developed IFI after a Transplant (BMT) procedure. Out of 32 pts, 7(22%) had proven pulmonary IFI (7/7 Aspergillosis) and 25(78%) had a probable IFI (defined according to international consensus), all 32 cases with pulmonary localization. 31/32 (97%) pts had less than 1000 granulocytes/mL at onset of infection. CAS was given at the dose of 70 mg on day 1, followed by 50 mg/daily. Median duration of CAS therapy was 20 days (range 8–72); 31/31 neutropenic pts (100%) received G-CSF. The overall response rate was 56% (18/32) with 12/18 complete responses and 6/18 partial responses; 2/32 pts had a stable disease. Twelve out of 32 pts (37.5%) did not respond and six of them (50%) died from mycotic infection. Univariate analysis showed that granulocytes recovery and status of haematologic disease were significantly associated to favourable outcome. No adverse clinical effects were reported and only a grade I-II transient increase of alkaline phosphatase and/or transaminases occurred in 4/32 (12%) pts. After CAS therapy six non-responders and 6 pts with a partial or stable response were rescued with voriconazole. Two out of 6 pts (33%) in the former group and 6/6 (100%) in the latter obtained a complete resolution of IFI. Our experience suggests an efficacy of CAS in combination with G-CSF, as first-line treatment of proven or probable IFI with lung localization. The drug was well tolerated and there were no significant hepatic adverse events even in pts receiving CAS with cyclosporin after a BMT. A significant proportion of non-responders or partial responders to CAS can be rescued with a subsequent voriconazole-based therapy.

P1223

Clinical analysis of cryptococcal meningitis (with 72 case reports)

G. Shi (Shanghai, CN)

Objectives: To analyse the clinical characteristics, prognosis and its related factors of patients with cryptococcal meningitis.

Methods: Clinical information of 72 patients with *cryptococcal meningitis* treated in Huashan Hospital from January 2000 to December 2004 were retrospectively analysed.

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Results: Among the 72 patients (46 men and 26 women; mean age, 38.7 years), 37 have a background disease: 22 with connective tissue disease, 6 with renal insufficiency, 4 with diabetes mellitus, 3 with tuberculosis, 2 with HIV/AIDS. 14 (19.4%) of them have a clear contact history with pigeons. 29 of them were diagnosed as tubercular meningitis at first, 9 as suppurative meningitis, 7 as lupus encephalopathy, 2 as viral encephalitis and 3 as other diseases. And 39 (54.2%) patients had recently used glucocorticosteroid and (or) immunosuppressor. Lab Findings: 57 patients (79.2%) were positive for CSF India ink smear, 21 (29.2%) positive for *Cryptococcus* culture, 66 (91.7%) positive for Latex agglutination test (LAT). And 18 patients had markedly elevated intracranial pressure (≥ 300 mmH₂O). Treatment: 37 patients with cerebral ventricle dilatation got lateral ventricle drainage, and 33 individuals implanted Ommaya. 58 patients were treated with a combination of Amphotericin B (AmpB) or its lipid formulations plus flucytosine. 52 individuals received fluconazole temporarily in induction course. 43 were treated with intrathecal AmpB injection. The average dose of AmpB is 3.06 g; length of therapy is between 12 weeks and 20 months. 54 patients (75%) were cured, 9 (12.5%) improved and 9 (12.5%) died.

Conclusion: High rate of misdiagnosis is common to cryptococcal meningitis. The application of immunosuppressor such as glucocorticosteroid and diseases such as HIV/AIDS cause immunocompromise, which is the major risk for cryptococcal meningitis. Besides, a contact history with pigeons is another main pathogenic factor. CSF LAT is still the most sensitive test for diagnosis. The keys to reduce mortality and improve prognosis include early diagnosis, combination antifungal therapy of AmpB with 5-FC, aggressive management of elevated intracranial pressure and intracerebral or intrathecal AmpB. Implantation of Ommaya and intra-Ommaya or intrathecal AmpB help to reduce the venal dosage of AmpB and to get improvement earlier, and accordingly shorten the length of therapy evidently.

P1224

The prevalence of aetiologic agents of dermatophytosis in wrestlers in Isfahan, Iran

S. Shadzi, A. Karbassian, N. Kassaian, M. Khademi, Z. Nokhodian (Isfahan, IR)

Objectives: Due to special conditions of wrestling sport, this group of athletes are at high risk of dermatophytic infections. These infections are easily transmitted to other populations either in gymnasium or home. The prompt diagnosis of infection and identification of the aetiological agents of dermatophytoses would help the prevention and treatment of this group of athletes which was the aim of this study.

Methods: Fifty-six male wrestlers suspected of having cutaneous dermatophytoses were studied during 2002–2004. Direct exam and culture were performed on the collected scraping specimens. The identification of fungal species were done by standard procedures.

Results: The wrestlers in this study were in 17–23 years of age and 80% of them stated in their medical history the lack of shower after exercises was the major factor of infection. On 12 patients only direct exam was performed and 44 specimens were cultured in which 38 (86%) *Trichophyton rubrum*, 4 (9%) *T. violaceum* and 2 (4.5%) *Epidermophyton floccosum* isolated and identified.

Conclusion: Regarding to resistance of *Trichophyton rubrum* to antifungal agents, the necessity of culture and identification of species involved in wrestlers is emphasized. Also, personal hygiene and shower after exercise are important. In addition, the

disinfectant of gymnasium and exercise equipments and sufficient ventilation of these places are recommended

P1225

Slowly progressing, and indolent pulmonary mucormycosis (zigomycosis) in a patient with an underlying multiple sclerosis, managed without immunosuppressive therapy

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

Introduction: Mucormycosis (M) is a infrequent filamentous fungal infection borne by a very elevated fatality rate despite prompt diagnosis and adequate therapy especially in its frequent rhinocerebral presentation and/or when decompensated diabetes mellitus, neutropenia or immunosuppression are of concern.

Case report: A 53-year-old female patient (p) with a multiple sclerosis previously treated with steroids-azathioprine (but controlled since 3 months without treatment), was hospitalized owing to hemoptoe in absence of other respiratory symptoms and fever. Laboratory testing did not disclose significant abnormalities (WBC, ESR and serum glucose were within limits) and tumoral markers tested negative but the detection of multiple lung infiltrates at chest X-ray and HRCT (predominant at right lobes, with an appreciable air bronchogram), prompted a bronchoscopy with biopsy-BAL involving the medial right lobar bronchus area. After an uncertain microscopy (with *Aspergillus* hyphae still suspected) and negative serum *Aspergillus* antigen search, cultures led to the isolation of *Mucor* spp., with tested *in vitro* susceptible to amphotericin B-posaconazole and resistant to itraconazole-voconazole. Liposomal amphotericin B (3 mg/kg/day) was delivered for 6 weeks predominantly on Day-Hospital basis with favourable tolerability: no hematological, blood chemistry and urinalysis alterations occurred. One month later our p completely recovered and a repeated HRCT and bronchoscopy confirmed a complete clinical, radiological and mycological cure.

Discussion: M is a rare occurrence especially when neutropenia and ketoacidosis are absent. However, anecdotal reports occurred after trauma and during COPD. Although the usual portal of entry of M is respiratory, however the rhinocerebral M remains the most frequent and life-threatening presentation. Clinicians should consider M even when obvious risk factors and an apparently slow progression are found. Diagnosis includes microscopic differentiation from *Aspergilli*, although mixed infections are not so rare. In pulmonary forms, percutaneous biopsy becomes sometimes needed. Liposomal amphotericin B remains the treatment of choice but surgery is sometimes necessary to debride extensive necrotic areas due to angioinvasion; some doubt remains about hyperbaric O₂ therapy. In our p, a slowly progressive pulmonary M was identified and cured in a reasonably short time, even in absence of underlying, active risk factors and an overwhelming clinical progression.

P1226

AIDS-defining fungal opportunism in the HAART era. Trend of frequency and reduced incidence when HIV protease inhibitors are administered

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

Background: Nine years after the introduction of HAART, opportunistic AIDS-related mycoses show a progressive drop of

incidence. Aim of our work is to assess the temporal trend of major AIDS-associated fungal infections during the last three-year period, and to relate our figures with HAART administration, and the different combinations of administered antiretrovirals, with attention focused on protease inhibitors (PI).

Methods: Through a retrospective analysis of clinical-microbiological records, 118 episodes of AIDS-defining mycoses were identified from 2001 to June 30, 2005.

Results: The great majority of the 118 episodes of visceral mycosis was represented by esophageal candidiasis (101 cases), followed by CNS-disseminated cryptococcosis (15 episodes), and candidemia (two episodes). The temporal trend demonstrated a progressive tendency to a reduction of diagnosed cases: 34 in the year 2001, 27 in the year 2002, 25 in the year 2003, 12 in the year 2004, and 20 in the first 6 months of 2005. In even 63 patients (p) of 118 (53.4%), visceral mycoses occurred concomitantly with the first positive HIV serodiagnosis: the so-called "AIDS presenters", who were never treated with antiretrovirals. In the remaining 55 episodes, fungal infections occurred as the first AIDS-related disorder in 31 cases, while in 24 p they represented a subsequent opportunistic complication interesting p already diagnosed with AIDS. Although all p suffered from an advanced HIV disease (as expressed by a CD4+ lymphocyte count of 127.3 ± 52.7 cells/ μ L), among the 55 patients who were taking antiretrovirals, PI were administered in 11 p only, while other combinations excluding PI were used in 44 p ($p < 0.001$).

Conclusions: Over 50% of episodes of visceral candidosis and cryptococcosis occur in "AIDS presenters", while the lack of adjunct of PI could contribute to explain the apparent increased predisposition to these opportunism versus other AIDS-defining diseases. Pathogenetic hypotheses cannot exclude the demonstrated direct *in vitro* antifungal effect exerted by PI.

P1227

Concurrent invasive cryptococcosis and candidiasis in AIDS presenters. Clinical epidemiology and features never observed also during the pre-HAART era

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

Background: The HAART regimens changed the course of HIV infection, first leading to a drop of opportunism related to a severe immunodeficiency, including visceral candidiasis-cryptococcosis. However, the incidence of the so-called AIDS presenters is increasing during HAART since patients (p) who are unaware of or neglect HIV cannot take advantage from HAART.

Methods: Two rare cases of concurrent visceral *Candida-Cryptococcus* co-infection occurred in p with a undiagnosed HIV disease are presented.

Results: Two p who were unaware of HIV disease, were referred with a far compromised clinical situation, including prolonged fever, dysphagia, pancytopenia and weight loss, Pneumocystis carinii-Mycobacterium kansasii-Staphylococcus aureus pneumonia in the first p, and persisting headache in the second p. A candidiasis was confirmed by esophageal biopsy in both cases, while the first p also had positive *Candida albicans* blood cultures. Cryptococcosis was the result of fungemia in the first p, and meningeal localization in the second one, whose CSF proved positive at culture-antigen search. The severe immunodeficiency was expressed by a CD4+ lymphocyte count of 44 and 13 cells/ μ L respectively. After the diagnosis of concomitant dual *Candida-Cryptococcus* infection, fluconazole, and concurrent AmB-isome in the first p, were administered, leading to mycological

and clinical cure. No relapses of yeast opportunism occurred during the 16–48-mo follow-up, while immune reconstitution took place thanks to HAART.

Conclusions: Multiple, concomitant-subsequent AIDS-defining illnesses were anecdotally described, but the concurrent detection of two different visceral and AIDS-related yeast diseases has no equivalents until now. Preventive-educational efforts are needed for each population target, since many p are at risk of suffering from a missed-delayed HIV recognition, and have an increased risk of advanced, life-threatening HIV disease including multiple AIDS-related disorders.

P1228

A 3-year study of resistance of *Candida* pathogens obtained from patients with fungaemia with emphasis on new antifungal agents

A. Koutsoukou, A. Stylianakis, S. Tsiplakou, M. Tachtatzis, E. Tsakona, A. Vourtsi, A. Sideri (*Athens, GR*)

Objective: The aim of the present study was to examine the resistance of *Candida* species to amphotericin-B, fluconazole, itraconazole, 5-flucytocine and to the new antifungal agents voriconazole and caspofungin.

Methods: 152 non duplicated *Candida* species, isolated from blood cultures (Bactec, Becton Dickinson) over a 3 year period (2003–2005), were identified and MIC values were determined. Identification was performed by VITEK II automated system (Biomerieux) and confirmed by the API 20C AUX (Biomerieux). MICs for amphotericin-B, fluconazole, itraconazole and 5-flucytocine were determined by ATB fungus 2 (Biomerieux). For the new agents voriconazole and caspofungin, MICs were defined by E-test (AB Biodisk, Solna, Sweden) and confirmed by broth microdilution method, according to NCCLS criteria (document M 27-A).

Results: *Candida parapsilosis* was the predominant species (64%) followed by *Candida albicans* (24%), *Candida tropicalis* (8%), *Candida glabrata* (2%), *Candida krusei* (1%). 77 (50%) strains were resistant to itraconazole 44 (33%) strains were resistant to fluconazole, 7 (5%) strains were resistant to 5-flucytocine while no strain was found resistant to amphotericin-B. The MICs of caspofungin were between 0.25–2 μ g/ml and the MICs of voriconazole were less than 1 μ g/ml. Only 2 strains of *Candida parapsilosis* had MICs higher than 2 μ g/ml to voriconazole and would be classified as resistant to the drug.

Conclusions: Our findings show that fungemia in our hospital is caused mainly by *Candida parapsilosis*. Moreover *Candida* blood stream infections had high-rates of resistance to itraconazole and fluconazole in contrast to voriconazole. All *Candida* strains were susceptible to caspofungin. Also, no resistance was observed to amphotericin-B which remains the more active antifungal agent in common use.

P1229

Candida embolic stroke and secondary fungal mycotic aneurysm

C. Garzoni, I. Uckay, L. Markham, R. Sztajzel, K. Bouchuigui-Waf, P. Temperli, J. Garbino (*Geneva, CH*)

Introduction: Embolic events are known complications of infective endocarditis but fungal secondary mycotic aneurysms rarely result. *C. parapsilosis* endocarditis with intra-cerebral embolism and secondary mycotic aneurysm formation was never described before.

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Case report: A 37 year-old i.v. drug abuser was first hospitalized on October 2004 with acute thromboembolic stroke and *C. parapsilosis* candidemia. Exhaustive research for a primary focus was negatives. The fungigram showed an intermediary sensitivity to fluconazole for the isolated strain and a treatment with voriconazole was started. The patient had favourable evolution and the treatment was discontinued after 3 months. Six weeks after the treatment was stopped he was hospitalized due to an acute hemiparesia. The MRI showed a cerebral artery occlusion and an intra-arterial thrombolysis was performed. *C. parapsilosis* yielded from the blood cultures with the same resistance pattern to the previous strain. Voriconazole was restarted. Due to an important aortic insufficiency with floating vegetation the aortic valve was replaced by a porcine xenograft. The neurological symptoms had a slight improvement. The new MRI done showed the cerebral media artery occluded with a fusiform aneurysm. Serial The stability of the lesion in the following months was documented by MRI done for control. After 9 months of voriconazole treatment the patient did not experienced any recurrence.

Discussion: Acute stroke is a feared complication of infective endocarditis. Up to 50% of patients with fungal endocarditis show arterial thromboembolic events and nearly 20% of them are intra-cranial. Interestingly, despite endocarditis is the major cause of intra-cranial bacterial aneurysms, secondary intra-cranial fungal aneurysm are very rare. A limited number of reports describe fungal mycotic aneurysm, but most are due to *Aspergillus* sp. To our knowledge there are only 2 reports of *Candida* intra-cranial aneurysms and none caused by *Candida* non-*albicans* species. Given the limited number of experience, therapeutic guidelines and experience with invasive therapeutic interventions are lacking.

P1230

Monitoring of voriconazole blood levels for prevention of serious neurological adverse events

A A. Pascual, S. Bolay, O. Marchetti (Lausanne, CH)

Background: Voriconazole (VRC) is a widely used broad-spectrum antifungal agent. Non-linear pharmacokinetics, polymorphism of cytochrome CYP2C19, drug interactions and hepatic dysfunction may result in inter- and intra-individual variations of VRC blood levels. We reported Serious Neurological Adverse Events (SNAE) probably associated with prolonged VRC overdosing (trough blood levels > 5.5 mg/L during >7 d) (ICAAC 2005, M-2164). This observation suggested that VRC dose adjustment based on monitoring of blood levels may help to prevent SNAE.

Objective: To prospectively evaluate the utility of monitoring VRC blood levels for prevention of SNAE.

Methods: VRC trough blood levels were measured by HPLC during the first week of therapy in 25 consecutive treatment courses during 2005. VRC dosing was adjusted if VRC trough blood levels were >5.5 mg/L. Clinical follow-up included surveillance for adverse events (NCI criteria). Occurrence of SNAE during VRC therapy in 2004 (no prospective dose adjustment based on VRC blood levels) and 2005 (prospective dose adjustment) was compared.

Results: Indications for VRC therapy were aspergillosis (60%), candidiasis (16%), and suspected mycosis (24%). Median VRC dose was 4 mg/kg bid (range 1.3–5.7). Median number of VRC trough blood levels measurements/treatment course was 2.5 (range 1–5). Median days to first measurement after starting VRC therapy were 2 (2–7). Nine pts (37%) presented transient self-limiting visual disturbances/hallucinations. Two patients

(8%) presented severe hepatotoxicity. Occurrence of SNAE in 2004 and 2005 is compared in table 1.

Table 1

	2004 No prospective dose adjustment	2005 Prospective dose adjustment
Patients with VRC trough level > 5.5 mg/L	8/32 (25%)	5/25 (20%)
Median VRC trough level, mg/L (range)	6.3 (5.6 to 11.2)	6.5 (5.6 to 11.5)
Median days > 5.5 mg/L (range)	12.5 (6 to 30) *	4 (2 to 7)
SNAE	4/32 (12%)	0/25 (0%)

* P = 0.05

Conclusions: These prospective data corroborate our preliminary report that rapid dose adjustment in patients with VRC blood levels exceeding 5.5 mg/L may help to prevent serious neurological adverse events. Further observations are needed to confirm this finding.

P1231

Influence of azoles on mixed long-term continuous flow cultures of *Candida albicans* and *Candida glabrata* under aerobic and anaerobic conditions

H. Bernhardt, M. Knoke, K. Zimmermann, G. Schwesinger, J. Bufler, K. Ludwig, J. Bernhardt (Greifswald, Karlsruhe, Rostock, DE)

Objectives: Around 3% of cultures obtained from candidaemia patients and 13% of those from oesophageal candidiasis patients grew both *C. albicans* and *C. glabrata*. A continuous flow system was used to investigate the effects of fluconazole (FLU) and voriconazole (VORI) on mixed *Candida* infections under aerobic and anaerobic conditions. This allows the simulation of the *in vivo* situation.

Methods: *Candida* strains were cultured at 37°C in double-wall vessels containing 110 ml medium. Anaerobic conditions were obtained by flushing with N₂/CO₂; aerobic conditions were maintained with pressurized air. Media flow rate was 6.0 ml/h. The system was adjusted to a generation time of 15–20 h. Media contained FLU (20 or 60 mg/l), VORI (20 mg/l) or no antifungal (controls). For the detection of morphologic alterations of the *Candida* cells half-size slides were kept in the culture vessels. At the end of the trials the biofilms on the slides were stained with BLANKOPHOR® (Bayer AG Leverkusen, Germany) and examined by fluorescence microscopy.

Results: Growth of *C. glabrata* was particularly strong in co-culture with *C. albicans*. *C. glabrata* consistently achieved higher densities than *C. albicans* (7.5 vs. 6.6 log₁₀ cfu/ml). In this setting, *C. albicans* was inhibited more effectively than *C. glabrata* by 20 and 60 mg/l FLU. At 60 mg/l, FLU inhibited *C. glabrata* more effectively under anaerobic conditions and after 96 h. Clinical isolates and type strains of both *C. albicans* and *C. glabrata* were inhibited more strongly by FLU and VORI under anaerobic conditions. The decrease in germ counts after addition of FLU or VORI to a *C. albicans* monoculture was more rapid and more profound under anaerobic (>99%; i.e. fungicidal) than aerobic conditions (90–99%; i.e. fungistatic). Both FLU and VORI strongly inhibited biofilm formation: in the absence of antifungals, a biofilm of budding yeast, mycelia and pseudomycelia developed on glass slides. In the presence of FLU or VORI, only few yeast cells and germ tubes were observed, but no mycelia or pseudomycelia.

Conclusion: In a long-term continuous flow culture system, the inhibition of various *Candida* strains by FLU or VORI was strongly enhanced by anaerobic conditions. This finding may be of relevance for the clinical setting, as mycotic abscesses and sequestered areas of infected tissue are hypoxic.

P1232

Investigation of synergistic effect of sertraline on fluconazole-resistant *Candida* spp. by microbiological methods and electron microscopy

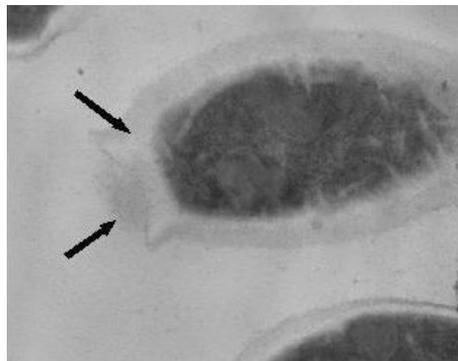
N. Nuhoglu, T. San, G. Soyletir (Istanbul, TR)

Objectives In neutropenic patients long-term antibiotic prophylaxis or treatment creates a selective environment for fungal growth and *Candida* spp. are the most frequently isolated agents. Fluconazole administration during neutropenic attacks may have the risk of selecting fluconazole-resistant *Candida* spp. which can further lead to serious infections. In order to overcome this resistance, we investigated the synergistic activity between fluconazole and achievable levels of sertraline, an antidepressant drug, which was previously shown to have antifungal activity in higher concentrations than that of serum levels.

Methods: Antifungal susceptibility testing (NCCLS M27-A) of nonalbicans *Candida* isolates were performed and five fluconazole resistant *Candida* (three *C. krusei*, two *C. glabrata*) strains which have MICs for fluconazole and sertraline in the ranges of 32–128 mcg/ml and 24–48 mcg/ml respectively, were tested for synergy between these two drugs. To do this checkerboard assay and electron microscopy were used.

Results: The MIC values of fluconazole dropped to 4–32 mcg/ml at sertraline's plasma achievable concentrations (0.09–0.18 mcg/ml) and fractional inhibition concentration (FIC) indices were found to be 0.06–0.5 indicating the presence of

synergy. We also observed irreversible changes of the cell morphologies including disruption of the cell wall and discharge of the cytoplasmic content, both at MIC levels of drugs and their synergistic concentrations under electron microscope (Fig 1).



Conclusion: In the case of candidiasis due to fluconazole resistant strains, routine dosages of sertraline can be added to treatment protocol, provided that these *in vitro* results are furtherly confirmed with *in vivo* studies. Our findings also suggest that, as a broad spectrum efflux inhibitor, sertraline may also be tested for synergistic effect against other microorganisms with efflux mediated resistance.

Antimicrobial resistance, permeability and fitness

P1233

Fitness costs of antimicrobial resistance associated with chromosomal mutations and plasmid-borne resistance genes in *Escherichia coli*: *in vitro* and *in vivo* studies

A. Petersen, F.M. Aarestrup, M. Bisgaard, J. Olsen (Frederiksberg, Copenhagen, DK)

Objectives: A reduced fitness, often measured by growth *in vitro*, is associated with antimicrobial resistance phenotypes. The fitness of antimicrobial resistant bacteria may influence their persistence and transmission. The question remains whether a reduced fitness *in vitro* can be confirmed by *in vivo* experiments.

Methods: *E. coli* BJ4 with different mutation- and plasmid mediated resistances were grown separately and in pair-wise competitions to estimate fitness cost *in vitro*. *In vivo* studies were performed by oral inoculation of equal numbers of two test strains in 4–5 weeks old chickens and colonization was followed for one week. At each sampling point, 4 chickens were sacrificed and content of the caecum was examined for the inoculated bacteria.

Results: *In vitro*: A single chromosomal mutation conferring resistance to streptomycin (str) did not impose a fitness cost to *E. coli* BJ4. An additional mutation leading to rifampicin (rif) resistance reduced relative fitness 19%. Introduction by conjugation of 53–60 kB plasmids with one or several resistance genes did not significantly reduce relative fitness of *E. coli* BJ4. *In vivo*: A co-introduction of a *E. coli* BJ4 harbouring a 53 kB plasmid and a str rif resistant *E. coli* BJ4 (*in vitro* fitness 1 and 0.82, respectively) in chicken resulted in average higher number of recoveries in the caecum of the former after 7 days but the difference was not significant. When a selection pressure in

favour of either of the two strains was introduced, the favoured strain was recovered in significantly higher numbers than the other.

Conclusions: Fitness costs associated with antimicrobial resistance was demonstrated under laboratory conditions. *In vivo* experiments only confirmed differences of *in vitro* relative fitness when a selective pressure was applied. The dynamics and epidemiology of antimicrobial sensitive and resistant bacteria in animals and in the environment may thus be difficult to predict based on *in vitro* studies.

P1234

The fitness cost imposed by plasmid RP1 on *Escherichia coli* differs from strain to strain

V.I. Enne, P.M. Bennett (Bristol, UK)

Objectives: Our previous studies have demonstrated a relatively low, and sometimes non-existent fitness cost imposed by antibiotic resistance plasmids on the *Escherichia coli* strain 345-2RifC. These results are in contrast to those found by other researchers who, using laboratory strains of *E. coli*, have reported much higher costs associated with plasmid carriage. These conflicting results may be due to variations between the strains used. We therefore investigated the overall fitness of cost incurred by carriage of the plasmid RP1 in five distinct *E. coli* strains.

Methods: The fitness impact of the IncP plasmid RP1 was studied in five *E. coli* strains: the K12 laboratory strain JM109; 99-24 and 99-40, two recent human clinical isolates and 345-8 and 343-9, two recent isolates from pig faeces. The study strains were selected on the basis that they are fully antibiotic

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susceptible and have different biochemical profiles and are therefore not related to each other. Plasmid RP1 was introduced into the strains by conjugation. The fitness cost of RP1 in each strain was assessed *in vitro* by pairwise growth competition in Davis minimal medium for six days, with six replicate experiments performed.

Results: In the laboratory strain JM109 plasmid RP1 imposed a fitness cost of $-5.8 \pm 1.0\%$ per generation. This was lower than the $-9.1 \pm 4.1\%$ cost it imposed on the clinical strain 99-24 and significantly lower than the $-9.7 \pm 1.4\%$ cost imposed on clinical strain 99-40 ($t = -5.48$, $p = 0.0003$). In contrast, there was little or no cost to carriage of RP1 by the animal strains 345-8 and 343-9. RP1 imposed a small fitness cost of $-1.8 \pm 0.8\%$ on 345-8, and conferred a slight benefit of $+0.8 \pm 0.9\%$ on 343-9. The fitness impacts of RP1 on 343-9 and 345-8 were significantly lower than the costs imposed on JM109, 99-24 and 99-40 ($p < 0.002$ in all cases).

Conclusion: The fitness cost associated with carriage of antibiotic resistance plasmids can vary considerably depending on the strain. It is therefore unwise to generalise results obtained with any one particular strain. In the case of RP1 and our five strains, the lowest costs were incurred by the strains of animal origin, while strains of human clinical origin incurred the highest costs.

P1235

Long-term persistence of resistant strains in the human intestinal microflora due to restored fitness

S. Löfmark, H. Billström, C. Jernberg, C. Edlund (Stockholm, SE)

Objectives: Antibiotic therapy often leads emergence of resistant intestinal bacteria mainly attributed to mutations or acquisition of resistance genes. Resistance traits are generally considered to lead to decreased fitness due to a cost for the bacteria. However, fitness may be restored by compensatory events, thus the level of resistance may not automatically revert to pre-antibiotic levels when the antibiotic is removed. The aim of the present study was to analyse fitness in consecutive isolates of two clindamycin resistant clones of *Bacteroides* spp, cultured from two clindamycin exposed subjects.

Methods: The fitness of serial isolates of two *B. tetaiotaomicron* clones, A and B verified by Rep-PCR, originally susceptible to clindamycin and lacking erm-genes was studied by an *in vitro* competition method. The strains derived from faecal samples of two healthy volunteers exposed for clindamycin (150 mg \times 4 for 7 days). Isolates belonging to clone A were analysed pretreatment (A:0-S), at the last day of administration (A:7d-R), after two weeks (A:2w-R) and 18 months (A:18m-R). The three latter isolates carried the ermG gene and expressed phenotypic clindamycin resistance. Clone B was isolated at corresponding intervals except for day 7 when the numbers of CFU were under the detection limit (B:0-S; B2w-R; B18m-R), post exposure isolates carried the ermF gene. Relative fitness was analysed *in vitro* by pair-wise competition experiments in triplicate, where R isolates were challenged against the S isogenic parent isolate.

Results: The imposed cost of resistance during clindamycin exposure was high for clone A, A:7d-R were competed out *in vitro* by its parental A:0-S. After 2 weeks no growth disadvantages was detected for the R isolates compared to the pre-exposed S isolates in any of the two studied clones. This regained fitness remained 18 months after administration.

Conclusion: The results indicate that the biological cost to carry a resistance gene can be compensated for and once the resistant clone has gained its resistance determinant it is difficult to eliminate. Most studies have focused on the fitness costs associated with resistances due to mutational events. Here we show that

the impact the acquired erm genes had on the fitness of the bacteria was low or non-existing, even though the initial cost was high. The fact that the clones persisted for at least 18 months is probable due to restored fitness in spite of the presumed extra burden of the resistance gene.

P1236

Mutators among UK CTX-M β -lactamase-producing *E. coli* pose a risk for the emergence of fosfomycin resistance

M.J. Ellington, D.M. Livermore, T.L. Pitt, L.M.C. Hall, N. Woodford (London, UK)

Objectives: Fosfomycin (FOS) is a possible oral treatment for lower urinary tract infections caused by the growing number of *E. coli* with CTX-M extended-spectrum β -lactamases. However, mutational resistance can occur and hypermutability among natural *E. coli* populations might increase this risk. We examined the prevalence of mutators amongst UK multi-resistant CTX-M producing *E. coli* urinary isolates and the risk they pose to the emergence of resistance.

Methods: Consecutive CTX-M positive *E. coli* clinical isolates from urinary tract infections ($n = 231$) were screened for a mutator phenotype by rifampicin (RIF) and FOS disc assays. MICs were determined by BSAC methodology. Wild-type and isogenic MutS- controls were also tested. Mutation frequencies were determined on agar in triplicate with $4 \times$ MIC or with 256 $\mu\text{g/ml}$ FOS.

Results: Eleven of 231 isolates were highly resistant to FOS or RIF and were not tested further. The MutS- strain and 10/231 (4.3%) CTX-M *E. coli* isolates yielded >70 and >10 colonies within the zones around both 50 μg FOS and 30 μg RIF discs indicating likely mutator phenotypes. The wild-type *E. coli* control and 80 clinical isolates gave <30 and <10 colonies in the zones around FOS and RIF discs, respectively, indicating non-mutators. The remaining 130/231 isolates gave 30-70 and <10 colonies in the zones, implying weak-mutator status at most. Mutation studies at $4 \times$ MIC were done on 20 isolates (including the hypermutator candidates). Mutation frequencies, for the MutS- control were 2.74×10^{-4} for RIF and 286×10^{-6} for FOS. Nine of 10 likely mutators from the screen had frequencies of $0.008-1.52 \times 10^{-4}$ for FOS and $0.1-2.26 \times 10^{-6}$ for RIF, and 2 of the 10 were confirmed as strong mutators to both RIF and FOS. The non-mutator control and 7 putative non-mutators selected from the screen had mutation frequencies of $2.99-6.42 \times 10^{-6}$ for FOS and $0.8-3.73 \times 10^{-8}$ for RIF, confirming non-mutator status. Only strong mutators (RIF frequencies $>8.86 \times 10^{-7}$) consistently gave single-step mutants at 256 $\mu\text{g/ml}$ FOS. No nitrofurantoin (NIT) resistant mutants were raised from any isolate or strain, except in only one of the three triplicated experiments with hypermutable MutS- control strain, (mutation frequency = 6.67×10^{-9}).

Conclusion: Of 231 multi-resistant urinary isolates of CTX-M *E. coli*, 4.3% were mutators with an enhanced risk for the emergence of FOS resistance. Whilst NIT is a far from ideal antibiotic it was less vulnerable to mutational resistance.

P1237

Ertapenem promoted the selection of totally resistant pathogens

F. Walsh, S. Bracher, P. Turner, S.G.B. Amyes (Edinburgh, UK; Cheshire, UK)

Objectives: The objective was to identify if ertapenem would encourage or inhibit the selection of a carbapenem-resistant

hospital-acquired pathogen from a mixed population. Thus, if a resistant isolate emerged to dominate the population *in vitro*, there would be a high possibility that this would happen within hospital settings and select for totally resistant pathogens.

Methods: The bacteria tested in these experiments were *Pseudomonas aeruginosa* blaVIM and blaIMP isolates with *P. aeruginosa* ATCC 27853, an ertapenem resistant and a susceptible *Klebsiella pneumoniae* from Scotland, *Acinetobacter baumannii* containing OXA-23, OXA-24 and OXA-58 competing with *A. baumannii* ATCC 19606. In each case, the resistant isolate and sensitive isolate were grown overnight in broth. The resistant isolate was mixed with the sensitive isolate in a ratio of 1:100. Ertapenem (8 mg/L) was added to the broth mixture at the recommended dosing interval of T0, T24 and T48. The broth was serially diluted and spread on plates containing imipenem, meropenem or ertapenem (8 mg/L in each) as well as plates with no antibiotic, at regular intervals. The colonies were counted and the percentage resistance development calculated for each strain and antibiotic. The competition studies were performed at least twice and the percentage resistances were averaged.

Results: The *P. aeruginosa* experiments identified that addition of ertapenem to a mixed population selected the blaVIM resistant isolate with cross-resistance to imipenem and meropenem but did not promote the emergence of the blaIMP isolate. Addition of ertapenem to a mixed population of *K. pneumoniae* promoted the growth of the resistant isolate over the susceptible isolate after 48 hours. The 3 OXA carbapenemase-containing *A. baumannii* were exposed to ertapenem using the same methods. However, the OXA-24 positive isolate differed from the OXA-23 and OXA-58 isolates. In both the OXA-23 and OXA-58 competition studies only isolates resistant to ertapenem emerged from the population. In the OXA-24 study, resistance to all 3 carbapenems emerged at T24 and initially at higher levels to imipenem and meropenem than ertapenem.

Conclusions: The results of this study indicated that exposure to ertapenem at the recommended dosing intervals promoted growth of strains resistant not only to ertapenem but also cross-resistant to imipenem and meropenem.

P1238

Convergent selection of ceftazidime resistance mutations at position 167 of CTX-M-3 beta-lactamase in hypermutable *Escherichia coli* strains

M. Stepanova, M. Edelstein (Smolensk, RU)

Objectives: In CTX-M beta-lactamases, mutations at position 167 confer increased ceftazidime (CAZ) hydrolyzing activity. Recently, we reported *in vivo* acquisition of P167T mutation in CTX-M-3 beta-lactamase rendering it into a ceftazidimase, CTX-M-42. The consecutive clonal isolates that produced CTX-M-3 and CTX-M-42 were found to be hypermutable. In this study, we used an *in vitro* selection with CAZ to simulate the evolution of CTX-M-3 in the mutator *E. coli* hosts and to characterize mutations increasing CAZ resistance.

Methods: The blaCTX-M-3 gene and its upstream promoter region were PCR-amplified from the original clinical isolate Irk1224, cloned in the pCC1 vector and introduced into the laboratory mutator strain GM2995 (mutD5). Both the Irk1224 and GM2995 carrying the blaCTX-M-3 were used in selection experiments. Mutants were obtained on agar plates containing CAZ at concentrations of 2x the MICs. Thirty-two randomly selected mutants of each strain were tested for their susceptibilities to CAZ and cefotaxime (CTX) by agar dilution method

and for the presence of mutations in the promoter and blaCTX-M ORF by sequencing. To confirm the role of particular mutations in CAZ resistance, wild type (WT) and mutant blaCTX-Ms were then recloned in *E. coli* EPI300 and susceptibilities of the resulting clones were determined.

Results: The total rates of mutations increasing CAZ resistance were 2E-8 and 2E-6 for the Irk1224 and GM2995, respectively. Both strains yielded two types of mutants. Those of the 1st type had CTX MICs \geq CAZ MICs, contained no changes in the sequences of blaCTX-M-3 and its 5' vicinity and comprised the majority of clones obtained. Their increased resistance to CAZ was apparently not related to the beta-lactamase and could have resulted from alterations in outer membrane permeability. Mutants of the 2nd type had CTX MICs < CAZ MICs. Out of them, 1 and 8 clones derived, respectively, from Irk1224 and GM2995 contained a single P167S substitution in the CTX-M. Another derivative of Irk1224 carried previously unidentified mutation, N136K. Comparison of CAZ MICs for isogenic strains expressing WT and mutant CTX-Ms clearly supported the involvement of S167 and K136 in CAZ resistance (Tab.1).

Table 1. Susceptibilities of *E. coli* strains expressing WT and mutant CTX-M β -lactamases.

Host strain	β -lactamase (mutation)	MICs, mg/L	
		CAZ	CTX
Irk1224	CTX-M-3 (WT)	32	\geq 256
Irk1224	CTX-M-3 (P167S)	\geq 256	64
Irk1224	CTX-M-3 (N136K)	\geq 256	64
GM2995	CTX-M-3 (WT)	1	4
GM2995	CTX-M-3 (P167S)	32	1
EPI300	CTX-M-3 (WT)	1	8
EPI300	CTX-M-3 (P167S)	16	1
EPI300	CTX-M-3 (N136K)	8	1

Conclusions: This study demonstrates the convergent *in vitro* selection of CAZ resistance mutations at position 167 of the CTX-M-3 which parallels its evolution *in vivo*. It also underscores the role mutator strains in the evolution of CTX-M beta-lactamases.

P1239

Mechanisms of high-level ciprofloxacin resistance in clinical isolates of *Escherichia coli* collected in the British Isles

S. Mushtaq, M.J. Ellington, D.M. Livermore, N. Woodford (London, UK)

Objectives: To investigate mechanisms and clonality in highly-ciprofloxacin-resistant clinical isolates of *E. coli* collected as part of a survey in 2001 – when this resistance was starting to expand rapidly.

Methods: Twenty-five highly ciprofloxacin-resistant (MICs 32–256 mg/L) isolates from 16 different centres were investigated. MICs were determined by the BSAC agar dilution method. Isolates were examined for relatedness by PFGE of XbaI digested DNA. The Quinolone Resistance Determining Regions (QRDR) of *gyrA*, *gyrB*, *parC* and *parE*, and the *marOR* regions of the *mar* operon were amplified by PCR and sequenced directly or after cloning into pCR2.1. Isolates were screened for *qnrA* by PCR. A fosfomycin/rifampicin disc method was used to screen for hypermutator phenotypes.

Results: PFGE banding patterns were obtained for 21/25 isolates; 3 pairs had similarities, all others appeared distinct. All 25 isolates had double mutations in *gyrA* resulting in Ser83

Abstracts

to Leu and substitution of Asp87 by Asn (n = 22), Tyr (n = 2), or Gly (n = 1); 24 also had known mutations at Ser80 in parC leading to substitution with Ile (n = 20) or Arg (n = 4). Eleven replaced Glu84 of ParC with Gly (n = 6), Val (n = 4) or Lys (n = 1) and 1 had Ala81 to Pro. A new ParC substitution was found -Gly78 to Cys (n = 2)- as was a mutation at novel site: Ser57 to Thr (n = 3). Fourteen of 25 gyrB QRDRs were sequenced, 13 had wild-type sequences, one had a novel Lys455 to Ile substitution. Seven of 25 had substitutions in ParE, 6 with Ser458 replaced by Thr (n = 3), Ala (n = 2) or Pro (n = 1) and one at a new site, changing Ile464 to Met. Twenty had Gly103 to Ser and Tyr137 to His substitutions in MarR, 4 also had a Lys62 to Arg substitution. No nucleotide mutations were found in the MarR binding sites or in the marbox of marO, but A1333C mutations (n = 19) were found in the accessory MarA binding site, and a T1328C mutation (n = 3) in the soxbox, of marO. All isolates were negative for qnrA; 5 had a hypermutator phenotype.

Conclusions: These ciprofloxacin-resistant *E. coli* were clonally diverse and their resistance was due to variable combinations of mutations, some of them novel. We conclude that high-level resistance emerged repeatedly and independently, and that hypermutability may be a facilitating factor in some isolates.

P1240

Evaluation of the accumulation of ^{99m}Tc-ciprofloxacin in *S. aureus* and *P. aeruginosa*

J.M. Sierra, D. Rodríguez-Puig, A. Soriano, J. Mensa, C. Piera, J. Vila (Barcelona, ES)

Objective: Gammagraphy with ^{99m}Tc-ciprofloxacin has been proposed as a useful test to diagnose infections. The objective of this study was to evaluate the intracellular accumulation of ciprofloxacin labelled with ^{99m}Tc in *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Methods: The accumulation of free ciprofloxacin, ^{99m}Tc-ciprofloxacin, and ^{99m}TcO₄⁻ was evaluated in two strains of *S. aureus*, 1199B and 1199 (with and without overexpression of an efflux system) and two strains of *P. aeruginosa*, PAOLC1-6 and KG2239 (with and without overexpression of an efflux system). Accumulation was measured at two different end-points, 5 and 30 minutes. Strains were cultured in LB overnight at 37°C. Cells were washed and resuspended in phosphate buffer to an OD₆₀₀ nm of 1.5. After that 490 ml were taken and 10 ml of ciprofloxacin were added to obtain an extracellular concentration of 10 mg/l (^{99m}Tc-ciprofloxacin presented an activity of about 555KBq/sample and free ^{99m}TcO₄⁻ presented an activity of about 555KBq/sample) and were incubated at 37°C during 5 or 30 min. Finally, samples were washed 3 times with PBS. Accumulation of ciprofloxacin (unlabelled) was measured by fluorimetry (previously, samples were lysed with glycine-HCl buffer) and accumulation of ^{99m}Tc-ciprofloxacin, and ^{99m}TcO₄⁻ was measured by a gamma counter.

Results: The accumulation of unlabeled ciprofloxacin in *S. aureus* and *P. aeruginosa* showing overexpression of an efflux pump was lower than for those without an efflux pump overexpressed. The accumulation of ^{99m}Tc-ciprofloxacin was not affected by the presence of an efflux system being the same in both pairs of strains at each end-points studied. Finally, ^{99m}TcO₄⁻ did not show accumulation in any of the strains.

Conclusion: The accumulation of ^{99m}Tc-ciprofloxacin was not affected by the overexpression of efflux systems in either *S. aureus* or *P. aeruginosa*. All the radioactivity detected in the cells was due to the accumulation of ^{99m}Tc-ciprofloxacin. Finally, ^{99m}Tc-ciprofloxacin may be useful as a radiopharmaceutical to

detect infection, regardless of the presence of an efflux system affecting quinolones.

P1242

Occurrence of MexCD-OprJ overexpressing efflux mutants of *Pseudomonas aeruginosa* isolated from hospitalised patients

K. Jeannot, D. Hocquet, P. Plésiat (Besançon, FR)

Objectives: Overexpression of the multidrug efflux system MexCD-OprJ in *P. aeruginosa* provides moderate-level resistance to fluoroquinolones (FQ) and "fourth generation" cepheems such as cefepime and ceftipime in mutants defective in nfxB, a gene that negatively controls the mexCD-oprJ operon. Since the first description of this resistance mechanism more than 10 years ago in *in vitro* mutants, clinical strains overexpressing MexCD-OprJ have only been reported in cystic fibrosis (CF) patients (Jalal S. Antimicrob. Agents Chemother. 2000, 44: 710-11). This study investigates the expression of the efflux system in strains exhibiting the nfxB susceptibility profile and recovered from non CF patients.

Methods: Forty four ciprofloxacin, cefepime resistant nfxB-like isolates of *P. aeruginosa* were isolated at the Teaching Hospital of Besançon between 2000 and 2005 (France). MICs of selected antibiotics were determined with the conventional agar dilution method (NCCLS). Expression levels of mexC and genes of other efflux operons (mexB, mexE, mexG, mexJ, mexY) were assessed by quantitative Real Time PCR after retro-transcription. Search for nfxB mutations was performed after DNA sequencing by alignment analysis.

Results: Only 4 strains (9%) were identified as MexCD-OprJ overexpressing mutants. These clonally unrelated isolates which exhibited baseline expression levels of the other known efflux systems (MexAB-OprM, MexEF-OprN, MexGH-OmpD, MexJK, MexXY) were resistant to ciprofloxacin (4 µg/mL) and cefepime (4-8 µg/mL), but hypersusceptible to most of the other β-lactams (e.g., aztreonam 0.5 µg/mL; imipenem 0.12 µg/mL), and to all the aminoglycosides tested (e.g., amikacin 0.5-1 µg/mL). The four strains harboured different deletions (from 2 to 17-pb) in the nfxB repressor gene.

Conclusion: Our results support the notion that MexCD-OprJ overproducing mutants are rather infrequent among non CF clinical strains of *P. aeruginosa*. Their high susceptibility to widely used antipseudomonal agents as well as their reduced virulence might explain such a low occurrence in the hospital setting. In agreement with this, the 4 nfxB mutants identified in this study were involved in patient colonisations.

P1243

Eight non-antimicrobial medicaments induce multidrug-resistance in *Escherichia coli* Ag100 strain

M.M. Tavío, V. Aquili, M. Perilli, L. Macià, C. Balagué, Z. González-Lama, G. Amicosante (Las Palmas de Gran Canaria, ES; L'Aquila, IT; Rosario, AR)

Objectives: Multidrug resistance induced in *Escherichia coli* by diazepam (a drug used in surgery) was described as equivalent to Mar phenotype resulting from marAB operon activation by salicylate (characterized by OmpF loss and enhanced active efflux). This work studied whether another eight non-antimicrobial medicaments used in surgery could also induce Mar phenotype in the susceptible *E. coli* Ag100 strain.

Methods: The effect of concentrations 1/512, 1/256, 1/128, 1/64, 1/32, 1/16, 1/8, 1/4 MICs of Chlorpromazine, Clonazepam, Dexamethosone, Diclofenac, Haloperidol, Ketorolac, Methimazol and Propacetamol in Ag100 was evaluated on antibiotic MICs, OmpF expression and norfloxacin intracellular accumulation. Results were compared with those of induction by salicylate. OmpF expression was analysed in SDS-PAGE. Active efflux was evidenced by carbonyl cyanide m-chlorophenylhydrazide (CCCP). Non-induced Ag100 and Ag112 (a marR mutant) were control strains. The inducing concentrations of medicaments were compared with their own levels in plasma in adult humans.

Results: Only those concentrations higher than or equal to 1/132 MICs of Clonazepam, Chlorpromazine, Haloperidol, Propacetamol, Ketorolac and Diclofenac, and concentrations higher than or equal to 1/128 MICs of Dexamethosone and Methimazol in the Ag100 strain, induced OmpF loss, increased 2–32 fold cefoxitin, nalidixic acid, norfloxacin, tetracycline and chloramphenicol MICs and decreased norfloxacin uptake in the Ag100 strain. 1/64–1/4 MIC of salicylate in the Ag100 strain led to equivalent results. Norfloxacin accumulation in Ag100 induced by salicylate or any of the eight medicaments was increased 2–4 fold by adding CCCP.

Conclusion: i) The tested medicaments like salicylate induced Mar phenotype only with concentration ranges between 1/128–1/4 of their MICs in the Ag100 strain. ii) Inducing concentrations of Mar phenotype of Clonazepam, Dexamethosone, Diclofenac, Haloperidol, Methimazol and Propacetamol might be found in plasma of patients in the course of treatments with them.

P1244

The role of the multi-drug efflux systems of *Pseudomonas aeruginosa* in tea tree oil tolerance

C.J. Papadopoulos, C.F. Carson, B.J. Chang, T.V. Riley (Perth, AU)

Objectives: *Melaleuca alternifolia* (tea tree) oil is an established topical antimicrobial agent with a broad spectrum of activity. *Pseudomonas aeruginosa* is substantially less susceptible than most bacterial species to the oil, with MICs ranging from 1 to 8%, compared with <0.5% for other Gram negative bacteria. The mechanisms by which *P. aeruginosa* attains resistance often involve efflux systems spanning the cytoplasmic membrane, periplasm and outer membrane. One pump commonly involved is MexAB-OprM, which has a diverse substrate profile including antibiotics and disinfectants. Previous work in our laboratory has indicated that an energy-dependant process, such as efflux, is involved in tolerance to tea tree oil in *P. aeruginosa*. The aim of this work was to establish the relationship between multi-drug efflux pumps and tea tree oil tolerance in *P. aeruginosa*.

Methods: The susceptibility of several types of efflux pump mutants of *P. aeruginosa* to tea tree oil and terpinen-4-ol, its major active component, was examined using time-kill assays and by determining MICs and MBCs. Complementation of mutants deficient in MexAB-OprM was achieved by transforming with pRSP17 (pRK415:mexAB-oprM).

Results: The tea tree oil MICs of MexAB-OprM deletion mutants were from 4 to 8 greater than times lower than their respective parental strains. MICs for the tea tree oil components terpinen-4-ol, cineole and alpha-terpineol were also decreased in these mutants. Mutants deficient in other pumps such as MexCD-OprJ, MexJK and MexXY did not show any increase in susceptibility. Time-kill assays demonstrated substantially increased killing rates in some efflux deficient mutants compared to isogenic parental strains. Sub-MIC concentrations of

the oil killed 90% of MexAB-OprM mutant cells after 30 minutes, compared with negligible death in the parent cells. Complementation studies showed that addition of the MexAB-OprM operon was able to successfully restore the tea tree oil and terpinen-4-ol susceptibility to that of parental strains.

Conclusion: The use of efflux mutants in susceptibility, time-kill and complementation assays indicates that the MexAB-OprM efflux operon is involved in the reduced susceptibility of *P. aeruginosa* to tea tree oil and some components.

P1245

Comparative analysis of *Salmonella typhimurium* proteomes from MAR mutants and following treatment with salicylate

N.G. Coldham, L.P. Randall, L.J.V. Piddock, M.J. Woodward (Surrey, UK; Birmingham, UK)

Objectives: The chromosomal multiple antibiotic resistance (mar) locus of *Salmonella enterica* and *Escherichia coli*, in cooperation with other stress response regulatory loci, play a key role in multiple antibiotic resistance (MAR). Mutations in these may give rise to MAR and are biologically important as they enable survival following exposure to sub-optimal concentrations of antibiotic. Microarray analyses of mutants that over express MarA have indicated differential expression of proteins which modulate molecular flux. The objective of the present study was to determine the role of the mar locus in MAR by comparison of proteomes following treatment with salicylate (a mar locus inducer) and from MAR mutants.

Methods: *S. typhimurium* (SL1344) was exposed to salicylate (5 mM) for 90 mins. Isogenic MAR mutants (n = 4) were selected after exposure to tetracycline (5 mg/ml). These mutants were also cyclohexane tolerant. All strains were grown to late logarithmic phase in LB - glucose media, cell envelope proteomes were extracted and digested with trypsin. The tryptic peptides were analysed by 2-dimensional HPLC-mass spectrometry.

Results: Relative to controls (100%), the expression of the efflux pump proteins AcrA, AcrB and TolC were increased in MAR mutants by 197% + 51, 182% + 30 & 216% + 31 (P < 0.01) respectively and following treatment with salicylate by 127% + 2.4, 139% + 12.4 & 199% + 12.3 (P < 0.05). Similarly, the outer membrane protein OmpF was significantly reduced (P < 0.01) in MAR mutants and with salicylate by 83.3% + 9.5 and 76.2% + 20 respectively compared to the parent. In contrast, OmpX was increased with salicylate by 2037% + 697 but reduced in MAR mutants 88.5% + 9.2. The putative omp COG3203 (16764875) was only expressed after treatment with salicylate.

Conclusion: Novel proteomic data was obtained that supports and extends published microarray data and reveals that other stress response loci, in addition to mar, also contribute to MAR.

P1246

Emergence of *Enterobacter aerogenes* strains resistant to imipenem mediated by the coexistence of metallo-beta-lactamase production and outer membrane permeability

M. Biendo, G. Laurans, B. Canarelli, D. Thomas, F. Hamdad Daoudi, F. Rousseau, C. Adjide, F. Eb (Amiens, FR)

Objective: We documented the emergence of imipenem resistant (IPM-R) strains after prolonged antibiotic therapy.

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Methods: 22 cases of *E. aerogenes* colonization or infection were monitored retrospectively (July 2003–May 2005). 62 strains were found in 22 patients consisted of 12 men (54.5%) and 10 women (45.5%) with a mean age of 67.1%. The antibiotic susceptibilities, envelope permeabilities, and molecular typing of all the clinical strains were studied.

Results: Of 62 strains, 22 (35.5%) were imipenem-susceptible (IPM-S) and 40 (64.5%) IPM-R. The following Beta-lactam resistance phenotype [FOX (R) CTX (R + I) FEP (R + I) CPO (R + I) ATM (R + I) IPM (R + I) CAZ (R)] 40/62 (64.5%) was found among the 40 IPM-R strains: The MBL production screened by Etest imipenem + EDTA (IPE) among the 40 IPM-R strains showed that 24 were producers [MICs IP: 16–64 µg/mL versus MICs IPE 4–24 µg/mL] (ratio *IP/IPE > 8 µg/mL). The AmpC enzyme production was investigated in the 40 IPM-R strains in Mueller-Hinton (M.H) + 500 µg of cloxacillin containing Cefotaxime (CTX), Ceftazidime (CAZ), Moxalactam (MOX) and Aztreonam (ATM) disks. Two strains were AmpC producers, 10 were ESBLs producers and 28 were AmpC + ESBL producers (mean diameter inhibition between M.H + cloxacillin and MH >5 mm). SDS-PAGE analysis showed 2 major proteins: 42 and 39 kDa presumed to be OmpC and OmpF like respectively. 25 IPM-R strains (IPM-MICs of 8–32 µg/mL) produced both Omps with the level of 42-kDa thinner than IPM-S strains and *E. coli* HB101. 15 IPM-R strains (IPM-MICs > 32 µg/mL) produced only a 39-kDa Omp. Pulsed-Field Gel Electrophoresis using XbaI, revealed 6 pulsotypes (A–F). The prevalent epidemiological pulsotype A included 77.4% of strains genetically related; 28 (58.4%) of them were IPM-R and 20 (41.6%) IPM-S.

Conclusion: The emergence of multidrug-resistant *E. aerogenes* strains involving the coexistence of ESBLs; AmpC Beta-lactamase; alteration of Omps and MBL-production is very disquieting in our hospital.*IP = imipenem

P1247

Resistance to cefoxitin and susceptibility to amoxicillin-clavulanate in clinical isolates of *Escherichia coli* not hyperproducing AmpC and lacking the OmpF porin

F. Fernández Cuenca, G. Amblar, L. Martínez-Martínez, A. Pascual (Seville, ES)

Objectives: To evaluate the role of AmpC hyperproduction and porin loss in the resistance to cephamycins in clinical isolates of *Escherichia coli* (Ec).

Methods: 13 Ec isolated from urine samples of outpatients attended in 2000 were submitted to the Clinical Microbiology Department, Univ Hosp Virgen Macarena, Seville, Spain. Genotyping was performed by REP-PCR. MICs of cefoxitin (FOX), cefotetan (CTT), cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP), amoxicillin (AMX) and amoxicillin-clavulanate (AC) were determined by microdilution (CLSI guidelines). The MICs of FOX were also determined in combination with BRL42715 (BRL) or clavulanic acid (CV). The isoelectric point (pI) and the inhibition profile of β-lactamases (BLs) with CV or cloxacillin (CX) were determined by isoelectric focusing. Hydrolysis of FOX was determined by spectrophotometry and by the cloverleaf assay. Mutations in the promoter and attenuator region of ampC were identified by DNA sequencing. Analysis of outer membrane proteins (OMPs) was performed by using the 10%SDS-PAGE system with 6 M urea.

Results: Seven REP-PCR patterns were observed. Isolates were resistant to FOX (MIC range: 32–128 mg/L) but susceptible to AC (4–16 mg/L) and CTT (1–8 mg/L), suggesting an AmpC non-hyperproducing phenotype. Seven isolates were resistant to

AMX (512–1024 mg/L) and 6 were susceptible to AMX (8–16 mg/L). All isolates were also susceptible to CTX, (<0.5–1 mg/L), CAZ (0.5–2 mg/L) and FEP (<0.125–0.5 mg/L). The MICs of FOX were not affected or were reduced 1 dilution by BRL or CV. A band of pI d9 (inhibited by CX but not by CV) was observed in all Ec. A band of pI 5.4 (inhibited by CV but not by CX) was also observed in AMX resistant isolates. Hydrolysis of FOX was not detected. Three point mutation profiles not associated with the hyperproduction of AmpC were observed in the regulatory region of ampC: -76, -56, +22, +26, +27, +32, +70, +80 (5 isolates); -88, -82, -18, -1, +58, +80 (5 isolates); and -56, +80 (3 isolates). Six different OMP profiles were observed. All these profiles were characterised by the expression of OmpC and OmpA, and the absence of Omp F.

Conclusion: Resistance to FOX in the Ec isolates of this study is not associated with resistance to CTT and AC. Resistance to cefoxitin is related to the absence of porin OmpF but not with β-lactamase production, including AmpC hyperproduction.

P1248

Are target gene mutations the major mechanisms of fluoroquinolone-resistance in salmonellae?

Y. Jin, J.M. Ling (Hong Kong, HK)

Objectives: 1. To study mechanisms of fluoroquinolone-resistance (FqR) in *Salmonella* sp. 2. To elucidate the importance of different mechanisms in contributing to FqR in these strains.

Methods: Fifty-nine single patient isolates of *Salmonella* sp. (out of 280) with ciprofloxacin (CIP) MICs >0.03 mg/l obtained during 2002 in New Territories East cluster hospitals of Hong Kong were tested for their susceptibilities to five other fluoroquinolones (Fqs) by determining the MICs using an agar dilution method recommended by the Clinical Laboratory Standards Institute. The quinolone resistance-determining region of gyrA, gyrB, parC and parE in these strains were amplified and mutations detected by single-stranded conformational polymorphism analysis and DNA sequence determination. Seven of these strains and 5 FqR mutants of *S. Typhimurium* (with or without target gene mutations) obtained after selection by Fqs (unpublished data), and a standard *Salmonella enterica* serotype *Typhimurium* strain (ATCC 13311) were tested for CIP accumulation and efflux in the presence and absence of CCCP (carbonyl cyanide m-chlorophenyl-hydrazone) spectrofluorometrically at excitation and emission wavelengths of 279 and 447 nm, respectively, acrB gene expression by real time RT-PCR and analysis of outer membrane proteins by SDS-PAGE.

Results: Of the 59 isolates tested, 10% did not harbour any detectable target gene mutations. However, 68% harboured 1 mutation (61% in gyrA [Asp87Asn/Gly or Ser83Phe/Tyr] and 7% in parC [Thr57Ser]), 15% harboured 2 mutations (8% with 2 gyrA [Ser83Phe + Asp87Asn] and 7% with 1 gyrA and 1 parC mutations [Ser83Phe + Ser80Arg or Asp87Asn/Tyr/Gly + Thr57Ser]), and 5% with 2 gyrA and 1 parC mutations [Ser83Phe + Asp87Asn + Thr57Ser/Ser80Arg]. There was 1 isolate with 4 mutations (2 in gyrA [Ser83Phe + Asp87Gly], and 1 each in parC [Ser80Arg] and parE [Ser458Pro]). Although CCCP enhanced accumulation of CIP in the tested strains, the amount accumulated was less than that in the control strain. Production of OmpA was decreased and acrB gene was overexpressed, indicating decreased permeability and overexpression of AcrAB-TolC efflux system, respectively in these strains.

Conclusions: The effects of efflux pump and decreased membrane permeability were probably as important as target gene mutations in contributing towards decreased Fq susceptibility or FqR in salmonellae.

P1249

Emergence of plasmid-mediated Ambler class A beta-lactamase in association with the plasmid-mediated quinolone resistance QnrS determinant in *Enterobacter cloacae*L. Poirel, C. Leviandier, A. Soares, P. Nordmann (*Le Kremlin Bicêtre, FR*)

Objectives: The plasmid-mediated quinolone-resistance determinant QnrS had been identified first in a *Shigella flexneri* isolate in Japan, then in *Enterobacter cloacae* from Vietnam (personal data) and is now identified in several enterobacterial isolates in hospital Bicêtre, France. Analysis of the genetic background of the qnrS gene in these latter isolates identified different self-transferable plasmids. Several QnrS-positive plasmids from clonally-unrelated *E. cloacae* isolates brought ampicillin resistance to *Escherichia coli* recipient strains that was not related to any known beta-lactamase.

Methods: PCR were performed using specific primers for known beta-lactamase genes (those encoding derivatives of TEM, SHV, and CARB enzymes). Then, cloning was performed to identify any novel beta-lactamase gene from a QnrS-positive *E. cloacae* clinical isolate. Pulsed-field gel electrophoresis (PFGE) allowed comparison of the *E. cloacae* isolates.

Results: A novel beta-lactamase gene was identified from a single QnrS-positive *E. cloacae* isolate. This beta-lactamase named LAP-1 (pI value of 6.7) shared low amino acid sequence identity with other known enzymes, being 60% identical with the most closely related TEM-1 penicillinase. Once expressed in *E. coli*, LAP-1 conferred resistance to penicillins that was inhibited by clavulanic acid and tazobactam. Its hydrolytic activity spared expanded-spectrum cephalosporins and carbapenems. PCR screening with bla (LAP-1)-specific primers using DNA of the six other QnrS-positive *E. cloacae* isolates (corresponding to four different clones by PFGE) showed that five isolates possessed this novel plasmid-borne beta-lactamase gene on the qnrS-positive plasmid. These plasmids differed in size and structures. A retrospective analysis showed that the QnrS-positive *E. cloacae* isolate we had identified from a Vietnamese isolate expressed also LAP-1, whereas a QnrA-positive *E. cloacae*, a QnrS-positive *E. coli*, a QnrS-positive *Serratia marcescens* and ten different Qnr-negative *E. cloacae* isolates from our hospital were negative for bla (LAP-1).

Conclusion: This work identified a novel Ambler class A beta-lactamase which gene is plasmid-mediated. This penicillinase gene was identified associated with the QnrS determinant.

P1250

Detection of plasmid-mediated low-level resistance to fluoroquinolones in *Enterobacter cloacae*P. Higgins, H. Seifert, H. Wisplinghoff (*Cologne, DE*)

Background: Low-level resistance to fluoroquinolones encoded by the qnr gene and mediated by plasmid pMG252 has recently been observed in clinical *Enterobacteriaceae* isolates, mainly *E. coli* and *K. pneumoniae*.

Methods: To determine the prevalence of pMG252 carrying the qnr, 110 clinical isolates from hospitals and private practices in the Cologne Metropolitan area were screened using a PCR based assay.

Results: Among 58 isolates from hospitalized patients with urinary tract infection (UTI) and 52 isolates from patients with community-acquired UTI, pMG252 was detected in one *Ente-*

robacter cloacae isolate from a hospitalized patient. The isolate displayed an intermediate susceptibility to ciprofloxacin (MIC, 2 µg/mL) and was susceptible to levofloxacin. Presence of pMG252 and the qnr gene was confirmed by DNA sequencing.

Conclusion: This is one of the first reports of pMG252 in a clinical *E. cloacae* isolate. Even though the prevalence of plasmid mediated resistance to fluoroquinolones in clinical isolates is low, pMG252 carrying the qnr gene has now been reported in most clinically relevant *Enterobacteriaceae*. Further studies monitoring the potential emergence of pMG252 positive strains may be warranted.

P1251

First characterisation of fluoroquinolone resistance in *Streptococcus suis*J.A. Escudero, A. San Millan, A.M. Catalan, A.G. De la Campa, M.A. Moreno, L. Dominguez, B. Gonzalez-Zorn (*Madrid, ES*)

Objectives: *Streptococcus suis* is a worldwide distributed gram positive bacterium that causes meningitis, endocarditis, septicemia, septic arthritis, pneumonia, and abortion both in humans and pigs. During the summer of 2005, 215 people were infected by *S. suis* in China, of which 39 (18%) ended fatally. Second generation quinolones (fluoroquinolones), such as enrofloxacin or ciprofloxacin, are one of the main families of antimicrobial compounds used for the treatment of *S. suis* infections. The use of fluoroquinolones against *S. suis* is causing the emergence of quinolone-resistant strains, with unpredictable consequences for pig production and Public Health. Here we present the molecular basis of fluoroquinolone resistance in *S. suis*.

Methods: Identification of *S. suis* isolates was performed using a species specific PCR of the gld (glutamate dehydrogenase) gene. All isolates were subjected to PFGE to assess phylogenetic relatedness. MICs were determined following the guidelines of the NCCLS. gyrA and parC genes were amplified using primers designed on the basis of conserved regions of other *Streptococcus* species.

Results: We have first identified the gyrA and parC genes in six unrelated fluoroquinolone sensitive *S. suis* isolates, including type strain ATCC 43765, a clinical isolate from Chile and four Spanish isolates. The QRDR of these genes was undistinguishable. Further, we amplified and sequenced the QRDR of eleven high-level resistant *S. suis* strains from different geographical origins. The parC gene of these bacteria possessed a S79Y mutation, known to confer resistance to fluoroquinolones in other bacteria. Sequence comparison of the gyrA gene revealed the E85D mutation conferring fluoroquinolone resistance, as well as novel mutations at S81K not yet described in other bacterial species.

Conclusions: We have identified and characterised the gyrA and parC genes of *S. suis*. Sequence analysis revealed mutations in these genes responsible for fluoroquinolone resistance in *S. suis*.

P1252

Step-wise parC and gyrA mutations in *Streptococcus pneumoniae*: the role of the Lys137 to Asn mutation in fluoroquinolone resistanceV. Allen, K. Green, D.J. Bast, J. Azavedo, C. Duncan, D.E. Low (*Toronto, CA*)

Objectives: Recent US data comparing 1994–1995 to 2002–2003 has found the emergence of parC mutations in *Streptococcus pneumoniae* (SP) isolates concurrent with a doubling of fluoro-

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quinolone resistance (Doern et al). The most commonly identified mutation in this series was Lys-137-Asp. The aim of this study is to determine the association between ciprofloxacin (C) and levofloxacin (L) prescription patterns and the development of the Lys-137-Asp parC mutation as a potential contributor to the step-wise fluoroquinolone resistance mechanisms in SP.

Methods: A total of 17,978 clinical isolates of SP were collected between 1993–2002 as part of a surveillance program throughout Canada. Specimens were stratified by C MIC and by year according to the fluoroquinolone (FQ) available and most commonly used in Canada; prior to 1998 C was the sole fluoroquinolone available, whereas L was the respiratory fluoroquinolone most commonly used during 2000–2002. The parC and gyrA genes were amplified by polymerase chain reaction for random isolates from each MIC stratification during each of these periods. C and L prescription patterns in Canada over time were also collected.

Results: C prescription rates peaked in 1999 at 1,536,402/yr but has remained stable since then. In contrast L prescription continued to increase from 16, 627 outpatient prescriptions in 1998 to 315, 467 in 2001. The rate of the Lys-137-Asn mutation in SP isolates was found in all MIC groups at a combined rate of 23.6%. There was a linear increase in the frequency of the Lys-137-Asn mutation as a function of the MIC in the 2000–2002 cohort. There was no such linear trend seen in the 1993–98 group. An increased frequency of combination parC and gyrA mutations was identified with MICs >4 µg/ml. 52% of isolates with an MIC >4 µg/ml demonstrated mutations in both of these genes, compared to 94% of isolates with an MIC >16 µg/ml.

Conclusion: The Lys-137-Asn parC mutation may be a harbinger of future resistance in SP as a first of a series of step-wise mutations. Unlike the previous US series, the overall rates of this mutation in our series did not increase as a function of F prescription patterns, and evidence of a linear increase in Lys-137-Asn as a function of higher MICs was only seen after 2000. Further details need to be elucidated regarding the clinical significance of the Lys-137-Asn mutation and whether the frequency and role of this mutation is altered by antimicrobial selection pressures created by newer fluoroquinolones.

P1253

Molecular characterisation of antibiotic resistance mechanisms in *Salmonella enterica* isolates in Ireland

D. Lee, S. Fanning, A. Coffey (Cork, Dublin, IE)

The primary objective of this study was to determine the level and range of antibiotic resistance among a broad collection of *Salmonella enterica* isolates from both human and animal sources in Ireland. The emergence of antibiotic resistance in *Salmonella* was highlighted over a decade ago. Many cases of *Salmonella* infection now involve multi-drug resistant strains. Quinolones, including Fluoroquinolones, are broad-spectrum antibiotics and are often the treatment of choice in cases of life-threatening Salmonellosis caused by multi-drug resistant strains. Resistance to the quinolones is mainly attributed to alterations of the target sites, caused by specific mutations within the chromosomal genes encoding DNA gyrase (gyr A, B) and topoisomerase IV (par C, E). These mutations are generally located within an area known as the Quinolone Resistance Determining Region (QRDR). The antibiotic resistance profile was determined for 167 *Salmonella* isolates, against a panel of 17 antibiotics representing those regularly used for clinical treatment of *Salmonella*

infections. Isolates with reduced susceptibility to quinolones were selected for further study with the aim of determining the correlation between specific mutations and resistance. The QRDR's of gyr A, B and par C, E were amplified by the Polymerase Chain Reaction (PCR) and the products sequenced. The minimum inhibitory concentrations for Nalidixic acid (Nal), Ciprofloxacin (Cip), and Norfloxacin (Nx) was determined using the E-test. Within the gyrA gene, two different mutations were identified. 9 isolates harboured a nucleotide substitution at codon 87 (GAC-TAC), all of animal origin. One isolate had a nucleotide substitution at codon 83 (TCC-TTC), of human origin. Both mutations correlated with observed high level resistance of >256 µg/ml to Nal, a reduced susceptibility to Cip and Nx was also observed. Two isolates harboured a single mutation located within the QRDR of par C at codon 57 (ACC-AGC) which was linked to intermediate resistance to Nal. All mutations resulted in amino acid substitutions. While treatment failure with quinolones has been reported in Asia and the United States, to date resistance to the quinolones in Ireland is rare, however constant screening for quinolone resistance among *Salmonella* isolates is vital.

P1254

Multidrug-resistant tuberculosis in Lisbon in 2003

I. Portugal, R. Macedo, E. Fernandes, J. Perdigão, I. João, E. Pereira, L. Brum (Lisbon, PT)

Objectives: Portugal remains the country with the highest rate of notified cases due to *Mycobacterium tuberculosis* in the Western E.U. In 2003, the Portuguese Health Authorities reported a tuberculosis (TB) incidence of 41.1/100 000 population nation wide, and 1.7% of multidrug resistant cases of tuberculosis (MDR-TB). Although strains isolated in hospital and public health laboratories are currently notified to Portuguese authorities, the true magnitude of the problem is unknown. In fact, even though only 17 MDR-TB cases were notified in 2003, we have collected more than 100 MDR-TB isolates in the Lisbon Health region. Our objectives were to evaluate the prevalence and clustering of MDR-TB strains isolated in Lisbon laboratories and hospitals, in particular a previous described family of strains, family Lisboa, and to investigate the mutations in genes associated with drug resistance to first line drugs.

Methods: MDR-TB strains were collected in several hospital and public health laboratories in Lisbon area. We performed mycobacterial interspersed repetitive units typing (MIRU) to all the isolated strains. Mutations in genes associated to rifampicin, isoniazid, streptomycin and pyrazinamide resistance were analysed.

Results: MIRU analysis revealed that family Lisboa strains are still responsible for 36% of all the MDR-TB strains analysed in this study. Nevertheless we could not find any relevant outbreak due to these strains. In fact, we did not find more than three strains in one particular hospital or public health laboratory. These results were in agreement with mutation analysis of some of the genes involved in resistance.

Conclusion: In view of the above findings, we can conclude that family Lisboa strains are still spread at least in Lisbon area and probably all over the country and continues to be responsible for the main resistant tuberculosis, although no significant outbreak was detected.

P1255

Genotypic assessment of rifampin resistance in *Mycobacterium tuberculosis* isolated in Belarus

V. Slizen, S. Zaker, L. Surkova, A. Bahrmand, M. Taghikhani, L. Titov (Minsk, BY; Tehran, IR)

Objectives: The mechanism of resistance to rifampin involves missense mutations in 81-bp fragment of *rpo B* gene with the majority of mutations occurring at codons 531, 526, 516, 511. Profile of mutations in *rpo B* gene depends on geographical region. The aim of the study was to identify mutations associated with rifampin resistance in strains isolated in Belarus.

Methods: Susceptibility to RMP and INH in 44 clinical isolates of *Mycobacterium tuberculosis* was determined by internationally accepted reference technique, their *rpo B* gene (305 bp region) was amplified and autosequenced.

Results: All 44 tested strains displaying resistance to isoniazid (0.1 µg/mL) and rifampin (2 µg/mL) had point mutations in 1–4 separate codons with the prevalence of double mutations. 1, 2, 3 and 4 mutations carried 11 (25%), 22(50%), 8 (18%), 4 (7%) strains respectively. Most of the mutations (80%) were located in 510, 526, 523, 531 codons accounted for resistance in 50, 45.5, 40.9, 29.5% of isolates correspondingly. The *rpoB* codons 531, 526, 516, 511 were reported to be the most frequently mutated codons worldwide. In Belarus, in contrast to other studies, most common codons affected by point mutations were 510 and 523. Point mutations in codons 508, 507, 512, 516, 520, 522, 521, 525 occurred in 2.3–9.1% of isolates. Most of the detected mutations led to alterations of coding aminoacids and only 7 mutations were silent. Mutations occurred in codon 510 resulted in replacement of Gln to Glu (59%) or Lys (13.6%) or generated stop codon. Mutations in codon 526 led to substitution His > Asp (85%) or His > Leu, in codon 523 there were revealed substitution Gly > Ala (78%) or silent mutations, in codon 531 – Ser > Leu (one strain displayed Ser > Lys).

Conclusion: It was revealed geographical variation in *rpo B* gene mutations profile in *Mycobacterium tuberculosis* isolates from Belarus, characterised by high frequency of double, triple, quadruple mutations and mutations localised in 510 and 523 codons.

P1256

Metronidazole heteroresistant *C. difficile* strains: morphotypes with cell-wall thickening

J. Martínez-Alarcón, T. Peláez, J. García-Bordas, P. Sandoval, L. Alcalá, E. Cercenado, E. Bouza (Madrid, ES)

Objectives: We previously reported a high prevalence of metronidazole-resistant (MTZ-R) *Clostridium difficile* strains in our institution. Resistance to MTZ seemed to be heterogeneous and unstable. We observed that strains which were heteroresistant to metronidazole suffered morphological changes depending on the presence or absence of metronidazole. We characterized the different morphotypes with and without MTZ. **Methods:** We studied 18 MTZ-R isolates (MICs: 64–16 mg/L) and a control strain (*C. difficile* ATCC 9689). Brain Heart Infusion broth tubes (BHI) with 4 and 8 mg/L of MTZ were inoculated with 100 µl of a 0.5 McFarland suspension of each isolate and incubated anaerobically at 37°C for 10 days. The cultures were later seeded onto Brucella agar without MTZ and examined. Plates were further incubated under the same conditions and observed for 10 days. Bacterial morphology was examined macroscopically (colonies) and by optical and electronic microscopy. Susceptibility testing of the different morphotypes was performed against vancomycin and teicoplanin using the E-test method.

Results: Subcultures from BHI with MTZ produced colonies with an atypical white appearance and round shape. These colonies seeded onto the *Brucella* without MTZ reverted progressively to characteristic *C. difficile* morphotypes (pleomorphic, yellow-green, ground-glass appearance). Macroscopic, optical and electronic microscopy demonstrated a heterogeneous morphology, consisting of a mixed population of small (cocci), medium (cocobacilli) and large (bacilli) colonies. The cocci and cocobacilli morphotypes showed significant cell-wall thickening that correlated with an increase in vancomycin and teicoplanin MICs. The control strain did not grow in the presence of MTZ.

Conclusions: We observed a mixed population of different morphotypes in heteroresistant *C. difficile* strains in the presence of MTZ. Two of the morphotypes presented cell-wall thickening that was associated with an increase in vancomycin and teicoplanin MICs.

P1257

Incidence and antimicrobial resistance pattern of anaerobic bacteria isolated from clinical cases

P. Giannopoulou, E. Kontekaki, A. Kyratsa, Z. Kyriazi, E. Trikka-Graphakos (Athens, GR)

Objectives: To evaluate the frequency of anaerobic bacteria from clinical specimens and their *in-vitro* activity to antimicrobials.

Methods: 95 isolates were collected from documented infections during a three year period (2002–2005). Processing of specimens was performed with conventional methods. Anaerobic culture media were as follow: Bactec bottles (Becton Dickinson), Brucella Blood agar enriched with vitamin K1&hemin, Neomycin-Vancomycin Laked Blood agar, Bacteroides Bile-Esculin agar and Cooked Meat Broth. Preliminary, bacteria were identified by Gram stain, colony morphology and susceptibility to high-potency disks (colistin, kanamycin, vancomycin, SPS). Species identification was performed by rapid ID 32A (Biomerieux) and B-Lactamase production by nitrocephin discs (Becton Dickinson). Antibiotic susceptibility was performed by Etest method (ABBioDisk, Sweden) in National Reference Center.

Results: From 95 isolates 66 (69.4%) were Gram negative (52 *Bacteroides fragilis* group, 6 *Prevotella* spp., 3 non-fragilis *Bacteroides* spp., 2 *Fusobacterium* spp., 2 *Veillonella* spp and 1 *Porphyromonas* spp.) and 29 (30.5%) Gram positive (10 *Clostridium* spp, 8 *Peptostreptococcus* spp, 8 *Propionibacterium* spp and 3 *Eubacterium* spp). Forty one strains were isolated from wounds and abscesses, 22 from blood cultures, 19 intra-abdominal infections, 8 pelvic infections and 5 diabetic foot ulcers. The most common isolate from blood cultures was *Bacteroides fragilis* group 13.9%, followed by *Clostridium* spp 12.6%. *Bacteroides fragilis* group was also the most frequent isolate from all the other specimens. Antimicrobial susceptibility testing was performed to penicillin, cefoxitin, clindamycin, metronidazole, piperacillin/tazobactam, and imipenem. *Bacteroides fragilis* group was resistant to penicillin 96.1% and clindamycin 25%, *Prevotella* spp to penicillin 83.3% and clindamycin 16.6%, *Clostridium* spp 13.2% and *Peptostreptococcus* spp 12.4% to clindamycin. Non-susceptible strains of gram-negative to cefoxitin was 25%. 3 isolates were resistant to metronidazole. Piperacillin/tazobactam and imipenem were highly effective to all isolates.

Conclusions: (a) *Bacteroides fragilis* group, the most common anaerobe, was highly resistant to penicillin followed by clindamycin (b) *Clostridium* and *Peptostreptococcus* spp showed low resistance to clindamycin (c) the evidence that metronidazole resistance may be emerging as well as high rates of resistance to cefoxitin and clindamycin, require closer surveillance.

Antimicrobial susceptibility testing

P1258

Analysis of the mechanism of *Acinetobacter baumannii* resistance to carbapenems in Jinan

D. Wu, Z. Geng, X. Yu, J. Hong (Jinan, Shandong, CN)

Objectives: *Acinetobacter* are important opportunistic pathogens causing a wide range of clinical complications. There is a growing on carbapenem-resistance *Acinetobacter* with the extended usage of carbapenems, and antimicrobial treatment of these clinical infections may be compromised. This study was conducted to analyse carbapenemase and out membrane proteins of *Acinetobacter baumannii* isolates to investigate the mechanism of *Acinetobacter* resistance to carbapenems in Jinan, China.

Methods: Extracted chromosome DNA of 5 *Acinetobacter baumannii* isolates resistance to carbapenems determined by E-test was subjected to polymerase chain reaction (PCR) with the specific primers of blaOXA-23 blaOXA-24 blaIMP and blaVIM, the PCR products were ligated with PMD18-T, transformed into DH5' and then sequenced. Out membrane proteins were prepared by supersound collected by ultra-centrifugation and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis SDS-PAGE© of which the concentrations of separation gel and stacking gel were 15% and 5.1% respectively.

Results: PCR with the presence of blaOXA-23-specific primers amplified a circa 1083-bp fragment with three isolates (11, 66 and 67), their DNA sequences were identical with blaOXA-23. One isolate (40) obtained a circa 1043 bp PCR product with the presence of blaOXA-24-specific primers, the DNA sequence was identical with blaOXA-72. A protein of 29 kDa decreased in one carbapenem-resistance isolate (66) and a protein of 24 kDa overexpressed in two carbapenem-resistance isolates (66 and 67).

Conclusion: Producing class D b-lactamase OXA C23 and OXA C72 was one of the mechanism of *Acinetobacter baumannii* resistance to carbapenems in Jinan. The decrease of a 29 kDa out membrane protein and the overexpression of the 24 kDa penicillin-binding protein were related to the resistance to carbapenems in *Acinetobacter baumannii*.

P1259

Grouping of CTX-M extended-spectrum beta-lactamases by pyrosequencingTM

S.D. Nyberg, K. Rantakokko-Jalava, P. Huovinen, J. Jalava (Turku, FI)

Introduction: During the last years the extended-spectrum beta-lactamases, especially the CTX-M producing strains, have increased in several countries in Europe (1). The CTX-M was first described in the early 1990s and since then over 40 different types has been found (<http://www.lahey.org/studies/webt.htm>). Enzymes of type CTX-M are typical ESBLs (2) and showing 40% identity with TEM and SHV-beta-lactamases. A rapid detection of the CTX-M enzymes enables faster identification of ESBL producers.

Methods: A large amount of CTX-M enzymes (K. Bush and G.A. Jacoby, 2005, <http://www.lahey.org/studies/webt.htm>) representing the four main groups; CTX-M-1, CTX-M-2, CTX-M-8 and CTX-M-9 were aligned and a specific part in the gene sequence was chosen in order to be able to separate the main groups from each other. A specific biotinylated reverse primer was designed for PCR. A specific sequencing primer was designed for the pyrosequencing reaction to be able to

distinguish the different groups of CTX-M. A total amount of 81 *E. coli* and *Klebsiella* spp. strains collected at Turku University Hospital were analysed. All strains were screened for ESBL according to CLSI standard with cefotaxime and ceftazidime alone and with clavulanic acid. All strains were sequenced by pyrosequencing according to the instructions by the manufacturer (Biotage Ab, Uppsala, Sweden).

Results: All strains had classical ESBL phenotypes according to CLSI breakpoints. Of all strains 50% belonged to the CTX-M-1 and 50% to the CTX-M-9 groups.

Conclusion: With pyrosequencing it is possible to separate the CTX-M subgroups with a short DNA sequence. The grouping of CTX-M by pyrosequencing TM is a simple and rapid method. It is also possible to recognize K1/OXY producing strains.

References

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P1260

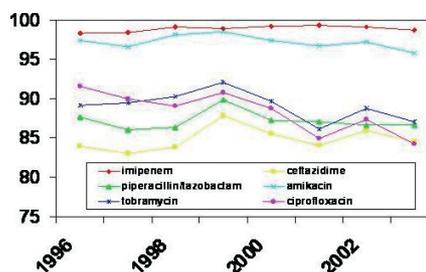
Phenotypic antimicrobial resistance patterns of *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. Multicentre Intensive Care Unit Surveillance Survey (ISS), 1996–2003

M.A. Abramson, G. Gallagher, S. Rawlins, H. Wilson (West Point, US)

Objectives: ISS is an ongoing surveillance study of antimicrobial susceptibility of nosocomial Gram-negative bacilli. A primary objective of ISS is to assess changes in resistance rates of these organisms over time.

Methods: 36 110 initial isolates of *Enterobacteriaceae*, 12 471 of *Pseudomonas aeruginosa*, and 3441 of *Acinetobacter* spp. from ICU patients were tested at 60–99 centres in the United States between 1996 and 2003. Cumulative susceptibility results for imipenem, ceftazidime, piperacillin/tazobactam, amikacin, tobramycin, and ciprofloxacin were recorded.

Results: Results for *Enterobacteriaceae* are graphed below. Susceptibility trends of *P. aeruginosa* and *Acinetobacter* spp. were similar to the *Enterobacteriaceae*, with decreases noted for all agents, and more pronounced for *Acinetobacter* spp. to ciprofloxacin (57.4–33.1%), ceftazidime (62.6–37.3%), and piperacillin/tazobactam (64.1–44.8%); and *P. aeruginosa* to ciprofloxacin (79.6–64.7%), imipenem (85.8–76.9%), and ceftazidime (82.2–81.2%).



Conclusion: In all years, imipenem and amikacin were consistently the most active agents against all isolates tested (maintaining greater than 85% susceptibility) with the exception of *P. aeruginosa*. Overall, susceptibility of all isolates to all agents trended downward over the 8-year time frame.

P1261

Antimicrobial activity of daptomycin tested against Gram-positive strains collected from European medical centres in 2005: results of the Daptomycin Surveillance Programme

H. Sader, J. Streit, T. Fritsche, R. Jones (North Liberty, US)

Objective: To evaluate the *in vitro* activity of daptomycin (DAP) tested against recent clinical isolates collected in Europe in 2005. DAP is a cyclic lipopeptide with activity against Gram-positive cocci (GPC) that displays no cross-resistance to other agents, making it ideal for treatment of multi-drug resistant (MDR) strains.

Methods: A total of 3,192 consecutive strains were collected in 21 medical centres located in 13 European countries. The main pathogens evaluated were: *S. aureus* [SA; 31% oxacillin (OXA)-resistant (R)]; coagulase-negative *Staphylococci* (CoNS; 76% OXA-R), *E. faecalis* [EF; 1% vancomycin (VAN)-R], *E. faecium* (EFM; 21% VAN-R), beta-haemolytic *Streptococcus* spp. (BHS; 228), and viridans group *Streptococcus* spp. (VGS; 102). The strains were susceptibility (S) tested by broth microdilution methods in Mueller-Hinton broth supplemented to 50 mg/L of calcium. Numerous comparators were also tested.

Results: DAP activity is summarized in the table: All *Enterococci* and *Staphylococci* were inhibited at DAP S breakpoint (BKP) established by the Clinical and Laboratory Standards Institute (formerly NCCLS); ≤ 4 and ≤ 1 mg/L, respectively). DAP and linezolid were the only compounds active against all *Enterococci* at the S BKP, and R to VAN did not adversely affect DAP activity. DAP was highly active against SA and CoNS (MIC₉₀, 0.5 mg/L) and independent of their S to OXA. BHS was highly S to DAP (MIC₉₀, 0.25 mg/L) and most comparison agents tested. DAP was also active against all VGS strains at the S breakpoint.

Organism (no. tested)	DAP MIC (mg/L)			%S
	50%	90%	Range	
SA OXA-S (1,232)	0.25	0.5	≤ 0.06 -1	100.0
OXA-R (554)	0.25	0.5	≤ 0.06 -1	100.0
CoNS OXA-S (114)	0.25	0.5	≤ 0.06 -1	100.0
OXA-R (362)	0.25	0.5	≤ 0.06 -1	100.0
EF (388)	1	1	≤ 0.06 -2	100.0
EFM VAN-S (155)	2	4	0.5-4	100.0
VAN R (11)	2	1	0.5-1	100.0
Other <i>Enterococcus</i> (18)	1	4	0.25-4	100.0
BHS (228)	≤ 0.06	0.25	≤ 0.06 -1	100.0
VGS (102)	0.25	1	≤ 0.06 -1	100.0

Conclusions: All GPC tested were S to DAP. R to other compounds did not influence the high DAP activity against *Staphylococci*, *Enterococci* or *Streptococci*. DAP showed significant potency and broad spectrum activity against recent clinical isolates of GPC isolated in European medical centres, including MDR subsets.

P1262

Antimicrobial activity of cefepime tested against ceftazidime-resistant Gram-negative clinical strains from North American hospitals: report from the SENTRY Antimicrobial Surveillance Program, 1998–2004

H. Sader, T. Fritsche, R. Jones (North Liberty, US)

Objectives: To evaluate antimicrobial spectrum and potency of cefepime (CPM) and selected comparators against ceftazidime

(CAZ)-resistant (R; MIC ≥ 16 mg/L) Gram-negative bacilli (GNB) collected in North America (NA) medical centres over a 7-year period (1998–2004).

Methods: Isolates were consecutively collected mainly from bloodstream (47%), respiratory tract (33%), urinary tract (9%) and skin/soft tissue (5%) infections in 48 major hospitals. Isolates were susceptibility (S) tested by reference CLSI broth microdilution methods in a central laboratory.

Results: A total of 42,061 GNB were collected during the study period. The most frequently isolated pathogens were *E. coli* (28.0%) > *P. aeruginosa* (PSA; 14.9%) > *Klebsiella* spp. (KSP; 14.0%) > *H. influenzae* (11.2%) > *Enterobacter* spp. (ESP; 7.8%) > *Serratia* spp. (4.0%). CAZ-R was observed in 8.5% of GNB and 5.6% of *Enterobacteriaceae* (ENT). The highest rates of CAZ-R were observed among *Acinetobacter* spp. (ASP; 40.3%) > ESP (20.9%) > PSA (16.9%) > *Citrobacter* spp. (CIT; 15.3%) > indol-pos. Proteae (10.0%). The activity of CPM against the most frequent CAZ-R organisms is summarized in the Table. Overall, 90% of CAZ-R ENT and 30% of CAZ-R PSA remained S to CPM. The activities (%S) of other antimicrobials tested against CAZ-R ENT and PSA were: amikacin 90 and 88%, ciprofloxacin 63 and 46%, ertapenem 93 and 6%, gentamicin 59 and 67%, imipenem 99 and 65%, levofloxacin 69 and 44% and piperacillin/tazobactam only 40 and 41%.

Organism (no. tested)	MIC ₉₀ % S			
	Cefepime	Ciprofloxacin	Gentamicin	Imipenem
<i>E. coli</i> (9,586)	$\leq 0.12/98.4$	>4/86.7	2/94.2	$\leq 0.5/99.9$
<i>Klebsiella</i> spp. (3,099)	8/91.9	1/90.0	>8/85.0	$\leq 0.5/99.7$
PSA (2,642)	>16/75.4	>4/70.8	>8/71.2	>8/79.6
<i>Enterobacter</i> spp. (1,805)	4/96.2	>4/82.0	8/88.9	1/99.3
<i>Acinetobacter</i> spp. (ASP; 1,093)	>16/48.2	>4/41.1	>8/42.1	>8/74.1

Conclusions: CAZ-R GNB exhibited high rates of R to other antimicrobials. CPM was very active against CAZ-R ENT, especially ($\geq 90\%$ S) ESP, CIT and indol-pos. Proteae, and showed activity similar to that of CAZ against all PSA and ASP isolated in NA medical centres. Continued R surveillance monitoring will be necessary to assess the effectiveness of widely used broad-spectrum antimicrobials.

P1263

Frequency of occurrence and antimicrobial susceptibility of bacterial isolates causing bloodstream infections in European medical centres: report from 9 years of the SENTRY Antimicrobial Surveillance Program in Europe (1997–2005)

H. Sader, D. Biedenbach, T. Fritsche, R. Jones (North Liberty, US)

Background: To evaluate the frequency of occurrence and antimicrobial susceptibility (S) of pathogens causing bloodstream infection (BSI) in Europe.

Methods: The first 20 unique and clinical relevant BSI isolates from 42 medical centres (10–31 years) were sent to a monitor each month. The isolates were tested for S (>30 antimicrobials) by broth microdilution methods and results interpreted according to the 2006 CLSI M100-S16 document. Selected resistance (R) mechanisms were characterized.

Results: 42,411 isolates 51.6% [Gram-negative bacilli (GNB)] were processed. The rank order for all years was (no. of isolates / % of total): *E. coli* (9,586/22.7%) > *S. aureus* (8,254/19.7%) > coagulase-negative *Staphylococci* (5,638/13.3%) > *Enterococcus* spp. (3,203/7.6%) > *Klebsiella* spp. (3,099/7.3%) > *P. aeruginosa* (PSA; 2,642/6.2%). This rank order and the frequencies were very stable through the years. The antimicrobial S rates of the key GNB are summarized in the table. ESBL phenotypes were observed in 4.8% of *E. coli*, 24.2% of *K. pneumoniae* and 9.1% of *P.*

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Organism (no. tested)	MIC90/% S			
	Cefepime	Ciprofloxacin	Gentamicin	Imipenem
<i>E. coli</i> (9,586)	<=0.12/98.4	>4/86.7	2/94.2	<=0.5/>99.9
<i>Klebsiella</i> spp. (3,099)	8/91.9	1/90.0	>8/85.0	<=0.5/99.7
PSA (2,642)	>16/75.4	>4/70.8	>8/71.2	>8/79.6
<i>Enterobacter</i> spp. (1,805)	4/96.2	>4/82.0	8/88.9	1/99.3
<i>Acinetobacter</i> spp. (ASP, 1,093)	>16/48.2	>4/41.1	>8/42.1	>8/74.1

mirabilis. Metallo-beta-lactamase (MBL) production was detected among *Enterobacteriaceae* (ENT) from Greece, Italy, Spain and Turkey. S rates for key antimicrobials against *S. aureus* were: 71.9% for oxacillin (OXA), 68.9% for ciprofloxacin, 47.7% for erythromycin, 81.3% for clindamycin, 98.1% for TMP/SMX, >99% for quinupristin/dalfopristin, teicoplanin, vancomycin (VAN), linezolid (LNZ) and daptomycin (DAP). VAN R was observed in 1.3% of *E. faecalis* and 10.7% of *E. faecium* with escalating rates in recent years, and no R to DAP or LNZ was detected among *Enterococci*. Among *S. pneumoniae*, S and R to penicillin were 74.5 and 12.4%, respectively, while 99.6% of strains were S to levofloxacin and gatifloxacin.

Conclusions: The main R problems detected among BSI strains collected in Europe by the SENTRY Program were: OXA R *S. aureus*, ESBL-producing ENT, multi-drug resistant PSA and ACB, and MBL-producing ENT and PSA.

P1264

Influence of serum-resistance properties and multidrug-resistance of *Pseudomonas aeruginosa* on mortality and duration of mechanical ventilation in patients with *Pseudomonas aeruginosa* pneumonia

A. Vitkauskienė, V. Dudzevičius, D. Adukauskienė, J. Braziulytė, R. Sabaniauskaite, R. Sakalauskas, H. Sahly (Kaunas, LT; Kiel, DE)

Aim: To evaluate the association between serum- and antibiotic-resistance of *P. aeruginosa* and the duration of mechanical ventilation (MV) and mortality of ICU patients with pseudo-monal pneumonia.

Materials and methods: Data of 149 ICU patients (mean age 54.8 ± 1.5 years) suffering from pneumonia caused by *P. aeruginosa* were analysed at the onset, after 5–7 and 21–30 days after the start of treatment. *P. aeruginosa* was isolated from patients' bronchoalveolar lavage fluid or bronchial secretions. The ability of the *P. aeruginosa* isolates to resist killing by human serum was tested and the strains were classified as serum-sensitive, intermediately resistant, or highly resistant to serum. Resistance to antibiotics was tested using the disc diffusion method (discs BBL) according to the NCCLS standards. Strains showing resistance to 3 or more antibiotics were considered as multi-drug-resistant (MDR). Initial treatment effectiveness, pneumonia type, and pathogen resistance patterns were assessed. Logistic regression was performed to detect factors associated with mortality 21–30 days after pneumonia onset. T-test and ANOVA were used for evaluation of difference in MV duration.

Results: After 5–7 days of treatment the early mortality was 20.9% (n = 31) and none of the analysed factors had significant influence on it. Mean MV duration was longer in patients infected with serum-resistant strains (16.9 ± 4.3 days, and 6.8 ± 1.1 days, respectively) (p < 0.05). Antibacterial resistance had no effect on the duration of MV. 21–30 days after the pneumonia onset overall mortality was 45.3% (n = 67), outcome was not evaluated in 12.8% (n = 19) of cases. Isolation of intermediately serum-resistant or highly serum-resistant strains

was significantly associated with increased late mortality 21–30 days after initiation of treatment (OR 9.00; CI 1.94–41.65 and OR 7.50; CI 0.93–60.42, p < 0.05). Mean duration of MV was longer in patients with MDR strains (13.5 ± 3.0 and 7.6 ± 1.5 days, respectively) (p < 0.05). Analysing all cases at all time-points showed that the MV duration was significantly longer in patients infected with MDR strains (15.1 ± 2.9 days and 8.1 ± 1.2 days, respectively) (p < 0.05).

Conclusions: 1. Serum-resistance property of *P. aeruginosa* increases patients' late mortality 21–30 days after the treatment initiation. 2. Serum-resistance property and multi-resistance of *P. aeruginosa* strains prolong the duration of mechanical ventilation in ICU patients

P1265

Antimicrobial potency and spectrum of activity for meropenem: report from the USA MYSTIC Programme (2005)

P. Rhomberg, T. Fritsche, H. Sader, R. Jones (North Liberty, US)

Objective: To monitor the activity of meropenem (MEM) and 10 broad-spectrum comparison agents against pathogens collected from hospitalized patients within United States (USA) medical centres participating in the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Programme, a global longitudinal surveillance network of >100 medical centres actively using carbapenems worldwide. In the USA, 15 sites participated by submitting up to 200 consecutive, non-duplicate clinical isolates from serious infections.

Methods: A total of 2910 isolates (97% compliance) including 1657 *Enterobacteriaceae* (ENT), 836 Non-fermentative Gram-negative bacilli (NFGB), and 417 oxacillin-susceptible *Staphylococci* were tested at a central monitoring laboratory using CLSI reference broth microdilution susceptibility (S) methods with interpretative criteria. Ribotyping (RT) and pulsed-field gel electrophoresis (PFGE) were performed on multi-drug resistant (R) strains to determine possible clonal dissemination contribution to R patterns.

Results: Against the ENT isolates, the carbapenems demonstrated the greatest susceptibility (S; >98.7%) and all other agents, except the fluoroquinolones (FQ; 83.9–84.9% S) showed >90% S. Sixty-six *E. coli* (EC) and *Klebsiella* spp. isolates producing ESBLs (7.0%) were submitted from 12 sites, and 24 clonally related (RT 105.497.1) *K. pneumoniae* strains were identified that produced a KPC carbapenemase, and 2 additional strains with a SME type carbapenemase were detected from two sites. FQ-R was most prevalent in indole-positive Proteae and EC strains with six epidemic/endemic clusters identified. Piperacillin/tazobactam, tobramycin and MEM were the most active agents (>87.6% S) against the 589 *P. aeruginosa* (PSA) isolates. Against the 125 *Acinetobacter* spp. isolates only tobramycin, imipenem and MEM demonstrated >85.6% S, all other agents were less than 72.0% S.

Conclusions: These 2005 MYSTIC Program results demonstrate the continued high activity of MEM against ENT, PSA, and oxacillin-susceptible *Staphylococci* (MIC90, 0.12 µg/ml), but the rising incidence of clonally-related carbapenemases (KPC-2 and -3) among ENT is a concern. Continued surveillance within these USA participant sites appears warranted to monitor the residual activity of the important carbapenem class as well as other broad-spectrum agents against key nosocomial pathogens compared to the 6 prior years, only FQ-R rates continued to significantly progress.

P1266

Difference in quinolone resistance in *Pseudomonas aeruginosa* from cystic fibrosis and non-cystic fibrosis patients

B. Henrichfreise, I. Wiegand, I. Noll, W. Pfister, B. Wiedemann (Bonn, Berlin, Jena, DE)

Objectives: German Network for Antimicrobial Resistance Surveillance (GENARS) data reveal for *P. aeruginosa* strains from cystic fibrosis patients (CFP) a higher rate but lower level of resistance to quinolones compared to strains from non-CF patients (nCFP). The aim of our study was to investigate this phenomenon on a molecular level.

Methods: 76 CFP and 207 nCFP GENARS strains from Jan 2002 to Jun 2004 with reduced susceptibility to ciprofloxacin (CIP) were included. For 12 CFP and 10 nCFP strains the QRDR of *gyrA* and *parC*, *mexR* and *nfxB* were sequenced. Clonal relationship among those strains was precluded by PFGE and a PCR screening for *qnr* was carried out.

Results: In Table 1 the MIC distributions of CIP for CFP and nCFP strains are shown. In CFP strains MICs ≤ 4 mg/L were the most frequent, single mutations in *gyrA* predominated and no mutation in *parC* was found. Multiple mutations and a 4-fold higher rate of MICs ≥ 16 mg/L was found in nCFP strains. *Qnr* was not detected.

Table 1. MIC distribution of CIP and mutations in strains from CFP and nCFP

		MIC (mg/L)			
		2	4	8	≥16
Strains from CFP	n=76	47.4%	34.2%	10.5%	7.9%
	n=12	1 (no mutation) 2 (<i>gyrA</i>)	4 (<i>gyrA</i>) 1 (<i>gyrA, nfxB</i>)	1 (<i>gyrA, mexR</i>) 1 (<i>gyrA</i>)	1 (<i>gyrA</i>) 1 (<i>gyrA, mexR, nfxB</i>)
Strains from nCFP	n=207	32.9%	21.7%	13%	32.4%
	n=10	-	2 (<i>gyrA</i>) 1 (<i>mexR, nfxB</i>)	1 (<i>gyrA</i>)	1 (<i>gyrA</i>) 1 (<i>gyrA, parC</i>) 1 (<i>gyrA, parC</i>) 1 (<i>gyrA, mexR</i>) 1 (<i>gyrA, parC, mexR</i>) 1 (2 <i>gyrA, parC, mexR</i>)

Conclusion: In several strains other mechanisms than the tested seem to contribute to CIP resistance. In CFP strains resistance to CIP was characterized by low-level resistance mostly due to single mutations in *gyrA*. In contrast, high-level resistance and multiple mutations were typically found in nCFP strains. Lower concentrations of quinolones in the mucus of the CF lung or biofilm formation may reduce the need for multiple mutations in CFP strains.

P1267

Molecular typing and alterations analysis of penicillin-binding proteins 1A, 2X and 2B and MurM protein and in 33 isolates of *Streptococcus pneumoniae* with vary penicillin and amoxicillin MICs

K. Kosowska-Shick, P.C. Appelbaum (Hershey, US)

Subject: Alterations in one or more of the conserved motifs of penicillin-binding proteins (PBPs) 1A (370STMK373, 428SRN430, 557KTG559), PBP 2X (337STMK340, 394HSSN397, 546LKSG549), and PBP 2B (385SVVK388, 442SSNT445, 614KTG616) as well as changes in murein (MurM) play an important part in resistance of pneumococci to β-lactams.

Methods: 33 strains from 10 countries with varying penicillin (PEN), amoxicillin (AMOX) and carbapenem MICs were selected from surveillance studies and PFGE analyses.

No. of strains	AMOX	PEN	DMYEN	MEROPEN	MurM allele	PBP2B			PBP1A		PBP2X		
						GROUP	411-413 -18T	514-609 KTGTG	370-373 STMK	337-340 STMK	394-397 HSSN	546-549 LKS	
4	1-4	1-2	0.125-0.25	0.25-0.5	1A*	1A'	SSNA	-	SAMK	SAMK	-	VBSG	
1	2	2	0.125	0.25	1A'	SSNA	-	SAMK	SAFK	-	-	VBSG	
3	8	4-8	0.125	0.25	MB1	1A'-6B'	SSNA	-	-	-	-	-	
2	2	4	0.25	0.5-1	4919Strain	1B'	SSNA	-	SSMK	-	LIDS	VBSG	
1	4	16	0.125	0.125	-	2A*	SSNA	-	-	SAMK	-	-	
3	1-2	1-4	0.03-0.125	0.125-0.25	-	or MB6	2A-62 C, 1C	SSNA	-	-	SMK	-	-
2	0.25-0.5	4-8	0.25	0.5-1	-	2B	SSNA	-	SSMK	SMK	-	-	
1	2	4	0.5	1	-	2B	SSNA	-	SSMK	SAMK	-	VBSG	
1	2	2	0.125	0.25	-	3*	SSNA	KTGTG	-	-	-	-	
1	4	4	0.125	0.25	MB6	3*	SSNA	KTGTG	-	SMK	-	VBSG	
3	4-8	2-4	0.125-0.25	0.25	-	or MB1	3*	SSNA	KTGTG	-	SMK	-	
3	4-16	2-8	0.25	0.5	MB1, MB6 or MB7	3*	SSNA	KTGTG	-	SAFK	-	-	
2	8	2-4	0.5	1	MB7	3*	SSNA	KTGTG	SAMK	SAMK	-	VBSG	
2	4	2-4	0.5	1-2	MB6	3*	SSNA	KTGTG	SSMK	SAFK	-	VBSG	
1	8	4	0.25	1	MB6	3*	SSNA	KTGTG	-	SAFK	-	VBSG	
1	8	4	0.25	0.5	4919Strain	3*	-	KTGTG	-	SAFK	-	-	

ted from surveillance studies and PFGE analyses. β-Lactam resistance mechanisms, sequencing analysis of *pbp* 1a, 2x and 2b regions encoding the 3 conserved motifs within the active penicillin-binding sites, and regions encoding the transpeptidase active site were performed in all strains. All strains with carbapenem MICs of 1 μg/ml were subjected to MLST.

Results: PEN, AMOX and carbapenems MICs and changes in the transpeptidase active site and flanking the conserved PBP motifs were as follows: The 14 strains with AMOX MICs higher than PEN all showed changes in 10 amino acid sequences in the PBP2B 590-640 region (group 3), which surrounds the 614KTG616 motif. The remaining 17 strains belonging to PBP2B groups 1 and 2 with AMOX ≤ PEN MICs had up to 4 of these changes, but none had a change at position 618, which may be the critical alteration resulting in the MIC changes seen in group 3. Altered MurM protein was present in all but two strains with elevated sulopenem MICs (1 μg/ml) and had the same set of alterations in PBP2B and PBP1B.

Conclusions: Alterations in MurM seemed to be associated with elevated sulopenem MICs. The 10 amino acid changes in the 590-640 region of PBP2B surrounding the KTG motif were associated with strains with higher AMOX than PEN MICs, with 618A→G likely to be the key substitution.

P1268

Molecular epidemiology of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* and *Serratia marcescens* in a neonatal intensive care unit

V. Crivaro, M. Bagattini, A. Di Popolo, F. Gentile, M.F. Salza, F. Rossano, P. Villari, M. Triassi, R. Zarrilli (Naples, Campobasso, Rome, IT)

Objectives: To investigate the molecular epidemiology of extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* and *Serratia marcescens* in the neonatal intensive care unit (NICU) of a university hospital in Italy.

Methods: Antibiotic susceptibility was evaluated by disc diffusion and E-test. ESBLs were identified by isoelectric focusing, PCR and DNA sequencing analysis. Genotyping was performed by pulsed-field gel electrophoresis (PFGE) and dendrogram analysis. Beta-lactamase gene transfer was performed by broth mating.

Results: The molecular epidemiology of ESBL-producing *K. pneumoniae* and *S. marcescens* was studied in the neonatal intensive care unit of University "Federico II" of Naples, Italy from September 2002 to September 2005. During the study period, ESBL-producing *K. pneumoniae* and ESBL-producing *S.*

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marcescens were responsible for 23 infections (15.6%) and 290 colonizations (37.4%), and 6 infections (4.1%) and 60 (7.7%) colonizations, respectively. The outbreak period began in September 2002 for ESBL-producing *K. pneumoniae* and in May 2004 for ESBL-producing *S. marcescens*. Molecular typing identified two distinct PFGE patterns for ESBL-producing *K. pneumoniae* isolates, one major PFGE pattern and two subtypes for ESBL-producing *S. marcescens*. The two *K. pneumoniae* epidemic clones showed an identical antibiotype, characterized by resistance to penicillins, monobactams, third generation cephe- m, kanamicin, netilmicin and gentamicin. *S. marcescens* strains of the major PFGE type and of the two subtypes were all resistant to penicillins, monobactams, third and fourth generation cep- hems, kanamicin, netilmicin and gentamicin. Molecular analysis of beta-lactamase genes identified blaTEM-1 in chromosomal and plasmid DNA and blaSHV-12 in plasmid DNA from *K. pneumoniae* strains of both PFGE types. All *S. marcescens* epidemic strains showed ampC in chromosomal DNA, blaTEM-1 in chromosomal and plasmid DNA and blaSHV-12 in plasmid DNA. Conjugation experiments demonstrated that resistance to third generation cephe- m, along with blaTEM-1 and blaSHV-12, was transferred from all *K. pneumoniae* and *S. marcescens* epidemic strains to a susceptible *E. coli* host at a frequency of about 1×10^{-6} CFU/recipient cells.

Conclusions: The conjugal transfer of blaSHV-12 ESBL gene was responsible for the selection of ESBL-producing *K. pneumoniae* and *S. marcescens* epidemic clones in the NICU of our university hospital.

P1269

Epidemiology of *Haemophilus influenzae* strains collected in 2004 in France and *in vitro* assessment of their susceptibility to beta-lactams

H. Dabernat, G. Faucon, M. Seguy, C. Delmas (Toulouse, FR)

Objectives: The objective of this study was to describe the epidemiology of *H. influenzae* strains collected in 2004 at the National Reference Center and to evaluate the activity of β -lactam antibiotics

Methods: Demographic characteristics and clinical specimens, capsular serotyping by slide agglutination with specific antisera, β -lactamase (Bla+) by a chromogenic cephalosporin test (Nitrocefin) and MICs of amoxicillin (AMX), co-amoxiclav (AMC), cefpodoxime (CPD), cefaclor (CEC), cefuroxime (CXM), and cefotaxime (CTX) by agar dilution method on *Haemophilus* Test Medium were determined for each strain.

Results: 808 strains of *H. influenzae* were isolated: 41.8% from bronchial secretions (BS), 16.2% from conjunctivitis, 6.6% from otitis media (OM), 4.2% from CSF and 8.6% from blood cultures. 95.6% of strains were not capsulated and 4.4% were of serotype b, e or f. 26.3% of strains were bla+producers (TEM-1 type), 31.4% of them being isolated from OM and 24.7% from BS. 185 isolates (22.8% of total strains) had reduced susceptibility to β -lactams due to modification of PBP 3A/3B (low BLNAR) associated or not with bla+. The MICs 50/90 (mg/L) were as follow: overall strains (n = 808): AMX 0.5/16 mg/L; AMC 0.25/2 mg/L; CEC 2/4 mg/L; CXM 1/2 mg/L; CPD 0.06/0.25 mg/L; CTX 0.015/0.06 mg/L. Susceptible (n = 471): AMX 0.25/0.5 mg/L; AMC 0.25/0.5 mg/L; CEC 2/4 mg/L; CXM 0.5/1 mg/L; CPD 0.06/0.12 mg/L; CTX 0.015/0.03 mg/L. Bla+ (n = 152): AMX 8/16 mg/L; AMC 0.25/0.50 mg/L; CEC 2/4 mg/L; CXM 1/2 mg/L; CPD 0.06/0.12 mg/L; CTX 0.015/0.03 mg/L. Low BLNAR Bla+ n = 62): AMX 16/>16 mg/L; AMC 1/2 mg/L; CEC 4/8 mg/L; CXM 2/4 mg/L; CPD 0.25/0.25 mg/L; CTX 0.03/0.06 mg/L. Low BLNAR (n = 123): AMX 1/2 mg/L; AMC

1/2 mg/L; CEC 4/8 mg/L; CXM 2/4 mg/L; 1CPD 0.25/0.25 mg/L; CTX 0.03/0.06 mg/L.

Conclusions: These results show that both bla+ and modifica- tions of PBP are widespread among *H. influenzae* strains isolated in France. CTX, and CPD remain the most active compounds against *H. influenzae*, whatever the resistance mechanisms.

P1270

Overdetection of imipenem resistance by VITEK 2 in blaVIM 12 producing *Klebsiella pneumoniae* with E-test low level carbapenem MICs

D. Tokatlidou, Z. Afkou, C. Papadopoulou, S. Pournaras, D. Sofianou (Thessaloniki, Larissa, GR)

The clinical significance of the carbapenem-hydrolysing metallo- b-lactamases (MBLs) is increasing. VIM-type MBLs are more common in non-fermenting gram negative bacteria. We describe 8 blaVIM-carrying *Klebsiella pneumoniae* isolates referred to as resistant to imipenem by Vitek 2 automated system (bioMerieux, france), and characterized as susceptible to carbapenems by E- test, according to the NCCLS breakpoints.

Methods: A total of 8 *K. pneumoniae* isolates recovered from blood cultures (2), from urine (1), from wound (1), from bronchial secretions (2), from sputum (1) and from drainage fluid (1). All isolates were identified and characterized as imipenem resistant by Vitek 2 automated system (bioMerieux, france). MICs of imipenem (IMP) and meropenem (MER) were determined by E-test. Susceptibility testing to other antimicro- bial agents was confirmed by disk diffusion method. The isolates were tested for production of metallo-beta-lactamase (MBL) by the IMP-EDTA double disk synergy test. MBL genes were detected by PCR, using VIM primers, and confirmed by DNA sequencing.

Results: The MICs of IMP and MER ranged from 1 μ g/ml to 4 μ g/ml and from 0.5 μ g/ml to 2 μ g/ml respectively by E-test and determine susceptibility to imipenem and meropenem. By Vitek 2 six isolates were characterized as intermediate to imipenem with MICs 8 μ g/ml. The remaining two isolates were characterized as resistant with MICs > 16 μ g/ml. The strains were resistant or intermediate to aztreonam and resistant to cefepime, ciprofloxacin, 3rd generation cephalosporins, ami- kacine and gentamicin. All strains exhibited synergy with EDTA in the IMP-EDTA double disk synergy test. The PCR for blaVIM was repeatedly positive using VIM primers and DNA sequen- cing confirmed blaVIM-12 carriage by all isolates.

Conclusions: These results confirm the overdetection of carbape- nem resistance by an automated system. The discrepancies between testing methods pose difficulties in the characterization of the isolates as susceptible or resistant to carbapenems and may have implications for the management of infections caused by them.

P1271

Viridans group streptococci in blood culture isolates, antibiotic susceptibility and identification of resistance genes

K. Westling, I. Julander, P. Ljungman, S. Jalal, C.E. Nord, B. Wretling (Stockholm, SE)

Objectives: Viridans group streptococci (VGS) cause severe infections, in immunocompromised patients antibiotic resistance is reported.

Methods: Patients with VGS isolated in blood cultures from four clinics 1998–2003, Karolinska University Hospital were

included in this study. Clinical data as septicaemia, haematological diseases, infective endocarditis (using Duke's criteria) were registered. Identification of the VGS species by using RNase P RNA gene (rnpB). Antibiotic susceptibility testing against ciprofloxacin, clindamycin, dalbavancin, erythromycin, linezolid, penicillin, tigecycline, TMP-SMX, vancomycin and daptomycin was performed using agar dilution method, reference values according to NCCLS 2003. Identification of ermB and mefA genes was performed in strains with a reduced susceptibility to erythromycin (MIC \geq 0.5 $\mu\text{g/ml}$).

Results: Antibiotic susceptibility testing results from 129 VGS blood cultures isolates (except for daptomycin). A reduced susceptibility to penicillin was documented in 18% (MIC \geq 0.25 $\mu\text{g/ml}$) of the isolates while resistance to penicillin (MIC \geq 4.0 $\mu\text{g/ml}$) was found in 4%, all patients with haematological diseases. 6% of the strains from patients with definite or possible endocarditis had a reduced susceptibility to penicillin. A reduced susceptibility to erythromycin was found in 25/129 (19%) of the isolates, nearly all patients with haematological diseases. ErmB and mefA gene were identified in 48% (12/25) and 80% (20/25) of the isolates respectively. The strains sequenced as *Streptococcus mitis* had a higher degree of non-susceptibility to penicillin and erythromycin. All the isolates were susceptible to vancomycin and linezolid.

Antibiotic susceptibility testing results from 129 VGS blood culture isolates from patients with septicaemia or endocarditis

Antibiotic	MIC (range)	MIC ⁵⁰	MIC ⁹⁰	Resistance (%)
penicillin G	0.016-4	0.064	0.25	4
ciprofloxacin	0.064->8	2	>8	A
clindamycin	0.016->2	0.032	0.125	2
dalbavancin	0.032-2	0.25	0.5	C
erythromycin	0.016->16	0.125	4	17
linezolid	0.064-2	1.0	1.0	0
tigecycline	0.016->2	0.125	0.5	B
TMP-SMX	0.016->4	0.25	2	0
vancomycin	0.25-1	0.5	0.5	0
daptomycin (n=70)	0.008-0.5	0.25	0.5	C

A NCCLS has no breakpoints for VGS and ciprofloxacin
 B Breakpoints for tigecycline are suggested to be S < 2 $\mu\text{g/ml}$ and R > 4 $\mu\text{g/ml}$ (Wyeth, 2005)
 C No breakpoints available

Conclusion: Penicillin and erythromycin resistance in VGS is primarily a problem in neutropenic haematological patients but a reduced susceptibility to penicillin exists in immunocompetent patients.

P1272

Evidence of an efflux pump mediating a mar phenotype in two ciprofloxacin-resistant marR-mutant *Escherichia coli* isolates

F. Fendukly, G. Kronvall, K. Dornbusch (Stockholm, SE)

Objectives: The marR gene of the multiple antibiotic resistance (mar) locus was sequenced for the detection of mutations in 25 ciprofloxacin-resistant *Escherichia coli* isolates from septicaemia patients admitted to the Karolinska University Hospital, Stockholm, Sweden. All isolates were previously shown to harbour at least one mutation involving the quinolone resistance-determining region (QRDR) of the gyrA gene, while the more resistant isolates harboured additional mutations involving the parC gene. The marR mutants detected were further analysed to assess a possible role of such mutations in the enhancement of quinolone resistance.

Methods: DNA amplification and nucleotide sequencing of the complete marR gene was performed using oligonucleotide primers designed according to the published *E. coli* K12

genome (accession number NC 000913). Testing for organic solvent tolerance was a modification of the agar overlay method described by Oethinger M, Kern WV, Goldman JD, Levy SB. Association of organic solvent tolerance and fluoroquinolone resistance in clinical isolates of *Escherichia coli*. J Antimicrob Chemother 1998; 41(1):111-114. Time-concentration studies of the two marR mutants and three other non-mutant controls in ciprofloxacin with and without the efflux pump inhibitor carbonyl cyanide m-chlorophenyl-hydrazone (CCCP) were then carried out to determine the possible involvement of an efflux pump in the quinolone resistance phenotype of the marR mutant isolates.

Results: A single point mutation involving the marR gene was detected in each of two isolates, namely a Val96→Ile and a Lys43→Arg. These two isolates were shown to be resistant to tetracycline and chloramphenicol in addition to ciprofloxacin and were also tolerant to the organic solvents hexane and cyclohexane. They lost their tolerance to cyclohexane when treated with the CCCP. Time concentration curve analysis of these two isolates demonstrated a more than 2 log reduction of bacterial counts after 8 hours of growth in ciprofloxacin and CCCP as compared to their growth in ciprofloxacin alone. This finding could not be confirmed in the reference strain *E. coli* ATCC 25922, or in three other non-marR mutant isolates exhibiting tolerance to cyclohexane which was not reversible by pretreatment with CCCP.

Conclusion: We propose that the two marR mutations observed in the two *E. coli* isolates may play a role in the enhancement of quinolone resistance.

P1273

First report of a linkage between tet(M) and erm(B) genes in *Clostridium difficile* clinical isolates

P. Spigaglia, F. Barbanti, P. Mastrantonio (Rome, IT)

Objectives: To investigate the possible linkage of tetracycline and erythromycin resistance determinants in a large number of *C. difficile* clinical isolates positive for both tet(M) and erm(B) genes

Methods: A sample of 100 *C. difficile* strains isolated from 1986 to 2002 was analysed by PCR assays to detect tet(M) and erm(B) genes. Forty-four isolates, positive for these genes, were examined for int and tndX, markers for the Tn916 and the Tn5397-like elements, respectively. Tetracycline, erythromycin and clindamycin MIC values were determined by the E-test method and the isolates were typed by PCR-ribotyping. Molecular analysis of the tetracycline and erythromycin resistance elements was performed by hybridization, PCR, PFGE assays and sequencing. Tetracycline and erythromycin resistance transfer was evaluated by filter mating experiments using *C. difficile* strain p881R as recipient.

Results: Nineteen isolates were positive for int and 25 for tndX. An increasing presence of Tn916-like elements was observed in recent *C. difficile* isolates. Forty-two per cent of the isolates positive for int were isolated in the early 2000s and belonged to PCR-ribotype R, predominant during the last decade in our Country. The erm(B) gene was detected in two isolates positive for int and in all those positive for tndX. All strains were naturally resistant to tetracycline, erythromycin and clindamycin, except one int positive isolate that resulted inducibly resistant to tetracycline. Both the int/erm(B) positive isolates showed two Tn916-like elements, whereas all the tndX positive showed only one Tn5397-like element. erm(B) and tet(M) were located in proximity only in one of the two Tn916-like elements

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harboured by the first two isolates, in the other isolates the genes were located in different elements. Sequence analysis indicate that the element carrying tet(M) and erm(B) has presumably arisen from a complex series of genetic events that combined DNA fragments from plasmids and transposons of different origin. This element seems to be not conjugative, as observed for other composite elements.

Conclusion: A genetic linkage between a tet(M) and a erm(B) gene was demonstrated for the first time in *C. difficile*, indicating that this bacterium has acquired the ability to cluster antibiotic resistance genes and that Tn916-like elements are probably implicated in this mechanism.

P1274

Dissemination of 16S rRNA methylase in AmpC producing *Enterobacter cloacae*, *Citrobacter freundii* and *Serratia marcescens* in Korea

S. Lee, Y-J. Park, J-K. Yu, K. Lee, Y. Arakawa (Seoul, KR; Tokyo, JP)

Objectives: A new type of mechanisms, methylation of 16S rRNA, conferred by plasmid, has been reported in a few clinical isolates of gram-negative bacteria and it can confer high-level broad-range resistance to aminoglycosides except neomycin and streptomycin in gram-negative human pathogens. In this study, we investigated (1) the prevalence of high-level aminoglycoside resistance and plasmid-mediated 16S rRNA methylase in *Enterobacter cloacae*, *Citrobacter freundii* and *Serratia marcescens* in Korea, and (2) its association with ESBLs.

Methods: A total of 413 consecutive, non-duplicate isolates, including *E. cloacae* (158), *C. freundii* (126), and *S. marcescens* (129), collected from clinical specimens at 11 clinical laboratories, were used in this study. For isolates showing high-level resistance (MICs of 512 µg/ml) to amikacin or arbekacin, a search for the 16S rRNA methylase genes (armA, rmtA, and rmtB) was performed by PCR amplification with the following sets of primers: rmtA, rmtB, armA. For detection of extended-spectrum β-lactamase genes, the pairs of primers specific for blaTEM, blaSHV, bla CTX-M-1, M-2, M-9, and blaPER-1 were used.

Results: Of the total of 413 isolates, 49 were highly resistant to both amikacin and arbekacin. One *E. cloacae* isolate was highly resistant to arbekacin but susceptible to amikacin (MIC of 16 µg/ml). The prevalence of the high level resistance to amikacin or arbekacin was 9.5% (15/158), 10.3% (13/126), and

17.1% (22/129) in *E. cloacae*, *C. freundii* and *S. marcescens*, respectively and almost all of them (14 *E. cloacae*, 12 *C. freundii* and 21 *S. marcescens* isolates) harboured armA. In *E. cloacae* and *C. freundii*, all but one of the armA producers also harboured ESBLs (13 out of 14 *E. cloacae*, 11 out of 12 *C. freundii*) but in *S. marcescens*, 14 out of 21 armA-producing *S. marcescens* harboured ESBLs. The most common type of ESBL was CTX-M (Table 1). One *C. freundii* isolate harboured rmtB gene and it also harboured CTX-M type ESBL. None harboured rmtA gene.

Conclusion: The prevalence of high-level aminoglycoside resistance was quite high (12.1%), and it was highest in *S. marcescens* and followed by *C. freundii* and *E. cloacae*. Co-existence of ESBL was higher in *E. cloacae* (92.9%) and *C. freundii* (81.8%) than in *S. marcescens* (71.4%). CTX-M type was the most common. One *C. freundii* harboured rmtB gene and it also harboured CTX-M gene.

P1275

Resistance mechanisms in *S. pneumoniae* in the Netherlands

J.W. Mouton, D. Klomberg, S. Meijers, C. Klaassen (Nijmegen, NL)

Background: In 1999, 2001–2002 and 2003 we performed surveys in The Netherlands (NL) to monitor antimicrobial resistance in *S. pneumoniae* (SP). We found increasing prevalence of macrolide resistance but virtually no resistance to quinolones. We here report the survey in 2005; we also determined the resistance mechanisms to macrolide and quinolone resistance, if present. In particular, we looked at the number of non-wild-type at the right tail of the MIC distribution of levofloxacin (Le).

Methods: 34 laboratories equally distributed throughout NL participated in the study. Each lab was asked to collect up to 25 strains of consecutive samples. Only blood or sputum (including lavage) was allowed. Strains were identified by participating laboratories using their own standard identification technique. MIC's were determined using the E-test on site for (Le), Moxifloxacin (Mo), Penicillin (Pe), Amoxicillin, Clarithromycin (Cl), Azithromycin, Cefotaxim (Ce), Cotrimoxazole and Doxycycline; control ATCC strains were included. Afterwards, strains were collected by the central lab for further analysis. Identification confirmation of SP was performed by bile solubility testing and also by LytA PCR for resistant strains. Resistance genes were identified using validated PCR-based methods for all strains with a MIC of >1 mg/L for Le, >0.5 mg/L for Cl and >0.06 for Pe.

Results: Overall, 12.7% of strains were Cl R, 0.5% Le R and 5.9% Pe I or R. 5.2% of strains had a MIC of 1.5 or 2 mg/L for Le. Of these, 32% were non SP (LytA-), comparable to results from earlier surveys in NL. Of the remaining strains, 25% had a known ParC or GyrA mutation, no mutations of ParE or GyrB were found. Of the Cl R strains, 41% was LytA-. Of the LytA + strains, 43% possessed the ErmB gene, 30% MefA, 5% MefE, 5% a 23S mutation, one strain a L4 mutation and no strains with ErmTR or L22 mutation. No mechanism was found for 6 strains. Interestingly, one Pe I strain was ErmB positive but susceptible to Cl. All strains were Ce susceptible.

Conclusions: Routine identification of true Sp remains problematic. Macrolide resistance in Pneumococci is still increasing in The Netherlands and approaches values that may prohibit blind prescribing. Analysis of less susceptible Le strains indicate that a ParC or GyrA mutation is present in a significant portion at the tail of the wildtype distribution. Given the PkPd characteristics, Le treatment may pose a risk for selection of double mutants.

Table 1. The occurrence of high-level amikacin/arbekacin resistance, 16S rRNA methylase production, and their association with ESBLs in *E. cloacae*, *C. freundii* and *S. marcescens*.

	<i>E. cloacae</i> (158)	<i>C. freundii</i> (126)	<i>S. marcescens</i> (129)
amikacin/arbekacin high level resistance (%)	15 (9.5%)	13 (10.3%)	22 (17.1%)
armA producer	14	11	21
ESBL producer	13	10	15
CTX-M type	9	7	14
TEM-type	1	0	0
SHV type	2	1	1
PER type	0	0	0
Undetermined	1	2	0
rmtA producer	0	0	0
rmtB producer	0	1	0

P1276

Fluoroquinolone resistance in *Streptococcus pneumoniae*: screening for first-step mutations

C. Ardanuy, L. Balsalobre, A.G. de la Campa, L. Calatayud, E. Bouza, R. Martín, R. Pallares, J. Liñares for the Spanish Pneumococcal Infection Study Network

Objective: Fluoroquinolone resistance (FQ-R) in *S. pneumoniae* (Spn) occurs mainly by mutations at the QRDRs of the DNA gyrase (gyrA and gyrB), and DNA topoisomerase IV (parC and parE) target enzymes. The aims of this study were: (1) To characterize 14 (3.9%) ciprofloxacin-R strains (CipR, MIC ≥ 4 $\mu\text{g/ml}$) obtained among 360 strains consecutively isolated in 2004 in a Spanish Multicenter Study. (2) To analyse the presence of first-step mutations among 157 Spn strains with Cip MICs of 1–2 $\mu\text{g/ml}$.

Methods: Using a PCR-RFLP method, 157 Spn strains (all 107 strains with Cip MIC of 2 $\mu\text{g/ml}$ and 50 selected strains with Cip MIC of 1 $\mu\text{g/ml}$) were screened for mutations known to confer resistance to FQ: ParC (S79, D83), ParE (D435) and GyrA (S81, E85). The QRDRs of 14 the CipR strains and of 8 strains with Cip MICs of 1–2 $\mu\text{g/ml}$ with presumed mutations as deduced by the PCR-RFLP method were sequenced. PFGE (SmaI) were performed in all CipR strains.

Results: No ParC or ParE mutations involved in CipR were found by the PCR-RFLP method among the 50 strains with Cip MIC of 1 $\mu\text{g/ml}$. Although the PCR-RFLP method predicted that seven (7.5%) of 93 strains with Cip MIC of 2 $\mu\text{g/ml}$ had ParC D83 mutation QRDR sequencing revealed a GAT to GAC polymorphism at D83. Only 1 of 93 strains (1%) with Cip MIC of 2 $\mu\text{g/ml}$ had a ParC mutation (S79F) involved in FQ-R. None of these 93 strains had ParE D435 mutations. The 14 CipR strains (6 with MIC of 4–8 $\mu\text{g/ml}$ and 8 with MIC of 16–64 $\mu\text{g/ml}$) were isolated from adult patients, and showed 14 different PFGE patterns. Among the 6 strains with low level of CipR, 3 had a single ParC mutation (S79F) and 3 had not mutations (suggesting an efflux mechanism). The 8 strains with high-level R showed double mutations at ParC (S79 or D83) and at GyrA (S81).

Conclusions: The prevalence of FQ-R in Spn in Spain was 3.9% and no clonal dissemination of these strains was observed. No mutations were found among strains with Cip MIC of 1 $\mu\text{g/ml}$. The frequency of first-step mutations among Spn with Cip MIC of 2 $\mu\text{g/ml}$ was low (1%). The PCR-RFLP method could be useful for screening of first step mutations, but the QRDRs sequencing of positive strains is necessary in order to discharge the presence of polymorphisms.

P1277

Telithromycin resistance in pneumococci

M. Rantala, P. Huovinen, J. Jalava and Finnish Study Group for Antimicrobial Resistance

Objective of this study was to test the telithromycin (TEL) susceptibility of pneumococci with known macrolide resistance determinant by disk diffusion method. Susceptibility testing to TEL of 196 erythromycin resistant (ERY MIC ≥ 0.5 mcg/ml) pneumococci with known macrolide resistance determinant [88 erm(B), 4 erm(B)+mef(E), 104 with mef(A/E)] and 47 ERY susceptible pneumococci was performed by disk diffusion technique according to CLSI guidelines. If growth was detected inside the inhibition zone, one colony was picked and susceptibility testing for zone isolate (N = 26) was performed. Whole erm(B) gene with its leader peptide, and genes coding L4 and L22 ribosomal proteins were sequenced, and mutations confer-

ring macrolide resistance were screened by pyrosequencing at the positions of 2058–2059 and 2611 of domain V, and loop 35 of domain II of 23S rRNA of 3 TEL resistant isolates and their respective zone isolates. In addition, whole 23S rRNA gene was sequenced from one TEL R isolate and its respective zone isolate. Twenty four erm(B) isolates, and two erm(B)+mef(E) isolates showed heterogenous resistance to TEL that means that one to several colonies grew inside the inhibition zone. In addition, two mef(A/E) positive isolates were grouped to intermediate category. The rest of the isolates carrying macrolide resistance determinant were susceptible to TEL, as well as 47 macrolide susceptible isolates. Of 26 zone isolates 23 were resistant to TEL (<15 mm), 2 were intermediate (16–18 mm) and 1 was susceptible (19 mm). Majority of the zone isolates showed high level resistance to TEL without any detectable inhibition zones. Nucleotide sequences of genes of TEL R isolates and their respective zone isolates were identical. No mutations known to confer macrolide resistance were detected in these isolates. 23S rRNA gene was identical between TEL R and zone isolate and was correspondent with wild type sequence. In conclusion, 14% of macrolide resistant pneumococci showed resistance to TEL, and most often the resistance was manifested as heterogenous growth pattern around the TEL disk. The results indicate that TEL resistance may be more frequent than previously reported. Thus, we suggest that susceptibility testing to TEL should routinely be performed with pneumococci known to be resistant to macrolides. TEL resistance is associated to erm(B), but the exact mechanism behind high level TEL resistance is not known.

P1278

Theoretical framework on the uses of standardised antibiotic phenotype and pattern indexes

I.C. Acuner (*Samsun, TR*)

Objectives: Acuner et al. have defined standard antibiotic phenotype and pattern indexes that are suitable for phenotype and pattern cataloguing using information derivable from probability distributions in event space. Here, I explore the theoretical framework of the indexes, compare their advantages to the possible alternatives in numerical taxonomy, and discuss the potential uses, especially in antimicrobial resistance surveillance.

Methods: The standard antibiotic phenotype index (SAPI) has been defined as: $I = c^{v/p}$ where; c = observed value of the different categorical case probabilities, v = total number of tested variables, p = specific position (location) of the tested variable in event space. $I = \sum_{i=1}^p (c^v \div p_i)$. The index is aimed at including and representing the information derivable from all of these defined dimensions that forms the event space, such that none of the index values obtained would match with other values. Wherever the derivable information is categorical, it has been digitized for use in the index. To define the dimensions that constructs the event space and use the index for the purposes of standardized antibiotic susceptibility phenotype (pattern) surveillance of bacteria; a. probable categorical case results; resistant, intermediate and susceptible have been digitized as 1, 2 and 3, respectively, and used as probable c values, b. total number of the tested antibiotics has been used as v, c. specific positions (locations) of the tested antibiotics have been digitized as 1, 2, ..., N in order. An example of a theoretical bacterial taxon at a specific taxon level (genus, species, subspecies) has been considered to be tested against 21 different antibiotics, and the index values have been calculated. The index values obtained for each of the sequential observable pattern probabilities are digitized as standard pattern index (SPI) values

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in the same order as 1, 2, ..., n. The numerical taxonomy alternative to the base-3 system has been compared.

Results: The index value for the theoretically most sensitive phenotype (i.e. susceptibility results of all tested antibiotics are sensitive) is $I = 3.645358705$; $SPI = 1$. The index value for the theoretically most resistant phenotype is $I = 38131739603$; $SPI = n$.

Conclusion: It has been concluded that SAPI and SPI may be useful in phenotype (including MIC) and pattern cataloguing for the purposes of resistance surveillance besides being a potential tool for the investigation of population dynamics of bacteria.

P1279

Trends in antibacterial resistance of *Streptococcus pneumoniae*: PROTEKT Global Years 1–5

D.J. Farrell, R. Canton, W. Hryniewicz (London, UK; Madrid, ES; Warsaw, PL)

Objectives: Data from Year (Y) 1–5 of PROTEKT (1999–2000 to 2003–2004) were analysed to compare the *in vitro* activity of telithromycin with that of other antibacterials against isolates of *Streptococcus pneumoniae* (SP) from patients with community-acquired respiratory tract infections.

Methods: *In vitro* antibacterial activity and susceptibilities of SP isolates from patients with CAP, AECB, sinusitis, otitis media and pharyngitis were determined at a central laboratory using CLSI methodology and interpretive criteria. Macrolide resistance genotypes were determined by PCR.

Results: For Years 1–5 a total of 3435, 4256, 6320, 6739, 7083 (Y1, Y2, Y3, Y4, Y5) SP isolates were collected from 166 centres in 42 countries. Resistance rates (%) by year were: erythromycin (ERY), 31.4, 32.7, 36.9, 36.4, 37.2; penicillin (PEN), 21.7, 18.0, 24.0, 22.3, 23.9. The prevalence of multidrug resistance (MDR; resistance to ≥ 2 antibacterial classes) by year was 36.0%, 34.9%, 41.4%, 39.7%, 40.0% of isolates. Most ERY-resistant SP (ERSP) exhibited the *erm(B)* genotype (57.0, 63.5, 57.9, 56.9, 55.0% of isolates). The prevalence of the *mef(A)* genotype decreased slightly over the first two study years (34.3%, 30.1%, 30.4%, 30.3%, 30.6%), while the proportion of *erm(B)+mef(A)* isolates ($MIC_{90} > 256$ mg/L) almost doubled (6.7, 5.0, 10.2, 10.5, 12.0%) over the five study years. The vast majority of *erm(B)+mef(A)* isolates were MDR [99.4% (955/961)]. Telithromycin retained potent *in vitro* activity against MDRSP, with 99.5% of such isolates fully susceptible to this antibacterial. Telithromycin *in vitro* activity and susceptibility data are shown below.

	Y1		Y2		Y3		Y4		Y5	
	S (%)	MIC ₅₀ (mg/L)								
All SP	99.9	0.12	99.9	0.12	99.8	0.12	99.7	0.12	99.8	0.25
ERSP										
<i>erm(B)</i>	99.5	0.5	99.6	0.25	99.0	0.25	98.9	0.25	99.0	0.25
<i>mef(A)</i>	100	0.25	100	0.25	100	0.25	100	0.25	100	0.5
<i>erm(B)+mef(A)</i>	100	0.5	100	1	99.2	0.5	98.8	0.5	99.4	0.5
Ribosomal	100	0.12	100	0.25	100	0.25	100	1	100	0.25
MDRSP	99.8	0.5	99.7	0.25	99.4	0.5	99.3	0.5	99.4	0.5
S, susceptible										

Conclusions: PEN and ERY resistance remained high in isolates of SP over the 5 study years. Telithromycin demonstrated potent *in vitro* activity against SP, including MDRSP isolates with the increasingly prevalent *erm(B)+mef(A)* genotype.

P1280

Activity of telithromycin against *Streptococcus pneumoniae* isolates resistant to other antibacterials: PROTEKT Year 5

G.C. Schito, D.J. Farrell, J.A. Ramirez (Genoa, IT; London, UK; Louisville, US)

Objectives: Continual monitoring of antibacterial resistance in common respiratory pathogens such as *Streptococcus pneumoniae* (SP) is essential to guide optimal antibacterial therapy for community-acquired respiratory tract infections (CARTIs). PROTEKT is a global, longitudinal surveillance study of antibacterial resistance among key respiratory tract pathogens, which was initiated in 1999. Data from PROTEKT Year 5 (2003–2004) were analysed to assess the susceptibility of SP isolates to the ketolide telithromycin and a range of other antibacterials.

Methods: Isolates were collected at 123 centres in 39 countries from patients with CARTIs. Antibacterial minimum inhibitory concentrations (MICs) were determined centrally using CLSI methodology. CLSI interpretive breakpoints were used to determine susceptibility (S).

Results: A total of 7083 SP isolates were collected in Year 5. Antibacterial activities [MIC₅₀ (mg/L)/MIC₉₀ (mg/L)/S (%)] were: amoxicillin-clavulanate (AMC), 0.03/2/92.6; azithromycin (AZI), 0.12/>128/62.7; cefuroxime (CEF), 0.06/8/68.1; clarithromycin (CLA), 0.06/>64/62.4; penicillin (PEN), 0.03/4/62.6; those for telithromycin are shown below. Susceptibility rates varied markedly between countries [e.g. AZI: 2.4% (Taiwan) to 100% (Colombia); PEN: 18.3% (Taiwan) to 96.6% (Netherlands)]. Of the 1696 (23.9%) PEN-resistant SP (PRSP) isolates, 1174 (69.2%), 1317 (77.7%), 1318 (77.7%) and 1695 (99.9%) were resistant to AMC, AZI, CLA and CEF, respectively. Of the 2638 (37.2%) erythromycin (ERY)-resistant SP (ERSP) isolates, 1325 (50.2%) were resistant and 549 (20.8%) were intermediately susceptible to PEN, 222 (8.4%) were resistant to AMC and 1587 (60.2%) were resistant to CEF. Overall, 18.7% (1325/7083) of

Phenotype	MIC ₅₀ (mg/L)	MIC ₉₀ (mg/L)	S (%)
All SP	0.015	0.25	99.8
ERSP	0.06	0.5	99.4
PRSP	0.06	0.5	99.1
ERSP+PRSP	0.12	0.5	98.9

isolates were co-resistant to PEN and ERY (PRSP + ERSPP).

Conclusions: Reduced susceptibility to beta-lactams and/or macrolides remains common worldwide among SP isolates collected from CARTI patients. Telithromycin continues to demonstrate strong *in vitro* activity against this common respiratory pathogen, including strains resistant to other antibacterials.

P1281

Evaluation of the VITEK 2 AST-N 041 card for detection of extended-spectrum beta-lactamase producing *E. coli* and *Klebsiella pneumoniae*

S.H. Koo, J.S. Goh, J.U. Kang, K.C. Kwon, J.W. Park (Daejeon, KR)

Objective: Increasing incidence of ESBL producing Gram-negative pathogens requires proper and rapid detection of resistance phenotype. The VITEK 2 AST-N 041 card (bioMérieux) is new and automated test for ESBL detection. The aim of this trial was to evaluate and compare equivalency between the bio-

Merieux VITEK 2 AST-N 041 card and confirmatory NCCLS methods for detection of ESBL producing *E coli* and *Klebsiella pneumoniae*.

Methods: A total of 178 clinically significant Gram negative bacterial pathogens (69 *Klebsiella* and 118 *E coli*), which were isolated from various material (blood, urine, stool, wounds) were included in this study. Detection for ESBL-producing strains was performed by VITEK 2 AST-N 041 card and a modified double disk diffusion test (MDDT) according to the latest NCCLS documents. For the quality control, ATCC 700603 for *K pneumoniae* and ATCC 25922 for *E coli* were used as reference strains.

Results: A high correlation was found between the results obtained by the VITEK 2 AST-N 041 card and by the NCCLS reference methods. The overall concordant rate between the two tests was 98.4%, the sensitivity was 96.6%, and the specificity was 98.7%. For *E coli*, the concordant rate of detection was 98.3%, sensitivity was 100% and specificity was 98.1%. The results for *K. pneumoniae* were 98.6%, 93.3% and 100% respectively.

Conclusions: The bioMerieux VITEK 2 AST-N 041 card represent an accurate and acceptable means for detection of ESBL producing gram-negative pathogens. It is very simple and fast for detection of ESBL and can be used as an alternative to disk diffusion test which is time consuming and labour-intensive.

P1282

Antibiotic susceptibility among invasive and non-invasive *Streptococcus pyogenes* isolates in Sweden; 2002–2004

B. Luca, J. Darenberg, B. Henriques Normark, A. Norrby-Teglund, C. Schalen, A. Jasir (Lund, Stockholm, Solna, SE)

Objectives: Since *Streptococcus pyogenes* is still susceptible to betalactams, but for other antibiotics highly variable resistance. A major objective was to study antibiotic susceptibility of invasive and none invasive isolates and type distribution of resistant strains in Sweden.

Methods: During active surveillance (2002–04) of invasive group A streptococcal infections in Sweden, 746 invasive isolates were collected from all microbiological laboratories (36) in the country. During the same period, 774 non-invasive skin- or throat control isolates were collected at 6 of the laboratories. *In vitro* susceptibility to antibiotics, MICs of resistant strains, T and emm typing and resistance gene detection were performed according to previously established methods.

Results: Erythromycin resistance was uncommon (0.2%), whereas an overall high rate of tetracycline resistance was found (25–30%). MIC for tetracycline resistant strains varied between 8 and 64 mg/L with a clustering at 24 mg/L. The tetracycline resistant strains belonged to more than 10 different T types, the majority being of T-type 3/13/B3264, 28, and 13/28 sub-divided into two large clusters of emm types 81 and 77. All tetracycline resistant isolates of emm type 81 carried tetM but among emm 77 both tetM and tetO were detected. Clonal relationships within sub-groups of isolates were investigated with pulse field gel electrophoresis (PFGE) and multi locus sequence typing (MLST).

Conclusion: Tetracycline is used in the treatment of chlamydial and mycoplasmal infections rather than streptococcal infections in Sweden; hence the level of tetracycline resistance among *S. pyogenes* clinical isolates, 25–30%, appeared comparatively high. To account for resistance development, the role of commensals bacteria, horizontal gene transfer from other *Streptococci* espe-

cially with regard to increasing veterinary usage of tetracycline have to be considered.

P1283

Evaluation of cefoxitin disk test in the detection of methicillin-resistant blood CoNS clinical isolates in a tertiary university department

R. Vorou, E. Giannitsioti, I. Galani, E. Koratzanis, H. Giamarellou (Athens, GR)

Objectives: Accurate phenotypical detection of methicillin-resistant (MR) in *Staphylococci* is difficult using standard media due to heterogeneous expression of MR in many strains. Detection of *mecA* gene is the gold standard for that purpose, however an equally reliable routine test has not been currently available especially for coagulase negative *Staphylococci* (CoNS) which usually are involved in serious infections like endocarditis and device associated infections. The present study was conducted to evaluate the role of cefoxitin diffusion test in the detection of MR CoNS.

Methods: All CoNS blood isolates that were collected during the last 1 year in our tertiary hospital were evaluated by 3 methods in order to assess MR. (1) Cefoxitin disc diffusion test (30 µg) was applied in Muller-Hinton agar and incubation lasted for 24–36 hours in 37°C. A cefoxitin zone <24 mm was interpreted as MR for CoNS. *MecA* gene was detected by PCR in all isolates. *MecA1* ('AAAATCGATGGTAAAGGTTGGC3') and *mecA2* (5'AGTTCTGCAGTACCGGATTTC3') primers were used. Minimal inhibitory concentration (MIC) for oxacillin was also performed according to the NCCLS standards (Oxacillin MIC > 2 µg/l indicates MR).

Results: In 63 blood isolates (44 *S. epidermidis* and 19 CoNS, non-epidermidis), MIC > 2 was found in 54 cases (85.7%). Both cefoxitin disk and *mecA* gene assessed MR in 100% of strains. Among the 17 isolates with MIC < 2 µg/l, only in one case of oxacillin MIC = 0.5 µg/l, cefoxitin disk revealed resistance.

Conclusions: Cefoxitin disk could be a good alternative to MIC oxacillin test for the detection of MR in clinical CoNS isolates as it is also easier, faster and cheaper. However, a larger number of observations could better confirm this statement for CoNS strains.

P1284

Quantitative antibiotic susceptibility of *Staphylococcus aureus* strains isolated from blood of septic patients with special attention to MRSA

F. Rozgonyi, N. Pesti, K. Kristóf, H. Lagler, G. Kotolácsi, E. Presterl, W. Graninger (Budapest, HU; Vienna, AT)

Objectives: Manifest bacteraemia and sepsis caused by *S. aureus* are life threatening infections particularly if the pathogen is MRSA. Their adequate treatment is of outmost importance. The aims of this study were to determine quantitatively the antibiotic sensitivity of 179 blood culture *S. aureus* strains and compare the results according to MRSA vs MSSA.

Methods: *S. aureus* strains were isolated from patients admitted to the Vienna University hospitals and identified by conventional tests including rapid slide agglutination. The presence of *nucA* gene was shown by PCR. Methicillin resistance was first detected with 1 µg oxacillin disc then confirmed by amplifying the *mecA* gene with PCR. Susceptibility to 10 antimicrobials was determined with the broth microdilution method using NCCLS/ICLS guidelines.

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Results: The *mecA* gene was present in 49 strains (27.4%). The vast majority of MRSA expressed high level oxacillin resistance (≥ 1024 mg/L), however a few strains was inhibited by as low as 2 mg/L oxacillin. Contrary, MBC for these strains proved to be high indicating oxacillin tolerance. Both clindamycin and clarithromycin were ineffective against MRSA (MIC ≥ 256 mg/L). MSSA strains were highly sensitive to clarithromycin but not to clindamycin. Most MRSA strains needed ≥ 16 mg/L ciprofloxacin, ≥ 8 mg/L levofloxacin and 4 mg/L moxifloxacin to inhibit growth, while MICs of them for most MSSA strains were 0.5 mg/L. MICs of gentamicin were over 32 mg/L for MRSA, while ≤ 2 mg/L inhibited the growth of most MSSA strains. Amikacin was equally effective against MRSA and MSSA ranging MICs 2–32 mg/L. Both MRSA and MSSA strains were equally sensitive to vancomycin. MRSA needed teicoplanin MICs of 1–8 mg/L, while MSSA did only 0.5–2 mg/L to inhibit growth.

Conclusions: Oxacillin tolerant *S. aureus* strains with low MIC high MBC have appeared in the Vienna patients with bacteraemia. For the treatment of MRSA sepsis, beside the glycopeptides, the new fluoroquinolons and amikacin or their combination should be considered.

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P1285

Antimicrobial resistance trends in invasive strains of *S. aureus*, *E. coli*, *Streptococcus pneumoniae*, and *Enterococcus* spp. in Romania – a 4-year participation in the EARSS

I. Codita, M.C. Balotescu, A. Israil, M. Popa, D. Tatu-Chitoui, V. Ungureanu, M. Pana, M. Ghita (Bucharest, RO)

Objectives: (i) To collect comparable and validated antimicrobial resistance data in Romania, following the EARSS protocols; (ii) to analyse trends in Romania during the 2002–2005 interval; (iii) to provide official national AMR data for policy decisions; (iv) to provide information on relevant antimicrobial resistance; (v) to encourage the implementation, maintenance and improvement of national antimicrobial resistance surveillance programmes; and (vi) to prepare and provide basic information for the Romanian national laboratories to be used for participating in scientific research programmes in Europe in the field of antimicrobial resistance.

Methods: 12–35 hospitals involved in the surveillance system (2002–2005, respectively; 58% population coverage in 2005). EARSS protocols used for collecting data and for laboratory testing strategy. Disc diffusion testing performed following the CLSI/NCCLS standards and MICs determined using E-test or agar dilution methods. Since 2004, PCR for *mecA* introduced in the National Antimicrobial Resistance Reference Laboratory.

Results: The total numbers of invasive strains completely investigated were: 296 *S. aureus* (80/2002, 85/2003, 92/2004, 39/2005), 160 *E. coli* (25/2002, 46/2003, 41/2004, 48/2005), 55 *S. pneumoniae* (10/2002, 22/2003, 11/2004, 12/2005), 37 *E. faecalis/faecium*. The proportions of resistant strains were generally increasing for MRSA (36.3/2002, 45.9/2003, 71.7/2004 and 61.5 for 2005), aminopenicillins resistant *E. coli* (50/2002, 69.6/2003, 78.6/2004, 76.9/2005), but showed irregular variations for fluoroquinolone resistant *E. coli* (20/2002, 14/2003, 17.1/2004, 4.2/2005) and PNSP (10/2002, 22.7/2003, 11.1/2004, 16.7/2005). ESBL production varied between 21.7 (2004) and 15.1 (2005). Rechecking MRSA strains using the ceftoxitin disc and the PCR for the *mecA* gene resulted in finding only 1 strain resistant

formerly reported as susceptible. Few strains of VRE were reported.

Conclusions: Though the total number of strains is generally low by comparison with other European countries participating in the project, partially due to the comparatively low number of blood cultures prescribed and analysed, we could conclude on the general trends for the majority of important pathogens. There are generally high rates of resistance and the ESBL production in *E. coli* strains is also high when compared to the European median. For *Enterococcus* species the total number of strains is very low and it is difficult to predict the actual resistance rate.

P1286

In vitro susceptibility of methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium* to vancomycin, daptomycin, linezolid, pristinomycin and other antibiotics

Z. Samra, O. Ofir, H. Shmueli (Petach-Tiqua, IL)

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is becoming increasingly prevalent as both a nosocomial and community acquired pathogen in addition to an ever-growing concern over development of vancomycin (VA) resistant. Similarly VA resistant *Enterococcus faecium* (VREF) is becoming a significant pathogen in the immunocompromised patients. The increasing prevalence of multi-drug resistant gram-positive pathogens highlights a critical need for newer antimicrobial agents. In this study we tested the *in-vitro* susceptibility of MRSA and VREF clinical isolates to VA, daptomycin (DA), linezolid (LI), pristinomycin (PR) and other antibiotics.

Methods: Susceptibility of 200 MRSA of 200 MRSA isolates (32-blood, 168-wounds and body fluids) and 90 VREF isolates (15-blood, 31-wounds and body fluids, 15-sputum and 29-stool). All isolates were recovered from hospitalized patients, one isolate from each patient. The minimal inhibitory concentration (MIC) of VA, DA, LI and PR was tested by E-test (Biodisc). Susceptibility to other antibiotics was tested by disc diffusion on Mueller-Hinton agar according to the NCCLS criteria. *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 and 19433 were used for quality control.

Results: The MICs results are summarized in the Table. All the MRSA isolates were sensitive to VA, DA and LI. 0.5% were resistant to PR. All the VREF isolates were sensitive to DA and resistant to VA. 14% were resistant to PR and 1% to LI. The sensitivity of the MRSA isolates to other antibiotics was: fusidic acid – 97.9%; trimethoprim/sulfamethoxazole – 96.9%; tetracycline – 91.7%; rifampicin – 86.6%; chloramphenicol – 46.9%; gentamicin – 37.6%; clindamycin – 22.2% and erythromycin – 20.6%. The sensitivity of the VREF isolates was: chloramphenicol – 96.7%; gentamicin – 66.7%; fusidic acid – 41%; tetracycline – 13%; ampicillin, penicillin and erythromycin – 4%.

Antibiotics	MRSA MICs (mcg/ml)			VREF MICs (mcg/ml)		
	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀	Range
VA	2	3	1-4	>256	>256	>256
DP	0.125	0.25	0.064-0.38	1	2	≤0.5-4
LI	0.5	1	0.25-2	1	1.5	≤0.5-4
PR	0.5	0.75	0.25-2	0.75	2	≤0.5-4

Conclusion: This study has shown that daptomycin, a new antibiotic agent with rapid *in-vitro* bactericidal activity given

once daily, have good *in-vitro* activity against a diverse collection of MRSA and VREF clinical isolates. Daptomycin can be considered as an alternative treatment for patients with VA

resistant MRSA strains or those who cannot tolerate VA. It may be useful in the management of infections caused by multi-resistant VREF isolates.

Antibacterial susceptibility studies – II

P1287

MBL mediated carbapenem resistance in *P. aeruginosa* and *A. baumannii* in cancer patients

E. Chinou, M. Georgouli, P. Giannakakos, M. Alexiou, H. Skouteli, P. Golemati (Athens, GR)

The use of carbapenem has increased over the last 3 years in our oncologic wards due to increased prevalence of ESBL producing strains.

Objective: This study was performed to determine the prevalence of metallo beta lactamase mediated (MBL) carbapenem resistance in clinical isolates of *P. aeruginosa* and *A. baumannii* from oncologic patients.

Methods: We examined fifty four strains of *P. aeruginosa* and 17 strains of *A. baumannii* isolated from urine, sputum and blood culture of patients with solid tumours hospitalized in one oncologic ward during the last year. The E-test metallo-beta lactamase strip method was used as a reference method. The MIC, was determined by Microscan semi automated method. The procedure and interpretation of E-test MBL results was carried out according to the manufacturer's recommendation (AB Biodisk).

Results: Twenty-two isolates (40%) of *P. aeruginosa* and nine isolates of *A. baumannii* (53%) were resistant to imipenem. All imipenem resistant *A. baumannii* isolates were also resistant to meropenem and gentamicin. 13 strains (60%) of the imipenem resistant *P. aeruginosa* isolates were resistant to meropenem and cefotaxime and 10 strains (45%) resistant to aztreonam. Seven carbapenem resistant *P. aeruginosa* isolates and five *A. baumannii* isolates were shown to produce an MBL activity by the E-test MBL, while the rest isolates had high MIC values to imipenem but were not MBL producer reflecting other mechanisms of resistance. The seven MBL producers *P. aeruginosa* strains were sensitive only to aztreonam.

P1288

Efficiency of cefoperazone, sulbactam alone and in two combinations against multidrug-resistant *Acinetobacter baumannii* strains isolated from nosocomial infections in seven different intensive care units in Turkey

A. Karadenizli, S. Akhan, S. Gundes, F. Kolayli, H. Vahaboglu (Kocaeli, TR)

Objectives: Multiresistance *A. baumannii* is an important therapeutic problem. Sulbactam is a beta-lactamase inhibitor which can be used in combination with beta lactams. In this study, the activity of cefoperazone, sulbactam alone and combined with two concentrations of sulbactam against sixty-nine isolates of multidrug-resistant *Acinetobacter baumannii* from Turkey were compared.

Methods: Sixty-nine multidrug-resistant *A. baumannii* strains isolated from clinical materials in seven different ICUs in Turkey. Strains were identified using colony morphology, Gram stain, oxidase reaction, aerobic growth and use of commercial identification panel (API 20 NE, BioMerieux, France). Minimal

inhibitory concentrations (MICs) using agar dilution method were determined according to NCCLS. Cefoperazone was accepted as susceptible if MIC level was ≤ 16 mg/L. Cefoperazone (cfp) and sulbactam (sul) combinations were investigated at two ratios as follows: cfp-sul (1:1), and cfp-sul (2:1).

Results: MICs were determined with each compound on its own and in combination with sulbactam. The table shows MIC50 and MIC90 values for sixty-nine isolates of *A. baumannii* (mg/L).

	Cefoperazone	Sulbactam	Cfp-Sul (1:1)*	Cfp-Sul (2:1)*
Minimum	2	1	1	2
Maximum	1024	256	128	256
MIC 50	1024	16	32	64
MIC 90	1024	64	64	128

*MIC levels belong to cefoperazone.

Conclusion: MIC50 and MIC90 to cefoperazone were found high. The addition of sulbactam did not enhance the activity of cefoperazone in both concentrations against multidrug-resistant *Acinetobacter baumannii* strains isolated from ICUs in Turkey.

P1289

High incidence of moxifloxacin resistance among *Bacteroides* spp. isolates in Greece. Results from the Hellenic Study Group on Gram-Negative Anaerobic Bacteria

A. Katsandri, D.P. Houhoula, G.L. Petrikkos, N.J. Legakis, J. Papaparaskevas, A. Pantazatou, A. Avlami (Athens, GR)

Objectives: The evaluation of the *in vitro* activity of moxifloxacin in comparison to penicillin, piperacillin + tazobactam, cefoxitin, clindamycin, metronidazole and imipenem against *Bacteroides* spp. isolates collected from 8 general hospitals, in Athens, Greece

Materials and methods: A total of 190 *Bacteroides* spp. clinical strains (162 *Bacteroides fragilis* group and 28 other *Bacteroides* spp. non-fragilis) isolated during the period 11/2002–10/2005 were tested using the E-test and the agar dilution methods, on brucella agar plates supplemented with 5% horse blood, vitamin K1 and haemin. All plates were incubated in a Bactron ChelLab Anaerobic Chamber for 48 hours. Interpretation of the results was according to CLSI guidelines M11-A6. For quality control the strains *B. fragilis* ATCC25285 and *B. thetaiotaomicron* ATCC29741 were used.

Results: Overall MIC90 to moxifloxacin, penicillin, piperacillin+tazobactam, cefoxitin, clindamycin, metronidazole and imipenem were >32 , >32 , 8, 64, >256 , 1 and 0.5 mg/L, respectively, whereas MIC50 were 2, >32 , 0.25, 16, 1, 0.5 and 0.064 mg/L, respectively. Overall non-susceptible to moxifloxacin (using a tentative MIC breakpoint of ≥ 4 mg/L, as no breakpoint has been established yet by CLSI or any other organization) were 44% of the isolates. *Bacteroides fragilis* group MIC90 were >32 , >32 , 4, 64, >256 , 1 and 0.5 mg/L, respectively, whereas MIC50 were 2, >32 , 0.25, 16, 1, 0.5 and 0.064 mg/L, respectively. *Bacteroides* non fragilis MIC90 were >32 , >32 , 16, 32, 32, 1 and 0.5 mg/L, respectively, while MIC50 were 4, >32 , 0.25, 8, 1, 0.5, and 0.125 mg/L, respectively.

Abstracts

Conclusions: Moxifloxacin exhibited high rates of resistance among the isolates tested, whereas piperacillin + tazobactam, imipenem and metronidazole were highly active. In that respect, it is recommended that the use of this newer fluoroquinolone in antimicrobial chemotherapy of anaerobic infections due to *Bacteroides* spp. isolates requires prior susceptibility testing.

Acknowledgement: Members of the Hellenic Study Group on Gram-Negative Anaerobic Bacteria are: Dr A. Avlami, Dr E. Papafrangas, Dr C. Koutsia-Karouzou, Dr C. Trikka-Graphakos, Dr C. H. Kontou-Castellanou, Dr H. Malamou-Ladas, Dr A. Pangalis, Dr M. Foustoukou, and Dr A. Vogiatzi.

P1290

Laboratory investigation of the combination effect of imipenem/colistin and sulbactam/colistin on clinical isolates of multidrug-resistant *Acinetobacter baumannii*

H-S. Leu, L-H. Su, J-W. Wang (Taoyuan, TW)

Objectives: Infections with multidrug-resistant *Acinetobacter baumannii* (MDRAB) has become a global problem. Carbapenem is usually the drug of choice in treating such infections. However, a rapid increase in the proportion of imipenem-resistant MDRAB (IR-MDRAB) isolates from 4% in 2002 to 22% in 2004 was observed in our hospital. The shortage of newer effective antibiotics has further deteriorated the problem. To provide an alternative therapeutic strategy, the *in-vitro* combination effect of imipenem (IPM)/colistin methanesulfonate (CM) and sulbactam (SB)/CM was evaluated.

Methods: By using a checkerboard method, 10 representative isolates of MDRAB/IR-MDRAB were used in the preliminary experiment to confirm whether any synergistic effect could be obtained by the proposed combinations. The effect of various combination ratios was determined by calculating the fractional inhibitory concentration index (FICI). The most frequent combination ratios showing a synergistic effect (FICI not greater than 0.5) were used in the subsequent screening of 90 isolates of MDRAB/IR-MDRAB using a standard agar dilution method.

Results: Synergistic effects were observed for both proposed drug combinations. For IPM/CM, when CM reached a concentration of 0.5 µg/ml, the associated MICs of imipenem reduced sharply no matter what the actual combination ratios of IPM/CM were. Thus a fixed concentration of CM at 0.5 µg/ml was added to a serial 2-fold dilutions of IPM for the subsequent screening. The proportion of IPM-non-susceptible isolates reduced significantly from 72.2% to 24.4% at the presence of 0.5 µg/ml CM ($P < 0.00000005$). For SB/CM, the best combination ratio of 1:4 (SB:CM) was used for the screening and the MICs reduced by 1–3 folds were observed in 34.8% of the isolates tested. However, the rate of CM-non-susceptible (MIC not less than 4 µg/ml) isolates reduced only slightly from 4.5% to 3.4% when SB was added at the fixed ratio.

Conclusion: Both drug combinations produced synergistic effects compared to when either drug was used alone. CM at a low level of 0.5 µg/ml appeared to increase greatly the *in-vitro* antibacterial effect of IPM in the IR-MDRAB/MDRAB isolates tested. However, the addition of SB to CM did not produce as promising results probably due to the low-level resistance to CM observed in the test population. For both drug combinations, no antagonism was found among the 90 isolates analysed.

P1291

Current state of antimicrobial resistance of *S. pneumoniae* isolated from children in Russia: results of multicentre study PEHASus

O. Sivaja, R. Kozlov (Smolensk, RU)

Objective: *S. pneumoniae* is one of the most common bacterial pathogens causing respiratory tract infections in children. Therefore it is of extreme importance to determine the antimicrobial resistance of that microorganism in order to optimize empirical therapy of pneumococcal infections.

Methods: This study was conducted in 1999–2005 in 25 cities of different regions of Russia. Identification of the strains was done on the basis of colony morphology, Gram stain, optochin susceptibility and bile solubility tests. Susceptibility to 15 antimicrobials was performed using cation-adjusted Mueller-Hinton broth (BBL, USA) with 2–5% lysed horse blood. Breakpoints were those of NCCLS/CLSI except for midecamycin (<1; >4 mg/L, SFM, 1996).

Results: A total of 840 non-duplicate clinical *S. pneumoniae* isolated from children of 1–18 years old were included in this study. The susceptibility testing results are presented in the Table.

Visit	Assessment	Linezolid n (%)	Vancomycin n (%)	P-value	95% CI (% Difference)
EOT	Effective treatment	53 (86.9)	37 (61.7)	0.0015	(+10.3, +40.2)
	Failed Treatment	8 (13.1)	23 (38.3)		
	Total Assessed	61 (100)	60 (100)		
F-U	Effective treatment	49 (83.1)	37 (64.9)	0.03	(+2.5, +33.8)
	Failed Treatment	10 (16.9)	20 (35.1)		
	Total Assessed	59 (100)	57 (100)		

Conclusions: Generally, non-susceptibility (I+R) to b-lactams remains to be comparatively low, varying from 0.2% to aminopenicillins to 8.3% to penicillin G. Resistance to all macrolides is not exceeding 6% with 16-membered macrolides and lincosamides being the most active with resistance less than 3%. Tetracycline and co-trimoxazole should not be used for empirical therapy of pneumococcal infections because of high resistance.

P1292

In vitro bacteriostatic and bactericidal activity of pristinamycin against *Streptococcus pyogenes*: influence of macrolide resistance mechanism

H.B. Drugeon, M.E. Juvin, C. Dib-Smahi (Nantes, Paris, FR)

Objectives: The objective of this study was to evaluate the *in vitro* bacteriostatic activity of pristinamycin (PRI) against 173 *S. pyogenes* isolated in 42 French hospital (year 2003) and the *in vitro* bactericidal activity of PRI against *S. pyogenes* isolates with different macrolides resistance phenotypes: erythromycin (ERY) susceptible, ERY-R mefA, ERY-R ermB, ERY-R ermA subtype ermTR (2 strains by each phenotype).

Methods: PRI and ERY MICs were determined by agar and broth dilution methods. The bactericidal activity was studied by using a killing curve method. PRI concentrations were used from 0.002 mg/L to 2 mg/L by following a two fold dilution. Survival bacteria were counted at T0, T15 minutes, T30 minutes, T1, T2, T4, T6, and T24 hours.

Results: 143 strains (82.7%) were erythromycin susceptible, 30 strains (17.3%) were erythromycin resistant with ERY-R ermB (n = 26), ERY-R ermA subtype ermTR (n = 2), ERY-R mefA (n = 3) and 1 isolate was ERY-R with no mechanism determined. All the isolates (ERY S and ERY R) had PRI MIC ≤0.06 mg/L.

PRI had a very rapid, concentration dependent bactericidal activity against wild-type strains and ERY-R *mefA*. After 15 minutes of contact with PRI concentrations ≥ 0.5 mg/L, the decrease of bacterial population was $> 2 \log_{10}$ cfu/ml; after 2 hours, a decrease $> 3 \log_{10}$ cfu/ml was observed with PRI concentrations ≥ 0.25 mg/L. With 0.06 mg/L, 24 hours of contact was necessary. With ERY-R *ermA* subtype *ermTR* and, with ERY-R *ermB* strains, the bactericidal activity of PRI was slower and time dependant. A 6 h-contact was needed with the first mechanism to obtain a bacterial population decrease of $> 2 \log_{10}$ cfu/ml with PRI concentrations ≥ 0.25 mg/L. With ERY-R *ermB*, a contact of 24 hours was necessary to reach a bacterial population of $> 2 \log_{10}$ cfu/ml with PRI concentrations ≥ 0.125 mg/L.

Conclusion: 100% of *Streptococcus pyogenes* isolates were susceptible to Pristinamycin. The mechanism of macrolide resistance didn't influence the bacteriostatic activity (MIC) of PRI. Pristinamycin had a very rapid concentration dependent bactericidal activity against wild-type and ERY-R *mefA* isolates. Pristinamycin had a time dependent bactericidal activity against ERY-R *ermA* subtype *ermTR* and ERY-R *ermB* genotype. Pristinamycin may be an alternative to beta-lactams in the treatment of *S. pyogenes* ERY-R.

P1293

In vitro activity of antimicrobial agent combinations against *Brucella melitensis* isolates by E test

S. Kaya Firat, B. Oral, F.S. Erdinc, M.A. Yetkin, N. Tulek, A.P. Demiröz (Ankara, Samsun, TR)

Objectives: Brucellosis is a zoonotic disease seen world-wide including Turkey. The management of the disease is difficult sometimes because of the intracellular localization of the *Brucella* species. Antibiotics that penetrates into the macrophages should be used in combination. In our study *in vitro* activities of streptomycin, tetracycline and rifampicin were investigated.

Methods: *In vitro* activities of antimicrobial agent combinations were tested against *Brucella melitensis* strains isolated from 30 patients treated at Infectious Diseases and Clinical Microbiology Department of Ankara Research and Training Hospital between 1999 and 2002. *In vitro* effectiveness of streptomycin-tetracycline, rifampicin-tetracycline and streptomycin-rifampicin combinations were tested by E test method. The interactivity of *in vitro* combinations were evaluated as synergism, indifference and antagonism depending on the conclusions of FIK index calculated for each strain.

Results: Synergism was found in 20 strains (66.7%) with streptomycin-tetracycline combination, in 26 strains (86.7%) with rifampicin-tetracycline combination and in 18 strains (60%) with rifampicin-streptomycin combination. Antagonism was found with rifampicin-streptomycin combination in only one strain. The rate of synergy of rifampicin-tetracycline combination was found higher compared to other combinations and this difference was statistically significant. We found that the rifampicin-tetracycline combination was the most effective combination *in vitro*.

Conclusion: Although routine antimicrobial susceptibility testing for *Brucella* isolates is not recommended, in case of treatment failure, relapses and reinfection synergy test with E test may be helpful for treatment approach.

P1294

Incidence and resistance patterns of pathogens from lower respiratory tract infections

O. Dorobat, A. Moisoiu, D. Talapan (Bucharest, RO)

Objectives: To evaluate the incidence and antibiotic susceptibility of pathogens in chronic LRTI and nosocomial infections in a clinical hospital of respiratory diseases.

Methods: A number of 938 strains isolated between September 1st 2004 and September 1st 2005 were identified by standard procedures and antimicrobial resistance was determined following CLSI approved standard. The majority of Gram-negative and Gram-positive bacteria were tested against 14 and 9 or 6 antimicrobials, respectively. Imipenem-EDTA Double Disk Synergy test and Etest were used for detection of metallo-beta-lactamase producing isolates of *P. aeruginosa*.

Results: There were isolated 744 Gram-negative strains: *H. influenzae* 34.6%, *P. aeruginosa* 17.7%, *H. parainfluenzae* 15.9%, *K. pneumoniae* 8.6% and another spp. and 194 Gram-positive strains: 54.1% *S. aureus* and 45.9% *S. pneumoniae*. Among *H. influenzae* and *H. parainfluenzae* isolates, the highest resistance rate was to trimethoprim/sulfamethoxazole (SXT 30.9% and 31.1%), followed by ampicillin (AMP 11.6% and 13.4%), chloramphenicol (CHL 4.5% and 5.1%) and clarithromycin (1.6% and 13.6%). *P. aeruginosa* strains showed a resistance rate between 7.9% to amikacin and 38.3% to cefoperazone. The resistance to imipenem (IPM) and meropenem (MEM) was close: 28.2% and 26.0%. From 36 *P. aeruginosa* IPM and multidrug resistant strains tested, 8 were probably producing of metallo-beta-lactamase. For *S. aureus* the highest resistance rate was to penicillin 93.3% followed by erythromycin (ERY 45.7%), oxacillin (OXA 41.9%) and CIP (33.3%); all strains were susceptible to vancomycin (VAN), teicoplanin and linezolid. From *S. pneumoniae* strains 13.4% were high resistant to penicillin and 39.3% were intermediate resistant. The resistance rate was 64.0% to SXT, 18.8% to ERY, 8.3% to CHL and all strains were susceptible to ofloxacin and VAN. *K. pneumoniae* strains were resistant to cefepime (11.3%), CIP (7.8%) and there was no resistant strain to IPM and MEM.

Conclusion: Our study indicate that: (1) *H. influenzae* and *H. parainfluenzae* the most common cause of chronic LRTI were most resistant to SXT, AMP, CHL. (2) *P. aeruginosa*, the second pathogen, was mostly resistant to cephalosporins; a higher resistance to IPM was observed in nosocomial infections. (3) From *P. aeruginosa* IPM resistant strains, 22.2% probably produced metallo-beta-lactamase. (4) *S. aureus* resistance rate to OXA was high. (5) From *S. pneumoniae* strains, 52.7% were resistant to penicillin.

P1295

Comparative study of some antibacterial agents in patients with shigellosis

M. Stoycheva-Vartigova, P. Koroljova (Plovdiv, BG)

Objectives: Treatment of shigellosis has been confounded by the increasing bacterial resistance to some antibacterial agents. The aim of this study was to outline a comparison between the effect of several antibacterial agents on the clinical course and early clearance of bacteria from faeces in patients with shigellosis and to suggest timely recommendations for empiric antibiotic therapy.

Methods: The study comprised 194 adult patients with culture proven shigellosis at the Clinics of Infectious Diseases, University Hospital-Plovdiv, Bulgaria. The antimicrobial susceptibility of *Shigella* isolates was tested by Kirby-Bauer disk diffusion method to gentamicin, amikacin, ciprofloxacin, ceftazidim and ceftriaxone. Antibacterial therapy was assumed as adequate or inadequate according to the *in vitro* susceptibility patterns.

Abstracts

Results: Adequate antibacterial therapy resulted in clinical cure in most of the treated patients (83%) whereas only 41% of patients were with clinical improvement after inadequate antibacterial treatment. Similarly good clinical effect was observed in 46% of patients without antibacterial therapy proving that in some patients shigellosis is a self-limiting infection. Elimination of *Shigella* from faeces was most frequent in patients (nearly 80%) with adequate therapy by amikacin, ciprofloxacin and ceftriaxone compared to those with inadequate or lacking antibacterial treatment.

Conclusion: These agents may be recommended for initial empiric antibacterial treatment in shigellosis.

P1296

Preliminary report on a specific anti-*Helicobacter pylori* microbicidal peptides produced by clinical strain of *Candida albicans*

E. Karczewska, P. Mak, A. Targosz, D. Trojanowska, M. Wcislo, A. Budak (Cracow, PL)

Objectives: The presented abstract demonstrate preliminary results of chromatographic fractionation of *Candida albicans* cells homogenate. The homogenate was obtained from a special clinical yeast-like fungus strain isolated from the stomach ulcer patient that simultaneously does not suffer from *Helicobacter pylori* infection. The results of fractionation prove, that the isolated *C. albicans* strain produce a proteinaceous factor that is able to selectively kill *H. pylori* cells.

Methods: *C. albicans* clinical isolate was grown on the Sabouraud medium, the cells were spun down, washed twice in the phosphate buffered saline and homogenized in the glass bead homogenizer. The homogenate was centrifuged, obtained clear supernatant was acidified, desalted on a solid-phase extraction cartridge and then fractionated on a C-18 reversed-phase column using high-performance liquid chromatography (HPLC) system and elution with a linear gradient of buffers containing mixture of water, acetonitrile and trifluoroacetic acid. Fourteen collected fractions were vacuum-dried, redissolved in water and subjected to SDS-PAGE electrophoresis as well as to *H. pylori* killing assay.

Results: Chromatographic separation of whole *C. albicans* cell lysate demonstrate that this material is especially rich in small proteins and peptides having molecular weights in range of 5 to 20 kDa. Two last fractions, containing most hydrophobic components, had pronounced killing activity toward *H. pylori*.

Conclusions: Obtained results prove that some strains of *C. albicans* produce a variety of proteinaceous factors that inhibit the growth and kill *H. pylori* cells. This observation explain a clinical phenomenon that many patients suffering from stomach ulcers and simultaneously from *C. albicans* infection simultaneously do not exhibit symptoms of *H. pylori* infection. We are conducting intensive research to purify these specific anti-*H. pylori* peptides in a homogenous form.

P1297

In vitro demonstration of synergy with two bacteriophage lytic enzymes (Cpl-1 and Pal), autolysin (LytA), and cefotaxime against *Streptococcus pneumoniae*

M. Gracia, V. Rodriguez-Cerrato, E. Garcia, P. Garcia, R. Lopez, G. del Prado, C. Martinez-Marin, L. Huelves, C. Ponte, F. Soriano on behalf of the Spanish Pneumococcal Infection Study Network G03/103

Objective: New therapeutic alternatives are currently explored to combat multi-drug resistant pneumococcal infections. We

perform an *in vitro* study to determine the activity of Cpl-1, Pal, LytA, and cefotaxime alone and in combination against two pneumococcal strains.

Methods: Cpl-1, Pal, and LytA were overproduced in *Escherichia coli* (strains HB101 [pCIP100], DH5alpha [pMSP11], and RB791 [pGL100], respectively) and purified by affinity chromatography in DEAE-cellulose columns. Two pneumococcal clinical isolates, one involved in meningitis (strain 3693, serotype 19F), and one in lower respiratory tract infection (strain AR-33118, serotype 3) were used. MIC determinations and synergy testing were performed by the checkerboard method in microtitre trays with cation-supplemented Mueller Hinton broth containing 4% lysed horse blood. Inocula were prepared by suspension of colonies to produce final inocula of approximately 5×10^5 cfu/mL and trays were incubated overnight at 35°C. MIC and Fractional Inhibitory Concentration (FIC) values were determined by standard methods and FIC results were interpreted as follows: ≤ 0.5 (synergy), > 0.5 to 4.0 (indifferent), and > 4 (antagonism).

Results: the MIC values of Cpl-1, Pal, LytA, and cefotaxime were 16, 128, 16, and 4 mg/L for strain 3693, and 16, 32, 8, and ≤ 0.015 for strain AR-33118, respectively. Cpl-1 and Pal combination showed synergism against both strains. LytA combined with Cpl-1 or with Pal showed indifference for both strains. Synergy was demonstrated with Cpl-1 or LytA combined with cefotaxime, but indifference was shown with Pal combined with cefotaxime when the highly-cephalosporin resistant isolate 3693 was tested. FIC indices indicating antagonism were not found.

Conclusions: The synergistic effect showed between Cpl-1 and Pal, and between Cpl-1 or LytA with cefotaxime in this study warrants the evaluation in *in vivo* infection models using antibiotic-resistant pneumococcal strains.

P1298

Activity of moxifloxacin and comparators against isolates of *Chlamydia trachomatis* from the UK

L. Williams, J. Northwood, I. Morrissey (London, UK)

Background: *Chlamydia trachomatis* (serovars A–K) is by far the most common sexually transmitted infection worldwide, especially amongst adolescents, and is a major cause of acute pelvic inflammatory disease (PID). Tetracyclines have been the basis of antichlamydial therapy for over 20 years, with quinolones becoming an important alternative. Moxifloxacin (MXF) is a fourth-generation fluoroquinolone with enhanced broad spectrum *in vitro* antimicrobial activity. In this study we compared the activity of MXF with levofloxacin (LFX), erythromycin (ERY), azithromycin (AZI) and doxycycline (DOX), against 51 *Chlamydia trachomatis* (CT) isolates.

Methods: Isolates collected between 1999 and 2004 from UK laboratories were included in the analysis. MICs of MXF and the 4 comparators were determined by a broth microdilution method, using McCoy cells in a supplemented McCoy Growth Medium.

Results:

		MIC (mg/L)			
		50%	90%	Range	
CT	MXF	0.06	0.06	0.03	- 0.12
51 isolates	LFX	1	1	0.25	- 1
	DOX	0.015	0.03	0.008	- 0.03
	ERY	0.12	0.25	0.06	- 0.25
	AZI	0.25	0.5	0.12	- 1

Conclusions: The data show that MXF produced lower MICs against all *C. trachomatis* isolates. MXF showed greater activity than LFX against this species with improved activity compared

with the macrolides ERY and AZI. This antimicrobial activity compliments the activity already shown against other bacteria, for the empirical treatment of genital tract infections and acute PID.

P1299

The antimicrobial susceptibility of bacteraemia isolates of Gram-negative pathogens in seven haematological centres in Russia

G. Kliasova, A. Mironova, L. Speranskaya, S. Ptitsin, A. Maschan, N. Yuritsina, S. Vereschagina, T. Kaporskaya, L. Krainova, T. Pospelova, O. Markina (Moscow, Samara, Irkutsk, Novosibirsk, Ekaterinburg, RU)

Objectives: To evaluate the antimicrobial susceptibility of GNP from blood in haematological centres (HC) of Russia.

Methods: Minimal inhibitory concentrations of antimicrobial agents were determined by the broth microdilution (NCCLS 2003).

Results: 288 GNP were isolated between January 2003 and October 2005. The predominant isolates were: *E. coli* (38%), *Klebsiella* spp. (16%), *P. aeruginosa* (16%), *Acinetobacter* spp. (9%), *E. cloacae* (7%). 42% enterobacteria strains were confirmed as ESBL producers. The susceptibility data (%) were for main species are summarized in Table:

	<i>E.coli</i> n=108	<i>Klebsiella</i> spp n=46	<i>P.aeruginosa</i> n=45	<i>Acinetobacter</i> spp. n=19
cefotaxime	79	39	11	23
ceftriaxone	80	39	8	19
cefazidime	78	41	24	11
cefoperazone/ sulbactam	91	70	64	88
cefepime	83	41	56	19
imipenem	100	100	67	77
meropenem	100	100	67	42
gentamicin	67	46	55	15
amikacin	80	83	69	31

Conclusion: *P. aeruginosa* indicated high levels of resistance to antimicrobial agents. The most active antibiotics against *Enterobacteriaceae* were carbapenems, cefoperazone/sulbactam, cefepime. *Acinetobacter* spp. showed the highest susceptibility rate to cefoperazone/sulbactam and high resistance to meropenem.

P1300

Extending observed susceptibilities with predicted susceptibilities based on a retrospective statistical analysis

A.D. Nielsen, S. Andreassen, M. Paul, L. Leibovici on behalf of the TREAT Study group

Objectives: *In vitro* testing of bacterial susceptibility is usually performed on a limited set of antibiotics, which may not necessarily include all relevant antibiotics. Clinically, coverage of untested antibiotics can sometimes be predicted from the *in vitro* results, using known cross-resistance properties for the antibiotics. The objective is to establish a statistically valid method for extending *in vitro* results to a larger set of untested antibiotics.

Methods: For each of several Gram negative bacteria the a priori coverage for 19 antibiotics, P(antibiotic1 = covering), and the conditional coverage given that another antibiotic was covering, P(antibiotic1 = covering | antibiotic2 = covering) or non-covering P(antibiotic1 = covering | antibiotic2 = non-covering), was calculated. Fisher's test (p s = 0.05) was used to test establish significant increases or decreases in susceptibility, given prior knowledge of susceptibility to the *in vitro* results.

Results: The susceptibilities of *Acinetobacter* sp., *Campylobacter* sp., *Citrobacter* sp., *Enterobacter* sp., *Escherichia coli*, *Haemophilus* sp., *Klebsiella* sp., *Pseudomonas* sp., and *Salmonella* sp. were evaluated using this method on a bacteraemia database (containing 11000 positive blood culture cases) from Rabin Medical Center, Israel. For example, for *Escherichia coli*, the observation of susceptibility to cefotaxime (a priori susceptibility: 89%) significantly increases the belief in susceptibility for an additional 4 antibiotics (ceftriaxone, aztreonam, ceftazidime, amikacin) to more than 95% (see table line 2). The observation of resistance to cefotaxime reduced the belief in susceptibility for an additional 5 antibiotics (ampicillin, cefazolin, piperacillin, cefuroxime, ceftriaxone) to below 5%.

Escherichia coli Coverage %	Ampicillin	Cefazolin	Piperacillin	Cefuroxime	Cefotaxime	Ceftriaxone	Aztreonam	Ceftazidime	Amikacin
P(covering)	31	32	43	81	89	89	88	95	93
P(cov cefotaxime covering)					1	1	98	1	1
P(cov cefotaxime non-cov)	0	0	0	0	0	3			

Conclusions: The use of statistically verified cross-resistance can be used to extend the predictions of coverage or non-coverage to a substantial set of antibiotics not included in the *in vitro* test. This typically expands the number of clinically acceptable choices by several appropriate antibiotic therapies, as well as eliminating several others as inappropriate.

P1301

Moxifloxacin in vitro activity against non-pneumococcal streptococci isolated from six recent clinical studies

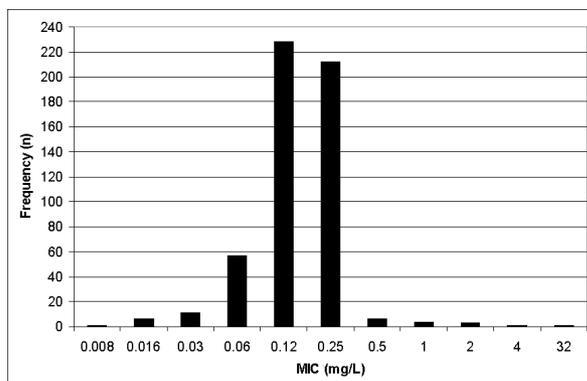
J.E. Ambler, S. Choudhri, D. Haverstock, J. Song, P. Reimnitz (West Haven, US; Leverkusen, DE)

Objectives: To determine clinically relevant susceptibility breakpoints for moxifloxacin against non-pneumococcal streptococci based on MIC frequency distributions.

Methods: Moxifloxacin MIC distributions were pooled for streptococci group A, B, C and D for four controlled, double-blinded, randomised, parallel group, phase III studies and two open-label studies. Indications were in uncomplicated and complicated skin and skin-structure infection and complicated intra-abdominal infection (2 of each indication).

Results: For moxifloxacin-treated patients (n = 217), and all patients (n = 530) the moxifloxacin MIC90 against all streptococci was 0.25 mg/L, the MIC50 0.12 mg/L and MIC range 0.008–32 mg/L. For beta- streptococci, for moxifloxacin-treated patients (n = 73), the moxifloxacin MIC90 was 0.25 mg/L, MIC50 0.12 mg/L and MIC range 0.03–2 mg/L; results were similar for all patients (n = 160), though the MIC range was 0.016–2 mg/L. For viridans streptococci, for both moxifloxacin-treated (n = 144) and all patients (n = 370), the moxifloxacin MIC90 was 0.25 mg/L, MIC50 0.12 mg/L and MIC range 0.008–32 mg/L. Using interpretative criteria of ≤1 mg/L for susceptible, 2 mg/L intermediate and >2 mg/L for resistant, the moxifloxacin MIC frequency distribution for all streptococci (see figure) shows 99.1% of strains susceptible, 0.6% intermediate and 0.4% resistant. Results were similar for moxifloxacin-treated patients only and for beta- and viridans streptococci separately. These results are similar to those of 5,160 non-pneumococcal streptococci pooled from published *in vitro* activity studies (MIC90 0.25 mg/L, MIC50 0.12 mg/L).

Abstracts



Conclusion: The moxifloxacin MIC frequency distribution of both clinical and *in vitro* isolates of streptococci groups A, B, C and D support a clinical breakpoint of susceptible ≤ 1 mg/L, intermediate 2 mg/L and resistant >2 mg/L.

P1302

In vitro* combination of rifampin with other antimicrobial agents against clinical isolates of *Stenotrophomonas maltophilia

S. Gandotra, M. El-Azizi, A. Adamski, J. Koirala, N. Khardori (Springfield, US)

Objectives: *Stenotrophomonas maltophilia*, often multi drug resistant, is a nosocomial pathogen of increasing importance especially in immunocompromised patients. We evaluated the effectiveness of double antibiotic combinations against MDR clinical isolates of *S. maltophilia*.

Methods: We tested the *in vitro* activity of Trimethoprim/Sulfamethoxazole (TMP/SMX), Ticarcillin/Clavulanic acid (TIC/CLV), Ciprofloxacin (CIP), Ceftazidime (CFZ) and Doxycycline (DOX) alone and in combination with Rifampin (RIF) against 37 clinical isolates of *S. maltophilia* recovered from blood, urine, wound and sputum. The MICs were determined by using the broth microdilution technique according to the Clinical Laboratory Standards Institute. Drug combinations were tested by using checkerboard and time kill assays.

Results: Synergistic effects were observed in combination of RIF with DOX (49%), CFZ (41%), CIP (31%), TIC/CLV (30%) and TMP/SMX (11%). Antagonism was observed in combination of RIF with CIP (10%), TMP/SMX (8%), and TIC/CLV (3%). No antagonism was observed in combination of RIF with either DOX or CFZ.

Conclusion: Our data show that combination of RIF with any of the tested antibiotics was more effective than using single drug alone against MDR *S. maltophilia*.

P1303

Activity of *Thymus vulgaris* L. essential oil against potential causative agents of premature delivery

L. Suvajdzic, M. Bogavac, B. Bozin, N. Mimica-Dukic, M. Mikov, N. Simin (Novi Sad, CS; Otago, NZ)

Objective: Reducing the number of preterm deliveries presents the main obstetric goal, especially in the area of Vojvodina, where we have been facing a negative rate of natural population growth since few decades. A number of organisms, which normally colonize vagina (*Escherichia coli*, *Klebsiella*, *Enterococcus*,

Streptococcus group B) were identified as causative agents of preterm premature rupture of amniotic membrane (PPROM) and premature delivery. Negative effects of most antibiotics and vaginal tablets/suppositories in the first trimester of pregnancy are well known. In that respect, there is a growing tendency towards developing new natural antibacterial substances and generating innovative pharmaceutical preparations on natural basis. In the present study, the results of biological activity of thyme (*Thymus vulgaris* L.) essential oil were presented.

Methods: The essential oil was isolated from dried aerial parts of thyme (cultivated in Vojvodina) by hydrodistillation. The composition of essential oil was evaluated by GC-MS. RSC was assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl)-method (Kirby and Schmidt, 1997) and measuring OH⁻ radical induced degradation of 2-deoxyribose. Inhibition on LP in both systems of induction was evaluated by TBA-test (Afanasev et al.1989) Antibacterial activity of essential oil was tested against cited bacterial strains, by standard antibiogram test.

Results: The examined essential oil strongly reduced free radical formation of DPPH (IC₅₀ = 0.19 μ g/ml). The inhibition 2-deoxyribose degradation was 34.38% (2.13 μ g/ml). In both examined systems of induction, essential oil (2.13 μ g/ml) expressed significant inhibitory effects on LP (54.10% in Fe²⁺/H₂O₂ system). The most effective antibacterial activity was observed against *Pasteurella* sp., *E. coli*, *Klebsiella*. Moderate activity was observed against *Enterococcus*, *Streptococcus* group B and *Staphylococcus aureus*.

Discussion and conclusion: Besides the well known use of aromatic plants and their essential oils as flavouring agents in a wide range of food, beverage and confectionery products, they have been known to support various biological activities such as antimicrobial (Mimica-Dukic et al., 2003) and hepatoprotective (Kuresh and Deans, 1999). The obtained results suggest that thyme could serve in prevention, treatment or supplement-therapy of vaginosis induced by the specified bacterial species. Its possible clinical application still requires further investigation.

P1304

Sensitivity testing and empirical treatment of pneumonia and acute exacerbations of COPD

S. Marinkovic, I. Marekovic, B. Balabanic-Kamauf, F. Pavicic, M. Jakopovic, M. Korsic (Zagreb, HR)

Objectives: Start of antimicrobial treatment of acute lower respiratory tract infections (pneumonia, acute exacerbations of COPD) is usually empirical. Most of the guidelines in patients with no co-morbidity or risk factors recommend per os macrolide or doxycycline, and in patients with risk factors with combination of β -lactam and macrolide or quinolone. The most important parameters for choosing appropriate antimicrobial agent is possible bacteria and it has sensitivity to antimicrobial agents at specific area. The aim is to estimate sensitivity of the most common respiratory pathogens to antimicrobial agents empirically used according to current guidelines in University Hospital for Lung Diseases Jordanovac.

Methods: Sensitivity to antimicrobial agents was followed during years 2003 and 2004 for isolates of: *S. pneumoniae* (N = 128), *H. influenzae* (N = 76), *K. pneumoniae* (N = 128) and *P. aeruginosa* (N = 198). Sensitivity was tested with diffusion method according to NCCLS standards. E-test was used to determine MIC, thus, *S. pneumoniae* isolates could be separated to moderately and highly resistant.

Results: In 2004 percentage of decreased sensitivity to penicillin *S. pneumoniae* slightly increased, although, this increase was due

to increase in moderately resistant *S. pneumoniae*. In the follow up period no highly resistant isolates were recorded. Resistance of *S. pneumoniae* to macrolides slightly decreased. During follow up period we found no resistant isolates to moxifloxacin, though, we recorded some isolates resistant to ciprofloxacin. *K. pneumoniae* resistance to co-amoxiclav and ciprofloxacin increase slightly. Resistance of *K. pneumoniae* to cephalosporines of 2nd and 3rd generation was less than 10%. *H. influenzae* resistance to amoxicillin was 14%, and all of the isolates are sensitive to co-amoxiclav and 2nd and 3rd generation of cephalosporines. During the follow up period resistance of *P. aeruginosa* to ceftazidime increased slightly and at the same time resistance decreased to carbapenems and ciprofloxacin.

Conclusion: Sensitivity of most common respiratory bacterial pathogens tested in University hospital for lung diseases is according to current guidelines for empirical treatment of respiratory infections. Future continuous follow up is obligatory because change in sensitivity dictate choice of empirical antimicrobial agent.

P1305

Comparative *in vitro* activity of levofloxacin against enterobacteria isolated from intra-abdominal infections

M. Mohseni Zadeh, S. Kopp, F. Jehl (Strasbourg, FR)

Objectives: Newer fluoroquinolons have seen their fields of action increased, especially with their recent use in intra-abdominal infections. In this study, conducted at the University Hospital of Strasbourg (France) we evaluated the sensitivity to levofloxacin of Enterobacteria isolated from abdominal infections, as well as other frequently used antibiotics, between June and October 2005.

Materials and methods: We prospectively collected 69 strains of Enterobacteria isolated from intra-abdominal infections and 1 strain of *Bacteroides* sp. All strains were obtained either from blood cultures or intra-abdominal specimens. Minimal inhibitory concentrations (MICs) of levofloxacin (LVX), ofloxacin (OFX), ciprofloxacin (CIP), amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (TAZ), imipenem (IMI), ceftriaxon (CRO), amikacin (AKN) and gentamicin (GN) for each strain were obtained by the agar dilution method and MIC50 and MIC90 were calculated.

Results: Enterobacteria collected were mostly *Escherichia coli* (n = 42; 60%) followed by *Klebsiella* sp. (n = 9), *Enterobacter* sp. (n = 8), *Proteus mirabilis* (n = 3), *Serratia* sp. (n = 3), *Salmonella* sp. (n = 2), *Citrobacter* sp. (n = 2) and *Bacteroides* sp. (n = 1). MIC50 and MIC90 of each antibiotic tested for all 70 strains and those for *E. coli* strains are summarized in table 1. 63 strains (90%) were sensitive to LVX and other fluoroquinolons (FQ). Only 4 strains of *E. coli* (5.7% of all strains collected), 2 *Enterobacter cloacae* strains and 1 *Serratia* sp. strain were resistant to FQ.

	LVX	OFX	CIP	AMC	TAZ	IMI	CRO	AKN	GN
<i>E. coli</i>									
strains	MIC 50	0.062	0.062	0.015	4	2	0.125	0.062	2
	MIC 90	0.25	0.5	0.125	64	2	0.125	0.062	4
	MIC 50	0.062	0.062	0.015	4	2	0.125	0.062	2
	MIC 90	0.5	1	0.25	128	16	0.5	0.5	4
All strains	%susceptible	90%	87%	90%	53%	87%	98%	91%	95%
	strains								96%

Conclusions: This study confirms the good activity of LVX against strains encountered in intra-abdominal infections.

P1306

Activity of beta-lactam/beta-lactamase inhibitor combinations against ESBL producing *Enterobacteriaceae*

B. Rybak, L. Naumiuk, M. Bronk, A. Samet (Gdansk, PL)

Objectives: Beta-lactam/beta-lactamase inhibitor are sometimes considered an useful alternative to carbapenems in treating infections caused by ESBL producing organisms. The aim of the study was to compare *in vitro* activity of amoxicillin-clavulanate (AMC), piperacilline-tazobactam (TZP) and cefoperazone-sulbactam (CSL) against ESBL (extended-spectrum beta-lactamase) producing *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter freundii* and *Serratia marcescens*.

Methods: Over twenty-one month study (January 2004–September 2005) we isolated 464 nonreplicate ESBL positive isolates of above mentioned species from patients hospitalized in Public Hospital No.1. All isolates exhibited ESBL phenotype in routine double-disk synergy test (DDS) with cefotaxime, amoxicillin-clavulanate and ceftazidime disks. Antimicrobial susceptibility testing was done according to disk diffusion CLSI guidelines. Bacteria were divided to 2 groups EK with *E. coli* and *Klebsiella* spp. and SEC with *S. marcescens*, *Enterobacter* spp. and *C. freundii*.

Results: The most active *in vitro* antibiotic in 372 EK isolates was TZP (56% susceptible) followed by CSL (53%) and AMC (16%). In much smaller SEC group (92 isolates) 35% were susceptible to TZP, 30% to CSL and 2% to AMC. Isolates were recovered from urine (34%), stools and rectal swabs (30%), respiratory tract (14%), blood culture (3.4%) and other (18.6%).

Conclusions: TZP and CSL were found active against half to one third of isolates depending of the species involved (EK and SCE respectively). AMC was active *in vitro* against a smaller fraction of EK group and inactive against SEC group due to AmpC enzyme production. TZP was only slightly more active against all isolates than CLS. Since they both contain different beta-lactam and beta-lactamase inhibitor it is difficult to point the factor which is responsible for their comparable activity.

P1307

Dynamic bactericidal activity of ceftazidime in combination with glycopeptides and azithromycin against *Pseudomonas aeruginosa*

B. Repetto, A. Palenzona, A. Marchese, E.A. Debbia (Genoa, IT)

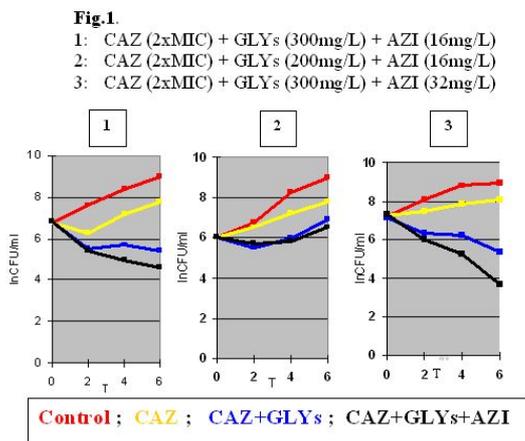
Objectives: *Pseudomonas aeruginosa* is an opportunistic pathogen. It is intrinsically resistant to many antibiotics and evolution toward resistance is very frequently. The low permeability of the outer membrane, due to the presence of lipopolisaccaride (LPS), is considered in part responsible of this resistance. It is assumed that some agents might disorganize the structure of LPS and allow the passage of other drugs into bacteria cell. In order to verify this hypothesis, ceftazidime (CAZ) has been tested in association with glycopeptides (GLYs) and azithromycin (AZI), which is a non anti-pseudomonal agents but has been shown to interfere with some virulence factors.

Methods: A bacterial suspension of about 109 CFU/ml was seeded on plates containing increasing doses of CAZ and a fixed concentration of GLYs (300 mg/l) and AZI (16 mg/l) Results were interpreted as synergism (99%), additivity (90%), and indifference (10%) of the CFU/ml reduction found in the drugs combination in comparison to the drug alone. Time-kill experiments were performed by adding the drugs to log-phase bacterial cultures diluted to 106–107 CFU/mL growing in 100 mL flasks at 37°C. Just before the compounds were added

Abstracts

and at 2, 4, 6 and 24 hr thereafter, bacterial counts were carried out. Survivors were evaluated by determining CFU on agar plates.

Results: CAZ in combination with GLYs reacted synergically in 35% of cases, additivity was found 52% of cases and indifference was noted in 13% of tests; the addition of AZI increased the incidence of synergisms to 42%, additivities to 49% and indifferences to 9%. The combinations that were tested through the dynamic bactericidal tests are CAZ (2xMIC)+GLYs (300 mg/l)+AZI (16 mg/l), CAZ (2xMIC)+GLYs (200 mg/l)+AZI (16 mg/l) and CAZ (2xMIC)+GLYs (300 mg/l)+AZI (32 mg/l). Preliminary results indicated that antimicrobial effect is always significant 6 hr thereafter, in particular at the presence of AZI; instead the lethal effect is reduced when GLYs is used at concentration of 200 mg/l and it is increased when AZI is used at concentration of 32 mg/l (fig.1).



Conclusion: The present findings demonstrated that CAZ favourably reacted with GLIs at the presence of AZI. Further studies to determine the best combinations among the drugs to achieve highest rate of synergism are under way.

P1308

Comparative antimicrobial susceptibilities of *Streptococcus pneumoniae* and *Haemophilus influenzae* in a university hospital in Turkey

A. Ilki, P. Sagiroglu, N. Elgormus, U. Over-Hasdemir, G. Soyletir (Istanbul, TR)

Objective: Increasing rates of resistance in both *Streptococcus pneumoniae* and *Haemophilus influenzae* to clinically useful antimicrobials are major concern worldwide. In this study we aimed to investigate the comparative susceptibility patterns of these pathogens to antimicrobials (Table 1.).

Materials and Methods: Clinical strains of *S. pneumoniae* and *H. influenzae* isolated between 2002 and 2005 were included. In pneumococci, E-test was used for determination of MIC of penicillin, and susceptibility to other antimicrobials were performed by disk diffusion test (CLSI, M2-A8). Nevertheless, ampicillin MIC determinations were also studied in *H. influenzae* strains showing resistance to ampicillin in disk diffusion test. Nitrocefin was used for detection of beta-lactamase activity in *H. influenzae*.

Results: *S. pneumoniae*; Overall resistance to penicillin was 36.0%. Intermediate and full resistance to penicillin were 25.3% and 10.7%, respectively. Percentages of fully penicillin resistant pneumococci gradually decreased by year: 14.3% in 2003; 10.7% in 2004; 6.3% in 2005. There were no marked

changes in MIC50 and MIC90 values over 3 years. Penicillin resistant pneumococci were also highly resistant to erythromycin, tetracycline and trimethoprim-sulfamethoxazole. No resistance was detected against ofloxacin and vancomycin (Table 1.). *H. influenzae*; Overall incidence of beta lactamase production was 2.8%. Ampicillin resistance was only determined in beta lactamase producers; except one beta lactamase negative isolate presenting increased MIC (2.0 µg/ml) of ampicillin. There was considerable resistance to chloramphenicol and trimethoprim-sulfamethoxazole in ampicillin resistant strains. None of the strains was found resistant to ampicillin sulbactam, azithromycin, and cefotaxime.

Table 1. Comparative resistance rates to various antimicrobials in *S. pneumoniae* and *H. influenzae* isolates

Antimicrobials	<i>S. pneumoniae</i> (n:150)		<i>H. influenzae</i> (n:387)	
	All isolates (n:150) n (%)	PK (n:54) n (%)	All isolates (n:387) n (%)	BL** positives (n:11) n (%)
Penicillin	54 (36.0)	54 (100)	-	-
Erythromycin	23 (15.4)	19 (35.2)	-	-
Tetracycline	21 (14.0)	17 (31.5)	-	-
Ofloxacin	0	0	-	-
Vancomycin	0	0	-	-
Trimethoprim-sulfamethoxazole	57 (38.0)	35 (64.8)	90 (23.3)	7 (63.6)
Chloramphenicol	8 (5.3)	6 (11.1)	7 (1.8)	4 (36.4)
Ampicillin	-	-	12 (3.1)	11 (100)
Ampicillin-sulbactam	-	-	0	0
Azithromycin	-	-	0	0
Cefaclor	-	-	3 (0.8)	1 (9.1)
Ceftriaxime	-	-	0	0

* Penicillin resistant isolates

** Beta lactamase

Conclusion: When compared with older studies in our hospital, full penicillin resistance in pneumococci reached to alarming levels with cross resistance to erythromycin, a commonly used alternative drug in respiratory tract pneumococcal infections. It is pleasant to observe no resistance to other alternative drugs including ofloxacin and vancomycin in pneumococci. Fortunately, ampicillin resistance in *H. influenzae* is still in its initiative levels and seems to be totally due to beta-lactamase production.

P1309

Reliability of daptomycin E-test strips for susceptibility testing

R. Cantón, J. Bille, A. MacGowan, C.E. Nord, G. Schito, H. Seifert, C. Soussy, M. Struelens, J. Verhoef, I. Morrissey (Madrid, ES; Lausanne, CH; Bristol, UK; Stockholm, SE; Genoa, IT; Cologne, DE; reitel, FR; Brussels, BE; Utrecht, NL; London, UK)

Objectives: Daptomycin (DAP) is a new cyclic lipopeptide antibiotic active against Gram-positive bacteria. Approval is being sought in Europe for the treatment of complicated skin and skin-structure infections. This study compared local laboratory DAP susceptibility testing by E-test (ET) with central reference broth microdilution methodology (BM).

Methods: *Staphylococcus aureus* (SA) and coagulase-negative *Staphylococci* (CNS), including methicillin resistant strains, *Enterococcus faecalis* (Efc) and *E. faecium* (Efm), including vancomycin (VAN) resistant strains, *Streptococci* (Str) and corynebacteria (Cor) were collected by 81 centres in Austria, Belgium, France, Germany, Ireland, Italy, Netherlands, Portugal, Spain, Sweden, Switzerland and UK (October 2004–March 2005). ET MIC for DAP (ET strip supplemented with Ca²⁺), teicoplanin (TEI) and VAN was determined using local methods. MIC was repeated in a central lab using CLSI BM. CLSI breakpoints were used but TEI EUCAST and SA CLSI breakpoints were applied for Str and Cor, respectively.

Results: Complete categorical agreement for DAP, TEI and VAN was 94.7%, 95.2% and 98.7% respectively. False susceptible (S) (DAP, TEI or VAN BM non-susceptible but ET susceptible)

and false resistant (R) (BM susceptible but ET non-susceptible) results are shown in the table. False-S results were low and less common for DAP than TEI or VAN. False-R was higher for DAP but most SA, CNS or Str had ET MIC of 1.5 or 2 mg/L (just above the DAP breakpoint of 1 mg/L). Efm was the most problematical for all agents, especially TEI.

		Number of isolates (%)					
		SA (711)	CNS (609)	Efc (318)	Efm (361)	Str (785)	Cor (83)
DAP	False-S	0	0	0	0	1 (0.1)	0
	False-R	43 (6.0)	43 (7.1)	0	33 (9.1)	29 (3.7)	2 (2.4)
TEI	False-S	0	6 (1.0)	6 (1.9)	23 (6.4)	0	0
	False-R	2 (0.3)	91 (14.9)	5 (1.6)	11 (3.0)	1 (0.1)	0
VAN	False-S	0	0	2 (0.6)	17 (4.7)	0	0
	False-R	0	5 (0.8)	6 (1.9)	5 (1.4)	3 (0.4)	0

Conclusion: ET can be relied upon to accurately ascertain susceptibility to DAP with high categorical agreement (>94%) and a very low false-S rate, this being a bigger problem than false-R.

P1310

In vitro antimicrobial activity of gatifloxacin against staphylococci

C. Tuchilus, A. Poiata, A. Vornicu, O. Gacea, D. Buiuc (Iasi, RO)

Gatifloxacin is in a class of drug called fluoroquinolone antibiotics. It eliminates bacteria that cause many infections, such as pneumonia and bronchitis, sinus, respiratory tract and urinary tract infections and sexually transmitted diseases. Gatifloxacin ophtalmic solution is used to treat bacterial conjunctivitis.

Objective: To assess trends in the susceptibility to ofloxacin and gatifloxacin of 634 staphylococci, communitary isolates.

Material and methods:

Microorganisms: A total of 634 staphylococci: 254 coagulase-positive staphylococci (CPS) and coagulase-negative staphylococci (CNS), colonising skin and nasal mucous, obtained from medical personal in 2005 year. Isolates were identified as CPS by demonstrating a positive coagulase test. Antimicrobial agents. Powders of ofloxacin and gatifloxacin, obtained from commercial sources were tested in concentration range from 0.125 to 128 mg/l. Sensitivity study: Minimum inhibitory concentrations (MICs) were determined by the agar dilution method established by NCCLS guidelines.

Results: The *in vitro* antibacterial activity of gatifloxacin and ofloxacin is shown in Table. In conclusion, gatifloxacin was more active than ofloxacin against coagulase-positive and coagulase-negative staphylococci.

Organisms	Antimicrobial agent	MIC 50 (mg/l)	MIC 90 (mg/l)	% R
CPS (254)	Ofloxacin	0,25	2	11.1
	Gatifloxacin	0.25	0.5	4
CNS (380)	Ofloxacin	0.125	4	20.6
	Gatifloxacin	0.125	0.5	0.5

Tigecycline In vitro studies - II

P1311

Sampling of Staphylococcus aureus and Enterococcus species isolates resistant to other drugs evaluated against tigecycline – T.E.S.T. Program Global Data

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

Background: Tigecycline (TIG), a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent activity against community and hospital acquired staphylococcal and enterococcal pathogens. The T.E.S.T. program determined the *in vitro* activity of TIG against strains resistant to 10 commonly prescribed antimicrobials for serious gram-positive infections: amoxicillin-clavulanic acid (AUG), piperacillin-tazobactam (PT), levofloxacin (LVX), ceftriaxone (CAX), linezolid (LZD), minocycline (MIN), vancomycin (VAN), ampicillin (AMP), penicillin (P) and imipenem (IMP). Study strains were collected from 80 laboratories globally throughout 2004–2006.

Methods: A total of 4440 clinical isolates (1735 *Enterococci*, 2705 *S. aureus*) were identified to the species level at each participating site and confirmed by a central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using broth microdilution panels. Antimicrobial resistance was interpreted according to CLSI breakpoints with TIG susceptible breakpoints defined as <0.25 mcg/ml for *Enterococci* and <0.5 mcg/ml for *S. aureus*.

Results: 231/1735 (13%) *Enterococci* and 379/2705 (14%) *S. aureus* (including MR + MS strains) were resistant to two or more drug classes. The multi-drug resistant (MDR) *Enterococci*,

resistance rates were LVX 97%, P 82%, AMP 75%, VAN 55%, MIN 21%, and LZD 1.3% rates for MDR *S. aureus* were P 100%, AMP 100%, AUG 72%, LVX 92%, PT 67%, CAX 58%, IMP 31%, LZD 0%, MIN 0.8% and VAN 0% TIG inhibited 98.3% of the MDR enterococci and 100% of the MDR *S. aureus*. TIG MICs were 0.06 and 0.12 mcg/ml for enterococci and *S. aureus*, respectively, against strains with or without resistant determinants.

Conclusions: TIG retained potent activity against MDR *S. aureus* and enterococci, inhibiting >99% of strains at the respective breakpoints. TIG should prove to be a useful empiric agent against these gram-positive pathogens whether they are determined to be resistant to other drugs or not.

P1312

Tigecycline in vitro activity against Staphylococcus aureus and Enterococcus strains resistant to other drugs in United States, 2004 – 2006

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

Background: Tigecycline (TIG), a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent activity against many Gram-positive and –negative organisms. T.E.S.T. program determined the *in vitro* activity of TIG against *S. aureus* and enterococci resistant to 10 commonly prescribed antimicrobials: amoxicillin-clavulanic acid (AUG), piperacillin-tazobactam (PT), levofloxacin (LVX), ceftriaxone (CAX), linezolid (LZD), minocycline (MIN), vancomycin (VAN), ampicillin

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(AMP), penicillin (P) and imipenem (IMP). Study strains were collected from hospitals in the United States throughout 2004–2006.

Methods: A total of 2776 clinical isolates (1081 *Enterococci*, 1695 *S. aureus*) from 77 labs in the United States were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using broth microdilution panels. Antimicrobial resistance was interpreted according to CLSI breakpoints with TIG susceptible breakpoints defined as <0.25 mcg/ml for *Enterococci* and <0.5 mcg/ml for *S. aureus*.

Results: 156/1081 (14%) *Enterococci* and 256/1695 (15%) *S. aureus* (including MR + MS strains) were resistant to two or more drug classes. The multi-drug resistant (MDR) *Enterococci*, resistance rates were LVX 99%, P 85%, AMP 82%, VAN 72%, MIN 14%, and LZD 1.3% rates for MDR *S. aureus* were P 100%, AMP 100%, AUG 71%, LVX 98%, PT 64%, CAX 51%, IMP 20%, LZD 0%, MIN 0.4% and VAN 0% TIG inhibited 99.4% of the MDR *Enterococci* and 100% of the MDR *S. aureus*. TIG MICs were 0.06 and 0.12 mcg/ml for *Enterococci* and *S. aureus*, respectively, against strains with or without resistant determinants.

Conclusions: TIG retained potent activity against MDR *S. aureus* and *Enterococci*, inhibiting >99% of strains at the respective breakpoints should prove to be a useful empiric agent against these gram-positive pathogens whether they are determined to be resistant to other drugs or not.

P1313

***In vitro* activity of multidrug-resistant *Acinetobacter* against tigecycline from the T.E.S.T. Program in Europe**

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

Background: The global increase in the emergence of multi-drug resistant (MDR) bacteria has created a dire need for new effective antimicrobial agents. Tigecycline (TIG) is a new first-in-class antimicrobial agent with expanded broad-spectrum activity against Gram-negative and -positive aerobes and anaerobes responsible for community and hospital acquired infections. The T.E.S.T. program determined the *in vitro* activity of TIG compared to piperacillin-tazobactam (PT), levofloxacin (LVX), ceftriaxone (CAX), cefepime (CPE), amikacin (AK), minocycline (MIN), ceftazidime (CAZ), and imipenem (IMP) against multi-drug resistant *Acinetobacter* strains collected from 33 investigational sites in 15 European countries throughout 2004–2006.

Methods: A total of 435 clinical *Acinetobacter* were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using broth microdilution panels. Antimicrobial resistance was interpreted according to CLSI breakpoints with TIG susceptible and resistant breakpoints defined as <2 mcg/ml and >8 mcg/ml, respectively.

Results: Resistance rates to the comparator drugs were CAX 35%, CAZ 35%, LVX 29%, CPE 23%, PT 28%, AK 18%, IMP 12%, and MIN 3.2% were grouped by presence of resistance to 0, 1, 2, 3, 4, or 5 drug classes. The percentage of strains falling into Groups 0 through 5 were 59%, 9%, 14%, 9%, 8%, and 1%, respectively. TIG inhibited >98% of strains in each Group, and 99.3% of all strains overall, with 0.7% intermediate to TIG and no resistant strains. MIC_{50/90} for Groups 0–5 were 0.12/0.25, 0.5/1, 0.5/1, 0.5/1, 1/1 and 1/2 mcg/ml, respectively.

Conclusions: It has been seen in some species that existing multi-drug efflux pumps may also pump TIG. In spite of this,

TIG remained effective although resistance to one or three drug classes increased the TIG MIC₉₀ by 4-fold, and resistance to 4 or more drug classes increased it by 8-fold, TIG remained active against the great majority of the multi-drug resistant strains, and 99.3% of all *Acinetobacter* isolates. *In vitro* activity against multi-drug resistant *Acinetobacter* should prove useful in therapy of infections caused by such strains.

P1314

Tigecycline *in vitro* activity against multidrug-resistant *Acinetobacter* from global population in the T.E.S.T. Program 2006

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

Background: The global increase in the emergence of multi-drug resistant (MDR) bacteria has created a dire need for new effective antimicrobial agents. Tigecycline (TIG) is a new first-in-class antimicrobial agent with expanded broad-spectrum activity against Gram-negative and -positive aerobes and anaerobes responsible for community and hospital acquired infections. The T.E.S.T. program determined the *in vitro* activity of TIG compared to piperacillin-tazobactam (PT), levofloxacin (LVX), ceftriaxone (CAX), cefepime (CPE), amikacin (AK), minocycline (MIN), ceftazidime (CAZ), and imipenem (IMP) against multi-drug resistant *Acinetobacter* strains collected from 120 investigational sites globally throughout 2004–2005.

Methods: A total of 1491 clinical *Acinetobacter* were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using broth microdilution panels. Antimicrobial resistance was interpreted according to CLSI breakpoints with TIG susceptible and resistant breakpoints defined as <2 mcg/ml and >8 mcg/ml, respectively.

Results: Resistance rates to the comparator drugs were CAX 43%, CAZ 44%, LVX 40%, CPE 34%, PT 27%, AK 14%, IMP 10%, and MIN 2.3%. Strains were grouped by presence of resistance to 0, 1, 2, 3, 4, or ≥ 5 drug classes. The percentage of strains falling into Groups 0 through 5 were 50%, 7.8%, 25.8%, 11.7%, 4.3%, and 0.3%, respectively. TIG inhibited >95% of strains in each Group, and 98.5% of all strains overall, with 1.1% intermediate to TIG and only 1 isolate with a resistant MIC of 8 mcg/ml. MIC_{50/90} for Groups 0–5 were 0.12/0.25, 0.5/1, 1/2, 1/2 and 1/2 mcg/ml, respectively.

Conclusions: It has been seen in some species that existing multi-drug efflux pumps may also pump TIG. In spite of this, TIG remained effective although resistance to one or two drug classes increased the TIG MIC₉₀ by 4-fold, and resistance to 3 or more drug classes increased it by 8-fold, TIG remained active against the great majority (98.0%) of the multi-drug resistant strains, and 98.9% of all *Acinetobacter* isolates. *In vitro* activity against multi-drug resistant *Acinetobacter* should prove useful in therapy of infections caused by such strains.

P1315

Multidrug-resistant *Acinetobacter* in the United States evaluated against tigecycline - T.E.S.T. Program 2006

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

Background: Tigecycline (TIG), a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent

expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the *in vitro* activity of TIG compared to piperacillin-tazobactam (PT), levofloxacin (LVX), ceftriaxone (CAX), cefepime (CPE), amikacin (AK), minocycline (MIN), ceftazidime (CAZ), and imipenem (IMP) against multi-drug resistant *Acinetobacter* strains collected from 77 investigational sites in the United States throughout 2004–2006.

Methods: A total of 921 clinical *Acinetobacter* were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using broth microdilution panels. Antimicrobial resistance was interpreted according to CLSI breakpoints with TIG susceptible and resistant breakpoints defined as <2 mcg/ml and >8 mcg/ml, respectively.

Results: Resistance rates to the comparator drugs were CAX 45%, CAZ 45%, LVX 45%, CPE 37%, PT 24%, AK 8%, IMP 6%, and MIN 2%. Strains were grouped by presence of resistance to 0, 1, 2, 3 and 4 or more drug classes. The percentage of strains falling into Groups 0 through 4 were 48%, 7.7%, 33%, 10%, and 1.4%, respectively. TIG inhibited ≥85% of strains in each group, and 98.4% of all strains, with 1.5% intermediate to TIG and one resistant strain with an MIC of 8 mcg/ml. TIG MIC_{50/90} for Groups 0–4 were 0.12/0.25, 0.5/1, 1/2, 1/2 and 2/4 mcg/ml, respectively.

Conclusions: It has been seen in some species that existing multi-drug efflux pumps may also pump TIG. In spite of this, TIG remained effective although resistance to one drug class increased the TIG MIC₉₀ by 4-fold, resistance to two or more drug classes increased TIG MICs by 8–16 fold but MICs still remain 1 doubling dilution below the conservative proposed resistant breakpoint of 8 mcg/ml. TIG remained active against the great majority of these resistant strains, and 98.4% of all *Acinetobacter* isolates in the United States. TIG's *in vitro* activity should prove a useful adjuvant in the armamentarium against infections secondary to multiply-resistant *Acinetobacter*.

P1316

Determining tigecycline's *in vitro* activity multidrug-resistant *Enterobacteriaceae* from the T.E.S.T. Program – Global Data

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

Background: Tigecycline, a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections including GN, GP, Anaerobic and resistant strains. The T.E.S.T. program determined the *in vitro* activity of tigecycline compared to amoxicillin-clavulanic acid, piperacillin-tazobactam, levofloxacin, ceftriaxone, cefepime, ampicillin, amikacin, minocycline, ceftazidime and imipenem against *Enterobacteriaceae* species collected from hospitals globally throughout 2004–2006. The objective of this study was to evaluate the activity of tigecycline against multi-resistant microorganisms, commonly associate with nosocomial infections.

Methods: A total of 9231 clinical *Enterobacteriaceae* were identified to the species level at each site and confirmed by the central laboratory. Minimum Inhibitory Concentration (MICs) were determined by each site using supplied broth microdilution panels and interpreted according to CLSI guidelines. Tigecycline breakpoint (FDA, 2005) is defined as susceptible MICs ≤2 mcg/ml.

Results: As expected, different resistance patterns were detected among *Enterobacteriaceae* sampled in this study. As shown in the table below, tigecycline presented excellent inhibitory activity against all resistance phenotypes encountered.

Resistant Phenotype	Tigecycline		
	%S	MIC ₅₀	MIC ₉₀
ESBL producing <i>E. coli</i> and <i>Klebsiella</i> isolates (n=415)	93.5	0.5	2
AmpC producing <i>Enterobacter</i> and <i>Serratia</i> isolates (n=206)	81.6	0.5	4
Fluoroquinolone resistant isolates (n=1053)	90.1	0.25	2
Aminoglycoside resistant isolates (n=15)	100	2	2
Isolates with reduced susceptibility to carbapenem (n=125)	90.4	1	2

Conclusion: Multi-drug resistance is often seen in health care acquired pathogens. The presented data suggest that tigecycline is highly potent against nosocomial or community pathogens regardless to the resistance patterns in these selected strains of *Enterobacteriaceae*.

P1317

Cross-resistance in clinical isolates of *Enterobacteriaceae* from the United States evaluated against tigecycline and 10 comparator antimicrobial agents

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

Background: Tigecycline (TIG), a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent broad spectrum activity against most commonly encountered species responsible for hospital acquired infections. Cross-resistance to several classes of antimicrobials is often seen in nosocomial pathogens. The T.E.S.T. program determined the *in vitro* activity of tigecycline against strains of *Enterobacteriaceae* cross-resistant to two or more drug classes that included the following antimicrobials: amoxicillin-clavulanic acid (AC), piperacillin-tazobactam (PT), levofloxacin (LV), ceftriaxone (CX), cefepime (CP), ampicillin (AMP), amikacin (AK), minocycline (MN), ceftazidime (CZ) and imipenem (IMP). The isolates were collected from 77 investigational sites in the United States during 2004–2006.

Methods: A total of 5760 clinical isolates were identified to the species level at each site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using broth microdilution panels. Antimicrobial resistance was interpreted according to CLSI breakpoints with TIG's FDA approved susceptible and resistant breakpoints defined as <2 mcg/ml and >8 mcg/ml, respectively.

Results: 392/5760 *Enterobacteriaceae* were multi-resistant to two or more drug classes. Of these resistant strains, 4.3% were resistant to TIG (MIC >8) compared to AMP 99%, AC 54%, CZ 37%, LV 72%, MN 44%, CX 23%, PT 23%, CP 13%, IMP 4.8% and AK 0.3%. The 2,239 *Enterobacter* spp. and *S. marcescens* collected, 106 presented resistance against CX and CZ but susceptible to CP suggestive of AmpC phenotype. 85/106 (80%) of these isolates demonstrated MICs <2 mcg/ml against TIG. TIG showed excellent inhibitory activity against members of *Enterobacteriaceae* that were resistant to AK (n = 1), LV (n = 630), and IMP (n = 59) inhibiting 100%, 90.8%, 93.2% of isolates, respectively, at <2 mcg/ml.

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

P1318

***In vitro* activity of tigecycline and comparators against community and hospital-acquired infections in the United States – T.E.S.T. Program 2006**

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

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

Methods: A total of 12,442 clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentration (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI guidelines.

Results: Results are in the table as follows*: *Tigecycline susceptibility defined according to FDA package insert (Tygacil®, 2005) where available. Tigecycline *Acinetobacter* susceptibility breakpoint are defined as ≤2 mcg/ml for comparative purposes only.

	<i>Enterobacteriaceae</i>				<i>Acinetobacter</i> spp.			
	In patients (n=4,174)		Out patients (n=1,236)		In patients (n=691)		Out patients (n=178)	
	%S	MIC ₉₀	%S	MIC ₉₀	%S	MIC ₉₀	%S	MIC ₉₀
Tigecycline	96.2	1	97.2	1	97.8	2	100	1
Amikacin	98.9	4	99.7	4	83.6	32	90.4	16
Cefepime	96	2	98.5	0.5	42.1	>32	68	>32
Ceftazidime	85.9	32	92.3	8	44.6	>32	66.3	>32
Imipenem	98.2	1	98.7	1	86.1	8	93.8	2
Levofloxacin	86.4	8	89.7	4	46.2	>8	66.3	>8
Minocycline	86.9	8	85.9	8	88.3	8	90.4	4
Pip-Tazo	89.9	32	94.7	8	74	>128	87.6	128

	<i>S. aureus</i>				<i>Enterococcus</i> spp.			
	In patients (n=1,233)		Out patients (n=363)		In patients (n=829)		Out patients (n=153)	
	%S	MIC ₉₀	%S	MIC ₉₀	%S	MIC ₉₀	%S	MIC ₉₀
Tigecycline	100	0.25	100	0.25	99.6	0.12	99.3	0.12
Levofloxacin	50.6	>32	62.3	32	40.8	>32	46.4	>32
Linezolid	100	4	100	2	97.1	2	96.7	2
Minocycline	99.2	0.5	99.2	0.5	52.4	8	41.8	>8
Vancomycin	100	1	100	1	74.5	>32	81.6	>32

Conclusions: Tigecycline's *in vitro* activity was comparable to or greater than most the commonly antimicrobials for both hospital and community acquired infections. The presented data suggest that tigecycline may be an effective and reliable therapeutic option against nosocomial or community pathogens.

P1319

***In vitro* activity tigecycline and 10 broad spectrum comparators against multidrug-resistant *Staphylococcus aureus*: a multi-nation evaluation**

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

Background: *S. aureus* are increasingly displaying resistance to multiple drug classes (MDR). Therapeutic options to MDR *S.*

aureus phenotypes are limited. Tigecycline, a new glycylcycline offers the potential of enhanced activity against MDR *S. aureus*. The tigecycline evaluation surveillance trial (T.E.S.T.) evaluated the activity of tigecycline and comparators to MDR *S. aureus* isolated worldwide.

Methods: 116 hospital sites in 25 countries between 2004–2006 collected 2705 clinically significant *S. aureus*. MICs were determined at each site using broth microdilution panels and results interpreted as specified by CLSI at each site.

Results: MIC₉₀ of tigecycline and comparators to MDR groups 0–4 are shown in the table. 1. Resistant to 0, 1, 2, 3 or 4 drug classes.

	MDR Group ¹ (N) / MIC ₉₀ (mcg/mL)				
	Group 0 (n=209)	Group 1 (n=1616)	Group 2 (n=689)	Group 3 (n=189)	Group 4 (n=2)
Tigecycline	0.25	0.25	0.25	0.5	0.5
AmoxClav	0.25	8	>8	>8	>8
Ampicillin	0.12	>16	>16	>16	>16
Ceftriaxone	4	16	>64	>64	>64
Penicillin	0.12	>8	>8	>8	>8
PipTazo	0.5	16	>16	>16	>16
Imipenem	0.25	0.5	16	>16	>16
Levofloxacin	0.25	4	>32	>32	>32
Linezolid	4	2	4	2	2
Minocycline	≤0.25	0.5	2	8	>8
Vancomycin	1	1	1	2	2

Conclusions: Tigecycline exhibited the lowest MIC₉₀ of all study agents against *S. aureus* strains worldwide without regard to multiple drug class resistance. Tigecycline MIC values remained at or below its susceptible breakpoint of 0.5 mcg/ml for *S. aureus*.

P1320

Continuity of mutant prevention concentration values for gatifloxacin, gemifloxacin and moxifloxacin against clinical isolates of *Streptococcus pneumoniae* (1994–2004)

J. Blondeau, S. Borsos (Saskatoon, CA)

Objectives: Mutant prevention concentration (MPC) defines the antimicrobial drug concentration threshold that would require an organism to simultaneously possess 2 resistance mutations for growth in the presence of the drug. We have previously shown that MPC testing identified differences between newer and older quinolones for their propensity to select for resistant subpopulations with clinical isolates of *Sp*. In order to assess potential changes to MPC values over time, we compared MPC measurements for gatifloxacin (GAT), gemifloxacin (GEM), levofloxacin (LFX) and moxifloxacin (MXF) against >550 clinical *Sp* isolates collected from 1994–2004.

Methods: For MPC testing, isolates were inoculated to blood agar plates, incubated overnight in ambient air and temperature and the next day transferred to 500 ml of Todd Hewitt Broth (THB) and incubated as described. The next day, the cultures were concentrated by centrifugation and then resuspended in 3 ml of THB. A total of 200 µl of THB containing >1 billion organism were inoculated to drug containing agar plates and incubated for 24–48 hours. The lowest drug concentration preventing growth was the MPC.

Results: A total of 488–559 clinical isolates were tested to all compounds. For GAT, GEM and MXF, MPC 50/90 values (µg/ml) remained constant over time at 1/2, 0.125/0.25, 0.5/1 respectively. For LFX, MPC 50/90 values (µg/ml) ranged from 2/2 to 2/4 being higher for more recently collected strains. The

number of strains with MPCs of 4 µg/ml or greater was as follows: GAT 19, GEM 1, LFX 108, MFX 6. GEM and MFX had the lowest overall MPC values. There were 12 Sp strains with MPC values of 16 µg/ml or greater to LFX as compared to 4 for GAT and none for GEM and MFX.

Conclusion: GAT, GEM and MFX had lower MPC values than did LFX. MPC90 values have not increased for the newer drugs. The number of strains with MPC values to LFX of 4 (µg/ml) or greater have increased over time as have the number of strains with MPC measurements greater than 16 µg/ml. Preferential use of more active compounds may slow the rate of quinolone resistance in Sp.

P1321

In vitro activity of tigecycline against tetracycline-resistant Gram-positive cocci isolated from patients with bacteraemia

S. Forsthuber, H. Lagler, K. Stich, S. Reichmann, A. Georgopoulos, W. Graninger, E. Presterl (Vienna, AT)

Objectives: The aim of this study was (i) to evaluate the *in vitro* activity of tigecycline against tetracycline-resistant *Streptococcus pneumoniae* (SPN), *Staphylococcus aureus* (SA) and viridans group streptococci (VGS) isolated from patients with bloodstream infections in Austria and (ii) to detect the genetic mechanism of tetracycline resistance.

Methods: A total of 153 tetracycline-resistant (TetR) gram-positive cocci (51 SPN, 54 SA, 48 VGS) were isolated from patients with bacteraemia. Isolates were collected between 2001 and 2004. MICs (minimal inhibitory concentrations) of tetracycline (TET), penicillin (PEN), erythromycin (ERY), clarithromycin (CLR), azithromycin (AZM), clindamycin (CLD), telithromycin (TEL), ciprofloxacin (CIP), levofloxacin (LVX), moxifloxacin (MXF), linezolid (LZD), gentamicin (GEN), fusidic acid (FUS) and vancomycin (VAN) were determined by the microdilution method according to the Clinical and Laboratory Standards Institute (CLSI). MIC of tigecycline (TIG) was tested by the Etest method (AB Biodisk). Tigecycline susceptible breakpoint was defined as ≤2. The presence of tetracycline resistance genes (tetM, tetO, tetK) was determined by PCR.

Results: The MICs of tigecycline ranged from 0.125 to 0.25 mcg/ml (MIC₅₀ = 0.25, MIC₉₀ = 0.5) for all isolates of TetR *S. pneumoniae*, including 34 tet(M) positive strains. Similar findings were observed for tetracycline-resistant VGS. The range of MICs was 0.125–1 mcg/ml, including 28 tet(M) and 1 tet(O) positive VGS. 30 *S. aureus* isolates contained tet(K) and two tet(M). The tigecycline MICs for tet(M/O) positive strains were higher (range, 0.25–1 mcg/ml) than for isolates which lacked tet determinants (range, 0.25–0.5 mcg/ml). All tested pathogens were sensitive to vancomycin and linezolid.

Conclusions: (i) The present study suggest that tigecycline may be an effective alternative to commonly prescribed antimicrobials in the treatment of gram-positive cocci. (ii) There was no relation between the presence or absence of tetracycline resistance determinants and the MICs of tigecycline in TetR VGS and SPN, whereas tet (K/M) positive *S. aureus* isolates showed higher MICs.

P1322

Benchmarking tigecycline activity against Gram-positive and Gram-negative pathogens with multidrug-resistance phenotypes derived from patient specimens from USA and Europe

D. Draghi, M. Jones, D. Sahn, C. Thornsberry (Herndon, US; Plaisir, FR)

Objectives: *Staphylococcus aureus* (SA), *Enterobacteriaceae* (EB) and *Acinetobacter* spp (AC) are frequently associated with infections in hospital-based patients. New agents, such as tigecycline (TIG), a glycylcycline with potent activity against both gram-positive and gram-negative agents, are essential to combat organisms with specialized resistance mechanisms. The activity of TIG was profiled against a recent, geographically diverse collection of organisms that exhibited resistance phenotypes to commonly used antibacterial agents.

Methods: EB [n = 1656; included *E. coli* (EC; n = 550), *K. pneumoniae* (KP; n = 551), *Citrobacter* spp (CT; n = 222), *Enterobacter* spp (ET; n = 222), *S. marcescens* (SM; n = 111)] AC (n = 265), and SA (n = 2,210) collected during 2000–2005 from US and Europe were tested by broth microdilution against TIG and several comparators according to CLSI guidelines. Baseline TIG activity was analysed according to multi-drug resistance (MDR), defined as resistance to ≥2 key comparator agents. MIC interpretative breakpoints were based on CLSI (M100–S15) for all agents, where applicable and FDA breakpoints for TIG.

Results: TIG MIC range/MIC₉₀ (mg/L) for all SA was 0.015–1/0.12. The TIG activity based on MIC₉₀, for MDR (0.25 mg/L) isolates was comparable to the non-MDR isolates (0.12 mg/L). TIG MIC₉₀s (mg/L) for MDR/non-MDR, respectively were as follows: 1/1 EC; 4/1 ET; 1/1 KP; 2/2 SM; and 0.5/0.25 EC. TIG susceptibility was >90% for all EB, regardless of resistance phenotype. Among AC, for ciprofloxacin non-susceptible (NS; n = 138), imipenem NS (n = 30), and cefepime NS (78) isolates, TIG maintained an MIC₉₀ of 2 mg/L.

Organism (n; all/tetracycline R)	tigecycline MIC _{50/90} (% S)	
	all	tetracycline (R)
<i>S. aureus</i> (280/12)	0.125/0.125 (100)	0.125/0.25 (100)
CoNS (279/67)	0.125/0.5 (97)	0.25/0.5 (90)
<i>S. pneumoniae</i> (274/20)	0.064/0.064 (-)	0.032/0.064 (-)
<i>S. pyogenes</i> (274/44)	0.064/0.064 (100)	0.064/0.125 (100)
<i>E. faecalis</i> (278/187)	0.125/0.5 (99)	0.125/0.25 (99)
<i>E. faecium</i> (256/107)	0.064/0.125 (-)	0.064/0.125 (-)
<i>E. coli</i> (280/83)	0.25/0.5 (100)	0.25/0.5 (100)
<i>K. pneumoniae</i> (276/170)	0.5/1 (97)	0.5/2 (96)
<i>E. cloacae</i> (270/123)	0.5/2 (97)	0.5/2 (93)
<i>P. mirabilis</i> (280/277)	2/8 (67)	2/8 (66)
<i>Serratia</i> spp (101/91)	2/2 (94)	2/2 (93)
<i>M. morgani</i> (88/35)	1/4 (86)	1/8 (77)
<i>Acinetobacter</i> spp (90/38)	0.25/1 (-)	0.5/2 (-)
<i>H. influenzae</i> (275/4)	0.25/1 (-)	0.5/1 (-)

(-) no breakpoint available

Conclusions: TIG exhibited potent *in vitro* activity against SA, EB, and AC, including MDR phenotypes from US and EU. These data serve as a benchmark to monitor the activity of TIG in both the US and EU.

P1323

A Nordic study exploring the activity of tigecycline against tetracycline-resistant Gram-positive and Gram-negative bacteria

L.E. Nilsson, T. Koernig, N. Frimodt Møller, G. Skov Simonsen, M. Vaara (Linköping, Solna, SE; Copenhagen, DK; Tromsø, NO; Helsinki, FI)

Objectives: Tigecycline is a glycylcycline developed as a parenteral extended-spectrum agent. This study shows the activity

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of tigecycline against tetracycline resistant isolates of Gram-positive and Gram-negative bacteria isolated in four Nordic countries.

Methods: A total of 1641 Gram-positive isolates and 1660 gram-negative isolates were collected from hospital laboratories in Denmark (n = 9), Finland (n = 11), Norway (n = 12) and Sweden (n = 24) during 2005. Susceptibility testing was performed with Etest using FDA breakpoints for tigecycline (*Staphylococci* S ≤ 0.5 mg/l, *Streptococci* except *S. pneumoniae* S ≤ 0.25, *E. faecalis* S ≤ 0.25, enterobacteriaceae S ≤ 2 mg/l) and the Swedish Reference Group for Antibiotics (SRGA) breakpoints for tetracycline (R > 2 mg/l). A total of 457 tetracycline resistant Gram-positive isolates and 821 tetracycline resistant gram-negative isolates were evaluated.

Results: Activity of tigecycline is summarized in the table. The susceptibility was high against all species with FDA-breakpoints except for *P. mirabilis* and *M. morganii*. The other species showed a high tigecycline susceptibility ≥90% for tetracycline resistant isolates. For tetracycline resistant isolates tigecycline MIC50 increased 2-fold for CoNS, *Acinetobacter* spp and *H. influenzae* and MIC90 increased 2-fold for *S. aureus*, *S. pyogenes*, *K. pneumoniae*, *M. morganii* and *Acinetobacter* spp

Conclusion: Tigecycline remained highly active against most tetracycline resistant isolates, probably due to stability to the commonly occurring tetracycline resistant mechanisms in Gram-positive and Gram-negative bacteria.

P1324

Antimicrobial activity of tigecycline against CTX-M extended-spectrum betalactamase-producing *Enterobacteriaceae* isolated from Austrian patients

A. Eisner, G. Feierl, L. Masoud, A. Grisold, E. Marth (Graz, AT)

Objectives: To evaluate the antimicrobial activity of tigecycline against *Enterobacteriaceae* that were found to be CTX-M producers isolated from Austrian patients in the period from 1999 to 2004. Tigecycline is a novel glycolcycline that inhibits protein translation in bacteria by binding to the 30S ribosomal subunit and blocking the entry of amino-acyl tRNA molecules into the A site of the bacterial ribosome.

Methods: A total of 49 non-related clinical isolates (38 *E. coli*, 11 *Klebsiella* spp.) that had previously been identified as CTX-M producers were tested for antibiotic susceptibility against tigecycline and tetracycline as a comparator agent. Isolates were tested using Etest. MIC results for tetracycline were interpreted using the criteria recommended by the National Committee for Clinical Laboratory Standards, results for tigecycline were interpreted using FDA interpretative criteria (≤2 mg/ml susceptible, 4 mg/ml intermediate, and ≥8 mg/ml resistant).

Results: Among the tested isolates, 76% (29 of 38) of *E. coli* and 54% (6 of 11) of *Klebsiella* spp. were found resistant to tetracycline. Tigecycline was highly active against *E. coli* (MIC50 and 90, 0.125 mg/ml and 0.25 mg/ml, respectively) and *Klebsiella* spp. (MIC50 and 90, 0.75 mg/ml and 1 mg/ml, respectively). All CTX-M-producing isolates were inhibited at ≤1 mg/ml of tigecycline, no intermediate or resistant isolates were detected.

Conclusion: Tigecycline demonstrated a high activity against CTX-M extended-spectrum betalactamase-producing isolates *in vitro*. Moreover, tigecycline was highly stable to resistance mechanisms responsible for resistance against tetracycline. Our data indicate that tigecycline may represent a reliable option for the treatment of infections due to multiresistant CTX-M-producing *Enterobacteriaceae*.

P1325

In vitro efficacy of tigecycline tested against community-associated methicillin-resistant *S. aureus* including analyses of 72 co-resistance patterns

R. Jones, M. Stillwell, H. Sader, T. Fritsche (North Liberty, US)

Objective: To determine if the activity of tigecycline (TIG) varies among prevalent susceptibility (S) patterns of CA-MRSA (1989 strains) isolated in North America between 2000–2004. The S patterns were defined by S and resistant (R; includes intermediate) criteria recommended by the CLSI (2005) for 10 drugs: chloramphenicol (CH), ciprofloxacin (CI), clindamycin (CL), erythromycin (ER), gentamicin (GT) rifampin (RF), tetracycline (TC), TMP/SMX, Synercid®, and vancomycin (VA).

Methods: CA-MRSA isolates were defined as *S. aureus* infections acquired in the non-healthcare setting or appearing in hospitalized patients during the initial 48 hours as determined by the demographic records of the laboratory and hospital infection control services. A total of 1989 MRSA met inclusion criteria and were categorized by S patterns and the S testing used the CLSI both microdilution method with fresh Mueller-Hinton medium for TIG. Patterns ranged from 1 to 8 drugs being R; the 7 most common patterns accounting for 81.6% of CA-MRSA isolates (72 total patterns). ER-R (94.2%) was highest followed by fluoroquinolones (CI-R, 84.4%)

Results: TIG-S rates varied from 89.7–100.0% among the most frequently isolated S patterns (see table). Across all CA-MRSA, TIG inhibited 98.2 and 100.0% of strains at ≤1 and ≤2 mg/L, respectively. All non-S TIG MIC results were at 1 mg/L only 2-fold greater than the US-FDA breakpoint for staphylococci. However, 1 mg/L was 2-fold lower than the TIG breakpoint applied to *Enterobacteriaceae*. The overall comparison agent S rates (%) were: CH (82.6), GT (86.6), linezolid (>99.9), Synercid® (>99.9), RF (93.7), TC (90.4), TMP/SMX (91.1), and VA (100.0). TIG activity versus nosocomial MRSA isolates (98.7% S) was comparable to that observed for CA-MRSA.

Strain origin/antibiogram (no. / %)	Tigecycline MIC (mg/L)		Cum %	
	MIC ₅₀	MIC ₉₀	≤0.5 mg/L	1 mg/L
CA-MRSA (1989)	≤0.12	0.5	98.2	100.0
None (58/2.9)	≤0.12	0.5	100.0	100.0
ER (162/8.1)	≤0.12	0.25	99.1	100.0
ER, CI (287/14.4)	≤0.12	0.5	99.3	100.0
ER, CI, CL (707/35.5)	≤0.12	0.5	98.3	100.0
ER, CI, CL, CH (259/13.0)	0.25	0.5	100.0	100.0
ER, CI, CL, GT (111/5.6)	0.25	1	89.7	100.0
ER, CI, CL, RF (42/2.1)	0.25	0.5	100.0	100.0
Nosocomial (1907)	≤0.12	0.5	98.7	100.0

Conclusion: TIG demonstrates high potency (MIC90, 0.25–0.5 mg/L) and breadth of coverage (98.2–98.7%) against CA-MRSA as well as nosocomial isolates. MDR patterns (72) did not adversely influence TIG activity and high-level R was not observed among nearly 4000 *S. aureus* isolates (all non-S MICs at 1 mg/L).

P1326

In vitro activity of tigecycline tested against an international collection of minocycline-resistant bacterial pathogens (2000–2004)

T. Fritsche, H. Sader, R. Jones (North Liberty, US)

Objective: To characterize the activity of tigecycline (TIG) against an international collection of minocycline (MINO)-susceptible (S) and -resistant (R) gram-positive and -negative

pathogens. TIG, a novel glycolcycline, is the 9-butylglycylamido derivative of MINO and has recently been approved by the US-FDA as a parenteral agent for use in treatment of skin and skin structure infections, and intra-abdominal sepsis. Glycolcyclines are inherently stable to most tetracycline-class resistance mechanisms.

Methods: Bacterial strains were acquired from recent (2000–2004) worldwide surveillance collections, and tested against TIG, MINO and other comparator agents using the NCCLS (2003) reference broth microdilution method. Interpretive criteria were from the TIG package insert or CLSI (2005).

Results: Activity of TIG is summarized in the table. TIG was highly active against all strains tested with defined resistance only being seen among the Proteae and SM (3.3–9.0%). Despite close structural similarities between the two molecules, TIG remained generally active against most MINO-S and -R organisms with MIC₅₀ values being 0.06–2 mg/L, and 0.06–4 mg/L (exception, PSA, MIC₅₀, 16 mg/L), respectively. Among MINO-R subsets, >93% of such critical pathogens as EC, SA, EF and EFM remained S to TIG. Decreases in TIG susceptibilities were noted among MINO-R SM (29.2%), PM (43.3%), IPP (64.3%) and ESP (63.6%), indicative of over-lapping binding sites.

Organism (no. MINO-S/MINO-R)	% TIG-R	TIG MIC ₅₀ in mg/L (%S)	
		MINO-S isolates	MINO-R isolates
<i>E. coli</i> (EC; 1821/271)	0.0	0.12/0.25 (100)	0.25/0.5 (100)
<i>Klebsiella</i> spp. (861/147)	0.0	0.5/0.5 (100)	0.5/4 (86.4)
<i>Enterobacter</i> spp. (ESP; 572/166)	0.0	0.5/1 (99.8)	2/4 (63.6)
<i>P. mirabilis</i> (PM; 7/221)	9.0	0.5/1 (100)	4/8 (43.4)
<i>Proteus</i> , indole + (IPP; 54/156)	4.9	1/1 (98.1)	2/8 (64.3)
<i>Serratia marcescens</i> (SM; 209/24)	3.3	1/1 (99.0)	4/8 (29.2)
<i>P. aeruginosa</i> spp. (189/676)	– ^a	2/4 (– ^a)	16/32 (– ^a)
<i>Acinetobacter</i> spp. (409/8)	– ^a	1/2 (– ^a)	1/4 (– ^a)
<i>S. aureus</i> (SA; 524/3/4)	– ^a	0.12/0.25 (99.9)	0.25/0.5 (94.1)
<i>E. faecalis</i> (EF; 302/475)	– ^a	0.06/0.25 (97.0)	0.12/0.25 (93.9)
<i>E. faecium</i> (EFM; 264/97)	– ^a	0.06/0.25 (99.6)	0.06/0.25 (99.0)

Conclusions: TIG offers advantages to existing agents including an enhanced spectrum of activity and stability to the commonly occurring tetracycline class R mechanisms (tetA or B and tetM or O). Although a derivative of MINO, TIG retains activity against many MINO-R isolates, making this first-in-class glycolcycline an attractive candidate for expanded clinical development.

P1327

Antimicrobial activity of tigecycline, a glycolcycline, tested against carbapenemase-producing *Enterobacteriaceae*

H. Sader, T. Fritsche, L. Deshpande, R. Jones (North Liberty, US)

Objectives: To evaluate the *in vitro* activity of tigecycline (TIG) and comparator agents tested against a well-characterized collection of carbapenemase (CPase)-producing *Enterobacteriaceae* (ENT) collected worldwide.

Methods: ENT strains collected through many surveillance programs from 2000–2005 were tested for susceptibility (S) against imipenem (IMI) and meropenem (MER). Isolates with MIC, d 2 mg/L for IMP and MER (except indole+ Proteae and *P. mirabilis*) were screened for production of metallo-β-lactamase (MBL) and Bush group 2f β-lactamases (2f BL) by disk approximation (DA) tests. Isolates with positive DA tests were evaluated by PCR using generic primers for IMP, VIM and SPM when DA-positive for MBL; and for KPC, SME, IMI and NmcA when DA positive for 2f BL. CPase gene sequencing and molecular typing were additionally performed to confirm CPase production and to evaluate clonality. All CPase-producers were S tested against TIG and >25 comparators by CLSI broth microdilution methods using fresh Mueller-Hinton broth.

Results: CPase production was characterized on 104 ENT isolates among >45 000 tested during the 2000–2005 period. The collection included *K. pneumoniae* (KPN; 52), *K. oxytoca* (KOX; 8), *Enterobacter* spp. (ESP; 24), *C. freundii* (CF; 9), *S. marcescens* (SM; 7) and *E. coli* (4) from the USA (79 strains, 11 medical centres (MC), 8 cities), Italy (2 strains, 2 MC, 2 cities), Turkey (11 strains, 2 MC, 2 cities), Greece (10 strains, 1 MC) and Spain (2 strains, 1 MC). The most frequent CPase was KPC–2/3 (73 strains), followed by VIM-1 (14), IMP-1 (11), SME-2 (5) and IMI-1 (1). All 2f BLs were detected in the USA while all MBLs were detected in Europe. The majority of KPC-2/3 isolates were observed among KPN from the New York City area (43 strains; ≥9 clones). However, KPC–2/3 were also observed in CF, ESP, *E. coli* and KOX from 7 MC in 5 USA cities. The antimicrobial S of the CPase-producers are summarized in the table: (See table)

Organism (no. tested)	MIC ₉₀ / %S			
	TIG	IMP	GEN	CPRO
<i>Neisseria</i> spp. (80)	1/100	8/22	2/98	>2/15
<i>Enterobacter</i> spp. (24)	0.25/100	4/64	8/48	0.5/62
<i>Citrobacter</i> spp. (9)	0.25/100	4/100	8/0	>2/17
<i>Serratia</i> spp. (7)	0.5/100	>8/0	<=2/83	<=0.25/63
<i>E. coli</i> (4)	0.12/100	2/100	8/0	>2/25
All ENT (104)	0.5/100	8/98	4/90	>2/32

Conclusions: CPase-producing ENT showed high rates of R to all antimicrobials tested except TIG, which was very active against this significant, contemporary collection of well characterized strains (MIC₉₀, 1 mg/L; 100% S). TIG appears to be an excellent option to polymyxins for treatment of infections caused by CPase-producing ENT.

P1328

Activity of tigecycline tested against an international collection (2000–2004) of AmpC-producing *Enterobacteriaceae*

T. Fritsche, H. Sader, R. Jones (North Liberty, US)

Objective: To evaluate the activity and potency of tigecycline (TIG) when tested against an international collection of *Enterobacteriaceae* (ENT) with chromosomal AmpC enzymes, including subsets predictive of extended-spectrum β-lactamase (ESBL) production. TIG is the first-in-class glycolcycline to be approved (US-FDA) as a parenteral agent indicated for intra-abdominal and skin and skin structure infections, and has demonstrated activity against a variety of Gram-positive and –negative pathogens, including anaerobes.

Methods: Non-duplicate clinically-significant bacterial isolates (2413) were collected from 2000 to 2004 in >60 medical centres participating in the global TIG surveillance program. Isolates included *Citrobacter freundii* (CF), *Enterobacter aerogenes* (EA), *E. cloacae* (EC) and *Serratia marcescens* (SM). All isolates were tested using NCCLS (2003) broth microdilution methods against TIG and representative comparator agents. Ceftazidime (CTZ) resistance (R) was used as a marker for stably derepressed AmpC production, and cefepime (CPM) elevated MIC values (≥4 mg/L) as a predictive marker for concomitant ESBL-production.

Results: TIG results for the R organism subsets are in the Table: Stably-derepressed AmpC- and ESBL-production were evident in, respectively: 22.2 and 3.1% of EC; 24.7 and 0.6% of EA; 17.2 and 2.7% of CF; and 2.0 and 0.2% of SM. Slightly decreased potency (MIC₉₀ values) of TIG among the CTZ-R subsets was noted and varied from 0- (SM) to 4-fold (EC and CF). Overall, 97.4 and 90.8% of CTZ-S and -R isolates, respectively, were inhibited by ≤2 mg/L of TIG. The presence of additional ESBL

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phenotypes among these isolates did not affect the potency of TIG, and >95% of CTZ- and CPM-R isolates remained S, indicative of the broad-spectrum of activity retained by this agent against highly-R ENT.

Organism (# tested)	MIC (mg/L)		% inhibited at MIC (mg/L)		
	50%	90%	≤1	≤2	≤4
EC (1246)	0.5	1	90	95	>99
CTZ-S (924)	0.5	1	96	97	>99
CTZ-R (276)	0.5	4	70	88	>99
CPM at < 4 mg/L (218)	0.5	4	71	87	>99
CPM at ≥ 4 mg/L (39)	1	2	71	94	100
EA (336)	0.5	1	93	97	>99
CTZ-S (236)	0.5	1	94	98	100
CTZ-R (83)	0.5	2	89	96	98
CPM at < 4 mg/L (77)	0.5	2	88	96	98
CPM at ≥ 4 mg/L (2)	1	-	100	-	-
CF (186)	0.25	1	94	98	100
CTZ-S (148)	0.25	0.5	96	99	100
CTZ-R (32)	0.5	2	87	96	100
CPM at < 4 mg/L (25)	0.5	2	88	100	-
CPM at ≥ 4 mg/L (5)	0.5	-	80	100	-
SM (645)	1	2	82	95	98
CTZ-S (618)	1	2	83	95	98
CTZ-R (13)	1	2	69	92	100
CPM at < 4 mg/L (12)	1	2	75	91	100
CPM at ≥ 4 mg/L (1)	2	-	0	100	-
Cumulative % Inhibited			88	96	>99

Conclusions: Among EC, EA, CF and SM, including those strains that express AmpC and ESBL- enzymes, 96% were inhibited by ≤2 mg/L of TIG (the current USFDA breakpoint) and >99% were inhibited by 4 mg/L. TIG is highly stable to most R determinants affecting multiple drug classes, and may represent a significant choice among parenteral agents for broad-spectrum coverage, including the most commonly occurring - and problematic - resistance phenotypes.

P1329

Evaluation of the *in vitro* activity of tigecycline against multidrug-resistant Gram-negative and Gram-positive isolates

F. Kontopidou, E. Koratzanis, A. Antoniadou, E. Giannitsioti, P. Evangelopoulou, S. Kanavaki, S. Karambela, L. Zerva, H. Giamarellou (Athens, GR)

Background: In order to evaluate the potent antibacterial activity of tigecycline (TGC) which is a new compound evolving

from the tetracycline family, we conducted the study of the *in vitro* activity of TGC against 400 multi-drug resistant (MDR) Gram-negative and gram positive pathogens.

Methods: Four hundred MDR clinical isolates provided by different ICUs Departments and our Department were collected from 2003 to 2005 and were tested both for their *in vitro* activity against TGC. This activity was compared to the *in vitro* activity of 12 and 11 drugs against gram (-) and gram (+) respectively. For these comparators drugs, MIC determination by broth microdilution method according to the NCCLS standards and E-test methodology for colistin, ampicillin/sulbactam and Quinopristin/Dalfopristin were applied.

Results: 1) Among 100 *Acinetobacter baumannii* strains provided by different ICUs, 95% were resistant to imipenem and 85% of them to ampicillin/sulbactam too. TGC MIC <1 µg/ml and <2 µg/ml accounted for 96% and 99% of all strains respectively. 2) All *Klebsiella pneumoniae* strains were MDR (producing in 32% ESBL, 15% VIM and 35% both ESBL and VIM, while 12% were resistant to colistin and 8% Pan-Drug resistant). MIC for TGC <1 µg/ml and <2 µg/ml were detected in 82% and 97% of strains, respectively. The lowest MICs (0.25 and 0.5 µg/ml) were exhibited by the most resistant strains, those producing ESBL and/or VIM). 3) Among *E. coli* strains tested, 90% ESBL producing) 96% and 100% presented MICs levels <0.5 µg/ml and <1 µg/ml. 4) 70% of MRSA strains tested were also resistant to levofloxacin while 99% of them had TGC MICs of <0.5 µg/ml; in all, TGC MICs were <1 µg/ml. 5) Among VRE strains, 7% were also resistant to linezolid and 8% resistant to Quinopristin/Dalfopristin. TGC MICs against VRE strains were in 100% of strains <0.12 µg/ml.

Conclusions: *In vitro* activity of TGC expressed by low MIC levels against MDR *K. pneumoniae*, *A. baumannii*, *E. coli* as well as MRSA and VRE probably makes this new drug a good treatment option for MDR bacteria (with the exception of *Pseudomonas aeruginosa*) taking also into account the PK/PD properties that reinforce *in vivo* the excellent *in vitro* activity of this new drug.

Nosocomial infection control: MRSA and VRE

P1330

Importance of premorbid and ICU-acquired nasal colonisation

A. Molnar, E. Hajdu, Z. Peto, R. Benko, A. Hortobagyi (Szeged, HU)

Objectives: Critically ill patients are at high risk to acquire nosocomial infections of which at least one-third could be prevented by infection control measures. Properly timed microbiological samplings are important in the early detection and treatment of these infections. As more than 50% of critically ill patients have already been colonised nasally at the time of intensive care unit (ICU) admission [1], the obtainance of nasopharyngeal samples could be of extreme importance. In the present study we aimed to screen nasal colonisation and

determine development rate of severe infections (pneumonia, sepsis) caused by identical bacteria.

Methods: We performed a prospective study between 2001–2004 at our 6-bed surgical ICU. All patients who were expected to stay over 48 hours at our ICU were routinely screened for nasal carriage of micro-organisms at admission and afterwards on every third day of stay. We obtained quantitative endotracheal aspirates on every third day from intubated patients and blood cultures in the presence of fever. Positive endotracheal aspirates and positive blood cultures were retrieved for those patients who had proven nasal colonisation and were on antibiotic course due to clinically evident nosocomial infections. Microbiologically positive endotracheal aspirates and blood cultures were analysed for the presence of identical bacteria previously found by nasal swabbing.

Results: Our results are summarized in the following table:

Number of patients with identical bacteria found in positive nasal cultures (NS+), quantitative endotracheal aspirates (EA+) and/or blood cultures (BC+)

	2001	2002	2003	2004	Total
NOP	118	116	125	128	487
NS +	21	55	66	77	219
NS+ and EA+	12	23	30	20	85
NS+ and BC +	0	2	1	0	3
NS+ and EA+ and BC +	1	2	1	3	7

NOP: Number of admitted patients with over 48 hour stay at ICU

Conclusion: Our survey shows that the pre-morbid and the ICU acquired nasal colonisation led to development of severe infections caused by identical bacteria and required antibiotic therapy in almost every second case (average: 47.3 ± 13.2). Nasal swabbing can be of help in the early start of targeted antibiotic therapy and therefore shorten length of stay and lower healthcare expenses. Further investigations are needed to determine the proper indication, frequency and predictive value of nasal screening.

Reference: Eggimann P, Pittet D. Infection control in the ICU. *Chest* 2001; 120(6):2059–93.

P1331

Management of an outbreak of vancomycin-resistant enterococci in a German university hospital hemato-oncology department

D. Mlangeni, H. Bertz, M. Bussmann, A. Conrad, J. Huebner, D. Jonas, M. Kist, A. Serr, M. Dettenkofer for the VRE Task Force, University Hospital Freiburg, DE

Objective: To investigate which infection control measures are efficient in controlling an outbreak of vancomycin-resistant enterococci (VRE).

Methods: At the peak of the outbreak in October 2004 in which 6 VRE-infected patients were identified, a VRE Task Force was formed and a multifaceted infection control policy was implemented. Empiric antibiotics for neutropenic patients with fever of unknown origin were changed by replacing ceftazidim with piperacillin/tazobactam. Use of vancomycin was restricted. All new admissions to the Hemato-Oncology Department were screened using rectal swabs and rapid identification of the van A and van B genes using PCR was established. Isolation of carriers (special ward) and cohorting of contacts was enforced. Environmental screening and intensified cleaning of the patient rooms was performed. Finally, sampling of health care workers hands (bag broth technique) on wards mainly affected was done.

Results: Between November 2004 and September 2005, 131 patients were VRE-positive (mainly van A genotype). Of these, 30 (23%) suffered from a VRE infection and 101 (77%) were VRE carriers as identified by screening. A total of 2549 screening tests were performed between November 2004 and September 2005. Of these, 309 (12%) were positive for VRE. Since the introduction of the measures of the VRE-Task Force the number of infected patients decreased significantly from 6 in October 2004 to 2 in January 2005. After April 2005 (1 patient infected), no additional primary infections occurred. The number of patients colonized with VRE was highest in December 2004 with 23 patients but subsequently decreased with 10 patients colonized in January 2005. Since then the number of colonized patients has further declined reaching an average of 6 (range 5–8) patients per month. Of the 236 environmental specimens, 16 (7%) were positive for VRE which was isolated from ultrasound equip-

ment, infusion equipment, ventilators, tracheal suction equipment, cardiac monitor, bedrails, curtains and bedside table. The hands of 68 health care workers were studied and five (7.4%) were positive for VRE. Educational seminars on hand hygiene were then held by the Infection Control staff on the wards affected by the outbreak.

Conclusion: The infection control measures initiated by the VRE-Task Force reduced the number of patients infected with VRE significantly. However, further screening is necessary to ensure timely detection of colonized patients.

P1332

The Uro-Quick system for detecting vancomycin resistance in enterococci

S. Roveta, C. Cassanelli, A. Marchese, E.A. Debbia (Genoa, IT)

Objectives: The automated Uro-Quick system has been employed to detect vancomycin-resistance in enterococci (VRE) in order to achieve full agreement between the antibiotic susceptibility results obtained by the reference method (CLSI) and the Uro-Quick system, the optimal experimental conditions (inoculum size, time of incubation, antibiotic concentration) were determined.

Materials and methods: A total of 71 clinical isolates of *Enterococcus* that belong to our Institute collection was tested: all microorganisms were identified by standard procedures with the API 20 STREP system (Bio-Merieux). The strains were chosen for the presence of VanA and VanB genes determined by PCR. *E. faecalis* ATCC 29212 and other 10 strains of *Enterococcus* spp. vancomycin-susceptible (MIC ≤ 2 mg/l) were used as glycopeptide-susceptible controls. Vancomycin susceptibility testing was performed by Mueller-Hinton broth microdilution method according to the procedures of the CLSI. The results obtained with the reference method were compared to those obtained using the Uro-Quick automated system. Two vancomycin concentrations (4 and 10 mg/l) were tested with the Uro-Quick system: the antibiotic was added in a vial containing 2 ml of broth suspension strain (standard inoculum: 0.1 ml, 10^6 cell/ml) and a drug-free vial was used as control. Broths employed in this study were Mueller-Hinton (MH) and Brain-Heart (BH) alone or supplemented with NaCl 2%. After incubation (5, 8 or 18 hours) the instrument printed the results: no growth and a growth curve like the control are representative of a susceptible and resistant strain respectively.

Results: Vancomycin resistance was correctly detected by the Uro-Quick system in all the VRE strains within 8 hours employing BH and vancomycin 4 mg/l. NaCl did not affect the results. The instrument printed the corrected results on susceptible strains under all the experimental conditions.

Conclusions: This study assessed the ability of the Uro-Quick system to detect VRE enterococci with the important advantage of a significant reduction of test-time. On the basis of the present findings the system appears to be useful in severe nosocomial infections due to *Enterococcus* spp.: the rapid evaluation of vancomycin-susceptibility allows a more direct treatment reducing the empiric therapy and the diffusion of resistant pathogens.

P1333

Impact of infection control interventions on hospital MRSA: a time-series analysis

A. Mahamat, F.M. MacKenzie, K. Brooker, D.L. Monnet, I.M. Gould (Nîmes, FR; Aberdeen, Elgin, UK; Copenhagen, DK)

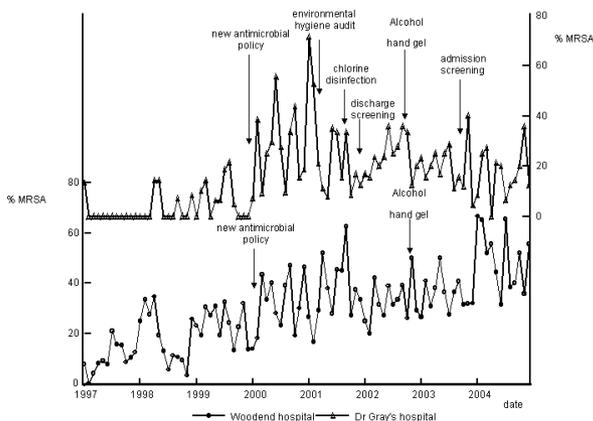
Objectives: Hospitals in the North-east of Scotland have experienced MRSA outbreaks since 1997. Several infection

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control measures and a new antimicrobial policy were introduced to control the incidence of MRSA. We evaluated the effects of these interventions on MRSA rates.

Methods: From January 1997 to December 2004, monthly percentage, non-duplicate *S. aureus* infections caused by MRSA and monthly antimicrobial drug use (defined daily doses/100 occupied bed-days) were collated from Dr Gray's hospital and a control hospital. Both hospitals introduced a common, new antimicrobial policy in January 2000 and the use of alcohol hand gel in November 2002. Furthermore, Dr Gray's introduced an environmental-hygiene audit in March 2001, chlorine disinfection of the environment in September 2001, discharge screening in December 2001 and admission screening in November 2003. Dynamic regression analysis with linear transfer functions and interrupted times-series analyses were used to estimate the effects of the interventions.

Results: At Dr Gray's hospital, % MRSA increased between January 1998 and January 2001 and then decreased. In the control hospital, % MRSA increased from January 1997 to December 2004. By multivariate analysis, introduction of the new antibiotic policy and the use of alcohol hand gel were associated with a decrease in %MRSA of 35% and 23% respectively for Dr Gray's and 23% and 26% respectively for the control hospital. At Dr Gray's, the environmental hygiene audit and admission screening were associated with decreases of 35% and 26% in %MRSA respectively, one month and 4 months later. The use of chlorine disinfection was associated with an increase in MRSA rates of 28% (but only for one month). Discharge screening did not significantly affect the MRSA rates.



Conclusion: Four infection control interventions were associated with a reduction in %MRSA, one was associated with a temporary increase in %MRSA and one had no impact

P1334

Reservoirs and transmission routes of methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* in a tertiary hospital: one-year surveillance

N.A. Faria, D.C. Oliveira, M.L. Amorim, E. Calado, C. Vasconcelos, A.P. Castro, J.M. Amorim, H. de Lencastre (Oeiras, Porto, PT; New York, US)

Objectives: To decrease the burden of nosocomial *S. aureus* infections and prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in Hospital Geral de Santo Antonio, characterized by a high frequency of *S. aureus* infections and a MRSA prevalence of 40%. A systematic study was designed in order to:

(i) identify the reservoirs of *S. aureus*; (ii) to characterize the transmission routes of *S. aureus*; (iii) to evaluate the prevalence of *S. aureus* colonization among outpatients; (iv) to make the monitoring of *S. aureus* clones.

Methods: To identify the *S. aureus* reservoirs, patients and healthcare personnel from the units with the highest risk of *S. aureus* infection, as well as diabetic foot outpatients, were screened for nasal colonization. To clarify the transmission routes, nasal swabs and infection samples were taken from patients with *S. aureus* infection. All strains were clustered in clonal types by pulsed-field gel electrophoresis. Clonal clusters were further characterized by spa typing and multilocus sequence typing (MLST). Staphylococcal chromosomal cassette (SCCmec) typing was also performed for all MRSA strains.

Results: In the vascular surgery and endocrinology units, active surveillance showed MRSA carriage rates of 11% and 9% among patients, and 8% and 0% among healthcare personnel, respectively. In the paediatric intensive care unit no MRSA carriers were found. Among diabetic foot outpatients, 9% were MRSA carriers. The percentage of methicillin-susceptible *Staphylococcus aureus* (MSSA) carriers was always higher than the MRSA carriers and was particularly high among health care personnel in the paediatrics intensive care and vascular surgery units (36% and 25%, respectively). Comparative analysis between infection and colonization isolates in the few available samples showed that the same strain was in both sites in 38% of the cases studied. Longitudinal analysis of carriage showed that most (72%) patients carried the same strain for long periods of time (≤ 6 months). Concerning the monitoring of MRSA clones, the previously dominant ST239-IIIa clone decrease its incidence to 16%, and clone ST22-IV has emerged with an incidence of 79%.

Conclusions: The vascular surgery and endocrinology units were shown to be the major reservoirs of MRSA among the hospital units studied. The infection control measures adopted by the paediatric intensive care unit were effective to curtail the MRSA transmission. Diabetic foot outpatients are important reservoirs of MRSA and may be vectors for MRSA dissemination between the community and the hospital.

P1335

A patient-centred approach to a clinical audit of the clinical practice in MRSA control

A. Guleri, J. Roberts (Liverpool, UK)

Introduction: This audit was conducted to assess the clinical practice in management of new and previously known hospitalised patients with Methicillin resistant *Staphylococcus aureus* (MRSA). The hospital MRSA control policy is based on national guidelines for control of MRSA. As a novel initiative, this audit essentially took on board the views of the patient on different aspects of their management and understanding of MRSA acquisition. The initial audit was conducted in June/July 2004 and this re-audit in June 2005.

Method: Over 1-week, 25 new and previously known MRSA patients in 13 wards of the hospital were assessed. Each questionnaire comprised of 3-sections, each to be completed by the staff nurse in-charge of the patient, the patient (or care nurse if patient unresponsive) and the assessor respectively.

Results: There was 100% compliance with easy availability and use of alcohol hand rubs/hand washing, aprons and gloves, documentation in case notes and awareness in nurses, doctors and domestic staff about MRSA status of patient. 24% patients were either unresponsive or confused from primary illness/treatment. 72% patients were nursed in isolation rooms, while the rest were cohort nursed and on decolonization procedure.

All of the 76% patients had been counselled about their MRSA status, had a positive attitude about its management and observed doctors, nursing and domestic staff taking care with hand hygiene and using aprons/gloves. 50% of doors of isolation rooms were found open. There was considerable improvement on all assessed parameters from the earlier audit (to be presented)

Conclusions: There is a range of improvement in compliance to the hospital MRSA control policy. However, some areas need further attention such as isolation rooms with closed doors, quality surveillance, a tiered system of education and training on infection control for all health care workers and ensuring that infection control and health hygiene is everyone's business.

P1336

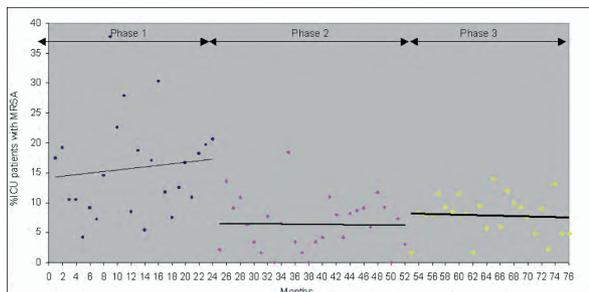
Patient decontamination to control MRSA in an intensive care unit

I.M. Gould, F.M. MacKenzie, G. MacLennan (Aberdeen, UK)

Objectives: To assess the effect of a change in patient decontamination policy on MRSA rates in a 16-bed, teaching/tertiary referral hospital ICU.

Methods: This cohort study used segmented regression analysis of time series data applied to monthly MRSA data from May 1999 to August 2005, split into 3 phases. In phase 1 (24 months) retrospective MRSA data were analysed. In phase 2 (28 months) all patients were screened on admission for MRSA and those found positive after 48–72 hours were barrier nursed. All patients underwent daily decontamination using 4% chlorhexidine body and hair washes and six hourly nasal application of neomycin/chlorhexidine or fucidin cream from admission to discharge. (Phase 2 was presented at ECCMID 2003, abstract O336). In phase 3 (24 months) the only difference from phase 2 was that body decontamination was discontinued in patients when MRSA screen-negative results were confirmed by the laboratory.

Results: Figure 1 details monthly % ICU patients identified as MRSA positive at any point during their stay. Phase 1: 1232 admissions and 192 new MRSA cases. Phase 2: 1675 admissions and 105 new MRSA cases. Phase 3: 1550 admissions and 123 new MRSA cases. Mean (SD) % MRSA per month are phase 1; 15.7 (8.23), phase 2; 6.6 (4.5) and phases 2 and 3 combined; 7.1 (4.0). Comparing phases 1 and 2, the change in slope was -0.08 ($p = 0.769$) and the change in levels (%MRSA) was -11.3 ($p = 0.006$). Comparing phases 2 and 3, the change in slope was -0.01 ($p = 0.952$) and the change in levels (%MRSA) was 2 ($p = 0.378$). Thus, during the individual phases, the % MRSA rates were constant. Between phases 1 and 2 there was a significant change in %MRSA and between phases 2 and 3 there was no significant change in %MRSA. Introduction of the interventions significantly reduced MRSA rates in the ICU. Despite stepping down the intervention between phases 2 and 3, there was no significant difference in rates of MRSA acquisition.



Conclusions: After 24 months of the modified intervention (phase 3), there continues to be no erosion of the efficacy of the intervention (phases 2 and 3). This suggests that the effect is due to decontamination of MRSA patients rather than prophylaxis against acquisition of MRSA.

P1337

Usefulness of axillar swab in the detection of methicillin-resistant *Staphylococcus aureus* colonisation

J. de Otero, C. Capó, M.A. Rada, E. Padilla, A. Serra (Manacor, ES)

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) control policies in Spain usually recommend nasal, axillary and inguinal swabs for colonisation screening. Our aim was to analyse the efficiency of performing the axillar swab in the screening of MRSA for colonised patients and contacts.

Methods: From January-2004 to October-2005 we examined patients positive for MRSA in any site and contacts assisted in our 207-beds regional Hospital in Manacor (Mallorca, Spain). Cultures were taken from nares, axillae, groins and other sites under suspicion to be colonised or infected. Patients with any positive culture for MRSA were considered colonised. Cultures were repeatedly performed after elimination treatment and during follow-up until 3 consecutive negative sets in 1-week intervals. Every complete set of nasal-axillar-inguinal swabs was considered as a case for analysis. Any uncomplete set was then excluded. Sensibility, specificity and positive and negative predictive values of axillar swab were calculated. Efficiency was evaluated considering every patient follow-up.

Results: A total of 214 triple sets corresponding to 86 patients were analysed. Of the 78 MRSA-colonised cases, 16 (20.51%) had a positive axillar swab culture (real positive) and 62 (79.48%) a negative one (false negative). All 136 negative axillar swab cultures corresponded to non-colonised cases (real negative). Sensitivity, specificity, positive predictive value and negative predictive value were 20.51%, 100%, 100% and 68.68, respectively. Only in 2 out of 78 (0.93%) MRSA-colonised cases, axillar swab culture was the only positive of the triple set. In one case, MRSA was simultaneously yielded from a skin ulcer culture. The second case was the screening set of a MRSA diabetic-foot infection and the following 3 consecutive sets in 1-week intervals remained negative after elimination treatment.

Conclusion: The finding of a positive axillar swab did not improve the identification of MRSA colonisation under our policy. Due to a very low sensitivity and negative predictive value it seems not to be an efficient technique. We conclude that axillar swabs should not be a part of routine MRSA screening.

P1338

A prospective study comparing IDI-MRSA real-time PCR assay (SmartCycler) versus chromogenic culture method for detection of methicillin-resistant *Staphylococcus aureus*

B. Ghebremedhin, W. König, B. König (Magdeburg, DE)

Objective: Nosocomial infections caused by Methicillin resistant *Staphylococcus aureus* (MRSA) increase the length of hospital stay, are responsible for rising health care costs and have a high attribution to mortality. Reliable and rapid detection of MRSA carriage is essential as part of epidemiologic investigation but is also necessary for the prompt implementation of barrier isola-

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tion of colonized patients. Real-time PCR allows an early detection of MRSA colonization, 2 hours compared to 1 or 2 days for culture and represents a rapid and powerful tool which can be used for the prevention of the spread of MRSA in healthcare setting.

Methods: We evaluated the feasibility of the IDI-MRSA amplification assay on the SmartCycler and compared the assay with in-house chromogenic culture plates. IDI-MRSA assay was performed according to the manufacturer's instructions. This study has been initiated in routine since March 2005. Specimen swabs from different sites of hospitalized patients and outpatients at high risk for MRSA carriage (medical and surgical ICU, general surgery, haemato-oncology, diabetology, dermatology and geriatry) from the University Clinic in Magdeburg and rehabilitation centre Flechtingen were examined.

Results: Over 2789 swabs of different sites were examined. IDI-MRSA assay revealed 2488 negative, 269 positive and 32 unresolved results. Culture procedure detected less MRSA positive swabs ($n = 230$) and in consequence more MRSA negative swabs ($n = 2559$). SmartCycler procedure (4–6 h depending on collection time of samples and transport logistic) was indeed shorter than that for routine culture diagnostic which took up to 60 h.

Conclusion: We conclude that careful consideration of IDI-MRSA method is more useful than routine-culture diagnostic method. These preliminary results obtained by SmartCycler for MRSA detection are promising. Despite 1.3% (32/2786) amplification failure or unresolved results, we consider that IDI-MRSA real-time PCR assay with an excellent sensitivity represents at our clinic a significant breakthrough in the detection of MRSA colonization and infection.

P1339

Molecular MRSA screening: finding negatives a.s.a.p.

E.J. van Hannen, P. Foppen, B.M. de Jongh (*Nieuwegein, NL*)

Objectives: To contain the development and spread of MRSA in the Netherlands there is agreement among health-care professionals to follow the policy of "Search and Destroy". This policy implies the need for screening for MRSA. With increasing prevalence of MRSA there is a rise in demand for screening with associated rise in number of isolation episodes. Especially in an outbreak setting there is a need for a sensitive and rapid MRSA assay with high volume capacity.

Methods: Traditional MRSA screening in our hospital consisted of overnight culturing of samples in oxacillin broth and subsequently incubation of these enrichments on CNA agar plates for 48 hours. This procedure generated negative outcomes in 3 to 5 days. During this time, patients with a history of contact with the MRSA index are kept in strict isolation. In addition of the extra costs of isolation, long isolation of patients is undesirable. To reduce the time to generate a negative outcome, we developed a multiplex real time PCR that detects *Staphylococcus aureus* and the *mecA* gene in overnight oxacillin enrichments after Magna Pure DNA isolation.

Results: In total, 1217 clinical samples were analysed by real time PCR and compared to culture. The assay had a negative

predictive value of 99.1%. This assay reduced the time to produce a negative outcome with 3 days and subsequently the total time of isolation of patients.

Conclusion: We developed a rapid and high throughput MRSA assay that gave a reduction of a total of 30 isolation days in an outbreak setting.

P1340

Effect of nose carrier screening in liver transplant candidates for preventing *Staphylococcus aureus* infections after transplantation: a retrospective study

V. Lamonaca, R. Di Stefano, G. Caruana, R. Volpes, M. Campanella, G. Vizzini, P. Grossi, B. Gridelli (*Palermo, IT*)

Background: Several studies suggest nasal colonization is an important risk factor for *Staphylococcus aureus* (*Staph. aureus*) infection.

Aims of the study: Evaluate efficacy of mupirocin topical therapy and impact of nasal carrier screening in preventing *Staph. aureus* infections after liver transplantation (OLTx).

Patients and methods: Review of 5-year follow-up in patients (pts) listed for OLTx and 6-month follow-up after OLTx, from July 1999 to November 2004. Rate of *Staph. aureus* colonization by nasal swab at listing, eradication after mupirocin therapy, rate and time of re-colonization, rate of colonization at the time of OLTx, and *Staph. aureus* infections after OLTx were evaluated.

Results: 377 listed pts were reviewed. At listing 237 pts (63%) had negative swab; 140 pts (37%) had *Staph. aureus*: 119 (31%) MSSA, 21 (6%) MRSA. After therapy, eradication rate was 98%: 122 pts (87.3%) after a single treatment, 15 pts (10.7%) after two. Failure was 2% (3 pts). Total re-colonization rate was 23% (32 pts): 0% by 6 months; 1.4% (2 pts) after 7–9 months; 8.6% (12 pts) after 10–14 months; 13% (18 pts) between 16 and 38 months. 162 patients underwent OLTx and received 183 grafts: 142 cadaveric, 7 split, 16 living-related, 18 re-OLTx. At the time of transplantation 127 pts (78%) had negative nasal swab, 35 pts (22%) were colonized: 30 (18.5%) with MSSA, 5 (3.0%) with MRSA. After mupirocin, eradication rate was 100%; 31 pts (88.5%) with a single treatment; 4 pts (11.5%) after two. Re-colonization was 5.7% (2 pts, MSSA) after 6 ± 2 months. De-novo nasal colonization was 2.1% (2 pts). After OLTx 8 pts (5%) developed *Staph. aureus* infections: 7 (4.4%) by MSSA, 1 (0.6%) by MRSA. 6 of them were colonized at admission and developed infection within 2 days after starting mupirocin. They had CVL infections (2 pts), CVL-sepsis (2 pts), pneumonia (1 pt), wound infection (3 pts), ascites infection (1 pt). The remaining 2 pts, though with negative nasal swab, had wound infections: 1 MSSA and 1 MRSA (probable personnel handling cross-colonization). All infections solved after specific therapy.

Conclusions: In our experience nose carrier screening and topical mupirocin therapy result very effective in eradicating *Staph. aureus* carriers and in preventing infections after OLTx. In the 34 re-colonized patients, in 97% of cases re-colonization occurred not earlier than 6 months post eradication.

Regional spread and outbreaks caused by nosocomial pathogens

P1341

European intercountry dissemination of a glycopeptide resistant *E. faecalis* clone

C. Novais, T.M. Coque, J.C. Sousa, M. Del Grosso, L. Peixe
(Porto, PT; Madrid, ES; Rome, IT)

Objectives: To establish the diversity of PFGE types, genes coding for antibiotic resistance and virulence and Tn1546 structure among representative vancomycin resistant *E. faecalis* (VREfls) isolates from Portugal (PT) and Italy (IT). These are two of the six European countries with highest VRE prevalence rates in Europe (<http://www.earss.rivm>).

Methods: Thirty-two clinical and environmental isolates that belong to a widespread clone (29 resistant and 3 susceptible to vancomycin) recovered from 3 different Portuguese regions between 1996–2002, and a bloodstream isolate from a hospital in the Center of Italy (2002) were studied. Clonal relatedness was established by PFGE. Antibiotic susceptibility was evaluated by agar dilution and microdilution methods (NCCLS). Characterization of antibiotic resistance genes (glycopeptides, macrolide, aminoglycosides), putative virulence factors and the backbone structure of Tn1546 were performed by PCR as previously described.

Results: Similar PFGE profiles were detected between PT and IT VREfls. The same PFGE type was also detected among clinical vancomycin susceptible *E. faecalis* (VSEfls) obtained from a PT hospital. Most of the isolates were resistant to erythromycin [erm(B)], gentamicin [aac(6′)-aph(2′)], streptomycin, kanamycin (aph-III), tetracycline, ciprofloxacin and all were susceptible to ampicillin and linezolid. All VRE isolates harboured vanA but located in a high diversity of Tn1546 (types PP2, PP4, PP5, PP15, PP17 among PT strains and the prototype Tn1546 in the IT one). Insertion of ISEf1 between vanX and vanY, was frequently and, exclusively observed in PT *Enterococci*. Variable presence of putative virulence traits was detected asa1 (PT+IT), gel (PT), cyl (PT) and esp (PT).

Conclusion: We reported the European intercountry dissemination of a VREfls clone causing severe clinical infections. The recovery of some isolates susceptible to glycopeptides and the diversity of Tn1546 and putative virulence traits harboured by different isolates belonging to this clone suggest spread of specific *E. faecalis* strains harbouring different genetic elements (as Tn1546 and putative virulence factors) probably selected among those available in local metagenomes.

P1342

Genomic diversity of vancomycin-resistant *Enterococcus faecium* isolated in France

L. Mihaila-Amrouche, J. Loubinoux, A. Doloy, G. Collobert,
N. Gaitch, A. Bouvet (Paris, FR)

Objective: Vancomycin-resistant *Enterococcus faecium* (VREF) has emerged as a nosocomial pathogen in the USA but remains rare in Europe. An increase of VREF incidence was observed in France (0.4% in 2003 to 2.9% in 2004). Thus, we compared 78 VREF strains isolated from 2004 to 2005 in different regions of France.

Methods: Clinical data from the colonised or infected patients were collected. Identification of VREF isolates was first performed with the rapid ID32 STREP commercialized systems (bioMérieux, Marcy l’Etoile, France) and then confirmed by species-specific PCR amplification of the D alanine-D alanine

ligase genes. Antimicrobial susceptibility to 11 antibiotics was determined by the agar diffusion method according to the guidelines of the ‘Comité de l’Antibiogramme de la Société Française de Microbiologie’ (<http://www.sfm.asso.fr>). For beta-lactams and glycopeptides, the minimal inhibitory concentrations (MICs) were also determined. The genes vanA, vanB, vanC1 and vanC2/3 responsible for resistance to glycopeptides were identified by multiplex PCR. Clonality of isolates was studied by pulsed-field gel electrophoresis (PFGE) after digestion of DNA with SmaI.

Results: VREF isolates were more often responsible for colonisation than infection. Most of the isolates (74/78) were resistant to penicillin G, amoxicillin, and piperacillin (MICs >256 mg/L). Intermediate susceptibility to amoxicillin was observed for 4 strains. Most of the strains (76/78) were resistant to macrolides. Resistance to aminoglycosides and tetracycline was variable. All strains were resistant to vancomycin (MICs 24–256 mg/L), and intermediate or resistant to teicoplanine (MICs 6–256 mg/L). All strains but one had the gene vanA. PFGE identified fourteen different clones.

Conclusions: Resistance to glycopeptides in VREF isolates was mainly due to the presence of the gene vanA. More than 10 different clones were identified by PFGE. These results demonstrate the genomic diversity of VREF isolated in France. A single clone was present in some hospitals. Thus, the transmission of VREF isolates between patients must be taken into account for the prevention of these nosocomial infections.

P1343

Molecular epidemiology of vanA-containing *Enterococcus faecium* in a university general hospital in Greece

M. Souli, I. Galani, L. Galani, A. Antoniadou, M. Pantelaki,
N. Siafakas, V. Sakka, F. Kontopidou, S. Athanasia, V. Veloni,
S. Tsiodras, L. Zerva, H. Giamarellou (Chaidari, GR)

Objective: The aim of this study was to determine the clonal relationship and the involved genotype of vancomycin-resistant *E. faecium* (VRE) isolates colonizing the gastrointestinal track of hospitalized patients in our hospital.

Methods: Prospective, point-prevalence surveys of faecal carriage of VRE in all hospitalized patients were performed with 10-day intervals during the 20 April–30 May period. Identification was performed by standard methodology and susceptibilities by E-test. Multiplex PCR for identification of the vancomycin resistance genes and the species was performed in 69 VRE isolated in various patients and in one isolated from the environment (light switch), using crude extracts as target DNA. *E. faecalis* ATCC 51299 (vanB) and *E. faecalis* (vanA) were included as control strains. All isolates were typed by PFGE using SmaI. PCR-RFLP analysis, of the 634 bp PCR amplicons corresponding to V domain region, with NheI was used to detect the G2576T mutation in 6 linezolid resistant isolates.

Results: From the 3 surveys 460 samples were evaluated from 367 patients. Total mean VRE carriage was 19.7% initially to decline gradually to 14.4% and 9.5%, subsequently, as strict multidisciplinary infection control measures were implemented. All isolates were identified as *E. faecium*, with vanA genotype. Six VRE strains had linezolid MIC of 12 µg/ml. Based on PFGE, 8 unique restriction profiles were identified. Two frequently presented clonal types (A and B) with 29 and 25 strains respectively were identified among these profiles. Based on

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PFGE five of the six linezolid resistant isolates were identical (clone type C), including the isolate from the light switch. The sixth isolate belonged to one of the predominant clone types (A). PCR-RFLP analysis of the 634bp amplicon from purified DNA of linezolid resistant isolates revealed the existence of at least one mutated copy of 23S rRNA gene.

Conclusions: This study revealed high VRE colonization rates among hospitalized patients in our hospital but we successfully managed to contain the VRE outbreak by applying the recommended infection control measures. This is the first outbreak due to a linezolid-resistant VRE strain in a tertiary care hospital. Of note, this multiresistant clone was identified during the first survey and completely disappeared thereafter as a result of infection control measures.

P1344

Establishment of polyclonal endemicity with hospital-adapted ampicillin-resistant *Enterococcus faecium* in a Dutch hospital

J. Top, R.J.L. Willems, H. Blok, A. Troelstra, M.J.M. Bonten (Utrecht, NL)

Objectives: A retrospective study on the occurrence of enterococcal infections in the University medical center Utrecht (UMCU) between 1994-2003 revealed an increase in ampicillin resistant *E. faecium* (ARE). The objective of current study was a) to determine whether this increase was due to complex-17 isolates, a hospital-adapted genetic subpopulation characterized by ampicillin resistance, and associated with clinical infections and hospital outbreaks world-wide. b) to determine the colonization prevalence on 2 wards (haematology and nephrology/gastroenterology) and an outpatient group and c) to determine risk factors for colonization with ARE.

Methods: Multiple locus variable number of tandem repeat analysis (MLVA) was used to genotype the ARE in the UMCU. Rectal swabs of 193 hospitalized patients and 650 outpatients were screened for ARE in the period March-October 2004. Twenty-two different variables were included in a risk factor analysis for rectal colonization with ARE among nephrology and gastroenterology patients.

Results: Genotyping of 130 clinical and 59 screening ARE isolates showed clonal spread of ARE and revealed that 31 of 38 different genotypes (189 isolates) belonged to complex-17. Colonization prevalence among nephrology patients (45.6%) was significantly higher than among gastroenterology patients (17.6%, $p < 0.001$), although patients were treated in the same ward, and was 28.1% among haematology patients. The prevalence in the outpatient group was significant lower compared to the two hospital wards (2.9% $p < 0.001$). Analysis of *E. faecalis* to *E. faecium* ratios in the first 20 enterococcal blood isolates per year (1994-2004) showed a relative increase of blood stream infections caused by *E. faecium* ($p = 0.001$). In multivariate analysis, diabetes mellitus (OR 8.59, 95% CI 2.08-35.44), three or more admissions (OR 14.84, 95% CI 3.44-64.10), beta-lactam antibiotics (OR 2.97, 95% CI 1.09-8.09) and quinolone use (OR 5.23, 95% CI 1.22-22.48) were associated with colonization with ARE.

Conclusions: Our findings demonstrate ecological replacement of *E. faecalis* by *E. faecium* and clonal spread of ARE belonging to the hospital-adapted complex-17. Antibiotic use, especially quinolones, appeared to be an important risk factor. Emergence of ARE in European hospitals will have important consequences for empirical antimicrobial treatment of immunocompromised patients.

P1345

Colonisation of vancomycin-resistant *Enterococci* in paediatric unit of a teaching hospital in Turkey

H. Guducuoglu, E. Aktas, F. Begendik Comert, K. Aygul, N. Ozlu, S. Baykal (Van, Zonguldak, TR)

Objectives: Upon a first time isolation of VRE from a urine sample of a 8 month-baby followed by another sample of a 5 month-baby, we decided to perform a VRE screening in our paediatric unit. After screening, isolated VRE strains were investigated by molecular methods to detect the resistance genotypes and possible clonal relatedness.

Methods: For this reason 88 samples (rectal swabs, skin of patients, surroundings of beds, hands of patients' mothers, staff, washbowl, stethoscope, and different environmental samples) were collected from Paediatric Unit of our hospital. The identification was performed by conventional methods and confirmed by PhoenixTM NMIC/ID, Becton Dickinson, USA kits. Vancomycin resistance genotypes were determined by PCR using VanA and VanB primers. Pulsed-field gel electrophoresis (PFGE) was used to evaluate the molecular relatedness of VRE isolates.

Results: 12 VRE strains were isolated from screening samples (8 of them were from rectal swabs, 3 of them were from surroundings of the beds and one of them was from skin of a patient). All of the isolates were identified as *Enterococcus faecium*. The isolated VRE strains were tested against vancomycin, teicoplanin, ampicillin, linezolid by disc diffusion method and also with PhoenixTM NMIC/ID panels. All of the strains were resistant to vancomycin, teicoplanin, ampicillin and were susceptible to linezolid by both methods. All the strains were found to be VanA genotype by PCR. According to PFGE band profiles, 2 different major PFGE band patterns (A, B) were detected. Five of the 14 isolates belonged to PFGE pattern A while 7 belonged to PFGE pattern B. There was one isolate closely related with PFGE pattern A (A1) and one isolate closely related with PFGE pattern B (B1).

Conclusion: After a first time isolation of VRE in our laboratory, the isolated VRE strains from the screening were important because of being the first isolation in our hospital and rarely reported incidence in our country. The detection of two major clones of VRE showed a nosocomial transmission according to contamination. Early detection of patients colonized or infected with VRE by performing surveillance cultures is an essential component of any hospital programme designed to prevent nosocomial transmission of VRE. We gave advices for the eradication of VRE to the paediatric unit like the rational use of antibiotics, education of the employees and taking serious preventions on controlling VRE colonisation.

P1346

A nosocomial VRE outbreak in the context of regional changes in VRE prevalence

D. Jonas, K. Biehler, A. Serr, M. Kist, A.-M. Fahr, M. Dettenkofer, J. Hübner, H. Bertz, F. Daschner (Freiburg, Heidelberg, DE)

Objective: A nosocomial outbreak of vancomycin-resistant *Enterococcus faecium* (VRE) and reports on an increasing prevalence of VRE in the South-West of Germany led to this investigation on the origin and the phylogenetic relation between strains from different health care facilities throughout Germany.

Methods: 188 VRE strains from a nosocomial outbreak and 82 VRE strains from 32 different health care facilities were assigned

to genotypes primarily by use of 'Multiple Locus Variant Number of Tandem Repeats' (MLVA). In addition, in esp+ strains the number of type A-repeats was determined. Results were confirmed by typing of selected strains by use of MLST and PFGE. Finally, the structure of the vanA encoding Tn1546 was investigated by RFLP in regionally predominating genotypes from different institutions.

Results: The outbreak investigation revealed one predominant MLVA genotype MT-159, which however could be distinguished in two PFGE types. Combining the numbers of type A-repeats in the esp gene with the MLVA types resulted in a concordant discrimination of the two predominating outbreak strains with three and four A-repeats. Accordingly, they are named 159_3 and 159_4 and corresponded to the MLST genotypes ST-203 and ST-194, respectively. Both ST were single locus variants of ST-74, a previous outbreak strain from the same hospital in the year 2002, which also belonged to the lineage of purK-1 epidemic *Enterococcus faecium* strains. The strains of both recent genotypes were detectable in different health care facilities in the South West of Germany, including hospitals more than 200 km apart. Moreover, strains of MT-159_4 are present as vanA+, vanB+ and vancomycin susceptible isolates, indicating that VRE of this MT have been independently arisen at least twice. The analysis of the Tn1546 by means of RFLP did not reveal any further differences in the structure of vanA+ MT-159 strains from different regions.

Conclusions: Determination of the number of esp A-repeats improves the discrimination of MLVA results. The close phylogenetic relation between the VRE outbreak strains in different surrounding regions supports the assumption that resistance can be regional problem rather than just a local problem in a single hospital.

P1347

Epidemiology and control of a cluster of USA300 methicillin-resistant *Staphylococcus aureus* (USA300-MRSA) skin/soft tissue infections in an inner-city haemodialysis unit

C. Manierski, K. Obeid, K. Reyes, S. Davis, M. Ajluni, A. Singh, M. Perri, S. Donabedian, D. Vager, M. Zervos (Detroit, US)

Background: The purpose of this study was to evaluate epidemiology, outcome and control measures for a cluster of USA300 infections in an outpatient dialysis unit. USA300 strains have been known to cause outbreaks of CA-MRSA, little information is available on outcome of infection and dissemination in the health care setting.

Methods: The outpatient haemodialysis has 42 stations with a capacity for 252 thrice weekly ESRD patients. The unit is geographically located on an acute care hospital campus. Rates of MRSA infection were 1.65/ 1000 patient dialysis days in 2005. A cluster of USA300 skin/soft tissue infections was identified and contact precautions were implemented. Anterior nares surveillance cultures of a sample of patients and cultures of environmental surfaces were obtained.

Results: Over a 22 week period, isolates were collected from all MRSA skin/soft tissue infections, patient nares and environmental surfaces. There were four patients and one health care worker with USA300, PVL positive isolates. Patients with USA300-MRSA (n 5) vs those with non USA300-MRSA (n 4) or which were negative for MRSA (n 21) had a mean age of 43, 68 and 61 years, respectively. Sites of culture were skin/soft tissue (80, 75, 0%) and nares (20, 25, 0%). 40, 50 and 29% had antibiotic use or hospitalization (60, 75, 48%) within 3 months prior to infection. HIV was present in 20, 0 and 0%. Duration of dialysis was 1.8, 3.6 and 4.8 years. USA300 isolates were susceptible in-

vitro to multiple agents with the exception of beta-lactams, clindamycin and erythromycin. USA300 infections were associated with recurrent disease (n 2), necrotizing pyomyositis (n 1) and death (n 1). Non USA300 infections were associated with panniculitis (n 1) and osteomyelitis (n 1). Patients with USA300 isolates were temporally and geographically clustered, environmental surface cultures (n 23) were negative for USA-300, two environmental surfaces yielded non-USA 300 MRSA. Further spread was controlled by contact isolation precautions. Follow-up surveillance cultures showed no further cases at 2 months.

Conclusion: USA300 isolates are increasingly common causes of CA-MRSA infection. Prompt identification and control are needed to prevent dissemination of isolates in the health care setting. Prolonged therapy may be needed for patients with recurrent disease.

P1348

Outbreak of surgical site infections in a cardiac surgical unit caused by an epidemic methicillin-susceptible *Staphylococcus aureus* clone

M. Pujol, A. Hornero, O. Arch, A. Manzur, M.A. Dominguez, A. Garcia, C. Peñalva, F. Gudiol, J. Ariza (Hospital Llobregat, ES)

Background: The ability of methicillin-sensitive *Staphylococcus aureus* (MSSA) for causing nosocomial infection outbreaks related to a single clone is poorly recognised. The aim of the study was to describe a surgical site infection outbreak caused by an epidemic MSSA strain.

Methods: Prospective surveillance and molecular typing.

Results: From July to September 2005, 6 patients who underwent cardiac surgery developed severe post surgical cardiothoracic infections caused by MSSA: 5 patients had mediastinitis and 1 a defibrillator infection, all of them had bacteraemia. Pulsed field gel electrophoresis showed that all isolates belonged to the same MSSA clone, resistant to penicillin and susceptible to the other anti-staphylococcal agents. The detection of Panton Valentin Leucocidine was negative. Screening for nasal carriage among the surgical team and ward personnel (n = 63 persons), revealed carriage of the same MSSA clone among 3 anaesthetists, 1 surgeon, and 5 ward nurses. Reinforcement of barrier measures during surgery and nasal decontamination with mupirocin of carriers of the epidemic strain stopped the outbreak.

Conclusions: We identified an epidemic MSSA clone as responsible for an outbreak of surgical infections among surgical cardiac patients. The identification of this strain among patients and sanitary personnel shows that invasive MSSA clones may cause nosocomial outbreaks as has been widely recognised for MRSA.

P1349

Food-borne nosocomial outbreak of *Salmonella enterica* serotype Enteritidis in a teaching hospital in Greece

A. Gikas, S. Maraki, E.I. Kritsotakis, D. Babalis, M. Roumbelaki, E. Scoulica, C. Panoulis, E. Saloustris, E. Kontopodis, G. Samonis, Y. Tselentis (Heraklion, GR)

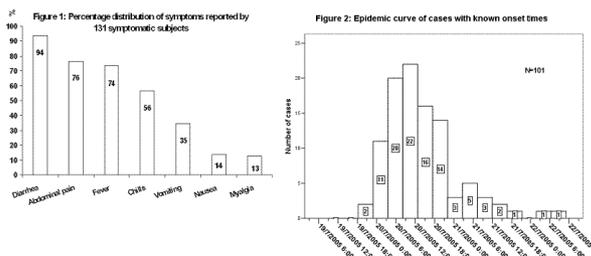
Objectives: To report a nosocomial outbreak of salmonella food-poisoning in a tertiary-care hospital in Crete, Greece.

Methods: Epidemiological (case-control study), microbiological (stool, blood cultures), environmental (food, water cultures) and molecular (PFGE, ribotyping of isolates) examinations were

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employed in order to investigate and control the outbreak. A 2-month clinical follow-up study was also carried out in order to identify complications.

Results: In total, 133 enteritis cases were detected: 86 inpatients, 16 staff members and 31 visitors. Cases were scattered in 20/32 wards and all developed symptoms from 19 to 22 July 2005. The epidemic curve suggested a point source (figures 1, 2). Statistically associated with illness were: roasted chicken (OR 23.9; $p < 0.001$), spaghetti (OR 13.4; $p < 0.001$) and crumbled cheese (OR 4.5; $p < 0.001$). Roasted chicken was considered as the probable transmission vehicle, since (a) 63% of cases did not eat cheese, 90% of whom had consumed chicken; (b) all 18 cases in the cardiology ward had consumed chicken, but not cheese. No leftovers from implicated foods were available and environmental sampling did not yield salmonella. *S. enterica* serotype enteritidis was cultured from 64 stool and 6 blood patient specimens. The strain was also cultured from 3 food-handlers (2 were asymptomatic) and sliced cheese stored in kitchen refrigerators, indicating cross-contamination of the roasted chicken due to inappropriate food handling. PFGE and ribotyping patterns of all isolates were identical, verifying the common source. Macroscopic inspection of catering facilities guided the interventions to food hygiene issues. Particular emphasis was given to improving the existing Hazard Analysis and Critical Control Point (HACCP) system. Quinolones were administered to all patients. Eleven patients presented serious complications: 7 suffered acute renal failure; 1 had a mild pancreatic reaction; 1 immunocompromised patient presented nosocomial pneumonia; whereas 2 presented decompensation of serious underlying diseases and subsequently died. The remaining patients were discharged from the hospital.



Conclusion: This outbreak serves as a reminder of the impact of *Salmonella* spp. food-poisoning in hospitals and underscores the importance of establishing and systematically reviewing HACCP systems in health-care institutions.

P1350

A *Chryseobacterium meningosepticum* colonisation outbreak in a neonatal intensive care unit

E. Galanakis, S. Maraki, E. Scoulica, A. Manoura, C. Giannacopoulou, Y. Tselentis (Heraklion, Crete, GR)

Objective: The aim of this study was to report epidemiologic, bacteriologic, and clinical features of a *Chryseobacterium meningosepticum* outbreak in a neonatal intensive care unit (NICU) of a referral teaching hospital.

Outbreak investigation: From April to October 2002, a strain of *C. meningosepticum* was isolated from four neonates in the NICU. All neonates were colonized in the endotracheal tubes and respiratory secretions but none of them progressed to clinical infection. *C. meningosepticum* was multiresistant. None of the neonates received specific antibiotic treatment. Pulse-field gel electrophoresis of isolates showed them to be representatives of a single strain. Environmental surveillance did not reveal the *C.*

meningosepticum source. The outbreak was only controlled by reinforcing of the usual measures, giving increased emphasis on routine hand hygiene among healthcare workers, without a need of restriction of further neonatal admissions or of thorough disinfection of the NICU. No additional colonization/infection was confirmed for more than a year after the last case.

Conclusion: This study suggests that *C. meningosepticum* colonization in neonates does not necessarily lead to clinical infection and that such colonization outbreaks may be controlled with emphasis on the standard precautions.

P1351

Spreading of beta-lactamase genes among nosocomial isolates collected from Moscow clinics

N.K. Fursova, A.N. Kruglov, I.V. Abaev, Y.N. Kovalev, E.I. Pecherskikh, E.I. Astashkin, D.M. Pachkunov, S. Pryamchuk, V.A. Anisimova, M.E. Mitzevich (Obolensk, Moscow, RU)

Objectives: Characterization of the *Enterobacteriaceae* nosocomial strain collection on the presence of Extended Spectrum Beta-Lactamase (ESBL) genes belonged to TEM, SHV, and CTX-M groups.

Methods: Identification of the strains was done using commercial panels for the automatic identification system MicroScan DadeBehring. Strain resistance profiles was determined using disc diffusion method. ESBL-test was done using Double-disc method. Minimal inhibition concentrations (MICs) of antibiotics were determined using microdilution method in Muller-Chinton media on the plates. ESBL genes have been studied to present by PCR.

Results: Collection of ESBL-producing *Enterobacteriaceae* strains from 12 inspected Moscow clinics have been collected during January-September, 2005. It contains: *Klebsiella* strains - 35.9% (including *Klebsiella pneumoniae* 34%), *E. coli*-29%, *Enterobacter* - 15%, *Citrobacter* - 10%, *Proteus* - 4.9%, *Morganella* - 3.5%, *Serratia* - 2%, *Hafnia alvei* - 1%. PCR analysis of 94 bacterial strains has shown presence of ESBL genes alone and in several combinations: TEM - 6.4%, CTX-M - 26.6%, SHV - 1%, CTX-M+SHV - 26.6%, CTX-M+TEM - 16%, TEM+SHV - 8.5%, CTX-M+TEM+SHV - 8.5%, and without any ESBL genes - 6.4% strains.

Conclusion: Epidemiology situation in some Moscow clinics was estimated using PCR screening. ESBL phenotypes of nosocomial strains under study are provided by different compositions of beta-lactamase genes depending on bacterial genus. Most *E. coli* have CTX-M or CTX-M+TEM (84%); *Klebsiella pneumoniae* - CTX-M or CTX-M+SHV (85%); *Morganella* - CTX-M (80%); *Proteus mirabilis* - CTX-M+TEM (100%); *Citrobacter freundii* - TEM (57%); *Enterobacter cloacae* - TEM+SHV (45.5%). Following studies will be concentrated on regulatory mechanisms of bacterial resistance in isolates collection under investigation.

P1352

An epidemic of hospital-acquired bacteraemia due to *Alcaligenes denitrificans*: true threat or fighting windmills?

H. Moraitou, M. Makarona, A. Gioga, I. Kousseris, E. Galani, N. Makrygiannis, S. Triantafyllou, A. Pefanis, S. Kanavaki (Athens, GR)

Objectives: We encountered 18 cases of bacteraemia, in different wards throughout our hospital, during a 10-month period (09/2004 - 07/2005), caused by a Gram(-), non-fermenting,

oxidase(+) bacterium, identified firstly as *Comamonas testosteroni*, by the Api NE system (Biomerieux, France). The unusual high frequency of bacteremias prompted us to ensure the accurate identification of the strains and the epidemiology of the clinical cases.

Methods: The strains were collected and retrospectively identified by Vitek2 updated GN cards (Biomerieux, France), as well as by Api 32 GN system (Biomerieux, France). The antibiotic sensitivity profile was tested by standard Kirby-Bauer method, and all strains were analysed by PFGE, to investigate the epidemiology of the infection. Bactec 9240 system was used to incubate and to identify positive blood cultures. Bottles with different lot numbers were used for sampling during the examined period.

Results: The strain formerly identified as *C. testosteroni*, was proved to be *Alcaligenes denitrificans* subsp. *denitrificans*, by all methods used. Disk susceptibility testing showed that all isolates were resistant to aminoglycosides and most cephalosporins, but were susceptible to piperacillin, imipenem, ceftazidime and trimethoprim-sulfamethoxazole. The PFGE proved identical for all strains analysed. Regarding the clinical status most patients showed no clinical signs of septicemia. All of them had peripheral venous catheters in place during the day of the reported bacteraemia. In all cases only one bottle, of the multiple blood culture sets, was positive per patient. No new cases of bacteraemia due to *A. denitrificans* were identified in our hospital since July 2005. Since the study was retrospective, it was not possible to further investigate the source of the bacteraemia.

Discussion: *A. denitrificans* is often considered as a colonizer, although several outbreaks of hospital-acquired infections have been reported in the literature, due to bacterial contamination of antiseptic solutions, central venous catheters, etc. Five cases of pseudo-bacteraemia due to contamination of ESR tubes have been also reported. In the present report PFGE results showed that all strains were identical, which is a clue of a true outbreak. However, the diversity of clinical signs and outcome, the widespread of the infection and the sudden ending of the 'epidemic' make us sceptical as whether we experienced a true bacteraemia or a pseudo-bacteraemia.

P1353

Infections with hepatitis B and C viruses in haemodialysed patients

A. Pisula, E. Janczewska-Kazek, A. Piskorowska-Plis, J. Wiczorek, A. Boron-Kaczmarzka (*Opole, Chorzow, Szczecin, PL*)

Objectives: Infections with Hepatitis B (HBV) and C (HCV) viruses are common in Poland. Patients treated with hemodialyses (HD) in hospital conditions are the ones most susceptible to the infection.

Methods: We analysed medical records of 297 patients with end stage renal failure, treated in the years 2000–2004 in 6 dialysis units which operate 59 hemodialysers in total. The retrospective analysis was made on the basis of medical documentation, analysis of medical procedures conducted, blood treatment and preventive vaccinations.

Results: Number of HBV infected among HD systematically decreases from 11% in year 2000 to 4.5% in year 2004. Such significant decrease in the number of infections was possible to achieve thanks to systematically conducted protective vaccinations, implemented in the pre-dialysis period or in the moment of first dialysis. Correctly performed vaccinations resulted in immunization of 77% HD. About 7.0% of HD required additional inoculations (anti-HBs titre <10 IU). The HD patients population susceptible to a possible infection had been limited

to 18.5%. HCV infection pose a decidedly more serious problem. The number of infected patients hasn't decreased in the years 2000–2004. HCV was newly diagnosed in 70 HD who may be a potential source of infection. The analysis of the new infections indicates a dialysis unit as the place of transmission in 9 of the cases. The reason for the spread of the infection in one of the dialysis stations was, most probably, the process of dialysers re-utilization (4 cases). In the material analysed, a connection between the duration of dialysis therapy, and the number of HCV infection in HD was confirmed (6% after 1 year, 45% after 10 years). No time relation between transfusions and the change in the patient's serologic status has been found. The use of erythropoietin (EPO) among HD increases systematically (an 208% increase from year 2000 till 2004) and replaces red blood cells concentrate (RBC) transfusions.

Conclusion: Transmission of an HBV infection among dialyzed patient systematically decreases due to effective immunization of the susceptible population. Number of hemodialysed patients infected with HCV does not decrease and stays on a high level. Transmission of HCV among HD is connected with inappropriate compliance with sanitary standards. Blood and its products possess a low risk of HBV/HCV transmission among HD in Poland.

P1354

Phenotypes and genotypes of slime positive - methicillin-resistant *Staphylococcus epidermidis* clinical isolates

A. Foka, V. Chini, E. Petinaki, F. Kolonitsiou, E.D. Anastassiou, G. Dimitracopoulos, I. Spiliopoulou (*Patras, Larissa, GR*)

Objectives: Methicillin-resistant-coagulase negative staphylococci (MR-CNS) constitute a major cause of hospital infections, especially in neonatal intensive care units (NICU). *Staphylococcus epidermidis* is the predominant species associated with clinically relevant infections. The role of biofilm formation as a major factor in CNS infections, especially among patients with invasive procedures, such as implanted prosthetic devices and intravascular catheters, is well established. The purpose of the present study was to correlate slime production with the presence of *ica* operon and clones among methicillin-resistant *S. epidermidis* (MRSE) isolated from different patients hospitalized in the NICU of our University Hospital.

Methods: The MIC of oxacillin was determined by the agar dilution method in Mueller-Hinton supplemented with 2% NaCl, according to the guidelines of NCCLS. The presence of *mecA* gene was investigated by Southern blot hybridization of *Claf* digests with a *mecA* DNA probe. Susceptibility profiles were performed, by the E-test method against: tobramycin (Tm), amikacin (Ak), gentamicin (Gm), netilmicin (Net), quinupristin-dalfopristin (Rp), linezolid (Lin), erythromycin (Em), clindamycin (Cm). The MICs of vancomycin (Va) and teicoplanin (Tp) were determined by the agar dilution method, according to the NCCLS guidelines. Slime production was detected by qualitative and quantitative methods. PCR was performed for the detection of *ica* operon, (genes *icaA*, *icaD*, *icaB*, *icaC*) and the insertion sequence element IS256. Clonal types were identified by PFGE of chromosomal DNA *Sma*I digests.

Results: All 115 MRSE isolates were *mecA* positive while 106 (92.2%) produced slime. Ninety two (87%) slime-producing isolates had all four *ica* genes tested, whereas the rest possessed different combinations. Seventy four (70%) slime-positive isolates belonged to the same PFGE type, named z. Four strains belonged to PFGE type g and the rest 28 (26%) were classified into 27 different PFGE types. All PFGE type z isolates exhibited

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the same resistance phenotype to Em, Cm, Ak, Gm, Tm. PFGE type g isolates showed a common resistance phenotype to Em and Cm, with the exception of one strain.

Conclusions: One dominant PFGE type z is characterized among the slime-producing MRSE tested, resistant to Em, Cm,

Ak, Gm, Tm. This multi-resistant slime-producing clone is spread in the nICU associated with bloodstream and catheter infections.

Multidrug-resistant Gram-negative organisms: survey and risk factors

P1355

Antimicrobial resistance of *Enterobacteriaceae* isolated from patients in Austrian intensive care units

C. Jebelean, H. Mittermayer (Linz, AT)

Objective: To investigate the epidemiology of the antimicrobial resistance of *Enterobacteriaceae* isolated from intensive care unit (ICU) patients in 43 Austrian hospitals.

Methods: 571 strains of *Enterobacteriaceae* (193 *E. coli*, 150 *Klebsiella* spp., 109 *Enterobacter* spp., 50 *Proteus* spp., 69 strains of other species) isolated in clinical laboratories from 8 different regions of Austria between January 2005 through September 2005 were tested for susceptibility to ampicillin/sulbactam (A/S), piperacillin/tazobactam (P/T), cefazolin (CZ), ceftazidim (FOX), cefuroxim (CXM), cefotaxim (CTX), ceftazidim (CAZ), cefepim (FEP), gentamicin (G), ciprofloxacin (CI) and imipenem (IM) using the agar dilution method as recommended by CLSI. The strains with CAZ or CTX MIC >1 mg/L were further investigated for the production of ESBL using 3 E-test strips: TZ/TZL, CT/CTL, PM/PML. For interpretation we used the breakpoints as recommended by CLSI.

Results: Overall, the rates of susceptibility were: A/S 51%, P/T 86%, CZ 37%, FOX 64%, CXM 65%, CTX 87%, CAZ 86.4%, FEP 95.8%, G 94%, CI 88%, and IM 100%. For A/S and CZ higher resistance rates were encountered in Salzburg: 62% and 69%; Carinthia: 58% and 73%; and Vienna: 53% and 66%, respectively, whereas CI resistance was more prevalent in Vienna (22%) and Upper Austria (13%). The CI resistance was higher in *E. coli* 17% and *Proteus* spp. 14%, as compared to *Klebsiella* spp. and *Enterobacter* spp. 11% and 9%, respectively. The rates of resistance to 3rd gen Cephs and CI were higher in the medical ICUs as compared to mixed and surgical ICUs. The rates of ESBLs in *E. coli* and *Klebsiella* spp. were higher in surgical ICUs (7.5%) as compared to medical (5.7%) and mixed ICUs (3%). The ESBL strains were concentrated in some hospitals where they reached 33% of *E. coli* or 25% of *Klebsiella* spp.

Conclusions: Although the overall antimicrobial resistance rates were moderate (with the exception of CZ, A/S and CXM), the emergence of CI resistance in isolates of *Enterobacteriaceae* (with rates as high as 43% in some ICUs) and the association with potent ESBLs in some strains is of great concern.

P1356

Risk factors for polymyxin-only susceptible *Pseudomonas aeruginosa* bacteraemia

P. Koletsi, P. Kopterides, A. Michalopoulos, Z. Mastora, K. Rellos, M. Falagas (Athens, GR)

Objectives: To identify risk factors associated with the development of polymyxin-only susceptible (POS) *P. aeruginosa* bacteraemia.

Methods: Case-control study performed in a tertiary-care hospital in Athens, Greece (01/2002-08/2005).

Results: Our study population consisted of 56 patients with *P. aeruginosa* bacteremia. In 16 of these patients (28.6%) the isolate was susceptible only to polymyxins (POS group) and in 40 (71.4%) the isolate remained sensitive to carbapenems (non-POS group). Mortality was 62.5% and 37.5% for the POS and non-POS group, respectively ($p = 0.09$). Bivariable analysis showed that exposure to carbapenems ($p = 0.001$), quinolones ($p = 0.043$), metronidazole ($p = 0.035$), and glycopeptides ($p = 0.018$) as well as admission to the intensive care unit (ICU) ($p = 0.023$), tracheotomy ($p = 0.035$), steroid treatment ($p = 0.008$) and parenteral feeding ($p = 0.008$) were associated with isolation of a POS strain among patients with *P. aeruginosa* bacteremia. The multivariable analysis showed that only prior use of carbapenem was an independent risk factor (OR, 9.0; 95% CI, 2.4–34.3; $p = 0.001$) for development of POS *P. aeruginosa* bacteremia.

Conclusion: The results of this study provide insight into the rising problem of POS *P. aeruginosa* bacteremia by showing that it is associated with a high mortality and highlighting that prior carbapenem use as an independent risk factor for its development.

P1357

Exposure to fluoroquinolones is a risk factor for polymyxin-only susceptible *Acinetobacter baumannii* bacteraemia

P. Kopterides, P. Koletsi, P. Morfou, K. Rellos, Z. Mastora, M. Rizos, M. Falagas (Athens, GR)

Objectives: Previous studies have examined risk factors for multidrug-resistant (MDR) *A. baumannii*, including carbapenem-resistant strains. However, there is an epidemic of infections due to *A. baumannii* resistant to all available antibiotics except polymyxins in several parts of the world. Thus, we aimed to explore the risk factors associated with bacteremia due to polymyxin-only susceptible (POS) *A. baumannii*.

Methods: We conducted a retrospective, cohort study of patients with *A. baumannii* bacteremia who were hospitalized during 01/Jan/2002–31/Aug/2005 at the "Henry Dunant" Hospital, Athens, Greece. To identify risk factors for development of POS *A. baumannii* bacteremia, the distribution of several variables between patients with bacteremia due to POS *A. baumannii* was compared with that of patients with bacteremia due to *A. baumannii* susceptible to other antibiotics besides polymyxins.

Results: Thirty-nine patients with bacteremia due to *A. baumannii* (35 ICU and 4 ward patients) were studied. Twenty-five patients (64%) had POS *A. baumannii* bacteremia while the rest (36%) had bacteremia due to *A. baumannii* susceptible to polymyxins as well as carbapenems. Mortality was 56% (14/

25) and 35.7% (5/14) for patients in the two groups, respectively ($P = 0.22$). The bivariable analysis showed that prior exposure to fluoroquinolones ($P = 0.01$) and antipseudomonal penicillins ($P = 0.004$) was associated with isolation of a POS strain among patients with *A. baumannii* bacteremia. The multivariable analysis using a backward logistic regression model showed that only exposure to fluoroquinolones was associated with development of POS *A. baumannii* bacteremia (OR, 11.6; 95% CI 2.4–55.9; $P = 0.02$).

Conclusion: The appearance of POS *Acinetobacter baumannii* infections represents a major threat to hospitalized patients. Exposure to fluoroquinolones is an independent risk factor for development of POS *A. baumannii* bacteremia.

P1358

Multiresistant strains of *Escherichia coli* isolated from dogs

M. Totaro, M. Corrente, A.L. Bellacicco, V. Martella, G. Greco, C. Buonavoglia (Valenzano, IT)

Objectives: To analyse the antimicrobial susceptibility of *Escherichia coli* isolates from ill and healthy pups.

Methods: Fifty-one strains of *E. coli* were isolated from 47 pups (mean age of 2 months). Thirty-four animals were from kennels, 9 were from private owners and 4 were free-ranging dogs. Thirty-one isolates were obtained from ill animals while 20 isolates were obtained from faecal samples of healthy dogs. All the strains were tested for susceptibility to 20 antimicrobial drugs by the agar diffusion method. The strains resistant to at least one extended-spectrum cephalosporin and/or augmentin were also analysed by the double-disk test and by a PCR specific for the blaAmpC, blaTEM, blaSHV and blaCTX-M-type genes that encode for beta-lactamases.

Results: Twenty-four out of 31 *E. coli* strains (77.3%) isolated from ill dogs displayed a multiresistant pattern, i.e. they were resistant to three or more drugs of different classes. In detail, 96.8% of the strains were resistant to sulfamethoxazole, 90.2% to streptomycin, 67.6% to co-trimoxazole and ciprofloxacin, 54.7% to doxycycline. One strain was resistant to imipenem. Only 2 isolates (6.4%) displayed resistance to a single drug. Seven strains were found to be resistant to cephalosporins and/or augmentin and positive to the double-disk test. By PCR, 3 such isolates were found to possess the blaAmpC, blaTEM and blaCTX-type genes, while 1 possessed only the blaCTX-type gene and 1 displayed only the blaAmpC-type gene. None of the isolate had the blaSHV-type gene. By contrast, multiresistance was detected only in 1 out of 20 *E. coli* isolates (5%) derived from healthy dogs. The rate of resistance observed in such strains was significantly lower than that of the strains isolated from ill dogs (Fisher exact test, $p < 0.001$).

Conclusion: Analysis of *E. coli* strains revealed resistance to new-generation molecules, that are used commonly in human therapy, such as ciprofloxacin, imipenem and cephalosporins, although most samples were from pups housed in kennels, suggesting frequent exposure of animals to humans microorganisms. Altogether, the findings of the present investigation highlight the need for surveillance of antibiotic resistance in *E. coli* strains of animal origin. In particular, the detection of multiresistant strains in family pets, that live in close contact with owners, underscores the risks of transmission to humans of *E. coli* strains bearing resistance to antimicrobials of new generation.

P1359

Clonal diversity and distribution of tetracycline resistance genes among *E. coli* strains isolated from aquatic environments

R. Cernat, V. Lazar, M.C. Balotescu (Bucharest, RO)

Introduction: The presence of more than one efflux-mediating tet gene in clinical and environmental *E. coli* strains is well documented.

Aim: to delineate the clonal diversity and transmission patterns and to investigate the occurrence and distribution of tet genes to antibiotic resistant *E. coli* strains isolated from aquatic sources.

Methods: 12 *E. coli* strains from fresh and polluted waters were studied for (i) antibiotic susceptibility by disc diffusion, double-disc and inductibility disc diffusion tests, and MICs values, (ii) plasmid DNA profiles, RAPD and Rep-PCR fingerprinting, PFGE restricted with XbaI and 16S rDNA sequences, and (iii) screening for the presence of tet genes using multiplex-PCR (tetA-tetG, tetH, tetJ, tetY, tetZ and tet30). PFGE, RAPD and Rep-PCR patterns were compared into clonal groups and variants using UPGMA algorithms. The 16S rDNA amplicons were directly sequenced and Blast searches against GenBank and RDP II databases were performed.

Results: All *E. coli* strains showed multiple antibiotic resistance phenotype without ESBL or inducible beta-lactamase production, with 100% resistance to AM, CM and TC, 80% to cephalosporins, 60% to aminoglycosides, 20% to quinolones. Plasmid DNA profiles showed variable number of plasmids ranging from 80 bp to >50 kb. Genomic DNA digested with XbaI produced an average of 30 fragments ranging between 1.0–70.0 kb. 4 of epidemiologically related strains were classified in two clusters by Rep-PCR and RAPD. Most informative profile was obtained with PFGE analysis, only 1 cluster with two strains being identified; the remaining 10 strains were not related to the clonal strains. 16S rDNA analysis identified 1 and 2 phylogenetic clusters grouping strains isolated from fresh and polluted waters. The aquatic *E. coli* strains are phylogenetically diverse from the clinical *E. coli* strains. Multiplex-PCR screening showed the prevalence of the tet (A) and tet (B) genes (100% of the isolates) followed by tet (D) gene (53%).

Conclusion: According with the isolation source, *E. coli* strains belonged to 2 genetically diverse populations, with only one cluster of related strains isolated from the polluted waters, 8 unrelated isolates having possible clonal relatedness. Both RAPD and PFGE are suitable for molecular typing of environmental *E. coli* isolates. All isolates carried more than one tet gene responsible for the active efflux-mediated resistance and therefore for the multiple antibiotic resistance phenotypes.

P1360

Laboratory identification and transmission of *Burkholderia cepacia* and *Stenotrophomonas maltophilia* in an intensive care unit

W. Pfister, W. Barclay, F. Gerlach, E. Straube (Jena, DE)

Objectives: Aim of our study is to assess identification and also transmission of strains of *B. cepacia* and *S. maltophilia* isolated from patients of an interdisciplinary ICU over a 5-year period by pulsed-field gel electrophoresis.

Methods: 32 clinical isolates of *B. cepacia* and 112 strains of *S. maltophilia* were cultivated from 144 patients. Strains were obtained from BAL of 98 patients (68.1%), from wound materials of 35 patients (24.3%) and from blood cultures of 11 (7.6%) patients. Copy strains were excluded. Identification of the

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isolates was performed phenotypically by means of the identification system api 20 NE (bioMérieux, Marcy l'Etoile, France). Genotypical identification of the *B. cepacia* strains was performed by determination of the rec A-gen by PCR followed by nucleotide sequencing. Antibiotic resistance of the strains was assessed by determination of MICs using microbougillon dilution technique (Micronaut S GENARS GN 3, Merlin, Bornheim-Hersel, Germany). For determination of the genotypes of the *B. cepacia* strains pulsed-field gel electrophoresis analysis using Spe restriction enzyme and GenPath group 3 reagents kit (Bio-Rad, Hercules, CA, USA) and for the DNA macrorestriction analysis of *S. maltophilia* XbaI restriction enzyme and GenPath group 6 reagents kit (Bio-Rad, Hercules, CA, USA) were used.

Results: 21.9 % of the *B. cepacia* strains tested were *in vitro* sensitive against ceftazidime (CAZ), 40.6% against ciprofloxacin (CIP) and 53.1% against cotrimoxazole (SXT). The *S. maltophilia* isolates had following sensitivity rates: CAZ 37.5%, CIP 22.9%, SXT 96.5%. All *B. cepacia* strains had the rec A-gen with identical nucleotide sequences. PFGE analysis of the *B. cepacia* strains showed the same genotype in all patients whereas PFGE analysis of the *S. maltophilia* isolates revealed for most of the patients different genotypes of this species. Only 25 of the 144 patients (17.4%) had the same PFGE type.

Conclusions: Determination of the rec A-gen improves identification of *B. cepacia* in clinical laboratory. Transmission of *B. cepacia* from one patient to another in ICU is obviously very common and has to be avoided. All infections observed in our ICU were hospital acquired. Infections in hospitals with *S. maltophilia* seem to be different. According to our results a transmission of strains in ICU occurs only in 17.4% of the patients. This means that most of the patients have already got their own *S. maltophilia* strain when coming to the ICU.

P1361

Is *Stenotrophomonas maltophilia* an emerging pathogen in German ICUs?

E. Meyer, F. Schwab, P. Gastmeier, H. Ruden, F. Daschner, D. Jonas (Freiburg, Berlin, Hannover, DE)

Objective: We analysed over a period of 4 years the proportion of *S. maltophilia* on all SARI isolates (Surveillance of Antimicrobial Use and Antimicrobial Resistance in Intensive Care Units) and the incidence density of nosocomial infections (NI) caused by *S. maltophilia* (data from KISS the German Hospital Infection Surveillance System).

Methods: The proportion of *S. maltophilia* on all SARI isolates (%SM) is defined by dividing the number of *S. maltophilia* by the total number of SARI isolates multiplied by 100, the incidence density of *S. maltophilia* (SM/1000 pd) by dividing the number *S. maltophilia* isolates by the total number of the patient days multiplied by 1000. The incidence density of NIs caused by *S. maltophilia* is defined by dividing the number of NIs caused by SM by the total number of the patient days multiplied by 1000.

Results: The data of 34 ICUs continuously participating in SARI from 2001 to 2004 were analysed. The data cover a total of 51,536 isolates and 576,446 patient days. Pooled mean %SM was 2.4 (median 2.0, range 0.7–6.9) and pooled mean incidence density of *S. maltophilia* was 2.2 (median 1.6, range 0.2–7.6). The incidence density of NIs caused by *S. maltophilia* ranged between 0–0.467 with a mean at 0.134. Over a period of 4 years no significant change in %SM (17 ICU had an increase, 17 an decrease) or in SM/1000 pd was observed. Antibiotic consumption was statistically not different in the two groups. Neither increased the incidence density of NIs caused by *S. maltophilia* significantly between 2001 and 2004.

Conclusion: *S. maltophilia* not revealed to be an emerging pathogens in 34 German ICUs over the last four years.

P1362

Achromobacter xylosoxidans from the airway secretions of patients with cystic fibrosis

H. Alexandrou-Athanasoulis, S. Doudounakis, A. Sergounioti, K. Halmouki, A. Pangalis (Athens, GR)

Achromobacter xylosoxidans (Ax) is a potential pathogen, which has been increasingly recovered from the sputum of patients with CF last years.

Objectives: The aim of our study was to determine trends in prevalence of this microorganism in patients with CF and to evaluate its resistance to the antibiotics. We reviewed retrospectively the clinical, demographic and laboratory data of the 388 patients with CF who have been attended our hospital between 01/2000-11/2005 for at least one year. During this period 6100 sputum or deep throat specimens were cultured. The identification was made by the API 20NE, as well as by the Vitek II automated system (Biomerieux). The antibiotic resistance was evaluated by the Kirby-Bauer and the Vitek II methods.

Results: 1) Positive for *A. xylosoxidans* were 53 cultures, corresponding to 19 patients (4.9 %) (12 males and 7 females) 2) The mean age of patients at the 1st isolation of *A. xylosoxidans* was 6.7 y (SD: 3.9).

3) *A. xylosoxidans* has been repeatedly isolated from seven of the patients (36.8%), whereas twelve of these patients (63.2%) had only one or two positive cultures.

4) *A. xylosoxidans* was the only microorganism isolated from 16 of the 53 positive for Ax specimens, while the other yielded two (26) or three (11) bacterial species. The most frequent co-isolated microorganism was *P. aeruginosa* (18 specimen), followed by *S. aureus* (4), *H. influenzae* (2), and *Pseudomonas* spp (2). In addition to Ax, seven cultures yielded *P. aeruginosa* and *S. aureus*, two *P. aeruginosa* and *Candida albicans*, one *P. aeruginosa* and *S. aureus* and one *Pseudomonas* spp and *S. aureus*

5) All strains were sensitive to piperacillin, piperacillin/tazobactam, whereas sensitivities to other antibiotics tested (%) were: ticarcillin (76.4), ticarcillin + clavulanic acid (88.2), ceftazidime (67.1) cefepime (29.4) imipenem (67.1), meropenem (64.7), cotrimoxazole (47.1) and colistin (76.4) respectively.

Conclusions: 1. *A. xylosoxidans* has been harboured by 19 (4.9 %) of our patients. 2. The mean annual incidence rate showed no changes. 3. Piperacillin and piperacillin/tazobactam were the most effective antibiotic for this species followed by ticarcillin + clavulanic acid.

P1363

Genotypic and phenotypic analysis of *Pseudomonas aeruginosa* isolates from sputum of patients with cystic fibrosis following antimicrobial treatment

O. Perovic, E. Marais, A. Duse, M. Mer, G. Richards, O. Perovic (Johannesburg, ZA)

Objectives: The study aimed to determine genotypic and phenotypic characteristics of *P. aeruginosa* strains isolated from sputum specimens before, during and after antibiotic treatment in cystic fibrosis (CF) patients who were routinely admitted for 14 days of therapy every four months for 1 year.

Methods: Only CF patients permanently colonized with *P. aeruginosa* were enrolled in the study. Sputum samples were collected "pre", "during" and "post" treatment with anti-

crobial therapy. Microscopy, culture, quantitative bacterial count (CFU) and susceptibility testing were performed. All isolates were evaluated phenotypically as mucoid or nonmucoid strains. Multiple drug resistance (MDR) was defined as resistance to more than two classes of anti-pseudomonal drugs. *P. aeruginosa* isolates were all identified using API 20 NE. Antibiotic susceptibility testing was performed using the disk diffusion method. Macrorestriction analysis using 30U XbaI and pulsed field gel electrophoresis (PFGE) was performed. The PFGE patterns were compared visually and Tenover criteria used to assess relatedness.

Results: A total of 163 phenotypically different strains of *P. aeruginosa* were identified from 14 patients. The number of mucoid strains decreased from 22 at the first to 10 at the third visit and 9 patients showed a more than 2 fold decrease in CFU (Table 1). Five of 7 patients with MDR isolates demonstrated the same susceptibility pattern throughout the course of treatment in all 3 visits. The PFGE patterns of 4 of 13 patients from sputum from 3 visits were indistinguishable. In another 4 patients, minor band differences were observed (less than 3 bands) between isolates over the treatment period, with 1 strain having 5-band difference. This shows that the strains were in all probably related, but have started to diverge. No interpatient strain similarities were found.

Table 1. Mucoid and nonmucoid phenotypes and decrease in colony count of *P. aeruginosa* isolates from sputum specimens from 14 patients

Visits	Pre treatment		During treatment		Post treatment		More than 2 fold decrease in CFU*
	Mucoid	Nonmucoid	Mucoid	Nonmucoid	Mucoid	Nonmucoid	
First	8	18	7	17	7	12	6
Second	7	16	5	15	1	11	7
Third	4	9	4	10	2	10	9

* CFU, Colony Forming Units

Conclusion: Although all the patients were colonized with genotypically indistinguishable strains of *P. aeruginosa*, these demonstrated phenotypic variability in the form of colony morphology and multi drug resistance. This reflects the ability of organism to survive and adapt to host immune mechanisms. Antimicrobial treatment decreased the total number of *P. aeruginosa* strains but had no effect on MDR isolates. The diversity of the strains between patients in this study suggests that transmission amongst patient did not occur and that the patients obtained their strains from different environments.

P1364

Genodiversity of resistant *P. aeruginosa* in ICUs varies in relation to antimicrobial usage and resistance rates

D. Jonas, E. Meyer, F. Schwab, B. Spitzmüller, F. Daschner, H. Grundmann (Freiburg, Berlin, DE; Bilthoven, NL)

Objectives: The prevalence of resistant strains in health care institutions depends on the selection of imported resistance traits and the rate of spontaneously arising mutants, but can also be due to expansion by cross-transmission of resistant strains. To improve our understanding of the underlying dynamics, the frequency of different genotypes was determined in *P. aeruginosa* isolates from ICUs and correlated with antimicrobial usage densities (AD) and resistance rates (RR). The numbers of indistinguishable genotypes found were considered as an indirect measure of cross-transmission events.

Methods: From the German SARI surveillance project, 273 ciprofloxacin- and 317 imipenem-resistant isolates of *P. aeruginosa* were obtained from 29 ICUs, together with unit - based antimicrobial consumption data (AD) and resistance rates (RR). Additionally, for one month in spring 2003, the participants

collected a cross sectional sample of 146 clinical *P. aeruginosa* isolates irrespective of the antimicrobial susceptibility. ICUs were grouped according to their relative positions above or below the median RR and AD of all ICUs. All strains were assigned ICU-based to genotypes by use of AFLP. ICU-specific diversity (Div) was expressed as the numbers of different genotypes per numbers of typed strains per ICU.

Results: In ICUs with high RR and low AD, the genodiversity of resistant *P. aeruginosa* - isolates was significantly lower (Fisher $P < 0.05$) than in those ICUs that featured low RR in the presence of high AD. No such differences could be shown in the cross sectional sample of *P. aeruginosa* isolates irrespective of their antimicrobial susceptibility. RR correlated with AD. However, in ICUs with a low diversity of resistant *P. aeruginosa* - isolates there was a stronger rise of RR with increasing AD than in those ICUs with a high diversity.

Conclusions: This study on resistant *P. aeruginosa* supports the assumption that a high RR in the presence of low AD results from more frequent cross-transmission events. A stronger rise of RR in ICUs with a low genodiversity indicates that RR in ICUs might be markedly determined by cross-transmission events beside AD.

P1365

Relationship between prior antibiotic exposure and multidrug-resistant *Pseudomonas aeruginosa*

T.P. Lodise, N. Patel, J. Graves, B.M. Lomaestro (Albany, US)

Background: The increasing emergence of multi-drug resistant *Pseudomonas aeruginosa* (MDR-PA) infections (infx) is a major public health concern. Despite the rising rates, MDR-PA risk factors have not been well defined & quantitative evaluations of the relationship between prior antibiotic (abx) exposure & MDR-PA have not been performed in patients (pt).

Objective: To examine the risk factors for MDR-PA & to quantify the relationship between prior abx exposure & MDR-PA.

Methods: Study period: 1/02–4/04. Inclusion criteria: (1) ≥ 18 yrs, (2) PA respiratory culture (met CDC infx criteria), (3) non-cystic fibrosis. Demographics, co-morbid conditions, abx (30 days (d) prior PA) were recorded. Recursive partitioning (CART) was used to identify the duration of abx exposure for each antipseudomonal class that was associated with an increased probability of MDR-PA. MDR was defined as resistance to ≥ 4 anti-pseudomonal abx classes. Logistic regression was performed to identify the predictor variables that were independently associated with MDR-PA. Variables that were associated with MDR-PA in the univariate analysis were included in the logistic regression analysis at model entry and a stepwise process was used to identify independent predictors.

Results: During study, 353 pts met criteria. Mean age: 60.6 ± 18.9 . Median LOS prior PA: 24 d [0–178]. Mechanical

The Univariate Analysis: Relationship Between Clinical Features and MDR-PA

Clinical Feature	MDR-PA	Non-MDR-PA	P-value
	N= 122	N= 231	
Length of stay prior PA ≥ 33 d (131 pts)	73 (59.8)	58 (25.1)	<0.001
Prior fluoroquinolone exposure ≥ 4 d (63 pts)	33 (27.0)	30 (13.0)	0.001
Prior cefepime exposure ≥ 9 d (30 pts)	19 (15.6)	11 (4.8)	0.001
Prior piperacillin/tazobactam exposure ≥ 12 d (78 pts)	41 (33.6)	37 (16.9)	<0.001
Prior carbapenem exposure ≥ 3 d (64 pts)	33 (27.0)	31 (13.4)	0.002
Prior aminoglycoside exposure ≥ 9 d (75 pts)	39 (32.0)	36 (15.9)	<0.001
Number of antipseudomonal agents prior to PA ≥ 3 (57 pts)	31 (25.4)	26 (11.3)	<0.001

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ventilation at PA onset: 80.2%. MDR-PA: 34.6%. 30-day mortality: 8.8%
Note for Table: Data are number (%) of pts with MDR-PA or non-MDR-PA. In logistic regression, prior carbapenem exposure ≥ 3 d (OR = 1.9, 95% CI: 1.1–3.4), prior piperacillin/tazobactam exposure ≥ 12 d (OR = 1.8, 95% CI: 1.1–3.1) & LOS prior PA ≥ 33 d (OR = 3.5, 95% CI: 2.2–5.8) were the only univariate predictor variables independently associated with MDR-PA.

Conclusion: Prior carbapenem exposure ≥ 3 d, prior piperacillin/tazobactam ≥ 12 d, & prolonged prior LOS were the strongest predictors of MDR-PA. Further epidemiologic and molecular studies are needed to ascertain the reasons for higher rates of MDR-PA associated with certain prior abx.

P1366

Multiresistant *Pseudomonas aeruginosa* outbreak caused by multiple sources and strains

C. Petignat, I. Federli, G. Zanetti, A. Wenger, J. Bille, P. Francioli, D.S. Blanc (Lausanne, CH)

Pseudomonas aeruginosa is an opportunistic pathogen. Multiple resistances (multi-R) to antibiotics is generally observed after a prolonged treatment, and their occurrences are generally sporadic. In some instances, the spread of the multi-R bacteria is responsible for outbreak. Between November 2002 and January 2003, we observed an unusual increase (15 cases) of patients with multi-R P.a. The majority of the patients had a cardiovascular surgical intervention or were hospitalised in the surgical ICU. All but two underwent a transoesophageal echocardiography during the intervention. The analysis of the procedure showed a potential contamination of the sound during its rinsing after disinfection. The sink where this rinsing was performed was found to be contaminated with the same P.a. strain as that of the patients. Retrospective analysis identified 4 other patients harbouring the same strains in 2002. Despite a new disinfection protocol, new cases of ICU patients with multi-R P.a. continued to be reported. In April 2003, an investigation in the ICU revealed that the siphons of several sinks were contaminated with multi-R P.a. A disinfection protocol of the siphons was set up and isolation precautions were taken for all patients with multi-R P.a. Despite these measures, new cases continued to be reported, a total of 39 patients were reported at the end of 2003. A molecular epidemiological investigation was undertaken including all ICU patients with P.a. The results showed that i) 2 strains were responsible for this epidemic, ii) one strain was found in the sinks of the operative room and the ICU (suggesting an environmental source) whereas the other was found only in patients (suggesting patient to patient transmission), iii) several P.a. isolates (10 patients) showing a non multi-R phenotype were found to be genetically identical to the epidemic strains. It was only after isolation precautions were enforced for all ICU patients with P.a. (multi-R or not) that the number of cases went back to baseline. This is the first report of a multi-resistant *P. aeruginosa* outbreak involving two strains and multiple sources of contamination (transoesophageal sound, siphons, other patients). The duration of the outbreak was probably due to the persistence of the epidemic strains in the environment, and to the lack of isolation of ICU patients harbouring the epidemic strains which did not showed a multi-R phenotype.

P1367

Nosocomial *Acinetobacter baumannii* vs. *Klebsiella pneumoniae* bloodstream infections: risk factors and outcomes

M. Paul, E. Robenstock, A. Fraser, L. Leibovici, S. Perez, S. Pitlik, I. Ostfeld, Z. Samra, M. Weinberger (Petah Tikva, IL)

Objectives: To assess mortality associated with nosocomial *Acinetobacter baumannii* (AB), bloodstream infections (BSIs), independent of other risk factors for mortality. We compared risk factors and outcomes for patients with nosocomial AB vs. nosocomial *Klebsiella pneumoniae* (KP) BSIs.

Methods: The study was conducted at Rabin Medical Center, Beilinson campus, a 900-bed primary and tertiary care hospital in Israel. All patients with nosocomial AB and KP BSIs between 2000–2003 were identified from an ongoing prospective database. Only first episodes were included. Detailed demographic, microbiologic, antibiotic treatment and other clinical data were collected retrospectively through patient chart review, using a standardized questionnaire. Nosocomial BSIs were defined as those developing more than 48 hours after admission. Mortality was defined as 30-day all-cause deaths. Variables significantly associated with mortality at the univariate level ($p < 0.1$) were entered into a multivariable logistic regression model.

Results: 112 patients with AB and 90 patients with KP nosocomial BSIs were included. AB was significantly associated with poorer performance status; burns; pneumonia, mechanical ventilation, arterial line and nasogastric tube prior to BSI; prior treatment with steroids and carbapenems, but not other antibiotics; pneumonia as source of infection; lower albumin and higher urea. KP BSIs were associated with urinary tract infections. Appropriate empirical antibiotic treatment was administered to 19.6% of patients with AB and 42.2% of patients with KP BSI ($p < 0.001$). AB BSIs were associated with a significantly higher percentage of septic shock and respiratory failure and resulted in longer hospital stay (32 ± 27 days vs. 22 ± 20 days for patients alive). Mortality was 61.6% with AB vs. 38.9% with KP ($p < 0.001$). Independent risk factors for mortality on multivariate analysis included AB BSI, diabetes, septic shock and urea (Table).

Multivariate model for 30-day mortality

	P-value	OR	95% CI	
Septic shock	<0.001	18.44	5.78	58.87
Age (continuous)	<0.001	1.08	1.04	1.12
Diabetes mellitus	0.033	2.88	1.09	7.59
<i>Acinetobacter baumannii</i> BSI	0.039	0.37	0.14	0.95
Urea	0.050	1.01	1.00	1.02
Blood products during last month	0.088	2.53	0.87	7.36
Mechanical ventilation at presentation	0.094	2.25	0.87	5.80
Any antibiotic during last month	0.151	4.91	0.56	43.17
Pneumonia as source of infection	0.160	0.46	0.16	1.36
Renal failure	0.333	0.60	0.21	1.69
Underlying pneumonia	0.437	1.61	0.49	5.31
Appropriate empirical antibiotic treatment	0.657	0.80	0.30	2.16
Urinary tract as source of infection	0.662	0.74	0.20	2.78
Corticosteroids during last month	0.861	0.92	0.35	2.39
Congestive heart failure	0.976	0.98	0.33	2.92
Number of days in hospital	0.979	1.00	0.97	1.03
Corthosis	0.999			

Conclusions: AB affects patients with more severe underlying conditions in hospital, when compared to a common nosocomial Gram-negative pathogen. However, the mortality associated with AB BSIs is higher than that of KP BSIs, after correction for underlying patients' conditions.

P1368

Neurosurgical meningitis due to *Acinetobacter baumannii*: comparison between different treatments

A. Rodríguez-Guardado, A. Blanco, J. Torres, V. Asensi, V. Pintado, F. Pérez, M. Lantero, E. Bustillo, J. Maradona, J. Cartón (Oviedo, Madrid, ES)

Background: The treatment of multidrug-resistant *Acinetobacter baumannii* meningitis is a serious therapeutic problem. We describe the outcome of nosocomial neurosurgical meningitis treated with different therapeutic options.

Methods: 64 patients with nosocomial postsurgical meningitis due to *Acinetobacter baumannii* diagnosed between 1990–2004 were retrospectively reviewed.

Results: Mean hospital stay before the infection was 26.5 [19.7] (limits 7–99 days); main underlying diseases were: intracerebral hemorrhage (45.3%), head trauma (29.7%) brain neoplasms (20.3%), and hydrocephalus (4.7%). 49 cases had intraventricular catheters, 4 had a ventriculoperitoneal shunt and 11 cases had CSF leakage. The most important treatment showed in table 1. Two patients dead without treatment. Although no patients treated with colistin dead and the mortality rate were lower in intrathecally treated patients (4 patients vs 18 p = 0.095) we have not observed statistically differences between the treatments. 22 patients dead due to the infection and the rest cure. The mortality was associated with no removal of CSF devices (p = 0.007, OR: 4.88 [1.27–19.48]), inadequate treatment (p = 0.0002, OR: 1.467; [1.102–1.951]), lack of stay in ICU (p = 0.041; OR: 3 [1.025–8.777]. and delayed in the beginning of treatment (p = 0.004)

Table 1. Characteristics of antibiotics treatments

Treatment	N	Adequate	Duration	Mortality
I.V. monotherapy - with CBP - whit AS	3			
	4			
	2	27	15.88[6.32]	11 (39%)
	8	5	22.20[16–38]	1(20%)
	5			
IV combined	5	5	14.6[6.45]	2(40%)
IV + Intrathecal -whit both colistin -whit aminoglycosides + CBP	1			
	9			
	7	7	20.85[4.25]	0(0%)
	7	12	20.66[11.82]	2(16.6%)
	2			

Conclusions: Nosocomial *Acinetobacter* meningitis have a high related mortality. This is lower in patients with intraventricular treatment. Colistin is a useful and safe option in the treatment of this entity.

P1369

Clinical epidemiology of biofilm production in *Acinetobacter baumannii* clinical isolates

J. Rodríguez-Bano, S. Martí, S. Soto, J.M. Cisneros, J. Pachón, A. Pascual, L. Martínez-Martínez, J. Vila on behalf of the GEIH

Objectives: *A. baumannii* is an important cause of device-associated nosocomial infections. We investigated the frequency and epidemiology of biofilm formation among clinical strains of *A. baumannii*.

Methods: *In vitro* biofilm formation was tested in 92 epidemiologically unrelated *A. baumannii* isolated from clinical samples during November 2000 in 25 Spanish hospitals participating in

the GEIH-Ab 2000 project. Biofilm formation was determined in an overnight culture, which was diluted 1:100 in LB broth and incubated without shaking at 37°C for 48 hours. The biofilm was stained with 1% crystal violet and quantified at 570 nm after solubilization with ethanol-acetone. The following variables were collected: age, sex, type and severity of underlying diseases, duration of hospital stay, invasive procedures, previous antimicrobials, types of infections and outcome. Univariate and multivariate analysis using logistic regression were performed.

Results: Among the 92 *A. baumannii* strains, 56 (61%) formed biofilm, 33 (36%) did not, and 3 could not be evaluated. Clinical and epidemiological data were available for 85 patients; 53 of them had a biofilm-forming strain (62%) and 32 (38%) a non-forming one. Biofilm-forming strains were less frequently epidemic (31% vs. 53%, p = 0.04), imipenem-resistant (25% vs. 47%, p = 0.03), ciprofloxacin-resistant (66% vs. 94%, p = 0.004) and isolated from the respiratory tract (25% vs. 52%, p = 0.01), but were more frequently isolated from urine (33% vs. 14%, p = 0.06) and blood (10% vs. 0, p = 0.07). Patients with biofilm-forming strains were less frequently in ICU (26% vs. 53%, p = 0.01) and had more frequently received aminoglycosides (43% vs. 20%, p = 0.07). There were only 3 pediatric patients, and all of them had biofilm-forming isolates. Multivariate analysis showed that ICU stay (OR = 0.1, 95% CI: 0.04–0.8), isolation from the respiratory tract (OR = 0.2, 95% CI: 0.05–0.9), and ciprofloxacin-resistance (OR = 0.06, 95% CI: 0.00–0.4) were associated with non-biofilm formation, while previous aminoglycoside use (OR = 13.1, 95% CI: 2.3–74.9) was associated with biofilm formation. There were no differences in mortality or duration of hospitalisation between patients with infections caused by biofilm forming and non-forming *A. baumannii*.

Conclusions: About 60% of unrelated *A. baumannii* strains were showed to form biofilm. ICU stay, isolation from the respiratory tract and ciprofloxacin-resistance were associated with biofilm non-forming *A. baumannii* strains.

P1370

Dissemination of tetracycline and chloramphenicol resistance genes in Portuguese *Salmonella* isolates carrying class 1 integrons

P. Antunes, J. Machado, L. Peixe (Porto, Lisbon, PT)

Objectives: In this study we evaluated the genetic background of tetracycline and chloramphenicol resistance in *Salmonella* carrying class 1 integrons.

Methods: The minimum inhibitory concentration for 10 antimicrobial agents was determined by the agar dilution method in 1511 isolates collected from human, food products and environment (2002–2004). Characterization of class 1 integrons was performed by PCR, RFLP (TaqI) and sequencing in the 331 sulfonamide resistant isolates. Screening for tetA, tetB, tetG, floR, cmlA and catA genes was searched by multiplex PCR assays. Conjugation assays and clonality analysis (PFGE-XbaI) were performed.

Results: 26% (386/1511) of the isolates were multiresistant (ranging from two to eight antibiotics). Class 1 integrons were found in 78% (259) of the sulfonamide resistant isolates, with 241 (93%) resistant to tetracycline and 188 (73%) to chloramphenicol. Resistance to tetracycline was related to tetA (46%), tetB (21%) or tetG (32%) and to chloramphenicol to floR (43%), catA (29%) or cmlA (24%). In contrast to the antimicrobial resistance determinants specific of the multiresistant DT104 serotype Typhimurium clone (Int1 1000: aadA2 + 1200: blaPSE-1, floR and tetG),

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class 1 integrons in the remaining isolates (85/182) were transferred by conjugation, mostly associated to tetracycline and chloramphenicol resistance genes. Gene cassettes conferring resistance to aminoglycosides (aadA1, aadA2 and aadA5), trimethoprim (dfrA1, dfrA12 and dfrA17) and β -lactams (blaPSE-1 and blaOXA-30) were found. Interestingly, a gene cassette encoding chloramphenicol resistance (cmlA) was also detected.

Conclusion: The presence of class 1 integrons is significantly associated with multidrug resistance including to tetracycline and chloramphenicol, which genes are not usually integrated in gene cassettes. Our results suggest that co-selection and maintenance of MDR in *Salmonella* might be the result of the use of several agents in food animals, namely sulfonamides, florfenicol and tetracyclines, which could difficult the reduction of antimicrobial resistance in this zoonotic bacteria.

P1371

Epidemiological types and integrons in multidrug-resistant *Acinetobacter baumannii* strains from intensive care units in Rome, Italy

S. D'Arezzo, A. Capone, N. Petrosillo, P. Visca on behalf of Gruppo Romano *Acinetobacter baumannii* GRAB

Introduction: *Acinetobacter baumannii* is a major opportunistic pathogen and a serious clinical challenge in Intensive Care Units (ICU). Molecular typing is essential for epidemiological tracing of *A. baumannii* infection and monitoring of multiple drug resistance (MDR). Studies on MDR in *A. baumannii* have demonstrated the presence of several antibiotic-resistance genes arrayed into integrons.

Objectives: 1) To investigate the epidemiology of *A. baumannii* isolates from ICU patients cared in six local Hospitals; 2) to evaluate the genetic relatedness between *A. baumannii* strains by random amplified polymorphic DNA (RAPD) analysis; 3) to characterize the structure of their integrons; 4) to correlate epidemiological types with integron carriage and MDR patterns.

Methods: Ninety *A. baumannii* isolates were obtained over a 12-month period from critically ill patients and the associated ICU environment in six public Hospitals in the Rome urban area. Four primers were employed for RAPD fingerprinting. Fingerprints were considered distinct if they diverged by more one band. Integrase and blaIMP genes were detected by PCR with appropriate primer pairs. For the detection of resistance genes in class-1 integrons, PCR was performed with the primers for the 5'- and 3'-conserved segments (CS). PCR products were sequenced to determine integron structure. Restriction fragment length polymorphism (RFLP) was used to compare integrons between isolates.

Results: Comparison of *A. baumannii* RAPD fingerprints showed six different types, with two main clusters. Most MDR strains were clustered in types I and II (cluster I: 70/90 isolates; cluster II: 15/90 isolates). The int1 gene was detected in 90/90 of the *A. baumannii* strains. Four integrons were identified, whose combination defined cluster I (carrying integrons A and D) and cluster II (carrying integrons A, B and C). Sequencing of the variable DNA region interposed between the 5'-CS and the 3'-CS

indicates that most MDR determinants in *A. baumannii* are assembled within class-1 integrons. RFLP confirmed the identity of similar sized integrons between strains belonging to the same cluster.

Conclusion: RAPD fingerprinting and search for integrons by PCR provided evidence of clonal distribution of two epidemic strains circulating for at least one year in the ICUs of six different Hospitals, and confirmed the high prevalence of class-1 integrons in MDR *A. baumannii* strains from both clinical and environmental ICU specimens.

P1372

Ward investigation after hospitalising a case with pandrug-resistant *Acinetobacter baumannii* infection

Y-H. Huang, Y-C. Huang, C-H. Lin, L-H. Su, C-T. Wu
(Kweishan, Taoyuan, TW)

Background: Pandrug-resistant *Acinetobacter baumannii* (PDRAB) emerged in Taiwan in early 2000s and spread rapidly throughout the whole island. This organism was not identified in our Children's Hospital (Hospital A) until March 2005 when a patient with pneumonia was transferred from a respiratory care hospital (Hospital B). PDRAB was subsequently recovered from her sputum specimen obtained on admission.

Methods: Being aware of the culture results, we placed the patient into a single room and implemented contact precaution immediately. We also conducted an investigation in the related wards of both hospitals. A total of 212 specimens were obtained from the 30 hospitalized patients (7 in Hospital A, 23 in Hospital B), their ward bed related facilities and environmental objects for the detection of PDRAB. All the AB isolates were analysed with antibiograms (imipenem, amikacin, gentamicin, ciprofloxacin, ceftazidime, cefepime, aztreonam, and piperacillin) and 2 genotyping methods.

Results: Of the 84 specimens obtained from Hospital A, 13 (15%) specimens were positive for AB, among which 6 were PDRAB. Of the 6 PDRAB isolates, 5 isolates were from the index case and her related facilities. Of the 128 specimens obtained from Hospital B, 23 specimens were positive for AB, among which 5 were PDRAB. The 5 PDRAB isolates were from 3 patients and their related facilities. One patient had 1 PDRAB isolate each while he stayed in Hospitals A and B, respectively. Of the 36 AB isolates, there were 9 IRS-PCR patterns, 12 PFGE patterns and 6 antibiogram patterns identified. 25 isolates belonged to a major IRS-PCR type (4 PFGE patterns) and presented with either pandrug resistance (all 11 PDRAB isolates clustered in this type) or multi-drug resistance (only susceptible to imipenem). From a single patient, the isolates were indistinguishable in 2 of 3 patients with multiple isolates. Compared to the isolate from a single patient, the isolates from its related facilities were indistinguishable in 2, highly related in 3 and distinct in 2 of 7 pairs of isolates.

Conclusion: *A. baumannii* is a ubiquitous organism that can be isolated from the patients and environmental objects. A clone of AB with multi- or pan-drug resistance was circulating in both Hospitals.

Epidemiology of resistance to antibiotics - I

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Increase of resistant *Enterobacter* isolates and correlation with antibiotic consumption at the ward level

P. Haller, S. Tschudin, M. Dangel, M. Simcock, R. Frei, H. Plagge, A.F. Widmer, M. Battegay, U. Fluckiger, S. Bassetti for the Multidisciplinary Antimicrobial Group University Hospital Basel, CH

Objectives: *Enterobacter* spp. (EB) are frequent causes of serious nosocomial infections. The incidence of EB resistant to 3rd generation (gen.) cephalosporins (ceph.) at the University Hospital Basel increased from 2003 to 2004. We analysed the relationship between antibiotic consumption and incidence of resistant EB at the ward level. EB have an inducible AmpC beta-lactamase. Under selective antibiotic pressure a stably derepressed mutant resistant to 3rd gen. ceph. can be selected. It has been shown that 3rd gen. ceph. and piperacillin/tazobactam (pip/taz) can select these resistant mutants. The role of 2nd gen. ceph. for the selection of resistant EB is controversial.

Methods: All patients with a resistant EB between 1/03 and 12/04 and the ward, where they were hospitalised at the time of EB isolation, were identified. The pharmacy provided antibiotic consumption data of the different wards (pip/taz, ceph, carbapenems, glycopeptids, aminoglycosides, quinolones) in DDD/100 patient-days for 2003 and 2004. With linear regression analysis we correlated resistance and antibiotic consumption of the wards.

Results: From 2000–2003 the incidence of resistant EB remained stable (0.1 per 1000 patient-days) with a sudden rise in 2004 to 0.227. In the linear regression analysis only consumption of 2nd gen. ceph. was significantly associated with incidence of resistant EB ($p < 0.0001$). The surgical ICU had the highest incidence of resistant EB (2003: 0.57 and 2004: 3.71) and the highest use of 2nd gen. ceph.: 20.1 DDD in both years. The ward with the 2nd highest incidence of resistant EB was the medical ICU with 0.57 in 2003 and 0.73 in 2004. This ward used only 2.4 DDD of 2nd gen. ceph. in both years, but had a high antibiotic consumption of 99.1 DDD in 2003 and 85.1 DDD in 2004. The ward with the highest antibiotic consumption was the bone marrow transplant unit (133.3 DDD in 2004). It had no resistant EB in both years and no 2nd gen. ceph. were used. Overall antibiotic consumption was not significantly correlated with resistant EB.

Conclusion: Use of 2nd gen. ceph. was associated with a higher incidence of resistant EB. This observation is supported by the high incidence of resistant EB in the surgical ICU, probably due to the ample use of 2nd gen. ceph. for perioperative prophylaxis, which is the only indication for these antibiotics at our institution. Our study adds more evidence to the hypothesis that also 2nd gen. ceph. may select derepressed EB mutants.

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Risk factors for carbapenem-resistant *Pseudomonas aeruginosa* among patients with respiratory tract infections

N. Patel, T.P. Lodise (*Albany, US*)

The prevalence of Carbapenem-resistant *Pseudomonas aeruginosa* (CR-PA) infections is increasing in many hospitals and is a major public health concern. Despite the rising rates, risk factors for CR-PA have not been well defined & quantitative evaluations of

the relationship between prior antibiotic exposure & CR-PA have not been performed in patients.

Objective: To examine the risk factors for CR-PA & to quantify the relationship between prior antibiotic exposure & CR-PA.

Methods: Study period: 1/02–4/04. Inclusion criteria: (1) ≥ 18 yrs, (2) PA respiratory culture (met CDC infection criteria), (3) non-cystic fibrosis. Demographics, co-morbid conditions, prior antibiotic exposure (30 days prior onset PA culture) were recorded. Recursive partitioning (CART) was used to identify the duration of abx exposure for each antipseudomonal class that was associated with an increased probability of CR-PA. Logistic regression (LR) was performed to identify independent predictors of CR-PA.

Results: During study, 351 pts met criteria. Mean age: 60.5 ± 18.9 . Male: 61.0%. Median LOS prior PA: 24 d [0–178]. Mechanical ventilation at PA onset: 80.2%. CR-PA: 48.1%. 30-day mortality: 8.3. Of the CR-PA, 84.6% and 71.0% of cultures were cross-resistant ≥ 4 classes of anti-pseudomonas antibiotics compared 10.4% of carbapenem susceptible pseudomonas cultures. In the logistic regression, prior mechanical ventilation for >9 days (OR = 4.1; 95% CI: 2.4–6.9), prior carbapenem exposure >2 days (OR = 4.1; 95% CI: 2.1–7.6) and prior fluoroquinolone exposure >3 days (OR = 2.4; 95% CI: 1.3–4.5) were independent predictors of CR-PA.

Univariate Analysis; Predictors of CR-PA

Predictor	CR-PA	Non-CR-PA	P-value
Mechanical ventilation $> 9d$	82.2	49.5	0.001
Prior aminoglycoside exposure $> 7d$	16.6	7.1	0.006
Prior fluoroquinolone exposure $> 3d$	25.4	11	0.001
Prior carbapenem exposure $> 2d$	29	8.2	0.001
Prior cefepime exposure $> 8d$	13	4.4	0.004
Prior piperacillin/tazobactam exposure $> 9d$	25.4	17.6	0.07

Conclusion: The overall prevalence of CR-PA was extremely high (48.1%) and the overall majority of CR-PA were cross-resistant to multiple classes of anti-pseudomonal antibiotics. Mechanical ventilation >9 days, Prior carbapenem exposure > 2 d, and prior fluoroquinolone exposure >3 days were the strongest predictors of CR-PA. Further epidemiologic and molecular studies are needed to ascertain the reasons for higher rates of CR-PA associated with certain prior antibiotic exposures.

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Risk factors associated with *S. pneumoniae* multiple resistance in Belgium over a 10-year period

J. Van Eldere, R.M. Mera, L.A. Miller, J. Verhaegen, J.A. Poupard (*Leuven, BE; New Orleans, Upper Providence, Philadelphia, US*)

Objectives: Resistance to two or more antibiotics has been increasing over time in many European countries. Factors associated with multiple resistance are investigated using 10-year surveillance data from Belgium.

Methods: Multiple resistance (MR) is defined as non-susceptibility to two or more antibiotic classes (penicillins, macrolides,

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tetracyclines, quinolones and sulfas). Surveillance data consists of 14,488 *S. pneumoniae* invasive isolates from 1994–2004, identified by postal code as well as clinical and demographic information. Risk factors associated with MR were analysed using multivariate logistic regression.

Results: Resistance to two or more antibiotic classes was 14.1% of all isolates in 1994, increasing to reach a peak of 30.7% in 2000, decreasing slightly to 28.8% in 2004. In 2004 out of all penicillin resistant isolates, 80% were erythromycin and 75% were cotrimoxazole resistant respectively. Similarly, 90.3% of all tetracycline resistant isolates were macrolide resistant. In a multivariate analysis, significant risk factors were age, source of the isolate, population density, serotype, location and previous antibiotic use. Isolates from children less than 5 years old were 1.55 times more likely to be multiply resistant (95% CI 1.3 – 1.7); ear isolates were 1.54 times more likely to be resistant than blood isolates (95% CI 1.4 – 1.8); for every 1,000 inhabitants per km², there is a 7% increase in resistance (95% CI 1.8% – 12.4%). 7 valent pneumococcal vaccine isolates were 14.3 times more likely to be MR than non-vaccine related serotypes (95% CI 12.5 – 16.4). Provinces that border France were 1.54 times more likely to be MR than those located by the Dutch border (95% CI 1.4 – 1.8). Subjects previously treated with antibiotics were 1.3 times more likely to harbor a multiply resistant strain (95% CI 1.2 – 1.5, $p = 0.01$) independent of location, time and other risk factors.

Conclusion: More than two thirds of all non-susceptible isolates in Belgium are resistant to two or more antibiotic classes. The proportion of all isolates that are MR has been increasing over time, while single resistant isolates have declined slightly, even though the overall trend has been toward a plateau in resistance. This study confirms the importance of population density even after adjusting for the effect of population structure, time, location and other known risk factors.

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Association between antimicrobial resistance and virulence in *Escherichia coli* blood and faeces isolates from humans in Denmark

L. Bagger-Skjøt, D. Sandvang, C.H. Lester, L.J. Porsbo, D.L. Monnet, N. Frimodt-Møller, A.M. Hammerum (Copenhagen, Bagsværd, Søborg, DK)

Objectives: The aim of this study was to investigate possible associations between resistance phenotype and virulence genes in *Escherichia coli* isolates obtained from the blood of bacteraemic patients and from the faeces of non-hospitalised humans. **Methods:** Between January and September 2003, 123 consecutive *E. coli* blood isolates were collected at the Clinical Microbiology Laboratory, Statens Serum Institut. During the same period, 85 *E. coli* faeces isolates from non-hospitalised human volunteers were obtained as part of the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP). Susceptibility to 17 antimicrobial agents was determined by micro-broth dilution using the Sensititre system (Trek Diagnostics Systems Ltd., East Grinstead, United Kingdom). The presence of the following 9 virulence genes was assessed by PCR: *iutA*, *fyuA* and *iroN* (siderophores); *hlyA* and *cnf1* (toxins); *sfaS* and *focG* (adhesins); *traT*, associated with serum resistance; and *malX*, a marker for a pathogenicity-associated island.

Results: The associations between antimicrobial resistance phenotype and virulence gene that were significant are presented in the Table. Table. Significant associations ($P < 0.006$, Bonferroni correction) among antimicrobial resistance phenotypes (S,

susceptible; R, resistant) and presence of virulence factor genes (VF+)

Antimicrobial agent	Virulence factor (VF) gene	Blood (n = 123 isolates)		Faeces (n = 85 isolates)	
		% VF+ among S	% VF+ among R	% VF+ among S	% VF+ among R
Ampicillin	<i>iutA</i>	29	71		
	<i>traT</i>	43	77	8	77
Sulphamethoxazole	<i>iutA</i>	30	76	11	70
	<i>traT</i>	44	80		
Streptomycin	<i>iutA</i>	32	74	9	70
	<i>traT</i>	48	74		
Tetracycline	<i>iutA</i>	38	78		
Trimethoprim	<i>iutA</i>	35	81		
	<i>traT</i>	49	81		

Conclusion: Clear associations were found between certain common resistance phenotypes and two virulence genes, i.e. *iutA* and *traT*, both in *E. coli* isolated from the blood of patients with bacteraemia and from the faeces of non-hospitalised humans. It is difficult to explain these associations and further studies are needed. However, such links between resistance and virulence are disturbing since the use of antimicrobial agents may result not only in the selection of resistant isolates, but also of more virulent ones.

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Antimicrobial resistance surveillance in 2004 in Korea: further increase of vancomycin-resistant *Enterococcus faecium*, expanded-spectrum beta-lactam-resistant *Klebsiella pneumoniae* and imipenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp.

K. Lee, C.H. Rhim, J.H. Cho, W.G. Lee, Y. Uh, H.J. Kim, D. Yong, Y. Chong for the KONSAR Group (Seoul, Korea)

Objectives: Monitoring temporal trends of antimicrobial resistance can provide useful information for empirical selection of antimicrobial agents to treat infected patients and for the control of nosocomial infections. Aim of this study is to determine resistance trends of important nosocomial pathogens in Korea.

Methods: Antimicrobial susceptibility test data in 2004 were collected from KONSAR program-participating hospitals. Data from 37 hospitals were analysed after excluding the hospitals with unreliable quality control performance. Resistance rates did not include intermediate category.

Results: Resistance rates in 2004 vs. in 2003 were compared. The rates were similar in oxacillin-resistant *Staphylococcus aureus* (68% vs. 68%) and oxacillin-resistant *Streptococcus pneumoniae* (68% vs. 70%). Slight increase of vancomycin-resistant *Enterococcus faecium* (25% vs. 20%) was noted. *Klebsiella pneumoniae* showed increased resistance to expanded-spectrum cephalosporin (34% vs. 25%) and cefoxitin (32% vs. 23%). Fluoroquinolone-resistance remained similar in *Escherichia coli* (32% vs. 32%), *Acinetobacter* spp. (56% vs. 58%), and *Pseudomonas aeruginosa* (38% vs. 40%). Rates of imipenem resistance further increased in *P. aeruginosa* (24% vs. 20%) and *Acinetobacter* spp. (17% vs. 13%). Amikacin resistance rate was similar in *P. aeruginosa* (24 vs. 25%), but slightly decreased in *Acinetobacter* spp. (46% vs. 54%). **Conclusion:** It is a concern that vancomycin-resistant *E. faecium*, expanded-spectrum cephalosporin-resistant *K. pneumoniae*, and imipenem-resistant *P. aeruginosa* and *Acinetobacter* spp. further increased in 2004 in Korea.

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Prevalence of antibiotic resistance and antibiotic resistance genes among *Enterococcus faecium* from pigs and poultry in the United Kingdom

V.I. Enne, E. Pleydell, P.M. Bennett (Bristol, Weybridge, UK)

Objectives: To determine the incidences of antibiotic resistance and expressed and silent resistance genes among *Enterococcus faecium* from healthy farm animals, reared on organic and conventional farms in the UK.

Methods: Faecal samples from broilers, turkeys and pigs reared on farms using either conventional or organic methods were collected. One colony displaying *E. faecium* morphology on Slanetz and Bartley agar was chosen at random per sample. Identity of the isolates as *E. faecium* was confirmed by PCR. Susceptibility of the isolates to chloramphenicol (CHL), erythromycin (ERY), gentamicin (GEN), quinupristin-dalfopristin (Q/D), tetracycline (TET) and vancomycin (VAN) was determined by disc diffusion. Presence of the resistance genes *cat*, *ermB*, *tetM*, *vanA* and *vatE* in resistant and susceptible isolates was determined by PCR. Unexpressed resistance genes were investigated by DNA sequencing.

Results: In total 304 *E. faecium* isolates were obtained. The prevalence of antibiotic resistance was high, with 87.8% of isolates resistant to at least one agent. Of these, 11.8% were multi-resistant (defined as resistance to three or more agents). Prevalence of resistance was 0.7% to VAN, 7.6% to Q/D, 7.9% to CHL, 55.9% to ERY, and 83.2% to TET. There was no resistance to GEN. Resistance was more common among isolates from conventionally than organically reared animals, with 72.4% of isolates from organically reared animals being fully sensitive or resistant to just one agent, while 81.1% of isolates from conventionally reared animals were resistant to at least two agents. In terms of gene frequencies both VAN resistant isolates had *vanA*, 91.3% of Q/D resistant isolates had *vatE*, 89.3% of TET resistant isolates had *tetM*, 74.1% of ERY resistant isolates had *ermB* and 20.8% of CHL resistant isolates had *cat*. Four isolates were PCR positive for *ermB* but susceptible to ERY. DNA sequencing showed that in three of these, the sequences of the *ermB* gene and its promoter were intact and wild type. In the fourth isolate, a mutation was found in the *ermB* promoter.

Conclusion: The incidence of antibiotic resistance among *E. faecium* isolated from healthy pigs and poultry is relatively high, although most isolates are susceptible to frontline therapeutic agents such as VAN or GEN. The genes investigated in this study accounted for most of the resistance detected, except for CHL. Three isolates were found to harbour apparently silenced *ermB* genes and warrant further investigation.

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Antibiotic susceptibility of bacteria isolated from hospitalised patients in Mauritius

M.I. Issack (Quatre-Bornes, MU)

Objectives: Microorganisms that cause nosocomial infections and their antibiotic susceptibility vary markedly according to region or hospital, and over time. We conducted a retrospective survey of bacteria recently isolated from hospitalised patients in Mauritius.

Methods: Specimens sent for bacteriological investigations from all government hospitals in Mauritius are processed at the Central Health Laboratory. Results of all specimens received in March 2005 were reviewed and analysed. Duplicate isolates from the same patient were excluded

Results: Information was available on antibiotic susceptibility of 541 gram-negative bacteria, of which the most common were *Klebsiella* spp. (98), *Pseudomonas aeruginosa* (86), *E. coli* (84), *Proteus* spp. (84) and *Acinetobacter* spp. (76). Most isolates originated from urine in the case of *E. coli* and from pus swabs and tracheal secretions for the other organisms. Percentage susceptibility of *Klebsiella* spp. to cefotaxime, ciprofloxacin, gentamicin, amikacin and meropenem was 56%, 63%, 54%, 90% and 100% respectively whereas the corresponding figures for *E. coli* was 83%, 75%, 83%, 86% and 100% respectively. Susceptibility of *Proteus* and *Acinetobacter* spp. were as follows: 50% and 13% respectively to cefotaxime, 46% and 21% to ciprofloxacin, 44% and 17% to gentamicin, 54% and 67% to amikacin, and 100% and 48% to meropenem. Most *Pseudomonas aeruginosa* were susceptible to ceftazidime (74%), piperacillin (67%), ciprofloxacin (57%), amikacin (80%) and meropenem (90%) but only 47% were sensitive to gentamicin. All *Klebsiella* spp. and *E. coli*, 99% of *Pseudomonas aeruginosa* and 97% of *Acinetobacter* spp. were reported as susceptible to colistin. Among the 85 *Staphylococcus aureus* isolates noted, only 5% were susceptible to penicillin but most were susceptible to erythromycin (84%), tetracycline (82%) and meticcillin (86%). Half of the MRSA isolates were obtained from specimens from the Burns Unit. Only one of 12 *S. aureus* isolates from blood cultures during the period surveyed was an MRSA. 89% of 53 enterococci were susceptible to ampicillin and no vancomycin-resistant *Enterococcus* sp. was isolated.

Conclusion: Resistance to broad-spectrum antibiotics among Gram-negative bacteria isolated from hospitalised patients in Mauritius is very common and seriously limits therapeutic options. On the other hand, resistance among *S. aureus* and enterococci are not a major problem, with the exception of MRSA in the Burns Unit.

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Antimicrobial resistance patterns in 14 neonatal intensive care units in Bogota, Colombia 2001–2004

G. Buitrago, G.A. Contreras, A.L. Leal, C.A. Alvarez, Grupo para el Control de la Resistencia Bacteriana de Bogotá (GREBO)

Objectives: Nosocomial infections are a challenge health problem and cause significant morbidity and mortality, specially in critical patients and the increase in antimicrobial resistance during the last years complicates the management of this type of infections. The objective of this epidemiologic surveillance was to analyse the bacterial isolates and the antimicrobial susceptibilities in the neonatal intensive care units (NICU) that belong to GREBO (Grupo para el Control de la Resistencia Bacteriana de Bogotá-Colombia, South America).

Methods: A descriptive retrospective study was performed. The data was collected from the microbiology laboratories from 14 high-complexity NICU in Bogotá, Colombia. The data was obtained from the automated systems (Vitek® and MicroScan®) and it was transferred to WHONET 5.3 with the aid of BacLink software. Quality assurance was performed by the National Institute of Health (Bogotá, Colombia). Duplicated isolates, more than one isolate per patient, were identified and deleted. Results were scored as susceptible, intermediate or resistant according to Clinical Laboratory Standards Institute (CLSI) criteria.

Results: 6051 microorganisms were isolated. The most important microorganisms were: *Staphylococcus coagulase* negative (37,91%), *Klebsiella pneumoniae* (10,94%), *Escherichia coli* (8,61%), *Staphylococcus aureus* (8,10%), *Enterococcus faecalis* (2,99%), *Serratia marcescens* (2,84%) and *Enterobacter cloacae* (2,68%). The

Antibiotic	S aureus		S coagulans negative		E faecium		E faecalis		E coli		K pneumoniae		E cloacae		S marcescens	
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Amoxicillin	166	100	166	100	166	100	166	100	457	12.7	166	100	212	12.7	166	100
Ampicillin	166	100	166	100	166	100	166	100	142	5.5	166	100	166	100	166	100
Ampicillin/Sulbactam	166	100	166	100	166	100	166	100	410	24.9	166	100	166	100	166	100
Ampicillin	166	100	166	100	166	100	166	100	157	15.2	166	100	166	100	166	100
Cefazolin	166	100	166	100	166	100	166	100	339	25.4	166	100	166	100	166	100
Cefepime	166	100	166	100	166	100	166	100	228	13.7	166	100	166	100	166	100
Cefepime	166	100	166	100	166	100	166	100	338	3.6	166	100	166	100	166	100
Ceftriaxone	166	100	166	100	166	100	166	100	436	6.2	166	100	166	100	166	100
Cefuroxime	166	100	166	100	166	100	166	100	235	7.2	166	100	166	100	166	100
Ciprofloxacin	375	31.4	167	25.3	89	38.8	162	5.3	458	3.7	603	3.7	148	6.8	335	9
Cloxacillin	467	3.4	1188	35.3	166	100	166	100	166	100	166	100	166	100	166	100
Doxycycline	293	35.4	1012	63.3	166	100	166	100	166	100	166	100	166	100	166	100
Gentamicin	395	38.1	1038	63.8	71	53.5	149	11.4	166	100	166	100	166	100	166	100
Gentamicin (High Level)	166	100	166	100	166	100	166	100	166	100	166	100	166	100	166	100
Imipenem	166	100	166	100	166	100	166	100	434	6.2	552	6.4	145	6.7	151	2
Levofloxacin	166	100	166	100	166	100	166	100	209	3.4	250	2	67	1.6	77	5.3
Meropenem	166	100	166	100	166	100	166	100	223	6	239	6.4	62	6	74	3.4
Morfloxacin	166	100	166	100	166	100	166	100	166	100	166	100	166	100	166	100
Oxacillin	442	31.7	1182	35.3	166	100	166	100	166	100	166	100	166	100	166	100
Piperacillin/Tazobactam	166	100	166	100	166	100	166	100	375	5.3	365	2.1	102	21.6	166	27.3
Rifampin	391	5	165	35.3	166	100	166	100	166	100	166	100	166	100	166	100
Tetracycline	203	22.5	957	35.7	166	100	166	100	166	100	166	100	166	100	166	100
Tosilopropylsulfamethoxazole	400	7	197	41	166	100	166	100	463	42	565	46.5	145	27.6	153	15.7
Vancomycin	413	6	1162	6	88	0	173	8	166	100	166	100	166	100	166	100

microorganisms were obtained from blood (47,09 %), catheter (11,94 %), urine (8,33 %) and feces (7,93 %). The chart illustrates the antimicrobial resistance for each microorganisms.

Conclusion: This study shows the great importance of the coagulase negatives *Staphylococcus* (CoNS) in the NICU, which have higher percentage of antimicrobial resistance, in special to oxacillin and ciprofloxacin. On the other hand, the gram-negative bacillus has compatible profiles with Extended Spectrum Betalactamase and AmpC and is worrisome specially in *K. pneumoniae*. The development of this type of studies in developing countries, will facilitate to guide the empirical antimicrobial treatment in our Institutions and establish control strategies of the bacterial resistance.

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Integrating susceptibility data from two surveillance programmes with unique methodological techniques: the Antibigram Resistance Method Or isolate-based Resistance monitoring (ARMOR) study

B. Epstein, J. Gums, P. Turner, for the Antibigram Resistance Method Or isolate-based Resistance Monitoring (ARMOR) Study Group

Objectives: The Antimicrobial Resistance Management (ARM) and Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) programs are ongoing antibiotic surveillance studies that operate by different methods. Our aim was to compare the susceptibility data generated by each program in order to determine if the two methods produce similar results.

Methods: The ARM Program is a web-based (www.armprogram.com), antibiogram-driven surveillance system, whereas MYSTIC tracks minimum inhibitory concentrations (MIC) by analyzing individual bacterial isolates. MYSTIC isolates undergo antimicrobial susceptibility testing via broth microdilution in a reference laboratory (for US institutions) in accordance with Clinical Laboratory Standards Institute (CLSI) methods. We compared national susceptibility rates during 1999–2004 from ARM and MYSTIC for *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. ARM captured data on 572,927 *E. coli*; 153,171 *K. pneumoniae*; and 207,454 *P. aeruginosa*. The corresponding isolate numbers from MYSTIC (US) were 2,333; 1,267; and 2,254.

Results: For *E. coli*, susceptibility rates were similar with the exception of levofloxacin, for which susceptibility was reported as 92.3% in the ARM Program and 83.0% in MYSTIC. (Table 1) Results for ciprofloxacin were 92.3% and 88.2%, respectively. For *K. pneumoniae*, susceptibility rates were comparable. Overall, for *P. aeruginosa*, susceptibilities were similar, though differences were evident for ciprofloxacin, cefepime, and gentamicin. Resistance was higher among isolates submitted to ARM compared to MYSTIC. Ciprofloxacin resistance was 26.6% in

MYSTIC and 36.7% in ARM. Resistance to cefepime was 25.5% and 16.2%, respectively. Likewise, for gentamicin, resistance was 29.5% and 14.8%, respectively. Similar patterns were apparent for other members of the fluoroquinolone, late generation cephalosporin, and aminoglycoside classes, but the differences were less marked.

Table 1. Cumulative susceptibilities for *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, 1999-2004

		AMI	AZT	CFP	CAZ	CRO	CIP	GEN	IMI	LEF	MER	PTZ	TOB
<i>E. coli</i>	ARM	99.7	96.1	99	97.6	98.6	92.3	95.8	99.7	92.3			96.1
	MYSTIC	99.9	98.2	99.6	98.4	98.6	88.2	94.2	100	83	100		95.8
<i>K. pneumoniae</i>	ARM	98	94.5	96.2	93.3	96.2	94.4	95.2	99.5	93.5			92.8
	MYSTIC	97.6	95.6	98.9	95.1	96.5	94.7	94.9	99.5	94.9	99.5		95.7
<i>P. aeruginosa</i>	ARM	91.3	66.9	74.5	81.6		63.3	70.5	62.3	88.8	88.2	87.1	87.1
	MYSTIC	96.9	66	83.8	83.7		73.4	85.2	67.9	87.8	89.2	91.3	91.3

Conclusion: The ARM program, an antibiogram-based surveillance system, and MYSTIC, an isolate-based initiative, produce nearly identical susceptibility data for *K. pneumoniae* and *E. coli*. For *P. aeruginosa*, susceptibility tended to be higher for ciprofloxacin, cefepime, and gentamicin in MYSTIC. These results suggest that surveillance of antibiotic activity patterns can be performed confidently with either method. Subtle changes in MICs and resistance mechanisms can only be elucidated by isolate-based surveillance.

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Antimicrobial resistance of major Gram-negative bacterial pathogens during a 7-year period

A. Slavcovi, M. Lupse, M. Flonta, V. Zanc, D. Tatulescu, A. Almas, D. Carstina (Cluj-Napoca, RO)

Objective: During studied period consumption of all major classes of antibiotics increased significantly. The aim of our study is to assess the prevalence of antibiotics resistance for *E. coli*, *Klebsiella* spp., *Enterobacter* spp. and *Pseudomonas aeruginosa* between 1998 – May 2005.

Methods: 1464 isolates were collected from ICU, medical and surgical hospitals in Cluj-Napoca: 736 *E. coli*, 271 *Klebsiella* spp., 194 *Enterobacter* spp., 263 *P. aeruginosa*. Bacterial identification and susceptibility testing were carried out following standard procedures (using API-bioMerieux system, disk diffusion method) in the Teaching Hospital of Infectious Diseases Cluj-Napoca. For *E. coli* and *Klebsiella* spp. extended-spectrum Beta-lactamases (ESBLs) production was confirmed by double-disc test.

Results: Of 736 *E. coli* isolates tested 13% were resistant to ceftazidime, 17.4% to ciprofloxacin and 7.8% to amikacin. The presence of ESBLs and multi-drug resistant isolates (MDR) in *E. coli* was detected in 8.4% respectively 7.4%. In *Klebsiella* spp. the prevalence of resistance was 45% for ceftazidime, 21% for amikacin, 30% for ciprofloxacin, 0.4% for carbapenems and 0.8% for colistin. The presence of ESBLs and MDR was detected in 28.7% respectively in 23.2% of *Klebsiella* spp. isolates. During the studied period the resistance rate increased significantly for ceftazidime (p = 0.0007) in correlation with ESBLs presence. In *Enterobacter* spp. the resistance frequency was 41% for ceftazidime, 23% for amikacin and ciprofloxacin, 3% for colistin. MDR strains were identified in 14.4%. Of 263 *P. aeruginosa* isolates, 67% were resistant for ceftazidime, 55% for ciprofloxacin, 30% for amikacin, 18% for carbapenems and 3.5% for colistin. MDR *P. aeruginosa* was identified in 26.9%.

Conclusions: Cephalosporins, ciprofloxacin and amikacin resistance is becoming increasingly common in *Enterobacteriaceae* and *P. aeruginosa*. The carbapenems and colistin are now the only reliable antibiotics against ESBL producing Gram-negative pathogens and MDR strains. There is necessary to limit the overuse of antibiotics and implementation of a new antibiotic policy.

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Serious developments of antimicrobial resistance in Europe: results from EARSS (European Antimicrobial Resistance Surveillance System)

N. Bruinsma, M.E.A. de Kraker, G. Kahlmeter, G. Cornaglia, F. Baquero, J. Monen, H. Grundmann and EARSS participants

Objectives: EARSS collects data from laboratories that serve over 30% of the European population, and for several countries the coverage amounts to 100%. This makes EARSS the most comprehensive public health effort describing and analysing geographic and secular trends in antimicrobial resistance in Europe. Recently, the EARSS data, irrespective of the base-line level of resistance in individual countries, showed increasing trends of antibiotic resistance across the board. In this study, resistance trends of indicator pathogens over time will be presented.

Methods: EARSS collects routinely generated antimicrobial susceptibility (AST) data for the indicator pathogens: *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli* and clinically relevant antibiotics. This data are regularly reported by over 800 laboratories serving 1300 hospitals in 31 European countries. We looked at resistance trends in Europe from 1999 to 2004. To determine significant trends the Cochrane Armitage test was used.

Results: MRSA-proportions are mainly increasing in countries that showed moderate to low prevalences over the past few years (central and northern European countries). In Scandinavian countries and the Netherlands that before long had MRSA rates well below 1% over the past 3 years a clear increase can be discerned. This trend must be taken seriously since a low threshold for losing control may exist but is not well defined. In *E. coli* the increase in fluoroquinolone resistance (FQ) continues unabated in most European countries; and, when including the trends for the ESBL expansion, this species treatment options are becoming slim. Vancomycin-resistance in *Enterococcus faecium* (VRE) remains below 10% in most European countries, but higher levels are reported from Portugal, Italy, Ireland, and Greece. Significant increases are observed for Germany, France, and Ireland.

Conclusions: Even for countries which successfully maintained low levels of MRSA for decades, EARSS data show a consistently rising trend, which call for a reappraisal of existing control strategies. The observed increase of VRE can be explained by the spread of strains belonging to a particularly hospital adapted clonal lineage, termed complex 17. The increasing trends of FQ resistance in *E. coli* is most probably the result of widely used fluoroquinolones, predictably posing an increasing challenge to European health care systems for years to come.

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***Stenotrophomonas maltophilia* surveillance in a Greek tertiary care hospital**

K. Koraki, P. Karapavlidou, D. Sofianou (*Thessaloniki, GR*)

Objectives: To determine the spectrum of infection and antimicrobial susceptibility of *Stenotrophomonas maltophilia*, a significant emerging pathogen that primarily affects patients who have been hospitalized for prolonged periods, have been subjected to invasive procedures or have received broad-spectrum antibiotics.

Methods: A total of 95 non-repeat *S. maltophilia* strains were isolated in our hospital from January 2002 to December 2004. Identification was done using the Vitek2 automated system (bioMérieux, France), while susceptibility testing was performed using the broth dilution method according to NCCLS guidelines.

Results: The most frequent source of isolation were bronchial secretions (23.1%), followed by wounds (14.7%), sputum (14.7%), blood (11.6%), intraperitoneal drainage catheters (10.5%), pus (8.4%), central and peripheral venous catheters (7.4%) and other sources (7.5%). Some of these isolates probably represent colonization rather than true infection. Intensive care units (ICUs) accounted for the majority of isolates (43.1%), followed by surgical clinics (16.8%) and the children outpatient department (14.7%) where all isolates derived from cystic fibrosis patients. *S. maltophilia* was found most often in bronchial secretions of ICU patients whereas it was most often isolated from wound swabs in the ward patients. The organism was isolated from blood more often in ICU (17.6%) than in ward patients (8.2%). Regarding the antibiotic susceptibility, 63.6% of isolates were susceptible to ticarcillin/clavulanate, 62.7% to ciprofloxacin and 43.6% to ceftazidime. Aminoglycosides inhibited less than 40% of isolates. All isolates were susceptible to trimethoprim/sulfamethoxazole while full resistance was reported for imipenem. A great variety of antibiotic resistance phenotypes were observed suggesting that there were no epidemic clusters of infection in the hospital.

Conclusion: *S. maltophilia* is a significant emerging nosocomial pathogen against which very few compounds showed reasonable *in vitro* activity. Resistance surveillance studies are necessary to guide empirical antimicrobial therapy and to assure effective control measures especially in critical hospital wards.

P1385

Activity of ertapenem and tigecycline against recent isolates from community-acquired lower respiratory infections in the UK and Ireland

R. Reynolds, D. Felmingham, *BSAC Working Party on Respiratory Resistance Surveillance*

Objective: To monitor the prevalence of resistance in the agents of community-acquired lower respiratory infection and to assess the activity of newer antimicrobial agents.

Methods: Community-acquired lower respiratory tract isolates of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* have been collected from 20 centres in the UK and Ireland between October and April each winter since 1999 and tested centrally by BSAC agar dilution methodology. Ertapenem and tigecycline were first tested in 2004–05.

Results: 93% of 750 *S. pneumoniae* were susceptible and 7% intermediate to penicillin; 9 and 14% were resistant to tetracycline and erythromycin, respectively. Beta-lactamase production (and corresponding ampicillin resistance), tetracycline resistance and erythromycin resistance was seen in 12, 1 and 4% of 888 *H. influenzae* and 93, 0.5 and 0% of 403 *M. catarrhalis* respectively; a further 94% of *H. influenzae* showed intermediate susceptibility to erythromycin. Ciprofloxacin resistance was rare, <4% in *S. pneumoniae* and <1% in *H. influenzae* and *M. catarrhalis*. Tigecycline MICs were ≤ 1 mg/L (mode 0.25 mg/L) for *S. pneumoniae* and *H. influenzae*, and ≤ 0.5 mg/L (mode 0.12 mg/L) for *M. catarrhalis*. Tigecycline MIC distributions were unaffected by the presence of penicillin-non-susceptibility, tetracycline resistance or beta-lactamase production, showing that the mechanisms involved in these resistances did not impinge on its activity. 92% of *S. pneumoniae* were susceptible to

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ertapenem at ≤ 0.03 mg/L, and 8% had intermediate susceptibility (0.06 – 1 mg/L). Ertapenem MICs increased from a mode of 0.004 and maximum 0.12 mg/L for penicillin-susceptible to mode 0.25 and maximum 0.5 mg/L for penicillin-non-susceptible *S. pneumoniae*; 7% had intermediate susceptibility to both agents. All *H. influenzae* and *M. catarrhalis* isolates were susceptible to ertapenem at ≤ 2 mg/L, with maximum MICs of 1 and 0.06 mg/L and modes of 0.03 and 0.015 mg/L respectively. Ertapenem MICs were slightly raised by beta-lactamase production for *M. catarrhalis* (mode 0.015 vs. 0.004 mg/L) but unaffected for *H. influenzae*.

Conclusion: The prevalence of resistance among community-acquired lower respiratory *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* remains relatively low in the UK and Ireland. Ertapenem and tigecycline showed very good activity against most or all of these isolates; ertapenem and penicillin susceptibility in *S. pneumoniae* were closely associated.

P1386

Trends in resistance of bacteraemia isolates in the UK and Ireland and current activity of ceftobiprole

R. Reynolds, R. Hope, BSAC Working Party on Bacteraemia Resistance Surveillance

Objective: To monitor the prevalence of resistance in the agents of bacteraemia and to assess the activity of newer antimicrobial agents, including ceftobiprole, a cephalosporin that binds staphylococcal PBP2'.

Methods: MICs were determined centrally, by BSAC agar dilution methodology, for bacteraemia isolates collected from 25 laboratories in the UK and Ireland each year since 2001. Ceftobiprole was included for the first time in 2004.

Results: There was little change in the prevalence of methicillin-resistance in *S. aureus* (43–48%) or in coagulase-negative staphylococci (62–80%), or vancomycin resistance in *E. faecium* (21–23%), whilst penicillin non-susceptibility in *S. pneumoniae* fell slightly (3–9%) over the 4 surveillance years. By contrast, there were sharp rises in the prevalence of ciprofloxacin resistance and ESBL production among *Enterobacteriaceae* (e.g. 8–18 and 0–6% respectively in *E. coli*); imipenem and ertapenem remained almost universally active against *Enterobacteriaceae* (<0.5% resistant in any species in 2004). Ceftobiprole, like daptomycin, linezolid and tigecycline, retained universal activity against staphylococci and streptococci. Modal MICs for methicillin-susceptible and resistant *S. aureus* were 0.5–1 and 2 mg/L, respectively, compared with 0.5–2 and 2 mg/l for corresponding groups of coagulase-negative staphylococci and 0.06 and 0.5 mg/l for penicillin-susceptible and non-susceptible *S. pneumoniae*. MICs >4 mg/L were seen for 3/139 *E. faecalis*, and for 63/80 *E. faecium*. MICs were from 8 to ≥ 128 mg/L for 5/9 *P. vulgaris*/penneri, 6/6 *K. oxytoca* hyperproducing K1 enzyme, 14/58 *Enterobacter* hyperproducing AmpC, and almost all ESBL-producers; otherwise MICs for *Enterobacteriaceae* were mostly ≤ 0.12 mg/L, with values of ≤ 0.12 to 4 mg/L for many with derepressed AmpC. MICs for *P. aeruginosa* were mostly (98%) 2–16 mg/L, whilst 29/32 *S. maltophilia* required MICs >4 mg/L (mode 64 mg/L).

Conclusion: The prevalence of methicillin resistance is high but stable among staphylococci while ESBL production and ciprofloxacin resistance are increasing in *Enterobacteriaceae*. Ceftobiprole had good activity against most organisms from bacteraemias in 2004, including MRSA, *S. pneumoniae*, *E. faecalis* and most *Enterobacteriaceae*, but not *E. faecium*, *S. maltophilia* nor ESBL-, K1- or many AmpC-producing *Enterobacteriaceae*; it may also have potential against *P. aeruginosa*.

P1387

Eight years of Belgian MYSTIC surveillance: 19982005

M. Ieven, M. Delmee, P. De Mol, H. Goossens, Y. Glupczynski, D. Pierard, M. Struelens, J. Verhaegen, G. Verschraegen (Edegem, Brussels, Liège, Yvoir, Leuven, Ghent, BE)

Objectives: As part of the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) surveillance, the activity of meropenem and six comparators was studied on a total of 11455 isolates from eight teaching hospitals in Belgium, collected from 1998 to 2005.

Materials and Methods: Centres collected consecutively up to 100 and 150 unduplicated, aerobic, Gram-positives and Gram-negatives respectively from patients admitted in ICU, neutropenia-, cystic fibrosis centres and general wards. CLSI methods and E-test were used for susceptibility testing. ESBL and AmpC production were confirmed by at least a 3 log₂ reduction in ceftazidime MIC in the presence of clavulanate and a ceftazidime MIC more than 8 mg/l not lowered by the presence of clavulanate respectively.

Results: Carbapenems were active against all Gram-positives: 98–100% susceptibility. Among the *Enterobacteriaceae*, there was no significant decrease in susceptibility from 1998–2005 for the antibiotics tested: 96.1–97.1% for imipenem, 99.6–99.4% for meropenem, 96.7–93.4% for ceftazidime, 83.3–83.9% for ceftazidime, 82.7–84.7% for piptazo, 93.9–94.1% for amikacin and 83.2–83.3% for ciprofloxacin. Resistance among *E. aerogenes* was significantly higher in Belgian isolates compared to other European countries, susceptibilities varying from 23–40% for both ceftazidime and ciprofloxacin and 40–66% for piptazo between 1998 and 2005. The occurrence of ESBL-producing *E. coli* and *K. pneumoniae* remained stable varying between 2–4% for *E. coli* and 6–15% for *K. pneumoniae*. ESBL-producing *E. aerogenes* isolates increased significantly. The % of AmpC producers among *Enterobacter* spp decreased from 50% in 1998 to 14.0% in 2005, for *Serratia* spp from 22.9% to 4.1% and for *Proteus* spp from 5.3% to 0%. Susceptibilities of ESBL and AmpC producers to carbapenems varied between 98–100%. Multidrug resistance in *P. aeruginosa* (R for ceftazidime, ciprofloxacin and gentamicin) was 9.4% among all isolates without significant trends. Piptazo and meropenem remain the most active agents against *P. aeruginosa* susceptibilities for piptazo between 73.0% in 1998 and 81.9% in 2005, for meropenem between 85.1% and 78.1%.

Conclusions: Resistance among *E. aerogenes* in Belgium is high. Prevalence of ESBL producers among *E. coli* and *K. pneumoniae* remained stable, prevalence of AmpC producers decreased. Carbapenems retain their activity against ESBL and AmpC producers. There is a trend towards decreased carbapenem susceptibility of *P. aeruginosa*.

P1388

Resistance of *Streptococcus pneumoniae* nasopharyngeal isolates from children in selected Russian orphanages: 2003 vs. 2004

R.S. Kozlov, O.U. Stetsiouk, J.A. Poupard, L.S. Stratchounski (Smolensk, RU; Philadelphia, US)

Objectives: Orphanages have been shown to be reservoirs of resistant strains of *Streptococcus pneumoniae* (SPN) which could spread to the community. A special survey was done to monitor resistance changes of SPN nasopharyngeal isolates from children living in orphanages in 2003 and one year later, in 2004.

Methods: This survey was performed as a part of the prospective study CORPUS in 12 orphanages (#1–12) from 5 cities (Moscow, Saint-Petersburg, Smolensk, Karachev, Bryansk) located in European part of Russia. Nasopharyngeal swabs were collected from 772 children < 7 years in 2003 and from 752 children in 2004 that yielded 399 and 397 SPN isolates respectively. Susceptibility to penicillin G (PEN), amoxicillin (AMO), amoxicillin/clavulanate (AMC), cefuroxime (CEF), cefotaxime (CTX), erythromycin (ERY), clindamycin (CLI), chloramphenicol (CHL), tetracycline (TET) and co-trimoxazole (SXT) was performed by CLSI (formerly the NCCLS) broth dilution methodology.

Results: SPN nasopharyngeal colonization rates were quite stable during the study period – 51.7% in 2003 and 52.8% – in 2004. There were no increase in SPN non-susceptibility (NS) or resistance (R) to PEN, AMO and AMC. However our data revealed a statistically significant increase of I and R SPN isolates to II and III generation cephalosporins (CEF and CTX) – 1.3/39.4 vs 17.9/31.2 and 3.8/2.5 vs 9.3/8.3 in 2003 vs 2004 respectively. It is noteworthy that NS SPN population to CEF increased due to the higher frequency of I isolates with the stable rate of R strains, while in the case of CTX NS – it was due to simultaneous (~2.4 fold and ~3.3 fold) increase of I and R isolates, respectively. At the same time no statistically significant changes in ERY, CLI and SXT susceptibility and statistically significant decrease in CHL R rates (15.0 vs 8.1%) were noted.

Conclusions: The results of the survey show stable rates of NS and R SPN nasopharyngeal isolates from children living in orphanages to penicillins, macrolides and lincosamides, tetracyclines and co-trimoxazole. Emerging resistance to II-III generation cephalosporins necessitates further evaluation of these alarming data to determine responsible risk factors and respective resistance mechanisms. Decrease of SPN resistance to chloramphenicol can be a positive example of possibility to diminish resistance following the restricted use of antimicrobials.

P1389

Geographical and temporal analysis of the changes in *S. pneumoniae* multiple resistance to antibiotics in Belgium over a 10-year period

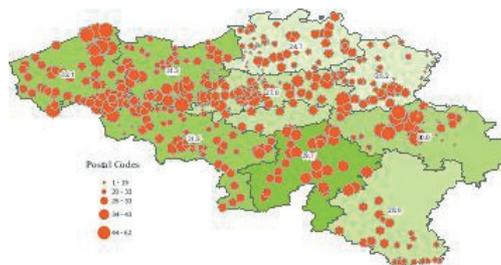
R.M. Mera, J. Van Eldere, L.A. Miller, J.A. Poupard, J. Verhaegen (New Orleans, US; Leuven, BE; Upper Providence, Philadelphia, US)

Objective: The evolution of resistance to two or more antibiotic classes in Belgium has a strong spatial component since it is located between countries with very high (France) or very low (Germany, The Netherlands) levels of antimicrobial resistance. Changes over time and space in Belgium are studied using a 10-year surveillance study.

Methods: Surveillance data consists of 14,488 *S. pneumoniae* invasive isolates by postal code in the period 1994–2004. Geospatial data for all postal codes by month is available. Spatial analysis uses a trend surface model, and temporal analysis is done with standard time series analysis techniques.

Results: 77% of all postal codes had at least one isolate in the database. There is a significant clustering of MR isolates near the border with France, which is independent of time. Postal codes located near the French border region of Hainaut-Namur are 1.95 times more likely to have multiply resistant isolates than those in the Dutch border region of Antwerp-Limburg (95% CI 1.7 – 2.3). Areas of high multiple resistance tend to cluster around main roads and rail lines connecting Lille (France) to Brussels and Liege to the west, Brugge to the north and Chaleroi to the southwest, independent of population density. Areas of

low MR density cluster around urbanized centers near the border with The Netherlands, and rural postal codes bordering Germany and Luxembourg. Time series analysis of monthly MR prevalence shows that there is a strong seasonal component with higher resistance in February and lower in August (7% average difference). There is also a strong statistically significant trend toward increasing prevalence over time that starts in the areas of West Flanders and Namur provinces, migrates to the main cities (Brussels, Liege) in the *centre* of the country and spreads roughly from the southwest to the northeast. There is a tendency toward a plateau since the year 2000, with some convergence in the prevalence rates, at levels of 32% in West Flanders and 25% in Antwerp in 2004 respectively.



Conclusions: Evolution of MR in Belgium since 1994 has followed a gradient that started at the postal codes bordering the French border and has spread through urban and rural postal codes following main roads to cities in the *centre* of the country. MR prevalence peaked in the year 2000, but the geographical differences have remained.

P1390

Selection and characterization of biocide resistant *Salmonella enterica* serovar Typhimurium

M. Webber, M. Woodward, L.J.V. Piddock (Birmingham, Weybridge, UK)

Objectives: To select biocide tolerant mutants from a panel of *S. Typhimurium* and determine whether exposure to biocides promotes antibiotic cross-resistance.

Methods: The strains used were SL1344 and derivatives lacking efflux genes *acrB* (L643) and *tolC* (L108), a laboratory *gyrA* (L696) mutant selected on ciprofloxacin, a ciprofloxacin resistant field isolate (L378), a representative DT104 isolate (L357) and a cyclohexane tolerant strain (L699). Mutants were selected with biocides Farm Fluid S, Virkon, AQAS and Superkill. Mutants were selected after exposure to 1X and 2X the MIC of each agent in agar and overnight incubation. Mutant colonies were retained and the susceptibility to a range of antibiotics determined as well as growth kinetics in both in the presence and absence of biocide.

Results: Mutants were selected for all biocides. Strains lacking efflux genes were more susceptible to biocides than all other strains. The MICs of biocide tolerant mutants were similar to those of parents or one dilution higher. However growth kinetic analysis revealed the tolerant strains were able to grow better in the presence of 1X the MIC of the selective biocide when challenged in mid logarithmic growth phase. Some mutants were also less susceptible to antibiotics when compared to their parent strain, notably mutants from L699 (cyclohexane tolerant strain). These were 8 fold less susceptible to ciprofloxacin than their parent and were inhibited by 4 mg/L, above the recommended breakpoint concentration for ciprofloxacin.

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Conclusions: Biocide exposure led to the selection of strains with increased tolerance to all biocides tested although the MIC changes were generally small. Biocide selected mutants demonstrated some antibiotic resistance, notably to ciprofloxacin after exposure to superkill. Further work to determine the mechanisms of resistance in these mutants is underway.

P1391

Polymyxin B resistance in multidrug-resistant *Pseudomonas aeruginosa*

J.L. Sampaio, C. Mendes, S.I. Sinto, P.C. Koga, A.C. Arruda, C. Kiffer (Sao Paulo, BR)

Objectives: To evaluate polymyxin B (POLB) resistance in multidrug resistant (MDR) *P. aeruginosa* clinical isolates by broth microdilution (BMD), Etest and agar screening (AS) methods.

Methods: The susceptibilities of 243 *P. aeruginosa* MDR clinical isolates previously determined to be resistant to at least three drugs among ciprofloxacin, cefepime, ceftazidime, meropenem or piperacillin-tazobactam were evaluated. A single isolate per patient was included. POLB minimal inhibitory concentrations were determined by BMD according to CLSI and by the Etest®. Isolates were also evaluated in regard to their ability to grow on Mueller-Hinton agar containing POLB (4 mg/L). Inoculum was approximately 1.5×10^6 CFU; plates were incubated in ambient air at 35°C, observed after 24 h and 48 hours and the presence of colonies recorded. For BMD and AS assays calcium and magnesium concentrations were adjusted to 20 mg/L and 10 mg/L, respectively. Antimicrobial concentrations ranges in BMD were 0.03 mcg/mL to 32 mcg/mL. Results were only considered if *P. aeruginosa* ATCC 27853, tested along with each batch tests, showed adequate results.

Results: Among 243 isolates, 27 (11.1%) showed skipped wells in BMD panels. All wells with higher drug concentrations where visible growth was detected after skipped wells were plated and grew again *P. aeruginosa*. Among these, 14 isolates with this feature were randomly assayed by BMD in triplicates, and skipped wells occurred in 9.7% (7/72) of tests. Bacterial population recovered from higher concentration wells did not show a stable POLB resistance phenotype, but presented again the pattern of skipped wells. In contrast, *P. aeruginosa* ATCC27853 tested 16 times did not show skipped wells. POLB MIC₅₀ and MIC₉₀ by BMD were 0.5 mcg/mL and 2.0 mcg/mL, respectively (liberal reading). However, if growth after skipped wells was considered (conservative endpoint), MIC₉₀ would increase to 4.0 mcg/mL and resistance rate would change from 2.0% to 11.5%. Four isolates with a MIC of 4.0 mcg/mL for POLB by BMD had MICs \leq 1.5 mcg/mL by Etest. AS with POLB detected four among five resistant isolates.

Conclusions: Etest should not be used as a confirmatory test to determine POLB resistance in MDR *P. aeruginosa*. AS with 4 mcg of polymyxin B/mL is not an adequate screening method to detect resistance in this bacterial population. The adequate endpoint (liberal or conservative) to be reported for isolates showing skipped wells is not defined.

P1392

Antibiotic resistance does not impede persistence of *Escherichia coli* in the infantile intestinal microbiota

N. Karami, E. Lindberg, A. Wold, I. Adlerberth, F.L. Nowrouzian (Göteborg, SE)

Objectives: The commensal intestinal microflora represent a large reservoir of bacteria which may carry antibiotic resistance

determinants, but the prevalence of antibiotic resistance among intestinal commensals and its ecological consequences has been poorly studied.

Methods: We have determined phenotypic resistance to eleven antibiotics in 271 *E. coli* strains obtained from 127 healthy Swedish infants followed longitudinally over their first year of life with quantitative cultures of the intestinal *E. coli* flora. Individual *E. coli* strains in each child were distinguished by random amplified polymorphic DNA (RAPD). The strains were characterized with respect to virulence factor genes, persistence in the gut microbiota, and faecal population numbers.

Results: *E. coli* strains were most commonly resistant to ampicillin (11%), tetracycline (9%) and trimethoprim (7%). Only 1% were resistant to 3 antibiotics. Resistant strains showed equally high population numbers as sensitive strain and persisted equally well in the microflora. Resistant intestinal *E. coli* strains often had p-fimbrial gene than sensitive *E. coli* strains ($p = 0.009$).

Conclusion: Antibiotic-resistant strains of *E. coli* seem to be as fit for competition in the large intestinal microbiota as susceptible strains. Carriage of certain virulence genes may contribute to their colonizing success.

P1393

Evaluation of daptomycin and other anti-staphylococcal antibiotics against clinical strains of community- and hospital-derived MRSA

M. Rybak, M. Amjad, C. Cheung, K. Lau, G. Kaatz (Detroit, US)

Objectives: Isolation of Methicillin-Resistant *Staphylococcus aureus* (MRSA) has increased dramatically. The increase in reports of community-associated MRSA (CAMRSA) will likely contribute to further complications associated with MRSA infections. We evaluated the antimicrobial activity of daptomycin (D), clindamycin (C), linezolid (L), erythromycin (E), doxycycline (Dx), vancomycin (V), teicoplanin (T), trimethoprim/sulfamethoxazole (TS) levofloxacin (Lv) gentamicin (G), and quinupristin/dalfopristin (QD), against 563 well characterized MRSA clinical isolates.

Methods: CAMRSA and healthcare-associated MRSA (HAMRSA) meeting clinical and SCCmec type definitions were evaluated. Multiplex PCR was used to determine SCCmec type, presence of Panton-Valentine Leukocidin (PVL) and accessory gene regulator (agr) type. The presence of inducible MLSB resistance was determined by disk diffusion. MIC and MBC values were determined using microdilution techniques. MIC determinations ($n = 20$) also were carried out in the presence of 50% serum for D and T to determine the impact of protein on susceptibility.

Results: 332 and 231 MRSA isolates met clinical and SCCmec typing definitions for CAMRSA and HAMRSA, respectively. CAMRSA were 100% SCCmec type IV, 85% agr type I, 83% PVL, and 5% iMLSB; 99.9% of HAMRSA were SCCmec type II, 79% agr type II, 15% PVL, and 50% iMLSB. Of interest, 92 of the 231 HAMRSA were found to be SCCmec type IV 61% agr type I, 58% PVL and 26% iMLSB. Against CAMRSA, MIC₅₀/MIC₉₀ for D:0.25/1, C:0.125/0.5, L:2/4, E:32/256, Dx:0.25/4, V:1/2, T:0.5/1, TS:0.125/0.25, Lv:0.25/4, G:0.5/2, QD:0.25/0.5 Against HAMRSA MIC₅₀/MIC₉₀ for D: 0.25/1, C: 64/64, L:2/4, E: 128/256, Dx: 0.5/4, V: 1/2, T: 0.5/2, TS: 0.125/1, Lv: 16/64, G: 0.5/1, QD: 0.25/1. Against HAMRSA (SCCmec type IV), MIC₅₀/MIC₉₀ D: 0.5/1,0, C:0.25/64, L:2/4, E: 64/128, Dx: 0.5/8, V:1/2, T: 0.5/1, TS:0.25/4, Lv:0.5/16,G:0.5/1, QD:0.25/0.5. MIC tests performed in 50% serum for D and T were 2-4 fold higher. MBC values followed MIC trends and were increased 1-4 fold for most antimicrobials.

Conclusions: Against CAMRSA and HAMRSA, MIC and MBC values were lowest for D, TS, V, T G and QD followed by Dx and L. Susceptibility to all antimicrobials tested was higher among CAMRSA compared to HAMRSA. Notably, HAMRSA SCCmec type IV had higher rates of iMLSB resistance and higher MIC₉₀ values for C, TS and Lv. compared to CAMRSA.

P1394

Susceptibility of recent European Gram-positive bacteria to daptomycin

C. Soussy, J. Bille, R. Cantón, A. MacGowan, C.E. Nord, G. Schito, H. Seifert, M. Struelens, J. Verhoef, I. Morrissey (Creteil, FR; Lausanne, CH; Madrid, ES; Bristol, UK; Stockholm, SE; Genoa, IT; Cologne, DE; Brussels, BE; Utrecht, NL; London, UK)

Objectives: Daptomycin (DAP) is a new cyclic lipopeptide antibiotic active against Gram-positive bacteria (GPB) and is licensed in the USA for the treatment of complicated skin and skin-structure infections. As marketing approval is being sought for Europe this study sought to determine DAP susceptibility for GPB from Europe

Methods: *Staphylococcus aureus* (SA), coagulase-negative staphylococci (CNS), *Enterococcus faecalis* & *E. faecium* (E), beta-haemolytic streptococci (BHS), 'viridans' streptococci (VS) and *Corynebacterium* spp. (COR) were collected from 81 laboratories in Austria, Belgium, France, Germany, Ireland, Italy, Netherlands, Portugal, Spain, Sweden, Switzerland and UK between October 2004 and March 2005 and CLSI broth microdilution MIC determined. CLSI breakpoints were used, except for teicoplanin against BHS & VS. SA CLSI breakpoints were used for COR.

Results: Susceptibility data for the collection (2867 isolates) are shown in the table [MR, methicillin-resistant; MS, methicillin-susceptible; VR, vancomycin-resistant; VS, vancomycin-susceptible; CTX, ceftriaxone; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; LEV, levofloxacin; LZD, linezolid; SYN, quinupristin-dalfopristin; EI, teicoplanin; VAN, vancomycin]. Only 8 isolates were found to be DAP non-susceptible: 1 MSSA, 1 MRCNS & 6 VS all with a DAP MIC of 2 mg/L (1 dilution higher than the susceptible breakpoint for these bacteria).

	Percent susceptibility:								
	MRSA	MSSA	MRCNS	MSCNS	VRE	VSE	BHS	VS	COR
N	444	267	400	209	195	484	566	219	83
DAP	100	99.6	99.8	100	100	100	100	97.3	100
CTX	-	96.9	-	96.7	-	-	99.8	92.2	21.7
CLI	55	92.9	63.3	91.4	-	-	89.9	90	6
ERY	29.7	82.8	30.3	67.5	1	10.3	81.6	64.4	10.8
GEN	75	94	62.8	93.3	-	-	-	-	33.7
LEV	16	90	32.5	85.2	17.4	52.9	98.6	96.4	13.3
LZD	100	100	100	100	100	99.8	100	100	100
SYN	98.7	99.6	98.8	99.5	47.7	30.6	99.6	91.8	98.8
TEI	100	100	96.5	66.7	25.1	100	100	100	100
VAN	100	100	100	100	0	99.4	99.8	100	100

Conclusion: DAP was very active against contemporary GPB in Europe including MRSA and VRE. Overall, 99.8% of staphylococci (n = 1320) and 100% of enterococci (n = 679) were susceptible to DAP independent of resistance to other drugs. Daptomycin should (subject to marketing authorisation) provide an excellent alternative therapeutic option for the treatment of infections caused by Gram-positive bacteria.

P1395

Exposure of *Staphylococcus aureus* isolates to daptomycin (DA) alone or DA plus vancomycin (VA) leads to a diminished macrophage inflammatory response (compared with VA alone)

B. English, E. Maryniw, A. Talati, E. Meals (Memphis, US)

Objectives: Treatment of *Staphylococcus aureus* (SA) infections with cell-wall active antibiotics such as vancomycin (VA) may lead to rapid bacterial lysis and trigger a pronounced host inflammatory response. Daptomycin (DA) is a cyclic lipopeptide antibiotic that exhibits bactericidal activity against SA but does not trigger rapid bacterial lysis. We hypothesized that exposure of SA isolates to DA or DA plus VA (compared with VA alone) would lead to a blunted macrophage inflammatory response with reduced accumulation of inducible nitric oxide synthase (iNOS) protein and decreased secretion of tumor necrosis factor (TNF).

Methods: Six bacterial strains were studied: two community-acquired (CA)- MRSA strains, two hospital-associated (HA)- MRSA strains, and two methicillin-susceptible (MSSA) strains. RAW 264.7 murine macrophages were stimulated with live bacteria (10⁶ or 10⁷ cfu/mL) and either DA 20 mcg/mL, VA 20 mcg/mL, or DA + VA. For studies of iNOS accumulation, cells were also exposed to recombinant murine interferon-gamma 10–25 U/mL. After incubation for 18 hrs, cell lysates were prepared and analysed for iNOS protein content by immunoblotting, and cell supernatants were collected and assayed for TNF concentrations by ELISA.

Results: VA-treated SA isolates stimulated large amounts of iNOS protein accumulation and TNF secretion by RAW 264.7 cells. Compared with VA, exposure of each of the six SA strains to DA led to marked reductions in iNOS protein accumulation (by densitometry, mean reduction was 51%, n = 19). Similarly, exposure of each of the six SA strains to DA (vs. VA) led to significantly lower TNF secretion (mean reduction 45%, n = 28). Finally, exposure of the SA isolates to combinations of DA plus VA (vs. VA alone) also resulted in substantially less macrophage iNOS protein accumulation (mean reduction 53%, n = 6) and TNF secretion (mean reduction 55%, n = 27).

Conclusion: Exposure of each of the six SA isolates to DA alone or DA plus VA resulted in substantially reduced macrophage iNOS accumulation and TNF secretion in response to these bacteria. These findings suggest that treatment of SA infections with DA (instead of or with VA) could result in a less intense host inflammatory response.

P1396

Further increase of fluoroquinolone resistance among *Escherichia coli* isolates in a Central European area

M. Kresken, D. Hafner on behalf of the Working Group for Antimicrobial Resistance of the Paul-Ehrlich-Society for Chemotherapy

Objectives: Since 1984 when the first fluoroquinolone (FQ) was introduced in Central Europe the consumption of the FQ has markedly increased. To evaluate the influence of the increasing consumption on the prevalence of resistance to FQ in clinical isolates of *Escherichia coli* we reviewed the susceptibility data from four collaborative studies conducted between 1995 and

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2004 by the Working Group for Antimicrobial Resistance of the Paul-Ehrlich-Society for Chemotherapy.

Methods: Isolates collected in 26 to 32 laboratories that participated in surveillance studies conducted in 1995, 1998, 2001 and 2004 were included. Every year each laboratory collected up to 30 consecutive, clinically significant isolates. MICs of isolates were determined by the broth microdilution method according to the standard of the German DIN. Ciprofloxacin was tested as reference FQ.

Results: A total of 2,930 isolates (1995, n = 783; 1998, n = 783; 2001, n = 619; 2004, n = 745) were tested. Resistance to FQ steadily increased during the study period from 5.2% in 1995 to 21.6% in 2004. FQ resistance was more prevalent in isolates from patients >60 years than in those from patients <21 years or patients aged 21–60 years. In isolates from patients >60 years, resistance to FQ jumped from 7.2% in 1995 to 27.7% in 2004. The level of resistance in patients aged 21–60 years raised from 4.2% to 18.5%, whereas it remained below 5% in patients <21 years. Of the 163 FQ-resistant isolates collected in the 2004 survey, 91.4%, 82.2% and 31.9% were cross resistant to ampicillin, cotrimoxazole, and gentamicin, respectively. FQ-resistant isolates also exhibited reduced susceptibility to third-generation cephalosporins. Susceptibility rates of FQ-resistant isolates for cefepime, ceftazidime, and ceftriaxone (85.3%, 82.2%, and 82.2%) were significantly lower than those for CIP-susceptible isolates (99.3, 99.3%, 98.3%) (P < 0.001). In contrast, ertapenem and meropenem retained *in vitro* activity against all FQ-resistant isolates.

Conclusion: Resistance to FQ has steadily increased among *Escherichia coli* isolates recovered from patients in hospitals located in Germany, Austria, and Switzerland. We strongly recommend a judicious use of FQ in order to combat the spread of FQ resistance in *Escherichia coli*.

P1397

Risk factors for invasive infection with fluoroquinolone-resistant *S. pneumoniae* and failure of oral outpatient fluoroquinolone therapy

K. Green, A. McGeer, D.E. Low, for the Toronto Invasive Bacterial Diseases Network TIBDN

Background: There is increasing concern about the emergence of fluoroquinolone (FQ) resistance in *S. pneumoniae*. We looked at FQ resistance rates and risk factors in patients with invasive pneumococcal disease (IPD) in Toronto, Canada.

Methods: From 01/00–12/04, TIBDN performed population-based surveillance for IPD in persons living in Toronto/Peel Region, Can. (pop 4M). IPD was defined as illness associated with isolation of SPN from a sterile site. Patients (pts) exposed to fluoroquinolones were classified as: treated with FQ for current infection before presenting with IPD (failure out-pt Rx) or received FQ for another infection in the previous 3 months (FQ prior 3 mos). Failures were defined as bacteremia while on or within 2 days of finishing oral FQ therapy.

Results: 1773/1879 (94%) case isolates were available for susceptibility testing; 28 (1.5%) had ciprofloxacin MIC \geq 4 (CipR), 21 (1.2%) had levofloxacin MIC \geq 8 (LevR) and 5 (0.3%) had moxifloxacin MIC \geq 4 (MoxR). 145 pts received FQ prior to presentation with IPD; 28 (1.5%) pts failed their out-patient FQ Rx for current infection while 117 (6.2%) had received a FQ for a different infection in the prior 3 months. Of the 21 LevR isolates identified, 6 (28.4%) were in pts failing FQ Rx, 8 (38%) were in residents of nursing homes and 4 (19%) were nosocomial isolates. Of the 5 pts with MoxR isolates, 2 were failing Ciprofloxacin out-pt Rx, 2 had received a FQ for another infection in the prior

Patient group	LevR	MoxR	Difference
No FQ exposure/No institutional exposure	4/1467 (0.3%)	0/1467 (0%)	NS
No FQ exposure	10/1629 (0.6%)	1/1629 (0.06%)	P=0.006
FQ prior 3 mths	5/116 (4.3%)	2/116 (1.7%)	NS
Failure FQ out-pt Rx	6/28 (21.4%)	2/28 (7.1%)	NS
Nosocomial	4/89 (4.5%)	0/89 (0%)	NS
Nursing Home	8/112 (7.1%)	2/112 (1.8%)	P=0.05

3 months and 1 isolate was from a nursing home resident with no FQ exposure. FQ resistance in these patient groups is shown in the Table below.

Conclusion: Patients who have been receiving a FQ for therapy and present to hospital with pneumonia or sepsis should be treated with a different class of antibiotics. FQ resistance in IPD in Toronto occurs almost exclusively in patients with recent direct exposure to fluoroquinolones, or recent exposure to a healthcare institution. In patients with none of these risk factors, all isolates were susceptible to moxifloxacin.

P1398

Mechanisms of resistance to quinolones in *Shigella* spp. causing traveller's diarrhoea

L. Mensa, E. Mendez-Arancibia, M. Navia, F. Marco, J. Vila, J. Gascón, J. Ruiz (Barcelona, ES)

Objectives: To determine the molecular mechanisms of resistance to quinolones in a series of *Shigella* spp. causing traveller's diarrhoea (TD), attending to their geographical origin.

Methods: The study includes all isolates of *Shigella* spp. recovered as a cause of TD during the period 1995–2004: 97 *S. flexneri*, 113 *S. sonnei*, 6 *S. dysenteriae*, 3 *S. boydii* and 7 *Shigella* spp. To analyse the evolution of the resistance, two sub-periods were considered (1995–2000 [125 strains] and 2001–2004 [101 strains]). Antimicrobial susceptibility to nalidixic acid (NAL) and ciprofloxacin (CIP) was established by the Kirby-Bauer method. Mutations in the *gyrA* and *parC* genes were detected by PCR and sequencing.

Results: From 229 *Shigella* spp. recovered, 12 isolates (5.2%) presented resistance or decreased susceptibility to quinolones. The resistant isolates were: *S. sonnei* (58%), *S. flexneri* (33%) and *S. dysenteriae* (8%). Four resistant isolates were recovered in the first period (3.3% of resistance) while 8 were recovered in the second one (7.9% of resistance). All strains with quinolone resistance were isolated from travellers returning from India (75%) or Western Africa (25%).

Specie	Year	Country	Nal	Cip	<i>gyrA</i> mutation	<i>parC</i> mutation
<i>S. sonnei</i>	1998	India	R	S	Leu83	-
<i>S. flexneri</i>	1998	Mali	R	S	Leu83	-
<i>S. sonnei</i>	1999	India	R	S	Tyr87	-
<i>S. flexneri</i>	1999	Mali	R	S	Leu83	-
<i>S. sonnei</i>	2001	India	R	S	Tyr87	-
<i>S. sonnei</i>	2002	India	R	R	Leu83, Asn87	Ik80
<i>S. flexneri</i>	2003	Eq. Guinea	R	S	Tyr87	-
<i>S. sonnei</i>	2003	India	R	S	Tyr87	-
<i>S. sonnei</i>	2003	India	R	S	Tyr87	-
<i>S. flexneri</i>	2004	India	I	S	Tyr87	-
<i>S. sonnei</i>	2004	India	R	S	Leu83	-
<i>S. dysenteriae</i>	2004	India	R	R	Leu83, Asn87	Ik80

Conclusions: An increase in the number of resistant strains isolated is observed in the last years mainly due to *Shigella*

strains isolated from patients to India. The most frequently found mechanism of resistance to quinolones are mutations in the *gyrA* gene.

P1399

Emergence of high-level fluoroquinolone-resistance in clinical isolates of group

G. streptococci

G. Brigante, F. Luzzaro, A. Bettaccini, G. Lombardi, B. Pini, G. Sokeng, A. Toniolo (Varese, IT)

Objectives: High-level resistance to fluoroquinolones (FQ) is an emerging problem in streptococci, including *S. pneumoniae*, *S. pyogenes*, and *S. agalactiae*. Group G streptococci (GGS) are involved in a wide spectrum of infections, including bacteremia, osteomyelitis, pharyngitis, skin and soft tissue infections. GGS are usually susceptible to most antibiotics, although resistance to macrolides and tetracycline (but not FQ) was reported. The purpose of the study was to evaluate susceptibility to different drugs (including FQ) in clinical isolates of GGS obtained over a five-year period.

Methods: Consecutive nonreplicate GGS isolates collected from March 2000 to March 2005 were investigated. Serotyping was obtained by a latex agglutination method (Strepto slide, Dienes, Siena, IT). Susceptibility to antibiotics (penicillin, erythromycin, clindamycin, tetracycline, ciprofloxacin, levofloxacin, and moxifloxacin) was determined by both Etest (AB Biodisk, Solna, Sweden) and microdilution methods.

Results: GGS were obtained from 202 patients, 82.2% of whom were non-hospitalized. Most frequently, isolates were obtained from skin or soft tissue infections. GGS were consistently susceptible to penicillin. Resistance to other drugs was as follows: erythromycin (6.9%), clindamycin (4.9%), and tetracycline (22.3%). Notably, 32/202 isolates (16.3%) showed high-level resistance (MIC, >32 mg/l) to ciprofloxacin, levofloxacin, and moxifloxacin. The first strain showing high-level resistance to FQ was recovered in March 2002; since then, every year we noticed an increasing number of patients affected with infections due to these pathogens.

Conclusion: High-level resistance to FQ is now emerging in GGS. As described in other species, this phenomenon could be due to mutations in target genes. Our results suggest that clinical microbiology laboratories should include FQ in their susceptibility testing of streptococci, in order to detect the presence of this particular resistance trait.

P1400

Comparative analysis of levofloxacin and ciprofloxacin activity against Gram-negative pathogens collected in five European countries grouped by site of infection, from GLOBAL Surveillance Study - 2005

M. Jones, D. Draghi, D. Sahn, C. Thornsberrry (Plaisir, FR; Herndon, US)

Objectives: Fluoroquinolones used empirically or targeted, provide an important therapeutic option in treating infections caused by gram-negative (GN) pathogens. This includes infections of the urinary tract, bloodstream, and respiratory tract. The GLOBAL Surveillance 2005 determined antimicrobial resistance trends among GN pathogens derived from different infection sites in five European (EU) countries. Data from this study

provides a benchmark of activity that may serve as a guide for future treatment regimens.

Methods: 1,493 non-repeat GN pathogens were isolated from the following specimens: urinary tract (UT), blood (BL), and lower respiratory (LRT) at hospital laboratories in five EU countries (United Kingdom [UK], France [Fr], Germany [Ger], Italy [It], and Spain [Sp]). Isolates were centrally tested by CLSI broth microdilution (M7-A6, 2003) against levofloxacin (LEV) and relevant clinical comparator agents for each site of infection. MICs were interpreted using the CLSI published interpretive criteria (M100-S15, 2005).

Results: *Escherichia coli* (EC) was the most prevalent organism isolated from UT, BL, and LRT sources. Among BL and UT isolates, LEV had superior activity compared with ciprofloxacin (CIP) based on resistance (R) rates. The %R rates for LEV and CIP were as follows, respectively: 25.4 and 29.9 for BL, 20.6 and 23.4 for UT, and 44.2 among both for LRT. Among *Klebsiella* spp. the %R rates for LEV and CIP were as follows, respectively: 7.5 and 14.9 for LRT, 6.2 and 10.7 for UT, and 11.7 among both for BL. *Pseudomonas aeruginosa* (PA) %R rates among BL were 22.0 for both LEV and CIP, among UT 41.3 for LEV and CIP, and among LRT 28.0 for LEV and 26.8 for CIP. Among *Proteus* spp. the %R rates for LEV and CIP were as follows, respectively: 8.7 and 13.0 for BL, 10.8 and 10.7 for LRT, and 6.3 and 8.4 for UT. The LEV %R ranged from 8.2 in Fr to 55.6 in Uk for EC; 3.6 in Ger to 21.2 in Uk for KL; 0 in Uk to 12.6% in It for PR; and 10 in Sp to 44.6 in It for PA.

Conclusions: Overall LEV showed slightly better *in vitro* activity than CIP against GN organisms tested, irrespective of the specimen source. Resistance rates varied significantly by country. Data derived from the longitudinal GLOBAL Surveillance initiative provides a useful tool to track and monitor antimicrobial resistance trends through time.

P1401

European surveillance study on the *in vitro* susceptibility of clinical bacterial isolates to telavancin

W. Jansen, A. Verel, A. Fleeer, J. Verhoef, D. Milatovic (Utrecht, NL)

Objective: To test the *in vitro* activity of telavancin, a novel semi-synthetic glycopeptide, against a total of 620 clinically relevant Gram-positive pathogens isolated from 25 European hospitals.

Methods: MICs were determined by broth microdilution according to NCCLS.

Results: Telavancin was highly active against *Staphylococcus aureus* and coagulase negative staphylococci (MIC_{50/90} 0.25/0.25 mg/L for both), with no difference in activity being observed for methicillin-susceptible and methicillin-resistant strains. It was the most active agent against *Staphylococcus aureus* being twice as active as daptomycin, 4-times more active than vancomycin and 8-times more active than teicoplanin and linezolid. Against *Enterococcus faecalis* and *Enterococcus faecium* telavancin showed high potency, the MIC_{50/90} values being 0.25/0.5 mg/L and 0.12/2 mg/L, respectively. The activity of telavancin was 4–8 times higher against vancomycin susceptible *enterococci* compared with VRE (0.25/0.5 mg/L versus 1/4 mg/L). Against VRE daptomycin, telavancin and linezolid were the most potent agents tested (MIC_{50/90} 1/2 mg/L, 1/4 mg/L and 2/2 mg/L, respectively). Telavancin exhibited excellent activity against both, penicillin susceptible and non-susceptible *Streptococcus pneumoniae*, all strains being inhibited by 0.06 mg/L. Compared with vancomycin, telavancin was 8-times more active

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against beta-hemolytic streptococci and 16-times more active against viridans streptococci (MIC₉₀ 0.06 versus 0.5 mg/L and 1 mg/L, respectively).

Conclusion: Telavancin has excellent activity against gram-positive cocci including methicillin-resistant staphylococci, penicillin-resistant pneumococci and vancomycin-resistant enterococci. It holds promise of becoming an important therapeutic option for infections caused by gram-positive bacteria, especially in view of the increasing resistance problems of certain species against other classes of antibiotics.

P1402

Molecular epidemiology of invasive high-level gentamicin resistance *Enterococcus faecium* isolates in Denmark

C.H. Lester, D. Sandvang, H.C. Schönheyder, J.O. Jarløv, D.S. Hansen, T.G. Jensen, N. Frimodt-Møller, A.M. Hammerum (Copenhagen, Bagsværd, Aalborg, Hillerød, Odense, DK)

Objectives: Enterococcal infections are often treated with a combination of an aminoglycoside and a cell-wall-active agent such as penicillin or a glycopeptide. In Denmark vancomycin resistant enterococci still have a very low prevalence, whereas high-level resistance to gentamicin (MIC > 500 mg/l) seems to be more common. In the other Nordic countries high-level gentamicin resistance (HLGR) is most frequently detected in *Enterococcus faecalis*. In Denmark more than half of the high-level

gentamicin isolates are *Enterococcus faecium*. In the present study the high-level gentamicin resistance was encoded by aac (6')-Ie-aph (2'')-Ia in all isolates. The aim of our study was to investigate the molecular epidemiology of these aac (6')-Ie-aph (2'')-Ia *E. faecium* isolates obtained from 5 clinical departments in Denmark.

Methods: A total of 62 invasive (mainly blood) HLGR *E. faecium* isolates were consecutively collected between January 2004 and December 2004. The strains were collected at four Danish Departments of Clinical Microbiology (Aalborg, Odense, Herlev, and Hillerød) and sent to the National Center for Antimicrobials and Infection Control at SSI. Only one isolate from each patient was included in this study. All isolates were typed by PFGE using SmaI as restriction enzyme. The PFGE patterns were then compared using BioNumerics 3.0 (Applied Maths, Kortrijk, Belgium). The PFGE patterns were designated indistinguishable if the patterns consisted of the same number of bands and the corresponding bands were of the same size. Only PFGE-profiles with indistinguishable PFGE-profiles were defined as the same PFGE-type.

Results: The 62 HLGR *E. faecium* isolates had 53 different PFGE-types. Despite the many PFGE-types clones did occur; five of the isolates from one of the Danish Departments of Clinical Microbiology belonged to PFGE-type 41. Five other clones consisted of two isolates each.

Conclusion: Spread of HLGR in *E. faecium* isolates in Denmark seems to be due both to horizontal spread (probably by plasmids) and to less intense clonal spread of the aac (6')-Ie-aph (2'')-Ia gene.

Beta-lactamases

P1403

CTX-M extended spectrum beta-lactamases in community-acquired infections caused by *Escherichia coli* in the North West of Spain, Pontevedra, Galicia

P. Álvarez, M. Álvarez, C. García-Riestra, V. Pulian, M. Hernández, M. García (Pontevedra, Vigo, Santiago de Compostela, ES)

Objectives: The aim of this study was to know the prevalence and type of CTX-M Beta-lactamases in community acquired *E. coli* infections in a population of 221.612 habitants in Pontevedra, Spain.

Methods: From April to December 2002, all non duplicate *E. coli* isolates were collected from patients with a suspected community acquired infection according to the criteria of the CDC. Identification and susceptibility testing was performed using WIDER system (Fco. Soria Melguizo, Spain). Any strain with a MIC \geq 1 to cefotaxime (CT) and/or ceftazidime (TZ) were screened for the presence of an extended spectrum beta-lactamase (ESBL) by the double synergy disk test and the ESBL-detecting E-test strip (AB Biodisk, Solna, Sweden). The identification of blaCTX-M genes was performed by PCR with specific primers.

Results: A total of 2.119 community acquired infections caused by *E. coli* were documented. Forty four *E. coli* strains with MICs \geq 1 to CT and/or TZ were identified, 24 isolates were suspicious of being ESBL producers according to the used criteria, 21/24 (87.5%) were positive for CTX-M-14 like-beta

lactamases. In one strain the coexistence of CTX-M-14 and CTX-M-2 like-beta lactamases was identified. The remainder 2 strains (8.3%) were negative for blaCTX-M genes. The urinary tract infection was the most common source where the strains were isolated representing the 90%.

Conclusion: CTX-M-beta lactamases were the most frequent ESBL identified in community acquired infections caused by *E. coli*. Being CTX-M-14 like-beta-lactamases the most frequent CTX-M subtype identified. An isolate producing both CTX-M-14 and CTX-M-2 like-beta lactamases was identified. Nevertheless the low prevalence of ESBL-CTX-M *E. coli* producers in our area 1.04% (22/2.119), continuous surveillance would be desirable in order to control this important emerging problem. On the other hand, the antimicrobial prescription of oral extended spectrum cephalosporins by clinicians could be inappropriate in some patients in the community setting.

P1404

Five-year evaluation of clonal dissemination and expansion of VIM-1 producing *Enterobacteriaceae* in Greece: a report from the SENTRY Antimicrobial Surveillance Program (2001–2005)

L. Deshpande, N. Legakis, R. Jones, H. Sader (North Liberty, US)

Objective: VIM-1 producing *Enterobacteriaceae* (ENT) emerged in 2000 and has become endemic in Greece. The objective of this

study was to characterize VIM-1 genotypes and to evaluate clonal relationships among VIM-1 producing ENT strains isolated in Greece during the course of the SENTRY Program.

Methods: All isolates received as part of the SENTRY Program were susceptibility tested by reference CLSI methods against >25 antimicrobials. ENT isolates (except *Proteus mirabilis* and indole-positive *Proteae*) with MIC \geq 2 mg/L for imipenem (IMI) and meropenem (MER) were screened for metallo-beta-lactamase (MBL) by disk approximation test followed by PCR. ENT isolates from Greece with positive MBL screen test results were further evaluated. MBL gene and its genetic context were revealed by PCR and sequencing techniques. The isolates were also epidemiologically typed by PFGE.

Results: In the 2001–2005 period 16 *K. pneumoniae* (KPN) and 2 *E. aerogenes* (EAE) isolates from a medical center in Greece were found to produce VIM-1. Multiple distinct clonal outbreaks of VIM-1 producing ENT were identified during this period. Susceptibility and molecular typing results are summarized in the table.

Organism	PFGE (no. of isolates)	Integron size (bp)	MIC range (mg/L) ^a						PFGE
			IMI	MEM	CAZ	P/T	ATM	CIPRO	
KPN	C (1)	>2700	>8	>8	>16	>64	>16	>4	A
	D (9)	2000, 1500, 1300, 800	4- >8	1->16	>16	>64	\leq 0.12->16	4->4	
	E (2)	800	8	2-4	>16	>64	>16	1	
	F (1)	>2700, 1000	>8	4	>16	>64	>16	2	
	G (1)	2000	4	4	>16	>64	\leq 0.12	\leq 0.03	
	H (1)	>2700	>8	>8	>16	>64	>16	4	
	I (1)	2000	4	2	>16	>64	\leq 0.12	>4	
	EAE	A (2)	1500, 1300	>8	>8	>16	>64	>16	

a. CAZ = ceftazidime, P/T = piperacillin/tazobactam, ATM = aztreonam, and CIPRO = ciprofloxacin

Conclusions: blaVIM-1 emerged and rapidly became endemic among ENT in Greece. The high mobility of blaVIM-1 was demonstrated by the finding of: i) different PFGE patterns among VIM-1 producing KPN; ii) variable sizes of the integron amplicons; and iii) the emergence of VIM-1 in EAE. Our results also demonstrated that blaVIM-1 is disseminating horizontally as well as vertically within the medical center. These findings represent a critical problem for the healthcare facility as carbapenems remain the last antimicrobial resort for treatment of infections caused by multi-drug resistant Gram-negative bacilli. In addition, this is the first report of EAE producing VIM-1.

P1405

VIM-1 producing *Enterobacter cloacae* strains from Madrid, Spain: report from the SENTRY Antimicrobial Surveillance Program 2005

L. Deshpande, H. Sader, M. Tato, F. Baquero, R. Jones, R. Canton (North Liberty, US)

Objective: To characterize *Enterobacteriaceae* (ENT) strains with reduced susceptibility (S) to carbapenems (CARB) isolated in Madrid, Spain.

Methods: As part of the SENTRY Program bacterial isolates are S tested by reference CLSI methods against >25 antimicrobial agents. ENT isolates (except *Proteus mirabilis* and indole + *Proteae*) exhibiting MIC results at \geq 2 mg/L to imipenem (IMI) and

meropenem (MER) were screened for metallo-beta-lactamases (MBL) and Bush-Jacoby-Medeiros group 2f carbapenemases by disk approximation (DA) and PCR. PCR amplicons were sequenced for epidemiological purposes as well as to reveal genetic context of resistance genes. Isolates were also typed by PFGE.

Results: Two *E. cloacae* (ECL) strains (2700A and 726C) showed elevated CARB MIC values as well as resistance to all beta-lactams, except aztreonam (AZT) in strain 726C (Table 1). The strains were isolated in March 2005 (12 days apart) from bloodstream and respiratory tract infections of patients hospitalized in two distinct hospital units. The strains showed distinct antibiograms and PFGE patterns (Table 1). Both strains showed positive DA test results and PCR screens positive for blaVIM with a Class 1 integron of approximately 2.5 kb. Sequencing of integron from the index strain (726C) revealed blaVIM-1 along with aacA4 and aadA1 genes.

Strain #	MIC (mg/L)							PFGE	
	IMI	MER	Ertapenem	AZT	Ciprofloxacin	Tetracycline	Tobramycin		TMP/SMX
726C	8	4	>8	8	>4	>8	4	1	A
2700A	4	4	8	>16	0.12	4	:2	\leq 0.5	B

Conclusions: This is the first report of VIM-1-producing ECL from Spain. VIM-1 has been recently reported in ENT (*K. pneumoniae* and *E. coli*) from Barcelona, Spain. The finding of blaVIM-1 in two clonally unrelated strains emphasizes the mobility of these genes. Thus, MBL may be emerging as a significant, geographically diverse resistance mechanism among ENT in Spain. It is imperative to screen ENT isolates with modestly elevated (not resistant) MIC values of either IMI or MER for carbapenemase production and to control the spread of this resistance mechanism in ENT isolates causing nosocomial infections

P1406

Occurrence and prevalence of CTX-M type beta-lactamases in Gram-negative bacteria from tertiary care hospital, North India

M. Shahid, V.M. Ensor, P.M. Hawkey (Aligarh, IN; Birmingham, UK)

Objectives: CTX-M beta-lactamases are increasingly reported worldwide. Although the ESBL phenotype has been reported from India, no further molecular surveys have been published with the exception of the first report of CTX-M-15 type. This study was designed to identify the presence, species distribution, and sub-types of CTX-M genes in a large tertiary hospital between 2003–2005.

Methods: Gram-negative bacteria from various clinical samples submitted to the department of Microbiology, J.N. Medical College & Hospital Aligarh, India, for routine culture and susceptibility testing were studied. 130 non-duplicate isolates resistant to a third generation cephalosporin were randomly selected. These were subjected to a Multiplex PCR designed to identify all geno-groups of a blaCTX-M. Further identification of the specific CTX-M types was done by Reverse Line Hybridization (RLH).

Results: A total of 130 bacterial isolates from 130 non-repeat clinical samples (Urine = 108, Bronchoalveolar lavage (BAL) = 16 and Pus = 6) were included in the study. Urinary isolates were *Escherichia coli* (n = 91), *Klebsiella pneumoniae* (n = 9), *Acinetobacter* (n = 6), *Klebsiella oxytoca* (n = 1) and *Citrobacter* spp. (n = 1). The isolates from BAL were *E.coli* (n = 11)

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and *K. pneumoniae* (n = 5) and from pus were *E. coli* (n = 2) and *K. pneumoniae* (n = 4). Eighty-two (63.1%) of the 130 isolates were found to be CTX-M Group-1 positive by PCR. RLH identified all of them as CTX-M-15 type beta-lactamase producers.

Conclusion: CTX-M Group-1 beta-lactamases are the only CTX-M type in our area, and only CTX-M-15 was found. It is surprising that no other CTX-M subtypes are present in this survey population in contrast with other countries. This is the first systematic survey report from India detecting CTX-M-15 type beta-lactamases.

P1407

Characterization of class 1 integrons carrying the genes for VIM- and IMP-type metallo-beta-lactamases in *Pseudomonas aeruginosa* strains from Russia

O. Shevchenko, M. Edelstein, V. Kretchikov, L. Strachounski (Smolensk, RU)

Objectives: To investigate the genetic context of metallo-beta-lactamase (MBL) coding genes in Russian strains of *P. aeruginosa*.

Methods: The six strains studied were isolated respectively in 6 hospitals of 3 cities of Russia as part of the RESORT national surveillance study in 2002–2004. Based on results of our recent study (O. Shevchenko, et al., 45th ICCAC, P. C2-105), five of them were found to produce VIM-2 beta-lactamase and were assigned to 3 distinct clonal groups. One unrelated isolate produced a single (E59K) mutation variant of IMP-1. A PCR with primers facing outward from the *intI1* and *blaVIM* or *blaIMP* genes was used to confirm the location of MBL genes in class I integrons. The entire cassette arrays were then amplified using the primers matching conserved sequences at the 5' (*intI1*) and 3' (*qacEdelta1*) ends of integrons, separated by gel electrophoresis and sequenced using the nested *intI1*-, *qacEdelta1*- and cassette-specific primers.

Results: All the MBL genes were associated with class I integrons. Sequence analysis demonstrated that the *blaVIM-2* gene cassettes were all identical to that reported from In58 in France (GenBank acc. no. AF263520), however the structure of the *blaVIM-2*-carrying integrons was different. Two out of 3 isolates representing the same clone broadly disseminated in Russia contained the cassette array: *aacA7*, *blaVIM-2*, *dhfr*, *aacC-A5*, whose sequence was 100% identical to that of the recently described outbreak strain from the USA (GenBank acc. no. AY943084). The genetic content of the *blaVIM-2*-carrying integron from the third clonally related isolate was distinct: *aadB*, *blaVIM-2*, and *aacA4* gene cassettes. The integron of one of the remaining unrelated strains contained only two gene cassettes: *blaVIM-2* and *aadA1*, and that of another one carried *blaVIM-2* and *aadB* cassettes separated by the unknown fragment of 315 bp that includes a 150-bp sequence 90% homologous to the *orfE*-like gene (GenBank acc. no. AY139595). The genetic environment of the novel *blaIMP* gene was also unique. There was an *aadB* gene cassette located upstream of *blaIMP*, and downstream of it there was a 305-bp unknown sequence followed by *cmlA* and *blaOXA* gene cassettes.

Conclusion: The results of our study demonstrate an increasing complexity and diversity of class I integrons carrying MBL genes in *P. aeruginosa*.

P1408

Prevalence of AmpC over-expression in bloodstream isolates of *Pseudomonas aeruginosa*

V.H. Tam, A.N. Schilling, M.T. LaRocco, L.O. Gentry, K. Lolans, J.P. Quinn, D.A. Melnick, K.W. Garey (Houston, Chicago, Wilmington, US)

Objectives: The prevalence of AmpC over-expression in *Pseudomonas aeruginosa* (PA) as a mechanism of beta-lactam resistance was reported to be 6% in France previously (De Champs, AAC 2000). However, its prevalence is not well established in the U.S. We examined the contribution of AmpC over-expression towards beta-lactam resistance in clinical isolates of PA obtained in our hospital.

Methods: All bloodstream PA isolates obtained in 2003 were screened for ceftazidime (CAZ) resistance with and without the presence of clavulanic acid (4 µg/mL). AmpC stable derepression was ascertained phenotypically by a spectrophotometric assay (with and without prior induction by imipenem) using nitrocefin as the substrate, and subsequently confirmed by real-time quantitative PCR of the *ampC* gene. The presence of AmpC was confirmed by isoelectric focusing (with cloxacillin inhibition). The clonal relatedness of the AmpC over-expressed mutants was assessed using PFGE. In addition, the *ampC* and *ampR* genetic sequences were determined by PCR. For comparison, 2 standard wild type (PAO1 and ATCC 27853) and 3 multi-drug susceptible PA isolates were used as controls.

Results: 76 non-repeat bloodstream PA isolates were available. Stable derepression of *ampC* [a single beta-lactamase inhibited by cloxacillin (pl = 8.7)] was confirmed in 14 isolates (prevalence rate = 18.4%), and consisted of 7 distinct clones. The most prevalent point mutations in *ampC* were found to be G27D, V205L, and G391A, respectively.

Conclusion: In contrast to the previous study, AmpC over-expression is a significant mechanism of beta-lactam resistance in PA. Understanding the prevalence and mechanism of beta-lactam resistance in PA may guide the choice of empiric therapy for nosocomial infections in our hospital.

P1409

Characterization of ESBL producing *Salmonellae* isolated in Hungary in 2000 – 2004

N. Nógrády, Á. Tóth, J. Pászti, M. Füzi (Budapest, HU)

In case of *Salmonella* infections caused by multiple resistant strains, for infants, elderly or immuno-compromised patients extended-spectrum cephalosporins (ESCs) are recommended for treatment. Consequently, since the 1990s outbreaks and cases of infections caused by *Salmonella* resistant to ESCs have been increasingly reported. The aim of the study was to screen the *Salmonella* isolates received by the 'Johan Béla' National Centre for Epidemiology, Hungary, during 2000–2004, for isolates producing extended-spectrum-beta-lactamase (ESBL) and to characterise the strains by molecular methods. An initial screening for cefotaxime resistant (CtxR) strains was performed by disk diffusion method. The ESBL phenotype of the CtxR strains was confirmed by double disk approximation test and ESBL Etest. The strains were investigated by PCR using conserved primer sets specific for *blaTEM*, *blaSHV* and *blaCTX-M* genes and also for class 1 integron. The PCR products were sequenced. The transferability of the identified ESBL genes

was tested by conjugational experiments; the clonal relationship of the strains was tested by PFGE. Eight *S. Typhimurium* and one *S. Enteritidis* have been found to show cefotaxime resistance and produce ESBL. Five *S. Typhimurium* strains harboured CTX-M-5 gene, which was transferred by an approx. 6.7 kb plasmid to the transconjugants. Two *S. Typhimurium* strains had CTX-M-15 and TEM-1 genes, both of which were transferred via an approx. 140 kb plasmid. These two strains plus one CTX-M-5 positive strain produced one or both of the 1.45 kb (aadB-catB3) and 2.05 kb (oxa1-aadA1) integrons. In a single *S. Typhimurium* strain SHV-5 and TEM-1 genes were identified which were not transferable. This strain was integron negative. The CTX-M positive strains belonged to the same genetic cluster; the SHV-5 positive strain represented a distinct cluster. The *S. Enteritidis* strain possessed an SHV-5 gene alone, which was located on and transferred by an approx. 90 kb plasmid, together with an approx. 3.2 kb integron (aacC4-aacC1-ORFX-ORFX'-aadA1a). ESBL producing of the Hungarian Salmonella strains appears to link mainly to plasmid-mediated CTX-M type genes. Emergence of the CTX-M-5, CTX-M-15 and SHV-5 genes in *S. Typhimurium*, as well as appearance of an ESBL producing *S. Enteritidis* in Hungary is reported for the first time.

Acknowledgement: Noémi Nógrády is holder of the Bolyai János stipend of the Hungarian Academy of Sciences.

P1410

Phenotypic and genotypic identification of metallo-beta-lactamases in *Pseudomonas aeruginosa* in a university hospital, Ankara, Turkey

A. Cakar, B. Sener, G. Hascelik (Ankara, TR)

Objectives: *Pseudomonas aeruginosa* (Pa) is a leading cause of nosocomial infections and is resistant to many antibiotics. Since carbapenem resistance due to metallo-beta-lactamases (MBL) is an increasing problem all around the world, identification of MBL in clinical microbiology laboratory is very important. The aim of this study is to identify MBL enzyme in clinical Pa isolates, by phenotypic and genotypic methods and also evaluate the performance of phenotypic tests in identification of the MBL.

Methods: Seventy-five clinical Pa isolates resistant to ceftazidime and/or imipenem were included in this study. Antibiotic susceptibilities were investigated by agar dilution method. For phenotypic identification of MBL, double disk synergy test, E-test, combined disk synergy test and Modified Hodge test were performed. Imipenem hydrolysis was screened in the crude extracts of the isolates. In order to confirm the results of the phenotypic tests, PCR analysis were performed with the specific primers for the conserved regions of blaVIM and blaIMP genes.

Results: Among the Pa isolates tested 50 (75%) of them were found as multiple drug resistant. According to the PCR analysis eight (10.6%) isolates were found blaVIM positive. Double disk synergy test identified three (37.5%) PCR positive isolates. Five (62.5%) isolates were positive with E-test, two (20%) of them were positive with Modified Hodge test and seven (87.5%) of the eight PCR positive isolates were also positive with combined disk synergy test. Four of the PCR positive isolates proved to be positive also with the hydrolysis test.

Conclusion: The results of this study revealed that MBL are prevalent among beta-lactam resistant Pa isolates in our hospital. Phenotypic tests alone are not always sufficient to detect MBL, since MBL genes are not always expressed. When PCR analysis based on the presence of MBL genes was taken as the gold standard, among the phenotypic tests the most reliable one seemed to be the combined disk synergy test.

P1411

Prevalence of CTX-M producing bacteria in Ireland

D. Morris, M. McManus, C. Slater, V. Buckley, L.E. Fenelon, G. Corbett-Feeney, M. Cormican (Galway, Dublin, IE)

Objectives: Extended Spectrum Beta Lactamase (ESBL) producing bacteria are a problem in hospitalized patients and are increasingly associated with community-acquired infection. The CTX-M class of beta-lactamases have emerged as a serious problem with a sharp increase in the occurrence of urinary tract infection (UTI) associated with CTX-M-15 producing *E. coli* noted in the U.K during 2003/2004. Dissemination within the UK of CTX-M-15 producing *E. coli* "strain A" has been reported. This aim of this study is to determine to what extent the strain A reported in the UK, or other CTX-M/ESBL positive *Enterobacteriaceae* are present in Ireland.

Methods: Three hundred and thirty two suspect ESBL producing *Enterobacteriaceae* were provided by 24 laboratories throughout Ireland. Isolates were collected from hospitalized and primary care patients between 1997 and 2005. Susceptibility testing to cefpodoxime, cefotaxime, ceftazidime and ceftioxitin was performed and interpreted in accordance with Clinical Laboratory Standards Institute (CLSI) disk diffusion methods. Suspect ESBL producers were assessed using three ESBL Etest strips (AB Biodisk, Solna, Sweden): ceftazidime/ceftazidime plus clavulanic acid (TZ/TZL); cefotaxime/cefotaxime plus clavulanic acid (CT/CTL); and cefepime/cefepime plus clavulanic acid (PM/PML). Confirmed ESBL producers and appropriate controls were examined for blaCTX-M, blaSHV, and blaTEM by PCR with sequencing of selected amplicons. Pulsed field gel electrophoresis (PFGE) using XbaI was performed.

Results: Three hundred and ten isolates (93%) were identified as suspect ESBL producers and 156 (47%) were confirmed ESBL positive. The blaCTX-M gene was detected in 92 isolates (68 CTX-M group-1, 23 CTX-M group-9, and 1 to be identified), the blaSHV gene in 58 and blaTEM gene in 84. DNA sequencing confirmed blaCTX-M-15 in 3 of the CTX-M group-1 isolates and blaCTX-M-14 in 1 of the group-9 isolates. One isolate harboring blaCTX-M-15 was indistinguishable from strain A from the UK. **Conclusions:** CTX-M producing *Enterobacteriaceae* have been present in Ireland since at least 2000 and were widely disseminated in Ireland before their presence was first confirmed in 2005. The clonal group designated strain in the UK is also present in Ireland.

P1412

Widespread dissemination of blaOXA-51-like among carbapenem-resistant *Acinetobacter* spp. isolates in Brazil

D.E. Xavier, A.C. Gales, A.S. Pereira, A.C.C. Pignatari, M. Castanheira (Sao Paulo, BR)

Objective: blaOXA-51 and variants have been recently described among carbapenem-resistant *Acinetobacter* spp. isolates from Argentina. In current study we evaluated the presence of blaOXA-51-like among carbapenem-resistant *Acinetobacter* spp. isolated from seven Brazilian hospitals.

Methods: A total of 30 non-duplicated imipenem-resistant *Acinetobacter* spp. clinical isolates were collected during the 2000–2001 period from seven distinct hospitals located in six Brazilian cities. The isolates were susceptibility tested by CLSI/NCCLS broth microdilution method against 12 antimicrobial agents. The genetic relationship among the isolates was deter-

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mined by RAPD. Gel Compar™ generated dendrograms for interpretation of RAPD results. The detection of blaOXA-51-like was performed by PCR with primers designed to align with the blaOXA-51 family. DNA sequencing of one amplicon was performed in both strands. Restriction digestion of the remaining amplification products confirmed the presence of blaOXA-51-like.

Results: All isolates showed high levels of resistance against most beta-lactam agents, including penicillins in combination with serine-beta lactamase inhibitors, broad-spectrum cephalosporins, imipenem, meropenem and aztreonam. The isolates were also resistant to aminoglycosides (amikacin and gentamicin) and fluoroquinolones (ciprofloxacin and gatifloxacin). The restriction profile of the PCR products showed that blaOXA-51-like was detected in 29 of 30 (96.6%) *Acinetobacter* spp. isolates studied. A great genomic variety was found among the *Acinetobacter* spp. isolates collected from different institutions but a predominant clone was observed in one of the hospitals.

Conclusions: Although recent reports have suggested that the OXA-type enzymes naturally occur among *Acinetobacter* isolates, specific OXA-encoding genes are only disseminated in particular regions. In the current study, the presence of blaOXA-51-like was widely detected among *Acinetobacter* spp. isolates collected from Brazilian hospitals located in Southern and Southeastern regions, which are closer to Argentina. The presence of OXA-51-like producing isolates might be one of contributing factors to justify the elevated carbapenem resistance rates found among *Acinetobacter* spp. isolated in Brazil.

P1413

Genetic analysis of metallo-beta-lactamase producing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolates in the intensive care unit of a university general hospital in Greece

E. Koratzanis, I. Galani, M. Souli, Z. Chryssouli, H. Giamarellou (*Chaidari, GR*)

Objective: During a study for the evolution and horizontal transfer of metallo-B-lactamase (MBL) genes from commensal to pathogenic gram-negative bacteria in patients of an intensive care unit of a tertiary care university hospital, we analysed the integrons carrying the MBL gene in *K. pneumoniae* and *P. aeruginosa* isolates.

Methods: Isolates from surveillance cultures as well as isolates considered pathogenic from the same patient that exhibited a positive EDTA-imipenem disk synergy test were studied. Susceptibility testing was performed by the disk diffusion technique and MICs were determined by the broth microdilution method according to NCCLS guidelines. EDTA-disc synergy test, was used to screen for MBL production. PCR and PCR-RFLP analysis and Southern hybridization were used to identify and analyse the blaVIM-containing integrons.

Results: All isolates exhibited reduced susceptibility or resistance to imipenem (0.5–8 µg/ml) and a positive EDTA-synergy test. Strains were resistant to ceftazidime, ceftriaxone and cefotaxime. Class I integrons associated with VIM-type MBL genes were found in all strains. Preliminary PCR-based experiments and RFLP analysis revealed the presence of 5 different integrons (1500–5500 bp) carrying blaVIM-1 in *K. pneumoniae* isolates. The predominant type is that of 1500 bp probably corresponding to an integron containing only blaVIM-1 gene cassette. A new integron containing blaVIM-1 and aac (6)-IIc that has been characterized by our group in an *Enterobacter cloacae* clinical isolate was also found in a *K. pneumoniae* isolate.

Interestingly, the same patient was also colonized by a *Citrobacter freundii* carrying the same integron. PCR and Southern hybridization revealed the presence of 2 integrons (both >2 kb) carrying blaVIM-2 in all of the *P. aeruginosa* isolates. The size and RFLP analysis revealed no similarities between the integrons of *P. aeruginosa* and those of *K. pneumoniae*.

Conclusions: MBLs of the VIM-type represent an emerging threat in Greek hospitals. All isolates of our study harboured blaVIM-1 (*K. pneumoniae*) or blaVIM-2 (*P. aeruginosa*), in class I integrons. We observed: 1) the diversity of blaVIM-1 containing integrons in different *K. pneumoniae* isolates of the same patient and the integration of the same class I integrons in *K. pneumoniae* nosocomial isolates of different patients and 2) no similarities of VIM-containing integrons from *P. aeruginosa* and *K. pneumoniae* isolates even those isolated from the same patient.

P1414

Detection of extended-spectrum beta-lactamases in *E. coli* and *Klebsiella* spp. by isoelectric focusing and PCR

L. Oksuz, N. Gurler (*Istanbul, TR*)

Objectives: The aim of this study was the detection of beta lactamases types in extended spectrum beta lactamases producing *E. coli* and *Klebsiella* spp strains. The study strains consisted of 12 *E. coli* and 32 *Klebsiella* spp (28 *K. pneumoniae* and 4 *K. oxytoca*) isolated from various specimens of patients hospitalized in different units (Intensive Care, Hematology, Oncology, Neonatology, Transplantation and Pediatric Surgery Units) of the Istanbul Medical Faculty between July 2002–March 2004.

Methods: Antimicrobial susceptibility tests were performed by disc diffusion according to the CLSI guidelines. MICs of cefotaxime and ceftazidime were determined by the agar dilution method and by the E-test ESBL. Isoelectric focusing was performed by the method of Matthew et al (*J Gen Microbiol*, 1976). Polymerase chain reaction was performed using primers specific for the bla TEM, blaSHV and blaCTX-M genes. Conjugation was carried out by broth mating. A rifampicin-resistant *E. coli* strain (J62-2) was used as the recipient. Plasmid extraction was performed by the alkaline lysis method.

Results: Resistance to imipenem or meropenem was not detected. Amikacin was the second most effective antibiotic against all strains, after carbapenems. All isolates demonstrated an ESBL phenotype using the double-disc synergy test. MIC50 and MIC90 for cefotaxime were found 16 µg/ml and 64 µg/ml in *Klebsiella* spp and *E. coli* strains, respectively. IEF results indicated that all isolates produced 1–4 beta lactamases. Isoelectric points of the isolates were between 5.4–9.0. Among *K. pneumoniae* and *E. coli* strains, rates of TEM, SHV, CTX-M beta lactamases were found 64.3%, 92.9%, 67.9%; 66.7%, 25%, 83.3%, respectively. The profiles generated with ERIC-2 PCR primer contained several bands, ranging in size from 170 to 1500 bp. Ten, three and six groups were separated for *K. pneumoniae*, *K. oxytoca* and *E. coli*, respectively. Thirty-one of the isolates (70.45%) were able to transfer their resistance to recipient *E. coli* strains by conjugation. Twenty of the transconjugants (64.5%) were found to carry only one plasmid (>48a). Eleven strains (35.5%) harbored more than one plasmid, with sizes ranging from 10 to 100 kb.

Conclusions: In conclusion, we found that CTX-M was more common than TEM and SHV beta lactamases in *E. coli* strains isolated from patients hospitalized in Istanbul Medical Faculty, however SHV was the most common beta lactamase in *K. pneumoniae*. The present work was supported by the Research Fund of Istanbul University. Project no T223/06032003.

P1415

Molecular characterization of *Enterobacteriaceae* producing CTX-M-1-like ESBL (CTX-M-1, -3, -15, and -32) in Madrid, Spain (2000–2004)

A. Novais, R. Cantón, R. Moreira, F. Baquero, T.M. Coque
(Madrid, ES)

Objectives: CTX-M enzymes are the most widespread class A extended spectrum beta-lactamases (ESBL), CTX-M-9-like and CTX-M-10 being the most commonly found in Spain. However, CTX-M-1-like variants have been recently detected. The aim of the study was to characterize the genetic diversity of these ESBL from our institution.

Methods: 20 *E. coli* CTX-M-1-like producing isolates from 14 patients were studied. ESBL characterization was performed by IEF, PCR and sequencing. Antibiotic susceptibility was determined by standard methods. Clonal relatedness was established by PFGE and *E. coli* phylogenetic groups determined by a multiplex PCR assay. Transfer of bla was carried out by broth and filter matings. Plasmid analysis was accomplished by the method of Barton, RFLP and characterization of the incompatibility group by the PCR assay based on replicon typing described by Carattoli et al. Presence of ISEcP1, IS26 and IS903 and their association with blaCTX-M genes were searched by PCR.

Results: Isolates were recovered from outpatients (45%) and from patients at medical (25%), ICU (25%) and surgical wards (5%). Nine CTX-M-1, 5 CTX-M-15, 5 CTX-M-32 and 1 CTX-M-3 producing isolates corresponding to 12 PFGE types (3 CTX-M-1, 3 CTX-M-15, 5 CTX-M-32 and 1 CTX-M-3) were studied. Most belong to phylogenetic group D (58,3%) followed by groups B1 (16,7%), A (16,7%) and B2 (8,3%). Resistance to quinolones, sulfonamides, and aminoglycosides was commonly observed (64%, 45% and 55%, respectively). Transfer of bla genes was achieved in most cases (83%). Plasmids containing blaCTX-M-15, blaCTX-M-1 and blaCTX-M-3 were diverse and assigned to incompatibility groups FIB, F and A/C. Plasmids carrying blaCTX-M-32 were related and belong to incompatibility group N. ISEcP1 was located upstream of all blaCTX-M studied although a copy of IS5 interrupting ISEcP1 was identified in all CTX-M-32 and 1/3 CTX-M-1 strains. Most isolates (10/12) were positive for IS26. IS903 was only detected among isolates containing blaCTX-M-15 and blaCTX-M-3.

Conclusions: The high clonal and plasmid diversity found among CTX-M-15 and CTX-M-1 isolates reflects a wide spread of these ESBL also in Spain. Dissemination of CTX-M-32 is linked to genetically related plasmids in our area. Association of ISEcP1, IS26 and IS903 to different bla genes and plasmids contributes to the spread, maintenance and amplification of a variety of resistance genes and elements in both community and nosocomial environments.

P1416

Repeated occurrence of extended-spectrum beta-lactamase CTX-M-9 in *Salmonella enterica*, serotype Virchow, isolated in Spain from 2002 to 2005

S. Herrera, R. Ramiro, R. Gonzalez-Sanz, S. Valdezate, R. Diez, A. Aladueña, M.A. Echeita (Majadahonda. Madrid, ES)

Members of a new group of extended-spectrum beta-lactamases (ESBLs), the CTX-M type, derived from chromosomal class A beta-lactamases, have been identified in the past 10 years. They have been detected in a variety of species of the family

Enterobacteriaceae in very distant regions and, although one specific enzyme could be detected in different regions, it seems that there are predominant enzymes in each region. In a one-year long study our group detected the presence of CTX-M-9 enzyme strongly associated to *Salmonella enterica*, serotype Virchow, phage-type 19.

Objectives: i) to survey the *Salmonella enterica*, serotype Virchow, phage type 19 strains submitted to the Spanish Reference Laboratory for *Salmonella* and *Shigella* (SRLSS) from 2002 to 2005 in order to determinate the rate of beta-lactam resistances. ii) to identify the ESBLs responsible for the resistances and iii) to further characterize the strains harboring the ESBLs to establish the genetic relationship among them.

Material and methods: The antimicrobial susceptibility for the *S. Virchow* isolates was determined by the disk diffusion method using Mueller–Hinton agar medium. ESBLs phenotype was detected by the double disk synergy test (DDST). A PCR and later sequencing of the obtained fragments were performed for the molecular ESBLs characterization. The genetic relationship among the strains was determined by PFGE.

Results and discussion: We found an association between *S. Virchow*, phage-type 19 and a resistance profile that affected not only to beta-lactams but also to other antimicrobial drugs. Regarding to the beta-lactams, the resistance found in most of the strains is due to the combined presence of TEM-1E and CTX-M-9 enzymes. Although different, some of the serotype Virchow PFGE fingerprints were highly related (similarity, 90%) which may indicate clonal spread of this resistance rather than horizontal transfer. Although the SRLSS is focus on the study of *Salmonella* isolated from human origin, some of the received strains had a non-human origin. It is interesting that *S. Virchow*, phage-type 19 is highly distributed in Spain but just in the human case.

Conclusion: We could be facing the spread of a clone of a multiresistant *Salmonella enterica*, serotype Virchow, phage-type 19 harboring a CTX-M-9 beta-lactamase distributed in most of the Spanish provinces.

P1417

High clonal and plasmid diversity of CTX-M-14 and SHV-12 producing isolates in the community (Madrid, Spain)

A. Valverde, T.M. Coque, A. Novais, J.C. Galán, F. Baquero, R. Cantón (Madrid, ES)

Objectives: Class A extended spectrum beta-lactamases producing *Enterobacteriaceae* have been increasingly recovered, being CTX-M-14 and SHV-12 the most common enzymes detected. The aim of this work is to study the epidemiological features and genetic diversity of these enzymes recovered in our institution since first description. **Methods:** We studied CTX-M-14 (76 isolates/67 patients) and SHV-12 (44 isolates/43 patients) producing isolates from 1999 to 2005. Clonal relatedness was established by XbaI- PFGE. Phylogenetic groups among *E. coli* isolates were identified by a multiplex PCR assay. Transfer of bla genes was searched by broth and filter mating. Plasmid characterization was accomplished by S1 nuclease and RFLP. Presence of ISEcP1, IS903, IS26 and association with blaCTX-M-14 or blaSHV-12 was elucidated by PCR.

Results: CTX-M-14 and SHV-12 were mainly recovered from the community setting (50%–70%, respectively) corresponding to outpatients, healthy volunteers and environmental samples. In the hospital, they were isolated from patients at medical (20/32%), surgery (5/11%) and ICU (2.5/7%) wards. Resistance to quinolones was higher among CTX-M-14-isolates than SHV-12

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(23/4.5%) but resistance to sulphonamide was more common among SHV-12 strains (75/54%). blaCTX-M-14 was mainly associated to *E. coli* (96%) while blaSHV-12 was linked to both *E. coli* (68%) and *K. pneumoniae* (31%). *E. coli* isolates belong to phylogenetic groups D (36.9%), A (36.9%) and B1 (24.3%). Among CTX-M-14 producing isolates, a high clonal diversity (index diversity (D) = 0.7) was detected. Transfer of blaCTX-M-14 was achieved in most cases, involving different plasmids (48.5 – 330 kb). This gene was mainly linked to ISEcp1 and occasionally to IS903. High clonal diversity among SHV-12-*E. coli* was also observed (D = 0.7) while most *K. pneumoniae* isolates belong to a single PFGE type (5 PFGE types/14 isolates) recovered from 2001 to 2003. Most isolates were able to transfer blaSHV-12 gene, located in plasmids ranging from 48–145kb. Presence of IS26 was detected in most of SHV-12-isolates.

Conclusions: Strains containing blaCTX-M-14 and blaSHV-12 are mainly associated to the community. The high clonal and plasmid diversity among *E. coli* isolates indicates a wide dissemination of these particular genes outside the hospital and suggests that this species plays a relevant role in the spread and maintenance of resistance genes linked to ISEcp1 or IS26 both in community and nosocomial settings.

P1418

Dissemination of acquired metallo-beta-lactamases in Gram-negative pathogens: first Italian countrywide survey

L. Pagani, F. Luzzaro, C. Mugnaioli, M. Spalla, F. De Luca, M. Perilli, G. Babini, G. Gesu, G. Amicosante, A. Toniolo, G.M. Rossolini (*Pavia, Varese, Siena, L'Aquila, IT; Eredebodgem, BE; Milan, IT*)

Objectives: The emergence of acquired metallo-beta-lactamases (MBLs) in gram-negative pathogens (*Pseudomonas aeruginosa*, *Acinetobacter* spp. and *Enterobacteriaceae*) is a worrisome phenomenon since MBLs can confer resistance to carbapenems and most other beta-lactams. MBL producers have now been reported worldwide. In Italy, both IMP- and VIM-type enzymes have been detected, mostly in *P. aeruginosa* but also in other gram-negatives. In this work we report the results of the first Italian nationwide survey on the prevalence of acquired MBLs in *P. aeruginosa* and other gram-negative nonfermenters (GNNFs), and in *Enterobacteriaceae*.

Methods: During a 4 month period (Sept.–Dec. 2004), consecutive nonreplicate isolates showing reduced susceptibility to imipenem (using ad hoc breakpoints, depending on species) were selected from 14 clinical microbiology laboratories distributed across Italy. Identification and susceptibility testing were carried out with the Phoenix ID/AST System (Becton Dickinson). MBL genes were screened by colony-blot hybridization and identified by sequencing.

Results: Of 2721 GNNFs and 12 245 *Enterobacteriaceae* nonreplicates isolated at the participating laboratories during the study period, 319 and 117, respectively, were selected for further investigation. Overall, 30 isolates were positive for acquired MBL genes including 27 *P. aeruginosa* (1.3% of total and 10% of carbapenem-resistant *P. aeruginosa* isolates, respectively), 1 *Pseudomonas putida*, 1 *Acinetobacter baumannii*, and 1 *Enterobacter cloacae*. MBL producers were detected in 10 of 14 sites. In 24 isolates the enzyme was of the VIM type, while in the remaining 6 it was of the IMP type.

Conclusions: *P. aeruginosa* producing acquired MBLs are broadly distributed in Italy, although their overall prevalence remains relatively low. In other gram-negative species acquired MBLs remain quite uncommon. Enzymes of the VIM type

appear to be more common than those of the IMP type. A financial contribution from Becton Dickinson Europe is acknowledged.

P1419

Phenotypic and molecular detection of metallo-beta-lactamases in imipenem-resistant *Pseudomonas aeruginosa* in a hospital centre, Coimbra, Portugal

A. Pena, A.F. Alves, R.M. Leitão, O.M. Cardoso (*Coimbra, PT*)

Objectives: *Pseudomonas aeruginosa* (PA) is one of the leading causes of nosocomial infections. Clinical isolates harbouring the metallo-beta-lactamases (MBLs) IMP and VIM have been increasingly reported worldwide, and VIM has been reported especially in Mediterranean Europe. Recently, another two MBLs have been described: SPM-1 in Brazil and GIM in Germany. The aim of this study was to determine the occurrence of these enzymes in Portuguese clinical isolates of PA imipenem (IP) resistant.

Methods: During two years (April 2003 to April 2005) 773 clinical isolates of PA were collected in Centro Hospitalar of Coimbra and were identified by MicroScan WalkAway (Dadebehering) system. The susceptibilities were determined with this system that uses the following antibiotics: Piperacillin (PIP), Piperacillin plus Tazobactam (TZP), Aztreonam (AZT), Ceftazidime (CAZ), Imipenem (IP), Meropenem (MP), Gentamicin (GN), Tobramycin (NN), Amicacin (AMK), and Ciprofloxacin (CIP). Interpretation was done according recommendations of NCCLS. In order to determine the occurrence of MBLs, 136 isolates IP resistant were studied. Screening was made with double disk synergy test. PCR using conditions and primers specific for blaVIM, blaIMP, blaSPM and blaGIM previously described. The amplicons obtained were sequenced to identify the MBLs genes.

Results: Among the 773 isolates of Pa collected 136 (17.6 %) were IP resistant. The susceptibilities to other antibiotics of the IP resistant isolates were: MP 20.3%, CAZ 56.7%, AZT 37.3%, AMK 73.1%, and CIP 53%. Double disk synergy test was positive in 40 (29.4%) isolates. By PCR, 24 isolates (60%) demonstrated the presence of blaVIM. The others MBLs tested were negative. Sequencing demonstrated the presence of VIM-2 in these isolates. Among these strains that harboured VIM-2, AZT inhibited 79.2%, followed by CAZ 66.7% and AMK 54.0%.

Conclusions: Twenty-four (17.6%) of the IP resistant isolates exhibited a MBL VIM-2, and were isolated from nosocomial infections. The emergence of MBL producing bacilli that are resistant to carbapenems, aminoglycosides, and fluoroquinolones is becoming a severe therapeutic problem, indicating the need of alternative antimicrobial therapies, and search of inhibitors of this kind of enzymes. A regular screening/monitoring system should be set up to prevent the wider spread of this resistance determinant.

P1420

Prevalence and molecular characterisation of beta-lactamases in Gram-negative rods isolated from oral flora

V. Toussaint, C. Voha, I. Madinier, T. Fosse (*Nice, FR*)

Objectives: Beta-lactam resistance in most gram-negative anaerobic bacteria is mediated by the production of class A extended-spectrum beta-lactamases. In the case of oral cavity

infections, a variable prevalence of CfxA-like beta-lactamase was described in *Prevotella* spp. strains. The aim of this study was to determine the prevalence of beta-lactamase producing Gram negative rods isolated from oral flora and to identify by PCR beta-lactamase genes according to species.

Methods: Over a period of 24 months 142 gingival samples were collected from 71 adult patients from dental department (Teaching University Hospital of Nice, France). Anaerobic or microaerophilic Gram negative rods presumptively resistant to beta-lactams were screened by culture on supplemented blood agar dishes with amoxicillin antibiotic disks. Beta-lactamase production was determined by the nitrocefin disk test. Antibiotic susceptibility was determined by Etest MICs. Beta-lactamase genes were determined by PCR (CblA-CepA, CfxA, Fus-1, TEM) and cfxA probe.

Results: Overall a very high prevalence (85%) of beta-lactamase producing strains was found. A total of 224 strains were identified (>3/patient). Most of the resistance was due to CfxA beta-lactamase. Main CfxA producing species were *P. buccae* (58 strains), *P. intermedia* (43), *P. denticola* (25), *P. oralis* (16) and *Capnocytophaga* sp. (9). A group of *Prevotella* sp. (25) cfxA negative producing a new extended-spectrum beta-lactamases was found. *Fusobacterium nucleatum* beta-lactamase positive strains were PCR Fus-1 positive (oxacillinase). TEM type beta-lactamase was only found in two *Hamophilus* sp. strains but not in *Prevotella* or *Capnocytophaga* strains.

Conclusions: With a sensitive culture screening method a very high prevalence of cfxA producing β -lactamase was found in oral flora. A new extended-spectrum beta-lactamases was found in *Prevotella* sp. and deserves further studies. This high prevalence was probably due to extensive horizontal transfer of mobile genetic element among oral flora.

Non-molecular diagnostics of MRSA

P1421

Implementation of BBL CHROMagar orientation for routine urine cultures in a high-volume clinical laboratory

S. Whittier, P. Della-Latta (New York, US)

The Clinical Microbiology Service of Columbia University Medical Center receives over 200 urine specimens per day for routine culture. Our current practice utilizes a bi-plate consisting of 5% sheep blood agar and MacConkey agar (Becton Dickinson, Sparks, MD). The purpose of this investigation was to determine the feasibility of replacing our existing bi-plate with the BBL CHROMagar Orientation agar, which was designed to detect and identify two of the most commonly isolated urinary tract pathogens, *E. coli* and *Enterococcus* species. We prospectively planted 1350 urine specimens on both the bi-plate and CHROMagar plate. Of these, 662 (49%) samples were determined to be positive by both methods, 634 (47%) were negative by both and there were 54 (4%) discordant sets of culture results. Of the 662 culture positive samples, 351 (26% of total specimens) grew clinically significant urinary tract pathogens. The remaining 311 (23% of total specimens) were either mixed (>3 organisms) or grew insignificant quantities (<10,000 CFU) of bacterial pathogens. Forty-two of the 54 discordant results were samples in which the bi-plate was positive for <10,000 CFU of nonpathogenic bacterial isolates while the CHROMagar plate demonstrated no growth. These discordant results were considered to be insignificant. The remaining 12 discordants were reported as >100,000 CFU mixed urogenital flora, however examination of the CHROMagar plate clearly demonstrated a predominance of *Enterococcus* species. This bi-plate "misses" were considered to be significant due to the implications of missing a potential uropathogen, particularly a VRE isolate. Identifications and susceptibilities were performed on the Vitek 2 system (bioMérieux, Durham, NC) from colonies isolated on the bi-plate as well as the CHROMagar plate. Results were consistent, independent of the media. Overall, the CHROMagar plate performed extremely well in our laboratory. The potential for cost-savings can be realized through the use of rapid identification and increased capacity to recognize mixed cultures, thus obviating unnecessary subcultures.

P1422

Evaluation of chromogenic Biomérieux MRSA ID medium for direct detection of methicillin-resistant *Staphylococcus aureus* from surveillance cultures and from clinical samples

C. Sánchez-Carrillo, E. Cercenado, M. Marín, J. Jensen, M. Guembe, M. Rivera, E. Bouza (Madrid, ES)

Objectives: Rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from surveillance cultures and from clinical samples is crucial in order to control the spread of MRSA. We evaluate a new selective and differential chromogenic medium, MRSA ID medium (Biomérieux, Marcy L'Etoile, France) which enables recovery and concomitant identification of MRSA strains directly from nasal swab specimens and from other clinical samples.

Methods: From May to June 2005, all samples received in our laboratory requesting MRSA search were studied. A total of 530 clinical samples were inoculated to MRSA ID and mannitol-salt agar (MSA, Tec-Laim, Spain): 452 surveillance nasal swab specimens obtained from the anterior nares and 78 other samples (57 wounds, 21 bronchial aspirates). Green colonies on MRSA ID and mannitol-positive on MSA at 24 hours and 48 hours were identified by the MicroScan system (Dade Behring) using Pos Combo 2SA panels and by detection of the *mecA* and *femA* genes by PCR.

Results: A total of 202 *S. aureus* isolates were recovered; of these 67 (33%) were oxacillin susceptible (MSSA) and 134 (66%) were oxacillin resistant (MRSA). On MRSA ID, 132/134, or 98.5% of MRSA were recovered, whereas recovery on MSA was 82.8% (111/134) ($p < 0.0001$). The overall sensitivity, specificity, positive predictive value and negative predictive value of MRSA ID were: 98.5%, 98.2%, 94.9%, and 99.4%, respectively; whereas, the corresponding values for MSA were: 82.8%, 99.7%, 99.1%, and 94.5%. Seven false positive readings on MRSA ID were detected (one nasal swab and 6 other samples) and corresponded to *Enterobacter cloacae* (3 isolates), MSSA (1), methicillin-resistant coagulase-negative *Staphylococci* (1), *Candida* spp. (1), and *Bacillus* spp. (1). Fifty-eight MRSA isolates (43%) were detected on MRSA ID at 24 hours; 42% at 48 hours, and in the remaining 15% of MRSA, readings were only performed at 48 hours. On MSA followed by conventional methods for the

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final identification of MRSA, identification was delayed to 72 hours in 14% of cases, and to more than 72 hours in 86%.

Conclusions: In this study, MRSA ID was superior to conventional methods for recovery of MRSA from nasal swabs and from other clinical samples, and shortened the detection time of MRSA to 24 or 48 hours. The enhanced recovery of MRSA using this medium and the identification of most MRSA isolates at 24 hours without additional susceptibility testing will benefit the patients and decrease costs associated to infections caused by MRSA.

P1423

Comparative evaluation of the chromogenic MRSA ID media with standard procedure for screening of methicillin-resistant *Staphylococcus aureus* carriers and detection of high-level resistance to mupirocin by disk diffusion

I. Mansoor, J. Brouillard, S. Cannoot, C. Ducastel (Baudour, Ath, BE)

Objectives: The procedure for screening patients with methicillin-resistant *Staphylococcus aureus* (MRSA) is very laborious. It includes the use of agar plates and selective enrichment broth (SEB). Results are available to clinicians only after 48–96 hours, thus delaying isolation measures needed for preventing nosocomial transmission of MRSA. In this study we evaluated the yield of direct plating specimens on MRSA ID® without SEB. Moreover high-level resistance to mupirocin was determined by disk diffusion (200 µg) and MICs.

Methods: A total of 523 specimens were cultured: 487 nasal and 36 wounds swabs. To overcome inoculation bias, a random table was used. Direct plating on MRSA ID® with a disk of mupirocin (200 µg) was compared with our procedure which includes a Columbia ANC blood agar (COL) and a NaCl 7.5% SEB. Plates were read after 24/48 hours and broths sub cultured on COL after 24 hours. All colonies resembling *S. aureus* on the COL and green colonies on MRSA ID® plates were identified with the Slidex Staph Plus® (SSP) latex. Oxacillin susceptibility was done with an oxacillin (6 µg/ml) agar screen method (OAS). Mupirocin inhibition zone diameters (IZD) were recorded for MRSA strains isolated from clinical samples and from 14 well-characterized *mupA* strains. Mupirocin MICs were done with E-test strips for MRSA strains with IZD <20 mm.

Results: A total of 39 (7%) MRSA were isolated by a combination of all methods. The overall sensitivities for primary plating were 77(87%) for COL and 90(100%) for MRSA ID® after 24(48 h) of incubation time respectively. The SEB in combination with COL gave a yield of 92%. The specificities for the MRSA ID® plates after 24 (48 h) incubation were 99.8(98.3%). No methicillin susceptible *Staphylococcus aureus* was isolated on the MRSA ID® while 65(12%) with our routine procedures. The number of SSP and OSA tests done were 281(54%), 149(28%) for routine procedure and 48(9%), 39(7%) for direct plating on MRSA ID® respectively ($p < 0.05$). The mupirocin IZD range from 6 to 50 mm and 6 to 14 mm for clinical and *mupA* strains respectively. Mupirocin MICs for strains (19) tested were > 1024 µg/ml.

Conclusions: The use of MRSA ID® with 24 hours of incubation time is as sensitive as standard procedure for MRSA screening. Green colonies appearing after 48 hours should be further identified and susceptibility testing done. High dose mupirocin disks could be a good alternative to E-test for detecting high-level resistance to mupirocin.

P1424

Direct identification and methicillin susceptibility of *Staphylococcus aureus* from blood-culture bottles using the Vitek 2 system

C. Flórez, E. López-Oviedo, C. Martín, J. Cabezas, D. Morilla, E. Martín-Mazuelos (Seville, ES)

Objectives: To assess the ability of The Vitek System in order to rapid identification of *S. aureus* and detection of methicillin resistance from blood-culture bottles.

Methods: Only blood cultures bottles showing Gram-positive cocci in clusters on direct Grams stain in more than one set of blood cultures were processed. The direct method was done using a suspension made by differential centrifugation of positive blood culture broth for inoculation of the Vitek ID-GPC, ID-GP and AST-536 cards (bio-Merieux). Standard identification was done using an inoculum made from and overnight culture on solid media and coagulase production (Staphaurex, Remel). The detection of methicillin resistance was evaluated with the oxacillin disk diffusion and PBP-2a latex test (Oxoid).

Results: A total of 51 consecutive *S. aureus* isolates from blood cultures were evaluated. The direct method resulted in 11.8% misidentifications or non-identifications. Methicillin resistance was detected in 6 isolates (11.8%) by direct method and the PBP-2a test and in 7 by disk diffusion.

Conclusions: The direct Vitek method could correctly report identification and susceptibility patterns within 8 hours in 88.2% of bacteraemic episodes with *S. aureus*. All direct results should be considered only preliminary reports and they should be contrasted with cultures and standard methods in order to detect mixed cultures or errors the identification or susceptibility.

P1425

Evaluation of BBL CHROMagar MRSA medium to detect methicillin-resistant *Staphylococcus aureus*

B.M.W. Diederer, M. van Leest, I. van Duijn, P. Willemse, P.H.J. van Keulen, J.A.J.W. Kluytmans (Tilburg, Breda, NL)

Objectives: All over the world, methicillin-resistant *Staphylococcus aureus* (MRSA) is an important and increasing problem. Optimal treatment and prevention is largely dependent on laboratory detection of this pathogen. The purpose of this study is to evaluate the *in vitro* sensitivity and specificity of a recently developed medium called BBL CHROMagar MRSA (BD Diagnostics, Le Pont de Claix, France).

Methods: A well-defined collection of *S. aureus* (251 methicillin susceptible (MSSA) and 270 MRSA) was used. The isolates were stored at -70°C until they were tested. The MRSA isolates were collected in The Netherlands and all had a unique typing pattern. BBL CHROMagar MRSA was supplied as prepoured culture plates by BD Diagnostics (Le Pont de Claix, France). The isolates were inoculated on Columbia agar-plates with 5% sheep blood and incubated for 24 hours at 35°C. From the resulting cultures a suspension of 0.5 MacFarland was made and subsequently 10 µl was streaked on a BBL CHROMagar MRSA plate using a sterile loop. The results were read after 24 and 48 hours of incubation at 35°C. Growth of colonies showing green coloration was considered to be positive (indicating MRSA). Results for the detection of MRSA were compared to the performance of a conventional mannitol salt agar plate with an added concentration of 4 µg/ml oxacillin (MSA). Discordant results were confirmed by molecular tests (e.g. *mecA*-gene PCR).

Results: In the current evaluation, 29 MRSA strains gave discordant results and on these isolates a PCR for the *mecA* gene was performed. A total of 27 (93%) of the MRSA strains had a negative result of the *mecA* PCR. These strains were removed from the analysis. The results obtained with BBL CHROMagar MRSA are shown in table 1. The sensitivity of BBL CHROMagar MRSA was 99.2% (241/243) after 24 hours and 99.6% (242/243) after 48 hours ($p = 1.0$). The specificity was 100% (251/251) after 24 hours and 99.2% (249/251) after 48 hours ($p = 0.5$). The sensitivity and specificity of MSA was statistically significant lower at both time intervals.

Table 1: Results of BBL CHROMagar MRSA medium and MSA medium after 24 and 48 hours incubation.

	No. of strains with a positive test result/total number of strains (%)	
	24 h	48 h
MSSA		
BBL CHROMagar MRSA	0/251 (0)	2/251 (0.8)
MSA	8/251 (3.2)	95/251 (37.8)
MRSA		
BBL CHROMagar MRSA	241/243 (99.2)	242/243 (99.6)
MSA	218/243 (89.7)	230/243 (94.7)

Conclusion: In conclusion, BBL CHROMagar MRSA is highly sensitive and specific to differentiate between MSSA and MRSA. Since the performance of BBL CHROMagar MRSA is already optimal after 24 hours of incubation, results can be obtained within a day.

P1426

Evaluation of MRSASelect medium to detect methicillin-resistant *Staphylococcus aureus*

B.M.W. Diederer, M. van Leest, I. van Duijn, P. Willemse, P.H.J. van Keulen, J.A.J.W. Kluytmans (Tilburg, Breda, NL)

Objectives: All over the world, methicillin-resistant *Staphylococcus aureus* (MRSA) is an important and increasing problem. Optimal treatment and prevention is largely dependent on laboratory detection of this pathogen. The purpose of this study was to evaluate the *in vitro* sensitivity and specificity of a recently developed medium called MRSASelect (BioRad, Marnes-la-Coquette, France).

Methods: A well-defined collection of *S. aureus* (249 methicillin susceptible (MSSA) and 271 MRSA) was used. The isolates were stored at -70°C until they were tested. The MRSA isolates were collected in The Netherlands and all had a unique typing pattern. MRSASelect was supplied as pre-poured culture plates by BioRad (Marnes-la-Coquette, France). The isolates were inoculated on Columbia agar-plates with 5% sheep blood and incubated for 24 hours at 35°C . From the resulting cultures a suspension of 0.5 MacFarland was made and subsequently $10\ \mu\text{l}$ was streaked on an MRSASelect plate with a sterile loop using a three-streak dilution method. The results were read after 24 and 48 hours of incubation at 35°C . Growth of colonies showing any pink coloration was considered to be positive (indicating MRSA). Results for the detection of MRSA were compared to the performance of a conventional mannitol salt agar plate with an added concentration of $4\ \mu\text{g/ml}$ oxacillin (MSA). Discordant results were confirmed by molecular tests (e.g. *mecA*-gene PCR).

Results: In the current evaluation, 30 MRSA strains gave discordant results and on these isolates a PCR for the *mecA* gene was performed. A total of 28 (93%) of the MRSA strains had a negative result of the *mecA* PCR. These strains were

removed from the analysis. The results obtained with MRSASelect are shown in table 1. The sensitivity of MRSASelect was 99.1% (241/243) after 24 hours and 100% (243/243) after 48 hours ($p = 0.5$). The specificity was 95.6% (238/249) after 24 hours and 94.8% (236/249) after 48 hours ($p = 0.8$). The sensitivity of MSA was statistically significant lower at both time intervals (91.4% and 96.3% respectively). The specificity of MSA was higher at 24 hours (96.8%), but not statistically significant. After 48 hours, the specificity of MSA was statistically significant lower (62.7%).

Table 1: Results of MRSASelect medium and MSA medium after 24 and 48 hours incubation.

	No. of strains with a positive test result/total number of strains (%)	
	24 h	48 h
MSSA		
MRSASelect	11/249 (4.4)	13/249 (5.2)
MSA	8/249 (3.2)	93/249 (37.3)
MRSA		
MRSASelect	241/243 (99.1)	243/243 (100)
MSA	222/243 (91.4)	234/243 (96.3)

Conclusion: In conclusion, MRSASelect is highly sensitive and specific to differentiate between MSSA and MRSA. Since the performance of MRSASelect did not differ significantly between 24 hours or 48 hours of incubation, optimal results can be obtained within a day.

P1427

Detection of nasal carriage of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in general population with a new chromogenic medium: MRSA ID agar

P. Ciragil, M. Gul, M. Aral, S. Alkis (Kahramanmaraş, TR)

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA), known as a nosocomial pathogen, has been isolated from community-acquired infections since 1980's. We assessed a new chromogenic medium MRSA ID agar (bioMérieux, France) for the prevalence of and risk factors for community associated MRSA and *Staphylococcus aureus* (*S. aureus*) nasal carriage in general population.

Methods: A total of 429 apparently healthy subjects who accompanied the patients from the community were evaluated for the prevalence of nasal *S. aureus* colonization and to identify risk factors (i.e., hospitalization, antibiotic therapy or underlying chronic disease) associated with *S. aureus* and MRSA colonization. Cultures of nares were performed and inoculated on Columbia with 5% sheep blood agar (CBA) and a new chromogenic medium: MRSA ID agar. Methicillin resistance of colonies isolated from CBA were evaluated by using mini API, ATB STAPH 5 (bioMérieux, France).

Results: Out of 429 samples, a total of 101 (23.5%) samples were positive for nasal *S. aureus* colonization. After 24 hours of incubation 13 (3%) of the samples were identified as MRSA with green colonies on MRSA ID agar. However, CBA was achieved to identify 12 (2.8%) MRSA isolates by combination of tests, including tube coagulase test and ATB STAPH 5 in 48 hours.

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Table. Agreement between MRSA ID agar and CBA for MRSA positivity

	MRSA ID agar		Total	
	Negative	Positive		
Blood agar	Negative	415	2	417
	Positive	1	11	12
Total	416	13	429	

Kappa: 0.88, p=0.000

Conclusion: There was an excellent agreement between the two media CBA and MRSA ID agar according to kappa test (Table). So, MRSA ID agar may be used instead of CBA for identification of MRSA strains, because identification of MRSA strains with MRSA ID agar are faster than CBA. Although no significant risk factors were observed among MSSA carriers and MRSA carriers ($p > 0.05$), further surveillance studies should be carried out in our region.

P1428

Evaluation of Bio-Rad MRSA-Select for the detection of methicillin-resistant *Staphylococcus aureus*

L. Louie, D. Soares, H. Gardner, H. Meaney, M. Vearncombe, A. Simor (Toronto, Ontario, CA)

Objectives: The prompt detection of MRSA is important for implementation of infection control measures and patient management. Newer chromogenic media for the detection of MRSA show promise for the rapid isolation of MRSA. We evaluated the use of MRSA-Select (Select) compared to mannitol salt agar with 8 µg/ml cefoxitin (MSFOX) for the detection of MRSA from clinical samples submitted for MRSA screening and surveillance.

Methods: A total of 6199 specimens from 1883 patients were tested, consisting of nares swabs ($n = 2483$), perianal/rectal swabs (2312), catheter exit sites (647), skin/soft tissue (632), sputum (58), and urine (67). Specimens were planted onto Select and MSFOX media, incubated in ambient air at 35°C, and read at 24 and 48 hours; the media were read independently by two technologists. Suspicious colonies (pink from Select; yellow from MSFOX) were worked up independently from both media for MRSA using standard methods, including a commercial latex agglutination test to detect PBP2a.

Results: A total of 181 MRSA were isolated. Select detected a total of 177/181 (98%) MRSA compared with 164/181 (91%) with MSFOX ($p = 0.006$). Of the 177 MRSA detected by Select, 169 (96%) grew after overnight incubation while only 132/164 (81%) were detected on MSFOX after overnight incubation ($p < 0.0001$). There were a total of 178 specimens planted on Select that yielded pink colonies after overnight incubation. All but one of these was confirmed as MRSA (177/178, 99.4%). The number of specimens with suspicious growth at 24 and 48 hours requiring further investigation and ultimately determined not to be MRSA was more often found with MSFOX (1302/6199, 21%) than with Select (468/6199, 8%) ($P < 0.001$).

Conclusion: Our results showed that MRSA-Select was superior to MSFOX for the detection of MRSA from clinical screening specimens, allowing greater yield, more rapid and easier detection after overnight incubation, and was less likely to support the growth of non-MRSA isolates.

P1429

Rapid detection of methicillin-sensitive and resistant *Staphylococcus aureus* direct from blood cultures

J. Montgomery, J. Bywater, B. Mayall (Melbourne, AU)

Objectives: Rapid detection of *S. aureus* (STAU) bacteraemia including those resistant to methicillin (MRSA) significantly improves outcomes in these infections, and may reduce empiric vancomycin use. Blood cultures with a Gram stain resembling *Staphylococci* had modified latex agglutination (MLA) testing if direct tube coagulase test (DTC) was positive on the day of detection (Becton Dickinson, USA). The aim was to determine the accuracy of DTC and MLA test directly from blood culture broth (BCB).

Methods: For the DTC: 0.1 ml of mixed BCB was added to 0.5 ml rabbit plasma, incubated at 36°C and read at 2, 4 and 24 hours. Incubation of bottles was continued at 36°C until DTC became positive. If positive, a modification of the PBP2a-Latex Test (Oxoid Ltd., England) was performed. A 10 ml aliquot of the BCB was centrifuged using a gel separator, the deposit washed twice in saline, and re-suspended in extraction reagent. The test was then performed as instructed by the manufacturer. *mecA* gene PCR was used as the reference.

Results: DTC were performed on 571 bottles with Gram-positive cocci resembling *Staphylococci*. Of those positive, 87% (186) were detected within four hours. There were 124 STAU and 91 MRSA. One false positive and one false negative were detected. Sensitivity (SN) and Positive Predictive Value (PPV) were 99.5%; specificity (SP) and Negative predictive values (NPV) were 99.7%. MLA was performed on 212 DTC positive bottles from 176 patients. Of these, 124 were *mecA* negative from 107 patients, and 88 *mecA* positive from 69 patients tested. Five false positive and five false positive MLA tests occurred. SN and PPV were 95% and SP and NPV were 96%. All false negatives occurred early in the study and may have been due to insufficient growth so extended incubation is important. Adequate washing of the bacterial pellet and adherence to the method is important to avoid false positive results.

Conclusion: The DTC and MLA test, performed as part of the routine processing of any blood culture with a Gram stain resembling *Staphylococci*, gives accurate phenotypic detection of MRSA at least twelve hours earlier than routine subculture. It has significant impact on the laboratory turnaround time and clinical relevance of results.

P1430

Optimisation of screening for methicillin-resistant *Staphylococcus aureus* by using the chromogenic medium MRSA ID

M.J. Bruins, M.J. Egbers, G.J.H.M. Ruijs, M.J.H.M. Wolfhagen (Zwolle, NL)

Objectives: In hospitals in the Netherlands, a strict search and destroy policy to prevent the spread of methicillin-resistant *Staphylococcus aureus* (MRSA) has so far kept the incidence of MRSA under 1%. To shorten patient isolation periods and control hospital and laboratory costs, screening methods need to be rapid, highly sensitive, specific and inexpensive. We prospectively analysed whether using the new chromogenic medium MRSA ID (bioMérieux, Marcy l'Etoile, France) instead of the currently used CHROMagar *S. aureus* (CHROMagar, Paris, France) improved our MRSA screening protocol with respect to turnaround time and workload.

Methods: Swabs submitted for MRSA screening were inoculated into a mannitol broth containing 2.5% NaCl. After 18–24 hours at 35°C broths were sub-cultured onto MRSA ID (MID) and CHROMagar *S. aureus* (CSA), which were incubated at 35°C. MID was read after 24 and 48 hours, CSA after 48 hours because in our experience 24 hours yielded insufficient growth and colony pigmentation. Green colonies on MID and mauve colonies on CSA were tested for DNase production and screened for methicillin resistance on Columbia sheep blood agar containing 6 mg/l oxacillin and with a methicillin strip on Mueller Hinton agar. Suspected MRSA isolates were confirmed by Vitek 2 followed by PCR for *mecA* and *sa442* genes.

Results: In total 449 cultures from 190 individuals were analysed. After 24 hours green colonies were found on MID in 43 cultures, of which 29 were positive for MRSA. After 48 hours, another 35 cultures on MID were suspect but MRSA negative. Of the 29 MRSA positives, 26 were found on CSA. Sensitivities and specificities of the media were 100% and 97% for MID after 24 hours, 100% and 88% for MID after 48 hours, and 90% and 55% for CSA after 48 hours respectively.

Media Tested	Nasal Swabs			Perineal Swabs		
	>18 h	48 h	Total (%)	>18 h	48 h	Total (%)
MSA-OXA	11	35	46 (75)	7	36	43 (86)
MSA-CFOX	48	12	60 (98)	35	15	50 (100)
CMRSA	53	5	58 (95)	30	5	35 (70)
MRSAS	60	-	60 (98)	48	-	48 (96)
Total	-	-	61 (100)	-	-	50 (100)

Conclusions: In this study, MID was found to be a 100% sensitive, easy to interpret medium with the highest specificity after 24 hours incubation. On the less selective CSA, three MRSA isolates were not found because of overgrowing (mauve) colonies of methicillin-sensitive *Staphylococcus aureus* and other flora. Changing to MID shortens the turnaround time of our MRSA screening protocol by 24 hours and its high specificity limits confirmative testing considerably, thus resulting in a decrease of cost.

P1431

Comparison of four commercial media for the detection of MRSA carriage from nasal and perineal specimens

L. Stoakes, R. Reyes, J. Daniel, G. Lennox, M. John, R. Lannigan, Z. Hussain (London/Ontario, CA)

Objective: The objective of this study was to compare four commercially prepared media for the rapid detection of MRSA on nasal and perineal specimens. The media included were Mannitol Salt Agar w/ 6 mg/l Oxacillin (MSA-OXA, Oxoid, Ottawa, Canada) Mannitol Salt Agar w/ Cefoxitin (2 mg/l, MSA-CFOX, Oxoid) and two chromogenic media CHROMagar MRSA (CMRSA, BD Diagnostic, MD, USA) and a new selective medium MRSASelect (MRSAS, Bio-Rad, Marnes-la-Coquette, France).

Method: Routine nasal and perineal swabs for MRSA were collected and plated on MSA-OXA, MSA-CFOX, CMRSA and MRSAS. Each medium was inoculated using 50 ml of a 400 ml prepared test suspension. Plates were incubated aerobically at 35°C. All media types were read after 18 and 48 hours incubation, with the exception of MRSAS, which were read at 18 to 24 hours. MRSA colonies were pink on the chromogenic media and yellow on mannitol salt media. Suspected colonies were confirmed by multiplex PCR for the presence of *mecA* with *nuc* and/or *femB* genes.

Results: A total of 2125 (1243 nasal and 882 perineal) consecutive swabs were tested. Of these, 111 specimens were positive for MRSA. Results are tabulated below according to specimen type and media incubation time: Overall, the sensitivity and specificity for MSA-OXA, MSA-CFOX, CMRSA and MRSAS are 80.2%/79%, 99.1%/84.8%, 82.9%/99.1% and 97.3%/99.8% respectively.

Conclusion: MSA-CFOX and MRSAS were equally sensitive in the detection of MRSA; however, MRSAS was more specific and allowed faster confirmation of MRSA, and earlier reporting of results. Pink colonies can be regarded as MRSA without additional testing or can be confirmed by simple tests such as gram smear, and/or coagulase test.

Antibiotic susceptibility testing: management, specific organisms and resistant traits

P1432

External quality assessment for antimicrobial susceptibility testing with ESBL-producing *Enterobacteriaceae*

C. Walton, D. Brown (London, Cambridge, UK)

Objectives: To assess the results from clinical diagnostic laboratories taking part in the United Kingdom National Quality Assessment Service antimicrobial susceptibility scheme with specimens containing extended β -lactamase (ESBL) producing strains of the *Enterobacteriaceae*.

Methods: EQA of antimicrobial susceptibility testing in 775 European laboratories was performed on a *Klebsiella pneumoniae* (SHV-5 and SHV-2) and four *Escherichia coli* (one CTX-M-14, one TEM-5, one TEM-10 and one TEM-26). Results were also analysed for four ESBL-negative *E. coli* and one *Citrobacter koseri*.

Results: The overall performance for all strain/antimicrobial combinations was good. On average 96% of the reports received were correct. However, detection of ESBL-mediated resistance to β -lactam agents by participants varied with the β -lactam tested and the ESBL present. Resistance to ceftazidime, cefotaxime and cefuroxime respectively was reported by 75, 97 and 99% participants with the CTX-M-14 *E. coli*; 93, 87 and 93% with the TEM-26 *E. coli*; 99, 86 and 89% with the TEM-10 *E. coli*; 96, 91 and 97% with the TEM-5 *E. coli*; and 95, 92 and 93% with the SHV-2 + SHV-5 *K. pneumoniae*. Between 32 and 83% laboratories did additional tests for ESBLs with ESBL-positive strains, the percentage increasing with time, and 88–97% correctly reported ESBL detection. 30% of laboratories reported additional tests for ESBL production on ESBL-negative organisms. There was no significant difference in reliability of detection of resistance by laboratories using BSAC, SRGA or CLSI methods.

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Conclusions: These data provide an insight into the ability of European laboratories to detect ESBL-mediated resistance to β -lactam agents in *Enterobacteriaceae*. In general for ESBL-producers resistance was more likely to be reported to ceftazidime than to cefotaxime, except for CTX-M-producers. False reporting of resistance to ceftazidime and cefotaxime was rare, but was more likely with cefuroxime. The number of laboratories performing additional tests for ESBL detection increased over time and there was little difference in reliability of standardized methods in detecting resistance or ESBL production.

P1433

Analysis of the comparative workflow and accuracy of two automated identification and susceptibility testing systems

Y. Gil, M.G. García, B. Barreda, J.L. Gómez-Garcés (Madrid, ES)

Objectives: The aim of this study was to perform a comparative study of the performance and productivity of two automated identification and susceptibility testing systems.

Methods: A total of 306 routine clinical isolates derived from urine specimens were tested: 279 Gram-negative bacilli (276 *Enterobacteriaceae* and 3 non-fermenting bacilli) and 27 Gram-positive cocci (17 *Enterococcus* spp., 5 *Streptococcus* spp., and 5 *Staphylococcus* spp.). All these isolates were processed by using both the WIDER (W), (F. Soria Melguizo Spain) and the VITEK 2 Compact (V2C), (bioMérieux France), according to manufacturer instructions.

Performance: Identification and susceptibility testing was performed on both systems.

Productivity: Chronometric measurements were made in three batches, each with 5 *E. coli* isolates.

Results: Of the 279 Gram-negative bacilli tested, 277 (99.3%) and 274 (98.2%) were correctly identified by the V2C and W systems, respectively. Excluding for this analysis the *E. coli* isolates ($n = 221$), we obtained 98.3% (57/58) and 93.1% (54/58) of correct identifications by the V2C and the W systems, respectively.

Performance: For Gram-positive cocci, we obtained a 100% of correct identification results by both methods. For AST, the overall category agreement was 99.0% and 98.4% by the V2C and the W systems, respectively. The minor error rates, major error rates, and very major error rates for all bacterial isolates tested were 0.9, 0.1, and 0.1 and 1.2, 0.2, and 0.2 for V2C and W, respectively.

Productivity: The mean time to prepare one isolate using V2C was 2.0 min and for W was 2.5 min. The mean time to results for all bacterial groups was 319 minutes for V2C and 1080 minutes for W.

Conclusion: Both systems give good identification results for all isolates. V2C showed better identification performance for *Enterobacteriaceae*, especially when *E. coli* isolates were excluded. For AST, both systems showed equivalent and optimal results in terms of category agreement. The V2C system required less manual manipulation time and less time than the W system to yield results.

P1434

A rapid DNA-based assay to detect *M. tuberculosis* resistance

S. Nikolaou, S. Anagnostou, A. Raftopoulou, F. Kontos, S. Karabela, S. Kanavaki (Athens, GR)

Objectives: The aim of our study was to evaluate the new Hain Lifescience Geno Type MTBDR test (Hain Lifescience GmvH,

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Nehren, Germany) for the detection of isoniazide (INH) and rifampicin (RIF) resistance of *M. tuberculosis* isolates.

Material and Methods: Our study included 93 MTB strains both resistant to INH, or RIF, or both and 50 strains susceptible to these drugs, which were collected from Greek patients and investigated by the Genotype assay. Our method is a nucleic acid based assay for multiplexed analysis of the *rpoB*-gene associated with resistance to RIF and the *katG*-gene position 315 associated with high-level resistance to INH. The test procedure starts from cultured MTB bacteria and the time to result is about five hours. DNA is amplified with the technique of PCR and the amplicons are hybridized in a 'reverse hybridization' strip format. All discrepant genotypes were confirmed either by DNA sequencing of the *rpoB* gene fragment, or for the *katG* 315 with sequencing and 'mass-PCR'.

Results: The 50 susceptible phenotypes were in full concordance to the Genotype result. RIF's resistance had a 91.6% concordance with culture (46/48) and INH high-level resistance had a 96.5% concordance with culture. From the 77 specimens showing resistant cultural phenotype, 20 exhibited only low-level resistance and showed no mutation in the *katG* region.

Conclusions: The Genotype MTBDR assay could be an appropriate addition to conventional drug resistance methods, to shorten time to result from weeks to one day. All strains tested by the Genotype assay showed highly concordant results compared to the phenotypic characterization.

P1435

Use of 2, 3, 5- triphenyl tetrazolium chloride for detection of drug resistance in *Mycobacterium tuberculosis*

J. Ghanaat, K. Ghazvini, T. Rashed, P. Farnia, A. Mohammadzadeh, M. Behdani (Mashhad, IR)

Objectives: Drug-resistant TB increases morbidity and the risk of mortality, as well as the costs associated with its management, thus poses a great health risk. Classical methods of detection of drug resistance in *M. tuberculosis* are expensive and time consuming, while the newer methods are not easily applicable in low-income countries.

Methods: In this study, we have evaluated the possibility of using colorimetric assay by mean of TTC (2, 3, 5- triphenyl tetrazolium chloride) to detect antibiotic susceptibility of *Mycobacterium tuberculosis* strains as a rapid and less expensive method. For this study 24 isolates of MTB were obtained from Iranian National Research Institute of Tuberculosis & Lung Disease (NRITLD). 12 isolates were resistant to RIF and INH and 12 isolates were susceptible to RIF and INH. The test was performed with a critical concentration of 0.2 $\mu\text{g}/\text{ml}$ for INH and 2.0 $\mu\text{g}/\text{ml}$ for RIF in 7H9GC broth with 1.25 mg of TTC. Each strain was incubated in these tubes and inspected daily for colour change up to 14 days.

Results: The sensitivity and specificity of this tested method were 100% and 92% for both INH and RIF, respectively. So, there was excellent agreement between colorimetric method using TTC and proportion method (kappa values, 0.93 for INH and RIF; $p < 0.001$). In this study the results were available in average of 4.9 days.

Conclusion: Considering rapidity, use of low technology and low cost, this assay that use tetrazolium-type compounds have the potential of becoming the methods of choice for drug susceptibility testing of *M. tuberculosis* isolates for most of the countries where tuberculosis is a major problem.

P1436

Direct E-test on lower respiratory tract samples: improving antimicrobial use in ventilator-associated pneumonia

E. Bouza, M.V. Torres, C. Radice, E. Cercenado, P. Muñoz, R. de Diego, C. Sanchez (Madrid, ES)

Introduction: Rapid microbiological assessment of Ventilator-Associated Pneumonia (VAP) can reduce morbidity and mortality. Conventional isolation, identification and susceptibility of bacteria can take no less than 48–72 hours for a final report. Direct E-Test on Lower Respiratory Tract Samples (eT-LRT) samples correlates well with conventional antimicrobial susceptibility tests (Cercenado E, et al. ABSTRACT 42nd ICAAC).

Objective: We prospectively assessed the impact of providing direct eT-LRT to clinicians in our institution in patients with clinical suspicion of VAP.

Methods: From December 2003 to June 2005, patients with suspicion of VAP and a positive direct Gram stain on LRT samples were randomised in two groups (2:1) for two different laboratory routines. Routine A included a direct eT-LRT antibiogram performed with 6 antibiotic strips (oxacillin, ciprofloxacin, amikacin, cefepime, imipenem and piperacillin-tazobactam). Results of this "preliminary" eT-LRT were sent to the attending physician a mean of 1.4 days afterwards with no further intervention followed by the traditional routine report. Routine B included only traditional routine identification and antibiogram when susceptibility test results were available. We measured the impact of Routine A and B in the management of antimicrobial agents and in the clinical evolution of both groups.

Results: Overall, 231 patients with confirmed VAP were included in the study (157 routine A and 74 routine B). Both populations had similar basal epidemiological and clinical characteristics. The comparison between Routine A and Routine B was as follows: days of fever (4.5 ± 5 vs. 8 ± 6 ; $P < 0.01$); DDDs of antibiotics (31 ± 24 vs. 43 ± 33 ; $P < 0.01$) days of antimicrobial therapy (15 ± 9 vs. 19 ± 10 ; $P: 0.03$); percentage of DDDs considered adequate (91% vs. 65%, $P < 0.01$); proportion of days on adequate therapy (95% vs. 74%, $P < 0.01$); *C. difficile*-associated diarrhoea (1.9% vs. 10.8%; $P < 0.01$); plain expenses on antimicrobials per episode (666 vs. 982; $P = 0.01$). Days on ventilation (18 vs. 19 ns) and ICU stay (24 vs. 27 ns).

Conclusions: A rapid susceptibility test performed directly on LRT secretions by 6 e-test strips significantly improves the accuracy of antimicrobial therapy of patients with VAP and reduces the rates of adverse effects.

P1437

Piperacillin-sulbactam: determination of zone diameter interpretive criteria using the new DIN recommendations

H. Grimm, W. Heizmann, H. Kruepe, A. Rodloff (Weingarten, Berlin, Fulda, Leipzig, DE)

Objectives: To obtain updated interpretative criteria for the agar diffusion test with piperacillin-sulbactam (PIS) using the new DIN-recommendations for susceptibility testing of β -lactamase inhibitor-protected penicillin. A 4 mg/l instead of an 8 mg/l fixed sulbactam-concentration is recommended in the newest supplement of DIN 58940.

Methods: 530 strains of various species of the *enterobacteriaceae*, preferable with reduced susceptibility or resistance against piperacillin were included in the study. The MICs of piperacillin alone as well as piperacillin with sulbactam (4 mg/l fixed

concentration) were determined by means of micro dilution technique as recommended in DIN 58940. In addition the agar diffusion test was performed with disks loaded with 30 mcg of piperacillin and 15 mcg of sulbactam according to DIN 58940. Inhibition zone breakpoints were calculated by regression analysis using the Excel® software.

Results: On the basis of MIC breakpoints according to DIN 58940, 52.5% of the strains were susceptible, 14.1% intermediate and 33.4% resistant to piperacillin. The respective figures for PIS were 74.3%, 12.3% and 13.4%. The regression analysis showed $x = -0.40y + 20.95$ and $y = -2.03x + 47.70$. Because of the moderate correlation ($r = -0.90$) between zone and MIC, the average equation from both regression equations shall be applied resulting in the following interpretation of PIS-inhibition zones: resistant up to 18 mm, intermediate 19–25 mm and susceptible more than 25 mm.

Conclusion: The calculated new PIS-inhibition zone breakpoints are 4–5 mm larger in comparison to the figures given in the DIN 58940 so far. This correction is necessary to avoid false susceptible results in susceptibility testing of piperacillin-sulbactam.

P1438

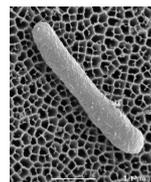
The culture and antibiotic sensitivity testing of pathogenic microorganisms on a highly porous ceramic

C. Ingham, P. Wever, P. Schneeberger (Den Bosch, NL)

Objective: To develop a fast and widely applicable, culture-based antibiotic sensitivity testing (AST) assay based around the growth and *in situ* imaging of micro-organisms on a porous ceramic support.

Methods: Sterile Anopore strips (an inert nanoporous ceramic derived from aluminium oxide, see figure) were placed upon appropriate agar plates (generally Mueller-Hinton or Sabouraud media) containing defined concentrations of test antibiotics. The strips were inoculated with the bacteria or fungi to be tested and were incubated in a CO₂ incubator at 37°C (for 40 minutes to a period of several hours). Subsequent staining of cells on the Anopore surface was with fluorescent dyes (Fun-1 for fungi, Syto 9 for bacteria; Invitrogen) through the pores from beneath. Stained microcolonies were imaged directly using an Olympus BX41 epifluorescence microscope. Image capture used an 8-bit Kappa CCD camera and TIFF files were converted to a binary image and then quantified using ImageJ software (<http://rsb.info.nih.gov/ij/>). Comparisons were made between the Vitek 2 and E-Test methods and this approach for 24 clinical isolates of the *Enterobacteriaceae*. Fungal pathogens, including *Candida albicans*, were also tested.

Results: Growth of *Enterobacter aerogenes* could be detected on anopore within 25 minutes and the earliest effects of the trimethoprim were noticed after 40 minutes (Figure: Scanning electron micrograph of a dividing *Enterobacter aerogenes* cell cultured on Anopore). AST testing against trimethoprim was performed with 2–3 hours culture and gave MIC values that were comparable with the Vitek 2 and E-Test methods. By the Anopore method, 5/5 trimethoprim-resistant and 19/19 trimethoprim-sensitive strains were assigned correctly. Additionally,



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AST testing of *Candida* was demonstrated with detection of sensitivity with 90 minutes culture and MIC values could be derived with 3 hours culture.

Conclusions: Microcolony imaging appears to be a rapid and effective approach to AST testing. Performing these tests on a porous ceramic facilitates staining and imaging *in situ* and has allowed rapid assessment of whether an antibiotic was inhibitory to growth.

P1439

Susceptibility pattern and toxigenicity of *Clostridium difficile* strains isolated from patients with diarrhoea in a tertiary hospital in Athens

M. Orfanidou, H. Kafkoulou-Alevizou, P. Karabogia-Karafilidis, E. Kontekaki, M. Theodoropoulos, A. Strouza, H. Malamou-Lada (Athens, GR)

Objective: To report the susceptibility pattern and toxigenicity of *Clostridium difficile* strains (*C. d.*), isolated from hospitalized patients suffering from *C. difficile* associated diarrhoea (CDAD), in a tertiary hospital in Athens, Greece, during 3 years period (03/02–03/05).

Methods: During the study period 1482 stool samples were examined for *C. d.* using cycloserin-cefoxitin-fructose agar with 5% egg yolk (CCFA) and cycloserin-cefoxitin blood agar (BD). The strains were identified by rapid ANA II (Remel, Lenexa) and latex test (Culturette, BD). Toxin A was detected from *C. d.* strains by an ELISA (Vidas, bioMerieux) and a chromatographic assay (ColorPac, BD). Both toxins A&B were detected by an EIA (Premier Toxins A&B, Meridian) and a chromatographic assay (Immunocard Toxins A&B, Meridian). Antibiotic susceptibility testing was performed by E-test (AB Biodisk, Solna) and β -lactamase production by a chromogenic test (Liofilchem).

Results: *C. d.* strains were isolated in 104/1482 (7%) stool specimens. Toxin A was detected in 90/104 (86.5%) strains, toxin B in 9/104 (8.7%) and A-B- in 5/104 (4.8%) strains. The resistance rate of the isolated *C. d.* strains to penicillin was 69% (MICs 1– > 32 $\mu\text{g/ml}$), clindamycin 52% (MICs 6– > 256 $\mu\text{g/ml}$), tetracycline 31% (MICs 8–64 $\mu\text{g/ml}$), while no resistance was observed to metronidazole (MICs \leq 0.38 $\mu\text{g/ml}$), vancomycin (MICs \leq 1 $\mu\text{g/ml}$) and piperacillin/tazobactam (MICs \leq 16 $\mu\text{g/ml}$). Only one strain, which produced toxin B, was found resistant to both meropenem and ertapenem (MICs 24 $\mu\text{g/ml}$ and > 32 $\mu\text{g/ml}$ respectively). The MIC's range of moxifloxacin and erythromycin for 34 *C. d.* strains was found to be 0.25– > 32 $\mu\text{g/ml}$ and 0.125– > 256 $\mu\text{g/ml}$ and their resistance rate was 23% and 62% respectively. All strains that produced toxin B were found resistant to penicillin, clindamycin and erythromycin (MICs 1.5– > 32 $\mu\text{g/ml}$, > 256 $\mu\text{g/ml}$, 12– > 256 $\mu\text{g/ml}$ respectively) and most of them 6/9 were found resistant to tetracycline (MIC 6–64 $\mu\text{g/ml}$). None of the strains produced β -lactamase.

Conclusions: (a) The most common toxin detected remains toxin A but there is an up going presence of toxin B, (b) There is a high resistance rate to penicillin, clindamycin, erythromycin, tetracycline and moxifloxacin, while there is still no resistance to metronidazole and vancomycin, and (c) The resistant mechanism to penicillin does not seem to be the production of β -lactamase.

P1440

Comparison of dalbavancin MIC values tested against Gram-positive organisms using E-test (AB BIODISK) and reference dilution methods

T. Fritsche, H. Sader, R. Jones, B. Goldstein (North Liberty, US)

Objective: To evaluate the comparative accuracy of the E-test (AB BIODISK, Solna, Sweden) method to determine dalbavancin (DAL) MIC results using the CLSI agar and broth micro dilution methods as the reference standards. A total of 100 organisms were tested, all Gram-positive species susceptible (S) to DAL.

Methods: DAL and control vancomycin (VAN) E-test strips were utilized, applying manufacturer technical and interpretive recommendations. CLSI agar dilution (AD) and broth micro dilution (BMD) methods were used as the reference method results and all control tests (15 replicates) were within published MIC ranges (M100–S15, 2005). 100 strains were processed as follows: 35 *S. aureus* (SA, 15 MRSA), 20 coagulase-negative staphylococci (CoNS) of 6 species (10 methicillin-resistant), 10 *Enterococcus* spp., 10 *S. pneumoniae* (SPN), 15 β -haemolytic streptococci (BHS, representing 5 groups), 10 viridians group streptococci and 3 QC strains. DAL was tested by BMD with 0.002% polysorbate 80 by CLSI methods.

Results: All E-test and CLSI reference QC results were within established limits as follows (E-test results only): SA ATCC 29213 (0.032–0.047 mg/l), *E. faecalis* ATCC 29212 (0.094–0.125 mg/l) and SPN ATCC 49619 (0.016–0.023 mg/l). DAL E-test versus reference BMD (table) demonstrated 66% identical results and 98% \pm one log₂ dilution, but versus AD the E-test MICs were generally 2-fold lower with 88 and 98% \pm 2-fold and \pm 4-fold from reference MIC values, respectively. Only BHS showed an E-test modal MIC one log₂ dilutions greater than the BMD MIC. The VAN E-test results compared to BMD (72% identical; 100% \pm one log₂ dilution) and to AD (72% identical; 99% \pm one log₂ dilution) MICs also showed excellent correlation. (See Table)

Organism (no.)	DAL Etest MIC variations (Log ₂ dilutions) from BMD MIC				
	-2	-1	0	+1	+2
<i>S. aureus</i> (35)	1	2	28	4	-
CoNS (20)	-	5	12	3	-
<i>Enterococcus</i> spp. (10)	-	2	8	-	-
Beta-haemolytic streptococci (15)	-	1	4	9	1
Viridians group streptococci (10)	-	-	5	5	-
<i>S. pneumoniae</i> (10)	-	1	9	-	-
Total	1	11 ^a	66 ^a	21 ^a	1

a. 98% +/- one log₂ dilution step

Conclusion: DAL E-tests provide an excellent and accurate alternative MIC method as demonstrated by these results. E-test could be applied with diagnostic confidence when used with concurrent QC determinations.

P1441

Evaluation of daptomycin 5, 10 and 15 mcg disks on IsoSensitest and Mueller Hinton agar against *Staphylococcus aureus*

L. Koeth, R. Smyth, G. Kahlmeter (Westlake, US; Vaxjo, SE)

Objectives: This study was performed in order to determine if the use of 5, 10 or 15 mcg daptomycin disks on either IsoSensitest agar or Mueller Hinton agar is a reliable disk diffusion methodology for detection of susceptible and non-susceptible *S. aureus*.

Methods: Four daptomycin/calcium disks at concentrations of 5/50, 10/50, 15/10 and 15/50 mcg were tested on 2 lots of IsoSensitest agar (ISA1 and ISA2) using semi-confluent growth according to BSAC and SRGA methods. Three daptomycin disks (2 manufacturers) at concentrations of 5, 10 and 15 mcg were tested on 2 lots of Mueller Hinton agar (MHA1 and MHA2). All disks were tested against 39 stock strains (including a challenge set consisting of 15 strains with daptomycin MIC = 2, 12 = 4 and 1 = 8 mcg/ml) and 49 clinical strains of *S. aureus*. The QC strain, *S. aureus* ATCC 25923 was tested on each day of testing.

Results: A non-susceptible breakpoint (NS BP) was chosen based on analysis of susceptible and non-susceptible zone sizes for each of the disk methodologies. All 12 strains with daptomycin MICs of 4 mcg/ml were reliably detected by all disks. The number of clinical (susceptible) strains that were categorized as non-susceptible and the number of stock strains with MICs of 2 mcg/ml (n = 15) that were categorized as susceptible are shown in the table.

Disk	NS BP	No. of Susceptible Clinical Strains = Non-susceptible				No. of Stock Strains MIC of 2 = Susceptible			
		BD Disks		Mast Disks		BD Disks		Mast Disks	
		MHA1	MHA2	MHA1	MHA2	MHA1	MHA2	MHA1	MHA2
5	<=14	0	0	13	4	1	1	2	0
10	<=16	1	0	1	4	2	1	1	1
15	<=17	0	0	2	4	2	2	1	1
		-	-	ISA1	ISA2	-	-	ISA1	ISA2
5/50	<=14	-	-	1	5	-	-	2	0
10/50	<=16	-	-	9	16	-	-	0	0
15/10	<=13	-	-	0	5	-	-	1	0
15/50	<=16	-	-	0	1	-	-	5	2

Conclusions: Daptomycin 10 or 15 mcg disks on MHA and for ISA a combination disc with daptomycin/calcium at 15/10 were best methods for discriminating between non-susceptible strains with MICs of 2 or more and susceptible strains of MICs of 1 or less. BD daptomycin disks on MHA were less likely to result in categorization of susceptible strains as non-susceptible compared to Mast disks, although Mast disks were slightly better at detection of non-susceptible strains compared to BD disks. Further assessment utilizing multiple test sites and media batches is necessary for method validation.

P1442

Evaluation of effect of low concentrations of calcium in daptomycin 30 mcg disks on IsoSensitest agar against *Staphylococcus*

L. Koeth, R. Smyth, G. Kahlmeter (Westlake, US; Vaxjo, SE)

Objectives: As a result of lack of detection of some daptomycin non-susceptible *S. aureus* using the daptomycin 30 mcg disk on Mueller Hinton agar, disk diffusion testing for daptomycin is currently not recommended by CLSI. Levels of ionised calcium in Mueller Hinton agar have been variable between manufacturers and batches, ranging from 17–64 mg/l. In contrast, calcium levels in IsoSensitest agar have been more consistent and at lower levels of approximately 10 mg/l. This study was performed as an initial screen to evaluate daptomycin 30 mcg disks containing calcium between 0 and 20 mg on IsoSensitest agar as a potential disk method.

Methods: Daptomycin 30 mcg disks containing 5, 10, 15 and 20 mg of calcium were utilized against a challenge set of *S. aureus* with elevated daptomycin MICs and against a set of recently isolated daptomycin susceptible *S. aureus*. The QC strains, *S. aureus* ATCC 25923 and *E. faecalis* 29212 were tested on each day of testing.

Results: Zone diameter ranges for each of the disks are shown in the table.

Challenge <i>S. aureus</i> by daptomycin MIC (mcg mL ⁻¹)	Calcium concentration (mcg) of daptomycin 30 mcg disk							
	20		15		10		5	
	Range (mm)	No. >=17 (mm)	Range (mm)	No. >=15 (mm)	Range (mm)	No. >=13 (mm)	Range (mm)	No. >=13 (mm)
1 (n=17)	13-16	0	11-15	6	11-15	11	9-14	7
2 (n=23)	11-17	1	10-15	2	10-15	4	8-14	3
4 (n=14)	10-14	0	9-12	0	8-12	0	7-11	0
4, 8 (n=3)	11, 16	0	9, 10	0	9, 9	0	8, 8	0
Recent clinical strains	Range (mm)		Range (mm)		Range (mm)		Range (mm)	
<i>S. aureus</i> (n=77)	17-20		15-18		13-18		13-18	
All staphylococci (n=177)	17-25		15-24		13-24		13-24	

Conclusions: The daptomycin 30 mcg disk with 5, 10, 15 or 20 mcg of calcium when used with IsoSensitest agar could potentially detect all strains with MICs of ≥ 4 mcg/ml. The 20 mcg calcium disk was the best disk for detection of non-susceptible strains with MICs = 2 mcg/ml. However, the 10 mcg calcium disk was superior at categorization of susceptible strains with MICs of 1. The introduction of a buffer zone could resolve some discrepancies observed at MICs of 1 and/or 2 mcg/ml. Additional testing of lower daptomycin concentration disks with similar levels of calcium may provide further separation of zones between susceptible and non-susceptible strains.

P1443

Evaluation of Cefoxitin Disk for detection of methicillin-resistant *Staphylococcus aureus*

M. Rahbar, S. Moulanaee, P. Islami, R. Atifeh, K. Pirayesh (Tehran, IR)

Objectives: Methicillin resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial and community acquired infections. Detection of MRSA in laboratories is very important for treatment and appropriate infection control. The aim of this study was to evaluate of cefoxitin disk diffusion method for detection of MRSA.

Methods: A total of 175 clinical isolates of *S. aureus* isolated from clinical specimens were studied. The isolates were identified by conventional laboratory methods. E-test MIC, cefoxitin, oxacillin disk diffusion methods and MAST ID Methicillin Strips were used for detection of MRSA. All disk diffusion methods performed as recommend by NCCL and manufactures guidelines.

Results: By using E-test MIC 53 (33.7%) of strains of *S. aureus* were resistant to methicillin. Disk diffusion method by using oxacillin disk showed 52 strains resistant to methicillin. In this method 8 strains had intermediate resistance to methicillin. In Cefoxitin disk diffusion method 52 strains were resistant to methicillin. This method had a good correlation with E-test MIC method. MAST ID Methicillin Strips showed 47 resistant to methicillin. Sensitivity and specificity for both cefoxitin and oxacillin disk diffusion methods were 98%and 100% respectively, however cefoxitin was better than oxacillin for detection of intermediate resistant strains of *S. aureus*. Sensitivity and specificity for MAST ID Methicillin Strips was 91%and 100% respectively.

Conclusion: Our study reveals that using cefoxitin is a good alternative method for oxacillin disk diffusion method for detection of MRSA. This method is reliable for identification of intermediate resistant strains of *S. aureus*.

P1444

Estimating methicillin resistance in *Staphylococcus* hospital strains: a comparison between Kirby-Bauer disc diffusion antibiogram and PBP2a detection with latex

A. Ksanthos, V. Kafarakis, E. Christoforaki, A. Vittoraki, D. Drygiannakis (*Rethymnon, Crete, GR*)

Objectives: (i) To evaluate methicillin resistance of several *Staphylococcus* hospital strains performing both PBP2a detection test with latex and Kirby-Bauer (K-B) disk diffusion antibiogram. (ii) To compare the results of the two methods.

Materials & methods: We examined 136 *Staph.* strains (46 *Staph. aureus*, 90 coagulase negative *Staphylococcus*-CNS) isolated in cultures of several materials (blood, catheters etc) from patients of our hospital. All the above strains were checked over the possibility of: (i) methicillin resistance, using the K-B disk diffusion antibiogram (according to NCCLS guidelines) (ii) PBP2a production, using the kit slidex MRSA Detection (Biomerieux), containing monoclonal antibody against PBP2a protein.

Results: According to the K-B method, methicillin resistance was detected in 8 of 46 *Staph. aureus* strains—for all of them, latex test was (+). From the rest of the strains, 36 were susceptible and 2 moderately susceptible to methicillin, while all of them were (-) to the latex. We also examined 90 CNS strains, out of which 59 proved to be methicillin resistant, according to the K-B, while to latex 45 were tested (+). The remaining 31 strains were sensitive to methicillin – 29 of them were (-) when tested with latex, while 2 strains were tested (+).

Discussion: (1). Regarding the *Staph. aureus* strains, the results of the two performed methods were in complete agreement. Thus, by using the latex test, a reliable preliminary estimation about the resistance of a *Staph. aureus* strain can be obtained within the first 24 hours, an information of major clinical interest, mainly in severe infections (e.g. bacteraemia). (2). As far as the CNS strains are concerned, we observed certain differences between the two methods, since there was a 24% (14/59) percentage among the methicillin resistant (according to K-B) strains, which gave a (-) latex test. In these cases, determination of Oxacillin MIC (through E-test) is highly recommended. Moreover, 6.5% (2/31) of the CNS strains with phenotypic sensitivity to methicillin, were tested (+) to latex. These bacteria may be carriers of *mecA* gene and, therefore, if K-B is the only method performed, a false negative result is considerably possible. (3). As a result, a preliminary examination of all *Staph* strains using the PBP2a detection latex test (before an antibiogram performance) can be proved invaluable since, in case of a positive result, it can lead to an immediate briefing of the attending physicians.

P1445

Detection of methicillin resistance in *Staphylococcus aureus* clinical isolates by phenotypic methods and PCR amplification of *mecA* gene

M.A. El-Sweify, M.F. Ahmed Fahmy Ali, A.A. Heiba, G.I. Gebreel (*Ismailiya, EG*)

The unique resistance of MRSA to all β -lactam antibiotics is primarily due to the acquisition of *mecA* gene, coding for PBP2a. The diagnostic methods that rely on phenotypic features of MRSA differ in reliability and sensitivity. Preferring a particular MRSA diagnostic method that suits a given laboratory requires

continuous assessment and evaluation of already available techniques. In the Suez Canal faculty of medicine microbiology dept., this work aimed to explore the most appropriate and applicable diagnostic assay by examining the available and popular methods. Sixty *S. aureus* clinical isolates were first tested for the presence of *mecA* gene by PCR and subsequent visualization on agarose gel, since this is the "gold standard" method for MRSA detection. All *mecA*-positive (36) and *mecA*-negative (24) isolates were further tested by agar disk diffusion using oxacillin 1 μ g and cefoxitin 30 μ g disks; agar dilution method to determine oxacillin MIC; MRSA-Screen latex agglutination test that detects PBP2a (Denka Seiken Co., Japan). Results showed that MRSA-latex was 100% concordant to the PCR assay. It was rapid, simple, and reliable. Agar disk diffusion showed sensitivity of 100% for cefoxitin and 83.3% for oxacillin, while specificity was 95.8% and 87.5%, respectively. The ranges for inhibition zone diameter for MRSA and MSSA were more distinct when cefoxitin was used, than with oxacillin disk. MIC assay by agar dilutions method showed 91.7% sensitivity and 95.8% specificity. Isolates showing MIC between 2 and 4 μ g/ml required retesting using closer serial dilutions of oxacillin. In conclusion, MRSA screen latex test was most appropriate as regards to cost, simplicity, timesaving, and reliability than all other phenotypic methods, with accuracy that almost matched PCR-assay accuracy. The latex test is therefore qualified to rely on as a single routine test for MRSA identification

P1446

Evaluation of diagnostic methods for detecting methicillin-resistant *Staphylococcus aureus* strains in clinical microbiology laboratories in Finland

A. Virolainen, A. Nissinen, A-M. Kerttula, J. Vuopio-Varkila and the Finnish Study Group for Antimicrobial Resistance

Objectives: The aim of this study was to evaluate diagnostic methods including cefoxitin disk vs. oxacillin disk tests to detect methicillin-resistant *S. aureus* (MRSA) in all clinical microbiology laboratories in Finland.

Methods: All 26 Finnish clinical microbiology laboratories were sent 20 isolates of *Staphylococcus aureus* strains previously characterized at the Hospital Bacteria Laboratory in National Public Health Institute. The study strains included high- and low-level methicillin-resistant MRSA strains, borderline oxacillin-resistant *S. aureus* strains (BORSA), and methicillin-sensitive *S. aureus* strains. The laboratories were asked to test oxacillin susceptibility of the strains according to their own routine methods and also by using cefoxitin 30 μ g disks, which were provided to them along with the test strains.

Results: All of the 26 laboratories used oxacillin 1 μ g disk diffusion test with confluent inoculum as the principal screening method for detecting MRSA. Four major variations of the testing conditions were used: Six laboratories preferred Mueller-Hinton agar (MH) without salt, incubation at 35–36°C for 18 to 24 hours as the CLSI standard suggests, six used MH with added NaCl (2–4%) at the same temperature, six used MH with NaCl at 30°C, and five used Iso-Sensitest agar (IS) at 35–36°C. The remaining three laboratories had a different approach each. For the cefoxitin disk diffusion-screening test for prediction of *mecA*-mediated resistance, 12 of the 26 laboratories used MH without salt, incubation at 35–36°C for 18 to 24 hours, and 13 used IS at 35–36°C. One laboratory preferred MH and incubation at 30°C. The *mecA*-mediated resistance was best detected by decreased susceptibility to oxacillin when using MH with extra NaCl,

incubation at 30°C for either 18 or 24 hours sensitivity 100%, specificity 78%. Compared to oxacillin testing, screening by cefoxitin was less sensitive but more specific, giving an accurate result with one BORSA strain but missing one low-level MRSA isolate.

Conclusion: Amongst the four major oxacillin testing conditions and cefoxitin screening, MH with extra salt and incubation at 30°C over night was the most sensitive alternative for detection of MRSA.

P1447

Efficacy of practised screening methods for selection of cephalosporin-resistant *Enterobacteriaceae*

R. Hope, N.A.C. Potz, M. Warner, D.M. Livermore (London, UK)

Objective: *Enterobacteriaceae* with extended-spectrum β -lactamases (ESBL) are now widespread in the UK, and simple phenotypic tests are required to identify them in diagnostic laboratories. The simplest is to screen with cefpodoxime and to do clavulanate synergy tests on isolates found resistant, but many laboratories screen with further cephalosporins in the initial screen. We investigated the screening methods used by 16 hospitals in Southeast England.

Method: Sixteen laboratories in the South-East of England submitted a total of 1195 consecutive *Enterobacteriaceae* isolates that they found to be resistant by their routine methods to any or all of cefpodoxime, ceftazidime and cefotaxime. These isolates were then tested centrally by the BSAC's agar dilution method using various cephalosporin/clavulanate combinations with multiplex PCR for blaCTX-M and blaAmpC alleles.

Results: Screening methods used were; cefpodoxime discs alone (1 site); cefpodoxime, cefotaxime and ceftazidime by discs (9 sites) or agar dilution (1 site); Phoenix (2 sites) Vitek 1 (1 site) and Vitek 2 (2 sites). Eight percent of isolates submitted based on disc tests proved fully cephalosporin sensitive, compared with 3% sent based on tests with automated systems (Phoenix, Vitek 1 and 2) and none of those sent based on agar dilution test. Among isolates submitted only on the basis of resistances to cefpodoxime 256/372 (69%) proved either cephalosporin susceptible or to have only borderline resistance with no clear mechanism demonstrable; this proportion fell to 18/122 (15%) for those submitted on the basis of resistance to cefotaxime, 28/160 (18%) for ceftazidime and 26/496 (5%) for those with both cefotaxime and ceftazidime resistance. The inference of ESBL production by Vitek 2 had the best agreement with reference results.

Conclusion: Many isolates found resistant only to cefpodoxime at the source sites proved not to have ESBLs or *AmpC*; screening with cefotaxime and ceftazidime allowed better specificity for identification of general mechanism based resistance, as did the automated systems; nevertheless cefpodoxime remains a useful single primary screen before confirmatory testing.

P1448

Accurate and rapid antibiotic susceptibility testing of *Haemophilus influenzae* by flow cytometry

M. O'Donoghue, W.L. Pong, M.V. Boost (Hong Kong, HK)

Objectives: *Haemophilus influenzae* continues to cause significant levels of morbidity and mortality and is responsible for serious infections such as pneumonia and meningitis. Susceptibility

testing was not required for this organism in the past as it was generally considered sensitive to certain antimicrobials. With the increasing development of resistance in recent years, previously employed empirical therapy is no longer adequate. The fastidious nature of this organism limits the options for susceptibility test methods and requires a minimum of 24 hours turn around time. This study aimed to develop a rapid antimicrobial susceptibility test method (AST) using a flow cytometer (FCM) and to compare the results with standard methods.

Methods: Effects of a range of concentrations of ampicillin and tetracycline on a *H. influenzae* control strain (ATCC 49247) were determined by FCM (Coulter EPICS Elite ESP flow cytometer) using the fluorescent probes DiBAC4 (3) and Propidium iodide (PI). A further 30 clinical isolates of *H. influenzae* obtained from several district hospitals in Hong Kong were then tested by FCM method to determine their susceptibilities to ampicillin and tetracycline and the results were compared to the Standard Broth Dilution method (SBD). Results obtained were categorized as sensitive, intermediate or resistant using a previously published formula.

Results: Treatment with antibiotics resulted in a reproducible and easily detectable change in fluorescence. The degree of change correlated well with sensitivity or resistance as determined by standard methods. There was no difference of categorization of 29 strains tested. The remaining strain being categorized as intermediately resistant by FCM and sensitive by SBD i.e. a minor error.

Discussion: Reliable results were obtained within 3 hours allowing the test to be completed within one working day, which may have a considerable clinical impact. The reduction of time for issuing of AST results would enable physicians to choose more appropriate, narrower spectrum and less costly antimicrobial therapy at the initiation of therapy. As reliable results were obtained for both static (tetracycline) and cidal (ampicillin) antibiotics suggesting the method can be extended to other antibiotics. As most hospitals already have a FCM for haematology purposes, it would not be necessary to purchase an additional machine.

P1449

Detection of *Helicobacter pylori* and clarithromycin resistance by real-time PCR in children

Y. Akyön, S. Tuncer, H. Demir, M. Misirlioglu, Y. Usta (Ankara, TR)

Objective: Our aim was to detect both Clarithromycin resistance and *Helicobacter pylori* from the biopsy sample, and to show the Clarithromycin resistance rate in children.

Methods: Four hundred and forty biopsy samples obtained from children who had dyspeptic complaints were included in the study. The age range was 2–19. Two hundred and forty seven were female, 193 were male. Chromosomal DNA was extracted by Cetyltrimethylammonium bromide (CTAB) method using Wilson's miniprep protocol. Real-time PCR assay on LightCycler (Roche Diagnostics, Germany) instrument was used for the detection of clarithromycin resistance associated point mutations in the 23S rRNA gene by using primers, probes and reaction conditions described by Chisholm SA et al (1). After amplification of 96-bp region clarithromycin resistance related mutations of A2143C, A2143G/A2144G were analysed by melting curve analysis on LightCycler Software version 3.5.3. Melting peaks of 82–86°C showed the detection of *H. pylori* in the sample. Probe melting peaks of positive samples has been

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analysed by the same method and sensitive strains, A2143C, and A2143G/A2144G gave peaks around 65°C, 60°C and 55°C, respectively.

Results: Of the 440-biopsy samples 46 (10.5%) of them were negative for *H. pylori*. From the 394 *H. pylori* positive patients 122 (31%) of them had clarithromycin resistance.

Conclusion: The rate of clarithromycin resistance was found to be significantly high, in this study. *H. pylori* prevalence is 70–80% in Turkey, but cure rate is about 60%. Our finding explains the reason of the low cure rate. Clarithromycin is one of the first choice drugs for *H. pylori* treatment. Therefore in countries, which have similar *H. pylori* prevalence, clarithromycin resistance rate should be known and if possible *H. pylori* antibiotic susceptibility testing, should be performed before treatment.

P1450

Comparison of E-test, disc-diffusion and Phoenix for antibiotic susceptibility assessment of *Stenotrophomonas maltophilia* strains

M. Mentasti, P. Morelli, G. Manno (Genoa, IT)

Stenotrophomonas maltophilia (*Sm*) is an emerging pathogen in immunocompromised patients (pts) and frequently isolated from airway samples of cystic fibrosis (cf) pts. Often multiresistant, *Sm* is intrinsically resistant to carbapenems and virtually resistant to all β -lactams, but at present only CLSI breakpoints for levofloxacin (L), minocycline (M) and trimethoprim/sulfamethoxazole (TS) are available. Agar dilution is the method recommended by CLSI to determine *Sm* antibiotic susceptibility pattern and E-Test (AB BioDisk) is described as a reliable alternative method.

Objectives: This work is aimed to assess the reliability of disc-diffusion with semi-automatic analysis by BioMIC Vision (Giles Scientific Inc.) and automated Phoenix System (BD) in *Sm* antibiotic susceptibility pattern determination, compared to E-Test results.

Methods: 70 non-repetitive *Sm* strains (38 from cf pts, 23 from nosocomial pts and 9 from nosocomial environment) identified by API ZONE and confirmed by species-specific amplification of 23S rRNA gene (Whitby PW, 2000), were tested by E-test and disc-diffusion on Mueller Hinton Agar (24 hours at 35°C) for L, M, TS, ceftazidime (C), gentamicin (G) and ticarcillin/clavulanate (TC), and tested by Phoenix with NMIC/ID-4 card containing C, G, L and TS. *Sm* ATCC 13637 and *Escherichia coli* ATCC 25922 were used as quality control strains. Discrepancies were classified as Very Major Error (VME), Major Error (ME) and minor Error (mE) as described in NCCLS M23-A2 (2000).

Results: MIN resulted active on all strains tested by E-test, followed by TS, L, TC, G and C. No discrepancy was detected between results of disc-diffusion with BioMIC Vision reading and E-Test for M and TS; for C, G and L 21 total mEs were detected, while for TC there were 6 VMEs. Phoenix generated 1 VME for L and 43 total MEs for C, G, L and TS.

Conclusions: M was more active than TS considered *Sm* drug of choice, which showed 2 resistant strains (1 from cf pts and 1 from non-cf pts). Disc-diffusion resulted a reliable method to test C, G, L, M and TS on *Sm* strains and BioMIC Vision an accurate system to assess disc-diffusion results for these antibiotics. Although Phoenix generated only 1 VME, it reported many more resistances (MEs) than E-test, consequently reducing therapeutical choices; moreover, it didn't test M and TC, which are very effective against these isolates. In our experience, Phoenix System seems not suitable to determine antimicrobial susceptibility of *Sm* isolates.

P1451

Prospective evaluation of imipenem/EDTA combined disk and E-test for detection of MBL producing *Pseudomonas aeruginosa*

L. Bergès, A. Deplano, H. Rodriguez-Villalobos, A. Mazzariol, M.J. Struelens (Anderlecht, BE; Verona, IT)

Objective: Nosocomial infection with multi-resistant *P. aeruginosa* is a growing problem worldwide. The recent emergence of metallo- β -lactamases (MBL) carried by integrons in this organism raises the issue of detecting this mechanism in multi-resistant strains. The aim of this study was to analyse the accuracy of imipenem-EDTA combined disk (MBL-CD) as part of the routine disk diffusion susceptibility test and imipenem-EDTA double E-test (MBL-E-test) for routine detection of MBL-producing *P. aeruginosa* clinical isolates.

Methods: From May 2004 to February 2005, all clinical *P. aeruginosa* isolates were prospectively screened for MBL production using a MBL-CD (ROSCO) test. The test is positive if the diameter of MBL-CD inhibition zone is > 6 mm wider than the IMI disk inhibition zone. All meropenem (MEM) and imipenem (IMI) resistant strains were tested by MBL-E-test (AB-Biodisk). This test is positive for a ratio MIC IMI/ MIC IMI-EDTA \geq 8. PCR for *bla* VIM and *bla* IMP gene detection and measure of hydrolysis of IMI with UV spectrophotometry were used as reference tests.

Results: Among 587 *P. aeruginosa* isolates during the study period, 42 (7%) were resistant to both IMI and MEM. MBL-CD was positive in 26 (62%) of these strains, 23 (55%) were MBL-E-test positive, 19 (45%) had VIM or IMP gene by PCR (16 VIM genes, 3 IMP genes) and 1 strain lacking VIM and IMP gene expressed carbapenemase activity confirmed by spectrophotometry. All carbapenem-resistant isolates with negative MBL-CD screening were confirmed as E-test and PCR negative. The sensitivity, specificity, positive and negative predicted values for MBL-CD was 100%, 73%, 77% and 100% respectively. MBL-E-test showed a better specificity (86%) and predictive positive value (87%) than MBL-CD.

Conclusion: In our setting MBL-CD test appeared to be an easy and sensitive tool for rapid screening of MBL producing *P. aeruginosa* isolates. MBL-E-test could be considered for confirmation of MBL production although PCR appeared necessary to provide confirmation and characterize the enzyme type.

P1452

Phenotypic detection of metallo- β -lactamases and extended-spectrum β -lactamases among Gram-negative bacterial clinical isolates

N. Fam, M. Diab, H. Gomaa, I. El-Defrawy (Giza, EG)

Objectives: Detection of metallo- β -lactamases (MBLs) and extended spectrum- β -lactamases (ESBLs) Gram negative *bacilli* (GNB) is crucial for the optimal treatment of patients and to

Antibiotic	E-Test (% of susceptibility)	Disc-diffusion vs E-test			Phoenix vs E-test		
		mE	ME	VME	mE	ME	VME
ceftazidime	28,5	11	-	-	17	7	-
ticarcillin/clavulanate	75,7	-	-	6			
gentamicin	50,0	7	-	-	15	21	-
levofloxacin	88,5	3	-	-	13	3	1
minocycline	100	-	-	-			
trimethoprim/sulfamethoxazole	97,1	-	-	-	-	12	-

control spread of resistance. However, NCCLS documents do not contain a method for detection of MBL producing isolates. Lack of sufficient reports from Egypt indicated the need for this study to determine the proportion of MBL producers among GNB isolated from clinical multi-drug resistant pathogens. We also attempted to assess the efficiency of several phenotypic tests for the rapid and convenient detection of MBLs between *Pseudomonas* and *Acinetobacter* spp. The efficiency of testing ceftazidime (CAZ) resistant versus imipenem (IMP) resistant pathogens was also compared.

Methods: A total of 70 CAZ intermediate/resistant GNB were identified and tested for antibiotic sensitivity by Vitek 2 Automated System (BioMérieux). Screening for ESBLs was performed by Oxoid combined test (CDO2) and confirmed by Vitek 2. The phenotypic detection of MBL production among *Pseudomonas* and *Acinetobacter* isolates was performed by modified Hodge test, EDTA-disk synergy test (DST), IMP-EDTA combined disk test (CDT) and E-test MBL strips. Negative control strain (*P. aeruginosa* ATCC 27853) was included in the tests.

Results: Of the 70 GNB pathogens, 25(35.7%) were ESBL producers mainly *E. coli* and *Klebsiella* spp., while 8 (11.4%) *P. aeruginosa* isolates were IMP resistant. Their MIC was 16 µg/ml as confirmed by E-test. None of the *Acinetobacters* showed resistance to IMP. Isolates were considered as MBL producers when three of the phenotypic tests were positive. Both DST and CDT (7/8) were superior to Hodge and E-tests (4/8) for detection of MBL production. One IMP resistant isolate was negative by all tests suggesting non-MBL production. None of the IMP resistant isolates was an ESBL producer.

Conclusion: The majority of our IMP resistant *P. aeruginosa* isolates seemed to be MBL producers. Genetic confirmation and analysis of MBL producers is mandatory for positive isolates screened by phenotypic tests. Between the latter DST and CDT proved to be more rapid and convenient tests for their detection in the clinical laboratory. Testing IMP resistant rather than CAZ resistant isolates could reduce screening work for MBL detection.

P1453

Reliability of carbapenem resistance in *Pseudomonas* spp. detected by automated systems

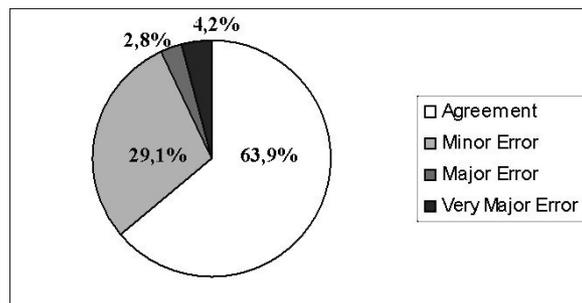
O. Karatuna, G. Soylerir (Istanbul, TR)

Objective: Ever since automated antimicrobial test systems took their places in microbiology laboratories, effort has been made to improve their accuracy. Even though antimicrobial susceptibility testing became much easier with automated systems, they are still far away from standard methods when it comes to accuracy of the test results. Micro organisms like *Pseudomonas* show multidrug resistance and accurate results for their last resort antibiotics such as carbapenems became of vital importance. In the literature false-resistant results have already been documented for carbapenems in *Pseudomonas*, which falsely limits therapy options.

Materials and Methods: In this study all *Pseudomonas* spp. (n: 133) isolated in our hospital between May and November 2005 have been evaluated for their carbapenem resistance. Identification and antimicrobial susceptibility testing were performed with Vitek2 system using Vitek2 GN and Vitek2 AST-GN09 cards (bioMérieux). No further tests were performed for isolates (n: 77), which were susceptible for carbapenems, imipenem and meropenem since our aim was to detect false resistance. For the remaining isolates (n: 36), which were, imipenem and/or meropenem resistant, both imipenem and meropenem MIC

values have been established using E-test strips (AB BIODISK) even when one of the carbapenems was susceptible according to Vitek2.

Results: Agreement and minor error rates between two methods for carbapenem susceptibility testing (n: 72) were 63.9% and 29.1%, respectively. We also observed discrepant results causing very major error rate of 4.2% in strains, which were intermediate or resistant to one of the drugs but susceptible to the other (Figure).



Conclusion: Our results exhibiting error rates out of acceptable ranges proved once again that laboratories, which use an automated system should consider using at least a second method to validate intermediate or resistant results for carbapenem group antibiotics when tested against *Pseudomonas* species. More importantly, finding an unacceptable rate of very major error even in a limited number of the tested strains strongly indicates that any result, either susceptible or resistant, obtained from an automated system should not be relied on.

P1454

Detection of extended spectrum β-lactamases in *Enterobacteriaceae* strains using four different methods in a Tunisian paediatric hospital

N.E. Jlili, H. Smaoui, S. Rejiba, A. Kechrid (Tunis, TN)

Objectives: The reliability of four phenotypic methods to detect extended-spectrum β-lactamases (ESBLs) was investigated in the present study.

Methods: A total of 55 non-duplicate clinical isolates of third generation cephalosporin-resistant *Enterobacteriaceae* species suspected of ESBL production were collected from January to September 2003. Medium and disc charge are used according to the CA-SFM guidelines. The isolates were screened for ESBL production by: (i) double-disk synergy test (DDST), (ii) modifying double-disk test (MDDT), performed with discs used in the DDST in addition to cefepime, aztreonam and *piperacillin-tazobactam* association, (iii) E-test ESBL (AB BIODISK) detection system, based on the reduction in the ceftazidime or cefotaxime MIC in the presence of clavulanic acid, and (iv) Cica-β test I/MBL (Kanto Chemical Co.Inc.) using the chromogenic cephalosporin HMRZ-86, which reacts ESBL directly and changing the colour rapidly.

Results: Among 55 strains (39 *Klebsiella pneumoniae*, 4 *Escherichia coli*, 6 *Enterobacter cloacae* and 5 *Enterobacter aerogenes*) studied, the classic DDST based clavulanate synergy classified only 36 (56.4%) ESBL positive, while, the MDDT detected the presence of ESBL in 47 (85.4%). Negative synergy affected all the species with DDST, whereas negative synergy spared the totality of *K. pneumoniae* with MDDT. Results of the E-test ESBL confirmed the ESBL production in 44 (80%) among the 47 ESBL producers screened by MDDT. The results of the E-test were indeterminate

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for the other eleven clinical isolates. The chromogenic test gave the highest ESBL detection rates with 52 (94.5%) strains. According to the study, the sensitivity and specificity of the HMRZ-86 for detecting ESBLs seemed to be the better. The combination of all ESBL detection methods showed agreement for 35 ESBL positive (32 *K. pneumoniae*, 3 *E. aerogenes*) and 3 ESBL negative clinical isolates, that belong to third bacterial group. At least, one method yielded a discordant result for each of the remaining 17 isolates.

Conclusion: Excepted the DDST, all the other methods conferred good rates to detect ESBL production. Chromogenic test seems to be potential and suitable tool to ESBL screening and it must be valuable in clinical laboratory.

P1455

The reliability of *in vitro* susceptibility tests for multi-resistant *Pseudomonas aeruginosa* isolates

B. Sener, S. Ak, G. Hascelik (Ankara, TR)

Objectives: Therapeutic approaches dealing with antibiotic-resistant *Pseudomonas aeruginosa* (Pa) infections depend on the results of *in-vitro* susceptibility testing. However, *in vitro* test results always are not successfully applied to *in vivo* conditions. This study was conducted to test the agreement between disk diffusion (DD), agar dilution (AD) and bactericidal testing which is considered to resemble more the *in vivo* conditions, for cystic fibrosis (CF) and non-CF Pa isolates.

Methods: Thirty-nine CF and 40 non-CF Pa isolates were tested for susceptibility against aztreonam (AZ), ceftazidime (CAZ), cefepime (CEF), ciprofloxacin (C), amikacin (A), tobramycin 4 (T4), tobramycin 200 (T200) mg/ml and meropenem (M) by DD and AD, and the results were evaluated according to CLSI breakpoints. The bactericidal activities were determined in microtiter plates by using single antibiotic concentrations based on estimates of the average peak levels in serum after single dose IV administration.

Results: DD and AD methods exhibited overall 83.5% inter-method agreement, discrepant results being more detected in CF (11/39) than non-CF (2/30) isolates ($p < 0.05$). % susceptibility according to bactericidal activity was less than the susceptibility rates obtained by AD and DD especially for β -lactam agents tested for CF Pa isolates.

	CF (n: 39)			Non-CF (n:30)		
	%S ^a (AD)	%S (DD)	% bactericidal	%S (AD)	%S (DD)	% bactericidal
AZ	48.7	46.2	25.6*	43.3	43.3	26.7
CAZ	48.7	46.2	25.6*	13.3	16.7	10.0
CEF	53.8	71.8	38.5*	30.0	30.0	26.7
C	89.7	92.3	76.9	20.0	20.0	16.7
A	87.2	89.7	74.4	73.3	73.3	73.3
T4	79.5	79.5	79.5	6.7	6.7	10.0
T200	-	-	92.3	-	-	-
M	79.5	79.5	74.4	33.3	36.7	30.0

^a: susceptibility
*: $p < 0.05$

Conclusion: Although AD and DD have acceptable agreement with each other, a significant discordance was observed when the results were evaluated according to bactericidal activity for CF Pa isolates. This discrepancy can be attributed to the presence of mucoid and slow growing variants for CF strains. Since bactericidal testing seems to mimic the *in vivo* conditions more than the other two tests, for the choice of effective antibiotic therapy especially for CF Pa infections, single bactericidal sensitivity testing can be taken into consideration. To assess whether the use of bactericidal testing improves the results obtained by accordingly directed antibiotic therapy for Pa infections, controlled clinical trials should be performed.

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P1456

Performance of cefoxitin in phenotypic susceptibility testing of cMRSA, ST80, and hMRSA, ST45

C. Cuny, W. Witte (Wernigerode, DE)

Objective: cMRSA of MLST ST80 which are widely disseminated in Europe and hMRSA of MLST ST45 exhibit a pronounced heteroresistance phenotype for oxacillin (MIC's 1–2 mg/l). Here we report about the performance of cefoxitin in microbroth dilution and in disk diffusion test.

Methods: Microbroth MIC according to CLSI standard 2005 for oxacillin and cefoxitin; disk diffusion test for oxacillin and for cefoxitin according to CLSI and to SRGA; screening tests by use of oxacillin-salt-agar (CLSI), tube test with oxacillin-sulbactam-salt (Cuny et al., Eur. J. Clin. Microbiol. Infect. Dis. 20 (2001) 683–6), and chromagar MRSA.

Results: For 63 isolates of MRSA (*mecA* positive) from independent cases of infections and belonging to lineages ST80 (n = 37) and ST45 (n = 30) low micro broth MIC's 1–2 mg/l for oxacillin but growth on oxacillin-salt-agar (incubation up to 48 hours essential), in oxacillin tube test and on chromagar MRSA was recorded. MIC's (mg/l) of cefoxitin were 2 (n = 2), 4 (n = 23), 8 (n = 30), and 16 (n = 8). In disk diffusion test with cefoxitin according to CLSI 35 from 63 isolates were classified as susceptible whereas performance according to SRGA correctly identified them as resistant.

Conclusion: Reliable detection of MRSA of ST80 and ST45 needs screening tests in parallel to micro broth MIC and disk diffusion test for oxacillin. For cefoxitin the MIC breakpoint should be 4 mg/l and above as resistant. For disk diffusion the interpretative criteria of SRGA should be used.

P1457

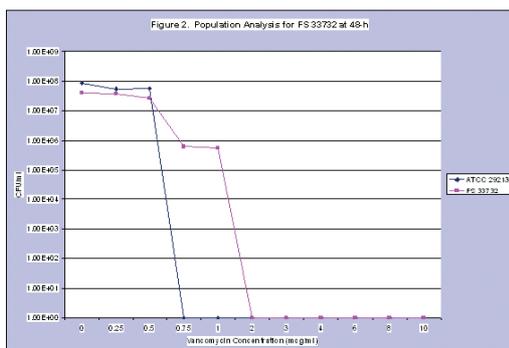
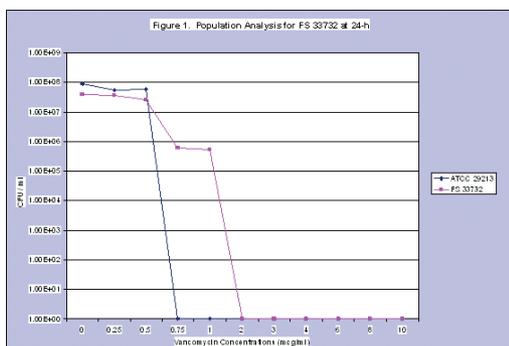
Phenotypic characterization of heterogeneous methicillin-susceptible *Staphylococcus aureus* correlates to patient outcome

V. Huang, M. Perri, D. Vager, M. Zervos (Atlanta, Detroit, US)

Objective: Thus far there has never been a case report of heterogeneous methicillin-susceptible *S. aureus* (hMSSA). We described a case of a 45-year-old African American female who presents with MSSA bacteraemia; cultures were taken from the peripherally inserted central catheter (PICC) line of the left arm. She was admitted for an infected PICC line. Patient was bacteraemic on vancomycin for 34 days, secondary to endocarditis with no abscess to drain. The patient was allergic to penicillin. She was given 1 dose of linezolid upon admission. Patient was sent for a new PICC line to complete her vancomycin therapy. In addition, blood cultures times 2 was ordered as well as a computed tomography (CT) scans of the abdomen and pelvis to find any sources of hidden infection. The CT scan is negative, however, patient cultures were positive for MSSA with vancomycin MIC of 2.0 by Vitek. Due to patient allergy and positive blood culture after 34 days of therapy, we sought to investigate this particular isolate.

Methods: Strains utilized were hMSSA (FS 33732) and ATCC 29213 as a control. MICs were performed according to CLSI. Subpopulation profiles were performed on MHA plates containing various concentrations (0.25–32 μ g/ml) of vancomycin (V) according to Pfetz *et al* procedure. Macro E-test was performed on BHI plate with a 2.0 McFarland as well as micro E-test on MHA plate with a 0.5 McFarland.

Results: FS 33732 and ATCC 29213 MICs were 2.0 and 1.0 respectively. Macro and micro E-test exhibited no colonies of subpopulation growth with MICs of 4.0 and 3.0. Population analysis for FS 33732 revealed various densities across 0.25–1.0 µg/ml, see graph in Figure 1 at 24 hours and Figure 2 at 48 hours.



Conclusions: We demonstrated FS 33732 to have characteristics of hMSSA. Discovery of hMSSA is of importance because it may provide further insight into treatment failures in those patients with penicillin allergy who require to be on vancomycin. Patient was sent to the intensive care unit (ICU) for desensitization of penicillin for her allergy. Patient tolerated well and nafcillin was initiated promptly to be completed for 8 weeks course.

P1458

Decreased susceptibility to glycopeptides in methicillin-resistant *Staphylococcus aureus*: a 20-year study in a large French teaching hospital, 1983–2002

J. Robert, R. Bismuth, V. Jarlier (Paris, FR)

Objective: To assess the evolution of glycopeptides' susceptibility in methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated in a large French teaching hospital from 1983 to 2002.

Method: Determination of glycopeptides' MICs by using the E-test method in Mueller-Hinton agar on a sample of randomly selected MRSA strains.

Results: A total of 1445 MRSA were tested, and one vancomycin-intermediate MRSA (VISA) and 31 teicoplanin-intermediate MRSA

(TISA) strains were detected. The first strains were detected in 1985, and all strains were gentamicin-resistant (GR). None of the gentamicin-susceptible strains has a glycopeptide MIC > 3 mg/L. In addition, there was a significant increase in glycopeptides' MIC geometric means over the years, and this increase was higher for teicoplanin than for vancomycin.

Conclusion: The higher increase in teicoplanin MICs and the good correlation between both glycopeptides' MICs, suggests systematic determination of teicoplanin MIC to screen for abnormal glycopeptide susceptibility between GR-MRSA.

P1459

Trends in resistance to some antibiotics in selected bacterial species-*S. aureus*, *S. pneumoniae*, *E. faecalis*, *E. faecium*-causing invasive infections in the Czech Republic in 2000–2004

B. Mackova (Prague, CZ)

Objectives: Invasive infections caused by *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecium* and *Enterococcus faecalis* can be serious due to widespread resistance to different antibiotics. Serious problem for some hospitals are MRSA (methicillin-resistant *Staphylococcus aureus*), PNSP (penicillin non-susceptible *Streptococcus pneumoniae*) and VRE (vancomycin-resistant *Enterococci*). The incidence of MRSA, PNSP and VRE infections in the Czech Republic varies widely with the regions and population groups.

Methods: Since 2000 the Czech Republic has been taking part in the European Antimicrobial Resistance Surveillance System (EARSS). EARSS monitored the incidence of seven pathogens in invasive isolates from blood and cerebrospinal fluid. Four of the causative agents under surveillance are *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecium* and *Enterococcus faecalis*. The incidence of MRSA, PNSP and VRE has been currently monitored from 92 hospitals of the Czech Republic. Data on patients, hospitals and phenotypes of antibiotic resistance in the bacterial strains isolated have been recorded. Basic statistical methods are used for epidemiological analysis.

Results: Between years 2000–2004, 5684 *S. aureus* strains and 781 *S. pneumoniae* were analysed. Between years 2001–2004, 1997 *E. faecalis* strains and 346 *E. faecium* strains were analysed. Out of these strains 6.5% were MRSA (increase from 3.8% in 2000 to 8.6% in 2004), 5.2% were PNSP (resistance oscillated around 5%), 0.6% were vancomycin-resistant *E. faecalis* (resistance oscillated around 0.5%) and 3.8% were vancomycin-resistant *E. faecium* (resistance oscillated around 3.6%). The incidence of resistant bacteria varied with types of hospitals, hospital wards, age groups and time. Thanks to collaboration of 48 laboratories, highly valid data covering 88% of the Czech population have been available.

Conclusion: Surveillance of MRSA, PNSP and VRE strains as a basis for active antibiotic policy has become of increasing concern to both health care providers in hospitals and community general practitioners. The incidence of MRSA infections in last years in the Czech Republic shows increasing trend. The incidence of PNSP and VRE infections have invariable trend. Very interesting is local distribution of resistant strains. The development of the incidence of infections caused by monitored agents in their spread will be the subject of further study.