# Antibiotic prescribing – quality indicators

## P1460

### Is self-medication with antibiotics in Europe driven by prescribed use?

L. Grigoryan, F.M. Haaijer-Ruskamp, J.G.M. Burgerhof, J.E. Degener, R. Deschepper, D. Monnet, A. Di Matteo, E.A. Scicluna, A. Bara, C. Stålsby Lundborg, J. Birkin *and the SAR group*

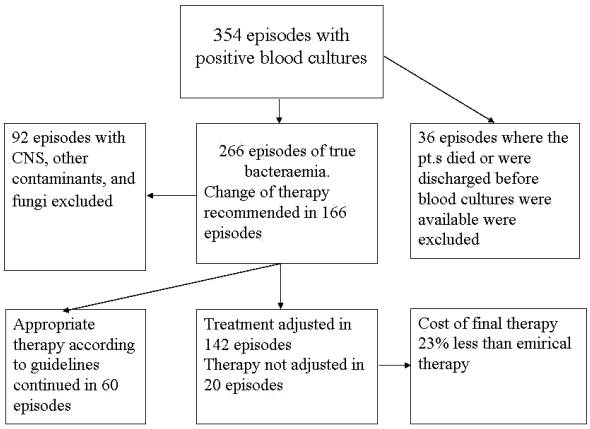
**Objectives:** The occurrence of self-medication with antibiotics has been described in the US and Europe, a possible contributing factor to increased antibiotic resistance. An important reason for using self-medication can be past experience with antibiotics prescribed by health professionals. We investigated whether self-medication in Europe follows the same pattern as prescribed use.
**Methods:** A population survey was conducted in: North and West of Europe (DK, AT, NL, SE, BE, UK, IE and LU), South (IT, MT, IL and ES) and East (SK, LT, CZ, HR, PL, RO and SL) with a self-administered postal questionnaire. A total of 15548 people completed the questionnaires. Using a multistage sampling design, we selected in each country a random sample of 1000–3000 adults equally distributed in urban and rural areas. The analyses were done on the individual level using multivariate logistic regression analysis. We tested possible interactions between the factors found to be significant.
**Results:** The use of prescribed antibiotics was independently associated with self-medication in the previous 12 months. A significant interaction was found between prescribed use and regions in Europe: the effect of prescribed use was the largest in North and West (odds ratio 7.6 95% CI 4.2–13.6) and smaller in South (OR 2.1, 1.2–3.7) and East (1.9, 1.3–2.7). When studying the association between prescribed use and self-medication for a specific symptom/disease or specific antibiotic strong associations were found. For example, respondents who used prescribed antibiotics for a throat symptom had 7.1 times (4.4–11.4) higher probability for self-medication for a throat symptom. For the other symptoms the odds ratios varied from 6.4 to 7.7. Prescribed use and actual self-medication were both significant predictors of intended self-medication. We found a significant interaction, indicating that prescribed use increased the risk of intended self-medication in those respondents who did not use actual self-medication. Respondents who used prescribed antibiotics for a specific symptom in the previous 12 months had 1.7–7.1 times higher probability of intended self-medication for the same symptom.
**Conclusion:** Self-medication with antibiotics in Europe is associated with prescribed use and may even be driven by it. Routine prescribing of antibiotics for minor ailments such as cold symptoms increases the risk of self-medication with antibiotics for such ailments.

## P1461

### Adjustment of antibiotic treatment according to the results of blood cultures leads to decreased antibiotic use and costs

D. Berild, A. Mohseni, L.M. Diep, M. Jensenius, S.H. Ringertz *(Oslo, NO)*

**Objective:** To investigate whether the results of blood cultures led to changes in antibiotic use and costs in a university hospital in Norway
**Methods:** Medical records from all patients with positive blood cultures in 2001 were analysed retrospectively. Factors predisposing to infections, results of blood cultures, antibiotic use, and outcome were recorded.
**Results:** The antibiotic use in 226 episodes of true bacteraemia were analysed. According to guidelines empirical antibiotic treatment should be adjusted in 166 episodes. Antibiotic use was adjusted in 146 (88%) of these 166 episodes, which led to a narrowing of therapy in 118 (80%) episodes. Compared to empirical therapy there was a 22% reduction in the number of antibiotics. Adjustment of therapy was more often performed in Gram-negative bacteraemia and polymicrobial cultures than in Gram-positive bacteraemia. In bacteraemia caused by ampicillin-resistant *E. coli*, ampicillin was mostly replaced by ciprofloxacin. The cost for 7 days adjusted therapy was 19800 EUR (23%) less than for 7 days of empirical therapy.




**Conclusion:** Adjustment of antibiotic use according to results of blood cultures led to improvement of antibiotic use and decreased costs.

## P1462

### Impact of an active surveillance system on antimicrobial use and resistance in a neurosurgical intensive care unit

E. Meyer, J. Buttler, F. Schwab, E. Strehl, P. Gastmeier, H. Ruden, K. de With, J. Zentner, F. Daschner *(Freiburg, Berlin, Hannover, DE)*

**Objective:** To analyse the impact of SARI (Surveillance of Antimicrobial Use and Antimicrobial Resistance in Intensive Care Units) on antibiotic use, resistance, device associated infection rates (NI) and costs in a neurosurgical university ICU.
**Methods:** Comparison of prospective unit and laboratory based surveillance data of 40 German ICUs from 2000–2004 with the study ICU. Antimicrobial consumption is calculated by WHO defined daily doses (DDD) per 1000 patient days (AD). NIs are reported according to CDC definitions. Benchmarking data were fed back to the study ICU and analysed there in a multidisciplinary team every three months between 2000 and 2003 and every 6 months since 2004.
**Results:** In the study a total of 1,004 SARI isolates were reported; this figure corresponds to 72.0 isolates/1000 pd.

Resistance rates (RR) over a period of 5 years were generally better than those reported for a total of 40 ICUs. The mean MRSA RR was 22.1%, for all the SARI ICUs, whereas it was only 2.7% in the study ICU. By the end of 2003 duration of treatment for pneumonia had been reduced to 5–7 days and written guidelines on empiric antibiotic treatment and prophylaxis were revised with respect to the resistance situation of the study ICU. The significant decrease between 2000 and 2004 in total antimicrobial AD from 1,099 to 607 in the study ICU resulted mainly from the reduced consumption of 2nd generation cephalosporins, carbapenems and imidazoles. NI did not change significantly over time. Compared to the year 2000, the costs for antibiotics were halved from €51,102 to 22,324, which corresponds to €18.7/pd and €6.6/pd, respectively. The percentage of antibiotics in the total ICU budget for pharmaceuticals decreased from 14.6% to 10.4%.
**Conclusion:** Surveillance and feedback of antibiotic use and resistance can serve as a valuable quality control instrument and can have an impact on antibiotic treatment. From 2000 to 2004, antibiotic use was reduced by 45% and costs for antibiotics/pd were cut by two third in the ICU study without any increase in device associated nosocomial infection rates. The resistance situation was generally better than in all SARI ICUs, but showed heavy fluctuations.

## P1463

## Similar illness burden but different antibiotic prescription to children: a population-based study

K. Hedin, M. Andre, A. Håkansson, N. Rodhe, S. Mölstad, C. Petersson *(Växjö, Falun, Malmö, Linköping, SE)*

**Objectives:** Respiratory tract infections are the most common reason for antibiotic prescription in Sweden as in other countries. The prescription rates vary markedly in different countries, counties and municipalities. The reasons for these variations in prescription rates are not obvious. The aim of the study was to find possible explanations for different antibiotic prescription rates in children. Therefore a prospective population based log book study was conducted in four municipalities which, according to official statistics, had high and three municipalities which had low antibiotic prescription rates.
**Methods:** During one month, parents recorded all infectious symptoms, physician consultations and antibiotic treatments, from 848 18-month-old children in a log book. The children's parents also answered a questionnaire about socioeconomic factors and concern about infectious illness.
**Results:** Antibiotics were prescribed to 11.6% of the children in the high prescription area and 4.7% in the low prescription area (crude OR 2.67 (95% CI 1.45–4.93)). After multiple logistic regression analyses taking account of socioeconomic factors, concern about infectious illness, number of symptom days and physician consultations, differences in antibiotic prescription rates remained (adjusted OR 2.61 (95%CI 1.14–5.98)). The variable that impacted most on antibiotic prescription rates although it was not relevant to the geographical differences was a high level of concern about infectious illness in the family.
**Conclusion:** The differences in antibiotic prescription rates could not be explained by socioeconomic factors, concern about infectious illness, number of symptom days and physician consultations. The differences may be attributable to different prescription customs, in which case physicians' prescription patterns are not always rational.

## P1464

## Decreasing outpatient antibiotic prescribing in Germany, 1995–2004, does not include newer macrolides, fluoroquinolones and extended-spectrum beta-lactams

W.V. Kern, K. de With, K. Nink, H. Schröder *(Freiburg, Bonn, DE)*

**Objective:** The ESAC (European Surveillance of Antibiotic Consumption, www.ua.ac.be/ESAC) project has shown that outpatient antibiotic prescribing in Germany has been comparatively low among European countries. We assessed trends over time and regional variation of outpatient antibiotic use in Germany, and wondered if the observable decreasing trend included all drug classes to a similar extent.
**Methods:** Prescription data (compulsory health insurance covering >90% of the population, sample of 0.4% until the year 2000, all prescriptions thereafter) were analysed using the ATC/WHO methodology and current DDD definitions. We specifically defined the following drug groups: ''basic'' penicillins (BPENs, oral penicillin or aminopenicillins), extended-spectrum betalactams (ESBLs, oral cephalosporins, staphylococcal penicillins, aminopenicillin/betalactamse inhibitor combinations, parenteral cephalosporins and broad-spectrum betalactams), newer macrolides (NMLs, roxithromycin, clarithromycin, azithromycin) versus older macrolides (OMLs). Quinolones (FQs), folate synthesis inhibitors (T/Ss) and tetracyclines (TETs) were also assessed. Data were expressed in yearly DDD/1000 persons covered by the insurance (DDD/1000).
**Findings:** Outpatient prescribing in 1995 was 6140 DDD/1000 (corresponding to 16.8 DID = DDD/1000 and day) and decreased to 5430 DDD/1000 in the year 2000 and to 4672 DDD/1000 in 2004. The decreasing trend over the last 4 years was observed in all regions. The decrease was most significant for OMLs (−55%), T/Ss (−48%), TETs (−36%), and BPENs (−13%) while there was no decreasing use of ESBLs (±0%) and increases in the rate of prescribing NMLs (+13%) and FQs (+43%). TETs and BPENs, however remained the most prescribed antibiotics in 2004. Regional variations in 2004 remained large for BPENs (>3-fold) with very low prescribing rates in the Eastern region, but were small for T/Ss, NMLs and FQs (<2-fold).
**Conclusions:** Over a decade we observed a 24% decreasing outpatient antibiotic prescribing that included relevant antibiotic drug classes except ESBLs, NMLs and FQs. The relative increase was most significant for FQs.

## P1465

## Severe community-acquired pneumonia admitted to the intensive care unit: impact of antibiotic therapy delay on hospital mortality

P.F. Laterre, T. Dugernier, X. Wittebole, C. Collienne, M.N. France, R. Wunderink *(Brussels, BE; Chicago, US)*

**Introduction:** Severe community-acquired pneumonia (CAP) admitted to the ICU remains associated with a high hospital mortality. Time to antibiotic treatment has been considered to influence patients (pts) outcome in severe infections. We thought to determine if time from hospital admission to antibiotherapy had an impact on hospital mortality in severe CAP.
**Materials and methods:** All consecutive pts with CAP from 2 institutions, admitted to the ICU and treated with a combination

antibiotic therapy were enrolled in the study. Pts were divided in 4 Groups according to time to treatment (<2 h GI, 2–4 h GII, 4–8 h GIII and >8 h GIV). Baseline severity scores (APACHE II, SOFA, PSI, CURB-65), microbiological documentation, and hospital outcome were compared for all groups.
**Results:** 152 pts were included in the study. Microbiological documentation was achieved in 80% of all pts, positive blood cultures in 54/152 (35.5%), *S. Pneumoniae* in 64/152 (42%). Mean age was 66 ± 13, APACHE II 25.8 ± 7.6, SOFA 8.8 ± 3.7, PSI 158 ± 40, CURB-65 3.2 ± 1, mechanical ventilation in 67.7% and vasopressors use in 63.1%. Overall ICU and Hospital all-cause mortality were 27.5% and 35.5%, respectively. Baseline severity scores were comparable in all 4 Groups and their respective Hospital mortality is provided in Table 1.

Table 1 : Hospital mortality according to time to treatment

| GROUP | GI | GII | GIII | GIV | All |
|---|---|---|---|---|---|
| (n) | 37 | 44 | 45 | 26 | 152 |
| Death (n) | 8 | 17 | 16 | 13 | 54 |
| (%) | 21,6 | 38,6 | 35,5 | 50 | 35,5 |

**Conclusions:** In severe CAP, treated with a combination therapy, time to treatment seems to have an impact on hospital all-cause mortality. Based on our results, antibiotic treatment should be initiated within the first 2 hours after hospital admission.

## P1466

## Antibiotics cycling in the intensive care unit: next stage – cefoperazone/sulbactam

D. Protsenko, S. Yakovlev, B. Gelfand, O. Romashov, A. Yaroshetskiy, O. Ignatenko *(Moscow, RU)*

**Purpose:** The aim of investigation was a study of changing antibiotic resistance of causative agents of VAP of the emergency municipal hospital as a result of antibiotic cycling of empirical treatment regimes.
**Methods:** The present study was based on the results of research conducted from October 2003 till September 2005 in the ICU department of the municipal emergency hospital. Within the period from October 2003 till December 2004 (Period I) cefepime (4 g a day) as monotherapy and from January 2005 till September 2005 (Period II) – cefoperazone/sulbactam (8 g a day) as monotherapy were used as the empirical antibacterial therapy of VAP. The rotation from cefepime to cefoperazone/sulbactam was performed due to our previous study demonstrated high frequency of ESBL producers among *Enterobacteriaceae*. The samples from the lower respiratory tract were obtained by mini-BAL. The sensitivity of microorganisms to the antibiotics studied (ceftazidime, cefepime, cefoperazone/sulbactam and carbapenems) was determined by the disk diffusion method.
**Results:** The main pathogens of VAP were *S. aureus* (20%), *P. aeruginosa* (19%), *Enterobacteriaceae* (23%) and this structure did not changed during both periods. The antibiotic sensitivity of *P. aeruginosa* and *Enterobacteriaceae* (*K. pneumoniae* and *E. coli*), was studied separately. A high level of resistance of *Enterobacteriaceae* to cefepime can be explained by the strains prevailing in the given ICU, which produced extended spectrum beta-lactamases (CTX-M). The resistance of *Enterobacteriaceae* to cefepime was 57.5% in I period and 80.5% in II period, to ceftazidime – 90.0 and 100%, meropenem – 0 and 0%, imipenem – 5.1 and 2.1%, cefoperazone/sulbactam – 10.9 and 20.8%, respectively. A change of cefepime for cefoperazone/sulbactam was not followed by any decrease of *Enterobacteriaceae* resistance level to cefepime during II period. The resistance level of *P. aeruginosa* to cefepime was 20.5% in I period and 24.1% in II period, to ceftazidime – 22.5 and 33.0%, meropenem – 44.7 and 39.5%, imipenem – 50.1 and 39.5%, cefoperazone/sulbactam – 16.9 and 12.5%, respectively.
**Conclusion:** The exclusion of cefepime for 9 months didn't improved the sensitivity of *Enterobacteriaceae* to this medication. The level of resistance of *P. aeruginosa* and *Enterobacteriaceae* to cefoperazone/sulbactam did not increased despite a wide use of this antibiotic during 9 months.

## P1467

## Antibiotic consumption in German acute care hospitals

M. Steib-Bauert, K. de With, E. Meyer, P. Straach, W.V. Kern *(Freiburg, Frankfurt, DE)*

**Objective:** Outpatient antibiotic use in Germany differs substantially between Eastern and Southern parts of the country (relatively low use) and Western part (relatively high use). There is no nationwide estimate of hospital antibiotic use and its geographic variation if any. The aim of the present study was to provide an estimate of recent hospital antibiotic use density in Germany and to identify basic unit/hospital characteristics associated with excess use.
**Methods:** Data on hospital consumption of systemic antibiotics in Anatomical Therapeutic Chemical (ATC) class J01 were obtained from a convenience sample of 145 acute care hospitals in Germany that participated in an IMS survey in the year 2003 and had complete data (dispensed drugs and patient-days per year) for at least one non-paediatric, non-psychiatric department or ward. A total of 275 non-ICU surgical departments/wards, 229 non-ICU non-surgical (general medicine, oncology-haematology, neurology/stroke) departments/wards, and 184 ICUs covering >16 million patient-days were analysed. Data were expressed in DDD (WHO/ATC definition version 2001) or ''prescribed/recommended daily doses'' (PDD, better reflecting [high] dosages given to hospitalized patients) per 100 patient days (DDD/100 and PDD/100).
**Findings:** The weighted mean over all departments/wards incl. ICUs was 49.8 DDD/100 (31.4 PDD/100). As expected, ICU antibiotic use density was much higher than use in non-ICU areas, and use in haematology-oncology was higher than in other non-surgical departments/wards. In univariate analyses, bed-size category and university affiliation (ICUs, surgical wards), region (ICU, surgical and non-surgical wards) and haematology-oncology as specialty (non-surgical wards) were associated with use density, but these associations were only partly confirmed in multivariate logistic regression analyses of factors associated with excess (≥75%) use density which showed university affiliation and haematology-oncology to be independently associated with high use.
**Conclusions:** Based on this hospital sample, antibiotic use in German hospitals shows little, non-significant regional variation and appears to be similar to what has been described from other European countries. Adjustment of the data at least for university affiliation and haematology-oncology is important in comparative analyses of hospital antibiotic consumption.

## P1468

## Impact of formulary change in medical intensive care unit on outcome of infection and antimicrobial resistance

V. Huang, J. Jinson, M. Zervos *(Atlanta, Detroit, US)*

**Objective:** Antimicrobial resistance in medical intensive care unit patients (ICU) is an increasing problem worldwide. We

sought to evaluate a formulary change and impact it has on infection and resistant.
**Methods:** Prospectively, all patients in a 20-bed ICU were followed for a period of 4 months in Phase I (390 patients per 2379 patient days) and to collect baseline data after a decrease in the use of piperacillin-tazobactam (PT) when substituted by cefepime for a period of 4 months in Phase II (383 patients per 2260 patient days).
**Results:** Total infections in Phase I vs. Phase II were lower respiratory tract (LRTI) 214 patients (55%) vs. 203 patients (53%); urinary tract infection (UTI) 94 patients (24%) vs. 96 patients (25%); and sepsis of undetermined aetiology 70 patients (18%) vs. 65 patients (17%), respectively. There were no significant differences in death (22% vs. 19%), cure or improvement of infection (53% vs. 56%), readmission to the unit (3.5% vs. 3.2%), hospital risk of death (29.8% vs. 30.2%), mean length of ICU stay (6.1 days vs. 5.9 days), or rates of nosocomial infection (6.3% vs. 5.1% for LRTI; 4.0% vs. 4.2% for UTI; 0.8% vs. 0.0% for soft tissue infection; 0.8% vs. 1.0% for bacteremia; 2.1 vs. 1.0 per 1000 patient days for intravenous catheter infection) in Phase I and II respectively. The cost of antimicrobial acquisition in Phase I and II were \$548 and \$433 per patient respectively ($p < 0.001$). The mean antimicrobial treatment costs per patient for PT were \$145 vs. \$100 and cefepime were \$80 vs. \$105 in Phase I and II respectively ($p < 0.01$). The in vitro susceptibility and rate of infection and colonization with *Escherichia coli* were unchanged in both study periods. There were 68 vs. 39 *Staphylococcus aureus* ($p < 0.001$); of these 94percnt vs. 87percnt were methicillin-resistant *S. aureus* and 11 vs. 9 *Enterococcus faecium* (82% vs. 77% vancomycin-resistant enterococci) in Phase I and II respectively. There were 73% vs. 31% *Pseudomonas aeruginosa* and 70% vs. 4% *Klebsiella pneumoniae* extended spectrum beta lactamases in Phase I and II respectively.
**Conclusion:** The implementation of formulary substitution of PT to cefepime in the medical ICU had resulted in a decrease in the use of PT. In addition, there were decreased costs and less *S. aureus* infections without adversely affecting the outcome of infection or antimicrobial resistance.

## P1469

## Intravenous antibiotic use in Scottish hospitals; evaluation of the Glasgow antimicrobial audit tool

R.A. Seaton, D. Nathwani, P. Burton, E. Douglas *(Glasgow, Dundee, UK)*

**Introduction:** There are few data on antibiotic prescribing within Scottish hospitals and a coordinated multisite point prevalence survey had not been performed before. There is concern that antimicrobials are overused in hospitals.
**Methods:** Antibiotic use in acute medical and surgical units in 10 Scottish hospitals across 5 trusts, was investigated using a point prevalence survey. Data were collected by pharmacists. Appropriateness of the IV route of administration was determined by review of data by an infectious diseases physician (IDP) and compared with a specifically designed computerised algorithm. The IDP also judged the appropriateness of the chosen IV agent against local guidelines. 3826 patients from 10 hospitals in 5 regions were surveyed on a single day. 1079 (28.3%) were receiving an antibiotic, 381 (35.3%) intravenously. 197 receiving oral antibiotics had received an IV previously. Median duration of IV therapy was 4 days (IQR 2–7 days) and time from IV to oral switch was 3.5 (2–6). The IDP judged appropriate IV route in 84% patients compared with 84.8% by the algorithm. The sensitivity of the algorithm was 93.4% and specificity 60.7%. The positive predictive value was 92.6% and the negative predictive value was 63.8%. The IDP judged IV agents to have been chosen and administered appropriately in 80%. Most frequently prescribed IV agents were 3rd generation cephalosporins (3GC) (28.3%), Co-amoxiclav (20.2%), Metronidazole (19.2%), Glycopeptides (18.6%). Significant regional differences were seen for most antibiotic groups including 3GCs (49.2% (site 3) vs 24.4% (sites 1, 2, 4, 5), $p < 0.001$) and Glycopeptides [31.8% (site 1) vs 9.3% (site 2, 3, 4, 5), $p < 0.001$]. It is possible to coordinate, collect and compare data from Scottish hospitals. The GAAT gives a good estimate of the appropriateness of IV therapy. Significant differences in prescribing patterns between similar patient groups across different hospital sites were demonstrated. Such data may usefully inform local and national audit and support prescribing initiatives.

## P1470

## Use of glycopeptides in 47 French hospitals

A.M. Rogues, C. Dumartin, H. Boulestreau, P. Parneix, N. Marty, J.P. Gachie *(Bordeaux, Toulouse, FR)*

**Objective:** To describe consumption of glycopeptides for systemic use in hospitals. Setting: 47 hospitals in south-western France (31 were public hospitals and 16 were private hospitals).
**Method:** Consumption of glycopeptides for systemic use (defined daily doses per 1,000 patient-days: DDJ per 1000 PD), number of methicillin-resistant *Staphylococcus aureus* per 1000 patient-days (incidence of MRSA), number of central-line per 1000 patient-days and hospitals characteristics (size, length of stay, number of beds: total and for each hospital areas) were recorded from January 2002 through December 2002. Associations between continuous variables were tested in univariate analysis with the Spearman correlation test (r). Multiple linear regression analysis was performed in a backward stepwise approach.
**Results:** The median rate of total hospital glycopeptides use was 4.11 (range 0.21 to 27.22) DDDs per 1,000 PD with higher consumption in large public hospitals. Consumption was higher in intensive care areas (median 46.51; range 7.19 to 134) than in surgery areas (median 4.5; range 0.17 to 24.76) and in medicine (median 4.26; range 0 to 41). Glycopeptides use correlated with number of central line per 1,000 PD (r: 0.44; p: 0.03) and with size of the various areas in the hospital (for intensive care, r: 0.50; for medicine areas, r: 0.33 and for surgery areas, r: 0.42; $p < 0.05$). Median incidence of MRSA was 0.87 per 1,000 PD. Incidence of MRSA explained a small proportion of the variation in hospital glycopeptides consumption (R2: 0.13). In a multivariate linear regression model, incidence of MRSA and number of beds in surgery areas were independent predictors of total glycopeptides use in the hospital (R2 adjusted: 0.39). After controlling for these factors, number of central-line per 1,000 PD was no more associated with glycopeptides use.
**Conclusion:** In our hospitals, total glycopeptides use was not heavily determined by incidence of MRSA. Although glycopeptides use in surgery areas was not the highest, the total number of surgery beds in the hospital explained a large variation of the total hospital glycopeptides use. Therefore we had to take it into account to interpret these consumption and to decide further evaluation.

## P1471

### Antibiotic management of acute lower respiratory tract infections among Dutch elderly patients in primary care

J. Bont, C. Birkhoff, T. Verheij, E. Hak *on behalf of ESPRIT*

**Objectives:** Acute lower respiratory tract infection (LRTI) can cause various complications leading to morbidity as well as mortality notably among elderly patients. Antibiotic treatment of LRTI is common, despite Dutch clinical guidelines recommending antibiotics only in case of pneumonia or high risk of serious complications. We assessed the course of illness and outcome of pneumonia, acute bronchitis and exacerbations of COPD or asthma among Dutch elderly patients in primary care and assessed whether GPs were inclined to prescribe antibiotics more readily to patients with potential risk factors for complications in acute bronchitis or exacerbations of COPD/asthma.
**Methods:** We retrospectively analysed medical data from 3,166 episodes of LRTI among patients ≥65 years of age presenting in primary care to describe the course of illness and outcome. The relation between prescriptions of antibiotics and patients with risk factors for a complicated course was assessed by means of multivariate logistic regression. Risk factors for a complicated course included heart failure, history of myocardial infarction, angina pectoris, diabetes, history of stroke, dementia, malignancy, and history of pneumonia or hospitalisation in preceding year.
**Results:** One or more complications arose in 17% of episodes of LRTI. Among these, 8% suffered from pulmonary complications, 5% had cardiovascular complications (heart failure, myocardial infarction etc.), 5% had a protracted course and 0.6% had a diabetes event. In 6.9% of the patients complications led to hospital admission and in 2.4% LRTI were fatal. Antibiotics were more readily prescribed to patients aged ≥90 years, when heart failure was present and in patients with diabetes. No significant association was observed in patients with other co-morbid conditions. Patients diagnosed with an exacerbation of COPD or acute bronchitis with a history of pneumonia or hospitalisation in the preceding year were not more likely to receive antibiotics.
**Conclusions:** A considerable part of elderly patients with a LRTI suffers from a severely complicated course in primary care. Although GPs are inclined to prescribe more readily antibiotics in the very old and those with heart failure or diabetes, other potential risk factors are not taken into account.

## P1472

### Prospective evaluation of consultations performed by infectious diseases trainees

O. Sipahi, M. Tasbakan, H. Pullukcu, B. Arda, T. Yamazhan, S. Mizrakci, S. Senol, S. Atalay, D. Koseli, G. Arsu, S. Calik, H. Sipahi, C. Buke, S. Ulusoy *(Izmir, TR)*

**Objectives:** In this study it was aimed to analyse the infectious diseases (ID) trainees' night/weekend shift consultation process in terms of patient and consultant characteristics, types of recommendations, and compliance with recommendations.
**Methods:** All consultations performed by ID trainees in night shift and at the weekends between June 10th–August 10th 2004 were analysed in terms of consultation type [treatment continuation (TC), consultation for surgical antibiotic prophylaxis (PA), and consultation with or without a request of a specific antibiotic (others)]. Appropriateness of recommendations was assessed the day after the consultation by infectious diseases specialists (IDS). Adherence to recommendations was assessed 3 days after the consultation by IDSs. Recommendations including antibiotics were considered appropriate, if they were appropriate according to national and international guidelines. Recommendations were considered complied, if they were done in up to 72 hours after the consultation (except the consultations in the emergency medicine and the consultations in which antibiotics were started by the counselling IDSs).
**Results:** Of 436 consultations 79 was for TC, 74 was for PA and 290 was for others. The clinic where ID consultations were requested mostly was general surgery clinic (152/436, 34.9%). In 2% of all consultations trainees consulted the specialists. Overall 146 consultations (74 for SP, 69 for a clinical infectious disease diagnosed clinically, 9 for an infectious disease diagnosed microbiologically) were for requesting spesific antibiotic(s). PA were approved in 68 of 74 consultations. Antibiotic was not recommended in 14 of 290 other consultations. In six of 74 consultations for PA antibiotic was changed for a clinically diagnosed infectious disease. In one of 79 consultations for TC antibiotic was changed due to lack of response to the given antibiotic, in others TC was approved. Inappropriate antibiotic recommendation rate was 2.4% (8/32, 4 inappropriate choice, 3 inappropriate dosage, one antibiotic unnecessary). Overall compliance to ID recommendations was 76.1% (476/608). Rate of compliance to antibiotic recommendations was evaluated in 240 consultations and was found 98.2% (277/282) and was higher than compliance to other (microbiology etc.) recommendations (60.8%, 199/327, chi square $p < 0.05$).
**Conclusion:** Methodologies to improve the compliance to non-treatment based recommendations and optimizing antibiotic selection is necessary.

## P1473

### Study of the influence of online practice guidelines on the appropriateness of antibiotic prescribing in a university-affiliated psychiatric hospital

J.F. Westphal, C. Nonnenmacher, D. Gregoire, M. Hittinger, C. Oulerich, F. Jehl *(Brumath, Strasbourg, FR)*

**Background:** Problems with the dissemination of guidelines are frequently cited as a major reason for failure to impact practice. Reviews of the effectiveness of various methods of guideline dissemination show that the most predictable impact is achieved when the guideline is made accessible through computer-based reminders that are integrated into the clinician's workflow. We report a time-series prospective investigation aimed at comparing the appropriateness of antibiotic (AB) orders for pneumonia at the treatment initiation level after vs. before having embedded our current AB guidelines for pneumonia in the computerized physician drug-order entry system of our teaching psychiatric hospital comprising 410 adult beds.
**Methods:** In total, 160 consecutive AB orders for pneumonia were evaluated by the pharmacy department, including 80 orders just before and 80 orders just after implementation of online AB guidelines. Appropriateness of AB orders relative to the guidelines was assessed according to 3 criteria: (1) the choice of AB with respect to the mode of acquisition (community- or hospital-acquired) of pneumonia or the presence of clinical risk factors for involvement of gram-negative bacilli, (2) the daily dosage, (3) the planned duration of treatment. Data were extracted from the computerized infection declaration system that recorded all AB-requiring infections in our hospital.

**Results:** The number of AB orders with at least 1 criterion of inappropriateness tended to decrease, yet not significantly ($p = 0.11$), after vs. before implementation of online guidelines: 30/80 (36.5%) and 40/80 (50.0%), respectively. The number of criteria of inappropriateness relative to all AB orders for pneumonia was significantly lower in the post-implementation period: 43.8% vs. 65.0% before implementation (difference 21.2%, 95% CI 6.1–36.3, $p < 0.01$), with a trend to a decreased number of orders containing more than 1 criterion of inappropriateness. Analyzed separately, the numbers of inappropriate orders for the choice of the AB, or the daily dosage, or the planned duration of treatment decreased, yet not significantly ($p > 0.1$ for each criterion), in the post- vs. pre-implementation period: 11 vs. 18, 12 vs.15, 12 vs.19, respectively.
**Conclusion:** In this study, the moderate impact on AB prescribing practices of online guidelines available at the time of drug order shows that additional types of intervention are needed to improve further the quality of AB prescribing.

## P1474

## Pilot implementation of multidisciplinary antibiotic management teams in Belgian hospitals: 2-year progress report, 2003–04

L. Sourdeau, M.J. Struelens, W.E. Peetermans, C. Suetens, M. Costers *for the Hospital Care Working Group of Belgium Antibiotic Policy Coordination Committee*

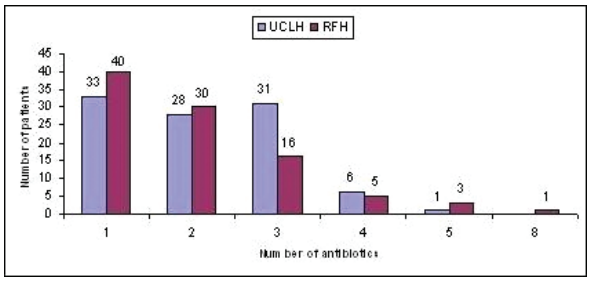
**Objectives:** To analyse the interventions undertaken by local Antibiotic Managers (AMs) and multidisciplinary Antibiotic Management Teams (AMTs) in Belgian hospitals during a 2-year pilot project.
**Methods:** Since 2002, the Belgian government subsidized the part-time activities of AMs in 36 hospitals with an operational AMT. The activity of AMTs from these hospitals and their impact on antibiotic usage were evaluated based on yearly progress reports for 2003 and 2004.
**Material:** The pilot hospitals had a median capacity of 654 (range, 154 to 1597) beds; their regional distribution was representative of population size; 18 were general hospitals, 8 teaching hospitals and 10 general hospitals with teaching beds.
**Results:** AMs were Internists (28), Microbiologists (13) and Pharmacists (13). AMTs included a mean of 10 members who met every 6 weeks on average. All hospitals irrespective of size or affiliation had undertaken a wide range of antibiotic management interventions in 2003, which increased in 2004; These included (in 2003 and 2004, respectively): major review of formulary (in 10 and 23 hospitals), development of clinical guidelines (69 and 215 topics), restricted access to selected antibiotics (carbapenems, glycopeptides, quinolones, new drugs; in 25 and 33 hospitals). In 2003, antibiotic consumption databases were established in 35 hospitals and antibacterial susceptibility databases in 31 hospitals. In 2004, cross-analysis of these databases was performed in 28 hospitals. In 2004, prescribing assistance, antibiotic stop orders, treatment streamlining and IV/PO therapy switch were implemented in 32, 21, 24 and 24 hospitals, respectively. In 2004, 26 hospitals reported a better use of target antibiotics, 15 hospitals a decrease in consumption of restricted antibiotics, 5 hospitals a decrease of total antibiotic consumption, 2 hospitals a decrease in high consumer departments.
**Conclusion:** All hospitals participating in the AMT pilot scheme have developed multiple antibiotic policy interventions and established monitoring and guidance of antibiotic prescription. Preliminary data from some hospitals indicated success in meeting self-defined targets of appropriate use and reducing the consumption of selected antimicrobial agents. More systematic evaluation using standard quantitative and qualitative indicators is planned.

## P1475

## Antibiotic prescribing practices at two linked London teaching hospitals

P. Panesar, G. Scott, P. Wilson, C. Kibbler, I. Balakrishnan *(London, UK)*

**Objectives:** To analyse and compare Microbiological isolates, their antibiograms and antibiotic prescribing patterns between two London teaching hospitals.
**Methods:** Patients admitted to hospital were identified prospectively over 3 months. Those initiated on antibiotics on admission were included in the study and followed throughout their hospital stay.
**Results:** Five hundred and fifty two patients were surveyed, of which 194 (35%) were initiated on antibiotics. Over the course of the study, a total of 402 patient antibiotics were prescribed during in-patient stay, of which 49% were intravenous (Figure 1). Cefuroxime was the most commonly prescribed in-patient antibiotic at UCLH and co-amoxiclav at RFH. On discharge, 53% of patients were given an antibiotic. Amoxicillin was the most frequently prescribed antibiotic on discharge at UCLH and co-amoxiclav at RFH. The main indications for antibiotic use were urinary tract infection (24% UCLH; 27% RFH), lower respiratory tract infection (29% UCLH; 42% RFH), and skin and soft tissue infection (24% UCLH; 21% RFH). Twenty-eight percent of these patients were already taking an antibiotic prior to admission - therapy was changed on admission in 19 patients (95%) at UCLH and 17 patients (65%) at RFH. Median duration of total antibiotic course was 7 days at both hospitals, and that of IV antibiotics was 3 days at UCLH and 3.5 days at RFH. The most frequent specimens sent at UCLH were urine (21% positive), blood (15% positive) and sputum (18% positive). At RFH the specimens sent were MRSA swabs (35% positive), urine (21% positive) and blood (20% positive). The predominant organisms isolated at both hospitals were *E. coli*, Enterococcus spp. and *S. aureus*. *E. coli* accounted for 50% of the total organisms from urine at UCLH and 89% at RFH. Resistance rates to *E. coli* were: trimethoprim 57% and 40%, amoxicillin 50% and 43% (UCLH and RFH respectively).




**Conclusions:** The study provides an insight into prescribing habits at two teaching hospitals, and highlights differences in resistance patterns and antibiotic use. The data derived may help inform local audit and aid in the assessment of prescribing initiatives. Further work is needed to establish accurate resistance patterns in order to guide local antibiotic policies.

## P1476

## Comparison of different antibiotic consumption measurement methods in large multidisciplinary hospital

E. Pujate, I. Apine, U. Dumpis *(Riga, LV)*

**Objectives:** Antibiotic selection pressure is determined by the total amount of antibiotics, number and density of patients treated with antibiotics in the particular geographical area. Several antibiotic consumption detection methods should be combined in the hospital setting. Our objective was to evaluate efficacy of different approaches in large multidisciplinary hospital.

**Methods:** Point prevalence studies were repeated annually at 2002–2004 in Stradins University Hospital (1000 beds) in Latvia. All patients receiving antibiotics on the day of the survey were identified and their medical records were reviewed. Data on antibiotics, dose and route of administration were collected. In addition, annual data on antibiotics dispensed to the departments were collected from pharmacy. Total used grams for each antibiotic were expressed into defined daily doses (DDD-WHO). Bed days (BD) and admission days (AD) were used as denominators.

**Results:** Table 1 Total use of antibiotics in Stradins University Hospital 2002–2004.

| Year | Point prevalence study | | | Pharmacy study | |
|---|---|---|---|---|---|
| | Patients on antibiotics / 100 patients | Antibiotics / 100 patients | DDD / 100 patients | DDD / 100 BD | DDD / 100 AD |
| 2002 | 23.75 | 30.72 | 34.54 | 54.86 | 438.49 |
| 2003 | 26.21 | 32.58 | 35.89 | 57.18 | 438.22 |
| 2004 | 23.38 | 30.92 | 35.51 | 57.25 | 450.19 |

The most commonly used antibiotic groups in the pharmacy study were 1st generation cephalosporins (13.35 DDD/100 BD in 2002, 11.6 in 2003, 10.8 in 2004) and penicillin's with extended spectrum (11.20, 11.98, 13.15) followed by fluoroquinolones (6.26, 8.13, 8.69) and metronidazole (4.42, 4.59, 5.61). There was no significant difference between distribution of different antibiotics from prevalence and pharmacy studies if calculated in DDDs. In contrast, distribution of antibiotics calculated per patient in the prevalence study was quite different; 1st generation cephalosporins (8.71%, 8.63%, 5.84% in 2002, 2003, 2004 respectively) and fluoroquinolones (3.05%, 6.69%, 5.63%) with smaller proportion of extended spectrum penicillins (4.36%, 3.88%, 3.93%) and metronidazole (4.03%, 3.56%, 4.68%).

**Conclusions:** There were no differences in the distribution of antibiotics calculated in DDDs per bed days and admissions. Distribution of antibiotics in annual pharmacy studies and point prevalence studies if calculated in DDDs were also similar. In contrast, the prevalence data expressed as a proportion of patients with selected antibiotics showed quite different distribution. Studies using only DDDs may overestimate use of certain antibiotic groups in our setting where WHO DDDs are significantly different from actual PDDs used.

## P1477

## A study of prescribing patterns and errors of antibiotics in a Saudi hospital

M. Al-Jamal, M. Al-Barrak *(Riyadh, SA)*

**Background:** The term ''prescribing patterns'' has been used extensively in studies to describe different aspects of the prescribing process. Antibiotics as well as other drugs are prescribed for the purpose of achieving definite therapeutic outcomes that improve a patient's quality of life while minimizing risk. In the clinical literature, the incidence of antibiotics prescribing errors ranges between 0.5% and 18.8%.

**Objective:** In this study we will address antibiotics prescribing patterns and the incidence of prescribing errors in a tertiary hospital and the potential relationship between them.

**Methods:** A prospective study of all prescriptions in a 3-month period (June to August 2003) in a tertiary hospital has been analysed. The hospital provides both primary and secondary levels of care. Criteria used include frequency of selected prescribed drugs, average number of items per prescription, compliance to the hospital formulary, frequency of prescriptions for antibiotics, generic prescribing and diagnosis. The prescribing patterns and the incidence of prescribing errors were performed.

**Results:** Total number of prescriptions for the 3-month study was 24,404. Emergency Room (ER) and primary care have the highest number of prescriptions (37.1%). The average number of items per prescription is 2.1. The most prescribed drugs by primary care (25.3% errors), emergency are antibiotics (28.2%), medicine (3.7), ophthalmology (22.3), gynaecology (7.8), and paediatrics (17.8). The prescription errors were 13.6% in primary care and 22.3% in emergency department.

**Discussions and conclusions:** Over 24000 prescriptions were included in this study. The incidence of prescribing errors was 18.8% the average number of items per prescription was 2.1. Total Prescription errors are also related to frequency of prescribing antibiotics. There was a relation between prescribing of antibiotics and prescribing of trade names ($p < 0.01$), and compliance to the hospital formulary ($p < 0.001$). Several factors influence prescribing patterns and variations in prescribing rates has been identified. These include general physician behavior, differences in morbidity and mortality patterns, social perception toward illness, and physician clinical skills, experience and qualification, as well as physician continuing education and training. Special antibiotic prescribing guidelines and restrictions should target primary care and emergency department physicians.

## P1478

## Effect of a policy for restriction of selected classes of antibiotics on antimicrobial drug cost and resistance

M. Falagas, I. Bliziotis, A. Michalopoulos, G. Sermaides, V. Papaioannou, D. Nikita, N. Choulis *(Athens, GR)*

**Objectives:** The introduction of a new policy for the restriction of selected classes of antibiotics offers the opportunity to study its effect on antimicrobial consumption and cost.

**Methods:** After the instructions of the National Organization of Pharmaceutical Agents a new policy regarding the use of antimicrobial agents was introduced in our hospital on July/01/2003. Specifically, quinolones, 3rd and 4th generation cephalosporins, carbapenems, monobactams, glycopeptides, oxazolidinones, and streptogramins were considered as ''restricted'' antibiotics that could be used with the approval of one of the Infectious Diseases specialists. We analysed the effect of the policy on the consumption and cost of antibiotics as a group and of specific classes, adjusted for the patient load, as well as on the antimicrobial resistance of the isolated bacteria.

**Results:** An 18% and 16% reduction of the adjusted consumption and cost, respectively, of the restricted antibiotics was accomplished during the first trimester after the implementation of the new policy. However, this was accompanied by a 54% and 44% increase of the adjusted consumption and cost, respectively,

of the non-restricted antibiotics. The logistic regression model we performed showed that the new policy had an independent positive effect on the in vitro antimicrobial susceptibility of *Pseudomonas aeruginosa* (p = 0.051) but not of Acinetobacter baumannii and *Escherichia coli* isolates.

**Conclusion:** Our data suggest that there are considerable limitations of the programs aiming to reduce the consumption of restricted antibiotics through the approval of their use by specialists, at least in a proportion of settings. Education programs that aim to involve the medical staff directly responsible for the care of patients in voluntary decisions regarding the appropriate use of antimicrobial agents may have more profound and sustainable success, and thus, deserve to be studied.

## P1479

## Estimating hospital versus ambulatory care consumption of antibiotics in southwestern Germany

K. de With, M. Steib-Bauert, H. Schröder, K. Nink, W.V. Kern *(Freiburg, Bonn, DE)*

**Objective:** Preliminary data from the ESAC (European Surveillance of Antibiotic Consumption, www.ua.ac.be/ESAC) project indicated that the proportion of hospital care (HC) antibiotic use on total antibiotic use in several European countries ranges between 5 and 20%. Only few countries, however, have so far been able to report representative countrywide information on both HC and ambulatory care (HC) antibiotic consumption. We estimated AC versus HC consumption of antibiotics for one of the 16 German federal states located in the Southwestern part of the country with a 10.5 million population.

**Methods:** Data on HC consumption (ATC class J01) were obtained from a convenience sample of acute care general hospitals (n = 42), extrapolated to state-wide consumption (using official statistics for the total state-wide 152 general plus 77 special non-psychiatric/non-paediatric/non-radiotherapy hospitals), expressed in defined daily doses per 1000 inhabitants and day (DID), and finally compared to ambulatory care antibiotic use density in the same region and period of time (years 2001 and 2002).

**Findings:** The estimated state-wide HC consumption of antibiotics was 2.1 DID (95% confidence interval, 1.9 to 2.2 DID) in both years. State-wide antibiotic consumption in the AC setting during the same time was 12 DID (~85% of total consumption). AC consumption of fluoroquinolones (1.1–1.2 DID, 79%) and macrolides/clindamycin (1.8 DID, 95%) made up a major proportion of total use of that drug classes.

**Conclusions:** Hospital antibiotic use in the Southwestern part of Germany can be estimated to contribute ~15% to overall antibiotic consumption in the general population.

## P1480

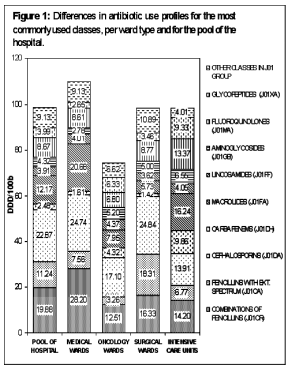
## Antibiotic use profile and temporal trends during a 5-year period at a Greek university hospital: implications for antibiotic policy changes

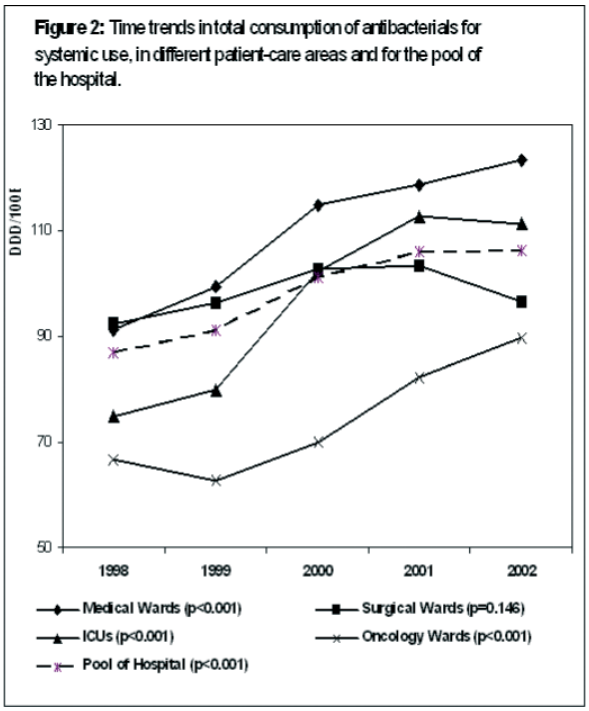
E.I. Kritsotakis, P. Assithianakis, P. Kanellos, N. Tzagarakis, M.C. Ioannides, A. Gikas *(Heraklion, GR)*

**Objectives:** To investigate the profile and temporal trends of inpatient antimicrobial use over a 5-year period at the University Hospital of Heraklion Crete, Greece. Further, to examine the way in which frequency of data collection and stratification by different patient-care areas provides guidance to antibiotic policy changes.

**Methods:** Retrospective monitoring of antimicrobial consumption was carried out according to the WHO anatomic therapeutic chemical classification (ATC) and defined daily dose (DDD) measurement methodology. Pharmacy records were used to obtain aggregate data of drug deliveries to individual wards. Results were expressed as usage density rates in DDDs per 100 bed-days (DDD/100BD). Linear regression was used in order to assess the statistical significance of a temporal trend in usage densities.

**Results:** During 1998–2002, hospital-wide antimicrobial use (ATC group J01) significantly increased by 22%, from 86.97 to 106.24 DDD/100BD. The annual average increase rate was 4.8 DDD/100BD. Stratification by clinical service demonstrated differences in the intensity and profile of class use, as well as varying temporal trends (figures 1, 2). Pooled usage rates in DDD/100BD, overall percentage increases and annual average increase rates were respectively 109.97, 35.6%, 8.1 for Medical wards; 98.21, 48.7%, 9.1 for ICU's; and 74.46, 34.3%, 5.7 for Haemato-oncology wards. Surgical wards had a fairly constant usage rate (98.36). A shift towards the newer broad-spectrum antibiotics to the detriment of the older penicillins and cephalosporins was noted in all hospital areas.



**Conclusion:** Surveillance of aggregate data on the consumption of antimicrobials using the ATC/DDD system provided a clear picture of the profile of hospital usage. Monthly data over a sufficient surveillance period allowed the assessment of temporal trends. Stratification of usage rates by clinical service allowed areas of concern to be specified. Thus, surveillance of monthly antimicrobial consumption rates stratified by patient-care area can provide a simple, rapid and efficient tool for triggering antibiotic policy changes in the hospital and targeting more detailed quality-of-use audits.

## P1481

## Appropriate use of aminoglycosides: the impact of an antibiotic control team

C. Rioux, P. Lesprit, J.R. Zahar, A. Hulin, A. Bernier-Combes, C. Brun-Buisson, E. Girou *(Créteil, Paris, FR)*

**Objectives:** Many factors are involved in the appropriate use of aminoglycosides (AG), such as modalities of administration, serum monitoring and duration of treatment. We assessed prospectively the risk factors and the impact of an antibiotic control team on the appropriateness of AG prescriptions.
**Methods:** In a setting of a restricted delivery system of AG in our hospital, we first performed an observational audit (OA) to assess the appropriateness of prescriptions including justification of prescribing, adequacy of drug choice, adequacy of administration modalities, modalities of serum monitoring and duration of treatment. After implementation of specific guidelines hospital wide, we then performed an interventional audit (IA) where an antibiotic control team could interfere when AG prescriptions were considered inappropriate. Appropriateness of AG prescriptions between the 2 audits was then compared.
**Results:** 200 prescriptions were analysed. During the IA, 32% of prescriptions were modified by the control team. As compared to the OA, prescriptions in the IA were significantly more appropriate with regard to treatment duration (73 vs 55%, p = 0.009) and serum monitoring (61 vs 40%, p = 0.05). Median treatment duration was shorter in the IA (4 d) than in the OA (6 d) (p < 0.0001). A logistic regression model showed that risk factors for appropriate treatment duration were (adjusted OR, 95% CI, p value): hospitalization in intensive care unit (4.39, 1.57–12.2, 0.005), polymicrobial infection (4.08, 1.38–12.08, 0.01) and antibiotic control team intervention (2.41, 1.23–4.72, 0.01).

**Table:**

| Appropriate criteria | OA (n = 100) | IA (n = 100) | P value |
|---|---|---|---|
| AG use justified | 93 | 92 | 0.79 |
| AG choice | 98 | 93 | 0.08 |
| Administration mode | 68 | 66 | 0.76 |
| Serum monitoring | 40 | 61 | 0.05 |
| Treatment duration | 55 | 73 | 0.009 |

**Conclusions:** Despite a restricted delivery system, AG use was frequently associated with excessive treatment duration and errors in monitoring modalities. Reinforcing practice guidelines through direct counselling improved appropriateness of prescriptions.

## P1482

## Hospital antibiotic consumption in southern and eastern mediterranean countries: preliminary results from the ARMed project

P. Zarb, M.A. Borg, H. Goossens, M. Ferech *for the ARMed Participants*

**Introduction:** ARMed is an international research project investigating antimicrobial resistance and consumption in 7 southern and eastern Mediterranean countries through the collection of comparable and validated antimicrobial resistance data as well as information about antibiotic consumption patterns and infection control initiatives.
**Objectives:** The consumption part of the study aims to collect data on antimicrobial use within participating hospitals in the region, which information is currently unavailable.
**Methods:** Data collection is planned over a 24-month period using Anatomical Therapeutic Chemical (ATC) classification, a validated methodology adopted by the European Surveillance of Antimicrobial Consumption (ESAC – www.ua.ac.be/esac). 23 hospitals are participating: Cyprus (5 hospitals); Egypt (7); Jordan (1); Malta (1); Tunisia (3); Turkey (6). Results are expressed in DDD/1000 bed-days.
**Results:** Data from 2004, the first year of data collection, indicates that Turkish hospitals seem to show the lowest overall consumption [230–480 DDD/1000Bed Days], whilst the Cypriot hospitals show highest values [2900–7500 DDD/1000Bed Days]. The most common antibiotics used are the beta-lactams, especially the penicillins although in Jordan and Turkey cephalosporin consumption is very close to the penicillins. Broad-spectrum penicillins [J01CA] are the mostly utilised penicillins in Cyprus, Jordan and Tunisia whereas in Malta and Turkey the combination penicillins [J01CR] are the most widely used. There is more variability where cephalosporin consumption is concerned. Cyprus utilises mainly first generation, Jordan and Malta the second generation. In Egypt, Tunisia and Turkey there is significant variability between hospitals; nevertheless use of third generation cephalosporins appears to be significant.
**Conclusion:** A significant variability was evident between countries. This is likely to be multifactorial depending on the antibiotics licensed in a country, the national and/or hospital formulary, the type of hospital as well as any antibiotic donations that are relevant in some of the study hospitals. Nevertheless, the preliminary results suggest that trends within hospitals of the same country tend to be similar. Furthermore, the region as a whole seems to utilise a considerable quantity of broad-spectrum antimicrobials. This can be a factor in the high prevalence of resistance already documented in the study.

## P1483

## Russian pharmacoepidemiology study of the antibiotic prescription during pregnancy

V. Rafalskiy, R. Tchilova, A. Ischenko, I. Toropova, I. Nedorezenyuk, E. Maneeva, O. Suplotova, N. Antonovitch, N. Shevchenko *(Smolensk, Moscow, Yakutsk, Vladivostok, Barnaul, Krasnodar, RU)*

**Objectives:** To evaluate the current clinical practice of prescription of antimicrobials in pregnancy women in Russia.
**Methods:** We carried out multicentre1 retrospective pharmacoepidemiological study based in 7 centers (Moscow, Smolensk, Vladivostok, Petropavlovsk-Kamchatski, Yakutsk, Krasnodar, Barnaul). The data were taking from obstetrics and gynecology clinics in 2003–04 years, 1464 cases of were analysed.

**Results:** Mean age of the patients was 25.6 ± 6.0 (min – 14, max – 52) years, mean gestational ages at admission to hospital was 27.0 ± 10.8 (3 to 42) weeks. Most often (77.5%) infection was community acquired and 2.1% – nosocomial, in 20% patients there was not to estimate origin of the infection. The most prevalent infections during pregnancy in Russia was urinary tract infections – 39.9%, STD – 19.3%, candidiasis – 17.4%, RTI – 6.6% Therefore the most interest was analysing the antibiotic prescription for UTI in pregnancy (table). In 28% cases were used topical (intravaginal) antimicrobial administration. Most often of topically administrated antimicrobials (19.02% of all prescriptions) were prescribed combined drugs included antibacterials and amtimycotics. In 80.98% cases antimicrobials were prescribed systemically. Mostly prescribed antimicrobials were beta-lactams (16.2% for outpatients and 57.7% for inpatients), ampicillin was prescribed more often (4.6% for outpatients and 31.5% for inpatients). Amoxicillin + clavulanic acid was prescribed in 6.4% of outpatients and 5.6% inpatients pregnant women with UTI. Cephalosporins were prescribed in 5.2% and 20.6% for outpatient and inpatient UTI (mainly III- and Ist generations). Mitroimidazoles – 1.8–7.4% (in general metronidasole), nitro-furanes – 8.2–15.0%, aminolglycosides – 4.6–3.8% were prescribed quite often but unjustified. Other antimicrobials (fluoroquinolones, doxicicline, antiviral drugs, antifungals) were prescribed relatively rarely. Despite the fact that most prescribed drugs were class B by FDA, 5.8% all antimicrobials prescribed to pregnancy were class C, 0.8% class D and 9.1% were unclassified.

| *Antimicrobials* | *Outpatient n=220* | *Inpatient n=360* |
|---|---|---|
| Ampicillin | 4.6 | 31.5 |
| Amoxicillin/clavulanate | 6.4 | 5.6 |
| Cephalosporins I | 1.9 | 9.7 |
| Cephalosporins II | 2.3 | 0.9 |
| Cephalosporins III | 1.0 | 10 |
| Aminolycosides | 4.6 | 3.8 |
| Quinolones | 6.8 | 1.5 |
| Nitrofuranes | 8.2 | 15.0 |
| Co-trimoxasole | 2.7 | 0.3 |
| Tetracyclines | 2.7 | 0 |
| Nitroxolonine | 8.6 | 4.7 |
| Chloramphenicol | 2.7 | 0 |
| Nitroimidasoles | 1.8 | 7.4 |
| Fosfomycin | 11.8 | 0 |
| Antifungals | 0 | 6.8 |
| Other | 0 | 2.7 |

**Conclusions:** Most often prescribed antimicrobials for UTI (the most prevalent infections during pregnancy in Russia) are beta-lactams and combined topical antibacterials. In 15.7% cases were prescribed antimicrobials of class C, D or unclassified by FDA. In 25% outpatient and 53.6% inpatient were used antibiotics with low in vitro activity for uropathogens.

## P1484

## Curbing pneumococcal resistance in orphanages: interventions on the basis of prospective surveillance of nasopharyngeal isolates

O.U. Stetsiouk, R.S. Kozlov, J.A. Poupard, L.S. Stratchounski *(Smolensk, RU; Philadelphia, US)*

**Objectives:** To study the dynamics of the antibiotic usage in children from orphanages located in different Russian cities as the result of interventions with the increased use of the most active antimicrobials and restrictions on use of the least active.
**Methods:** The study was performed in 12 orphanages (1–12) from 5 cities of European Russia (Moscow, Saint-Petersburg, Smolensk, Karachev, Bryansk). Use of antimicrobials during the previous 12 months was analysed upon reviews of medical records of 743 children <7 years in 2003. Appropriate recommendations on predominant use of selected beta-lactams (e.g. amoxicillin/clavulanate – AMC) with restriction of antimicrobials of other classes (e.g. co-trimoxazole – SXT) were made where applicable on the basis of the expert analysis of antibiotic usage and pneumococcal nasopharyngeal resistance rates. Repeated antibiotic usage analysis was performed 7 months later in 2004 upon reviews of medical records of 752 children <7 years.
**Results:** Total usage of antimicrobials increased 1.4 times in 2004 (1,065 courses per 752 children in 7 months equivalent to 2.44 courses per child per year) in comparison with 1,301 courses per 743 children in 2003 (1.75 courses per child per year). Beta-lactams were the most frequently prescribed antibiotics (70.6% and 72.1% of all prescriptions in 2003 and 2004 respectively). Among these the predominant ones in 2003 were: ampicillin/oxacillin – AM/OX (17.9%), AMC (16.1%), cefazolin (14.1%), cefotaxime (13.0%) and ampicillin (11.2%); in 2004: AMC (18.4%), AM/OX (13.2%), cefazolin (10.4%), penicillin (PEN) (8.8%) and cefotaxime (7.7%). Usage of AMC increased from 19 to 45 courses/100 children/year, cephalosporins from 42 to 67 courses/100 children/year. Usage of macrolides decreased 2 times: from 20 to 10 courses/100 children/year; at the same time usage of aminoglycosides and SXT increased almost twice: from 10 to 19 and from 10 to 18 courses/100 children/year in 2004 vs 2003.
**Conclusions:** The recommended intervention resulted in 2.4 times increase of AMC usage in 2004 vs 2003 with no detectable increase of resistance to PEN and aminopenicillins. Enhanced use of cephalosporins led to increase of resistance to these drugs. In spite of recommendations to restrict usage of AM/OX, aminoglycosides and SXT, the analysis showed that these antimicrobials still accounted for 13.2%, 7.8% and 7.5% of all prescriptions, respectively, thus dictating the need for further enforcement measures.

## P1485

## Antibiotic consumption in ambulatory care in Latvia, 2004

S. Berzina, M. Ferech, G. Ozolins, H. Goosens *(Riga, LV; Antwerp, BE)*

**Objectives:** To collect data on antibiotic consumption in ambulatory care (AC) in Latvia according to the ESAC data collection protocol. ESAC (European Surveillance of Antimicrobial Consumption, granted by DG SANCO of the EC) is an international network of national surveillance systems, aiming to collect reliable and comparable data on antibiotic consumption in Europe.
**Methods:** The data on AC antibiotic consumption for 2004 have been collected using ATC/DDD classification (WHO, version 2005) and expressed in Defined Daily Doses per 1000 inhabitants per day (DID). Data were obtained from the State Medicinal Agencybased on the reports of the wholesalers for AC.
**Results:** The overall use of antibiotics in AC was 11.7 DID in 2004, which positions Latvia to countries with comparatively low antibiotic consumption in Europe. The mostly used class of antibiotics in AC were penicillins with extended spectrum (mainly amoxicillin) – 3.97 DID (33.9%). Other frequently used antibiotics were tetracyclines (mainly doxycycline), representing 2.41 DID (20.6%), combinations of penicillins/with beta-lactamase inhibitors (essentially co-amoxiclav) – 1.18 DID (10.1%), macrolides (mainly clarithromycin) 0.85 DID (7.26%), fluoroquinolones (essentially ciprofloxacin) – 0.84 DID (7.17%)

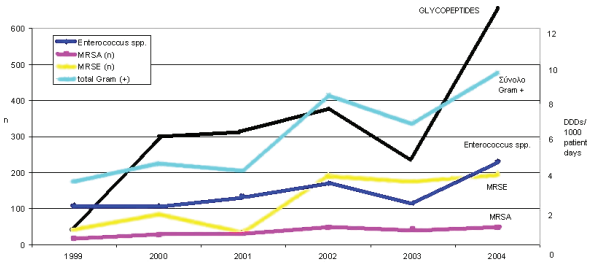
and combinations of sulphonamides and trimethroprim, incl.derivatives – 1.02 DID (8.71%). The most frequently used antibiotics in AC in Latvia, in 2004, were amoxicillin (3.97 DID), doxycycline (2.41 DID), and co-amoxiclav (1.18 DID).
**Conclusions:** Valid data on outpatient antibiotic use in Latvia has been for the first time collected and delivered to European Surveillance of Antimicrobial Consumption. This allows international comparison of the pattern of antibiotic consumption in Latvia with other European countries.

## P1486

## Trends in glycopeptide antibiotics consumption over a 6-year period in a general hospital, Athens, Greece

C. Loupa, G. Kouppari, H. Papadaki, E. Divari-Katsiki, I. Koti, K. Papaefstathiou, M. Tsimoraga, A. Karaitianou, M. Zoumberi, M. Lelekis *(Athens, GR)*

**Introduction:** Glycopeptide use is under restriction in Hellenic hospitals since late '80s. The aim of our study was to record trends in their consumption over the last 6 years in our Hospital (''A. Fleming'' General Hospital – 300 beds) and to correlate these data with the numbers of important Gram (+) strains isolated in our hospital during the same time period.
**Methods:** We measured glycopeptide use for the period 1999–2004 by using data from the pharmacy computer. Consumption was expressed as DDDs/1000 patient days (ABC calc 3.0). Furthermore we correlated these data with data from the microbiology department concerning numbers of MRSA, MRSE and enterococci isolated during the same period.
**Results:** Glycopeptide consumption was 1.3, 6.1, 6.3, 7.9, 4.5 and 13 DDDs/1000 patient days for the years 1999, 2000, 2001, 2002, 2003, 2004 (900% increase). At the same time the cumulative number of MRSA, MRSE and enterococci isolated were 175, 224, 207, 415, 338, 479 respectively (170% increase). When both types of data were put on the same graph, glycopeptide consumption correlated well with the number of important Gram(+) strains isolated (figure). Furthermore VRE percentage among enterococci was 0, 0, 3.0, 0.6, 3.5, 1 for the study years respectively. It is worth noting that 87% of our MRSA strains were sensitive to rifampin, 83% to clindamycin, 74% to cotrimoxazole, 80% to clindamycin + rifampin and 74% to cotrimoxazole + rifampin. Linezolid has not been introduced in our hospital yet.
**Conclusions:** (A) Despite the restriction policy, a tremendous increase in glycopeptide use was recorded in our hospital during the study period and this correlated to the number of the important Gram(+) strains isolated; (B) Nevertheless, VRE is not a significant problem for our hospital yet; and C. The huge increase in glycopeptide use could be avoided at least in part, since other, older and simpler antibiotics could substitute for glycopeptides in many cases.




## P1487

## An audit of linezolid use in a university teaching hospital, Galway, Ireland

S. Mcnicholas, A. Barber, G. Corbett-Feeney, M. Cormican *(Galway, IE)*

Linezolid is the first of a new class of antibacterial drugs, the oxazolidinones. It has inhibitory activity against a broad range of Gram positive organisms, including meticillin-resistant *Staphylococcus aureus* (MRSA), glycopeptide-intermediate *Staphylococcus aureus* (GISA), vancomycin-resistant enterococci (VRE) and penicillin-resistant *Streptococcus pneumoniae*. Linezolid is licensed for the treatment of pneumonia and skin and soft tissue infections.
**Objective:** To audit linezolid use over a six-month period among the in-patient population of a 504-bed teaching hospital that includes most medical and surgical specialties with the exception of nephrology, rheumatology and orthopaedics.
**Methods:** A prospective audit was carried out of the prescribing of linezolid to in-patients from October 2004 to April 2005. The ward pharmacist recorded the details of all patients who were prescribed linezolid. A chart review was performed to assess the profile of patients prescribed linezolid, clinical and microbiological indications for treatment, adherence to treatment guidelines and documented adverse events.
**Results:** Over the 6-month period 53 courses of linezolid were prescribed. Fifty two percent of the patients for whom linezolid was prescribed were from surgical specialties; half of these patients were under the care of one surgeon. Pneumonia was the clinical indication for use in 32% of cases and soft tissue infection in 36% of cases. The microbiological indication was clear in 64% of cases where MRSA or VRE had been isolated. In 36% of cases therapy was either (1) empiric with no significant organisms isolated prior to prescription of linezolid or (2) therapy was directed against an organism that could have been treated with an alternative agent. Duration of treatment exceeded 10 to 14 days in 40% of courses. An adverse event was recorded in the case of only one course of linezolid.
**Conclusion:** In more than a third of cases linezolid use was prescribed without clear justification. Avoidable use of linezolid is associated with increased costs and risks of acquired resistance.

# Professional and public health issues

## P1488

## The future of clinical microbiology in Belgium

W. Laffut, J. Van Eldere *(Lier, Leuven, BE)*

**Objective:** In winter/spring 2003–2004, the Belgian Society for Infectiology and Clinical Microbiology (BVIKM/SBIMC) conducted an inquiry on the future of infectiology and clinical microbiology (CM). The aim was: to obtain data on the current organisation of infectious diseases management in Belgium in and outside the hospitals, to identify shortcomings, opportunities for better organisation, to map the aspirations and view of the future of clinical microbiologists, to identify key issues in the formation of future clinical microbiologists.
**Methods:** The inquiry was done by specialists in training in clinical biology. An extended questionnaire was sent to all

participants prior to on an oral interview during which the interviewer filled in the answers.
**Results:** A total of 62 CM specialists completed the inquiry. This represents approximately 25% of the national quorum. Mean age was 48 years (32–63). 22 of the 62 interviewed CM specialists worked full-time with more full-time employment in hospital labs (HL). Next to routine microbiology, other activities performed by CM specialists are mainly the other domains of clinical biology, hospital hygiene and to a lesser extent quality control and lab management. Almost two thirds of the interviewed CM specialists believes that their training hasn't prepared them properly for the tasks they are performing now. Most desired changes include more emphasis on the clinical aspect of infectious diseases and on antibiotic treatment counselling. CM does not exist as a separate speciality in Belgium but is included in the 'clinical biology' speciality training. The majority of the respondents thinks that CM should become a sub-speciality (still part of clinical biology) but with a specific minimal training that needs to be defined. The majority of the CM specialists also believes that CM can share lab infrastructure with other disciplines and that the essential aspect of CM lies predominantly in the medical expertise.
**Conclusion:** CM training should put more emphasis on the clinical aspect of infectious diseases and on antibiotic treatment counselling. The majority of the respondents feels that CM should become a sub-specialty (still part of clinical biology), with a well defined training curriculum.

## P1489

## Analysis of 6871 infectious disease consultations

Y. Wiener-Well, Y. Schlesinger, D. Raveh, A.M. Yinnon
*(Jerusalem, IL)*

**Objectives:** Since more than 10 years, all infectious disease consultations have been recorded in a computerized database (Epi Info 6.04d, CDC). Here we report on 1163 consultations of a fellow, conducted during 1 year, compared with 5708 consultations conducted by two veteran board-certified infectious disease consultants during the same period.
**Methods:** We analysed computerized consultation records, including demographic details of patients; referring department; initiative for, route and purpose of the consultation; and recommendations; and compared between the different consultants.
**Results:** A larger percentage of veterans' compared to the fellow's consultations, were requested by attending physicians (72% vs. 50%, $p < 0.001$), while follow-up (15% vs. 23%, $p < 0.001$), laboratory results (11% vs. 23%, $p < 0.001$) or prescription for a restricted antimicrobial agent (1.3% vs. 3.3%, $p < 0.001$) were more prevalent in fellow consultations. The fellow had a higher rate of additional consultations (in which the patient was seen more than once) (40% vs. 30%, $p < 0.001$), and performed more bedside consultations (68% vs. 47%, $p < 0.001$) or consultation by curb side discussion (25% vs. 13.2%, $p < 0.001$), and less consultations by telephone (6.6% vs. 40.1%, $p < 0.001$). Diagnosis and prophylaxis were more often the purposes of the veterans' consultations (44.8% vs. 36.1%, $p < 0.001$, 3.9% vs. 2.1%, $p < 0.01$, respectively), and they also offered new diagnoses more frequently ($p < 0.005$). The veteran consultants more often conducted consultation for community-acquired infections (53% vs. 44%, $p < 0.001$), and more often started antibiotic treatment (19% vs. 8.9%, $p < 0.001$).
**Conclusions:** Significant differences were detected between consultations conducted during the first year of a fellow compared to those of veteran infectious disease consultants. These differences reflect the changing demands and activities in the consultant's work as experience and knowledge accumulate. Periodic analysis of computerized data of consultations facilitates supervision as well as direction of consultants' work, addressing issues such as antibiotic use and patterns of microbial resistance.

## P1490

## Bridging the gap between health care and public health; capacity building in infectious disease control

J. Doosje, M. Jambroes, A. Timen, J. van Steenbergen, V. van Casteren *(Utrecht, Bilthoven, NL; Brussels, BE)*

**Objectives:** In recent years, the European Union (EU) has developed and supported many activities in the field of communicable diseases. These activities not only concern surveillance networks of specific infectious diseases (e.g. Enter-net for Salmonella and *Escherichia coli* infections, EWGLI for Legionella infections, EISS for Influenza infections), but also EU training programmes like EPIET (European Programme for Intervention Epidemiology Training) and a EU communicable disease bulletin. Even more recent are the EU's initiative BICHAT to improve preparedness and response to bioterrorism, and the development of a EU CDC. Moreover, a major part of the new programme of Community action in the field of public health (PH) (2003–2008) concerns ID, with not only a commitment to improve information and information exchange, but particularly to strengthen the international rapid response capacity.All this, to illustrate the importance of IDC on the EU agenda.
**Methods:** The European Public Health Association (EUPHA) is an umbrella organisation for PH associations in EU. In 2003 EUPHA has created an EUPHA section IDC bringing together EUPHA members with expertise in this field and representatives of the various above mentioned EU initiatives in order to: promote and strengthen research in the field of IDC; provide a platform for the exchange of information, experience and research in IDC; bring together researchers, PH practitioners and policymakers active in IDC; encourage joint activities in IDC; and improve IDC training.
**Results:** By now the section has 307 members from 50 different countries. As of 2005 the section is represented in the ECDC advisory forum. Different section activities: Organising 8 workshops, a breakfast meeting and a pre-conference meeting on timely IDC topics during EUPHA conference. Organising a section workshop during ESCMID conference 2006. Participation in different EU projects: SPHERE (=Strengthening Public Health Research in Europe) project, INPHECT (Intelligent Network for Public Health by using Enhanced Communication Technologies) proposal. Editorial in the EU journal of PH.
**Conclusion:** Since 2003 the EUPHA section on IDC has become a mature multidisciplinary platform of IDC specialists in Europe. For an adequate response on emerging infectious diseases it is of utmost importance to closely collaborate with different specialists in EU. Therefore we promote a strong collaboration between the EUPHA section on IDC and the ESCMID.

## P1491

### EUREGIO MRSA-net Twente/Münsterland: fighting (CA-)MRSA international under different cultural perspectives

F. Verhoeven, J.E.W.C. van Gemert-Pijnen, M.G.R. Hendrix, A.W. Friedrich, M.F. Steehouder *(Enschede, NL; Munster, DE)*

**Objectives:** To establish a cross-border Dutch-German network (www.mrsa-net.org) providing a user-friendly knowledge centre for hospitals, public health authorities, GPs, nursing homes and laboratories. Primary purpose is to aid in the reduction of MRSA-rates and limit the cross-border transmission of MRSA. Guidelines and their implementation play a significant role in reaching these aims. Cross-border (CA-) MRSA guidelines will be redesigned according to international standards and socio-cultural differences between the nations.
**Methods:** Based on quality standards for safety and healthcare documentation used in high risk chemical organizations, a framework for a systematic content analysis of national MRSA-guidelines was developed. National guidelines were analysed on the basis of this framework.
**Results:** A content analysis of the current national MRSA-guidelines showed five dominating MRSA-perspectives: rule-, expert-, risk-, demand- and community-driven. German guidelines are mainly dominated by the rule- and expert-driven perspectives (guidelines are literally derived from law and follow the infection transmission route), in contrast to the Dutch which focus on the demand of the user and the community (addressed to public health and acceptability of guidelines by users).
**Conclusion:** The analysis showed that the fact that there are different guideline-perspectives results in an enormous, confusing set of guidelines. The management and use of guidelines becomes uncontrollable and leads to an illusory organisation where healthcare workers don't act in accordance with the guidelines and start applying their own insights. This might lead to cost-increasing and contrasting situations. To implement guidelines successfully in a cross-border situation, a cultural and technical synchronisation alongside an integrated approach of the different perspectives of guidelines is necessary, inline with the current disease management models. Further research about the redesign and the evaluation of those guidelines in practice will help achieving this.

## P1492

### Prevention of rabies in Georgia

R. Tsiklauri *(Tbilisi, GE)*

**Background:** Animal bites are a common but under recognized public health problem. It has been estimated that there are 8–10 000 bites each year in Georgia, and based on an average visit and post-exposure treatments cost at list $120 000 per year. Despite the frequency and expense of these injuries, there is little information about the incidence of animal bites because of a lack systematic reporting and a lack of measurement of the quality and completeness of reported data.
**Objectives:** To investigate animal bites and rabies reported cases, revealed unreported cases, analyse and based on study results find more effective epidemiological measures of animal bites and deaths (due to rabies) prevention in Georgia.
**Methods:** The capture-recapture method was used, along with log-linear modelling. For sources were used to identify victims: policlinic/ambulatory reports, hospital reports, animal control reports and victim reports.
**Results:** In 1980–2001 years 150 700 dog and other animal bites were reported. The capture-recapture method estimated that there were 146 200 unreported bites. During these period 118 deaths due to rabies was registered in Georgia and 67 (57%) cases among them have been registered during the last 6 years. The reasons of fatal cases were untreated (47%), uncompleted treated (34%) and late began post-exposure treated (19%) cases of bites (mostly dog bites). About 56% of bitted persons did not know about rabies and it's prevention measures. About 32% had incorrect information about prevention and only 12% of them knew epidemiological and clinical aspects of disease. About 16% of physicians who were responsible on quality post-exposure treatment had not an adequate knowledge.
**Conclusion:** Dog and other animal bites are common but preventable injuries. To improve surveillance and prevention of rabies in Georgia, the focus should be on educating the general public about the serious consequences of animal bite injuries and developing the animal's vaccination strategy.

# Pharmacoeconomics and electronic resources

## P1493

### The expected economic burden of methicillin-resistant *Staphylococcus aureus* in complicated skin and skin structure infections: a modelling approach

A. Kuznik, R. Mallick, D. Weber *(Collegeville, Chapel Hill, US)*

**Objective:** To model the expected rate of clinical failure of initial empiric therapy and economic burden likely to be associated with the increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in patients hospitalised with complicated skin and skin structure infections (cSSSI) in the United States.
**Methods:** Using published data on (1) the prevalence of MRSA and other bacterial pathogens causing cSSSI in the US, (2) the in-vitro susceptibility rates of commonly used regimens in cSSSI in the US in relation to the most pervasive pathogens identified above, and (3) estimated costs of failure of initial, empiric treatment from a recent study of a large US multi-hospital database, we developed a model to predict the expected clinical and economic impact of increasing prevalence of MRSA. Specifically, clinical failure of 5 of the more commonly used initial regimens in cSSSI was modeled in terms of their in-vitro susceptibility rates with respect to MRSA, weighted by MRSA prevalence. Varying the rate of MRSA further yielded projected clinical failure rates and costs attributable to increasing levels of methicillin resistance over time.
**Results:** Given current 55% prevalence of *S. aureus* pathogens in cSSSI, half of them methicillin-resistant (base case MRSA = 27%), the model projected an overall clinical failure rate of 35.9% for 5 of the more commonly used initial regimens, with an expected overall treatment cost (in US dollars) of $5,492 per patient (range, $4,566–5,633). If none of the *S. aureus*

pathogens were resistant (MRSA = 0%), clinical failure rate was projected to be 18.4% and treatment cost to be $4,869 per patient. The differences in the two scenarios translated to an expected clinical failure rate of 17.5%, an incremental cost of $623 per patient, and for the 800,000 patients hospitalised for cSSSI annually in the US, an expected health care system burden of $498 million attributable to MRSA. Under a "worst-case" scenario in which MRSA was the only causative pathogen (MRSA = 100%) in cSSSI, clinical failure rate was projected to be 79.3%, and treatment cost per patient was expected to be $7,035.
**Conclusions:** Going beyond existing estimates, our model generated a substantial expected clinical failure rate and economic impact attributable to current MRSA levels, as well as simulations of the expected impact of increasing MRSA prevalence over time, varying levels of MRSA across regions and choice of initial empiric regimens.

## P1494

## Treatment of complicated skin and skin structure infections in the US: expected cost differences between tigecycline and vancomycin/aztreonam

R. Mallick, A. Kuznik, D. Weber *(Collegeville, Chapel Hill, US)*

**Objective:** To compare tigecycline and vancomycin/aztreonam in terms of treatment-related costs for patients hospitalised in the United States with complicated skin and skin structure infections (cSSSI).
**Methods:** We conducted a retrospective analysis of pooled data from US centres in two randomized, double-blind clinical studies comparing tigecycline and vancomycin/aztreonam in the treatment of cSSSI. Using regression analysis, we estimated the effect of tigecycline treatment on hospital length of stay (LOS), controlling for other significant predictors. Using published estimates of daily hospitalisation cost of cSSSI in the US from a multi-hospital audit, we then translated the estimated impact on LOS into economic terms. This analysis was repeated for the subgroup of patients in which the primary pathogen was methicillin-resistant *Staphylococcus aureus* (MRSA). Clinical efficacy (tigecycline 81%, vancomycin/aztreonam 83.1%; p = 0.376) was similar across treatments and was not included as a model parameter.
**Results:** Our retrospective analysis of the pooled clinical data from US centres found that tigecycline was associated with a shorter LOS [−1.85 days (p = 0.015)] compared with the combination of vancomycin/aztreonam in the treatment of patients with cSSSI. At a mean daily hospitalisation cost (in US $) of $794, excluding antibiotic costs, this translated into expected medical cost savings of $1,469 per patient for tigecycline compared with vancomycin/aztreonam. In the MRSA subgroup, comprising 26% of the clinical study sample, tigecycline was associated with a greater reduction in LOS [−2.82 days (p = 0.011)] compared with vancomycin/aztreonam, translating to expected medical cost savings of $2,239 per patient treated with tigecycline. These expected medical cost savings more than offset the higher average daily drug acquisition costs of tigecycline ($118/day) relative to the vancomycin/aztreonam combination ($110/day).
**Conclusion:** In a retrospective analysis of pooled clinical data of patients with cSSSI treated at US centres, tigecycline was associated with a significantly reduced length of hospital stay relative to vancomycin/aztreonam; this translated into substantial cost savings, especially in the subset of cSSSI patients with MRSA.

## P1495

## The economic impact of linezolid in the treatment of skin and soft tissue MRSA infections in Italy

M. Eandi, P. Dale, S. Sorensen, T. Baker, M. Procaccini, S. Duttagupta *(Turin, IT; London, UK; Bethesda, US; Rome, IT; New York, US)*

**Objective:** Linezolid has been shown to be highly effective against infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) in patients with complicated skin and soft tissue infections (cSSTI). The objective of this study was to evaluate the clinical and economic consequences of using linezolid for the empiric treatment of cSSTI from the Italian hospital perspective.
**Methods:** A decision-analytic model was developed to calculate the clinical and cost outcomes of empiric treatment of hospitalized patients with cSSTI in Italy prescribed linezolid, vancomycin or teicoplanin. Efficacy data were derived from clinical trials. Costs from published sources were applied to tests, adverse events, and days of intravenous and oral (linezolid only) treatment and hospitalization by ward type (general, intensive-care). Resource use and utilization patterns were obtained from a combination of clinical trial data and expert opinion. Outcomes included total costs per patient, cost per cure and cost per death avoided. Uncertainty surrounding the CE ratio was tested using one-way sensitivity analysis.
**Results:** Starting empiric treatment with linezolid resulted in 98.4% of patients cured from MRSA compared to 98.0% with vancomycin. The average cost per patient treated with linezolid was €6,305 versus €6,228 for patients treated with vancomycin. This resulted in a cost per cure of €22,404. In a separate analysis more patients were cured using linezolid (97.6%) compared to teicoplanin (94.7%). The average total cost per episode was €6,401 for linezolid treated patients versus €5,897 for teicoplanin treated patients, resulting in a cost per cure of €17,100. The most sensitive parameters included hospital LOS and MRSA resistance rate.
**Conclusions:** In the treatment of cSSTI due to suspected MRSA in Italy, the empiric use of linezolid is cost-effective when compared to vancomycin and teicoplanin

## P1496

## Outpatient and home parenteral antimicrobial therapy for the treatment of cellulitis: evaluation of efficacy and cost

H. Ziglam, R. Tilley, C. Wootton, J. Morrison, D. Nathwani *(Dundee, UK)*

**Objective:** Outpatient and home parenteral antibiotic therapy (OHPAT) programmes are effective, well tolerated and economically advantageous in carefully selected patient populations. Skin and soft tissue infections represent a high burden disease which in amenable to treatment by OPHAT programmes. We retrospectively analysed our outcomes registry to evaluate the clinical and health economic impact of treating cellulitis in this setting.
**Methods:** We have reviewed 465 patients with cellulitis and erysipelas who were treated with OHPAT. Each patient treatment has a full integrated care pathway (ICP). The ICP documents the microbiological outcome, drug and vascular access complication rates, impact on drug costs and in-patient bed days on the 465 number of patients treated from April 1998 to March 2005 are presented here. We also reviewed using the SMR1O inpatient discharge diagnosis data from the Information Statistics Division Scotland (ISD) and the Dundee Infectious

Diseases Units (DIDU) Outcomes Registry Database. The key diagnosis (ICD 10 codes) groups considered were cellulitis (LO30, 31, 32, 33, 38, 39) and erysipelas (A46X) over eight consecutive years (1997–2004).

**Results:** The patients received intravenous antibiotic therapy for a mean duration of 5.64 days. The two primary agents administered were once-daily ceftriaxone in 84%of patients and teicoplanin in 9.8% of patients. Of the 465 patients, 431 (92.6%) were cured or improved; 5 worsened and required surgery. Tinea pedis was found in 31% of patients treated for cellulitis. Economic benefits were realized despite use of more expensive agents. Data from the Dundee outcomes registry revealed a mean reduction in length of hospitalization from 5.8 days (1996/1997) to3.2 in 2003–2004- a reduction of 48% compared to Scottish data from ISD which did not show any changes in length of hospitalization between year 1996/1997 (5.33 days) and year 2003–2004 (5.92 days).

**Conclusions:** We have found that OHPAT is clinically effective and can be administered safely and successfully in an outpatient setting. The majority of complications were minor, and 93% of patients were cured. Tinea pedis and were found to be significant risk factors for acute cellulitis and indicate that improved awareness and management of toe web intertrigo might reduce the incidence of cellulitis. This analysis also supports the premise that an adult OHPAT programme can substantially reduce healthcare resource use in the European healthcare setting.

## P1497

## Cost-effectiveness analysis of intravenous moxifloxacin compared to levofloxacin in hospitalised elderly patients with community-acquired pneumonia

A. Lloyd, D. Battleman, S. Choudhri, A. John, M. Kubin *(London, UK; Arlington, West Haven, Kenilworth, US; Wuppertal, DE)*

**Objective:** To evaluate the cost-effectiveness of moxifloxacin compared to levofloxacin in hospitalised patients aged ≥65 with community acquired pneumonia (CAP).

**Methods:** A randomised double-blind parallel group study was conducted in 47 US hospitals. Patients had radiological evidence of bacterial pneumonia confirmed by at least 2 other signs, were aged ≥65 years and were managed as inpatients on initiation of treatment. Patients initially received moxifloxacin 400 mg IV O.D. or levofloxacin 500 mg IV O.D., and once stabilised were switched to oral therapy with the same agent. The effectiveness endpoint for the economic analysis was the percentage of patients successfully treated, defined as patients with marked improvement, resolution or clinical cure at test of cure visit after 7–14 days of therapy who did not experience a serious cardiac adverse event. Total costs were estimated from the perspective of the treating hospital and included antibiotic drugs, hospital stay, hospital re-admission within 28 days and cost of managing treatment failures.

**Results:** 394 patients were included in this analysis, 195 randomised to moxifloxacin and 199 to levofloxacin. 82% (95% CI: 77–88%) of moxifloxacin and 75% (69–81%) of levofloxacin treated patients were successfully treated (resolution, clinical cure at TOC and no serious drug related AEs). In the moxifloxacin group patients reported a mean of 3.5 days of IV antibiotic treatment and 5.7 days inpatient stay with 3.4 days IV antibiotic treatment and 6.1 days inpatient stay in the levofloxacin group. Mean per patient drug cost was $257 in the moxifloxacin group, $222 in the levofloxacin group. Mean total cost was $8,339 in the moxifloxacin group and $8,354 in the levofloxacin group. Findings were consistent across a range of patient subgroups. Costs were sensitive to length of hospital stay.

**Conclusions:** Patients in the moxifloxacin group had higher rates of successful treatment at slightly lower average costs than the levofloxacin group. This confirms the results of the TARGET study where moxifloxacin showed superior clinical efficacy in comparison to co-amoxiclav with or without clarithromycin in hospitalised CAP patients. Antibiotic costs were slightly higher in the moxifloxacin group than the levofloxacin group but total costs were slightly lower, due to reduced hospital stay.

## P1498

## Economic impact of invasive fungal infections in ICU patients in a tertiary care hospital in Switzerland

A. Imhof, W. Zingg, R. Laffer, C. Ruef *(Zurich, CH)*

**Objectives:** Invasive fungal infections (IFI) cause significant morbidity and mortality. The management of invasive fungal infections is currently undergoing important changes due to the availability of new therapeutic agents with improved safety profiles but the acquisition costs of these new agents are high. We evaluated the average overall cost of management (microbiological diagnosis and treatment) of invasive fungal infection in critically ill patients at a large university hospital.

**Methods:** A retrospective (2003–2004), pairwise-matched cohort study was performed on 4 surgical ICUs and one medical ICU at our university hospital. ICU patients with documented IFI (n = 23) were matched with control subjects (n = 46) on the basis of disease severity, sex and age (±5 years). Clinical outcome was principally evaluated by in-hospital mortality. The economic impact of microbiological studies and antibiotic treatment was assessed. Calculations were based on the period between admission and diagnosis of IFI in cases and the duration of hospital stay in controls, respectively.

**Results:** The median length of hospital and ICU stay differed significantly between cases and controls (43 vs 14 days, 19 vs 3 days, $P < 0.001$, respectively). IFI occurred after a median hospital stay of 22 (range 2–95) days. The mortality rate for patients with IFI and matched control subjects were 30.4% and 10.9%, respectively ($p = 0.04$). There was no significant difference between cases and controls for Charlson index, McCabe and SAPS II score. Median number of antibiotic treatment courses was 3 for cases and 1 for controls ($p = 0.003$), with a median duration of therapy of 6 days vs 1 day ($p < 0.0001$), respectively. Microbiological studies (MiS) were conducted 78 times/100 patient-days (pd) in cases and 17 times/100 pd in the control group ($p = 0.03$). The most frequent samples were bloodcultures in both groups. Swabs were ordered significantly more frequently in cases (median 25 (range: 1–150) vs. 2 (0–23); $p = 0.04$). The cost for MiS was 13'728 Euro/100 pd in cases vs. 3742 Euro/100 pd in controls, and the costs for antifungal therapy 4'373 Euro/100 pd vs. 99 Euro/100 pd, respectively.

**Conclusions:** IFI is associated with excess length of ICU and hospital stay, increased use of antibiotics and microbiological diagnostics. The microbiological studies have a significant economic impact on the treatment of IFI.

## P1499

### Cost-effectiveness of voriconazole to amphotericin B deoxycholate in early and late treatment of invasive aspergillosis

R. Greene, J. Mauskopf, C. Roberts, T. Zyczynski, H. Schlamm *(Boston, Research Triangle Park, New York, US)*

**Objective:** We estimate the cost-effectiveness of alternative initial drug treatments of invasive pulmonary aspergillosis (IPA) in suspected earlier and later lung involvement, based on the presence or absence of the halo sign on thoracic computed tomography (CT).
**Methods:** We constructed a decision analysis model comparing 12-week treatment outcomes for a subset of patients enrolled in a clinical trial of initial treatment of IPA with amphotericin B dexoycholate (AmBd) vs voriconazole (VOR). Patients included those with suspected lung involvement who underwent a baseline thoracic CT. The subset was subdivided into two groups based on the presence or absence of a characteristic CT halo sign, a perimeter of ground glass CT opacity surrounding a solid lung nodule ≥1 cm diameter, known as an early indicator of IPA. Healthcare resource use and survival data were obtained directly from the clinical trial. US unit costs for drugs and health care services were applied from standard data sources. Cost and survival at 12-weeks were estimated for those with and without a halo sign at baseline. Incremental cost-effectiveness ratios comparing VOR to AmBd were calculated for both patient subgroups. Sensitivity of results to uncertainty in health care use and cost estimates was tested.
**Results:** Patients in the halo subgroup had better survival than those in the no-halo subgroup (70.6% vs 54.4%), with lower total treatment cost ($44,352 vs $47,077). Survival was higher for VOR than for AmBd in both patient subgroups (halo: 75.3% vs 65.2%; no-halo: 68.3% vs 39.5%). In the halo subgroup, total costs were lower for those treated with VOR than for those treated with AmBd ($40,380 vs $48,985). In the no-halo subgroup, total cost per patient was slightly higher for those treated with VOR ($48,133 vs $45,938). Accounting for the difference in survival, the incremental cost-effectiveness ratio for VOR compared to AmBd was $7,625 per additional 12-week survivor in this subgroup.
**Conclusions:** Earlier identification and treatment of IPA appears to result in better survival and potentially lower costs than later treatment. Initial treatment of IPA with VOR improves survival in patients with early or late disease compared with AmBd, is cost saving in the halo sub-group, and is cost-effective in the no-halo subgroup, within the constraints of our analysis.

## P1500

### A multi-centre comparison of nursing staff time required for the preparation and administration of liposomal amphotericin B and amphotericin B deoxycholate to voriconazole

E. Flynn, B.A. Oppenheim, P. Ribaud, G. Barbabietola, V. Démarez, A. Marciniak, C. Roberts, K. Barker *(Auburn, US; Manchester, UK; Paris, FR; Foligno, IT; Creteil, FR; Sandwich, UK; New York, US)*

**Objective:** To describe and compare the nursing labor time required for preparation and administration of liposomal amphotericin B (L-AmB), amphotericin B deoxycholate (AmBd), and voriconazole (VOR).
**Methods:** Activities associated with nurse preparation and administration of the three study drugs were timed by trained observers at five hospitals (one in Italy, three in France, and one in the United Kingdom). Target tasks were classified as those likely to be affected by the difference between the drugs and excluded those tasks likely to differ because of site-specific factors (e.g., travel time to a patient room in different hospitals). Target tasks included: obtain supplies and medications; prepare medications; educate patient; administer medications; monitor for adverse events; and prepare follow-up medications. The mean times for administration of a single day of study drug were summarised and compared, accounting for a single daily dose of L-AmB and AmBd and 2 daily doses of VOR IV or oral.
**Results:** Sixty-nine patients were observed receiving 256 doses of study medications at the five hospitals. Time of administration in minutes per day was 20, 16, 14, and 3 for L-AmB, AmBd, VOR IV, and VOR oral, respectively. Administration time was significantly lower for VOR IV compared with L-AmB ($P < 0.05$) and for VOR oral compared to all IV regimens ($P < 0.05$). The task of preparation of medications required the most time for IV formulations, and was longer in the L-AmB group than the others (L-AmB: 12 mins vs AmBd: 7 mins; VOR IV: 9 mins). AmBd required more time for patient monitoring and administration of follow-up drugs than other formulations (AmBd: 7 mins vs L-AmB: 4 mins; VOR IV: 2 mins).
**Conclusion:** VOR IV required significantly less time to prepare and administer on a daily basis compared to L-AmB. Measurements of IV antifungal versus oral VOR administration suggest the opportunity to save 10–17 minutes per day by switching to oral therapy when possible.

## P1501

### Need of cost-effectiveness investigation focused on diagnosis, management and prevention of osteopenia and osteoporosis in the setting of HIV disease treated with HAART: when to act, how to act, which patients are the first target of intervention

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

**Background:** Osteopenia/osteoporosis are emerging untoward effects of HIV infection/HAART. The pathogenesis is multifactorial, involving all classes of anti-HIV drugs, although protease inhibitor use, overall HAART duration, and the male sex, seem related to a greater risk.Epidemiological-clinical data. In an ongoing study at our Centre where >1000 HIV-infected patients (p) are followed, bone mineral density was assessed in lumbar spine/femural head by a dual energy X-ray absorptiometry (DEXA) exam to estimate the prevalence of osteopenia/osteoporosis. In a screening of ~100 p, the frequency of osteopenia and osteoporosis (based on lumbar T-score) was ~38% and ~10%, respectively. An increased risk was found in p treated with protease inhibitors versus those receiving non-nucleoside reverse transcriptase inhibitors or triple nucleoside/nucleotide combinations.
**Discussion and future insights:** Prospective studies of extensive p samples are needed, to elucidate the epidemiology, pathogenesis, clinical issues and evolution of HIV-associated bone metabolism anomalies. When planning strategies for their early diagnosis, prevention and management also cost-effectiveness issues should be considered, since no pharmacoeconomic data still exist in this setting. Although severe consequences (e.g. pathological fractures, prosthetic implants) are expected to be infrequent their consequences in terms of length and intensity of hospitalization, related costs,

and especially severe consequences on the p's quality of life, play a notable role. Anyway, the most reliable diagnostic procedure (DEXA) has affordable costs (around Eur 43.40 for a total-body scan which also offers a body composition assessment), as well as the first-line drugs for osteopenia, e.g. supplementation with calcium (Eur 5–6.5/month), and vitamin D (Eur 7/month). These costs cannot be compared with the costs of a standard care of an asymptomatic HAART-treated p (Eur 471 to 774/month) and the immunological, virologic, laboratory and clinical controls made at least quarterly. Like postmenopausal osteopenia/osteoporosis (burdened by a greater risk of bone mass anomalies) also HIV disease should be investigated from multiple cost-effectiveness points of view to establish which p are the preferred candidates for a DEXA screening when this examination is more useful during HIV disease course and therapy, when the exam should be repeated and when and how to intervene pharmacologically to prevent serious and potentially invalidating complications.

## P1502

## A comparative study on the cost of new drugs in different therapeutic categories

M. Falagas, K. Fragoulis, G. Zouglakis, I. Karydis *(Athens, GR)*

**Objectives:** Drug treatment is becoming more expensive due to the increased cost for the introduction of new drugs and there seems to be an uneven distribution of medication cost for different therapeutic categories. We hypothesized that the cost of new antimicrobial agents may differ from that of other therapeutic categories and this may play a role in the stagnation of development of new antibiotics.
**Methods:** We performed a pharmaco-economical comparative analysis of the drug cost of treatment for new agents introduced in the United States drug market in various therapeutic categories. We calculated the drug cost [in US dollars (USD)] of a 10-day treatment of all new drugs approved by the FDA during the period between January 1997 and July 2003, according to the 2004 Red Book Pharmacy's Fundamental Reference.
**Results:** New anti-neoplastic agents were found to be the most expensive drugs in comparison to all other therapeutic categories with a median 10-day drug-treatment cost of 848 USD compared to the median 10-day drug-treatment costs of all other categories ranging from 29 to 301 USD (Table). On the other hand, new antimicrobial drugs were found to be much less expensive with a median 10-day drug-treatment cost of 137 and 85 USD for all anti-microbial agents and for anti-microbial agents excluding anti-HIV medications, respectively.
**Conclusion:** The drug-treatment cost of new medications varies considerably by different therapeutic categories. This fact may influence industry decisions regarding the development of new drugs and may play a role in the shortage of new anti-microbial agents in the fight against the serious problem of anti-microbial resistance.

Table. Number of original new drugs (January 1997 – July 2003) and ten-day treatment cost of different therapeutic categories (in USD - 2004).

| Therapeutic category | Number of new drugs | *Ten-day treatment cost (in USD)* | | |
|---|---|---|---|---|
| | | *median* | *mean* | *range* |
| Antineoplastic | 13 | 848 | 1,455 | 41 - 4,182 |
| Respiratory/Allergy | 8 | 301 | 264 | 7 - 1,300 |
| Cardiovascular | 16 | 184 | 969 | 14 - 7,912 |
| Anemia/Water/Electrolytes | 5 | 138 | 294 | 43 - 959 |
| Infectious diseases | 18 | 137 | 468 | 14 -3,682 |
| Endocrinology | 6 | 129 | 547 | 11 - 1,685 |
| Skin diseases | 4 | 120 | 102 | 12 - 158 |
| Gastrointestinal | 7 | 65 | 113 | 43 - 390 |
| Ophthalmology | 9 | 51 | 334 | 32 - 1,687 |
| Central nervous system | 23 | 50 | 64 | 9 - 180 |
| Gynecology/Urology | 10 | 38 | 106 | 12 - 532 |
| Musculoskeletal | 5 | 29 | 81 | 23 - 202 |

## P1503

## Usage and expenditure of 4f-quinolones in a tertiary hospital in 2003 and 2004

T.A. Peppas, K. Malengou, N. Zachos, D. Voutsinas, O. Kosmopoulou, N. Galanakis *(Piraeus, Athens, GR)*

**Objective:** Our aim was to assess 4-F-quinolone (4FQ) usage, distribution and expenditure over 2 years.
**Material and methods:** All pharmacy 4FQ prescriptions towards all departments of a 616 bed tertiary hospital for 2003 and 2004 were entered in PC databank. Analysis was performed, by SPSS, by drug, defined daily dose (DDD) and retail cost (in €). Under the Greek legislation, as advanced agents 4F-quinolones consist restricted antimicrobials.
**Results:** An overall of 16083 DDDs of 4FQ was dispensed in 2003 and 30378 during 2004, reflecting 0.097 and 0.19 4FQ DDDs/patient-day, respectively. Expenditure for 4FQ was 434351 € for 2003 (20.4% of total hospital antimicrobial cost) and 520052 € [21.1%] for 2004. In partial analysis most usage involved ciprofloxacin, under 5 brand-names, which consisted 79% of all 4FQs dispensed. Most ordering forms were poorly completed to justify choice. Huge discrepancies were observed among similar departments. The significant increase (+91%) seen in 2004 was due to greater percentage of bid 4FQ choice and poorer moxifloxacin representation.
**Conclusions:** Close monitoring of antimicrobial usage, particularly 4FQ, the most commonly used (and abused) among restricted antimicrobials pinpoints the priorities of Infection Control Team regarding urgent intervention planning. Full data recording is of paramount importance for both medical and administrative purposes.

## P1504

## Usage and expenditure of antimicrobials in a tertiary hospital in 2003 and 2004

T.A. Peppas, N. Zachos, K. Malengou, O. Kosmopoulou, I.F. Hager, M. Savvala, E. Dimitrakopoulou, N. Galanakis *(Athens, GR)*

**Objective:** Our aim was to assess antimicrobial (A) consumption, overall and regarding A under restriction (AUR), and also their expenditure over 2 years.
**Materials & methods:** All pharmacy A prescriptions towards all departments of a 616 bed tertiary hospital for 2004 and 2005 were entered in PC databank. Analysis was performed, by SPSS, by drug category, defined daily dose (DDD) and retail cost (in €). Special analysis was performed for AUR [Under the Greek legislation, advanced agents (3rd and 4th gen.cephalosporins, carbapenems, 4F-quinolones and glycopeptides-linezolid) consist restricted antimicrobials and non-restricted As (NRA).

**Results:** An overall of 103884 DDDs of As was dispensed in 2003 and 111376 during 2004, reflecting 0.63 and 0.71 DDDs/patient-day, respectively. Expenditure for As was 2128223 € for 2003 (20.3% of total hospital drug costs) and 2456465 € [20.1%] for 2004. In partial analysis usage was higher, more advanced and costlier in surgical compared to medical departments, but huge discrepancies were observed among departments in both sectors. Usage of AUR was quite high, grossing 30766 DDDs and 1197473 € in 2003 and increasing to 32885 DDDs and 1487239 € respectively in 2004. A certain department achieved expenditure of 23.9€/pt-day for AUR alone in 2004, and a total of 256350 €, far exceeding the respective ICU cost.
**Conclusions:** Close monitoring of antimicrobial usage and selecting areas of urgent intervention planning are among the primary duties and targets of Infection Control Team. Full data recording is of paramount importance for both medical and administrative purposes.

## P1505

## Antibiotic utilisation in Croatia, 2004

D. Štimac, I. Vukušic, J. Culig, Z. Šostar, S. Tomic *(Zagreb, HR)*

**Objective:** To analyse antiobiotic utilization in Croatia using Anatomical-Therapeutic-Chemical (ATC) drug classification system and number of defined daily doses (DDD).
**Methods:** Data on the number of packages and purchase price were collected for each individual drug. These data were used to calculate the number of defined daily doses (DDD) and DDD per 1000 inhabitants per day (DDD/TID). Data obtained from 80% of pharmacies and 65% of hospitals were extrapolated to the total number of pharmacies and hospitals in Croatia. Drug utilization 90% (DU90%) segment was used as a prescribing quality indicator.
**Results:** In 2004, the overall utilization of antibiotics in Croatia amounted to 23.35DDD/TID. According to drug groups, penicillins (J01C) showed highest utilization (12.24 DDD/TID), predominated by the subgroup of penicillin combinations (including beta-lactamase inhibitors, J01CR) with6.86 DDD/TID, within which the combination of amoxicillin + clavulanic acid accounted for99.9% with 6.85DDD/TID. Broad-spectrum penicillins (J01CA) accounted for 43.2%(5.28 DDD/TID) of total penicillin utilization, with a 97.7% predominance of amoxicillin (5.16 DDD/TID). Cephalosporins (J01D) ranked second with 4.00DDD/TID, followed by macrolides and lincosamides (J01F) with 2.41 DDD/TID, with an 86.84%predominance of macrolides (J01FA) with 2.09DDD/TID. Among the latter, azithromycin showed highest utilization with 1.44 DDD/TID, accounting for 68.66% of total macrolide utilization. Tetracyclines (J01A) ranked fourth with 2.02DDD/TID, accounting for 8.65% of overall antibiotic utilization, followed by quinolones with 1.65 DDD/TID, other antimicrobials with 0.91 DDD/TID, and aminoglycosides with 0.16DDD/TID. Sulfonamides (J01E) accounted for a negligible proportion of overall utilization. DU 90% segment included 9 of 43 antibiotics registered in Croatia, with amoxicillin + clavulanic acid as the leading one, followed by cephalexin with 1.83, cefuroxime with 1.53, azithromycin and norfloxacin with 1.3each, nitrofurantoin with 0.56 and clarithromycin with 0.53DDD/TID. Hospital utilization accounted for 7.1% of overall antibiotic utilization expressed in DDD/TID and 22.7% of the respective financial cost, predominated by aminoglycosides (J01G) with 72% and 94.2%, and lowest proportion of tetracyclines (J01A) with 4.3%, and 2.4%, respectively.
**Conclusion:** The utilization of antibiotics in Croatia is among the highest in Europe, mostly due to overuse of amoxicillin + clavulanic acid, which has no rational ground in professional guidelines.

## P1506

## A comparative analysis of prescriptions for antibiotics issued by outpatient doctors in urban areas in 1999 and 2005

W. Drzastwa, E. Drzastwa, W. Lukas, E. Mizgala, G. Golczyk, H. Trzeciak *(Zabrze, PL)*

**Objective:** The objective of the research was to analyse antibiotics prescripsion behavior by Family doctors and specialists treatments prior to and after the introduction of the health care reform in Poland.
**Materials and methods:** Prescriptions from the first six months of 1999 and 2005 were compared. The data was collected from two randomly chosen pharmacies in the city of Zabrze that supply the citizens of the Silesian agglomeration from various social backgrounds. Taking into account the value of a single antibiotics package and the price a patient has to pay for it an average price of medications prescribed by family and specialist doctors was calculated.
**Results:** A total of 30793 prescriptions were analysed out of which 24834 dated from 1999 and 5959 from 2005.In the first half-year of 1999 the percentage of prescriptions for antibiotics reached 7.76% on average, and in the year 2005 – the average was 11.6%. In the first half-year of 1999 family doctors mostly prescribed: penicyllins (43%), makrolids (26%), cephalosporins (16%), tetracyclins (11%), chinolons (3%). In the same period spcialist doctors prescribed: penicyllins (41%), cephalosporins (17%), makrolids (15%), tetracyclins (13%), lincozamids (9%), chinolons (4%). In the first half-year of 2005 family doctors most often prescribed penicyllins – (44.8%), makrolids – (27.1%), cephalosporins – (12.5%), tetracyclins – (9.4%) and lincozamids–based (3.1%) treatments Specialist doctors, on the other hand, prescribed penicllins (41.7%), makrolids (17.9%), cephalosporins (17.7%), tetracyclins (12.1%), lincozamids (5.2%) and chinolons (3%). The average prices of the prescribed medications in the years 1999 and 2005 were, respectively: for family doctors-EU 3.08 and 4.01, for specialist doctors EU 4.44 and 3.87.
**Conclusions:** There has been a considerable increase in the percentage of prescriptions for antibiotics from 7.45% (in 1999) to 11.3% (in 2005). The tendency towards prescribing antibiotics in the specific groups of doctors has not changed significantly. In both years prescriptions for antibiotics were in line with the recommendations. Also, prices of medications prescribed by family doctors have risen.

## P1507

## Internet guide on antimicrobial resistance

A. Sosa, F. Traub, S. Valovic, P. Chea *(Boston, US)*

**Objectives:** (1) To organize the plethora of information available, providing clinicians the tools to easily access available online resources that include academic institutions, professional societies as well as sites maintained by private individuals; (2) to inform clinicians of new advances in the epidemiology, diagnosis, treatment, and prevention of most common infections; (3) to inform on subjects such as clinical trials in antimicrobial resistance, information about specific pathogens and their infections, genomic resources, culture collections, electronic images of pathogens and antimicrobial agents, antimicrobial resistance lecture and teaching materials, environmental health and safety information, and a listing of

websites of infectious disease and clinical microbiology professional societies.

**Methods:** We defined four inclusion criteria after extensive consulting with APUA staff and scientific advisory members: (1) Recognized/reputable source; (2) High quality of information presented; (3) Potential usefulness to medical professionals and the general public; and (4) Ease of navigation. Ideal parameters were determined for the guide's scope and the appropriate sources identified online were subsequently reviewed. A set of broad categories was established to organize the topics and the online resources. Sites reviewed included those maintained by the federal government, academic institutions, nonprofit organizations, and commercial entities. Some personal websites were included because of their quality and their association with academic institutions. This review is intended as an introduction to AMR websites.

**Results:** With use of popular search engines, such as Google, Yahoo!, and AltaVista, we initially identified a great number of websites. Using broad search terms, such as "antibiotic resistance," we identified 1,310,000 web addresses. The term "antimicrobial resistance" generated 578,000 hits, and the term "drug resistance" generated 6,190,000 hits.

**Conclusions:** Websites found were classified following a systematic topic structure. Each website listed describes: (1) Full citation of the resource: author/editor and title of website; (2) Date of publication or last revision; (3) Name of sponsor; (4) Date of access (i.e.: 3 Sep 1999); (5) URL of resource with date; (6) Description of resource; (7) Outstanding information related to criteria.

## P1508

## An educational web portal on febrile neutropenia: www.febrilnotropeni.net

H. Akan, M. Akova, T. Çalikoglu, K.H. Gülkesen *on behalf of the Turkish Febrile Neutropenia Study Group*

**Objectives:** Countless health portals on the internet are available for healthcare personnel. www.febrilnotropeni.net, a Turkish health portal dedicated specifically to febrile neutropenia related problems, has been run by the Turkish Febrile Neutropenia Study Group (TFNSG) since May 2002. We analysed an 11-month period (December 2004–October 2005) of usage patterns of the Portal.

**Methods and results:** The portal is free to use but requires registration and has more than 1850 registered users as of November 2005. Of these, 20% are in-training positions, 28% hold a faculty position in infectious diseases (ID), microbiology or hemato-oncology, 24% are specialists in a non-university, but a teaching setting. The remaining are a mixed group of primary physicians, other speciality doctors and pharmaceutical company workers. Running costs of the portal are partially covered by educational grants from pharmaceutical sponsors who have no role in organization of the site, but their names are acknowledged. Articles chosen by the two faculty members, one ID physician and one haematologist, are sent to registered users by daily e-mail postings. These are selected from toc alerts of various core clinical journals and well known educational web sites (e.g. CDC, WHO, Medscape). A short Turkish summary is provided and the reader is referred to the abstract and if available, to the free full text of original article with a link. Other materials included guidelines, free slide sets, study protocols and updates from the Group, CME activities and meeting announcements. Registrants may also use the site for expert opinion. During the trial period, the site has been visited 30740 times with 282239 hits. Approximately 5.5 Gb material was downloaded. The frequency of readings are related with the time (highest between 9.00–11.00 am during weekdays and lowest during weekends), the type of documents (i.e. educational materials and guidelines), popularity of the news (e.g. peaked during an epidemic of avian influenza when related news and articles announced). The pages are most frequently visited by ID specialists followed by clinical microbiology, haematology and pharmaceutical company workers (33%, 19%, 9% and 8% respectively).

**Conclusion:** Timely published medical data have high attraction rates among physicians. Our results also indicated that, a web page gets ''old'' after about a month of publishing, emphasizing the importance of well-timed announcements of the Portal material.

## P1509

## NeLI and NRIC survey: information needs of infectious disease professionals

P. Kostkova, S. D'Souza, G. Madle, J. Mani-Saada, S. Wiseman, A. Roy, J. Weinberg *(London, UK)*

Healthcare professionals are increasingly facing the problem of information overflow. It is getting impossible to keep up-to-date with the latest research findings, care guidelines and pathways, government strategies and national and local policies. Internet enables an instant information dissemination enabling access to the latest results at any time as well as informal knowledge exchange by using chat rooms and discussion forums. However, it is getting increasingly difficult for busy professionals to find reliable quality-assured information on the Internet when they need it. National Internet libraries in the UK are addressing this problem: the umbrella portal National electronic Library of Infection NeLI (http://www.neli.org.uk) providing a single access portal to quality-assured information on treatment, diagnosis, prevention and management of infection diseases, and the National Resource for Infection Control NRIC (http://www.nric.org.uk) - a single-stop shop for policies, guidelines and research around infection control, hosted by NeLI. To better meet the information needs of these Internet portals, accessed by 2000 unique users per month, we are conducting an information needs study to explore clinical questions, user needs and disease priorities of users seeking answers on NeLI and NRIC. A pilot qualitative online questionnaire-based study revealed that our users come from the variety of professionals: clinical scientist, consultant, registrar, psychotherapist, lecturer, GP, medical librarian, information scientist, health protection. These have questions mainly around HIV, tinea, molluscum contagio, meningitis, cold, MRSA, Lyme, Toxoplasma, chicken pox, Influenza, diarrhoea and vomiting, rash, *Staph. aureus*, traveller infections antibiotics resistance, malaria, MMR, meningitis, viral myocarditis, anthrax, smallpox, and TB. This is in line with our quantitative weblog-based evaluation of the most commonly access topics on NeLI by NHS-based users: Antimicrobial resistance and HAE (10.27%), TB (9.54%), meningitis (9.47%), HIV (8.95%), chlamydia (6.31%), *E. coli* (5.54%), *Staph. aureus* (5.26%), adenovirus (4.84%), blood borne infections (4.44%). The results of the ongoing analysis of google search keywords that brought users to NeLI and NRIC will be discussed. Further results identifying the needs specific to the infection disease professions will be discussed in relation to differences in the national variations in information needs and priorities.


ISSN: 1470-9465

## P1510

### Training in infection

S. D'Souza, P. Kostkova, F. Cooke, A. Holmes *(London, UK)*

Specialists today require prompt access to quality information in order to work effectively. The diversity of specialist interests in the field of infection has led to the formation of a large number of professional and scientific societies. These play an increasingly important role in ensuring that the trainee is effectively supported, not only during the period of training, but also in longer-term personal development. Details of relevant societies, conferences, grants etc are, on most society websites, confined to those for either that society or others in that specialty only, and knowledge of the numerous places in which to look for this information is necessary to find out the latest information. Training In Infection (TII – www.trainingininfection.org.uk) is an online resource, primarily aimed at infection specialist trainees but useful throughout the career path, which brings together this information into one central access point, so that users from all infection specialties can find the appropriate information for their specialty quickly and easily. It identifies and links to the key relevant resources covering a broad range of infection related disciplines in a dynamic database structure. Information on societies, conferences, grants, journals, textbooks and more are available on the site, and have been put together to create a one-stop infection training portal. Online discussion forums to be implemented will allow trainees' to share ideas and make the most of their combined expertise, and users will be able to receive alerts on new information in their specialty as well as be reminded of conference deadlines, journal submission deadlines etc. The ability to discuss regional issues online within specialties also aims to promote greater local and international collaboration. Training in Infection is endorsed by the National electronic Library of Infection (NeLI – www.neli.org.uk), an established digital library bringing together the best available online evidence-based resources on the investigation, treatment, prevention and control of infectious diseases.

## P1511

### Research designs and statistical methods in medical abstracts

M. Kompoti, M. Matsagoura, A. Koutsovasilis, A. Koutsovasili, S. Drimis *(Athens, GR)*

Statistical methods used in biomedical research articles are being increasingly scrutinized in medical journals. However, no such strict policy is generally applied in abstracts presented in medical congresses.

**Objective:** This study aimed at assessing the frequency of research designs and statistical methods reported in abstracts presented in two successive years of the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID).

**Material and methods:** We reviewed all abstracts included in the abstract book of the 14th ECCMID (Prague 2004) (PG) and the 15th ECCMID (Copenhagen 2005) (CP). All abstracts of original research studies but no abstracts of lectures were included in our study. Two independent investigators read all abstracts and extracted information concerning origin, type (clinical, laboratory, animal model), research design, sample size and statistical methods used in the study. Data analysis was performed with logistic regression and Pearson's chi-square test for categorical variables and Student's *t*-test for continuous variables. Statistical significance level was set at $p < 0.05$.

**Results:** A total of 4178 abstracts were included in the analysis according to eligibility criteria (2110 from PG and 2068 from CP). Laboratory studies prevailed (61%) followed by clinical studies (35%) and experimental studies with animal models (4%). The majority (79.3%) of the studies were observational (retrospective, prospective, cross-sectional) of which 6.3% concerned diagnostic accuracy testing of laboratory methods and 2.3% were pharmacological studies, 3.8% were randomized controlled trials. Statistical evaluation was clearly described in 26.5% of abstracts (26.1% in PG and 19.8% in CP, $p < 0.001$), while the rest of abstracts included only descriptive statistics or no statistics at all. The proportion of statistical methods reporting varied according to the type of the study (animal model studies 49.4%, clinical studies 38.9% and laboratory studies 12.0%, $p < 0.001$). Multicentre research studies reported statistics more frequently than single-center studies (26.5% vs. 21.5%, respectively, $p = 0.005$).

**Conclusions:** Statistical analysis is an inseparable part of original research. Research design as well as the implemented statistical methods should always be reported in an adequate manner, thus improving the scientific quality of abstracts.

# Antimicrobial PK/PD

## P1512

### NXL103-Oral Streptogramin: a phase I, double-blind, single escalating oral dose study to evaluate safety, tolerability and pharmacokinetics in healthy adult male volunteers

M. Rangaraju, J. Rey, J. Hodgson *(Romainville, Vitry sur Seine, FR)*

**Background:** NXL 103 (formerly XRP 2868) is a novel semi-synthetic oral streptogramin that consists of a 30/70 (w/w ratio) association of a pristinamycin IA (PI) derivative and a pristinamycin IIB (PII) derivative. NXL 103 is being developed for the treatment of respiratory tract and skin and skin structure infections.

**Methods:** 60 healthy male subjects were enrolled in this study. 10 subjects in each of 6 cohorts (125 mg, 250 mg, 500 mg, 1000 mg, 1500 mg and 2000 mg) received either NXL103 (8) or placebo (2). An additional cohort of 10 subjects received a single dose of 500 mg NXL103 in fasting and fed conditions. Blood and urine samples for PK analysis were collected at multiple time points. Safety was assessed via adverse events, physical examination, clinical laboratory data, ECG and cardiac monitoring.

**Results:** NXL103 administered as 125 mg capsules at single doses from 125 mg to 2000 mg was well tolerated and safe. There was no serious or severe adverse event, no dose-dependency in the number of AEs or their severity, no significant variation in blood pressure or heart rate, no

abnormality on ECG recording, and no clinically significant changes compared to baseline for laboratory parameters. Both components were rapidly absorbed; PI being slightly more rapidly absorbed than PII. The Cmax and AUC (0-t) increased approximately in proportion with dose. The proportion of PI and PII components estimated on mean exposure values was approximately comparable to that administered (30/70), indicating that the relative bioavailabilities of PI and PII are simila. Elimination half-life ranged from between 2 to 3 hours for PI to 4 to 6 hours for PII. Food increased the bioavailability of PI and PII by approximately 20%.

**Conclusions:** NXL103 is safe, well tolerated and exhibits predictable PK properties in healthy volunteers in doses up to 2000 mg administered as a single dose.

## P1513

## Correlation of vancomycin and daptomycin susceptibility in *Staphylococcus aureus* in reference to accessory gene regulator polymorphism and function

W. Rose, M. Rybak, B. Tsuji, G. Kaatz, G. Sakoulas *(Detroit, New York, US)*

**Objective:** Polymorphism at the accessory gene regulator (agr) locus in *S. aureus* (SA) defines 4 groups (I–IV). Agr group II SA have been associated with glycopeptide treatment failure in patients. SA with loss of agr function appear to have a higher tendency to become vancomycin (V) resistant. It is unknown whether this association only pertains to glycopeptides. We examined the effect of varying V and Daptomycin (D) against agr+ and agr null pairs in an *in vitro* pharmacodynamic model (IVPM).

**Methods:** Agr group I and II wild-type prototype and knockout (tetM::agr) pairs were evaluated. MIC values were determined according to Clinical Laboratory Standards Institute. IVPM glass and hollow fibre models were used to simulate dosages and AUC/MIC exposures for V ranging from 62.5 mg–1 g q 12 h fAUC/MIC range 31–665 mg/L*h, and D 0.75 mg/kg–6 mg/kg/day fAUC/MIC range 37.5–346. The dosage regimen and AUC/MIC breakpoints that produced resistance was then evaluated in the hollow fibre IVPM. All IVPM simulations were performed in duplicate over 72 h. Resistance was evaluated using 3 and 6 x MIC screening plates at 0, 8, 24, 48, 56 and 72 h.

**Results:** Pre-exposure MIC values for agr I± and agr II± were 0.75/1 and 1 for V and 0.25 $\mu$g/ml for D respectively. V-intermediate resistance (MIC = 8 mg/L) was detected in both agr I and II null strains at a simulated V dosage of 62.5 mg q 12h (AUC/MIC 31), representing an MIC increase of 8–10 fold. This breakpoint for resistance was verified in the hollow fibre model. Although significant regrowth was noted with suboptimal dosing of D, no resistance was detected on D screening plates for any daptomycin regimen evaluated.

**Conclusions:** Exposure of SA to V approximating 1/6 of optimal serum concentrations resulted in the development of heteroresistance in the agr null group I and II. Loss of agr function did not correlate with the development of D resistance despite suboptimal simulations of D exposures. These results implicate loss of agr function important to the development of glycopeptide resistance but not to loss of susceptibility to D.

## P1514

## Teicoplanin efficiently penetrates into the rabbit infected vitreous but may enhance expression of virulence factors at sub-inhibitory concentrations

E. Forestier, F. Jehl, C. Gallion, R. Andres, S. Bronner, L. Leininger, G. Prévost *(Strasbourg, FR)*

**Objectives:** Fluoroquinolones are the antibiotics that most efficiently penetrate inside vitreous. However, alternative treatments for endophtalmitis may be required in some cases for example resistant bacteria. We used a rabbit experimental model of endophtalmitis to evaluate the penetration of teicoplanin in different conditions. The influence of sub-inhibitory concentrations of teicoplanin was also evaluated on the expression of *S. aureus* virulence factors.

**Methods:** New Zealand rabbits (>3 kgs) received one or repeated doses of intra-venous (IV) teicoplanin (60 mg/kg) every 12 hours for 3 days plus one dose a day for 2 more days. Another group of rabbits was infected by 1000 CFU of a methicillin resistant *S. aureus* IBS 3284 (CMI = 1.5 mg/L) producing Enterotoxin A, Panton-Valentine leucocidin and LukE-LukD. They were administrated 24–36 hours later with 60 mg/kg teicoplanin, as a single dose or as to reach the steady state. Vitreous (200 $\mu$L) was sampled before new injections of teicoplanin or at indicated time as well as blood before or 15 min after teicoplanin injection. Teicoplanin concentrations were measured by HPLC. Bacterial counts were recorded and expression of virulence factors was semi-quantified by dedicated competitive RT-PCR tests.

**Results:** In safe eyes, teicoplanin penetration remains moderate reaching about 4 mg/L within about 6–8 h after one IV injection. The half-life of teicoplanin in the rabbit vitreous is about 25 hours. After 5 days of repeated injections, intra-ocular concentration stabilises around 4 mg/L while residual blood concentrations were comprised between 30–40 mg/L. In infected eyes, teicoplanin, when repeatedly administrated after the beginning of clinical signs, i.e. 24 h post-infection = 106 CFU/mL, reaches intra-vitreal concentrations of 9.3 ± 2.3 mg/L 40 h post-infection, and increases to 14.3 ± 5.4 mg/L 84 h post-infection and 12 h after a fourth injection. However, at sub-inhibitory concentrations (~2 mg/L), it may be responsible for a significant increase of agr, gamma-hemolysin hlgA, lukED and Panton-Valentine leucocidin luk-PV expressions with ratio ranging from 15 to 100 folds.

**Conclusion:** These preliminary results strongly suggest that teicoplanin IV administration constitutes an interesting alternative therapy for endophtalmitis provided high intra-ocular concentrations are rapidly obtained. Investigations now concern optimisation of teicoplanin dosage regimen.

## P1515

## Pharmacokinetics of temocillin in intensive care patients and Monte Carlo simulations to evaluate susceptible breakpoints

J.W. Mouton, R. DeJongh, V. Basma, P. Tulkens, S. Carryn *(Nijmegen, NL; Genk, Brussels, BE)*

**Background:** Temocillin (TMO) is a narrow spectrum penicillin with good activity against Gram negative micro-organisms including ESBL and AmpC producers. Little pharmacokinetic data are available however. We performed a pharmacokinetic study in 6 ICU patients receiving TMO 2g q12h. Parameter estimates were used to predict concentrations during continuous infusion (CoInf) and compared with data obtained

from 6 other ICU patients receiving CoInf to validate the model. The model was then used to perform Monte Carlo Simulations (MCS) to determine Probabilities of Target Attainment (PTAs) for pharmacodynamic indices (PDI) in order to evaluate and suggest clinical breakpoints.
**Methods:** Blood samples were taken from ICU patients prior to (t = 0) and after (t = 1, 2, 3, 6, 12 h) a 30 m infusion of 2 g TMO (n = 6) or after 48 h during CoInf with 4 g/24 h (n = 6), and then cooled, centrifuged and stored at −70°C until analysis by HPLC. Protein binding was determined using an ultrafiltration method. Results were used to estimate population pharmacokinetic parameters by Winnonmix including the covariance matrix. Miclab233 was used to perform simulations for CoInf as well as to perform MCS (10000 cycles) and obtain PTAs for the unbound fraction including 95% confidence intervals (CI) for the target concentrations. fT>MIC was chosen as the PDI because of the pharmacodynamic properties of TMO.
**Results:** Protein binding was 75%. A one-compartment model best fitted to the data, with estimates (se) of Vc = 14.3 (0.87) L and k10 = 0.172 (0.059) 1/h corresponding to a mean half-life of 4.03 h. Using these estimates, the predicted unbound concentration during CoInf was 16.9 mg/L, while the mean concentration in the 6 other patients was 17.03 mg/L, a bias of less then 1%. The breakpoint MIC for a mean fT>MIC of 50% was 16 mg/L. However, MCS -taking the variation in the population into account - indicated that 100% PTAs of a 2g q12h dose were obtained at 4, 4, and 2 mg/L for 40, 50 and 60% fT>MIC, respectively. The 95% CI at 50% fT>MIC indicated a clinical breakpoint of 8 mg/L. The 95% CI was relatively large, as expected from data obtained in patients rather than volunteers.
**Conclusion:** The population pharmacokinetic estimates from 6 ICU patients were very well in agreement with the validation study, with a bias of <1%. The MCS indicate a susceptible breakpoint for temocillin of ≤8 mg/L provided an administration of 2 g q12h is used.

## P1516

## Tissue penetration and pharmacokinetics of moxifloxacin in diabetic foot infections:an interim analysis

J. Majcher-Peszynska, K. Karrasch, M. Saß, R. Mundkowski, A. Gussmann, P. Kujath, B. Ruf, W. Schareck, H. Koch, B. Drewelow *(Rostock, Bad Saarow, Lubeck, Leipzig, Beeskow, DE)*

**Objectives:** With its broad spectrum of activity against grampositive, gramnegative and anaerobic organisms moxifloxacin covers the pathogens of the mainly polymicrobial infections associated with the diabetic foot. Inflammatory and fibrotic processes in diabetic foot infections (DFI) contribute to impaired tissue penetration of antibiotics. In addition, diabetic patients represent a pharmacological risk population, physiological changes in diabetic patients may alter the pharmacokinetics of antibiotics. The study was designed to investigate the penetration of moxifloxacin into perinecrotic tissue in 60 patients with DFI and the pharmacokinetic properties of moxifloxacin in diabetic patients.
**Methods:** The interim analysis of this open, multicentre study included 30 adult, hospitalized male and female patients (mean age: 69.8 years) with type 2 diabetes mellitus and DFI. The pharmacokinetic parameters of moxifloxacin and penetration into DFI tissue at steady state (day 4 to 8) following once daily administration of 400 mg IV or PO were evaluated. Correlations between penetration of moxifloxacin and clinical and laboratory parameters were examined.
**Results:** In all 30 patients the moxifloxacin concentrations measured in infected diabetic foot wounds 3 hours after administration exceeded the *in vitro* MIC90 values of susceptible *Staphylococci* (0.125 mg/l). The moxifloxacin concentrations achieved in DFI tissue correlated more strongly with the AUC0-24 (r = 0.659; p < 0.01) than with the corresponding plasma concentrations (r = 0.492, p < 0.01), but not with the extent of the systemic inflammation and the blood glucose level. Taking into account the predictive PK/PD parameters for moxifloxacin (based on an *in vitro* MIC90 value of 0.125 mg/l for *Staphylococcus aureus*) a therapeutic success can be expected (AUC24/MIC: 204.3; Cmax/MIC: 22.9). Significant differences between the routes of administration (IV vs PO) were only observed for tmax (p < 0.01) and t1/2 (p < 0.05), but not for other clinically relevant parameters (AUC0-24, Cmax, moxifloxacin tissue concentration). This allows sequential therapy i.v./p.o. in this indication.

| | | Plasma | | | | | | Tissue |
|---|---|---|---|---|---|---|---|---|
| Adm. | | $C_{max}$ (mg/l) | $T_{max}$ (h) | $AUC_{0-24}$ (mgxh/l) | $t_{1/2}$ (h) | $V_{ss}$ (l/kg) | $Cl_{tot}$ (ml/min) | DFI conc. (mg/kg) |
| PO (n=16) | mean | 2.43 | 2.69 | 25.02 | 9.04 | 2.62 | 289.14 | 1.73 |
| | SD | 0.96 | 1.62 | 12.34 | 4.29 | 1.18 | 166.03 | 1.01 |
| IV (n=14) | mean | 3.39 | 1* | 26.17 | 14.3* | 2.23 | 206.77 | 1.78 |
| | SD | 1.45 | - | 9.31 | 7.95 | 0.61 | 95.60 | 1.56 |

SD: standard deviation; $C_{max}$: maximum plasma concentration; $t_{max}$: time to maximum plasma concentration; $AUC_{0-24}$: area under the concentration-time curve from 0 to 24 h; $t_{1/2}$: terminal half-life; $V_{ss}$: volume of distribution at *steady state*; $Cl_{tot}$: total body clearance
*p<0.05 versus PO.

**Conclusion:** Based on adequate plasma concentrations in diabetic patients, the sufficient penetration into DFI tissue and the possibility of a sequential therapy, moxifloxacin represents - from a pharmacological point of view - a valuable therapeutic option in the treatment of diabetic foot infections caused by susceptible organisms.

## P1517

## Fluoquinolones effects on patient lymphocytes during prolonged treatments

E. Bertazzoni Minelli, A. Benini, D. Doria, P. Franceschetti, M.E. Fracass *(Verona, IT)*

Fluoroquinolones (FQ), widely used in clinical practice, are well tolerated. The most common adverse reactions are those affecting gastrointestinal tract, phototoxicity and allergy. The aim of the study is to evaluate the possible cellular damage in lymphocytes of patients treated with different FQs according to pharmacokinetic data. Blood samples obtained from thirty-six patients treated with ciprofloxacin (CPX, 14 pts), levofloxacin (LVX, 15 pts.) and moxifloxacin (MFX, 7 pts) at different doses were analysed. Patients treated with CPX and LVX were in therapy with other drugs (diuretics, cardiovascular drugs, omeprazole, antiinflammatory drugs, etc.). MXF treated patients were not in therapy with other drugs. Samples were collected at time 0 (before FQs administration) and after 3 and 9 days of treatment. Serum levels of FQs were determined with microbiological method and HPLC. Comet test was performed on lymphocytes, to evaluate DNA damage. GSH levels were determined as efficiency marker in metabolic process of detoxification. CPX showed good serum concentrations; its

levels increases proportionally with administered doses (from 250 to 1000 mg). LVX concentrations resulted in good inhibitory levels after 3 treatments (500 mg) both per os and i.v. Patients orally treated with 250 mg showed similar serum levels (from 0.8 to 2.1 mg/l). MFX levels were between 3.6 and 1.6 mg/l after 3 and 9 days. Repeated CPX administration induced a dose-dependent increase in all DNA damage parameters, with statistical differences after 9 treatments. MFX (400 mg) and LVX administration didn't induce DNA damage after 3 and 9 days. Intracellular levels of GSH were similar in all treated groups, even if CPX treated patients showed the lowest concentrations. No statistical correlations were found between all parameters studied. These data indicate that CPX induce DNA damage in lymphocytes in combination with a reduced efficiency in detoxification system. This effect does not seems to depend on high intersubject variability for FQs administered doses, co-administration of other drugs, different ages of patients and low samples numbers. Effects of single FQs molecules seems to be structure-specific and selective.

## P1518

## Penetration of ertapenem in human pancreas. Results of a prospective clinical trial

S. Motamedi, M.H. Schoenberg *(Munich, DE)*

**Objectives:** Ertapenem is a carbapenem commonly used to treat intra-abdominal infections. The antibacterial spectrum includes the major causative pathogens. Clinical trials proved excellent clinical and microbiological efficacy in peritonitis. On the other hand in inflammatory pancreatic diseases sufficient antibiotic concentration in the inflammatory tissue is vital for the outcome of the disease. We therefore investigated ertapenem concentrations in pancreatic tissue and juice in comparison to the plasma levels measured at the same time.
**Methods:** In a prospective clinical trial ertapenem was given in a dosage of 1 g i.v. 30 minutes prior to operation in patients (18–70 years) suffering from chronic pancreatitis or pancreas carcinoma undergoing pancreas resection. Blood samples were collected every 60 minutes during the operation. Moreover we collected pancreatic tissue and pancreatic juice shortly before resection and shortly before finalisation of the anastomosis. The samples of ertapenem (blood, juice, tissue) were determined by HPLC.
**Results:** In 13 patients (5 female, 8 male, mean age 54.8 ± 11.2 years) ertapenem blood concentrations were determined and demonstrated intraoperatively high concentration (20 ± 8 mg/l) above MIC90 values for major expected pathogens. Concomitantly in 10 of these patients ertapenem concentration was determined also in pancreas tissue and pancreas secretion (in further 3 patients in pancreas secretion only). In 7/10 patients sufficiently high ertapenem levels were detected in pancreatic tissue. In 3 patients with chronic pancreatitis no accumulation was seen. Mean pancreas tissue concentration was 0.8 ± 0.4 μg/g tissue. 5 of 6 patients with pancreas carcinoma had increased ertapenem levels in pancreas secretion but only 2 of 6 patients with chronic pancreatitis.
**Conclusion:** In patients with pancreas carcinoma, ertapenem levels were measured in pancreatic tissue as well as in pancreatic secretion and penetration seems to be similar to imipenem. Due to chronic inflammation and possibly altered microcirculation only in one half to one third of chronic pancreatitis patients ertapenem levels were detected.

## P1519

## Bacterial strain-independent pharmacodynamics of linezolid/doxycycline combinations with *Staphylococcus aureus*: 5-day simulations using an *in vitro* dynamic model

M. Smirnova, I. Alferova, I. Lubenko, Y. Portnoy, S. Zinner, A. Firsov *(Moscow, RU; Cambridge, US)*

**Objective:** To delineate the possible advantages of linezolid (L)/doxycycline (D) combinations over monotherapy, the pharmacodynamics of L, D and L+D were studied with *S. aureus*.
**Methods:** *S. aureus* ATCC 43300 and a clinical isolate *S. aureus* 479 were exposed to twice-daily L (half-life 6 h) and once-daily D (half-life 15 h), alone and in combination (1:3 ratio based on 24-h AUC/MICs), for five consecutive days. To provide simultaneous mono-exponential elimination of L and D with different half-lives, a previously described dynamic model was modified according to Blaser and Zinner. The MICs of L were 1.56 and 1.56 mg/L and MICs of D were 0.1 and 0.05 mg/L for *S. aureus* ATCC 43300 and *S. aureus* 479, respectively. Nine dosing regimens were simulated with each organism exposed to different AUC/MICs (in hours): L30, L60 and L200; D90, D180 and D520; L30 + D90, L60 + D180 and L200 + D520. The cumulative antimicrobial effect was expressed by its intensity (IE) measured from the start of treatment to the time after the last antibiotic dose when numbers of antibiotic-exposed bacteria reached at least 108 CFU/ml. Emergence of resistance was monitored daily by quantitating surviving organisms on agar plates containing 2x and 4xMIC of L or D.
**Results:** With both *S. aureus* ATCC 43300 and *S. aureus* 479 exposed to L or D, IE increased with increasing simulated AUC/MIC ratios, although significantly higher IEs were produced with L30, L60 and L200 treatments relative to D90, D180 and D520 treatments. Each of the combined treatments, i.e., L30 + D90, L60 + D180 and L200 + D520, produced much greater IEs than the sum of L and D IEs observed in the respective mono-treatments with both *S. aureus* strains. Based on population data, a pronounced selection of *S. aureus* resistant to D occurred in all mono-treatments with D. It was also observed with L30 + D90 and, to a lesser extent, with L60 + D180 but not with L200 + D520. No resistance to L was observed with L mono- or combination treatments.
**Conclusions:** These data predict a synergistic interaction of L with D against *S. aureus*.

## P1520

## Anti-staphylococcal effects of telavancin in an *in vitro* dynamic model: impact of different half-lives and initial concentrations

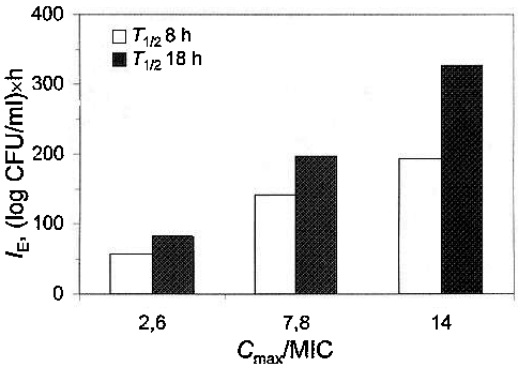
I. Lubenko, M. Smirnova, E. Strukova, S. Vostrov, S. Zinner, A. Firsov *(Moscow, RU; Cambridge, US)*

**Objective:** Telavancin is a lipoglycopeptide antibiotic with excellent activity against gram-positive bacteria including methicillin-susceptible and methicillin-resistant strains of *Staphylococcus aureus*. Telavancin pharmacokinetics are linear and dependent on renal function, with distinctly different half-lives (T1/2 s) in healthy volunteers (8 h) and in patients with severe renal insufficiency (18 h). To demonstrate the possible impact of these pharmacokinetic features on the pharmacodynamics of telavancin, its anti-staphylococcal effects were studied in an *in vitro* one-compartment dynamic model

that simulates normal (NEK) and impaired elimination kinetics (IEK).

**Materials and methods:** A glycopeptide intermediately susceptible strain of *S. aureus* (GISA) Mu-50 with a telavancin MIC of 0.5 mg/L was selected for the study. With both NEK and IEK simulations at a starting inoculum of 6 log cfu/mL, GISA Mu-50 was exposed to different ratios of the peak concentration (Cmax) to the MIC of telavancin (as a single dose), i.e., 2.6, 7.8 and 14. Based on time-kill data, the intensity of the antimicrobial effect (IE - the area between control growth and time-kill curves) was determined from time zero to the time when the effect no longer could be detected, i.e. the time after the last dosing at which the number of antibiotic-exposed bacteria reached 7 log cfu/mL.

**Results:** In each treatment, bacterial regrowth followed gradual reduction in the starting inoculum during the first 12 h (similar in NEK and IEK simulations) that led to significantly lower minimal numbers of surviving organisms in IEK simulations compared to NEK simulations. Despite similar rates of initial killing, times to regrowth were much longer in IEK than NEK simulations. At a given Cmax/MIC ratio, the IEs observed in IEK were greater than in NEK simulations (figure).




**Conclusions:** These findings demonstrate pharmacokinetic-dependent pharmacodynamics of telavancin with *Staphylococci*.

## P1521

## Pharmacokinetics of amoxicillin in pregnant women with pre-term premature rupture of the membranes

A.E. Muller, J. DeJongh, P.M. Oostvogel, R.A. Voskuyl, P.J. Dörr, M. Danhof, J.W. Mouton *(The Hague, Leiden, Nijmegen, NL)*

**Objectives:** Amoxicillin is widely used during pregnancy, in particular to treat group B *Streptococcus*. Insufficient knowledge on the pharmacokinetics just before and during delivery, could pose patients with preterm premature rupture of the membranes (PPROM) at serious risk for under dosing. We investigated the pharmacokinetics in patients with PPROM in this critical situation.

**Methods:** Seven healthy women at 34–37 weeks of gestation were included. They received 1 g (first dose 2 g) amoxicillin for PPROM according to local guidelines. From each patient 14–28 blood samples were taken. Antibiotic serum concentrations were determined by a validated HPLC method. Pharmacokinetic parameters were estimated by population PK modeling using NONMEM. To discriminate between various models the minimum value of the objective function (MVOF) was used. A reduction of >10 in MVOF was considered significant.

**Results:** A three-compartment pharmacokinetic model best described the time course of amoxicillin. The clearance and volume of distribution of the central compartment (Vc) were estimated at respectively 23 ± 1.8 L/h and 6 ± 0.8 L (mean ± SE). Estimates of the parameters and model discrimination improved when we assumed the size of the third compartment to be equal to the first compartment. The residual error was found to be proportional to the serum concentrations. Most of the inter-individual variability could be explained by variation of clearance. The mean volume of distribution at steady state (Vss) and terminal half-life were 23.9 L and 1.34 h respectively. Estimated values of elimination and distribution rate constants were: k10 = 3.9 h-1, k12 = 1.7 h-1, k21 = 0.8 h-1, k13 = 8.8 h-1 and k31 = 8.8 h-1. As was to be expected due to the small population size, no significant relationship was observed between the individual posthoc estimates for clearance and patient characteristics.

**Conclusion:** Here we describe the pharmacokinetics of amoxicillin in pregnant women with PPROM. It was found that the pharmacokinetics clearly differs from that in non-pregnant individuals. Clearance and Vss were significantly higher and the terminal half-life was shorter. Furthermore, a 3-compartment model was found to describe the data better than a 2-compartment model. It is an intriguing question whether this 3rd compartment is a unique feature associated with pregnancy. These data offer a theoretical basis to make proper dose-adjustments in a particular patient group in a critical condition.

## P1522

## Penetration of piperacillin and tazobactam in severe acute pancreatitis

S. Foerster, E. Eckleben, U. Werner, E. Klar, B. Drewelow, R. Wacke *(Rostock, Wismar, DE)*

**Objectives:** Acute necrotizing pancreatitis is still related to an extremely high mortality rate, based on local infectious complications, particularly in necrotizing areas. Limited penetration of antimicrobial drugs in these areas is considered to be a major cause for failure of therapy of severe infections. Combinations of beta-lactamase inhibitors (BLI) and beta-lactam antibiotics like broad-spectrum penicillines (BSP) have antibacterial activity against most of the common pathogens in severe necrotizing pancreatitis. Co-administration leads to an increase of antibacterial activity due to an inhibition of beta-lactamases. On that score, the penetration of co-administrated PIP and BLI into inflamed or necrotic pancreatic tissue has not been investigated yet.

**Methods:** Adressing the penetration capability of BSP and BLI a clinical trial was designed to investigate the penetration of piperacillin (PIP) and tazobactam (TAZ) in patients with severe necrotizing pancreatitis undergoing pancreas surgery. Samples (n = 21) were taken from plasma (Pl), necrotic areas of pancreatic tissue (PN), peripancreatic fatty tissue (PFT) and bursa secretion (BS) following intravenous administration of 4.0 g PIP and 0.5 g TAZ. Concentrations of PIP/ TAZ were determined by HPLC/ UV.

**Results:** Mean plasma concentrations at 1.5 h after application were 56.4 ± 29.5 mg/l (PIP) and 16.9 ± 15.9 mg/l (TAZ). Corresponding mean concentrations were in PN 3.4 ± 4.9 mg/kg (PIP) and 2.3 ± 1.8 mg/kg (TAZ), in PFT 2.1 ± 2.9 mg/kg (PIP) and 1.4 ± 1.0 mg/kg (TAZ), in BS 44. 8 ± 28.2 mg/l (PIP) and 15.1 ± 13.7 mg/kg (TAZ). The penetration rate into PN was 7.5% (PIP) and 13.4% (TAZ), into PFT 4.7% (PIP) and 8.5% (TAZ), into BS 79.5% (PIP) and 89.5% (TAZ). The aimed concentration for full enzymatic effect of TAZ (4 mg/l) was

exceeded in Pl and BS, nearly reached in PN but not in PFT. The concentration of PIP in combination with TAZ exceeded or reached the MIC90 in Pl, PN and BS against *E. coli*, *Klebs*. spp., *Enterobacter*, *Proteus* spp. and *Clostr*. spp., in Pl and BS even against *Pseudomonas* and *Bacteroides*.

**Conclusion:** Given in combination both - PIP and TAZ - have been demonstrated to reach rapidly effective inhibitory concentrations in inflamed and necrotic compartments of pancreatic and peripancreatic tissue. Co-administration of piperacillin and tazobactam may have a potential clinical benefit in prevention and treatment of local infectious complications of severe necrotizing pancreatitis.

## P1523

## PK/PD challenges of *in vitro* time-kill curves – a new modelling approach

S. Schmidt, O. Burkhardt, W. Treyaprasert, H. Derendorf
*(Gainesville, US; Bangkok, TH)*

**Objective:** *In vitro* PK/PD models, based on time-kill curve data, have become a powerful tool to predict the in vivo situation. Up to date, several modelling approaches have been undertaken to develop suitable PK/PD models that fit *in vitro* data sufficiently well. Widely used simple sigmoid Emax models meet these criteria only partly. A further approach was undertaken to address the weak points of currently used models and applied to model the effects of ceftriaxone against *Escherichia coli*.

**Methods:** Constant concentration time-kill curves were performed in Mueller-Hinton broth (MHB, DIFCO) with and without bovine serum albumin (BSA) 40 g/L. Using concentrations of ceftriaxone, ranging from 0.25 MIC to 16 MIC, the change in number of bacteria (CFU/ml) versus time was linked to its effect. *Escherichia coli* ATCC 25922 was employed as the test organism. Samples were taken at 0, 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours. The data were modelled simultaneously, using a modified sigmoid Emax model and the software Scientist® for Windows™.

**Results:** A differential equation, characterized by growth rate constant (k0) times the starting number (N) of bacteria represent the simplest case. Barging from log-growth phase to stationary phase can be described by an additional Nmax term. However, bacteria do not necessarily start growing in the log-growth phase. This delay in onset of growth can be modeled by an exponential term, characterized by a factor beta (b) and time (t). To describe the overall change in number of bacteria not only growth but also concentration (C) dependent kill has to be taken into account. From certain drug specific concentrations on, a maximum effect is reached, described by the maximum kill rate constant kmax. However, it may be necessary to model a delay in the onset of kill with an additional exponential term, characterized by a factor alpha (a) and time (t). Finally, a Hill factor/shape factor (h) is necessary to smooth the predicting curves out. As shown in figure 1 this new model meets the *in vitro* time-kill curve data sufficiently well. The final equation including all parameters described above is:

$$\frac{dN}{dt} = \left( k_0 \cdot \left(1 - \frac{N}{N_{max}}\right) \cdot \left(1 - e^{-\beta t}\right) - \frac{k_{max} \cdot C^h}{EC_{50}^h + C^h} \cdot \left(1 - e^{-\alpha t}\right) \right) \cdot N$$

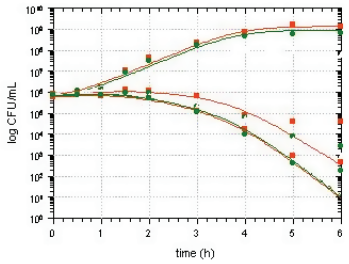



Figure 1: Ceftriaxone against Escherichia coli ATCC 25922 with BSA 40g/L (growth control, 0.016-1.024µg/mL)

**Conclusion:** The proposed model was able to describe the observed data much better than a simple Emax-model. Incorporating two additional terms into the model, the *in vitro* situation could be described much better, taking the delay in onset of growth and kill into account.

## P1524

## Pharmacodynamic profiling of ceftobiprole for the treatment of complicated skin and skin structure infections and nosocomial pneumonia

T.P. Lodise, L. Ma, R. Pypstra, J. Kahn, G.L. Drusano, G.L. Noel *(Albany, US; Basel, CH; Raritan, US)*

**Background:** Ceftobiprole (BPR), an investigational broad-spectrum cephalosporin with robust activity against MRSA & Gram-negatives (GNB), is in phase III trials for complicated skin and soft tissue infection (cSSTI) & nosocomial pneumonia (NP). Objectives of this study were (1) to describe the pharmacodynamic (PD) profiles of BPR 500 mg IV Q8H as a 2-hr infusion & 500 mg IV Q12H as a 1-hr infusion; (2) to determine the overall probability of target attainment (PTA) by weighting for the expected distributions (dis) of renal function (Rfx) in the populations (pop) of interests; (3) to determine the organism-specific PTA against the pathogens encountered in phase II trials.

**Methods:** 150 subjects (total 2443 samples) were studied (Phase I/II subjects). Samples were analysed using BigNPOD. To assess the impact of differing degrees of RFx impairment on PTA, CrCL (CrCL-Cockcroft & Gault method) was employed as a covariate in the pop PK analysis. Monte Carlo Simulation (MCS) (9999 subjects) was performed with ADAPT II. Overall PTA was calculated for 30–60% fT>MIC. Weighting for the expected dis of RFx in the pop of interests was accomplished by using the dis of CrCL observed in 2 previous registration studies of the same indications (cSSTI and NP). Dis of MICs for pathogens was supplied by sponsor.

**Results:** In the pop PK analysis, the pop mean (SD) values for volume, CLslope, CLintercept, Kcp and Kpc were:7.65 (3.89) L, 0.51 (0.32) L/hr, 2.35 (1.08), 3.05 (5.14) hr-1 and 1.10 (0.95) hr-1, respectively. The obs-pred plot was obs = 1.003 x pred + 0.627; r2 = 0.947 after the Bayesian step. In the MCS analysis of BPR 500 mg IV Q12 H, the PTA of achieving 30% fT>MIC & 50% fT>MIC exceeded 90% for MICs values ≤2 mg/L & ≤0.5 mg/L, respectively. For BPR 500 mg IV Q8H, the PTA of achieving 60% fT>MIC exceeded 90% for MICs values ≤2 mg/L. In the organism-specific analysis, the PTA of a static effect (30% fT>MIC) exceeded 90% for both MSSA & MRSA for BPR 500 mg IV Q12H. BPR 500 mg IV Q8H provided a >90% PTA of a cidal effect (50% fT>MIC) for both MSSA & MRSA. For GNB, the PTA of BPR 500 mg IV Q8H in achieving a cidal effect (60% fT>MIC) exceeded 90% for non-AmpC-bearing GNB. For AmpC-bearing GNB, the PTA of achieving a cidal effect was 84.8%.

**Conclusions:** An extensive evaluation of the PD of BPR was performed to estimate the overall activity of BPR against target pathogens. These findings need to be validated in the clinical trial arena.

## P1525

### Investigation of different levofloxacin regimens in patients with acute complicated urinary tract infections

P. Tenke, R. Benko *(Budapest, Szeged, HU)*

**Objective:** In the present study we aimed to find out if a continuous or an intermittent levofloxacin (1 × 500 mg) treatment is more advantageous for patients with acute complicated urinary tract infection (UTI) caused by urinary obstruction. We investigated if levofloxacin adsorbs to the surface of the foreign body, which was inserted with the aim of temporary resolution of ureteral obstruction. Preventive effect of levofloxacin on bacterial biofilm formation and incrustation was also evaluated.
**Methods:** We enrolled and randomised 24 patients who had acute UTI caused by urinary obstruction. Obstruction was resolved with double J stent (DJS) insertion or percutaneous nephrostomy (PCN) and meanwhile, antibiotic treatment was started in all patients. 12 patients (group 1) were on antibiotics till the day of definitive curative operation when all foreign bodies were removed. In the other 50% of the patients (group 2) the antibiotic therapy was stopped 7 days after the DJS or PCN insertion. Short term antibiotic course - which is advisable for prevention of UTI before invasive endoscopic treatment - was administered in both groups from the day of the operation (after DJS or PCN removal) and it was continued until the removal of all possible urinary foreign bodies used during the operation. In both groups of patients we recorded and evaluated early and late clinical and microbiological recovery. Retrieved stents were sectioned for further laboratory examinations. Adsorbed levofloxacin in the conditioning film layer and on the stent surface was detected by HPLC. Rasterelectron microscopy (REM) was used to examine biofilm formation and encrustation.
**Results:** We did not find any significant differences between the two groups of patients, neither in clinical (presence of fever, back pain, flank pain, leukocyte count) nor in microbiological recovery. Statistical analysis showed that significantly greater amount of levofloxacin adsorbed to the conditioning film than to the stent surface in both groups of patients (0.72 ± 0.83 vs. 0.20 ± 0.34 in group 1 and 0.24 ± 0.28 vs. 0.1 ± 0.15 in group 2). No viable, adherent bacteria were recovered by sonication and culture in any of the patients, and no biofilms or encrustation were seen under REM either.
**Conclusion:** Our data prove the hypotheses that continuous antibiotic treatment does not have any clinical or microbiological advantages in patients with indwelling ureteral stents compared to intermittent therapy.

## P1526

### Penetration of linezolid into sternal bone of patients undergoing routine cardio-pulmonary bypass surgery

S. Metallidis, N. Charokopos, E. Alexiadou, A. Tsona, M. Chatzidimitriou, G. Lazaraki, O. Vasilaki, J. Nikolaidis, G. Theodoridis, P. Nikolaidis *(Thessaloniki, GR)*

**Objective:** The prophylaxis of bacterial infections during cardiac surgery is widely used in clinical practice. *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus* spp are the pathogens most frequently involved in infective complications of cardio-pulmonary bypass (CPB) surgery. It is generally agreed that the success of prophylaxis is dependent on the ability to reach and maintain free antibiotic concentration in tissues higher than the MICs for the most common pathogens. So we estimated the tissue concentrations of linezolid into sternal bone of patients undergoing CPB surgery.
**Methods:** Six patients undergoing routine CPB surgery were given 600 mg linezolid as a 30 min IV infusion along with conventional prophylaxis of 1.5 g of cefuroxime immediately before surgery. Two hours after the end of infusion blood samples and sternal bone tissues were collected. The local medical research ethics committee approved this study and all patients gave written informed consent. Samples were assayed for the presence of linezolid by a high-performance liquid chromatography (HPLC) method.
**Results:** Following a 600 mg infusion of linezolid, mean serum concentration for the six patients were 8.48 mg/L (range 7.3–12.36 mg/L) 2 hours after the end of infusion. The concentration of linezolid into sternal bone was 4.35 mg/L (range 3.9–5.2 mg/L) 2 hours after the end of infusion. The penetration of linezolid into sternal bone was 52.2%.
**Conclusion:** The penetration of linezolid into bone was 52.2% of the simultaneous blood levels. In all bone samples the concentration of linezolid exceeded the MIC for susceptible pathogens (<4 mg/L). Although these data have been obtained from healthy, well-perfused bone the values suggest that linezolid may be a useful agent in the management of multidrug-resistant Gram-positive bone infections.

## P1527

### The antibacterial effect of daptomycin, teicoplanin and vancomycin against *S. aureus* studied in an *in vitro* pharmacokinetic model of infection

A. Noel, K. Bowker, A. MacGowan *(Bristol, UK)*

**Objectives:** Daptomycin (DAP) is the first cyclic lipopeptide antibiotic approved for parenteral use in Gram-positive infection. As yet, no comparative pharmacodynamic studies have been performed using DAP and the two most common iv therapies teicoplanin (TEI) and vancomycin (VAN). We used a pharmacokinetic (pK) model to study the antibacterial effect (ABE) of these agents against two typical MRSA strains (UKEMRSA15 & 16) and a hetero vancomycin intermediate MRSA (hVISA).
**Methods:** An *in vitro* dilutional model was used to simulate the free drug concentration over 48 h associated with doses of - DAP 6 mg/kg 24 hrly (Cmax 6.4 mg/L, t 1/2 8 h); TEI 400 mg 24 hrly (Cmax 4.5 mg/L, t1/2 24 h); VAN 1 g 12 hrly (Cmax 13.5 mg/L, t1/2 6 h). An inoculum of $10^6$ CFU/ml was used and the experiments performed in triplicate. ABE was assessed by area-under-the-bacterial-kill-curve 0–24 h (AUBKC24) and 0–48 h (AUBKC48; logCFU/ml.h). Three strains of MRSA were used; strain 15841 VAN MIC 0.38,TEI MIC 0.25, DAP MIC 0.12 mg/l; strain 33024 VAN MIC 0.38, TEI MIC 0.5, DAP MIC 0.12 mg/l; strain 19898 (hVISA) VAN MIC 2, TEIC MIC 8, DAP MIC 1 mg/l.
**Results:** The AUBKC24 and AUBKC48 are shown in the table. The smaller the value, the greater the ABE. Comparison of ABE by ANOVA indicated that DAP had a superior ABE to TEI and VAN, VAN a superior ABE to TEI. DAP or VAN MIC had no effect on the ABE observed. TEI had poor ABE against the hVISA ($p < 0.05$).
**Conclusion:** The ABE of DAP was superior to TEI and VAN at simulated serum free drug concentrations using an *in vitro* pK model.

| | Log CFU/mL.h | MRSA 15841 | MRSA 33024 | hVISA 19898 |
|---|---|---|---|---|
| DAP | AUBKC24 | 6 ± 3 | 3 ± 1 | 6 ± 1 |
| | AUBKC48 | 6 ± 3 | 27 ± 7 | 6 ± 1 |
| TEI | AUBKC24 | 62 ± 1 | 68 ± 7 | 95 ± 3 |
| | AUBKC48 | 122 ± 1 | 132 ± 27 | 220 ± 2 |
| VAN | AUBKC24 | 42 ± 2 | 43 ± 2 | 50 ± 10 |
| | AUBKC48 | 92 ± 28 | 73 ± 5 | 55 ± 11 |

## P1528

## No effect of vitamin B6 co-administration for the prevention of linezolid-induced myelosuppression

D. Plachouras, E. Giannitsioti, F. Kontopidou, S. Athanasia, S. Savanis, K. Pontikis, A. Antonopoulou, A. Papadopoulos, H. Giamarellou *(Haidari, GR)*

**Objectives:** Linezolid, the first oxazolidinone, is active against methicillin resistant *Staphylococcus aureus* and has been effective in a variety of acute infections. However long- term administration, although desirable in bone infections caused by resistant Gram-positive organisms, is hampered by the occurrence of anaemia and thrombocytopenia. Administration of vitamin B6 has been reported to prevent myelosuppression.
**Methods:** Patients attending the infectious disease clinic with bone infections caused by resistant Gram-positive bacteria and treated with linezolid (600 mg b.d. orally), received vitamin B6 (125 mg o.d. orally) for the period of administration of linezolid. Full blood counts were followed-up weekly. Linezolid treatment was discontinued if haemoglobin declined below 9 mg/dl or platelets below 120000/$\mu$l. Data from sixteen patients with osteomyelitis and 8 with prosthetic joint infections were evaluated. Comparisons were performed with 20 matched historical controls receiving linezolid without B6 by Kaplan-Meyer curves with the log-rank test.
**Results:** The median follow-up of patients receiving B6 was 3.5 weeks and of controls 4 weeks. In the B6 group 63% of the patients discontinued while in the control group 44% of the patients discontinued treatment because of side effects (p NS). 47% of patients receiving B6 discontinued due to thrombocytopenia and 20% due to anaemia. Respective percentages in the control group were 21% and 38% (p NS for all comparisons). Mean time to the occurrence of thrombocytopenia was 4 weeks in the patients who received B6 and 3.25 weeks in the control patients. Respective times to occurrence of anaemia were 3.4 and 3.85 weeks. All cases of myelosuppression were reversible.
**Conclusions:** Administration of B6 failed to prevent or delay both thrombocytopenia and anaemia in patients receiving linezolid. Other methods should be investigated to facilitate longer administration of linezolid in this group of patients.

## P1529

## Therapeutic drug monitoring of colistin – a 7-year review from a UK clinical antibiotic assay service

K. Bowker, A. Noel, S. Tomaselli, A. MacGowan *(Bristol, UK)*

**Objectives:** Over the last 5 years there has been increased use of colistin (Col). However, little clinical data is available on the therapeutic levels of colistin. Monitoring of Col is useful in terms of therapeutic levels and avoidance of toxicity for patients with cystic fibrosis and complicated Gram-negative infections. Previous *in vitro* data has shown that Col is bactericidal at Col concentration is ≥3 mg/L. Here we assessed Col data collected from our antibiotic assay service from the last seven years in order to establish if such levels were obtained.
**Methods:** Col levels were determined by bioassay. Data was retrospectively collected from the hospital information management system. Data was assessed collectively and stratified by known cystic fibrosis patients, sex and age.
**Results:** From March 1998–April 2005, 220 serasets from 119 individual patients (59 male, 60 female) were received from 52 requester locations. 37 were denoted as cystic fibrosis patients, 22 were aged ≤16 y and 90 ≥ 16 y. Of the 110 pre dose requests received the mean Col concentration was 3.71 mg/L (95% CI 2.47–4.18), post dose (n = 123) 9.36 mg/L (CI 8.28–10.44). The mean cystic pre dose Col (n = 32) 3.16 mg/L (CI 3.26–4.17), post dose mean (n = 36) 9.36 mg/L (8.28–10.44). Mean female pre dose 4.73 (3.11–6.34), post dose 10.05 (8.1–12.01); male pre dose 3.78 (3.18–4.39), post dose 9.00 (7.56–10.45) Mean ≤16 y pre dose Col (n = 19) 3.3 mg/L (CI 2.17–4.49), post dose mean (n = 22) 9.09 mg/L (5.51–12.68). Mean ≥16 y pre dose Col (n = 83) 3.55 mg/L (CI 3.09–4.02), post dose mean (n = 90) 9.55 mg/L (5.86 –10.78). No significant differences were seen between the pre and post dose concentrations for the different subsets Student's *t*-test $p < 0.05$.
**Conclusions:** These data show that the pre dose levels are adequate for achieving clinical efficacy for *Pseudomonas* spp strains that have MICs below the NCCLS b.pt of ≤2 mg/L. However for resistant strains increased serum concentrations maybe required.

## P1530

## Antibacterial effect of moxifloxacin and ceftriaxone, first doses, on *Streptococcus pneumoniae* studied in an *in vitro* model

K. Bowker, A. Noel, A. MacGowan *(Bristol, UK)*

**Objectives:** ßlactams like ceftriaxone (cfx) and quinolones such as moxifloxacin (mox) are widely used to treat pneumococcal infection. We studied the antibacterial effect of (ABE) after the first dose exposure to free drug serum concentrations of iv cfx 2 g and po mox 400 mg against *S. pneumoniae* strains with typical MICs at low and high inocula.
**Methods:** A hollow fibre *in vitro* model was used to simulate free drug concentrations over 24 h of cfx (2 g 24 h iv, Cmax 26 mg/L, AUC 56 mg/L·h, T1/2 6 h) and mox (400 mg, 24po, Cmax2.0 mg/L, AUC20 mg/l·h, T1/2 10.5 h). The cfx MIC was 0.06 mg/L (T>MIC cfx 100%) and mox MIC 0.06 mg/L (AUC/MIC 334). Initial ABE was measured by the slope of the log viable count 0–5 h and total ABE over the dose interval (24 h) by log reduction in viable count at 24 h (d24) and the area-under-the-bacterial-kill-curve (AUBKC24). Inocula of 106 cfu/mL and 108 cfu/mL were used.
**Results:** The initial and total ABE at low and high inocula were: Given the pK/pD indices modelled both drugs showed a maximal effect. Clearance from the model occurred at 12 h (106 inoculum) and 24 h (108 inoculum). There were no significant differences in speed or extent of ABEs comparing cfx and mox.
**Conclusion:** The ABEs of iv cfx and po mox against *S. pneumoniae* is similar in the first 24 hrs of drug exposure.

| | $10^6$ inoculum | | $10^8$ inoculum | |
|---|---|---|---|---|
| | cfx | mox | cfx | mox |
| Slope | -0.36 ≤ 0.17 | -0.56≤ 0.17 | -0.61≤ 0.5 | 0.35 ≤ 0.08 |
| d24 | >4.0 | >3.8 | >5.8 | >5.9 |
| AUBKC24 | 16.1 ≤ 7.2 | 18.7 ≤ 4.1 | 4.1 | 47.1 ≤ 3 |

## P1531

### Emergence of resistance in *E. coli* and *Ent. cloacae* after exposure to ceftriaxone or ertapenem in an *in vitro* model of infection

K. Bowker, A. Noel, A. MacGowan *(Bristol, UK)*

**Objectives:** Emergence of resistance (EoR) is an emergent factor in therapeutic choice. We studied EoR to ceftriaxone (cfx) and ertapenem (ert) in *E. coli* (Ec) and *Ent. cloacae* (Entclo), a more challenging species within inducible Blactamases.

**Methods:** An *in vitro* dilutional model was used to simulate free drug concentrations associated with 1 g 24 hrly cfx (Cmax 22 mg/L, t½ 8 h) and ert (Cmax 13 mg/L, t½ 5 h) over 72 h. Two inocula 106 and 108 CFU/ml were used and EoR assessed by population-analysis-profiles (PAP). The area under the PAP (AUC-PAP) was used to measure EoR. Ert MICs were 0.018 mg/L Ec and 0.25 mg/L Entclo, cfx MIC 0.14 mg/L Ec and 0.5 mg/L Entclo. Experiments were performed in triplicate and mean values presented.

**Results:** Observations at 72 h were similar to those at 48 h, hence data to 48 h is given. At 106 and 108 CFU/ml, Ec viable counts were reduced by 2 → 4 logs, there was no EoR. Against Entclo inoculum 106 and 108, cfx resulted in an initial 0–2 log drop, then regrowth, ert produced a >4 log reduction. EoR as measured by the mean AUC-PAP (n = 3) with Entclo is shown below: Dosing with cfx resulted in EoR to cfx and ert at high and low inoculum. Dosing with ert resulted in no EoR at 106 inoculum, at 108 resistance emerged to both cfx and ert.

| Drug dosed | Initial inoc (log CFU/mL) | Drug for AUC-PAP | Mean AUC-PAP at | | |
|---|---|---|---|---|---|
| | | | 0h | 24h | 48h |
| cfx | 6 | cfx | 5.5 | 32.7 | 32.8 |
| | | ert | 4.7 | 22.8 | 20 |
| | 8 | cfx | 7.4 | 20.9 | 25.8 |
| | | ert | 5.5 | 8.6 | 15.0 |
| ert | 6 | cfx | 5.5 | <1 | <1 |
| | | ert | 4.7 | <1 | <1 |
| | 8 | cfx | 7.4 | 13.6 | 19.9 |
| | | ert | 5.5 | 1.4 | 10.3 |

**Conclusions:** EoR depends on species (Entclo > Ec); duration of exposure (long > short) and agent (cfx > ert). Ert appears to induce less EoR both to itself and cfx than cfx does to itself and ert. However, initial use of cfx may reduce the effectiveness of ert.

## P1532

### Comparative serum activity of telithromycin, azithromycin, and amoxicillin/clavulanate against aerobic and anaerobic respiratory pathogens

G. Stein, S. Schooley, E. Goldstein, K. Tyrrell, D.M. Citron *(East Lansing, Santa Monica, US)*

**Objectives:** The purpose of this investigation was to study the clinical potential of telithromycin, a new ketolide antibiotic, for the treatment of mixed aerobic/anaerobic respiratory infections. In this study, we compared the pharmacodynamics (duration of inhibition/killing) of telithromycin (Tel) to azithromycin (Azi) and amoxicillin/clavulanate (A/C) against aerobic and anaerobic pathogens associated with mixed respiratory infections.

**Methods:** Following written informed consent, ten healthy adult subjects (ages, 20–47 yrs) received single doses of Tel (800 mg), Azi (500 mg), and A/C (875 mg) one week apart following a 12-h fast. Venous blood samples were obtained at 2, 6, 12, and 24 h after the dose and stored at −70°C. Inhibitory and bactericidal titre s were determined by microdilution (*S. pneumoniae* & *H. influenzae*) and agar dilution (*Peptostrep. magnus*, *Peptostrep. micros*, *Prev. bivia*, & *Prev. melaninogenica*) procedures following Clinical & Laboratory Standards Institute methodology. Bactericidal titres in serum endpoint was determined as the highest dilution of serum yielding 99.9% killing. The median titres at each time point were calculated and the duration of activity was used for comparison of these agents.

**Results:** Against 2 Azi-resistant (MICs = 8 & 16 μg/ml) strains of *S. pneumoniae*, both Tel (MICs = 0.25 & 0.5 μg/ml) and A/C (MICs = 0.03 & 2.0 μg/ml) exhibited prolonged inhibitory (6–12 h) as well as cidal (2–12 h) activity in our subjects. All three antibiotics provided 12–24 h of inhibitory activity against a beta-lactamase (BL) positive (Tel MIC = 4.0 μg/ml; A/C MIC = 0.5 μg/ml; Azi MIC = 2.0 μg/ml) and negative (Tel MIC = 4.0 μg/ml; A/C MIC = 4 μg/ml; Azi MIC = 2.0 μg/ml) strain of H. influenzae. Both Tel and A/C exhibited prolonged inhibitory (24 h for Tel; 6–12 h for A/C) activity against each of the anaerobes studied (Tel MICs = 0.03–0.25 μg/ml). Furthermore, both Tel and A/C provided prolonged (12–24 h) cidal activity against the two Prevotella species.

**Conclusions:** In this *ex-vivo* study, we found that Tel can provide prolonged (50% of the dosing interval) inhibitory activity in serum against macrolide-resistant strains of *S. pneumoniae*, BL pos. and neg. strains of *H. influenzae*, and common respiratory anaerobic pathogens. These findings suggest that Tel could have clinical utility in the treatment of community-acquired mixed aerobic-anaerobic respiratory tract infections, including sinusitis, bronchitis, and pneumonia.

## P1533

### Urinary bactericidal activity of levofloxacin (750 mg) against fluoroquinolone-resistant uropathogens

G. Stein, S. Schooley, D. Nicolau *(East Lansing, Hartford, US)*

**Objectives:** Increasing resistance in isolates of *E. coli* (12%) and *P. aeruginosa* (40%) to fluoroquinolones (FQ) is a concern since these antibiotics are commonly used in the treatment of complicated urinary tract infections (UTIs). Currently, no interpretive standards exist for ''susceptible'' isolates in urine for the newer FQ. The purpose of this investigation was to evaluate the activity of high-dose levofloxacin against FQ-resistant urinary pathogens.

**Methods:** In this study, we determined the serum and urine levels of high dose (750 mg) levofloxacin (L) as well as its bactericidal activity in urine (UBA) against L-resistant isolates of *E. coli* (MICs = 8 to 64 μg/ml) and *P. aeruginosa* (MICs = 8 to 64 μg/ml). Following written informed consent, blood and urine samples were collected from 10 healthy adult (ages, 20–47 y/o) fasting subjects (5 M and 5 F) prior to and at 1.5, 4, 8, 12, and 24 hours after a single 750 mg dose of L. Serum and urine concentrations were measured by a validated HPLC assay (0.9–2.1% CV). The testing methodology for UBA was similar to the

microdilution assay used for serum bactericidal testing (CLSI) with the exception that antibiotic-free urine was used to dilute these samples. The median titre (1:2–1:32) at each time point for the 10 subjects was used to determine UBA.

**Results:** The mean serum pharmacokinetic parameters were similar to previously published values: Cmax = 9.2 μg/ml, AUC = 95 mg·h/L, and T1/2 = 7.6 h. Mean urine concentrations ranged from 475 μg/ml (4 h) to 186 μg/ml (24 h). UBA (titres >1:2) was maintained for at least 12 hours in all subjects for *E. coli* isolates with MICs = 8, 16, and 32 μg/ml. For the *E. coli* strain with a MIC = 64 μg/ml, 8 subjects exhibited UBA at 8 h but only 2 subjects exhibited UBA at 12 h. Similar results were observed against the *P. aeruginosa* isolates.

**Conclusions:** The results from this ex-vivo pharmacodynamic study in healthy volunteers found that 750 mg of L provides prolonged (at least half the dosing interval) UBA against L-resistant strains of *E. coli* and *P. aeruginosa* up to 32 μg/ml. This suggests that a separate urinary susceptibility breakpoint is indicated for urine isolates treated with 750 mg doses of L.

## P1534

## Meropenem and cloxacillin are active against MRSA clinical isolates (including VISA) in acidic broth and in THP-1 macrophages

S. Lemaire, F. Van Bambeke, P. Tulkens, M.P. Leclercq, J. Coyette, Y. Glupczynski *(Brussels, Liège, Yvoir, BE)*

**Objectives:** Exposure of Methicillin-resistant *S. aureus* (MRSA) to acid pH restores its susceptibility to beta-lactams (Sabath and al., AAC, 1972). In macrophages, *S. aureus* is mainly confined within phagolysosomes where the pH is acidic. We showed that meropenem (MEM) displays similar intracellular activity against MRSA ATCC 33591 and MSSA ATCC 25923 in macrophages. In the present study, we have investigated the intraphagocytic activity of MEM and cloxacillin (CLX) against 3 MRSA clinical isolates (including one VISA strain), in comparison with the reference MRSA ATCC 33591 and MSSA ATCC 25923 strains.

**Methods:** MIC's were determined in MHB (plus NaCl 2%) by micro-dilution method. mecA expression was examined at neutral and acidic pH by a semi-quantitative RT-PCR (16 S rRNA as housekeeping gene). Intracellular activity was assessed in human THP-1 macrophages exposed to extracellular concentrations equivalent to human Cmax (total drug; MEM: 50 mg/L; CLX: 8 mg/L) by examining the decrease in cell-associated CFU after 24 h from the original, post-phagocytosis inoculum (controls [no antibiotic]; approx. 1 log CFU increase).

**Results:** The table shows the MICs (in broth) at neutral and acid pH and the intracellular activity for the 5 strains studied. In ATCC 33591, mecA expression was similar for bacteria maintained in broth at pH 7.4 or 5.5 (O.D. mecA/O.D. 16 S rRNA: 0.20 ± 0.1 vs. 0.24 ± 0.1).

| Strains | | MEM | | | CLX | | |
|---|---|---|---|---|---|---|---|
| | | MIC (mg/L) | | ΔCFU | MIC (mg/L) | | ΔCFU |
| | | pH 7.4 | pH 5.5 | | pH 7.4 | pH 5.5 | |
| MSSA | ATCC 25923 | 0.125 | 0.125 | -0.9 ± 0.1 | 0.125 | 0.03 | -0.7 ± 0.1 |
| MRSA | ATCC 33591 | 16 | 0.125 | -0.6 ± 0.1 | 128 | 0.06 | -0.5 ± 0.1 |
| | N4120032 | 2 | 0.06 | -0.8 ± 0.1 | 1 | 0.06 | -0.6 ± 0.1 |
| | N4120210 | 2 | 0.06 | -0.6 ± 0.1 | 1 | 0.06 | -0.4 ± 0.0 |
| | VISA NRS 18 | 8 | 0.06 | -0.6 ± 0.1 | 8 | 0.03 | n.d. |

**Conclusions:** The intracellular environment markedly enhances the activity of beta-lactams against MRSA, probably through exposure to acid pH, although the latter does not affect mecA expression.

## P1535

## Comparative activity of dalbavancin against European Gram-positive isolates

I. Morrissey, C. Dencer, J.W.T. Dallow, J. Childers, A. Brook, J. Cowan *(London, UK)*

**Objectives:** Dalbavancin (DAL) is a new semisynthetic lipoglycopeptide with a half-life of 8.5 days, enabling once-weekly dosing. This study compared the activity of DAL with other agents against Gram-positive isolates from Europe.

**Methods:** Isolates from Belgium, the Czech Republic, Denmark, Finland, France, Germany, Hungary, Italy, the Netherlands, Poland, Spain, Sweden and the UK were included. The CLSI broth microdilution method was used to determine MIC using dried microtitre plates. The following antimicrobial agents were evaluated: DAL, vancomycin (VAN), teicoplanin (TEI), daptomycin (DAP), linezolid (LZD), dalfopristin/quinupristin (SYN), erythromycin (ERY), levofloxacin (LEV) and tetracycline (TET).

**Results:** Selected data are shown in the Table (BHS, beta-haemolytic streptococci; CNS, coagulase-negative staphylococci; MR, methicillin-resistant; MS, methicillin-susceptible; SA, *S aureus*; VS, 'viridans' streptococci). DAL was very active against all isolates including MRSA (except for 25 of 204 enterococcal isolates that were VanA). Excluding the VanA, DAL was the most active agent tested, including newer agents such as DAP.

| Organism | MIC range (mg/L) [% susceptible] | | | | | | |
|---|---|---|---|---|---|---|---|
| | MSSA | MRSA | CNS | BHS | VS | Efc | Efm |
| N | 351 | 201 | 206 | 640 | 114 | 66 | 138 |
| DAL[a] | 0.015-0.12 [100]* | 0.008-0.25 [100] | 0.015-0.25 [100] | <0.004-0.25 [100] | <0.004-0.25 [100] | 0.015-8 [99] | 0.015->16 [83] |
| VAN | 0.25-2 [100] | 0.5-4 [100] | 0.5-4 [100] | 0.25-1 [100] | 0.25-1 [100] | 1->64 [99] | 0.25->64 [82] |
| TEI | <0.12-2 [100] | <0.12-4 [100] | 0.12-64 [98] | <0.12-0.25 [NA][b] | <0.12-0.25 [NA][b] | 0.12->64 [99] | 0.12->64 [83] |
| DAP | 0.12-1 [100] | <0.12-1 [100] | <0.03-1 [100] | <0.12-1 [100] | <0.12-1 [100] | 0.25-4 [100] | 0.25-4 [100] |
| LZD | 0.5-2 [100] | 0.5-2 [100] | <0.5-2 [100] | <0.5-2 [100] | <0.5-2 [100] | 1-2 [100] | 1-2 [100] |
| SYN | <0.25-1 [100] | <0.25-1 [100] | <0.25-1 [100] | <0.25-1 [100] | <0.25-4 [92] | 0.5->16 [2] | 0.25-8 [61] |
| LEV | <0.12->16 [93] | <0.12->16 [13] | <0.12->16 [53] | 0.12-8 [99.8] | 0.12-2 [100] | 0.25-16 [62] | 0.12->16 [41] |
| ERY | 0.12->32 [87] | 0.12->32 [14] | <0.12->32 [37] | <0.12->32 [81] | <0.12->32 [74] | <0.12->32 [20] | 0.12->32 [5] |
| TET | <0.25->16 [89] | <0.25->16 [59] | <0.25->16 [78] | <0.12->16 [52] | <0.12->16 [62] | 0.25->16 [32] | 0.25->16 [56] |

[a]For dalbavancin projected susceptible breakpoints of ≤1 mg/L for staphylococci and enterococci; and ≤2 mg/L for streptococci were used; [b] NA, CLSI breakpoints unavailable

**Conclusion:** Dalbavancin demonstrates potent activity against the majority of Gram-positive pathogens including MRSA and represents a potential treatment option for serious Gram-positive infections.

## P1536

## Population pharmacokinetics of dalbavancin and pharmacodynamic simulations of a weekly regimen

J.A. Dowell, B.P. Goldstein, M. Buckwalter, M. Stogniew, B. Damle *(New York, US)*

**Objectives:** Dalbavancin (DAL) is a next generation lipoglycopeptide antibiotic in development for the treatment of complicated skin and skin structure infections (cSSSI). A population pharmacokinetic (PK) analysis was performed to estimate patient parameters and to determine significant covariates. Incorporating the PK model, pharmacodynamic (PD) parameters were simulated to support the effectiveness of a weekly dosage.

**Methods:** The PK analysis included 1668 DAL concentrations from 532 patients across 3 clinical trials. Most patients received 1000 mg on Day 1 and 500 mg on Day 8. Possible covariates

examined included demography and concomitant medications, including medications that are considered inhibitors, inducers, and substrates of cytochrome P450 enzymes. The PK-PD analysis employed Monte Carlo simulations of time-dependent and concentration-dependent parameters. Distributions of MICs were obtained directly from clinical studies, and were also simulated to explore the effect of higher MICs.
**Results:** DAL PK fit a 2-compartment model with interpatient variability (IPV) on all parameters. The typical value and IPV (CV%) of clearance (CL) was 0.0571 L/h (18.0%), influenced by body surface area (BSA) and creatinine clearance (CLCR). Volume of distribution (V1) was 4.15 L (24.5%) and influenced by BSA. The inter-compartmental clearance and peripheral volume were 0.476 L/h and 11.4 L, respectively. Free drug concentrations were simulated using a DAL protein binding of 93%. For a weekly 2-dose regimen, free DAL remained above 1 mg/L for the majority (>90%) of patients for more than 14 days. Using previously described area under the curve (AUC)/MIC targets for *Staphylococcus aureus*, a proposed MIC of at least 0.5 mg/L was associated with a greater than 90% probability of target attainment.
**Conclusions:** DAL PK were predictable, demonstrating low IPV. BSA and CLCR were the only sources of variability, but described less than 25% of the IPV. PD simulations support the use of dalbavancin in a weekly regimen.

## P1537

## Distribution of radioactivity in bone and related structures following administration of [14C]-dalbavancin to New Zealand White rabbits

E. Solon, J.W. Dowell, J. Lee, S.P. King, B. Damle *(Newark, New York, US)*

**Objectives:** Methicillin-resistant *Staphylococcus* joint infection due to peri-operative contamination is a complication after arthroplasty. The objective of this study was to assess the distribution of radioactivity in bone and related structures using quantitative autoradiography after administration of [14C]-dalbavancin in rabbits.
**Methods:** New Zealand White male rabbits were given a single intravenous (IV) bolus dose of 20 mg/kg [14C]-dalbavancin (n = 18) or control vehicle (n = 3). Plasma, cerebrospinal fluid (CSF), bone, bone marrow, and nucleus pulposus were collected at 12, 24, 72, 120, 168 and 336 h post-dose by necropsy, homogenized, combusted, and analysed for total drug-derived radioactivity using liquid scintillation counting (LSC). In addition, the left hindlimb from 1 rabbit/time point was flash-frozen and cryosectioned for quantitative autoradioluminography.
**Results:** [14C]-dalbavancin-derived radioactivity was rapidly and widely distributed into bone, bone marrow and, to a lesser extent, in CSF and nucleus pulposus. Autoradioluminography data indicated that concentration of radioactivity was highest in bone marrow, whole blood, articulate cartilage, ligament, epiphyseal plate, periostium, and meniscus. At 336 h post-dose, [14C]-dalbavancin-derived radioactivity was measurable in all tissues, and remained at relatively high concentrations in bone marrow (30.27 $\mu$g equiv/g), epiphyseal plate (8.42 $\mu$g equiv/g), periostium (5.18 $\mu$g equiv/g), and articular cartilage (4.84 $\mu$g equiv/g). In homogenized bone using LSC, mean concentration after 336 hours was 1.96 $\mu$g equiv/g.
**Conclusion:** [14C]-dalbavancin-derived radioactivity rapidly penetrated knee joint tissues and persisted at relatively high concentrations for at least 336 h after a single IV dose in rabbits.

## P1538

## Telavancin transmembrane clearance during *in vitro* continuous venovenous hemofiltration

J.H. Patel, M.C. Grio, M.D. Churchwell, J. Seroogy, S. Barriere, B.A. Mueller *(Ann Arbor, South San Francisco, US)*

**Objectives:** Telavancin (TLV), a bactericidal lipoglycopeptide with multiple mechanisms of action, is in Phase 3 trials for the treatment of hospital-acquired pneumonia (HAP) with a focus on infections due to methicillin-resistant *S. aureus*. TLV is primarily eliminated by the kidneys and requires dosage adjustment for renal dysfunction. TLV is highly protein bound (93%) in healthy subjects which would suggest that it would not be removed by dialysis, but its small volume of distribution (0.15 L/kg) means that it may be removed by CVVH. CVVH is widely used in the management of critically ill patients. The objective of this study was to determine CVVH telavancin transmembrane clearance (Cl) with 2 commonly used hemofilters (AN69, polysulfone) at conventional ultrafiltrate flow rates.
**Methods:** TLV Cl was assessed in our *in vitro* CVVH model using citrate anticoagulated bovine blood and B. Braun Diapact machine. Experiments were run using AN69 (M100, Gambro) and polysulfone (F160NR, Fresenius) hemofilters. Ultrafiltrate (UF) flows were 1, 2, 3 and 6 L/hr with sufficient blood flows [(Qb) 200–350 mL/min] to maintain UF rates. Blood samples were collected from the pre-filter line and UF samples from the post-filter UF port. Concentrations of TLV in plasma and UF samples were assayed using validated LC-MS/MS methods. TLV Cl was determined using the following formula:Cl = (UF flow rate) [TLV]UF/[TLV]Arterial. Cl differences between the filter types were compared using a two-tailed, unpaired t-test.
**Results:**

| Flow Rates | Mean (± SD) CVVH TLV Cl (mL/min) | | p-value |
|---|---|---|---|
| | AN69 (n = 3) | Polysulfone (n = 5) | |
| Qb 200 mL/min, UF 1 L/hr | 4.98 ± 0.52 | 5.04 ± 0.84 | 0.91 |
| Qb 200 mL/min, UF 2 L/hr | 9.13 ± 2.5 | 9.97 ± 0.88 | 0.63 |
| Qb 300 mL/min, UF 3 L/hr | 14.56 ± 1.42 | 13.6 ± 2.6 | 0.53 |
| Qb 350 mL/min, UF 6 L/hr | 30.8 ± 7.45 | 24.9 ± 1.6 | 0.30 |

**Conclusion:** TLV is substantially cleared by CVVH and Cl increases significantly with increasing UF rate. Cl did not differ by hemofilter type. CVVH Cl at higher UF flows exceeds the total Cl reported in patients with normal renal function. TLV likely will require dose adjustments in patients receiving CVVH.

## P1539

## Telavancin pharmacokinetics during *in vitro* continuous venovenous haemodialysis

J.H. Patel, M.C. Grio, M.D. Churchwell, J. Seroogy, S. Barriere, B.A. Mueller *(Ann Arbor, South San Francisco, US)*

**Objectives:** Telavancin (TLV), a bactericidal lipoglycopeptide with multiple mechanisms of action, is in Phase 3 trials for the treatment of hospital-acquired pneumonia (HAP) with a focus on infections due to methicillin-resistant *S. aureus*. TLV is primarily eliminated by the kidneys and requires dosage adjustment for renal dysfunction. TLV is highly protein bound (93%) in healthy subjects which would suggest that it would not be removed by dialysis, but its small volume of distribution (0.15 L/kg) means that it may be removed by CVVHD. CVVHD is used in the management of critically ill patients. The objective of this study was to determine CVVHD TLV transmembrane clearance (Cl) with 2 commonly used hemodialyzers at conventional CVVHD dialysate flow rates.

**Methods:** TLV Cl was assessed in our *in vitro* CVVHD model using citrate anticoagulated bovine blood and B. Braun Diapact machine. Experiments were run 5 times using AN69 (M100, Gambro) and polysulfone (F160NR, Fresenius) hemodialyzers. Dialysate flows were 1, 2, 3 and 6 L/hr with sufficient blood flows [(Qb) 200–350 mL/min] to maintain appropriate transmembrane pressures. Blood samples were collected from the pre-filter port (A) and post-filter port (V), and spent dialysate samples (D) from the post-filter D port. Plasma TLV concentrations (arterial and venous) and dialysate samples were assayed using validated LC-MS/MS methods and TLV Cl was determined using the following formula: Cl = (D flow rate) [TLV]D/(([TLV]Arterial+[TLV]Venous)/2). Dialytic Cl between filter types was compared using a two-tailed, unpaired t-test.
**Results:**

| Flow Rates | Mean (SD) CVVHD TLV Cl (mL/min) | | p-value |
|---|---|---|---|
| | AN69 | Polysulfone | |
| Qb 200 mL/min, D 1 L/hr | 4.62 (1.84) | 7.10 (2.35) | 0.10 |
| Qb 200 mL/min, D 2 L/hr | 8.31 (0.58) | 12.2 (3.23) | 0.054 |
| Qb 300 mL/min, D 3 L/hr | 8.95 (1.43) | 15.0 (1.35) | 0.00014 |
| Qb 350 mL/min, D 6 L/hr | 10.2 (1.68) | 23.8 (4.51) | 0.0013 |

**Conclusion:** TLV is effectively cleared by CVVHD. The higher permeability polysulfone dialyzer was associated with significantly increased Cl vs. the AN69 dialyzer as dialysate flow increased. The degree of TLV Cl seen with CVVHD suggests that dose adjustments will be necessary in patients receiving CVVHD.

## P1540

## Carbapenems – differences in their antibacterial activity due to their protein binding

S. Schmidt, O. Burkhardt, S. Schubert, A. Dalhoff, H. Derendorf *(Gainesville, US; Kiel, DE)*

**Objective:** It is still a subject of controversy that only free, unbound drug is responsible for antibacterial activity of antibiotics. To provide further proof, that only free drug contributes to antimicrobial efficacy a comparative, dose-ranging time-kill curve study was performed. To exclude influence factors resulting from different mechanisms of action this was done within the antibiotic class of carbapenems, using compounds with different serum protein binding.
**Methods:** Constant concentration time-kill curves were performed in 50% serum for the slightly serum protein bound mer-openem (~2%) and imipenem (10%) as well as for the highly serum protein bound ertapenem (95%) and faro-penem (95–96%), ranging from 1 × MIC to 16 × MIC. The change in number of bacteria (CFU/mL) versus time was linked to their effect. *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* Bay 63, *Staphylococcus aureus* Bay 133 and *Streptococcus pneumoniae* ATCC 6303 were used as the test organisms. Samples were taken at 0, 0.5, 1 2, 4, 6 and 8 hours. The data were modelled simultaneously using the software Scientist® for Windows™ and a modified sigmoid Emax model characterized by growth rate constant (k0), maximum kill rate (kmax), and concentration at half maximum effect (EC50).
**Results:** For all four bacterial strains investigated, there were dramatic increases (400-700%) in EC50 for the highly se-rum protein bound carbapenems (ertapenem, faropenem) in the presence of serum proteins (Fig. 1). For both substances no significant differences in k0 and kmax were determined. In contrast, imipenem and meropenem showed only minor differences in EC50 in the presence and the absence of 50% serum.

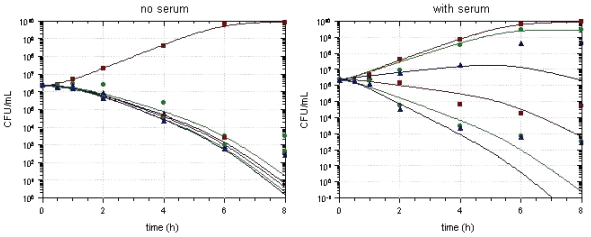


Figure 1: Staphylococcus aureus Bay 133 with and without 50% human serum

**Conclusion:** Only free, unbound drug is responsible for the antimicrobial activity. Analysis of these time-kill curves clearly showed that the antibacterial efficacy was significantly decreased in the presence of 50% serum for the highly bound ertapenem and faropenem while being unaltered for the slightly bound meropenem and imipenem.

## P1541

## Experimental pitfalls in protein binding measurements

S. Schmidt, O. Burkhardt, M. Sahre, H. Derendorf *(Gainesville, US)*

**Objective:** Numerous *in vitro* experiments have shown that Protein Binding (PB) is an important factor for antimicrobial activity, especially for highly bound antibiotics. However, the experimental conditions that simulate the in vivo situation best are still subject of controversy. Therefore, an *in vitro* microdialysis experiment was performed that evaluates various influence factors on the PB of the highly bound beta-lactams ceftriaxone (PB 95–98%).
**Methods:** A comparative, dose-ranging *in vitro* microdialysis study was conducted to determine free, unbound ceftriaxone concentrations in Lactated Ringer's solution and Todd Hewitt Broth (THB) both with and without bovine serum albumin (BSA; Sigma, St. Louis) 40 g/L and human plasma at 37°C. Furthermore, *in vitro* constant concentration time-kill curves were performed, using *Escherichia coli* ATCC 25922, *Streptococcus pneumoniae* ATCC 6303 and *Streptococcus pneumoniae* ATCC 49619 as the test organisms. The Data was analysed using an appropriate PK/PD model, characterized by growth rate constant (k0), maximum kill rate (kmax), and concentration at half maximum effect (EC50) and correlated to free ceftriaxone THB concentrations determined by HPLC-UV.
**Results:** There were only minor differences in both unbound drug concentrations and anti-infective activity when BSA 40 g/L was added to either Lactated Ringer's (PB 0% and 12.5±2.5%, with and without BSA respectively) or THB (PB 27% and 25.5±2.5%, with and without BSA respectively). No significant changes in k0, kmax and EC50 were observed. However, using human plasma, unbound concentrations (PB 60%−88%) were altered dramati-cally.
**Conclusion:** Only free, unbound drug is responsible for the antimicrobial activity. However, one cannot rely on that binding to commercially purchased BSA is consistent with reported protein binding values. Unbound concentrations should be measured under the respective experimental conditions to be able to correctly interpret the experimental results.

## P1542

### *In vitro* postantibiotic effect of faropenem on penicillin-resistant *Streptococcus pneumoniae* and beta-lactamase-producing Haemophilus influenzae

C.L. Young, I.A. Critchley, U.A. Ochsner, N. Janjic *(Louisville, US)*

**Objectives:** Faropenem (FAR) is an oral penem with potent activity against respiratory pathogens such as penicillin (PN)-resistant *Streptococcus pneumoniae* (SP) and beta-lactamase (BLA)-producing *Haemophilus infleunzae* (HI). The postantibiotic effect (PAE) is a pharmacodynamic (PD) parameter that monitors suppression of bacterial growth following short exposure and removal of the drug. PAEs are clinically important for agents such as FAR with short half-lives (1 h). The aim of the study was to determine the PAE of FAR on resistant phenotypes of SP and HI.
**Methods:** Nine clinical isolates of SP, 3 PN-S, 3 PN-I, and 3 PN-R and six clinical isolates of HI, 3 BLA-negative and 3 BLA-positive were tested in PAE studies. PAEs were determined in cation-adjusted Mueller-Hinton broth with 3-5% lysed horse blood for SP and Haemophilus Test Medium for HI. Exponential cultures (106 CFU/mL) were exposed to FAR at 1, 4 and 10 x MIC. FAR was removed by serial washing (10,000-fold dilution) prior to transfer to fresh media. Control cultures were treated in the same way. Bacteria were incubated with shaking and viable CFUs determined at 1, 4, 6, 8, 16 and 20 h. Counts of log10 CFU were plotted against time and PAE defined as the difference is the time required for count in test culture and control (untreated culture) to increase 1 log10 above the count observed immediately after removal.
**Results:** Significant PAEs of >0.5 h were observed for all strains of SP at 4 and 10 x MIC. However, the PAE was more prolonged on the PN-R strains with mean PAEs of 3.9 h at 4 and 10 x MIC. Among HI, little or no PAE was observed on BLA-negative strains but a significant PAE was observed on the BLA-positive isolates (mean PAEs of 5.9 h and 8.9 h at 4 and 10 x MIC respectively).
**Conclusions:** FAR demonstrates a prolonged PAE on key resistant phenotypes of SP (PN-R) and HI (BLA-positive) compared with susceptible strains. The observation of PAE in BLA-positive HI is unique in the class of beta-lactams. FAR exhibits *in vitro* PD properties that may contribute to its clinical efficacy against PN-R SP and BLA-positive HI.

## P1543

### Telavancin is more efficacious than vancomycin in a murine model of bacteraemic peritonitis induced by methicillin-resistant *Staphylococcus aureus*

S. Hegde, N. Reyes, B. Benton, R. Skinner *(South San Francisco, US)*

**Objective:** Telavancin (TLV) is a novel lipoglycopeptide that operates through multiple mechanisms to produce potent and rapid bactericidal activity against clinically relevant gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA). The present studies evaluated the in vivo efficacy of TLV vs vancomycin (VAN) in a model of MRSA induced peritonitis in neutropenic mice.
**Methods:** Female NSA immunocompromised mice were inoculated intraperitoneally with ATCC MRSA 33591 and treated, beginning at 4 h post-infection, with 2 subcutaneous doses (q 12h) of vehicle (VEH) or test compound. Mouse pharmacokinetic data were generated and used to choose doses of TLV (40 mg/kg) and VAN (110 mg/kg) in order to equate clinical exposures (AUC's (free drug) of 44 and 132 $\mu$g.hr/mL, respectively). In survival studies, deaths were recorded for 14-days post-infection and survival curves were compared using log-rank test. In bacterial titer determination studies, designated groups of control and drug-treated surviving animals were humanely euthanized at various times post-treatment and their blood and spleen were harvested to determine bacterial titers.
**Results:** MICs of TLV and VAN were 0.5 and 1.0 $\mu$g/mL, respectively. Mortality was 100% in animals treated with VEH or VAN. Mortality was 7% in TLV-treated animals ($p < 0.05$ vs VEH and VAN). The pre-treatment bacterial titres were 4.3 log CFU/mL and 8.7 log CFU/g in the blood and spleen, respectively. Analysis of the time kill curves for both blood and spleen revealed that TLV exhibited significantly greater killing activity than VAN ($p < 0.05$, two-way ANOVA). At 6 hrs after the first dose, the titers in the blood were reduced to a greater extent by TLV (−2.5 log CFU/mL) compared to VAN (−1.2 log CFU/mL). At 6 hrs after the second dose, the splenic titers were reduced to a greater extent by TLV (−3.8 log CFU/g) when compared to VAN (−1.4 log CFU/g).
**Conclusions:** The data described here demonstrate that TLV's in vivo bactericidal activity is superior to that of VAN against MRSA and results in successful infection resolution and, consequently, improved survival in the murine peritonitis model.

## P1544

### Proper use of carbapenems for blood-derived clinical isolates of *Pseudomonas aeruginosa*

Y. Kobayashi *(Tokyo, JP)*

**Methods:** Regimens of carbapenems were given to 1500 healthy adult subjects. Changes in their blood concentrations of carbapenems were compared by using pharmacokinetic parameters (two-compartment model analysis) of meropenem (MEPM), imipenem (IPM), and panipenem (PAPM) and by applying the lognormal distribution to the probability distribution of distribution volume and plasma half-life with Monte Carlo simulation (MCS). Based on the data on distributions of the minimal inhibitory concentrations (MIC) of various carbapenems for 77 blood-derived clinical isolates of *Pseudomonas aeruginosa* isolated/identified at Keio University Hospital between October 1997 and October 2004 (MIC90: MEPM 8 mcg/mL, IPM 16 mcg/mL, PAPM 32 mcg/mL), the MICs in the 1500 subjects were obtained with MCS. From the changes in blood concentrations and MICs in the subjects, the probability of achieving T>MIC was calculated for each carbapenem regimen, using the formula reported by Kuti et al.: %T>MIC = ln (Dose/Vd*MIC)*(T1/2/0.693)*(100/DI) <<ln: natural logarithm, Dose: 1 dose (mg), Vd: distribution volume (L), T1/2: plasma half-life (h), DI: dosing interval (h)>>. Based on Craig's data, the maximum bactericidal effect on Gram negative bacilli is attained when %T>MIC is approximately 50%. We focused on this information and analysed our data.
**Results:** The probability of achieving T>MIC50% was 43.2% for MEPM 500 mg bid, followed by 4.2% for IPM 500 mg bid and 3.6% for PAPM 500 mg bid. When the dose was increased from 500 mg to 1000 mg, it was 60.7% for MEPM 1000 mg bid, followed by 10.3% for IPM 1000 mg bid and 9.9% for PAPM 1000 mg bid. When the dose remained at 500 mg and the dosing frequency was increased to three times daily, it was 76.5% for MEPM 500 mg tid, followed by 43.0% for IPM 500 mg tid and 18.8% for PAPM 500 mg tid. Regarding MEPM, it was 85.9% for

500 mg qid? and 86.7% for 1000 mg tid showing higher probabilities.
**Discussion:** In severe sepsis caused by *Pseudomonas aeruginosa*, remarkably higher T>MIC50% was achieved with carbapenems at 500 mg tid, although the daily dose (1500 mg) was lower, compared to 1000 mg bid. Carbapenems with a low MIC distribution, i.e. a superior antibacterial activity, showed higher probability of achieving T>MIC. Therefore, the optimal treatment for such sepsis is MEPM 500 mg tid. MEPM 500 mg qid appeared to provide comparable therapeutic effects with those at 100 mg tid, the usual dose in foreign countries.

## P1545

## Penetration of moxifloxacin into normal and infected subcutaneous tissue in patients with spinal cord injury measured by microdialysis

J. Barth, D. Jäger, M. Müller, O. Burkhardt, H. Derendorf *(Halle, DE; Vienna, AT; Gainesville, US)*

**Background:** Skin breakdowns, also termed decubitus ulcers or pressure sores, are a major complication associated with spinal cord injury, resulting in infection and tissue death. Moxifloxacin (MFX) is approved for the treatment of SSSI. Our objective was to construct a population PK model for MFX disposition in plasma, normal and infected subcutaneous tissue in spinal cord injured patients with infected decubitus ulcer.
**Methods:** 4 patients receiving 400 mg MFX orally daily were enrolled in this study. Blood, saliva and interstitial tissue fluid samples (microdialysis in normal and infected tissue) were collected over a time period of 8 hrs. MFX concentrations were measured by a validated HPLC. Concentration-time data obtained in the present study were pooled with previously published MFX data (n = 12). Population PK modelling was performed with NONMEM.
**Results:** The concentrations of MFX achieved in plasma, saliva, normal subcutaneous tissues and infected decubitus ulcers showed parallel profiles versus time. The PK was best described by a 2-compartment model with a link to interstitial tissue fluid. The population PK parameters were as follows (given as estimate with percent interindividual variability in parentheses): CL 10.2 L/h (3%); central VD 83.2 L (15%); intercompartmental CL 31.9 L/h (16%); peripheral VD 92.2 L (17%); and elimination rate constant for interstitial tissue fluid 1.57 h−1 (17%). With a conservative MIC90 of 0.25 mg/L, the Peak/MIC ratios were higher than 10 and the AUC24/MIC ratios were higher than 100 for plasma, saliva and interstitial tissue fluids.
**Conclusions:** This study showed the good diffusion of MFX into subcutaneous tissue in spinal cord injured patients with decubitus ulcers. The interstitial tissue fluids reached bactericidal levels for common bacteria found in infected skin lesions.

## P1546

## Postantibiotic effect and postantibiotic subminimum inhibitory concentration effects of ciprofloxacin, levofloxacin and moxifloxacin on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

I. Kaleli, M. Demir, N. Cevahir, E. Mete, U. Yildirim, S. Tikvesli *(Denizli, TR)*

**Objective:** Investigations of pharmacodynamic parameters such as postantibiotic effect and postantibiotic subminimum inhibitory concentration effect have been employed for design of dosing schedules of antimicrobial agents. In this study we compared postantibiotic effect and postantibiotic subminimum inhibitory concentration effect of ciprofloxacin, levofloxacin, and moxifloxacin for clinical isolates of methicillin susceptible *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* and *Pseudomonas aureginosa*.
**Methods:** The following strains were tested in this study: methicilline-susceptible *Staphylococcus aureus* (n:4), methicilline resistant - *Staphylococcus aureus* (n:2) and *Pseudomonas aeruginosa* (n:2). The PAE was determined by viable plate count method using Mueller Hinton broth. Tubes containing 5 ml of broth and the antibiotic to be tested at 1, 2, 4 and 8x the MIC were inoculated with approximately $5 \times 106$ CFU/ml. Growth controls with an inoculum but not antibiotic were included with each experiment.
**Result:** Postantibiotic effects of ciprofloxacin, levofloxacin and moxifloxacin increased with increasing concentration of the drug. The longest postantibiotic effect was observed for moxifloxacin. Moxifloxacin showed no postantibiotic effect one *P. aureginosa* at all concentration and had no post antibiotic effect to another *P. aureginosa* at x2MIC and MIC. In our study the longest postantibiotic subminimum inhibitory concentration effect against MSSA was determined with moxifloxacin. Similarly the moxifloxacin induced the longest effect against MRSA. However, this time frame was shorter than that of MSSA.
**Conclusions:** All three antibiotics, showed for longer postantibiotic subminimum inhibitory concentration effect in all subMIC concentrations, immeasurable within the study period i.e. 24 hours.

## P1547

## Lack of horizontal transmission of fluoroquinolone resistance between *S. mitis* and *S. pneumoniae*

S. Campbell, C. Howley, R.J. Davidson *(Halifax, CA)*

**Objectives:** Fluoroquinolone (FQ) resistance can arise in *S. pneumoniae* through acquisition of DNA from *S. mitis* and subsequent homologous recombination. The frequency at which this occurs is unknown, and while likely a rare event, increases in FQ resistance among *S. mitis* may increase the rate at which horizontal transmission occurs. We sought to determine the frequency at which FQ resistance could be transferred from *S. mitis* to *S. pneumoniae* or from *S. pneumoniae* to *S. mitis*.
**Methods:** *S. mitis* (either FQ^R,Tetracycline[Tet^S], FQ^R,Penicillin[Pen^S], or FQ^S,Pen^R) and *S. pneumoniae* (either FQ^S,Tet^R, FQ^S,Pen^R or FQ^R,Pen^S) were grown in co-culture using a pharmacodynamic model in the presence of either moxifloxacin (MXF) or levofloxacin (LFX) at salivary drug concentrations. After incubation, aliquots were plated onto either Tet or Pen containing SBA plates to select for the recipient strains. FQ susceptibility was performed using microbroth dilution. The entire parC and gyrA genes were amplified and sequenced to determine if horizontal transmission occurred.
**Results:** In initial experiments Tet was used as the selective agent. However Tet resistance was transferred and therefore Pen^R was used as a selective marker. An increase in the LFX MIC in was observed in 3 *S. pneumoniae* and 1 *S. mitis* strain. Sequencing of the parC gene revealed the selection of 2 Ser79Phe and 1 Ser79Tyr mutations in *S. pneumoniae* and Ser79Phe in *S. mitis* consistent with FQ resistance. Sequencing of the entire gene failed to uncover evidence of horizontal transmission. No mutations were detected in gyrA. Selection of 1st step parC

mutations occurred only after exposure to LFX. MXF eradicated both *S. mitis* and *S. pneumoniae* and failed to either select for resistance or support horizontal transmission.
**Conclusions:** Although 1st step parC mutations were selected in 4 strains (3 *S. pneumoniae*, 1 *S. mitis*), we failed to find evidence of horizontal transmission between *S. pneumoniae* and *S. mitis* under our laboratory conditions. The phenomenon of horizontal transfer resulting in FQ resistance has been described, however, based on our results, we must speculate that it is an extremely rare event and not likely to be a major driver of FQ resistance. Of interest, the parC mutations were selected only under the selective pressure of LFX. MXF completely eradicated both *S. pneumoniae* and *S. mitis* and did not select for the development of FQ^R mutations.

## P1548

## Activity of ertapenem and metronidazole against *Bacteroides fragilis* and *Escherichia coli* in an *in vitro* pharmacokinetic/pharmacodynamic model employing pure and mixed cultures

R. Schaumann, C. Vorwerk, R. Habt, J. Forberg, A. Rodloff *(Leipzig, DE)*

**Objectives:** The aim of the present study was to assess the killing activity of ertapenem (ERT) and metronidazole (MTR) against four selected *Bacteroides fragilis* strains with different MIC values in an *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) model. Since anaerobes are often present in mixed infections, kill kinetics were also established for mixed inocula employing the *B. fragilis* strains together with four selected *Escherichia coli* strains. The killing activity was analysed for kinetic concentrations of the antimicrobial agents simulating human serum kinetics.
**Methods:** A PK/PD *in vitro* model was established by adding appropriate amounts of broth every half hour. At the same time intervals samples were obtained and plated. After incubation colony forming units were counted. Human serum concentrations were simulated with Cmax = 100 mg/L and t1/2 of 5 hours for ERT and Cmax = 14.0 mg/L and t1/2 of 7 hours for MTR. Mann trend test was used for statistical analysis.
**Results:** As to be expected the *E. coli* strains were not killed by MTR both in pure as well as in mixed cultures whereas the susceptible *E. coli* strains were effectively killed by ERT. In pure cultures the *B. fragilis* strains were effectively killed by MTR and the growth of the susceptible *B. fragilis* strains was reduced by ERT by about two to four logs. However, in some mixed cultures the killing activity of MTR against the *B. fragilis* strains was significantly reduced.
**Conclusion:** The in part moderate *in vitro* activity of ERT against the *B. fragilis* strains and the reduced activity of metronidazole in mixed cultures against the *B. fragilis* strains may explain some of the difficulties in treating mixed aerobic/anaerobic infections.

## P1549

## Penetration of ciprofloxacin into human cerebrospinal fluid and brain tissue

A. Tsona, S. Metallidis, E. Koumentaki, J. Nikolaidis, P. Kollaras, G. Lazaraki, P. Nikolaidis *(Thessaloniki, GR)*

**Objectives:** The aim of the present study was to determine the penetration of ciprofloxacin into cerebrospinal fluid (CSF) and brain tissue of humans.
**Methods:** A total of 10 patients undergoing brain tumor excision were evaluated. The patients received a single intravenous dose of 400 mg ciprofloxacin. Samples of blood, cerebrospinal fluid and brain (brain-adjacent tumour tissue) were collected during surgery 2 h after drug administration. Ciprofloxacin concentrations in serum, cerebrospinal fluid and brain homogenate were analysed by means of a validated HPLC method.
**Results:** Ciprofloxacin concentrations in plasma (mcg/ml), cerebrospinal fluid (mcg/ml) and tissue homogenate (mcg/g), respectively, after 2 h ranged 0.87–2.67 mcg/ml, 0.07–0.37 mcg/ml and 0.65–4.02 mcg/g. CSF-to-serum ratio ranged between 0.06 and 0.14. Tissue-to-serum ratio ranged between 0.41 and 4.25. Mean (±S.D.) CSF/serum concentration ratios and brain tissue/serum concentration ratios were respectively 0.10 ± 0.03 and 1.70 ± 1.17.
**Conclusion:** These findings suggest that valuable informations on brain tissue penetration can be obtained only from brain material. Data from CSF penetration cannot be extrapolated to the brain since the blood: SF barrier differs from the blood:brain barrier. Concentrations of ciprofloxacin in cerebrospinal fluid were lower than those in serum, in contrast to the brain tissue concentrations that exceeded serum concentrations. The achieved concentrations in brain tissue were generally above the MIC90 of common pathogens in central nervous system infections (*H. influenze*, *N. meningitidis*, *S. pneumoniae*, *L. monocytogenes*, *Escherichia coli*, aerobic gram-negative bacilli, Group B *Streptococci*, MSSA). Cerebrospinal fluid concentrations exceed the MICs of *Neisseria meningitidis* and most gram-negative aerobic bacilli. Our findings suggests that ciprofloxacin may be an acceptable alternative for the treatment of meningitis due to susceptible gram-negative aerobic organisms and for the treatment of brain abscesses.

## P1550

## Pharmacodynamic comparison of three carbapenems against extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella* spp. from the MYSTIC Program in Brazil

C. Kiffer, C. Mendes, K.J. Eagye, J.L. Kuti, D. Nicolau *(Sao Paulo, BR; Hartford, US)*

**Objective:** To model the performance of imipenem (IMI), meropenem (MEM), and ertapenem (ETM) against ESBL producing *E. coli* and *Klebsiella* spp in order to identify possible PD differences among compounds.
**Methods:** Minimal inhibitory concentrations (MICs) were generated for 133 randomly selected ESBL producing isolates of EC (n = 29) and KL (n = 104) collected during 2004 from Brazilian hospitals as part of the MYSTIC program. MIC testing for IMI, MEM, ETM, ceftazidime (CTZ), and cefotaxime (CTX) were done by E-test methodology. ESBLs were confirmed via CTZ/clavulanate and CTX/clavulanate E-Test. PD exposure, measured as percent time above the MIC for free drug (fT>MIC), was modelled via a 5000 subject Monte Carlo simulation for the following 30-minute infusions:IMI 1 gram every 8 hours, MEM 1 gram every 8 hours, and ETM 1 gram every 24 hours, using pharmacokinetics from healthy volunteers. The bactericidal cumulative fraction of response (CFR) was calculated for each regimen against the populations of EC, KL, and against all ESBL isolates together. Bactericidal CFR was defined as 40% fT>MIC for all agents. Results are reported as CFR (95% Confidence Interval).

**Results:** Isolates were 100% susceptible (S) to IMI and MEM (MIC range 0.125–1.5 and 0.023–4 mg/L, respectively), and 97% S to ETM (MIC range 0.008–32 mg/L). Although all 3 agents achieved high bactericidal CFR against all ESBL isolates, ETM [97.06% (96.55–97.49)] was less likely than IMI [99.96% (99.85–99.99)] and MEM [99.90% (99.77–99.96)] to attain this target ($p < 0.05$). Similar results were observed when simulated against only KL: ETM: 96.04% (95.46–96.55); IMI: 99.96% (99.85–99.99); and MEM: 99.88% (99.74–99.94); $p < 0.05$. Against EC, all compounds performed comparably, with CFRs close to 100%.
**Conclusions:** These findings support other data that although ETM is likely to be an effective empiric agent against most ESBL producing EC and KL, its ability to achieve high bactericidal PD exposure will be dependent on the presence of less susceptible organisms in the population. IMI and MEM should remain first line for ESBL infections.

## P1551

## Activity of moxifloxacin, levofloxacin and azithromycin physiological concentrations in serum and epithelial lining fluid against *in vivo* selected *Streptococcus pneumoniae* mutants and its parental strain

D. Sevillano, L. Aguilar, L. Alou, M.J. Giménez, F. Cafini, O. Echeverría, E. Pérez-Trallero, J. Prieto *(Madrid, San Sebastián, ES)*

**Objectives:** This study analyses eradication and resistance selection in *Streptococcus pneumoniae* with moxifloxacin, levofloxacin and azithromycin, using a parental serotype 3 infecting strain (A) and subsequent resistant step-mutants (isolates B, C and D) selected in vivo in a patient with pneumonia.
**Methods:** Moxifloxacin, levofloxacin and azithromycin MICs were 1, 2 and 0.5 μg/ml for the parental strain, 4, 16, and 4 μg/ml for isolate B, and 4, 16 and >128 μg/ml for isolates C and D, respectively. A pharmacokinetic computerized device was used to simulate serum and epithelial lining fluid (ELF) concentrations. Initial inocula was approx. 108 cfu/ml. Population analysis profiles were performed using plates with increasing antimicrobial concentrations on a MIC basis.
**Results:** In serum, moxifloxacin eradicated the parental isolate (isolate A), with an AUC0-24 h/MIC value of 39.6. Serum AUC0-24 h/MIC values of 26.5 and 5.5 for levofloxacin and azithromycin, respectively, were not able to eradicate isolate A. In ELF, moxifloxacin showed a bactericidal pattern against all isolates with a minority (approx. 100 cfu/ml) of the survival population (isolates B, C and D) growing in plates with moxifloxacin concentrations higher than those obtained in ELF. Levofloxacin and azithromycin showed a bactericidal pattern only against isolate A, with the whole population of isolates B, C and D growing in plates with levofloxacin concentrations higher (16–64 μg/ml) than those obtained in ELF, and in plates with azithromycin concentrations as high as 2048 μg/ml (for isolates C and D). In ELF, moxifloxacin AUC0-24 h/MIC values were 201.0 for isolate A, and 50.3 for isolates B, C and D. Levofloxacin AUC0-24 h/MIC values were 94.0 for isolate A, and 11.8 for isolates B, C and D. Azithromycin AUC0-24 h/MIC values were 8.0 for isolate A; 10.0 for isolate B; and 0 for isolates C and D.
**Conclusion:** If prevention of resistance depends more on the eradication of possible emerging mutants in pulmonary tissues than of the parental susceptible strain, moxifloxacin concentrations in ELF may provide advantages over previous quinolones and macrolides in preventing clinical failures.

## P1552

## Effects of levofloxacin, ciprofloxacin and azithromycin on the competitive growth of *Streptococcus pneumoniae*, as a model approach to selection of resistant populations

L. Alou, M.J. Giménez, D. Sevillano, L. Aguilar, O. Echeverría, F. Cafini, E. Valero, A. Fenoll, J. Prieto *(Madrid, Majadahonda, ES)*

**Objectives:** To explore how antimicrobial pressure influences the evolution of *Streptococcus pneumoniae* populations sharing the same ecological niche.
**Methods:** An *in vitro* computerized pharmacodynamic model simulating physiological concentrations obtained over 24 h after 500 mg o.d levofloxacin, 750 mg b.i.d ciprofloxacin, and 500 mg o.d azithromycin was used to investigate its effect on a mixed culture of five *S. pneumoniae* serotypes (S) as an approach to ecology of population dynamics. Resistance patterns were: S12 was susceptible to study drugs, S31 was low-level macrolide-resistant (efflux phenotype), S11 was high-level macrolide-resistant (erm genotype), S9V was low-level quinolone-resistant, and S3 was high-level quinolone-resistant. Initial mixed inocula (time 0) included similar percentages of each serotype.
**Results:** Mean colony counts in antibiotic-free plates (whole pneumococcal population) increased (from 0 to 24 h) from log10 6.9 to 8.8 in drug-free simulations (control), from log10 6.8 to 7.9 in levofloxacin simulations, from log10 6.8 to 8.6 in ciprofloxacin simulations, and from log10 7.1 to 8.8 in azithromycin simulations. At 24 h of control drug-free experiments, dominant strains were S9V (57.4%) and S12 (41.8%) with marginal populations of S31, S3 and S11. Azithromycin selected in a much higher extent the strain with low-level resistance to macrolides (S31) than the strain with high-level resistance (S11) (accounting for 99.9% vs. 0.1% of total population at 24 h). Ciprofloxacin selected in a higher extent low-level (S9V) than high-level (S3) quinolone resistance (72.4% vs. 27.6%). Levofloxacin decreased the proportion of the predominant S9V in controls to 22.2% (an intermediate-resistant strain with MIC = 4 μg/ml), and unmasked the high-level resistant strain (MIC = 32 μg/ml) up to 77.8%.
**Conclusion:** Strain distribution in antibiotic-free environment depends on bacterial fitness in mono- and multi-strain niches. The selective pressure of antimicrobial regimens eradicate some populations and unmask minor populations, thus redistributing the whole population. Selective potential only for resistance phenotypes with very low prevalence (as high-level quinolone resistance) in the community should be preferred to that selecting more prevalent resistance phenotypes.

## P1553

## Re-evaluation of the role of broad-spectrum cephalosporins against staphylococci applying contemporary *in vitro* results and pharmacokinetic-pharmacodynamic principals

H. Sader, S.M. Bhavnani, P.G. Ambrose, R. Jones *(North Liberty, US)*

**Objectives:** To re-evaluate the current *in vitro* activity and to assess the PK-PD target attainment of cefepime (CPM), ceftriaxone (CRO) and ceftazidime (CAZ) against *Staphylococcus* spp.
**Methods:** The potency of CPM, CRO and CAZ against staphylococci was accessed through the SENTRY Antimicrobial Surveillance Program database, worldwide.

During the 1998–2004 period 41,883 *S. aureus* (SA; 63% oxacillin [OXA]-susceptible [S]) and 14,349 coagulase-negative staphylococci (CoNS; 22% OXA-S) were S tested against CPM, CRO, CAZ and numerous comparators by CLSI broth microdilution methods. Using volunteer PK data and a linear intermittent intravenous infusion model, and an animal-derived PK-PD target of 25% time above MIC, expected probabilities of target attainment (PTA) for cephems were evaluated using Monte Carlo simulation. PTA were determined for the following dosing regimens: CPM 1gm q12 and q8 hours, CAZ 1 gm q8 hours and CRO 1 gm q24 hours, each representing the most common dosing patterns applied clinically. Cephem susceptibility (%S) was calculated based on the current CLSI (2006) breakpoints (BKPs) and also on BKPs derived from a PTA >90%.

**Results:** Against OXA-S SA, MIC50/90 values were (in mg/L): 2/4 for CPM, 4/4 for CRO and 8/16 for CAZ, respectively; and against OXA-S CoNS MIC50/90 values were (in mg/L) 0.5/2 for CPM, 2/4 for CRO, and 4/8 for CAZ, respectively. The calculated %S of these cephems are summarized in the Table: Twenty year-old CLSI BKPs would rank the tested agents CPM ≥ CRO > CAZ and by PK-PD PTA CPM ≥ CAZ > CRO. CPM has a potency advantage over CAZ (4- to 8-fold) and superiority at the usual dosing over CRO (22.7–66.1%) for OXA-*S staphylococci*. CAZ PK overcomes by-weight activity disadvantages, while a low proportion (<5%) of active free-drug penalizes CRO in the PTA calculations. PTA remained at >90% to a BKP of 16 mg/L for CPM (1 gm q8) and CAZ and to a BKP of 2 mg/L for CRO.

| | % S | | | | | | |
|---|---|---|---|---|---|---|---|
| | CLSI BKPs (mg/L) | | | BKPs (mg/L) based on PK-PD PTA [regimen] | | | |
| Organism (no. tested) | CPM (<=8) | CRO (<=8) | CAZ (<=8) | CPM (<=8) [1gm q12] | CPM (<=16) [1gm q8] | CRO (<=2) [1gm q24] | CAZ (<=16) [1gm q8] |
| OXA-S SA (26,431) | 100 | 99.8 | 89.4 | 100 | 100 | 33.9 | 99.6 |
| OXA-S CoNS (3,191) | 100 | 99.4 | 95.3 | 100 | 100 | 77.3 | 99.5 |

**Conclusions:** Regardless of applied BKP (CLSI or PK-PD), CPM has the widest and more potent anti-staphylococcal activity among commonly used ''third- or fourth-generation'' cephems. When used at doses ≥ 3 gm/day, CPM assures maximal coverage of OXA-*S staphylococci* whether using existing (CLSI) or modified (PK/PD) BKPs. CRO should be used with caution.

## P1554

## Pharmacokinetic-pharmacodynamic modelling of *in vitro* activity of azithromycin against four different bacterial strains

W. Treyaprasert, U. Suvanakoot, H. Derendorf *(Bangkok, TH; Gainesville, US)*

**Objectives:** To investigate the pharmacodynamic effect of azithromycin by evaluation of *in vitro* kill curves against four bacterial strains (*Streptococcus pneumoniae*/penicillin-intermediate, *Streptococcus pneumoniae* /penicillin-sensitive, *Haemophilus influenzae* and *Moraxella catarrhalis*), and to establish a mathematical model to describe the PK-PD relationship of azithromycin.

**Methods:** The MIC for all strains were determined by serial two-fold macrodilutions. An *in vitro* kinetic model was used to investigate the antibacterial efficacy of constant drug concentrations during 6 hours. The selection of the doses of azithromycin tested in each bacterial strains was based on their MIC values. Bacterial counts were determined on appropriate agar plates using an adapted drop-plate method. Twelve different PK/PD models were fitted and compared to the time-kill data by using non-linear regression.

**Results:** A simple PK-PD model was not sufficient to describe the pharmacodynamic effects for the four bacterial strains. Appropriate models that gave good curve fits included a saturation term for the number of bacteria (Nmax), delay terms (1-e-zt) for the initial bacterial growth phase and/or the onset of anti-infective activity as well as a Hill factor (h) to capture the steepness of the concentration-response relationship. Azithromycin had high potency against *S. pneumoniae* strains and *M. catarrhalis* while the potency of azithromycin against *H. influenzae* was poor.

**Conclusions:** The developed PK/PD models are suitable for describing the pharmacodynamics of azithromycin. Applications of these PK-PD models will eventually provide a tool for rational antibiotic dosing decisions.

## P1555

## Dose optimisation of enrofloxacin for use in chickens against *Salmonella enterica* serovar Typhimurium DT104 to improve efficacy and minimise selection of resistance

L.P. Randall, S.W. Cooles, K. Stapleton, N.G. Coldham, L.J.V. Piddock, M.J. Woodward *(New Haw, Birmingham, UK)*

**Objectives:** Optimal antimicrobial dosage regimens aim to achieve successful clinical outcomes without drug toxicity or emergence of bacterial resistance. For concentration dependent antibiotics, such as the fluoroquinolones, in humans a Cmax:MIC ratio of >10 is considered more important for efficacy and reduced selection of resistance than prolonged antibiotic concentrations just above the MIC. Fluoroquinolone resistance in zoonotic bacteria is a matter of public health concern, and fluoroquinolone treatment of poultry can rapidly select for bacteria with reduced fluoroquinolone susceptibility. In this study we compared basic pharmacokinetic parameters for the recommended dose of Baytril (enrofloxacin) 10% oral solution in poultry to 2.5x this dose for birds dosed by continuous water (standard) compared to pulsed water treatments and dosing by gavage.Methods. For the pulsed versus continuous water treatments, groups of chickens received Baytril 10% oral solution at 50 (recommended) or 125 ppm continuously in the water or at 10 (recommended) or 25 mg/kg pulsed in the water. For each group, three birds were killed at 0, 2, 4, 6, 8, 10 and 24 hours after start of antibiotic treatment and caecal contents, liver, lung and sera were taken and the concentration of fluoroquinolone determined by fluorescence HPLC. For gavage treatment, dosing was at 10 and 25 mg/kg by crop intubation and four birds were killed in each group at 2, 6 and 24 hours after gavage; caecal contents, liver and sera were taken and analysed as above. Basic pharmacokinetic parameters were determined using PK solutions software.

**Results:** The mean fluoroquinolone Cmax in caecal contents (and sera) for gavage, pulsed water and continuous water treatments respectively was 78.01 (1.81), 53.19 (1.17) and 24.73 (0.73) mg/ml after the recommended dose and 115.86 (3.86), 115.63 (2.74) and 68.02 (1.17) mg/ml after 2.5x the recommended dose. Cmax of antibiotic in liver and lung was increased by the modified regimens in similar proportions to above. Both pulsed water and gavage treatment not only resulted in higher Cmax values, but also a faster rate of fluoroquinolone clearance than continuous water treatment (Figure 1).

**Table 1**. Efficacy of recommended and modified enrofloxacin treatments against experimental *Salmonella* Typhimurium infections in chickens and selection of resistance.

| Dosage method | Exp. No. | Dose level | No. chicks per group | Days of Dose | Mean log cfu / gram caecal contents [a] (± SEM) | P value [b] | No. colonies replica plated during / post treatment [c] | % colonies with reduced susceptibility during / post treatment |
|---|---|---|---|---|---|---|---|---|
| Water continuous | I | Control | 10 | NA | 6.8 (0.76) | NA | 400 / 187 | 0 / 0 |
| | | 50 ppm [d] | 30 | 5 | **-0.4 (1.12)** | **< 0.001** | 1328 / 752 | 0.4 / 43 |
| | | 125 ppm | 30 | 2 | **0.6 (1.11)** | **< 0.001** | 1824 / 3859 | 0 / 96 |
| | | 250 ppm | 25 | 1 | 4.3 (0.77) | 0.213 | 969 / 3089 | 0 / 14 |
| Water pulsed | III [e] | Control | 13 | NA | 5.12 (0.25) | NA | ND / 206 | ND / 0 |
| | | 10 mg/kg | 15 | 5 | **1.10 (0.27)** | **< 0.001** | ND / 33 | ND / 0 |
| | | 25 mg/kg | 15 | 2 | **< 1.0 (0)** | **< 0.001** | ND / 293 | ND / 0 |
| | | 50 mg/kg | 15 | 1 | 1.92 (0.25) | < 0.001 | ND / 335 | ND / 0 |
| Gavage | II | Control | 24 | NA | 8.2 (0.69) | NA | 394 / 1242 | 0 / 0 |
| | | 10 mg/kg | 51 | 5 | **3.2 (0.71)** | **< 0.001** | 2920 / 2474 | 42 / 100 |
| | | 25 mg/kg | 41 | 2 | **3.0 (0.74)** | **< 0.001** | 1206 / 2010 | 0 /0 |
| | | 50 mg/kg | 34 | 1 | 5.2 (0.69) | 0.051 | 1455 / 1999 | 0 / 14 |
| | III [e] | Control | 13 | NA | 5.12 (0.25) | NA | ND / 206 | ND / 0 |
| | | 10 mg/kg | 15 | 5 | **< 1.0 (0)** | **< 0.001** | ND / 62 | ND / 0 |
| | | 25 mg/kg | 15 | 2 | **0.78 (0.29)** | **< 0.001** | ND / 206 | ND / 0 |
| | | 50 mg/kg | 15 | 1 | 2.41 (0.25) | < 0.001 | ND / 128 | ND / 0 |

NA, not applicable. ND, not determined
**Bold, counts reduced by at least four logs**.
*a* Counts were one day post last antibiotic dose to reduce the effect of any re-colonisation following the end of therapy.
*b* P value denotes significance of antibiotic therapy in reducing *Salmonella* counts compared to control.
*c* Colonies were replica plated from many representative plates (and hence different birds) from all time points for up to four weeks post antibiotic treatment.
*d* Current and recent datasheet recommended treatment is 10 mg/ml or 50 ppm for five days supplied continuously in the water.
*e* In experiment III birds received a lower challenge of *Salmonella* which resulted in a lower level of colonisation.

**Conclusion:** Dosing by gavage is not practical for thousands of chickens. However, pulsed dosing at 2.5x the recommended dose can increase Cmax values about fourfold and so could improve efficacy and reduce selection of resistance, compared to the current recommended treatment regime.

## P1556

## Nephrotoxicity of intravenous colistin: a prospective evaluation

M. Falagas, K. Fragoulis, S. Kasiakou, G. Sermaides, A. Michalopoulos *(Athens, GR)*

**Objectives:** Nephrotoxicity is the major concern arising with the use of intravenous colistimethate sodium.

**Methods:** A prospective cohort study was performed at ''Henry Dunant'' Hospital, a 450-bed tertiary care center in Athens, Greece. Patients who received intravenous colistin for at least 7 days for the treatment of multidrug resistant Gram-negative bacterial infections were included in the study. The development of nephrotoxicity through evaluations of serum creatinine, blood urea, serum electrolytes, urinalysis, and creatinine and sodium in 24-hour urine collection during intravenous colistin therapy was the primary end point of the study.

**Results:** Twenty-six patients were included in the study, 21 of whom received colistimethate sodium (CMS) for at least 7 days and were evaluated further. The mean (± SD)/median daily dose, cumulative dose, and duration of treatment of intravenous CMS was 5.5 (± 1.9)/6 million IU, 90.2 (±52.0)/72 million IU, and 17.7 (±11.7)/15 days (range 7–54 days), respectively. Three of the 21 evaluable patients (14.3%) developed nephrotoxicity during the intravenous treatment with CMS. The cumulative dose of the administered CMS was statistically correlated with the difference between the end and start of CMS treatment values of serum creatinine ($r = 0.6$, $p = 0.004$ by Spearman's test). A statistically but not clinically significant decrease of the mean baseline serum sodium concentration was observed between start and end of treatment [mean 144.2 (±6.9) to 142.1 (±6.1) mmol/L, $p = 0.04$]. No other toxic events were noted during the intravenous administration of colistimethate sodium.

**Conclusion:** Although this is an evaluation of a small number of patients, our prospective study shows that nephrotoxicity was not commonly observed in this group of patients who received intravenous colistimethate sodium. However, caution should be taken to avoid the prolonged administration of the antibiotic.

# New and not so new antimicrobials

## P1557

## Molecular design of novel antimicrobial agents on the base of 4-thiazolidone derivatives

A. Artemenko, E. Muratov, V. Kuz'min, A. Fedtchuk, N. Mykhaylovska, R. Lesyk, B. Zimenkovsky *(Odessa, L'viv, UA)*

**Objectives:** The objective of the present work is Quantitative Structure-Activity Relationship (QSAR) analysis of antimicrobial activity of the 4-thiazolidone derivatives and consequent computational design of new antimicrobials.

**Methods:** For the achievement of the formulated objectives the QSAR investigation has been carried out using computational chemistry approach based on Simplex Representation of Molecular Structure (SiRMS). On the framework of SiRMS it is possible to develop the molecular design of the new effective antimicrobials.

**Results:** Systematic researches of relationship between antimicrobial activity (*Staphylococcus aureus* – methicillin-sensitive (MSSA) strain, *Pseudomonas aeruginosa* – R and S strain, *Klebsiella pneumoniae*, *Candida albicans* S and Ñitrobacter *freundii*) and a structure of about one hundred fifty compounds (4-thiazolidone derivatives and analogs). The elucidation of structure-activity relations allows predicting biological properties of such compounds, to execute their direct synthesis and to receive the indispensable information for research of mechanisms of their biological effect. Completely adequate statistical partial least squares models (R2 = 0.841–0.990, Q2 = 0.600–0.814) have been obtained for all of the studied cultures. On the base of the first ones the molecular fragments both promoting and interfering the given antimicrobial activity have been determined. They give a possibility to realize the computer high throughput screening and molecular design of active compounds. The results of prognosis are verifying by the experimental investigations. Also the influence of heterocycle system evolution on antimicrobial activity has been revealed.

**Conclusion:** QSAR analysis of antimicrobial activity of 4-thiazolidone derivatives allows us to discover that the presence of naphthalene-substituted fragment (independently on its location in molecule) has distinctly negative influence on antimicrobial action. The requirements to molecular design have been formulated. For example, high active compounds must include 3-indolyl fragment.

## P1558

## Computational design of the new antimicrobials based on the substituted crown ethers

E. Muratov, A. Artemenko, V. Kuz'min, I. Konup, L. Konup, S. Kotlyar, G. Kamalov, A. Fedtchuk, N. Mykhaylovska *(Odessa, UA)*

**Objectives:** The objective of the present work is Quantitative Structure-Activity Relationship (QSAR) analysis of antimicrobial

activity of the row of substituted crown ethers and consequent molecular design of new antimicrobials.
**Methods:** The well-known Hierarchic System of QSAR Models based on simplex representation of molecular structure has been used for the solution of the formulated problem, within the framework of which one it is possible to develop the molecular design of the new effective antimicrobial agents.
**Results:** We tried to conduct systematic researches of relationship between antimicrobial activity (*Planococcus citreus*, *Streptococcus lactis*, *Micrococcus lysodeiktious*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtillum*) about two hundred fifty crowns ethers including aromatic, cyclic and heterocyclic etc. fragments and a structure of these molecules, in particular – macro cycle size, it dentacy, lipophily, nature of the substituents, and other factors. The elucidation of similar relations allows predicting biological properties of crown compounds, to execute their direct synthesis and to receive the indispensable information for research of mechanisms of biological effect of such kind of compounds. Completely adequate QSAR models (R2 = 0.841–0.995, Q2 = 0.660–0.954) have been obtained using partial least squares method for all of the studied cultures. On the base of the first ones the molecular fragments with positive or negative influence on the explored properties have been determined. They give a possibility to realize the virtual screening and molecular design of compounds with the high level of target activity. The results of prognosis are verifying by the experimental investigations.
**Conclusion:** QSAR analysis of antimicrobial activity of crown ethers allows us to suppose the presence of two different mechanisms of their antimicrobial action. It is discovered that the presence of diphenyloxide and tert-butyl fragments promotes; diphenyl-sulphide and diamino-biphenyl – prevents the antimicrobial action. It is shown that the hexadenthal crown ethers containing aromatic fragments with a tert-butyl group are the most perspective antimicrobials.

## P1559

## Enzymatic characterisation of methionyl tRNA synthetase inhibition by REP8839

T. Jarvis, J. Bullard, L. Green, W. Ribble, J. Bertino, U.A. Ochsner, I.A. Critchley, N. Janjic *(Louisville, US)*

**Objectives:** Methionyl tRNA synthetase (MRS) catalyses the covalent attachment of methionine to its cognate tRNA. REP8839 is a synthetic inhibitor of MRS with potent antibacterial activity against *Staphylococcus aureus* including clinically-relevant resistant strains (MIC90 equals 0.06 to 0.5 $\mu$g/mL). We determined the biochemical potency and mechanism of action of REP8839 and related compounds with respect to *S. aureus* MRS enzymatic activity. We also evaluated the enzyme kinetic properties of mutated forms of *S. aureus* MRS.
**Methods:** The metS gene from *S. aureus* was expressed in *E. coli* and MRS was purified to near homogeneity by ammonium sulfate fractionation and anion exchange chromatography. Aminoacylation of tRNAmet was measured using scintillation proximity assays (SPA). The kinetics of the ATP:PPi exchange were determined using thin layer chromatography (TLC). Mutants of *S. aureus* MRS were selected by serial passage and spontaneous resistance in the presence of REP8839.
**Results:** REP8839 exhibited strong inhibition of *S. aureus* MRS in the aminoacylation reaction, having an IC50 limited by the enzyme concentration. In order to estimate the true inhibition constant (Ki), we utilized an ATP:PPi exchange assay. REP8839 showed potent inhibition of *S. aureus* MRS, with a Ki of 10 pM. Related inhibitors were analysed, and a correlation was observed between the Ki for MRS and the MIC for *S. aureus*. REP8839 was found to be competitive with methionine binding, but uncompetitive with ATP binding (i.e., increasing the ATP concentration resulted in tighter binding of REP8839). The majority of analogs exhibited comparable mechanism of action; altered mechanism of action was observed with a subset of analogs. Mutated *S. aureus* MRS variants (derived from strains with elevated MICs) showed substantially weaker binding by REP8839. All of the mutated enzymes exhibited impaired tRNA aminoacylation activity, with defects ranging from reduced turnover rates to weaker affinities for one or more substrates.
**Conclusions:** REP8839 is a potent inhibitor of *S. aureus* MRS. Enzymatic potency of this class of inhibitors correlates with microbiological potency. Mutations that confer resistance to REP8839 result in functionally impaired MRS, encompassing a wide variety of enzymatic phenotypes.

## P1560

## Antimicrobial activity of some new thioureides of 2-(4-chlorophenoxy)-benzoic acid

C. Limban, M.C. Balotescu, V. Missir, I. Chirita, G. Mihaescu, A. Israil *(Bucharest, RO)*

We report here the antibacterial and antifungal activity of 8 newly synthesized and physico-chemically characterised thioureides of 2-(4-chlorophenoxy)-benzoic acid. The new compounds were prepared in three stage. Firstly, the 2-(4-chlorophenoxymethyl)-benzoic acid was prepared by treating the phtalide with p-chlorophenol potassium salt in xylene. The second stage was the synthesis of 2-(4-chlorophenoxymethyl)-benzoyl chloride by treating the corresponding acid with thionyl chloride using anhydrous 1,2-dichloroethane as solvent, followed in the third stage, by the treatment of the above-mentioned chloride with ammonium thiocyanate. The 2-(4-chlorophenoxymethyl)-benzoyl isothiocyanate resulted after refluxing the reaction mixture in dry acetone. The new compounds were prepared by refluxing the isothiocyanate with primary aromatic amines in dry acetone. The obtained compounds have been characterized by their physical properties and their chemical structures were confirmed using the spectral analysis. The aim of this study was also to evaluate the *in vitro* antimicrobial activity of the new compounds. The *in vitro* antimicrobial testing was performed by binary microdilution method, in 96 multi-well plates, in order to establish the minimal inhibitory concentration (MIC), against Gram-positive (*Listeria* (*L.*) *monocytogenes*, *Staphylococcus* (*S.*) *aureus*, *Bacillus* (*B.*) *subtilis*), Gram-negative (*Psedomonas* (*P.*) *aeruginosa*, *Escherichia* (*E.*) *coli*, *Salmonella* (*S.*) *enteritidis*), as well as *Candida* sp., using both reference and clinical, multidrug resistant strains. Our results showed that the tested compounds exhibited a specific antimicrobial activity, depending on the nature of the substituents and their position on the benzene ring, both concerning the microbial spectrum and the MIC value. The MICs values widely ranged between 1024 mcg/ml and 32 mcg/ml. The most active proved to be N-[2-(4-chlorophenoxymethyl)-benzoyl]-N′-(2,6-dichloro-phenyl)-thiourea and N-[2-(4-chlorophenoxymethyl)-benzoyl]-N′-(4-bromo-phenyl)-thiourea, showing a large spectrum of antimicrobial activity against enterobacterial strains (*E. coli* and *S. enteritidis*), *L. monocytogenes*, *S. aureus* and *Candida* sp. All the tested compounds were highly active against *S. aureus* (MIC = 32 mcg/ml). Four of the tested compounds exhibited antifungal activity (MIC = 256–32 mcg/ml), and *P. aeruginosa* as well as B. subtilis were resistant to all tested compounds.

## P1561

## *In vitro* antimicrobial activities of novel dianthraquinones produced by a marine *Streptomyces* sp. against clinical *Staphylococcus aureus* and *Enterococcus faecium* isolates

K.L. LaPlante, K. Lor, A. Socha, D.C. Rowley *(Providence, North Kingston, US)*

**Objectives:** The escalation of antibiotic resistance among gram-positive pathogens presents increasing treatment challenges and requires the development of new therapeutic agents. Recently we discovered a new class of dianthraquinone antibiotics produced by a marine streptomycete. The inhibitory and bactericidal activity of four dianthraquinone secondary metabolites and four semi-synthetic derivatives were measured against clinical strains of vancomycin resistant *E. faecium* (VRE), methicillin susceptible and methicillin resistant *S. aureus* (MSSA and MRSA, respectively). Two compounds, DAQ550a and DAQ552, were tested against an expanded panel of pathogens.
**Methods:** Thirty-two clinical strains of VRE (n = 10), MSSA (n = 12) and MRSA (n = 12) were obtained from patients at the Veterans Affairs Medical Center in Providence, RI. MIC's were performed using methodologies described by CLSI. Control isolates were ATCC52923 and ATCC29213. The bactericidal activity of each antimicrobial agent was evaluated with time-kill experiments using 6 randomly selected MSSA (n = 2), MRSA (n = 2), and VRE (n = 2) isolates tested at 4 times the respective MIC.
**Results:** Overall, DAQ550a and DAQ552 demonstrated significant *in vitro* activity. Over the 24 h time kill, DAQ552 demonstrated bactericidal activity (99.9%) against MSSA, MRSA and VRE. DAQ550a demonstrated bactericidal activity against MSSA and MRSA.

| Isolates | DAQ550a $MIC_{50}$ (Range) in mcg/ml n=12 | DAQ550a Δ in 24-h $\log_{10}$ CFU/mL n=2 avg. | DAQ552 $MIC_{50}$ (Range) in mcg/ml n=12 | DAQ552 Δ in 24-h $\log_{10}$ CFU/mL n=2 avg. |
|---|---|---|---|---|
| MSSA | 0.063 (0.063 – 0.5) | -3.37 ± 0.244 | 0.063 (0.063 – 0.125) | 3.14 ± 0.275 |
| MRSA | 0.25 (0.125 – 0.5) | -2.98 ± 0..04 | 0.125 (0.063 – 0.125) | 3.17 ± 0.424 |
| VRE | 4 (1 – 4) | -2.59 ± 0..63 | 0.5 (0.125 – 0.5) | 3.00 ± 0.294 |

**Conclusions:** The potent activities and unusual structures of the dianthraquinones tested here suggest that these may provide a new molecular scaffold for the development of novel antimicrobial agents. More biological testing is warranted to more fully explore the clinical potential of these antibiotics.

## P1562

## Efficacy of the novel antimicrobial peptide plectasin to staphylococci

D. Sandvang, P.H. Mygind, S. Jenabian, D.R. Segura, H. Kristensen *(Bagsværd, DK)*

**Objective:** The purpose of the investigation was to investigate the *in vitro* efficacy and kill kinetics of Plectasin against *Staphylococcus aureus*. Plectasin is a newly discovered defensin-type antimicrobial peptide found in the fungus *Pseudoplectania nigrella* which showed activity against several Gram-positive bacteria including drug resistant strains (Mygind PH. et al. Plectasin is a peptide antibiotic with therapeutic potential from a saprophytic fungus. Nature 2005; 437:975–980).
**Methods:** All experiments were determined according to CLSI/NCCLS guidelines. Bactericidal activity was characterized by time kill experiments at 2 and 10 times the MIC. *Staphylococcus aureus* (*S. aureus*) ATCC29213 were used as the test organism and vancomycin was used for comparison. The kill kinetics and post antibiotic effect (PAE) were evaluated by CFU determination. Inoculum sizes ranging from 10E4 to 10E7 cells were used to test the inoculum effect. 10E10 cells were employed for determination of mutant prevention concentration (MPC) and the frequency of spontaneous resistance.
**Results:** Plectasin is bactericidal as evidenced by kill kinetics showing a 2.1 log reduction in CFU/ml after 1 hour of incubation and a reduction of 3.1 log CFU/ml after 2 hours. This is superior compared to the activity of vancomycin. No inoculum effect was observed in the employed range of cells. The observed PAE had a duration of 2 hours and 42 minutes. No spontaneously resistance mutation was observed among 10E10 cells of staphylococci and the MPC were determined to be 8 times MIC.
**Conclusions:** Plectasin is a novel antimicrobial peptide that shows potent antimicrobial activity against Gram-positive bacteria including drug-resistant organisms. The potent, excellent bactericidal activity *in vitro*, lack of cross-resistance to clinical used antibiotics, low spontaneously resistance mutation frequency and good PAE properties, suggest that Plectasin may have potential as a therapeutic agent against staphylococci.

## P1563

## *In vitro* antimicrobial activity of the novel polymeric guanidine Akacid plus®

C. Kratzer, S. Tobudic, W. Graninger, A. Buxbaum, A. Georgopoulos *(Vienna, AT)*

**Objectives:** Cationic antimicrobials are widely used for disinfection within clinical settings. In the present study the bactericidal and fungicidal activity of Akacid plus®, a novel polymeric compound of the cationic family of disinfectants, was evaluated against quality control strains of *Staphylococcus aureus*, *Enterococcus hirae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* in comparison to chlorhexidine digluconate.
**Methods:** The *in vitro* activity of Akacid plus® and chlorhexidine was determined by quantitative suspensions tests according to the European Committee for Standardization at concentrations of 0.01–0.5% against bacterial strains and *C. albicans* and at concentrations of 0.5–4% against *A. niger* after exposure for 5, 15 and 60 min in the presence and absence of 0.3% bovine albumin and dilution in distilled and hard water.
**Results:** In the basic quantitative suspension test Akacid plus® destroyed all bacterial pathogens at a concentration of ≥0.1% in ≤5 min contact time. Chlorhexidine was also highly active against *S. aureus*, *E. coli* and *P. aeruginosa*, but failed to eliminate *E. hirae* within 5 min. Under high organic burden, the bactericidal activity of both disinfectants was slightly reduced. Akacid plus® showed fungicidal activity against *C. albicans* within 15–60 min and eliminated *A. niger* at a concentration of ≥1% in 5 min contact time. Chlorhexidine was fungicidal against *C. albicans*, but did not achieve biocidal activity against *A. niger*.
**Conclusion:** The novel polymeric guanidine Akacid plus® when compared to chlorhexidine digluconate showed similar bactericidal activity against *S. aureus*, *E. coli* and *P. aeruginosa* and superior biocidal activity against *E. hirae* and *A. niger*.

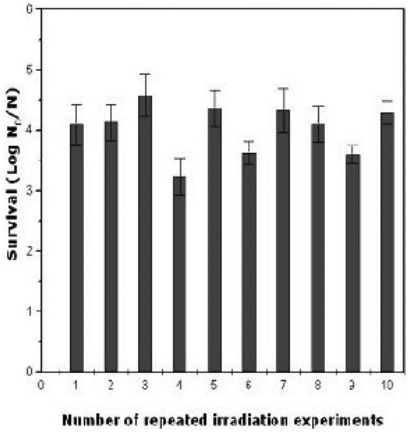
## P1564

## Investigation of emergence of bacterial resistance to the novel antibacterial photodynamic agent XF-42

L. Borsetto, E. Reddi, W. Rhys-Williams, W.G. Love *(Padua, IT; Brighton, UK)*

**Objectives:** The emergence of bacterial resistance to antibiotic therapies is a major threat to modern healthcare. The XF drugs

are novel, light activated antibacterial agents (1) active against Gram-positive bacteria, which have greater potency than antibiotics. The emergence of resistance to XF-42 has been investigated.
**Methods:** 0.382 mg/L of XF-42 was added to 108 cells/mL of MRSA. After 5 minutes incubation in the dark the unbound XF-42 was removed and the culture illuminated with 13.7 J/cm$^2$ of light at 422 nm and CFU analysis undertaken to determine the number of viable cells remaining. 5 surviving clones of the treatment were cultured and subjected to further treatment. 10 cycles were undertaken to determine whether the number of surviving cells increased, suggesting resistance build up to XF-42.
**Results:** The survival of methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC BAA-44) is expressed as log N0/N, where N0 and N are the CFU of untreated and treated suspensions, respectively.




**Conclusions:** The results demonstrate that no detectable resistance build up to the activity of XF-42 was seen after 10 successive treatments. A low propensity for emergence of resistance is a valuable attribute for new anti-bacterial agents. XF-42 might be effectively employed in the clinical setting for prophylactic use to decolonise skin and nares and therapeutic use to treat infected wounds/ulcers.
**Reference**
(1) Maisch T et al. Photodynamic effects of novel XF porphyrin derivatives on prokaryotic and eukaryotic cells. Antimicrob Agents Chemother 2005; 49: 1542–52.

## P1565

## Efficacy testing of the topical antimicrobial XF-73 using a novel *ex vivo* porcine skin model

T. Maisch, R.M. Szeimies, N. Lehn, W. Rhys-Williams, W.G. Love *(Regensburg, DE; Brighton, UK)*

**Objectives:** The XF drugs are novel, light activated antibacterial agents (1) active against Gram-positive bacteria which have superior potency to antibiotics but possess a low propensity to induce resistant bacterial strain emergence. A novel *ex-vivo* porcine skin model has been developed to test the antibacterial activity of XF-73 on the surface of skin.
**Methods:** 10x7 cells of methicillin-resistant *Staphylococcus aureus* (MRSA) were inoculated onto a 1.32 cm$^2$ area of *ex-vivo* porcine skin samples, immobilised in agar. After drying, solutions of XF-73 were applied and after 60 minutes, the samples were illuminated for 15 minutes with blue light (422 nm) with various total light doses using a LumaCare$^{TM}$ LC-122M lamp. CFU analysis were undertaken to determine the number of viable cells remaining after treatment. Controls of drug alone and light alone were included.
**Results:** Using 7.64 mg/L of XF-73, CFU analysis demonstrated that at a total light dose of 5 J/cm$^2$, there was ~90% kill of bacteria. At 10 J/cm$^2$, there was 99.9% kill of bacteria, and 99.99% at 20 J/cm$^2$ and 40 J/cm$^2$. At a total light dose of 20 J/cm$^2$, it was found that there was a <99% kill by XF-73 at concentrations of 0.76, 1.53 and 3.8 mg/L. At a concentration of 7.64 mg/L, there was a >99.9% kill. This kill did not significantly increase at 22.93 and 76.4 mg/L.
**Conclusions:** The results demonstrate that XF-73 has exceptional activity at low concentrations against MRSA on the surface of porcine skin. XF-73 and light are non-toxic to skin at therapeutic concentrations. Work is in progress to clinically evaluate the effectiveness of this compound in eradicating staphylococcal nasal carriage.
**Reference**
(1) Maisch T et al. Photodynamic effects of novel XF porphyrin derivatives on prokaryotic and eukaryotic cells. Antimicrob Agents Chemother 2005; 49: 1542–52.

## P1566

## Novel antimicrobial photodynamic agents active against epidemic methicillin-resistant *Staphylococcus aureus*

G. French, A. King, K. Shannon, W.G. Love, W. Rhys-Williams *(London, Brighton, UK)*

**Objectives:** The rise of epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA) and the emergence of mupirocin resistance means that it is essential to develop new therapies that cannot be readily overcome by microorganisms. The XF series of novel light activated antibacterial agents (1) active against Gram-positive bacteria addresses this issue and have superior levels of activity to antibiotics but with less likelihood of resistance emergence. The antibacterial activity of the XF Drugs against EMRSA has been investigated.
**Methods:** MIC and MBC assays were used to investigate the antibacterial activity of XF-73, a novel antimicrobial photodynamic agent against a range of *Staphylococcus aureus* strains. A concentration range of 2–0.001 mg/L was investigated. 15 minutes of 420 nm light activation (13 J/cm$^2$) was applied. Light alone had no effect.
**Results:**

| Organism | XF-73 MIC (mg/L) | XF-73 MBC (mg/L) |
|---|---|---|
| ATCC BAA-44 (MRSA) | 0.06 | 0.12 |
| NCTC 11939 (EMRSA-1) | 0.06 | 0.5 |
| EMRSA-15 | 0.03 | 0.25 |
| EMRSA-16 | 0.03 | 0.25 |
| NCTC 6571 (MSSA) | 0.06 | 0.06 |
| ATCC 25923 (MSSA) | 0.12 | 0.12 |

**Conclusions:** The results demonstrate that XF-73 has exceptionally low MIC and MBC values against all of the *S. aureus* strains tested. The results also demonstrate that XF-73 is equally effective against MRSA and methicillin-sensitive *Staphylococcus aureus* (MSSA) indicating its mode of action is independent of antibiotic resistance. XF-73 may therefore be useful in prevention and treatment of EMRSA. XF-73 is non-

toxic to skin at prophylactic/therapeutic concentrations and has potential for the treatment of skin sepsis and the eradication of nasal and skin MRSA carriage. Work is in progress to evaluate the effectiveness of this compound in eradicating staphylococcal nasal carriage.

**Reference**

(1) Maisch T et al. Photodynamic effects of novel XF porphyrin derivatives on prokaryotic and eukaryotic cells. Antimicrob Agents Chemother 2005; 49: 1542–52.

## P1567

## The *in vitro* bactericidal activity of NXL101, a novel bacterial topisomerase inhibitor, against Gram-positive cocci

M. Borgonovi, C. Delachaume, A.M. Girard, J. Lowther *(Romainville, FR)*

**Objective:** NXL101 is a novel antibacterial currently in pre-clinical development. The mechanism of action is directed against topoisomerase, and the spectrum of activity is exclusively against Gram positive organisms. The goal of the study was to characterise the activity and time/kill kinetics against common aerobic cocci in comparison to currently marketed molecules: linezolid (LIN), vancomycin (VAN), quinupristin/dalfopristin (Q/D) and moxifloxacin (MOX).

**Methods:** (i) *In vitro* susceptibility tests: The strains used were from the culture collection of Novexel and were of clinical origin. MICs were determined by an agar dilution technique. Mueller Hinton agar medium was used, supplemented with 5% horse blood for Group A streptococci (GAS), Group B streptococci and *S. pneumoniae*. Overnight cultures were diluted to obtain the final inoculum of $10^4$ CFU/spot. The MIC was the lowest concentration which inhibited all visual growth (3 or less colonies were ignored). (ii) Time/kill kinetics: Experiments were performed against strains of *S. aureus* (n = 8) and *S. pneumoniae* (n = 4) in 20 ml volumes of appropriate growth medium with initial inoculum of around $10^6$ CFU/ml of logarithmically growing culture. Timed samples over a 24 hour period were enumerated using a spiral plating method. NXL101 was compared to linezolid and vancomycin and the concentrations tested were 4, 8 and 16-fold the MIC90 for both species.

**Results:** (i) The MIC90s of NXL101 versus comparators are shown in the table. (ii) Time kill experiments showed that NXL101 was bactericidal against *S. aureus*, including methicillin resistant strains (>3log10 reduction within 6–8 hours) compared to a slowly bactericidal effect for vancomycin (24 hours). NXL101 and vancomycin were both bactericidal against *S. pneumoniae* within 6–8 hours. Linezolid was bacteriostatic against all strains tested.

| Isolates | DAQ550a MIC$_{90}$ (Range) in mcg/ml n=12 | DAQ550a Δ in 24-h log$_{10}$ CFU/mL n=2 avg. | DAQ552 MIC$_{90}$ (Range) in mcg/ml n=12 | DAQ552 Δ in 24-h log$_{10}$ CFU/mL n=2 avg. |
|---|---|---|---|---|
| MSSA | 0.063 (0.063 – 0.5) | -3.37 ± 0.244 | 0.063 (0.063 – 0.125) | 3.14 ± 0.275 |
| MRSA | 0.25 (0.125 – 0.5) | -2.98 ± 0.104 | 0.125 (0.063 – 0.125) | 3.17 ± 0.424 |
| VRE | 4 (1 – 4) | -2.59 ± 0.163 | 0.5 (0.125 – 0.5) | 3.00 ± 0.294 |

**Conclusion:** NXL101 exhibits bactericidal activity against common Gram positive cocci, including strains which exhibit resistance to methicillin, vancomycin and fluoroquinolones. NXL101 warrants further investigation.

## P1568

## Protein targets of N-chlorotaurine in bacteria

R. Arnitz, B. Moser, B. Sarg, H.W. Ott, H. Lindner, M. Nagl *(Innsbruck, AT)*

**Objectives:** The aim of this study was to identify bacterial proteins as targets of the endogenous antiseptic N-chlorotaurine (NCT), which is a promising microbicidal agent for topical treatment of infections. In addition, a combination of NCT with ammonium chloride which enhances the microbicidal activity significantly was investigated.

**Methods:** *Escherichia coli* and *Staphylococcus aureus* were treated with NCT and NCT plus ammonium chloride for different incubation times between 1 and 30 min – a period where killing takes place. To find out protein changes, 2D-PAGE of bacterial proteins followed by mass spectrometry was performed.

**Results:** Incubation in 1% NCT revealed a change of the charge and a separation of numerous proteins into a series of spots with a different isoelectric point. Moreover, in *E. coli* heat shock protein 60 appeared, while ribosome releasing factor, D-ribose periplasmic binding protein, and malonyl-CoA transacylase spots decreased. In *S. aureus*, enolase and a translation elongation factor decreased. These changes appeared more rapidly in the presence of ammonium chloride, which can be explained by formation of the more lipophilic and microbicidal monochloramine. Molecular mechanisms of attack comprised mainly oxidation of thio and amino groups as confirmed with model peptides.

**Conclusion:** These results fit very well to previous preclinical and clinical findings. They indicate both surface attack and penetration of oxidation capacity into the bacteria and destruction of essential proteins by NCT and NCT plus ammonium chloride, respectively.

## P1569

## Anti-staphylococcal activity of ceftobiprole in recent clinical trial isolates

K. Bush, R. Strauss, K. Amsler, M. Heep, S. Bajaksouzian, A. Windau, M. Jacobs *(Raritan, US; Basel, CH; Cleveland, US)*

**Objectives:** Ceftobiprole is a new extended-spectrum cephalosporin with activity against methicillin-susceptible and methicillin-resistant *Staphylococci*, as well as against most *Enterobacteriaceae*. In this study the anti-staphylococcal activity of ceftobiprole is reported from a set of isolates from a recent clinical trial.

**Methods:** Consecutive clinical isolates of *Staphylococci* from 340 patients enrolled in a multicentre clinical trial involving complicated skin infections were examined for their susceptibility to ceftobiprole and selected anti-Gram-positive agents. MICs were determined using CLSI methodology.

**Results:** Among these isolates, 525 *Staphylococcus aureus* and 84 coagulase-negative *Staphylococci* (CoNS) were identified. The percentages of methicillin-resistant strains were 43% for *S. aureus* and 50% for CoNS. All strains (except one CoNS with a linezolid MIC of 8 mg/L) were susceptible to vancomycin and linezolid, with MICs <2 mg/L.Against methicillin-susceptible *S. aureus*, ceftobiprole MIC50 and MIC90 values were 0.25 and 0.5 mg/L, respectively, and against methicillin-resistant *S. aureus*, ceftobiprole MIC50 and MIC90 values were 0.5 and 2 mg/L, respectively. Ceftobiprole MICs ranged from ≤0.06 to 1 mg/L against methicillin-susceptible-CoNS (MS-CoNS) and ≤0.06 to 4 mg/L against methicillin-resistant CoNS (MR-CoNS). Ceftobiprole MIC50 and MIC90 values were 0.25 and 0.25 mg/

L, respectively, against MS-CoNS and 0.5 and 2 mg/L, respectively against MR-CoNS. Only one staphylococcal strain (MR-CoNS) had a ceftobiprole MIC of 4 mg/L.
**Conclusions:** The potent anti-staphylococcal activity of ceftobiprole was demonstrated in this collection of recent clinical trial isolates, where >99% of the strains had MIC values ≤2 mg/L.

## P1570

## Potency of garenoxacin tested against an international collection of *Staphylococcus aureus* isolates, including oxacillin- and ciprofloxacin-resistant subsets (2004–2005)

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

**Objective:** To characterize the antimicrobial activity of garenoxacin (GRN), a novel des-F(6)-quinolone in late stage clinical development, against a large international collection of *S. aureus* (SA), including oxacillin (OXA)- and ciprofloxacin (CIPRO)-susceptible (S) and resistant (R) subsets, collected in 2004–2005.
**Methods:** Consecutive, non-duplicate bacterial isolates (10,068 strains) acquired from patients with bloodstream, respiratory, and skin and skin structure infections both nosocomial and community acquired were submitted from >70 medical centres in Europe, the Americas and the Asia-Pacific region. All isolates were tested using CLSI/NCCLS broth microdilution methods against GRN, the currently marketed fluoroquinolones (FQ) including CIPRO, levofloxacin (LEVO), gatifloxacin (GATI) and representative comparator agents. OXA- and CIPRO-S and -R subsets were included. A GRN-S breakpoint of ≤0.12 mg/L was applied for comparative purposes only and was based upon the MIC population distributions of strains that included quinolone-resistance determining region (QRDR) mutations.
**Results:** Potency for GRN and comparator FQs tested against SA: (See Table). Key resistance patterns (%) among this SA collection included OXA (40.3), CIPRO (36.4), erythromycin (47.5), clindamycin (13.4), tetracycline (9.7), and trimethoprim/sulfamethoxazole (4.8%); Gram-positive-targeted comparator including vancomycin, linezolid, daptomycin and quinupristin/dalfopristin all remained >99% S. Compared with currently marketed FQs when tested against all SA, GRN was 2- to 16-fold more active (MIC50, ≤0.03 vs. 0.06 or 0.5 mg/L). Against both OXA-S and -R SA, GRN displayed markedly enhanced potency compared with CIPRO and LEVO (≥4-fold), and GATI (2- to 4-fold). Among CIPRO-R isolates, GRN also maintained ≥4-fold greater potency (MIC50, 1 vs. ≥4 mg/L) although overall S for all FQs was 0–1%.

$MIC_{50}$(mg/L)/%S

| Organisms (no. total) | GRN | CIPRO | LEVO | GATI |
|---|---|---|---|---|
| | S<=0.12 mg/L | S<=1 mg/L | S<=1 mg/L | S<=0.5 mg/L |
| All SA (10,068) | <=0.03/64 | 0.5/63 | 0.12/64 | 0.06/64 |
| OXA-S (6,009) | <=0.03/95 | 0.25/94 | 0.12/94 | 0.06/95 |
| OXA-R (4,059) | 1/18 | >4/17 | >4/18 | 4/18 |
| CIPRO-S (6,312) | <=0.03/>99 | 0.25/100 | 0.25/>99 | 0.06/>99 |
| CIPRO-R (3,667) | 1/1 | >4/0 | >4/<1 | 4/1 |

**Conclusions:** Compared to the FQ agents tested against SA, GRN was the most potent agent and maintained the broadest coverage against OXA- and CIPRO-R strains even when applying a very conservative epidemiologic breakpoint. When a FQ is indicated for staphylococcal coverage, this des-F(6) quinolone may represent a superior alternative among FQ class agents, while minimizing selection of resistance.

## P1571

## Garenoxacin activity tested against *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* with elevated or resistant fluoroquinolone MIC values

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

**Objective:** To assess the garenoxacin (GRN) potency against a vast number of international respiratory tract infection (RTI) pathogens, especially versus phenotypic (high MIC) or genotypic (sequence change) QRDR mutants. A total of 40,423 isolates from 6 continents were analysed (1999–2005).
**Methods:** *S. pneumoniae* (SPN; 18,887 strains), *H. influenzae* (HI; 15,555) and *M. catarrhalis* (MCAT; 5,981) were susceptibility (S) tested by CLSI both microdilution methods against GRN and 25 comparison agents. Phenotypic QRDR mutants (PQMs) were defined by a ciprofloxacin (CIPRO) or levofloxacin (LEVO) SPN MIC at ≥4 mg/L; or CIPRO MIC of ≥0.25 mg/L for HI and MCAT. 124 SPN strains in 3 S groups (1. CIPRO and LEVO MIC ≥4 mg/L; 2. CIPRO ≥ 4 mg/L and LEVO ≤2 mg/L; 3. CIPRO and LEVO ≤2 mg/L) had QRDR sequences determined (gyr A,B; par C,E).
**Results:** Penicillin, macrolides and ceftriaxone resistance (R) rates for SPN were 18.1, 28.4 and 0.8%, respectively. CIPRO (2.6% R) and LEVO (0.9% R) rates were low as were PQM occurrences among HI (19 strains, 0.1%) and MCAT (15 strains, 0.2%). GRN remained the most active fluoroquinolone (>90% S) against all mutant isolates, but HI isolates were 78.9–84.2% S (see table). QRDR sequencing demonstrated average numbers of mutations at 2.7, 1.5 and 1.1 for S groups 1, 2, and 3, respectively; GRN MIC 50/90 results are 0.5/2, 0.06/0.12 and 0.06/0.12 mg/L. Highest GRN MIC results (≥1 mg/L) were associated with ≥3 QRDR mutations. (See Table)

**Table: LZD versus SA isolates.**

| Region (nations/sites/strains) | MRSA % (range) | LZD $MIC_{50/90}$ mg/L (% S) |
|---|---|---|
| Canada (1/5/182) | 22 | 1/1 (100) |
| South America (4/10/355) | 46 (32 – 61) | 2/2 (100) |
| Europe (6/14/467) | 23 (3 – 42) | 1/2 (100) |
| Asia-Pacific (5/12/303) | 47 (15 – 79) | 2/2 (100) |

**Conclusions:** GRN maintains clinically usable activity (MIC, ≤1 mg/L) against important community-acquired RTI pathogens having R to presently marketed fluoroquinolones and against those isolates with documented QRDR mutations. Continued development of this novel des-F(6) quinolone agent appears desirable.

## P1572

## Evaluation of garenoxacin activity tested against all patterns of multiresistant *S. pneumoniae*: multi-centre studies of nearly 15, 000 isolates

R. Jones, M. Stillwell, H. Sader, T. Fritsche, D. Johnson *(North Liberty, US)*

**Objective:** To determine the possible co-resistance (R) patterns among contemporary worldwide (1999–2005) isolates of *S. pneumoniae* (SPN) and the effect on a new des-F(6) quinolone, garenoxacin (GRN). Multi-resistant (MDR) patterns were defined by CLSI breakpoint criteria (2005) for six agents (penicillin [PEN], cefuroxime axetil [CROX], erythromycin [ER], clindamycin [CL], tetracycline [TC], TMP/SMX [T/S]).

**Methods:** A total of 14,665 SPN strains were susceptibility (S) tested by CLSI broth microdilution methods, with isolates originally cultured in laboratories in Europe, Asia, Australia, Africa and the Americas. Comparison fluoroquinolones (FQs, 4) were ciprofloxacin, gatifloxacin (GATI), levofloxacin (LEVO) and moxifloxacin (MOXI). All S patterns were determined and then the S rates calculated for non-pattern agents determined in each group having ≥100 occurrences. See table for prevalence of ≥2%.
**Results:** Fifty-two distinct patterns were detected, of which 10 (81.8% of all strains) predominated. Among MDR SPN strains (R at ≥2 agents; 39.6% of isolates), GRN remained very active with 99.88% S at ≤1 mg/L (MIC90, 0.06 mg/L). SPN with R to only one drug or a complete S pattern had 99.93% S to GRN ($p > 0.05$). This potency for GRN was greater than GATI (MIC90, 0.5 mg/L), LEVO (MIC90, 1 mg/L) and MOXI (MIC25, 0.5 mg/L), all providing a 99.00% S rate. Patterns with R to four agents had the lowest GRN-S rate at 99.55%. Strains R to all agents were completely GRN-S (see table).

**Table: LZD versus SA isolates.**

| Region (nations/sites/strains) | MRSA % (range) | LZD $MIC_{50/90}$ mg/L (% S) |
|---|---|---|
| Canada (1/5/182) | 22 | 1/1 (100) |
| South America (4/10/355) | 46 (32 – 61) | 2/2 (100) |
| Europe (6/14/467) | 23 (3 – 42) | 1/2 (100) |
| Asia-Pacific (5/12/303) | 47 (15 – 79) | 2/2 (100) |

**Conclusion:** GRN was highly active (99.91% at ≤1 mg/L) against all SPN R patterns including MDR phenotypes (5,811 strains). This activity was greater than comparison marketed FQs (4), macrolides, and beta-lactams, but comparable (near 100% S) to glycopeptides, e.g. vancomycin (data not shown). Continued development of GRN for serious MDR SPN infections appears warranted.

## P1573

## Garenoxacin activity and potency against *S. pneumoniae* and *H. influenzae* respiratory tract isolates (2004–2005): report from a Worldwide Surveillance Network

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

**Objective:** To evaluate the comparative activity of garenoxacin (GRN), a novel des-F(6) quinolone, tested by reference methods against recent community-acquired respiratory tract (CARTI) and CA pneumonia (CAP) isolates. *S. pneumoniae* (SPN) and *H. influenzae* (HI) strains from Latin America (10 sites), USA (23), Europe (20), and the Far East (11) were sampled in 2004–2005.
**Methods:** Consecutive, non-duplicate cultures of SPN (3,042) and HI (965) were tested by CLSI reference broth microdilution methods with concurrent QC and interpretative criteria (M7-A7 and M100-S16, 2006). Comparison antimicrobials numbered >25, including: penicillin (PEN), clarithromycin (CLAR), ceftriaxone (CTRI), and 4 fluoroquinolones (FQ), ciprofloxacin (CIPRO), levofloxacin (LEVO), gatifloxacin (GATI), and moxifloxacin (MOXI). GRN susceptibility (S) was defined as MIC at ≤1 mg/L for comparison purposes only. CARTI isolates came from 23 nations and CAP strains from hospitalized patients (HCAP) in 9 countries.
**Results:** The following table lists key study results: (See table) GRN activity remained unchanged compared to 1999–2003 results (2005 ECCMID abstract 1555 and 1565) with 99.9 and 100.0% inhibition at ≤1 mg/L for SPN and HI, respectively. GRN was more potent than LEVO (16-fold), GATI (8-fold) and MOXI (4-fold) against SPN, and HCAP isolates were slightly more S than CARTI strains to nearly all agents. CTRI (96.0–98.8% S), cefepime (92.8–96.4%) and amoxicillin/clavulanate (90.5–91.8%) were the most active beta-lactams against SPN. PEN- and macrolide (CLAR)-R was elevated (26.9–37.3%) in SPN and nearly 24% of HI produced a beta-lactamase (ampicillin-R). Possible QRDR mutations (CIPRO MIC, ≥4 mg/L) in SPN were noted for 1.2–2.8% of isolates.

| Organism/source (no.) | MIC90 / %S | | | % non-S (R) | | |
|---|---|---|---|---|---|---|
| | GRN | LEVO | GATI | PEN[a] | CLAR | CTRI |
| SPN | | | | | | |
| HCAP (84) | 0.06/100.0 | 1/98.8 | 0.5/98.8 | 34.5 | 26.9 | 1.2 |
| CARTI (2,958) | 0.06/99.9 | 1/98.0 | 0.5/98.9 | 37.3 | 32.5 | 4.0 |
| HI | | | | | | |
| CARTI (965) | 0.016/100.0 | <=0.03/100.0 | <=0.03/100.0 | 23.9 | 11.1 | 0.0 |

a. Ampicillin result for HI.

**Conclusions:** GRN continues to exhibit the greatest activity (4- to 16-fold) compared to FQs tested against an updated (2004–2005) collection of CARTI and CAP isolates of SPN and HI. As FQ resistance evolves due to QRDR mutations, GRN MIC values generally remain well below potentially R levels, minimizing further selective pressure.

## P1574

## *In vitro* activity of garenoxacin tested against ciprofloxacin-susceptible and -resistant *Enterobacteriaceae* and *Acinetobacter* spp. strains collected worldwide by the SENTRY Antimicrobial Surveillance Program (2004–2005)

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

**Objective:** To evaluate the contemporary activity of garenoxacin (GRN) against ciprofloxacin (CIPRO)-susceptible (S) and CIPRO-resistant (R) *Enterobacteriaceae* (ENT) and *Acinetobacter* spp. (ASP). Unlike recently marketed fluoroquinolones (FQ), GRN, a des-F(6) quinolone lacks the C-6 fluorine.
**Methods:** A total of 9,017 isolates (8,247 ENT and 770 ASP) were consecutively collected from > 70 medical centres from bloodstream, respiratory, urinary and skin and soft tissue infections and tested by reference broth microdilution methods according to CLSI/NCCLS methods and interpretative criteria. A GRN S breakpoint of ≤1 mg/L was applied for comparison purposes only.
**Results:** The results of the major organism groups tested: (See table). GRN showed excellent activity against this large collection of ENT (MIC50, 0.12 mg/L) and 82.7% of isolates

| | GRN ($MIC_{50}$ in mg/L/% S) | | |
|---|---|---|---|
| Organism (no. CIPRO-S/R) | CIPRO-S | CIPRO-R | All strains |
| *Citrobacter* spp. (173/20) | 0.06/85.5 | >4/0.0 | 0.12/76.7 |
| *E. cloacae* (675/103) | 0.12/93.3 | >4/1.9 | 0.12/81.2 |
| *E. coli* (3,040/679) | <=0.03/98.9 | >4/0.4 | <=0.03/80.9 |
| *K. pneumoniae* (1,308/230) | 0.12/95.9 | >4/3.5 | 0.12/82.1 |
| *P. mirabilis* (340/94) | 0.25/92.6 | >4/1.1 | 0.25/72.8 |
| *Salmonella spp.* (134/0) | 0.06/99.3 | . | 0.06/99.3 |
| *Serratia* spp. (459/31) | 1/59.0 | >4/0.0 | 1/55.3 |
| All ENT (6,985/1,262) | 0.06/94.0 | >4/1.2 | 0.12/79.8 |
| ASP (503/267) | <=0.03/97.0 | >4/3.6 | 4/36.0 |

were inhibited at ≤1 mg/L. The activity (MIC50/%S) of other oral antimicrobials tested against ENT was: amoxicillin/clavulanate 8/62.2%, cefuroxime 4/58.2%, gatifloxacin ≤ 0.5/85.9%, levofloxacin ≤ 0.5/85.9%, moxifloxacin 0.06/84.7%, trimethoprim/sulfamethoxazole ≤ 0.5/74.4%. The *in vitro* activity of GRN was most similar to that of CIPRO. Among CIP-S ENT (6,985 isolates), 97.2% (86.3–100.0%) where S to GRN, while among CIPRO-S ASP, 98.1% were S to GRN. 91.6% of CIP-R ENT isolates were also R to GRN.
**Conclusions:** GRN *in vitro* activity was superior to other tested orally administered agents representing several different drug classes when tested against contemporary (2004–2005) ENT and ASP isolates and was similar to that of the several commonly used FQs.

## P1575

## Microbiologic efficacy of garenoxacin vs. comparators in complicated skin and skin structure infections

D. Krievins, C. Le Devehat, G. Herrera *(Riga, LV; Nevers, FR; San Jose, CR)*

**Objectives:** Garenoxacin (GRN) is a novel, broad-spectrum des-F(6)-quinolone with activity against Gram-negative and Gram-positive aerobes and anaerobes including quinolone-resistant *Staphylococcus aureus*. The objective of this analysis was to compare the microbiologic efficacy of GRN to that of comparators against common pathogens involved in complicated skin and skin structure infections (cSSSI).
**Methods:** Two multinational, double-blind, randomized studies were conducted. In the first study, subjects received GRN (600 mg IV to PO qd) or piperacillin/tazobactam (3.375 g IV q6h) with transition to PO amoxicillin/clavulanate (500 mg PO q8h). In the second study, subjects received GRN (600 mg PO qd) or ciprofloxacin/metronidazole (500 mg q12h/500 mg q8h). All antimicrobials were administered for 7 to 14 days. Subjects were adults (≥18 y) newly hospitalized or ambulatory outpatients with evidence of cSSSI who did not have underlying osteomyelitis. Microbiologic efficacy was determined 5 to18 days post-therapy.
**Results:** A total of 567 subjects were microbiologically evaluable (GRN, n = 283; comparators, n = 284). The disease diagnosis was similar between GRN and comparators and included infected pressure sore (5% vs 3%), infected diabetic foot ulcer (18% vs 19%), major abscess (66% vs 67%), or postsurgical wound infection (11% vs 12%). The majority of common skin pathogens were eradicated by GRN and comparators. Eradication rates for *S. aureus* were higher in the GRN group [88% (120/137)] than in the comparator group [74% (108/146)]. Eradication of methicillin-resistant strains of *S. aureus* was 72% (13/18) and 60% (12/20) for GRN and combined comparators, respectively. GRN effectively eradicated quinolone-resistant *S. aureus* [73% (8/11); comparators 50% (5/10)]. Eradication rates for anaerobic pathogens were 86% (92/107) and 92% (100/109) for GRN and comparators, respectively. GRN effectively eradicated the Bacteroides fragilis group [82% (18/22)], *Peptostreptococcus* species [86% (32/37)] and *Prevotella* species [92% (23/25)]. Eradication rates were higher for GRN than comparators against Gram-negative pathogens [88% (137/156) vs 81% (143/176)]. This included activity against *Escherichia coli* [82% (23/28)], *Pseudomonas aeruginosa* [83% (24/29)] and *Proteus mirabilis* [82% (9/11)].
**Conclusion:** GRN demonstrated excellent, broad spectrum activity against Gram-positive, Gram-negative, and anaerobic pathogens including resistant organisms involved in cSSSI.

## P1576

## Dual action of gemifloxacin in acute bacterial sinusitis in patients with allergic rhinitis: antibacterial and immunomodulatory effects?

H. Lode, J. Garau, T. File, P. Ball, G. Tillotson, I. Morrissey *(Berlin, DE; Barcelona, ES; Akron, US; St Andrews, UK; Waltham, US; London, UK)*

**Background:** Acute bacterial sinusitis (ABS) is a common infection world-wide, with many patients having an associated an allergic component/history. However the role of antibacterials in these patients (pts) has not been examined. As some fluoroquinolones (FQ) have an *in-vitro* immunomodulatory effect (IE) the clinical efficacy of GEM was compared other agents in ABS pts with or without allergic rhinitis (AR).
**Methods:** 5 phase 3 clinical trials were pooled and pts where allergic rhinitis was identified (584 pts) were compared with pts not reporting AR (1834 pts). Clinical response (success or failure) at end of therapy (EOT) & at follow-up (FU, approx. 1–3 weeks after treatment) was studied. Comparators (CMP) were cefuroxime (CEF) and trovafloxacin (TRO).
**Results:** % Success based on clinical outcome at EOT and FU for AR and non AR pts are shown in the table. For all treatments EOT success was high for the non AR pts, but at FU this was reduced, especially with both FQs. In contrast, GEM retained a high clinical success rate in pts with AR unlike CEF or TRO.

| | | % success (number of pts) | | | |
|---|---|---|---|---|---|
| Group | Treatment | EOT | | FU | |
| Non AR | GEM | 93.00% | (1408) | 85.20% | (1408) |
| | All CMP | 93.70% | (428) | 88.30% | (426) |
| | CEF | 93.70% | (252) | 90.40% | (251) |
| | TRO | 93.80% | (176) | 85.10% | (175) |
| AR | GEM | 90.90% | (473) | 82.50% | (473) |
| | All CMP | 82.00% | (111) | 71.80% | (110) |
| | CEF | 82.80% | (87) | 73.30% | (86) |
| | TRO | 79.20% | (24) | 66.70% | (24) |

**Conclusion:** GEM has been shown to be very efficacious in a sub group of problematic ABS pts. This advantage may be due to the high antibacterial activity of GEM vs key ABS pathogens and/or a stimulatory IE. Both being important with pts having decreased local immune defences. These data also show that not all fluoroquinolones have immuno-stimulatory properties.

## P1577

## Garenoxacin efficacy against multidrug-resistant *Streptococcus pneumoniae*: retrospective analysis of community-acquired pneumoniae isolates obtained from nine phase II and III clinical studies (1999–2003)

T. Black, H. Waskin, R. Hare *(Kenilworth, US)*

**Objective:** Garenoxacin (GRN) is a novel, des-F(6)-quinolone with excellent activity against *S. pneumoniae*, one of the most common pathogens causing community-acquired pneumoniae (CAP). The incidence of infections caused by antibiotic-resistant isolates of *Streptococcus pneumoniae* is on the increase, therefore information regarding the activity of new anti-infective drugs against populations of *S. pneumoniae* that are multi-drug resistant (MDR) is critical. MDR *S. pneumoniae* (MDRSP) includes isolates previously known as PRSP (penicillin-resistant *S. pneumoniae*), as well as strains resistant to two or more of the following antibiotics: second-generation

cephalosporins, macrolides, tetracyclines, and trimethoprim/sulfamethoxazole.

**Methods:** Pretreatment sputum and blood isolates collected worldwide during GRN phase 2/3 clinical CAP trials (1999–2003) were retrospectively analysed for the MDRSP phenotype. Of the 352 *S. pneumoniae* isolates originally identified, 208 from 180 subjects were subjected to secondary MDR susceptibility testing by central laboratories. Confirmed MDRSP isolates were matched to individual subjects to assess clinical and microbiological outcomes for MDRSP-infections treated with GRN.

**Results:** Expanded susceptibility testing identified 53/208 MDRSP isolates from 44 unique subjects. The lowest MIC50 and MIC90 values for MDRSP isolates tested against a panel of representative drugs were observed for GRN (Table 1; 0.03 μg/ml and 0.06 μg/ml, respectively). The incidence of resistance to the five classes of drugs was 11%, 12%, 21%, 19% and 18% for penicillin, 2nd Generation Cep., macrolides, tetracycline and Tri/Sulf, respectively. No isolates were resistant to GRN using a proposed susceptibility breakpoint value of ≤1 μg/ml. Thirty-five percent, 28%, 15%, 9% and 13% of isolates were resistant to 1, 2, 3, 4 and 5 drug classes, respectively. The worldwide incidence of MDRSP was 18% with an equivalent geographic distribution of 19%, 21% and 16% among North America, Europe and the Rest of World. Overall, GRN provided clinical and bacteriological success for 32/35 (91%) CAP evaluable subjects with MDR infection, which was similar to clinical success for evaluable subjects with non-MDRSP CAP infections 151/165 (92%).

| Drug Class | Number of Isolates | $MIC_{50}$ (ug/ml) | $MIC_{90}$ (ug/ml) | MIC Range |
|---|---|---|---|---|
| Penicillin | 53 | 2 | 4 | 0.03 - 4 |
| Cefuroxime | 52 | 4 | 8 | 0.12 - 16 |
| Erythromycin | 50 | 8 | 16 | 0.06 - 128 |
| Tetracycline | 50 | 16 | 16 | 0.12 - 32 |
| Trimethoprim/Sulfamethoxazole | 50 | 8 | 8 | 0.12 - 16 |
| Levofloxacin | 52 | 1 | 1 | 0.25 - 1 |
| Garenoxacin | 52 | 0.03 | 0.06 | 0.015 - 0.12 |

**Conclusions:** These data demonstrate the ability of GRN to successfully eradicate MDRSP associated with CAP.

## P1578

## Clinical outcome of community-acquired pneumonia infections treated with gemifloxacin: the effect of patient risk factors

J. Garau, T. File, P. Ball, H. Lode, G. Tillotson, I. Morrissey
*(Madrid, ES; Akron, Waltham, US; St Andrews, London, UK; Berlin, DE)*

**Background:** Due to increased resistance to many traditional therapies the respiratory fluoroquinolones have become important agents in the management of patients with community-acquired pneumonia (CAP). Gemifloxacin is a new potent fluoroquinolone with excellent activity against CAP pathogens. Pooled data from 6 phase III CAP gemifloxacin clinical trials were evaluated.

**Methods:** Patients (1352 in total) were grouped based on risk factors according to the IDSA guidelines [Mandell et al.2003 CID 37:1405]. Clinical response (success or failure) at end of therapy (EOT) & at follow-up (FU, approx. 1–3 weeks after treatment) was evaluated. The main comparators in the pooled studies were cefuroxime & clarithromycin (171 patients), trovafloxacin (145 patients) & amoxicillin-clavulanate (98 patients). Per cent success is shown in the table (AB, antibiotics, COPD, chronic bronchitis and obstructive lung disease, HD, heart disease).

**Results:** Although gemifloxacin showed lower % success than comparator against CAP patients with no defined risk factor, gemifloxacin was considerably more successful than comparator against patients associated with risk factors, especially diabetic patients where comparator success was low. This advantage was often more prominent at FU than at EOT. Patients with other comorbidities such as renal failure or malignancy were not recruited in sufficient number for analysis.

| Percent success (N) | | EOT | | FU | |
|---|---|---|---|---|---|
| None defined | All Gemifloxacin | 85.6% | (757) | 81.1% | (757) |
| | Comparators | 87.8% | (378) | 82.0% | (378) |
| All combined | All Gemifloxacin | 87.2% | (797) | 81.7% | (797) |
| | Comparators | 86.8% | (555) | 80.5% | (555) |
| Prior AB | All Gemifloxacin | 86.6% | (402) | 82.1% | (402) |
| | Comparators | 86.6% | (262) | 79.0% | (262) |
| Diabetes | All Gemifloxacin | 87.4% | (143) | 81.1% | (143) |
| | Comparators | 85.5% | (83) | 75.9% | (83) |
| COPD | All Gemifloxacin | 85.6% | (209) | 78.0% | (209) |
| | Comparators | 84.3% | (198) | 78.8% | (198) |
| HD | All Gemifloxacin | 87.6% | (387) | 81.7% | (387) |
| | Comparators | 85.8% | (303) | 79.5% | (303) |

**Conclusions:** These data support the use of gemifloxacin in the treatment of CAP, especially where the patient has recognised IDSA risk factors.

## P1579

## Microbiologic efficacy of garenoxacin vs. comparators against common pathogens associated with community-acquired pneumonia

M. De Salvo, H. Vasconcelos Lopes, P. Kasemets, H. Waskin *(Buenos Aires, AR; Sao Paulo, BR; Tampere, FI; Kenilworth, US)*

**Objectives:** Garenoxacin (GRN) a novel, broad-spectrum des-F(6)-quinolone is active against many clinically important respiratory pathogens including penicillin-resistant strains of *Streptococcus pneumoniae*. GRN has dual sites of inhibition (DNA gyrase and topoisomerase IV) and may be less likely to promote resistance. The objective of this analysis was to compare the microbiologic efficacy of GRN to that of comparators against common pathogens involved in community-acquired pneumonia (CAP).

**Methods:** Two multinational, double-blind, randomized studies were conducted. In the first study, subjects received GRN (400 mg PO qd for 5 d) or amoxicillin/clavulanate (A/C; 500 mg PO q8h for 7–10 d). In the second study, subjects received GRN (400 mg PO qd for 7–10 d) or levofloxacin (LEV; 500 mg PO qd for 7–10 d). Adults (18 years of age or older) were enrolled with clinical and radiologic evidence of CAP [new infiltrate(s) on chest radiograph and fever, leukocytosis, cough, chest pain, auscultatory findings, or sputum production]. The majority of subjects were Fine class I/II in both studies. Bacteriologic eradication was assessed 5 to18 days post therapy.

**Results:** A total of 377 treated subjects had pretreatment pathogens (GRN, n = 179; comparators, n = 198). The overall eradication rate in all treated subjects was 91% (129/142) for GRN and 85% (123/145) for the comparators. Eradication rates for *S pneumoniae* were 92% (24/26) for garenoxacin and 96% (49/51) for the comparators. Eradication of *S pneumoniae* was 100% and 89% for A/C and LEV, respectively. In strains with reduced susceptibility to penicillin eradication rates were 86% (6/7) vs

67% (2/3) in favour of GRN. Eradication rates for *H. influenzae* were 89% (40/45) and 75% (30/40) for GRN and comparators, respectively. LEV eradicated 71% of *H. influenzae* isolates and A/C eradicated 77% of the strains isolated. There were very few isolates (4) of *Moraxella catarrhalis* in the 2 studies. In 1 study GRN was 100% effective against 3 strains of *M. catarrhalis* and in the other A/C was 100% effective against the 1 strain isolated. GRN eradicated 95% (19/20) of the *Staphylococcus aureus* isolates vs 100% (11/11) for the comparators.
**Conclusions:** GRN was highly active against pathogens commonly associated with CAP including drug-resistant strains of *S pneumoniae* and represents an effective therapeutic option for this patient population.

## P1580

## Efficacy of garenoxacin in the treatment of community-acquired pneumonia caused by multidrug-resistant *Streptococcus pneumoniae*

G. Dien, J. Gaillat, L. Dunbar, L. Vives, H. Waskin *(Hyeres, Annecy, St Gaudens, FR; New Orleans, Kenilworth, US)*

**Objectives:** Garenoxacin (GRN) a novel, broad-spectrum des-F(6)-quinolone is active against many clinically important respiratory pathogens including penicillin-resistant strains of *Streptococcus pneumoniae*. There is a growing problem of resistance in strains of *S pneumoniae*, with multi-drug-resistant *S pneumoniae* (MDRSP) becoming increasingly more common. The objective of this study was to evaluate the clinical and microbiologic efficacy of GRN in the treatment of community-acquired pneumonia (CAP) caused by MDRSP.
**Methods:** This was a multinational, open-label, non-comparative study. Subjects were adults (≥18 and <75 y) with clinical (clinical signs, sputum production), radiologic (new infiltrates on chest radiograph), or microbiologic (predominance of Gram-positive cocci in pairs on sputum Gram-stain or a positive blood culture for *S. pneumoniae*) evidence of CAP caused by *S. pneumoniae*. Subjects received GRN 400 mg PO qd or GRN 400 mg IV with transition to 400 mg PO qd for 7 to 14 days. Clinical and microbiologic responses were determined at a test-of-cure visit 7 to 14 days posttherapy.
**Results:** A total of 121 subjects were enrolled. Of these, 47 (17 PO only, 30 IV to PO) were clinically and microbiologically evaluable. Clinical and microbiologic success rates were 91% (43/47) and 89% (42/47), respectively. Clinical success rates were 94% (16/17) and 90% (27/30) for PO and IV to PO, respectively. Documented *S. pneumoniae* bacteremia was present in 28% (n = 13) of subjects with a clinical success rate of 92%. Among evaluable subjects, resistance rates for *S. pneumoniae* were penicillin 13%, second-generation cephalosporin 17%, macrolides 21%, tetracyclines 21%, and trimethoprim/sulfamethoxazole 17%. Twelve evaluable subjects had pneumonia caused by MDRSP. Clinical success rate was 92% (11/12) in subjects with MDRSP and 91% (32/35) in non-MDRSP subjects. Clinical success of GRN for strains resistant to 2, 3, 4, or 5 antimicrobial drug classes, were 100% (5/5), 100% (1/1), 100% (2/2), and 75% (3/4), respectively. Microbiologic success was 83% (10/12) and 91% (32/35) for MDRSP and non-MDRSP (susceptible or resistant to 1 class) strains, respectively. GRN was generally well tolerated with drug-related adverse events (AE) reported in 14% (8/58; PO) and 21% (13/63; IV to PO) of subjects.
**Conclusions:** GRN (PO or IV to PO) is an effective treatment for CAP caused by MDRSP and non-MDRSP. GRN is well tolerated.

## P1581

## *In vitro* bactericidal activity of daptomycin against *Staphylococcus aureus* and *Enterococcus* spp.: comparison with vancomycin, teicoplanin and linezolid

H. Drugeon, M. Juvin *(Nantes, FR)*

**Objectives:** The aim of this study was to evaluate the bactericidal activity (by killing kinetics) of daptomycin (DAP) against *Staphylococcus aureus* (SA) clinical isolates with different teicoplanin MICs and against *Enterococcus faecalis* (EFL) and *E. faecium* (EFM) with different mechanisms of glycopeptide resistance. DAP has been compared with teicoplanin (TEI), vancomycin (VAN) and linezolid (LIN).
**Methods:** 6 SA strains (2 MSSA and 4 MRSA) with TEI MIC distributed from 0.5 to 8 mg/L, 6 *Enterococcus* (3 EFL and 3 EFM) with glycopeptide phenotypes [S, R-vanA, R-vanB] were studied using a killing curve method. 7 antibiotic concentrations were used from 1 mg/l to 64 mg/l in two fold dilutions. Surviving bacteria were counted at T0, T30′, T1, T3, T6, T12 and T24 hours using agar plates with inhibitors to prevent antibiotic carry-over. Antibiotics tested were daptomycin (DAP), teicoplanin (TEI), vancomycin (VAN) and linezolid (LIN).
**Results:** All the SA isolates were susceptible to DAP (MIC = 0.25–1 mg/l), to LIN (MIC = 1–2 mg/L), to VAN (MIC = 1–2 mg/L) regardless of susceptibility to methicillin. DAP showed the same strong concentration dependent bactericidal activity with MSSA and MRSA: at T30′ bactericidal activity (BA) (decrease of 3 log10 cfu/mL) was observed with 8–16 mg/L of DAP; at T3 hours, 1–4 mg/L of DAP was sufficient and at T6 hours, BA was obtained with 1 mg/L of DAP. The other antibiotics showed a time dependent bactericidal activity but BA was observed only with long exposure (≥12 hours) and with high concentrations. All the *Enterococcus* isolates were susceptible to DAP (MIC = 1–2 mg/L) and to LIN (MIC = 1–2 mg/L) regardless of the resistance to glycopeptides. BA of DAP was also concentration dependent. BA was obtained with 4–8 mg/L after 6 hours of contact and with 1 mg/L after 12 hours of contact for EFL. BA was observed with 8–16 mg/L after 6 hours of contact and with 1–2 mg/L after 24 hours of contact for EFM. The other antibiotics had a time dependant activity but didn't show bactericidal activity with concentrations 32 mg/L.
**Conclusion:** The bactericidal activity of daptomycin was very strong, concentration dependent, and not influenced by the level or mechanism of glycopeptide resistance The bactericidal activity of linezolid was time dependent and observed only with the highest concentration and the bactericidal activity of vancomycin and teicoplanin was time dependent but was influenced by the mechanism of glycopeptide resistance.

## P1582

## Comparative *in vitro* activities of telavancin and other antibacterial agents against selected Gram-positive bacteria

J. Brauers, M. Kresken, S. Bagel *(Rheinbach, DE)*

**Objectives:** Telavancin (TLV) is a bactericidal lipoglycopeptide with multiple mechanisms of action that is in phase 3 clinical trials for the treatment of complicated skin and skin structure infections and hospital-acquired pneumonia with a focus on infections due to methicillin-resistant *Staphylococcus aureus* (MRSA). This study evaluated and compared the antibacterial

activity of TLV with that of other antibacterial agents against recent gram-positive clinical isolates from Germany.
**Methods:** A total of 363 aerobic gram-positive bacterial strains recently collected were included. Antibiotics tested were TLV, vancomycin (VAN), teicoplanin, penicillin, oxacillin, ampicillin, cefuroxime, ceftriaxone, daptomycin (DAP), linezolid (LZD), quinupristin-dalfopristin, clindamycin, ciprofloxacin, levofloxacin, gentamicin, streptomycin, erythromycin, telithromycin, co-trimoxazole and tetracycline. MICs were determined by the broth microdilution procedure according to the guidelines of the CLSI.
**Results:** TLV exhibited potent activity against all gram-positive bacteria including resistant isolates such as MRSA, VAN-resistant enterococci, pneumococci (including multiple resistant strains with various antibiotic resistance phenotypes) and other streptococcal species. TLV showed excellent *in vitro* activity against the species irrespective of the antibiotic phenotype tested. For methicillin-susceptible *S. aureus* (MSSA, n = 33) and MRSA (n = 42) MIC90 of TLV for both phenotypes were 0.5 mg/L. For coagulase-negative *Staphylococci* (n = 72, incl. MSSE, MRSE, MSSH, MRSH and others) MIC90s were 0.5 or 1 mg/L. MIC90s of TLV for *Enterococcus faecalis* (n = 30) and *E. faecium* (n = 32) were 1 and 2 mg/L, respectively. For VAN-resistant strains of *E. faecalis* (n = 2) or *E. faecium* (n = 7) MICs for TLV ranged from 0.12 to 4 mg/L. Against *Streptococcus pneumoniae* (n = 60) TLV MICs ranged from ≤0.001 to 0.03 mg/L. All *Streptococcus pyogenes*, *Streptococcus agalactiae* and all viridans group streptococci (n = 94) had MICs of ≤0.125 mg/L.
**Conclusion:** Based on MIC90, TLV was more potent than VAN, DAP or LZD against *Staphylococci*, streptococci and *E. faecalis*. It was superior to DAP and LZD against *E. faecium* and at least as active as DAP or LZD against most VAN-resistant enterococci. TLV appears to be a promising new antimicrobial agent for the treatment of infections caused by gram-positive organisms including multiply resistant isolates.

## P1583

## Evaluation of daptomycin, vancomycin, teicoplanin and linezolid against *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium* utilising *in vitro* time-kill methodology

J. Brauers, M. Kresken, A. Menke, A. Orland, I. Morrissey
*(Rheinbach, DE; London, UK)*

**Objective:** The cyclic lipopeptide daptomycin (DAP) exhibits rapid, concentration-dependent bactericidal activity against Gram positive bacteria (GPB). In this study we assessed the bactericidal activity of DAP by time-kill methodology against GPB in comparison with vancomycin (VAN), teicoplanin (TPL) and linezolid (LZD) using drug peak serum concentrations (PSC) and free serum concentrations (FSC).
**Methods:** The activity of DAP, VAN, TPL and LZD was evaluated by the CLSI time-kill method. For our experiments we used the following PSCs/FSCs (mg/l): VAN 40.0/18.0; TPL 45.0/4.5 and LZD 15.0/10.4. As PSC of DAP 60.0 mg/l was chosen. The extent of protein binding (PB) of DAP is still under investigation and data available so far indicate PB of either 92% or 63%. Therefore we tested two FSCs: 22.0 (corresponding to 63% PB) and 4.8 (corresponding to 92% PB). The activity of DAP was determined in Mueller-Hinton broth supplemented with 50 mg/l calcium. Viability counts were performed at 0.25, 0.5, 1, 3, 6, 12 and 24 h. One methicillin-susceptible *Staphylococcus aureus* (MSSA), two methicillin-resistant *S. aureus* (MRSA), one vancomycin-susceptible (VAN-S) and one VAN-resistant (VAN-R) *Enterococcus faecalis*, one VAN-S and one VAN-R *Enterococcus faecium* were tested. Bactericidal activity was defined as >99.9% killing during incubation.
**Results:** DAP was bactericidal at concentrations of 60.0 mg/l and 22.0 mg/l in all seven strains. The concentration of 4.8 mg/l was bactericidal against the two MRSA and against the VAN-S *E. faecium*. In the other four strains the maximum reduction of initial inoculum ranged from 1.52 to 2.53 log10 CFU/ml. In six strains a bactericidal effect at 60.0 mg/l and 22.0 mg/l of DAP, respectively, occurred between 15 minutes and 3 h and after 6 h in the VAN-S *E. faecalis*. VAN at 40.0 mg/l or 18.0 mg/l was bactericidal in only two strains after 24 h (1 MSSA, 1 MRSA). Against the other five strains, VAN was bacteriostatic with maximum reduction of initial inoculum between 1.91 and 2.78 log10 CFU/ml at 40 mg/l after 24 h, respectively. Both TPL and LZD were consistently bacteriostatic against the test strains.
**Conclusion:** DAP at PSC of 60.0 mg/l as well as at FSC of 22.0 mg/l showed a pronounced bactericidal effect within 3 h in 6/7 strains. VAN was bactericidal in only 2/7 strains after 24 h. Compared to VAN bacterial killing by DAP was very rapid. TPL and LZD were bacteriostatic only.

## P1584

## The effect of human serum on the bactericidal activity of daptomycin and comparators against *Staphylococcus aureus* and *Enterococcus* spp.

I. Morrissey, A. Leakey, R. Maxfield, T. Veltman, R. Woods, M. Robbins *(London, UK)*

**Background:** Daptomycin is a new cyclic lipopeptide antibiotic that shows rapid bactericidal activity and has high protein binding when assessed by standard methodology. This study investigated the bactericidal activity of daptomycin and the effect of protein binding by the addition of 50% human serum (HS).
**Methods:** Exponentially-growing methicillin-susceptible and –resistant *S. aureus* (MSSA, MRSA) and vancomycin-susceptible *Enterococcus faecium* (VSE) and -resistant *Enterococcus faecium* (VRE) (ca. 106 CFU/mL) were exposed to daptomycin (DAP), vancomycin (VAN), teicoplanin (TEI), piperacillin-tazobactam (PTZ) or linezolid (LZD) at peak (P) and trough (T) serum concentrations in Mueller Hinton broth supplemented with Ca2+ to 50 mg/L with or without HS. Viable count was determined at 0.25, 0.5, 3, 6 & 24 h. Plots were made of log reduction in viable count over time and the area-under-the-curve measured to calculate bactericidal indices (BIs) from these plots (J Antimicrob Chemother 1997, 39: 713–717).
**Results:** Daptomycin reduced viable count of MSSA & MRSA by approx. 5 logs or more within 0.25 h and VSE or VRE within 3 h at P. Other agents either did not achieve this or required 24 h to do so (not shown). BI data are shown below (>represents kill beyond the limit of detection). HS had little effect on DAP kill, except against the VRE at T. Nevertheless, DAP at T against VRE was more bactericidal than any other antibacterial except DAP at P.

| (Conc. mg/L) | | BI | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DAP | | VAN | | TEI | | LZD | | PTZ | |
| Serum | Strain | P (99) | T (9) | P (66) | T (8) | P (30) | T (9) | P (21) | T (6) | P (368/19) | T (21/1) |
| 0 | MSSA | >113.5 | >109.4 | 57.9 | 48.7 | 49.3 | 47.6 | 22.5 | 13.4 | 59.2 | 40.5 |
| | MRSA | >115.4 | >111.5 | 59.6 | 57.9 | 73.4 | 63.2 | 19.5 | 8.8 | 54.4 | 0 |
| | VSE | >105.8 | 42.3 | 13.3 | 12.5 | 41.1 | 35.3 | 10.4 | 0 | 55.9 | 40.3 |
| | VRE | >104.2 | 78.1 | 0 | 0 | 14.6 | 0 | 17.8 | 11.1 | 0 | 0 |
| 50% | MSSA | >94.7 | >90.2 | 46.3 | 46.8 | 21 | 17.3 | 8.3 | 0 | 35.7 | 14.6 |
| | MRSA | >98.8 | >92.2 | 57.5 | 52 | 40.3 | 34.5 | 6.1 | 0 | 52.4 | 0 |
| | VSE | >106.1 | 35.9 | 21.7 | 20.1 | 46.1 | 45.5 | 8 | 3.1 | 71.8 | 15.2 |
| | VRE | 83.5 | 26.4 | 0 | 0 | 0 | 0 | 22.2 | 10.8 | 0 | 0 |

**Conclusions:** DAP was the most bactericidal agent tested as measured either by BI or rate of kill. Dap at P reduced MSSA and MRSA to below detection within 15 min. The effect of HS was minimal which suggests that protein binding is either weak or highly reversible. These data support the use of DAP in the treatment of infections caused by these organisms.

## P1585

### Daptomycin activity against multi-resistant *Staphylococcus haemolyticus* bloodstream isolates from severe infections

F. Campanile, M. Venditti, S. Borbone, D. Bongiorno, A. Penni, S. Stefani *(Catania, Rome, IT)*

**Objectives:** Daptomycin, a new cyclic lipopeptide with activity against multidrug-resistant Gram-positive pathogens including MRSA, is approved for use in cSSST infections (US-FDA) and is being reviewed by EMEA for approval in EU member countries. The rapid bactericidal activity of daptomycin, due to its unique mechanism of action, makes it an attractive antibiotic for serious Gram-positive infections. The study was performed: (i) to evaluate the activity of daptomycin and other drugs against 50 multi-resistant clinically relevant *Staphylococcus haemolyticus* (MRSH), isolated from bloodstream infections in various hospitals in Italy (ii) to determine epidemiologic and genetic correlation among strains, and (iii) to characterize the SCCmec DNA of these strains.
**Methods:** The MRSH strains were tested against a panel of antimicrobial agents, by broth microdilution method performed according to CLSI (Clinical Laboratory Standards Institute) guidelines, including supplementation of 50 mg/L calcium for daptomycin. Moreover, phenotypic tests and antibiotic susceptibility profiling were carried out and the results compared with molecular typing analysis by using SmaI-PFGE fingerprints and PCR to characterize the mec-complex.
**Results:** All isolates were resistant to erythromycin, gentamicin, ciprofloxacin, 16 strains showed reduced susceptibility to vancomycin (MICs 2 mg/L), 24 strains were resistant to cotrimoxazole, 16 strains to clindamycin, 6 strains to chloramphenicol and 3 strains to tetracycline. Almost all isolates were inhibited by ≤1 mg/L of daptomycin, and only four strains exhibited a MIC value of 2 mg/L. PFGE analyses showed the existence of at least two multi-resistant *S. haemolyticus* clones widespread in different hospitals. Methicillin-resistance was correlated to the presence of the mecA and preliminary results regarding the genetic element carrying the gene, showed an organization of the mec-complex of class A and class C.
**Conclusions:** Our results suggest that daptomycin has excellent activity against multiresistant MR *S. haemolyticus* isolates, which represent a serious threat in catheter-related bloodstream infections. Furthermore, the emergence of *S. haemolyticus* exhibiting reduced susceptibility to vancomycin is of particular concern, probably due to the common use of vancomycin as initial therapy for such infections. Moreover, the use of additional molecular techniques to fingerprint isolates makes this study of clinically important CoNS more accurate.

# Resistance, linezolid, glycopeptides and inhibitors of protein synthesis

## P1586

### Mechanism of action of ceftobiprole: structural basis for anti-MRSA activity

A. Lovering, F. Danel, M.G.P. Page, N.J. Strynadka *(Vancouver, CA; Basel, CH)*

**Objectives:** Ceftobiprole is a new cephalosporin with a broad spectrum of action including methicillin-resistant *Staphylococci* (MRS) as well as many other Gram-positive and Gram-negative pathogenic bacteria. This study investigates the structural basis for the good activity against MRS.
**Methods:** The primary beta-lactam resistance determinant of MRS, penicillin-binding protein PBP 2′ (or 2a) has been cloned and expressed as a soluble form in which the amino-terminal residues forming a membrane-anchor have been deleted. The soluble form has been crystallized and the structure of the complex formed after soaking crystals in a solution containing ceftobiprole has been determined at 2.8 Angstrom resolution. Additional data on the structure of the ceftobiprole-PBP2′ complex formed in solution has been obtained using spectroscopic methods such as UV-circular dichroism.
**Results:** Ceftobiprole reacts rapidly with PBP2′ to form a stable acyl-enzyme complex. The ceftobiprole moiety is positioned deep within the active site of the acyl-enzyme complex formed with PBP2′, where it forms several hydrogen bonds and hydrophobic interactions. In particular, the 7-aminothiadiazolylhyroxyiminoacetyl side chain of ceftobiprole sits more deeply within the side-chain binding pocket of PBP 2′ than does the 7-acylamino side chain of nitrocefin in the previously determined complex structure. The additional interactions probably add to the enhanced stability of the acyl-enzyme complex formed with ceftobiprole, compared to complexes formed with other beta-lactams that are inactive against MRS. Significant structural rearrangements between apo-enzyme and acyl-enzyme are evident in the crystal structure and in solution.
**Conclusion:** Ceftobiprole readily forms a stable inhibitory acyl-enzyme complex with the PBP 2′, the beta-lactam resistance determinant of MRS. This, together with potent inhibition of the normal complement of beta-lactam sensitive penicillin-binding proteins, accounts for its excellent activity against *Staphylococci* and probably accounts for the low rates of resistance development observed in experimental conditions.

## P1587

### Incidence of *Staphylococcus aureus* with reduced susceptibility to glycopeptides in a French hospital (November 2004–April 2005)

C. Morate, A. Charron, C. Bebear, J. Maugein *(Bordeaux, FR)*

*Staphylococcus aureus* are a major cause of nosocomial infections around the world. Glycopeptides remain the drug of choice for severe infections caused by MRSA. However, after the emergence of vancomycin resistance in *Enterococcus* and in the

coagulase negative staphylococcus, strains of *Staphylococcus aureus* with reduced susceptibility to glycopeptides (GISA) have been reported in different countries like Japan, France, Spain, the UK and the United States. The aim of our study was to determine the proportion of vancomycin resistance in clinical *S. aureus* isolates in a french university hospital, between November 2004 and April 2005, then we wanted to define if there was an epidemic clone and study the clinical impact of these GISA strains. The protocol of detection was, first, a screening test on BHI agar containing 4 mg/L of teicoplanin, then, the vancomycin and teicoplanin MICs were determined by the method of Etest with an inoculum of 2.0 McF on the selected strains. Finally, the isolates with MIC of the teicoplanin ≥8 mg/L and MIC of the vancomycin ≥4 mg/L or MIC of the teicoplanin ≥12 mg/L and MIC of the vancomycin ≤4 mg/L were studied on population analysis. After that, pulsed-field gel electrophoresis (PFGE) was performed on the different isolates and the pulsotypes were compared. From November 2004 to April 2005, 468 *S. aureus* isolates were collected from 331 patients and screened for glycopeptide resistance on an initial agar screening test containing 4 mg/L of teicoplanin. The teicoplanin MIC was >4 mg/L for 65 isolates (13.9%) from 59 patients and these strains were selected for the determination of the MICs by ''macromethod'' Etest. By this technique, 39 strains were selected and studied by population analysis. All the profiles were compared to the reference strain Mu3 profile. This procedure detected 5 isolates (from 5 patients) with heterogeneous reduced susceptibility to glycopeptides (hGISA). So the incidence of *Staphylococcus aureus* with reduced susceptibility to glycopeptides in our hospital was found to be 1.1%. Four strains were resistant to methicillin and 3 were also resistant to gentamicin. The diversity of the strains was confirmed by PFGE: there was not an epidemic clone in the hospital. The clinical history showed that 4 patients had received a prior treatment with vancomycin, and that 3 patients had a failure in treatment: 2 of them had cystic fibrosis.

## P1588

## Distribution of different aminoglycosides resistance genes among the Iranian population of enterococci

M.M. Feizabadi, S. Khatibi, A. Asgharzadeh, S. Gharavi *(Tehran, IR)*

**Objectives:** *Enterococcus faecalis* was the most prevalent organism (72.5%) involved in enterococcal infections at Tehran Hospitals followed by *E. faecium* (22.5%). Due to widespread expansion of aminoglycoside modifiying Enzymes (AGMEs) genes, the rate of resistance to high level concentration of aminoglycosides has increased in these years. The rate of high level gentamicin resistant isolates of enterococci (HLGR) is high in Iran (46%). The aim of this study was to determine the genes encoding resistance to aminoglycosides among enterococci in Iran.
**Methods:** Disks containing 120 $\mu$g gentamicin were used to detect HLGR isolates. Primers specific for aac (6′) aph (2") and aph (3′) IIIa genes were used in PCR to possibly detect acetyltransferases and phosphotransferas, the common AGMEs among 113 isolates of enetrococci. Theses isolates were resistance to different concentration of gentamicin.
**Results:** A 222 bp region of the aac (6′)-aph (2") gene was amplified by PCR in 95% HLGR isolates as well as in 40% of low level getamicin resistant isolates (LLGR). Moreover the gene aph (3′) IIIa was detected in 92.5% and 72% of isolates of HLGR and LLGR respectively. Differences between isolates of *E. faecalis* and *E. faecium* were found in term of prevalence of aph (3′) IIIa gene.
**Conclusion:** The bifunctional enzyme AAC (6′)-APH (2") is the main cause of resistance to high concentration of aminoglycosides in our collection of enterococci. This enzyme confers resistance to all clinically useful aminoglycosides with the exception of streptomycin. In the absence of AAC (6′)-APH (2"), gentamicin could be used in combination therapy.

## P1589

## Prevalence and genetic analysis of methicillin-resistant *Staphylococcus aureus* expressing high-level and low-level mupirocin resistance

M. Kural, T. Us, Y. Akgun *(Eskisehir, TR)*

**Objectives:** To investigate the genetic location of mupA gene which encoded mupirocin resistance and characterize mupirocin-resistant methicillin resistant *Staphylococcus aureus* (MRSA) isolated from patients in a Turkish University hospital by polymerase chain reaction (PCR) and plasmid analysis.
**Methods:** Methicillin and mupirocin resistance were detected by disk diffusion (Oxoid, UK). The Etest (AB Biodisk, Sweden) was performed to determine mupirocin minimum inhibitory concentrations (MICs). The presence of mupA and mecA were detected by PCR using specific primers. Plasmid analysis were used to study the genetic location of mupA gene.
**Results:** A total of 595 (44.9%) MRSA strains were identified by disk diffusion in 1324 *S. aureus*. Of the 595 clinical isolates 394 (66.2%) were from wound, 91 (15.3%) from blood, 41 (6.9%) from catheter, 36 (6.1%) from lower respirator tract (bronchoalveolar lavage, pleural fluid and transtracheal aspirates), 13 (2.2%) from sputum, 12 (2.0%) from urine and 8 (1.3%) from other (serebrospinal fluid, parasynthesis fluid, peritoneal fluid, and bone marrow) clinical samples. Among the MRSA isolates, mupirocin resistance was detected in 35 (5.9%) strains with disk diffusion and Etest. Of the 35 mupirosin-resistant isolates 23 (3.9%) expressed high-level (MuH) and 12 (2%) expressed low-level (MuL) mupirocin resistance. All isolates were vancomycin, teicoplannin susceptible and chloramphenicol resistant with disk diffusion. Isolates with high-level and low level mupirocin resistance due to the mupA gene were also detected with PCR. Plasmids were detected in all of the 35 isolates. However only the MuH isolates contained a 38 kb plasmid that encoded high-level resistance. All of the isolates contained a 4.4 kb plasmid and resistant to chloramphenicol.
**Conclusion:** Our results indicated that the MRSA clones detected in the hospital had acquired a high-level mupirocin resistant plasmid. The past observations and recent studies suggested that the numbers of such strians have increased following extensive topical use of mupirocin. The usage of mupirocin in our hospital has not yet been systematically implemented. It is frequently prescribed for the treatment of staphylococcal skin infections and less to eliminate nasal carriage of MRSA. In our hospital we should be aware of the possible emergence and increase of mupirocin highly resistant MRSA strains in the future so that we should be considered when using mupirocin to control the spread of MRSA in hospital.

## P1590

## Emergence and spread of acquired fusidic acid resistance in *Staphylococcus aureus*

A. J O'Neill, F.B. McLaws, I. Chopra *(Leeds, UK)*

**Objectives:** A major route to fusidic acid resistance (fusR) in *S. aureus* involves acquisition of fusB, a resistance determinant first

identified on plasmid pUB101. Here we show that (i) the two currently-circulating major clones of fusR *S. aureus* identified to date have acquired fusB from pUB101 (or from the same ancestral source as pUB101), and (ii) that the pUB101-encoded FusB is only one of at least three lineages of this protein that appear to have evolved since recruitment of the original, ancestral fusB to the *Staphylococci*.

**Methods:** Plasmid purification, DNA sequencing, PCR amplification, and cloning in *S. aureus* RN4220 using shuttle-vector pCU1, were all performed using established methods. Antibiotic susceptibility testing was performed by agar dilution.

**Results:** The Epidemic European Fusidic acid-resistant Impetigo Clone (EEFIC) and community-acquired MRSA strain ST80 have been shown to carry chromosomal and plasmid-encoded fusB, respectively. DNA sequencing of fusB and its surrounding regions in these backgrounds revealed that they are identical to sequences on pUB101. However, acquired fusR does not always result from acquisition of the prototypical fusB gene. A gene encoding a FusB homologue was recently identified during sequencing of *S. aureus* strain MSSA476, and we identified an additional homologue encoded in the genome of *S. saprophyticus* strain ATCC15305. The products of these genes exhibit ~40% homology to FusB and to each other. Cloning of PCR amplicons corresponding to these genes and their upstream expression signals into *S. aureus* established that they both confer resistance to fus. Since these functional homologues are more closely related to each other than to those from other Gram-positive organisms, it is highly likely that they evolved from an ancestral FusB after its recruitment to the *Staphylococci*.

**Conclusions:** The three members of the staphylococcal family of FusB proteins appear to have evolved from the same ancestral protein, which, based on the low level of sequence homology between fusB genes at the nucleotide level, clearly occurred well before the introduction of fus into the clinic. Of the three, the FusB protein encoded by pUB101 is by far the most successful, and this gene/plasmid represents the source of (or shares a source with) the major fusR strain lineages.

## P1591

## Telithromycin activity is reduced by efflux in *Streptococcus pneumoniae*

C. Benvenuti, R. Koncan, G. Bahar, A. Mazzariol, G. Cornaglia *(Verona, IT; Ankara, TR)*

**Objectives:** Telithromycin shows an excellent activity against M-type erythromycin-resistant *Streptococcus pneumoniae*, thus is commonly regarded as being capable of overcoming the efflux resistance mechanism. Nevertheless, telithromycin MIC values in those strains appear to be distinctly higher than in the erythromycin-susceptible ones. The possibility of telithromycin acting as an actual efflux substrate, as it was already demonstrated in *Streptococcus pyogenes*, seemed worth investigating.

**Methods:** Telithromycin MIC distribution was analysed in a collection of 423 Italian *S. pneumoniae* strains originating from multi-centre studies (2000–2003). The effect of an efflux mechanism was investigated using [3H]-telithromycin.

**Results:** Telithromycin MIC ranges were ≤0.002–0.12 mg/l (MIC50 0.008 mg/l and MIC90 0.03 mg/l) in erythromycin-susceptible strains (lacking both mef and erm genes) and 0.016–1 mg/l (MIC50 0.25 mg/l and MIC90 0.5 mg/l) in strains endowed with the M phenotype. A distinct telithromycin efflux was detected in the strains expressing the mef gene, but not in those expressing the erm(B) gene, nor in the susceptible strains lacking mef or erm genes. Efflux reversibility by addition of an inhibiting compound (sodium arsenate) was demonstrated. An msr-like sequence was also found in all strains effluxing telithromycin, but not in the others.

**Conclusions:** This is the first time that telithromycin has been shown to be effluxed by *S. pyogenes* isolates. That the efflux is related to the presence of both the mef and the msr-like genes is clearly demonstrated, but – owing to the increasingly evident complexity of *S. pneumoniae* efflux systems – other genes might also contribute to the efflux.

## P1592

## An unusual phenotype of *Enterococcus faecalis* in Greece expressing low-level resistance to clindamycin and dalfopristin but susceptibility to quinupristin-dalfopristin

M. Maniati, F. Kontos, P. Liakos, E. Petinaki, I. Spiliopoulou, A. Maniatis *(Larissa, Patras, GR)*

**Objectives:** To investigate the resistance mechanism of a new described phenotype among *Enterococcus faecalis* expressing low-level resistance to clindamycin and dalfopristin but susceptibility to quinupristin-dalfopristin (Q-D).

**Methods:** In Greece, during 2005, three *Enterococcus faecalis* isolates, expressing this unusual phenotype, were recovered from urine samples. The isolates were studied by PCR for the lsa-gene and by PFGE. Nucleotide sequencing analysis of lsa and 309 bp of the upstream region was performed. The isolates were also tested by RT-PCR for the expression of the lsa-gene.

**Results:** The isolates belonged to three distinct clones and carried the lsa-gene. No stop codons were found in any strain, while some point mutations in the lsa-gene were detected. Comparing the lsa mRNA production of these unusual strains with that obtained from fully Q-D resistant ones no quantitative differences were found.

**Conclusions:** The findings of the present study clearly show that the resistance mechanism of quinupristin-dalfopristin is not only correlated with the presence and the expression of the lsa-gene. Some mutations detected in the lsa gene probably are responsible for the production of an Lsa protein with decreased activity, resulting to the Q-D susceptibility.

## P1593

## The presence of erm TR gene is responsible for the macrolide-resistance of *Streptococcus agalactiae*

A. Pratti, E. Akrivoussi, V. Karavassilis, E. Petinaki, A. Maniatis *(Larissa, GR)*

**Objectives:** To investigate the mechanism of resistance to macrolides in strains of *Streptococcus agalactiae* in the area of Thessalia, Greece during the period 2000–2005.

**Methods:** The subject of this study were 147 strains of *S. agalactiae* which were collected from clinical specimens (90% vaginal swabs) from pregnant and non pregnant women. The strains were identified by Gram stain, the Lancefield B antigen, and by API Strep System (BioMerieux, France). Susceptibility to macrolides, lincosamides and streptogrammines B was studied by the disk diffusion method. The MICs were also measured by the use of E-test. The differentiation between M and MLSB inducible type was tested by the Double Disk Synergy Test (DDST). The detection of the genes mef A, erm TR, and erm B was performed by Polymerase Chain Reaction (PCR). The clonality of the resistant strains was studied by pulse-field gel electrophoresis.

**Results:** Of the 147 strains, 15 were resistant to erythromycin, lincosamid and streptogrammines B. None was found to be resistant to erythromycin only (M-phenopype). 60% of the strains were MLSB constitutive phenotype, while 40% were MLSB inducible. All strains were found to carry the erm TR gene. Only one strain was found to carry both erm TR and erm B genes. PFGE analysis revealed the emergence of multiple resistant clones.
**Conclusions:** The resistance of *S. agalactiae* to MLSB antibiotics is related with the presence of erm TR gene in Central Greece.

## P1594

## Emergence of novel clindamycin resistance phenotype among invasive *Streptococcus pyogenes* isolates in Sweden

A. Jasir, B. Luca, C. Schalen *(Lund, SE)*

**Objectives:** In some recent throat group A streptococci (GAS) isolates from our diagnostic laboratory total resistance to clindamycin but susceptibility to erythromycin and other 14- as well as 15-membered macrolides was found. The isolates were susceptible to 16-membered macrolides and streptogramin B. These atypical strains thus did not agree with previously known MLS resistance phenotypes. The main objective was to characterize theses resistance phenotype and genotypes.
**Method and results:** The isolates were examined for resistance genes by PCR. Out of 14 strains one harboured an ermA gene. The gene was sequenced and showed a mutation in regulatory part and was localized on a transposons. All other strains were negative for any erm genes and were also tested for 23s rRNA mutations with negative outcome. Strains were T-and emm typed and showed to belong to different types.
**Conclusions:** GAS account for common human infections such as acute pharyngotonsillitis and impetigo, which untreated may be followed by the nonsuppurative complications rheumatic fever and acute poststreptococcal glomerulonephritis GAS may also give rise to invasive, often life-threatening acute disease, such as scarlatina, erysipelas, endometritis, necrotising fasciitis and sepsis, often accompanied by toxic shock. Without known exceptions, GAS are fully susceptible to betalactams, which are first-choice drugs for treatment. In cases of allergy or intolerance to penicillins, macrolides are most used, and possibly as a consequence, a significant resistance development to these agents has evolved in many parts of the world. Though the role of clindamycin for treatment of streptococcal disease is more limited this drug was shown to be particularly effective in eradicating streptococci after penicillin treatment failure of pharyngotonsillitis. Clindamycin, often as a supplement to betalactams, also may have a life-saving effect in the treatment of fulminant streptococcal infections. Due to its important role in the treatment of invasive streptococcal disease, resistance development to clindamycin in GAS is considered highly undesirable. The alarming finding of a possibly new phenotype of selective clindamycin resistance in GAS will motivate a thorough analysis of the phenotype as well as identification of its resistance determinants.

## P1595

## Resistance mechanisms of telithromycin-resistant clinical isolates of *Streptococcus pneumoniae* in Europe

A. Al-Lahham, M. van der Linden, R.R. Reinert *(Aachen, DE)*

**Objectives:** Telithromycin is a novel ketolide antibiotic with significant *in-vitro* activity against *Streptococcus pneumoniae*. The aim of this study is to characterize the resistance mechanisms of clinical isolates of *S. pneumoniae* with reduced susceptibility to telithromycin (>1 mg/l) and to perform the time-kill kinetics with telithromycin.
**Methods:** Determination of MICs was performed by the microbroth dilution method according to the CLSI and the serotyping by the Neufeld Quellung reaction. Multilocus sequence typing, sequencing of the 23S rRNA, sequencing of genes encoding ribosomal proteins (L4 and L22), and ermB were performed according to standard methods. Four isolates were selected for time-kill, two of which with a telithromycin MIC 2 mg/l and two strains with a telithromycin MIC of 8 mg/l.
**Results:** In two nation-wide studies and one European surveillance study (n = 6604) performed at the National Reference Center for Streptococci (NRCS) in Germany, reduced susceptibility to telithromycin (>1 mg/l) was detected in 17 isolates (0.3%). MIC50/MIC90 (mg/L) of the strains to other antibiotics were as follows: Telithromycin 2/8, penicillin G 2/2, cefuroxime 8/8, erythromycin A >32/>32, clindamycin >32/>32, tetracycline 32/32, and gatifloxacin 0.25/0.25. Two major serotypes were observed, serotype 14 (58.8%) and serotype 19A (29.4%). All isolates possess the cMLSB phenotype (ermB positive). The isolates showed a wide range of combinations of resistance determinants including multiple alterations in the 23S rRNA (A138G, C150T, A260G, A1745T, and C2216T), a S20N alteration in the ribosomal protein L4 (n = 9), and a N100S alteration in the erm(B) gene (n = 14). The predominant clone was serotype 14 sequence type 143 (8 of 17 isolates), which was seen in France (n = 7) and Germany (n = 1). Telithromycin-resistance has also spread to the Spain23F-1 clone (ST 81; n = 1) and its serotype 19A variant. *In vitro* time-kill showed a minimal kill from 0–8 hours and then regrowth. Bactericidal activity was achieved only with 8 times the MIC in all strains.
**Conclusions:** Although the incidence of telithromycin resistance remains rare world-wide, the spread of telithromycin resistance to multi-drug resistance clones with world-wide distribution is worrisome.

## P1596

## Genotypes and serotypes correlation among macrolide-resistant *Streptococcus agalactiae* isolates in Zaragoza, Spain (2002–2004)

M.E. Llaneza, C. Seral, F.J. Castillo, E. Duran, C. García, I. Millán, J.A. Sáez-Nieto, M.C. Rubio, R. Gómez-Lus *(Zaragoza, ES)*

**Objective:** To analyze the phenotype-genotype as well as the genotype-serotype correlation among macrolide-resistant *Streptococcus* group B (GBS) isolated in Zaragoza, Spain, from May 2002 to April 2004.
**Methods:** A total of 562 GBS were isolated from May 2002 to April 2004 at the University Hospital of Zaragoza, Spain: 354 GBS obtained from vaginal swabs of pregnant women and 208 GBS obtained from non-pregnant women. The erythromycin resistant-GBS were identified, phenotypically analysed, screened by PCR for mre(A) gene and for erythromycin resistance genes: erm(B), erm(TR), mef(A) and mef(E), and serotyped with type specific antisera for serotypes Ia, Ib, II, III, IV, and V.
**Results:** Among the total of GBS, 161 (28.65%) were erythromycin-resistant: 86 (24.29%) erythromycin–resistant GBS were isolated from vaginal swabs of pregnant women and 75 (36.06%) from non-pregnant women. The frequency of serotypes in 151 erythromycin-resistant GBS tested, the distribu-

tion of their resistance genes and the distribution of serotypes among the different genotypes are illustrated in the table. NT, nontypeable, The mre(A) gene was found in all the GBS strains tested. MICs of erythromycin in erythromycin-resistant GBS were: MIC50 and MIC90, >128 mg/L; range, 4 to >128 mg/L for GBS harbouring erm(B) and erm(B)+erm(TR) and MIC50 and MIC90, 8 mg/L and >128 mg/L, respectively; range, 0.5 to >128 mg/L for GBS harbouring erm(TR).

| Genotype | N° of isolates of serotypes | | | | | | | Total N°(%) isolates |
|---|---|---|---|---|---|---|---|---|
| | Ia | Ib | II | III | IV | V | NT* | |
| *erm*(B) | 5(22.7) | 0 | 14(45.2) | 31(48.4) | 0 | 7(53.8) | 3(37.5) | 60(39.7) |
| *erm*(TR) | 8(36.4) | 7(58.3) | 13(41.9) | 19(29.7) | 1(100) | 3(23.1) | 2(25) | 53(35.1) |
| *erm*(B)+*erm*(TR) | 2(9.1) | 1(8.3) | 1(3.2) | 10(15.6) | 0 | 3(23.1) | 0 | 17(11.3) |
| *erm*(B)+*mef*(A) | 0 | 0 | 1(3.2) | 0 | 0 | 0 | 0 | 1(0.7) |
| *mef*(A) | 0 | 1(8.3) | 0 | 0 | 0 | 0 | 0 | 1(0.7) |
| *mef*(E) | 1(4.5) | 0 | 0 | 0 | 0 | 0 | 0 | 1(0.7) |
| None/other gene | 6(27.3) | 3(25) | 2(6.5) | 4(6.3) | 0 | 0 | 3(37.5) | 18(11.9) |
| Total N°(%) isolates | 22(14.6) | 12(7.9) | 31(20.5) | 64(42.4) | 1(0.7) | 13(8.6) | 8(5.3) | 151(100) |

**Conclusion:** Erm(B) was the erythromycin-resistant gene most prevalent among the GBS isolates and these isolates showed the highest MICs of erythromycin. The commonest serotypes among erythromycin-resistant GBS isolated were III, II and I, and showed genotypic variability harbouring either of the two most prevalent genes, erm(B) or/and erm(TR).

## P1597

## Distribution of serotypes in tetracycline-resistant *Streptococcus* group B isolated in Zaragoza, Spain

M.E. Llaneza, C. Seral, F.J. Castillo, S. Salvo, A. Martinez, M. Pardos, M.C. Rubio, J.A. Sáez-Nieto, R. Gómez-Lus *(Zaragoza, Madrid, ES)*

**Objectives:** To analyse the tetracycline-resistant genotypes in erythromycin-resistant *Streptococcus* group B (GBS) isolated in Zaragoza, Spain collected from April 2002 to May 2004, and their serotype distribution.
**Methods:** We studied the rates of resistance to tetracycline and minocycline among 161 erythromycin-resistant GBS strains isolated at the University Hospital Lozano Blesa of Zaragoza, Spain. Isolates were subsequently phenotypically analysed by means of the disk diffusion method and screened by PCR for erythromycin and tetracycline resistance genes [erm(B), erm(TR), mef(A/E), tet(M) and tet(O)]. The susceptibility to erythromycin, josamycin, tetracycline and minocycline was tested by the agar dilution method according to the NCCLS. The strains were serotyped with type specific antisera for serotypes Ia, Ib, II, III, IV, and V.
**Results:** Among the total of 161 isolates of macrolide-resistant GBS collected from May 2002 to April 2004 in our hospital (28.6% of the total SGB isolated), 124 (78.85%) were tetracycline-resistant. The distribution of tet(M) and tet(O) among the erythromycin-resistant GBS harbouring erm(B) was (66.1% and 54.2%, repectively) and harbouring erm(TR) was (63.8% and 34.5%). The distribution of tetracycline resistance genes and serotypes among the different genotypes in 121 GBS are illustrated in the table.*NT: non typable Isolates carrying tet(M) or tet(M)+tet(O) presented the following MICs: tetracycline (MIC90 64, MIC50 32, range 16–128 mg/L); minocycline (MIC90 32, MIC50 16, range 4–32 mg/L). Isolates carrying tet(O) presented the following MICs: tetracycline (MIC90 64, MIC50 32, range 32–64 mg/L); minocycline (MIC90 16, MIC50 16, range 4–32 mg/L).
**Conclusion:** The majority (61.1%) of tetracycline-resistant GBS harboured tet(M) alone or in combination with tet(O). The most prevalent serotypes among the total of tetracycline-resistant GBS was the serotype III (38%) and the serotype II (28%). Serotype III was more prevalent among the GBS harbouring the tet(M) gene and serotype II was more prevalent among the GBS harbouring the tet(O) gene.

| Genotype | N° of isolates of serotypes | | | | | | | Total N°(%) isolates |
|---|---|---|---|---|---|---|---|---|
| | Ia | Ib | II | III | IV | V | NT* | |
| tet(M) | 13 | 5 | 9 | 20 | 1 | 8 | 3 | 59(47.9) |
| tet(O) | 3 | 5 | 14 | 9 | 0 | 3 | 1 | 35(28.9) |
| tet(M)+tet(O) | 1 | 0 | 13 | 9 | 0 | 1 | 2 | 16(13.2) |
| None/other | 0 | 2 | 2 | 8 | 0 | 0 | 0 | 12(9.9) |
| Total N°(%) isolates | 16(13.2) | 12(9.9) | 28(23.1) | 46(38.0) | 1(0.8) | 12(9.9) | 6(4.9) | 121 |

## P1598

## Prevalence, type and genetic elements involved in macrolide resistance in Viridans streptococci

M.S. Hernández Iglesias, J.L. Muñoz-Bellido, J.A. García-Rodríguez *(Salamanca, ES)*

**Objectives:** To know the prevalence of resistance to macrolides in viridans streptococci, its mechanism and the genetic elements which are involved.
**Methods:** We studied 89 viridans *Streptococcus pharyngeal* isolates from different patients. MICs for macrolides were determined by the agar dilution method. The presence of mef and ermA, ermB and ermTR genes, and the presence of MEGA (Macrolide Efflux Genetic Assembly) or Tn1207 in resistant, mef + isolates was determined by PCR with specific primers. The similarity to mef genes first described in pneumococci (mefE) and *Streptococcus pyogenes* (mefA) was determined by sequencing.
**Results:** 48 viridans streptococci isolates were resistant to macrolides (53.9%). 42 out of the 48 resistant isolates harboured mef genes (87.5%), one harboured ermB (2.1%), and 5 isolates harboured both mef and ermB genes (10.4%). No isolates harboured ermA or ermTR genes. We studied genetics elements which harbour mef genes in other streptococci, in 15 mef (+) isolates. We found MEGA insertion element in 14 of 15 isolates (93.3%), all of them harbouring mefE. The only isolate in which we found mefA, did not harbour MEGA, but Tn1207.
**Conclusions:** M phenotype is frequent in viridans streptococci, and all of them harbour mef genes. Most MLSB phenotype viridans streptococci do not harbour erm genes alone; most of them combine erm and mef genes. Most isolates contained the mef sequence corresponding to mefE, and the genetic element (MEGA) usually described in pneumococci as harbouring this gene. One isolate contained the sequence corresponding to mefA, and the genetic element usually described in *S. pyogenes* (Tn1207). The increasing presence of macrolide-resistant pneumococci harbouring MEGA element might be related with its wide presence in viridans streptococci. The acquisition of MEGA by pneumococci from viridans streptococci through transformation is being studied.

## P1599

## Characterisation of a *Streptococcus pneumoniae* strain carrying erm(A) isolated in Italy

R. Camilli, M. Del Grosso, F. Iannelli, M. Monaco, A. Pantosti *(Rome, Siena, IT)*

**Objectives:** The principal mechanism of macrolide resistance in *Streptococcus pneumoniae* in Italy is target site modification

mediated by erm(B). The erm(A) gene is common in *Streptococcus pyogenes* but rare in *S. pneumoniae*, even if recent studies have demonstrated an increased detection of this resistance determinant. Recently, a clinical *S. pneumoniae* isolate carrying erm(A) has been obtained from a patient with meningitis in Italy. The aim of this study is the molecular characterization of this isolate.
**Methods:** Antimicrobial susceptibility tests were determined by Etest. The presence of erythromycin resistance determinants was detected by PCR assays. Genotyping was performed by PFGE and MLST. The flanking regions of erm(A) were analysed by sequencing a fragment amplified by inverse PCR. Transformation and conjugation experiments were carried out. Transformants were analysed by PFGE and hybridization with an erm(A) probe.
**Results:** The isolate belonging to serotype (st) 11A, was resistant to erythromycin, inducibly resistant to clindamycin and susceptible to penicillin and tetracycline. By PCR, the only macrolide resistance determinant detected was erm(A). PFGE analysis revealed the genetic correlation of this strain with other st 11A *S. pneumoniae* Italian isolates. MLST confirmed this data since the isolate belonged to ST2003, which is a single-allele variant of ST62, the most common ST among Italian st 11A isolates. A 5200 bp DNA fragment, obtained by inverse PCR and containing the erm(A) gene, was sequenced. This fragment contains an orf upstream erm(A), the gene erm(A) identical to that described in *S. pyogenes* and 3 ORFs, downstream erm(A), one homologous to a hypothetical kinase and the other two to transposases of other Gram-positive species. In transformation experiments the gene erm(A) was transferred to an erythromycin susceptible recipient. Hybridization analysis of one transformant revealed that the size of the transferred DNA fragment was approximately 50 kb. No transconjugant was obtained in mating experiments.
**Conclusions:** This is the first Italian report of an *S. pneumoniae* isolate carrying erm(A). erm(A) appears to be contained in a genetic element that includes two transposases, although the gene is not transferable by conjugation.

## P1600

## Linezolid resistance in three clinical isolates of coagulase negative staphylococci isolated from bloodstream infections

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

**Objective:** Treatment with the first oxazolidinone antibiotic, linezolid, of infections caused by staphylococci has proved effective in most cases. In the present study we present the first three cases of linezolid resistant staphylococci in our hospital.
**Methods:** We examined three coagulase negative *Staphylococcus* strains isolated from blood cultures (Bactec, Becton Dickinson). Identification and susceptibility testing were performed by the Vitek II automated system (Biomerieux) and the results were confirmed by the API system (Biomerieux) and E-test (AB Biodisk, Solna, Sweden), according to NCCLS guidelines.
**Results:** Three linezolid resistant staphylococci were isolated from blood cultures. Identification showed that all three isolates were *Staphylococcus cohnii* subsp. urealitycum and the MIC values were 12 $\mu$g/ml (n = 1) and 24 $\mu$g/ml (n = 2) which are much higher than the value of 4 $\mu$g/ml that characterizes sensitive strains. The isolates derived from three patients in different wards of our hospital. The first two isolates were recovered from two ICU patients in April and August 2005 and the last *Staphylococcus cohnii* was isolated from a patient in the neurosurgery ward, who is still hospitalized. All patients received prolonged treatment with linezolid.
**Conclusions:** Although six linezolid resistant clinical isolates of *S. aureus* were previously reported in the literature, these three isolates are the first coagulase negative Staphylococcus isolates resistant to linezolid. It is imperative to screen for resistance to linezolid all *Staphylococci* and take the necessary precausions in order to prevent the spread of a linezolid resistant strain in other wards of our hospital.

## P1601

## Correlation between MIC and number of mutated 23S rRNA genes in oxazolidinone-resistant *Staphylococcus aureus*

S.E. North, M.J. Ellington, A.P. Johnson, D.M. Livermore, N. Woodford *(London, UK)*

**Objectives:** To determine the number of mutated 23S rDNA alleles present in clinical and laboratory-generated linezolid-resistant *Staphylococcus aureus* isolates.
**Methods:** 36 linezolid-resistant isolates were tested, 9 of them clinical isolates (MICs 16–32 mg/L) and 27 mutants selected *in-vitro* (MICs 8–64 mg/L). The mutants were raised by repeated passage on increasing linezolid concentrations and their parentage was verified by PFGE. MICs were determined by agar dilution. Genomic DNA was digested with EcoRI and hybridized with a 420 bp probe corresponding to domain V of the genes encoding 23S rRNA, to determine gene copy number. Pyrosequencing was used to quantify the proportions of wild-type and mutated alleles present; assays were designed to detect the presence of 7 mutations conferring oxazolidinone resistance. Pyrosequencing and hybridization data were combined to determine the number of mutated alleles present.
**Results:** Resistance selected *in-vitro* proved less stable than that in the clinical isolates. Pyrosequencing showed that all 9 clinical isolates had the G2576T mutation, 16 of the *in-vitro* selected mutants had G2576T, 7 had T2504C, 2 had T2500A, 1 had A2503G and 1 had G2505A. The 23S rDNA copy number in the oxazolidinone-resistant clinical isolates varied from 4–6, and from 5–9 in the laboratory-generated mutants; 14/27 laboratory-selected mutants had changes in copy number, compared with their parent strains, and 3 had changes in fragment size, but not number. The number of mutated copies in lin-resistant isolates ranged from 1–5 in laboratory-selected mutants and from 2–4 in clinical isolates. An increasing number of mutated genes correlated with increasing linezolid MIC.
**Conclusions:** In combination, pyrosequencing and hybridization successfully determined the number of mutated 23S rDNA alleles. Exposure to linezolid selected changes in 23S rRNA gene copy number as well as sequence in 50% of *in-vitro* selected mutants. There was a positive correlation between both the number and proportion of mutated 23S rDNA copies and MIC, previously unproven for *Staphylococci*.

## P1602

## Rapid emergence of resistance to linezolid during linezolid therapy of an *Enterococcus faecium* infection

J. Seedat, G. Zick, I. Klare, C. Konstabel, N. Weiler, H. Sahly *(Kiel, Wernigerode, DE)*

**Objectives:** Linezolid (LZD) is an important antibiotic for the treatment of enterococcal infections, especially when the corresponding strain possesses multiresistance including

resistance to vancomycin (VAN). We report the emergence LZD resistance in clonally related VAN-susceptible and VAN-resistant *Enterococcus faecium* isolates originated from an ICU patient only 12 days after initiation of linezolid therapy.
**Patient and Methods:** VAN-resistant *E. faecium* was repeatedly isolated from intraabdominal cultures of a 76-year-old female ICU-patient with infected necrotizing pancreatitis after pancreaticoduodenectomy (Whipplés procedure). Antibiotic susceptibility testing of the bacteria was performed by E-tests; vanA gene were detected by PCR. The possible LZD resistance mechanism (mutation in the 23S rDNA of one or more of the six 23S rRNA alleles of *E. faecium*) was examined by a PCR-based method. Molecular typing of the strains was performed by SmaI macroresticton analysis.
**Results:** VAN-resistant but LZD-sensitive *E. faecium* (VRLSE) were initially detected in intraabdominal cultures, however, already twelve days after initation of LZD therapy, VAN- and LZD-resistant *E. faecium* (VRLRE) strains were detected. Resistance to LZD was confirmed: MICs ranged from 16 to 32 mg/l. All *E. faecium* isolates showed identical or closely related PFGE patterns. Throughout the ICU period, VAN- and LZD-susceptible *E. faecium* (VSLSE) strains were repeatedly detected in the same specimens from which the VRLSE and VRLRE were isolated. Additionally, VAN-susceptible *E. faecium* isolates with resistance to LZD (VSLRE) were detected. Mutations in the 23S rDNA of three out of six alleles led to LZD resistance in the *E. faecium* isolates examined. Two weeks after termination of the LZD therapy, no LZD-resistant strain could be detected in follow-up swabs.
**Conclusions:** Resistance to LZD in *E. faecium* can occur already shortly after the initiation of LZD therapy. Assessment of antibiotic susceptibilities of all isolates at the start of therapy and regularly during the therapy is advisable, especially during therapy of severe infections. The epidemiological and clinical repercussions of resistance to LZD among enterococci cannot be predicted at this time. Attention to proper dosing and prompt removal of infected devices, when feasible, could limit occurrence and spread of LZD-resistant *E. faecium*.

## P1603

## Different prevalence of the main tet determinants among *S. sonnei* and *S. flexneri*

L. Mensa, E. Pisos, S. Capilla, F. Marco, J. Vila, J. Gascón, J. Ruiz *(Barcelona, Las Palmas de Gran Canaria, Zaragoza, ES)*

**Objectives:** To investigate the mechanisms of resistance to tetracycline in *Shigella* spp.
**Methods:** One hundred and eleven tetracycline-resistant *Shigella* spp strains (67 *S. sonnei*, 44 *S. flexneri*), 6 were isolated as a cause of enteritis in our geographical area and the remaining recovered from patients with traveller's diarrhoea. Antimicrobial susceptibility to tetracycline was determined by the Kirby-Bauer method. Presence of tetA, tetB and tetG genes was established by PCR. Sequencing of amplified products were used to corroborate the reliability of the PCR results. Maentel Haenszel test was used to establish the statistical significance.
**Results:** The statistical analysis showed that the tetA gene was more frequent in *S. sonnei* ($p < 0.05$), while tetB was more usual in *S. flexneri* ($p < 0.0005$). Although without statistical significance (p:0.07), presence of non-determined mechanisms of tetracycline-resistance seems to be more frequent among *S. sonnei*.
**Conclusions:** Species-specific differences in the distribuition of the tetA and tetB genes has been shown. Moreover 22.5% of the analysed strains did not show any of the analysed determinants of tetracycline-resistance. The concomitant presence of more than one of the analysed genes is a rare event.

| Spp. | origin | Tet determinants | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | B | G | A+B | A+G | B+G | A+B+G | none |
| S.flexneri | America | 0 | 8 | 0 | 2 | 0 | 2 | 0 | 2 |
| | Africa | 3 | 11 | 0 | 1 | 0 | 2 | 0 | 1 |
| | Asia | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 2 |
| | Non established* | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 1 |
| | TOTAL | 5 | 26 | 0 | 3 | 0 | 4 | 0 | 6 |
| S.sonnei | America | 3 | 8 | 0 | 0 | 0 | 3 | 0 | 6 |
| | Africa | 12 | 4 | 0 | 0 | 0 | 0 | 0 | 8 |
| | Asia | 9 | 1 | 2 | 0 | 1 | 0 | 0 | 3 |
| | Non established* | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 2 |
| | TOTAL | 26 | 16 | 2 | 0 | 1 | 3 | 0 | 19 |

* Authocthonous, Multiple destinations, and destination non determined

## P1604

## Distribution and genetic determinants of tetracycline resistance in *Laribacter hongkongensis* isolates from humans and fish

S.K.P. Lau, P.C.Y. Woo, M.W.S. Li, G.K.M. Wong, K.Y. Yuen *(Hong Kong, HK)*

**Objectives:** To study the distribution of tetracycline resistance and to clone and characterize a tetracycline resistance determinant in *Laribacter hongkongensis*, a recently discovered bacterial genus and species associated with community-acquired gastroenteritis.
**Methods:** Twenty-four *L. hongkongensis* strains isolated from patients with community-acquired gastroenteritis and 24 *L. hongkongensis* strains isolated from freshwater fish in Hong Kong were used in this study. Genetic determinants for tetracycline resistance were looked for by screening a genomic DNA library of *L. hongkongensis*. The prevalence of tetA gene in other strains of *L. hongkongensis* was studied by PCR using laboratory-designed primers. The presence of the tetracycline resistance determinants in plasmid was examined by Southern blot analysis.
**Results:** Among 24 human and 24 fish isolates tested, 3 human and 1 fish isolates were tetracycline-resistant. A 3566-bp gene cluster, which consists of 2 putative transposases, a tetR and a tetA gene, was cloned by inserting restriction fragments of genomic DNA from a resistant strain, HLHK5, into pBK-CMV. The 1266-bp tetA and 636-bp tetR genes shared significant nucleotide sequence homology with known tetA and tetR genes. While the flanking regions and 3' end of the tetA were identical to the corresponding regions of a tetC island in Chlamydia suis, the tetA was almost identical to that of transposon Tn1721 and plasmids found in many gram-negative bacteria, suggesting that illegitimate recombination may have occurred to produce the present tetracycline resistant determinant. Southern hybridization suggested that the tetA gene of HLHK5 was plasmid-encoded. The tetracycline resistance in *L. hongkongensis* was associated with tetA. PCR amplification of the tetA gene in the 48 isolates of *L. hongkongensis*, including HLHK5, showed the presence of tetA in all the four tetracycline resistant isolates but none of the tetracycline susceptible ones. In contrast to strain HLHK5, the tetA of two strains were identical to that of Tn1721, while that of the other strain was more closely related to other gram-negative bacteria plasmids.
**Conclusion:** Our results indicate that horizontal transfer of genes, especially through Tn1721 and related plasmids, between *L. hongkongensis* and other gram-negative bacteria is probably a frequent event and is an important mechanism for acquisition and dissemination of tetracycline resistance in *L. hongkongensis*.

## P1605

## Succesful treatment of infective endocarditis with linezolid

T. Hryniewiecki, U. Lopaciuk, J. Stepinska *(Warsaw, PL)*

**Objectives:** There is an increasing proportion of resistant strains causing infective endocarditis in recent years. It has changed the approach to choice of antibiotic therapy. Linezolid (Zyvoxid®) is a new bacteriostatic antibiotic with a wide spectrum of activity against Gram-positive organisms and with good efficacy in experimental animal models of endocarditis. Unfortunately clinical experience with linezolid in the treatment of endocarditis is limited. The aim of the study was to observe efficacy of linezolid in the treatment of infective endocarditis.
**Methods:** The study group consisted of 8 patients hospitalised in Institute of Cardiology in Warsaw (2003–2005) due to clinically resistant infective endocarditis. The diagnosis of endocarditis was established according to the Duke criteria by clinical examination, echocardiography, laboratory investigations and positive blood cultures with vancomycin MIC estimation (in 5 pts). All patients were treated surgically (valve replacement, artificial material removal) in conjunction with different conventional antibiotics and afterwards with 600 mg of linezolid every 12 hours intravenously.
**Results:** Infective endocarditis was diagnosed as caused by MRCNS in 2 pts, MSSA in 1 pt, *Enterococcus faecalis* in 1 pt and *Staphylococcus epidermidis* MR in 1 pt. Vancomycin MIC vary from 2 to 4 mg/l. In 3 pts culture-negative endocarditis was diagnosed. All patients were treated with linezolid intravenously 2 to 4 weeks (average 3,1). Clinical response and eradication of bacteremia were achieved in all patients. Leukopenia nad thrombocytopenia as an adverse reaction occurred in 1 patient.
**Conclusions** 1. Linezolid is effective in patients with Gram-positive endocarditis. 2. Linezolid could be also effective in some patients with culture-negative endocarditis. 3. Linezolid may provide an alternative in the treatment of infective endocarditis due to multi-resistant bacteria, in patients with resistant course or with adverse reaction to conventional antibiotics.

## P1606

## Linezolid for the treatment of infections caused by Gram-positive organisms in China

Y. Zhang, D. Lin, J. Wu, F. Wang, J. Zheng, J. Miao, L. Zheng, R. Sheng, X. Zhou, H. Shen, M.M. Ijzerman, R. Croos-Dabrera, W. Sheng *(Shanghai, Hangzhou, Beijing, CN; Ann Arbor, US)*

**Objectives:** To evaluate the safety and efficacy of LZD in a Chinese population.
**Methods:** This randomized, double-blind, multi-centre study was conducted in China. After obtaining written informed consent, patients from 18 to 75 years of age with pneumonia (PNEU) or skin and soft tissue infection (SSTI) known or suspected to be caused by a Gram-positive pathogen were randomized 1:1 to receive either LZD, 600 mg, or vancomycin (VAN), 1 g, each given IV q12h. Patients were to be treated for 7 to 21 days, and outcomes were assessed at end-of-treatment (EOT) and follow-up (F-U) visits.
**Results:** One hundred forty-two patients were enrolled and received study medication, 80 with PNEU and 62 with SSTI. Clinical assessments (Effective = ''cured'' plus ''marked improvement'') for patients in the fully evaluable population are summarized in the table. The most frequently isolated pathogen was *Staphylococcus aureus*: all isolates were susceptible to both study drugs. The eradication rates for all pathogens in evaluable patients at the F-U evaluation were 54/58 (93.1%) in LZD-treated patients and 42/56 (75.0%) in VAN-treated patients (p = 0.0072). All patients receiving study drug were evaluated for safety. Drug-related adverse events (AEs) were reported in 18 (25.4%) LZD-treated and 12 (16.9%) VAN-treated patients. The most commonly reported drug-related AEs in LZD-treated patients were mild abnormalities in liver function tests and leucopenia (4.2% each); rash (7.0%) was the most commonly reported AE in VAN-treated patients. Seven (9.9%) LZD-treated and 10 (14.1%) VAN-treated patients discontinued study drug because of an AE.

| 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:** Linezolid is an effective drug for the treatment of infections caused by Gram-positive pathogens and is well-tolerated.

## P1607

## Eradication in one patient, by rifamycin-linezolid, of a methicillin-resistant *Staphylococcus aureus* producing Panton-Valentine leukocidin, responsible for 7 relapses over 18 months, and decolonisation of her family by mupirocin

L. Hocqueloux, M. Niang, L. Martin, J-F. Aumaitre, J.-L. Vaur, J. Etienne, D-M. Poisson *(Orléans, Lyon, FR)*

**Background:** From October 2003 through March 2005, 9 strains of methicillin-resistant *Staphylococcus aureus* producing Panton-Valentine leukocidin (PVL-MRSA) were isolated in the hospital of Orléans, among which 4 were isolated in an Ethiopian family of 6.
**Objective:** We report the case of the mother who experienced 7 relapses over the period.
**Methods:** PVL-MRSA were isolated on routine and MRSA agars (BioRad). Antibiotypes were studied by disk diffusion method. Genetics and pulsotypes were studied by the French Centre National de Référence des Staphylocoques in Lyon (CNR).
**Results:** Mrs KYM had her 4th child on October 8, 2003 in the hospital of Orléans. She was healthy and presented no risk factor for delivery. Three weeks later she was addressed for surgical treatment of an abscess on buttocks. Cultures yielded the special antibiotype: methicillin-R, kanamycin-R and tobramycin-S, of the PVL-MRSA currently spreading across Europe and Maghreb. The CNR found the lukS-PV and lukF-PV-genes.Through March 2005, she relapsed 7 times and was treated by pyostacin for a total of 32 weeks. Two of her children were addressed for abscesses (1 buttocks, 1 thumb) yielding the same bacteria. In March 2005, Mrs Kym was addressed to the infectious diseases ward because of nasal furonculosis. Samples yielded a PVL-MRSA with MLS-B phenotype. Treatment by rifamycin-linezolid 14 d was initiated. The whole family was screened. The father and the boy, 9, who had the infected thumb 17 months earlier, were carriers. The girl, 12, who had an abscess on buttocks 8 months earlier was not. In April the whole

family accepted an attempt for decolonisation by 2% nasal mupirocin 2 or 3/d for 5 d. Cure and decolonisation were confirmed by nares and cutaneous folds samples in May and June. They missed an additional appointment in the beginning of term, but a phone call to the social worker confirmed none of them relapsed. The CNR studied 7 strains (3 from Mrs KYM 2003, 2004, 2005, 2 from children abscesses in 2003 and 2004, 2 from boy's and father's nares 2005), and confirmed that they were all identical along the period and across the family.
**Conclusion:** Short treatment with linezolid-rifamycin in the relapsing case associated with familial decolonisation by nasal mupirocin was an effective strategy to stop a time-prolonged familial outbreak of PVL-MRSA infection.

## P1608

## Multiple brain abscesses and purulent meningitis by *Listeria monocytogenes* in an otherwise healthy man. Favourable linezolid response, hampered by a suspected early drug myelotoxicity

R. Manfredi, S. Sabbatani, G. Marinacci, E. Salizzoni, F. Chiodo *(Bologna, IT)*

**Introduction:** *L. monocytogenes* CNS infection in immunocompetent adults remains rare. Meningitis is the most common CNS manifestation, with brain abscesses being <1% of overall episodes. Anecdotal episodes of CNS *L. monocytogenes* infection were reported from immunocompetent patients where both diagnosis and treatment may be hampered by low clinical suspicion and a frequent non-specific presentation. In a 15-year-long survey conducted in Dallas (US), only 4 cases of non-neonatal *L. monocytogenes* meningitis were found (estimated incidence rate: 0.3%).
**Case report:** A 50-year-old male with a negligible history and no obvious exposure to *L. monocytogenes* was hospitalized owing to dizziness. A brain CT scan showed a small, late ischemic lesion. A few days later hyperpyrexia, headache, vomiting and altered mentation occurred. The CSF study detected an elevated albumin content (217 mg/dL), low glucose (4 mg/dL) and a WBC count of 256 cells/$\mu$L (60% neutrophils) so that ceftriaxone-chloramphenicol were immediately started. Clinical-neurological conditions deteriorated while *L. monocytogenes* was cultured from the CSF so that treatment was changed towards high-dose ampicillin-gentamicin. The persistence of severe clinical-neurological conditions and altered CSF assay prompted the introduction of rifampicin-cotrimoxazole after 10 days, but 8 days later other focal neurological deficits appeared and a MRI showed small, hyperintense focal lesions involving the medulla oblongata, interpreted as multiple abscesses. The introduction of linezolid-meropenem, despite anemia (requiring 2 RBC transfusions after 7–14 days) led to a progressive clinical-CSF improvement. Our patient recovered completely and a control MRI carried out 1 month after discharge confimed the complete disappearance of the multiple brain *Listeria abscesses*.
**Discussion:** The *L. monocytogenes* meningitis and multiple subtentorial abscesses (including rare localizations at cerebellum, bulb, and pons Varolii), had an evolving cumbersome presentation. Despite the *in vitro* activity of a broad spectrum of agents, multiple therapeutic changes became necessary, until the last linezolid-meropenem combination, which was proved very effective, although it was affected by relapsing anemia probably attributable to linezolid. Linezolid, due to its elevated CSF-brain penetration, and its activity against a broad spectrum of CNS pathogens (including the intracellular *L. monocytogenes*), is expected to become a key antimicrobial compound, waiting for RCT.

## P1609

## Discrepancy between favourable *in vitro* microbiological data and a severe clinical course of a staphylococcal knee and soft tissue infection responsive to oxazolidinone linezolid only after failure of all other therapeutic attempts

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

**Introduction:** To offer therapeutic alternatives for the emerging, multiresistant, serious Gram-positive infections, novel molecules (quinupristin/dalfopristin, linezolid, daptomycin) were introduced and are made available when multiresistant Gram-positive cocci are documented as no more susceptible to all available drugs including glycopeptides. However, inezolid encompasses unique tissue penetration and diffusion features (regarding soft tissues, lungs, joints and central nervous system) which make this last drug extremely promising in all circumstances where the penetration rate into infectious foci becomes critical.
Clinical experience: A very intriguing case report of a severe, staphylococcal knee arthtiris associated to an extensive local cellulitis/fasciitis and haematogenous dissemination occurring after a surgical curettage was characterized by a complete lack of response to a prolonged vancomycin/teicoplanin plus rifampicin therapy based on the apparently favourable *in vitro* sensitivity assays of methicllin-resistant *Staphylococci*, but rapidly responded to i.v. (followed by oral) linezolid administration. The complete lack of clinical activity of a 2-week glycopeptide-rifampicin administration cannot be explained by the *in vitro* measured MIC90 values of isolated pathogens which showed complete sensitivity of *Staphylococcus aureus* against vancomycina/teicoplanin and rifampicin and susceptibility of a concurrent hematogenous *S. epidermidis* strain to glycopeptides-rifampicin. Since an abscess formation and an underlying osteomyelitis were carefully excluded by adequate instrumental examinations, from a theoretical point of view the active glycopeptide-rifampicin molecules should have been provided appropriate cure. On the other hand, from a strictly clinical issue, only a 2-week administration of i.v. linezolid followed by one more week of oral linezolid allowed to obtain a complete clinical-bacteriological cure and a complete function recovery without any sequelae after a 1.5-year follow-up.
**Conclusions:** When the management of severe, multiresistant Gram-positive infections is of concern, the *in vitro* activity of single drugs and therapeutic classes should be carefully evaluated in relation with the expected penetration and diffusion rates of these drugs into the relevant organs and tissues involved by the ongoing infectious localizations. Otherwise, apparently unexplained failures may occur also when *in vitro* studies point out a complete activity of the tested compounds.

# Epidemiology of resistance to antibiotics – II

## P1610

## Contemporary prevalence of BRO beta-lactamases in *M. catarrhalis*: report from the SENTRY Antimicrobial Surveillance Program (USA; 1997–2004)

L. Deshpande, H. Sader, R. Jones *(North Liberty, US)*

**Objectives:** To evaluate the prevalence of BRO-1 and BRO-2 among B-lactamase (Bl)-producing *M. catarrhalis* (MCAT) in the USA. Although the Bl-mediated penicillin (PEN) resistance (R) in MCAT has been stable at 95%, the BRO-1 and -2 occurrence has not been determined in USA isolates since 1998. BRO-2 rates have been reported at <15 (1980s), 4.8 (1984–94), 2.1 (1994–95) and 3.1% (1997–98).

**Methods:** Community-acquired MCAT isolates (SENTRY Program 1997–2004) were tested by CLSI broth microdilution methods including: 7,860 worldwide and 3,671 in North America (NA). BRO-1 and -2 was detected by PCR methods (Levy and Walker; 2004), compared to epidemiologic tests, and MIC values. 100 B-lactamase-positive (BL+) MCAT samples per year from USA (39 sites) and Canada (CA; 7 sites) were tested for the odd-numbered years.

**Results:** The BRO-2 rate was 4, 4, 3, and 3% for 1997, 1999, 2001 and 2003, respectively; rates in CA (8 isolates) > USA (6). Several agents remained active: amoxicillin/clavulanate (MIC90, ≤0.25 mg/L), ceftriaxone (CTRI; 0.5), cefuroxime (2), erythromycin (≤0.25–0.5), levofloxacin (≤0.03–0.06), tetracyclines (2) and trimethoprim/sulfamethoxazole (TMP/SMX; ≤0.5/9.5). PEN MIC distribution was tri-modal (≤0.03, 1–2, >4 mg/L) and CTRI bi-modal (0.016, 0.5), yet BRO-1 and -2 MIC/zone distributions overlap (best discriminated by methicillin (mean zone, 10.6 vs. 19.4 mm) and PEN (13.9 vs. 24.1) disks). Possible BRO-2 epidemic clusters could not be excluded due to a very common ribotype in 3 centres (CA, 2 sites; USA, 1).

$MIC_{50}$ (mg/L) for:

| Antimicrobial agent | Worldwide (7,860) | NA (3,671) | PCR sample (400) |
|---|---|---|---|
| PEN | >4 | >4 | >4 |
| Amoxicillin/Clavulanate | <=0.25 | <=0.25 | <=0.25 |
| CTRI | 0.5 | 0.5 | 0.5 |
| Cefuroxime | 2 | 2 | 2 |
| Erythromycin | <=0.25 | 0.5 | 0.5 |
| Levofloxacin | 0.06 | <=0.03 | 0.06 |
| Tetracycline | <=2 | <=2 | <=2 |
| TMP/SMX | <=0.5 | <=0.5 | <=0.5 |

**Conclusions:** This BRO-1 and -2 enzymes NA prevalence update in MCAT isolates (1997–2003) shows stability at 96–97% and 3–4%, respectively. Phenotypic tests (zones or MICs) cannot easily distinguish between these B-lactamase types, necessitating the use of molecular applications.

## P1611

## Penicillin susceptibility of beta haemolytic streptococci: a 6-year surveillance

O. Karatuna, S. Karavelioglu, G. Soyletir *(Istanbul, TR)*

**Objective:** Although resistance to penicillin in beta haemolytic streptococci has not been reported yet, increasing resistance rates for alternative drugs, such as erythromycin, clindamycin or tetracycline is an emerging concern which brings the necessity to carefully monitor penicillin susceptibility.

**Materials and Methods:** In order to detect any changes in penicillin MICs, we performed antimicrobial susceptibility testing for all isolated beta haemolytic streptococci in our hospital between January 2000 and November 2005. Identification to serogroup level was done using a commercial latex agglutination kit (Avipath Strep, Omega Diagnostics Ltd., Scotland, United Kingdom).

**Results:** A total of 1890 isolates were identified, distribution of groups for serogroup A, B, C and G were 75.6%, 18.9%, 2.8% and 2.7%, correspondingly. Penicillin susceptibility was determined using Etest (AB BIODISK Solna, Sweden) strips according to manufacturers' instructions. When results are evaluated in 2-year periods, MIC90 increased from 0.012 to 0.016 mg/ml for group A, from 0.064 to 0.094 mg/ml for group B, from 0.012 to 0.064 mg/ml for group C and from 0.016 to 0.023 mg/ml for group G (Table).

Table. Periodical distribution of penicillin MICs against beta hemolytic streptococci between 2000-2005

| | PERIODS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Serogroups | 2000-2005 | | 2000-2001 | | 2002-2003 | | 2004-2005 | |
| A, B, C, G | n = 1890 | | n = 676 | | n = 577 | | n = 637 | |
| | MIC 50 | 0.012 | MIC 50 | 0.008 | MIC 50 | 0.012 | MIC 50 | 0.012 |
| | MIC 90 | 0.047 | MIC 90 | 0.032 | MIC 90 | 0.064 | MIC 90 | 0.064 |
| A | n = 1428 | | n = 565 | | n = 388 | | n = 475 | |
| | MIC 50 | 0.008 | MIC 50 | 0.008 | MIC 50 | 0.008 | MIC 50 | 0.012 |
| | MIC 90 | 0.016 | MIC 90 | 0.012 | MIC 90 | 0.016 | MIC 90 | 0.016 |
| B | n = 358 | | n = 79 | | n = 141 | | n = 138 | |
| | MIC 50 | 0.047 | MIC 50 | 0.047 | MIC 50 | 0.064 | MIC 50 | 0.064 |
| | MIC 90 | 0.094 | MIC 90 | 0.064 | MIC 90 | 0.064 | MIC 90 | 0.094 |
| C | n = 53 | | n = 11 | | n = 32 | | n = 10 | |
| | MIC 50 | 0.016 | MIC 50 | 0.012 | MIC 50 | 0.016 | MIC 50 | 0.023 |
| | MIC 90 | 0.064 | MIC 90 | 0.012 | MIC 90 | 0.047 | MIC 90 | 0.064 |
| G | n = 51 | | n = 21 | | n = 16 | | n = 14 | |
| | MIC 50 | 0.016 | MIC 50 | 0.012 | MIC 50 | 0.016 | MIC 50 | 0.016 |
| | MIC 90 | 0.023 | MIC 90 | 0.016 | MIC 90 | 0.023 | MIC 90 | 0.023 |

**Conclusions:** Even though highest MIC90 values were to be found in group B (0.094 mg/ml), our results indicate the steady increase in penicillin MIC for all serogroups. Three group A and six group B isolates with penicillin MIC of 0.125 mg/ml, reaching susceptibility breakpoint concentration according to CLSI, and also highly elevated MIC90 concentrations for group B streptococci may be messengers of possible forthcoming resistant strains.

## P1612

## Trends in macrolide resistance among pneumococcal carriage strains in French day-care centres: 1999 to 2004

H. Carsenti-Dellamonica, M. Roussel-Delvallez, B. Dunais, C. Pradier, P. Bruno, P. Touboul, C. Laurans, E. Bonnet, P. Dellamonica *(Nice, Lille, Paris, FR)*

**Objective:** To study trends in macrolide resistance rates among *S. pneumoniae* isolated from children aged 3 to 40 months attending day-care centres in France following implementation of prudent antibiotic use campaigns (Alpes Maritimes 2001, 2003, France 2003) and pneumococcal conjugate vaccine (PCV) (2003).

**Method:** Nasopharyngeal aspirates were obtained from a random 2-stage cluster sample of children attending day-care centres in the Nord (N) and Alpes Maritimes (AM) areas during 3 consecutive surveys between January and March 1999, 2002 and 2004. Susceptibility to erythromycin and clindamycin and

resistance phenotype were analysed by disk diffusion method. Serotypes were determined using the quelling reaction. Pneumococcal immunization status and antibiotic prescriptions over the 3 previous months were recorded.
**Results:** SP was isolated from 278/548, 289/534 and 269/567 children in 1999, 2002 and 2004, respectively ($p < 10^{-3}$). Resistance to macrolides declined overall from 77.0% to 64.3% of strains between 1999 and 2004 ($p < 10^{-2}$). Among erythromycin-resistant (E-R) isolates, percentage of erm-B phenotype increased from 81.0% to 90.2% ($p = 0.001$). While the proportion of penicillin non-susceptible strains declined from 33.6% to 24.7% of SP isolates ($p < 10^{-3}$), erythromycin resistance remained stable among these strains at 92.0%. Overall proportion of treated children fell from 64.3% to 48.7% ($p < 10^{-3}$) between 1999 and 2004; in AM this reduction was observed in 2002 (16.0%; $p < 10^{-3}$), while in N it occurred in 2004 (24%; $p < 10^{-3}$) and the percentage of macrolides among prescriptions fell from 13.0% to 9.0% ($\chi^2$ for trend: $p = 0.06$). Serotype distribution showed most E-R isolates were 6B, 14, 19F and 23F. A 50% reduction in serotype 23F was observed in AM in 2002 and in N in 2004. Immunisation with PCV concerned at least 33.5% of children in 2004.
**Conclusion:** Macrolide resistance has followed a parallel decline with penicillin resistance as a result of antibiotic-prescription reducing campaigns and pneumococcal immunization against the most prevalent macrolide-resistant serotypes.

## P1613

## Macrolide-resistant *Streptococcus pneumoniae* in Slovenia 1999–2004: correlation with macrolide use

M. Cizman, S. Zad-Pecar, M. Paragi *for the Slovenian Meningitis Study Group*

**Objective:** To evaluate the prevalence of resistance of invasive strains of *S. pneumoniae* to erythromycin after decline in macrolide consumption.
**Methods:** The number of packages of antibiotics was obtained from the Institute of Public Health of Slovenia. For the period 1999–2004 the data on outpatient antibiotic consumption were collected using the ATC/DDD classification (WHO version 2005) and the results were expressed in DDD/1000 inhabitants per day (DID). All invasive strains of *S. pneumoniae* isolated from sterile body fluids in all Slovenian hospitals were included in the study. Susceptibility testing was performed using NCCLS approved disk diffusion test.
**Results:** During 1999–2004 the total use of antibacterials in Slovenia decreased for 17.4% from 20.23 DID to 16.71 DID. The consumption of macrolides which constituted 17.1–19.4% of total use of antibacterials decreased for 21.3% (3.81 to 3.0 DID). Short-acting (erythromycin, miocamycin), intermediate-acting (midecamycin, roxithromycin, clarithromycin), and long-acting (azithromycin) decreased for 33.4%, 25.8% and 13% respectively. In all years the use of intermediate-acting macrolides was the most prescribed subclass of macrolides corresponding for 1.50–2.21 DID, followed by long-acting (1.25–1.47 DID) and short-acting (0.07–0.12 DID). The resistance of *S. pneumoniae* strains to erythromycin increased from 5.4% (6/110) to 11.2% (19/170); in children from 2.9% (1/34) to 18.1% (6/33) and in adults from 6.5% (5/76) to 9.5% (13/137) respectively. Rates of the isolates resistant to erythromycin and at least 2 of the following agents: penicillin, tetracycline, TMP/SMX, chloramphenicol increased from 0.9% (1/110) to 7.7% (13/169); in children from 0% (0/34) to 15.1% (5/33) and in adults from 1.3% (1/76) to 5.8% (8/137) respectively.
**Conclusion:** Despite a reduction of macrolide consumption in outpatients the resistance of invasive strains of *S. pneumoniae* was increasing during the observation period especially in children.

## P1614

## Multiple drug resistance explains best the changes in *S. pneumoniae* resistance in a ten-year surveillance study in Belgium

J. Van Eldere, R.M. Mera, L.A. Miller, J. Verhaegen, J.A. Poupard *(Leuven, BE; New Orleans, Upper Providence, US; Philadelphia, US)*

**Objective:** Belgium is located between countries with very high and very low antibiotic resistance rates. Modeling how resistance changes over time and place in Belgium provides insights into correlates of *S. pneumoniae* resistance at the population level.
**Methods:** Surveillance data consists of 14,488 *S. pneumoniae* invasive isolates from 1994–2004, identified by postal code as well as clinical and demographic information. Antimicrobial consumption (IMS Health Services) is expressed in defined daily doses (DDD) per 1000 inhabitants per day. Changes in resistance by month and postal code were evaluated using mixed effects models for repeated measures, using mathematical models of transmission for the curve shape, and taking into account seasonality. Resistance to penicillin, erythromycin, tetracycline, and ofloxacin was considered in the analysis.
**Results:** Resistance to penicillins, macrolides and tetracyclines peaked in the year 2000, and their levels in 2004 were 11.6%, 35.6% and 28.7% respectively. The shape of the curves is similar for most of the antibiotics studied, with a steep rise from 1994 to 2000 and a plateau thereafter. Resistance to two or more antibiotic classes corresponded to 72% of all resistant isolates and in a multivariate model explains most of the variability through time and place of the antibiotics studied. Resistance to only one antibiotic (any) decreased from 16.3% in 1994 to 11.7% in 2004, while resistance to two or more increased 2.5 times (95% CI 1.95–3.12, $p < 0.0001$) from 14.1% in 1994 to 28.8% of all isolates 10 years later. More than nine out of ten isolates that were macrolide or tetracycline resistant were also multiply resistant (MR). MR increases 0.57% for each DDD of overall cumulative antimicrobial consumption, and out of all antibiotic classes, macrolides and broad-spectrum penicillins are most associated with resistance.
**Conclusion:** Resistance to two or more antibiotics is the most important factor in understanding the changes over time for all studied antibiotic classes in Belgium. The cumulative impact of antimicrobial exposure of separate antibiotic classes at the population level facilitates the survival and transmission of any isolate that is resistant to two or more antibiotic classes.

## P1615

## Molecular mechanisms of antimicrobial resistance in *Salmonella typhimurium* isolates, causing bacteraemia in children under 5 years of age in southern Mozambique

J. Ruiz, I. Mandomando, P. Esquivel, J. Vila, J. Gascon, P.L. Alonso *(Barcelona, ES; Manhiça, MZ; Resistencia, AR)*

**Objectives:** To analyse the molecular mechanisms of resistance to 7 antimicrobial agents in 85 *Salmonella typhimurium* causing bacteremia in children under 5 years of age in Mozambique.

**Methods:** The isolates were identified by biochemical tests and specific serotyping. Antimicrobial susceptibility to ampicilllin (Amp), amoxicillin plus clavulanic acid (AUC), cloramphenicol (Cm), gentamicin (Gm), cotrimoxazole (Sxt), nalidíxic acid (Nal) and tetracycline (Tc) were established by the method of Kirby Bauer. The presence of beta-lactamases encoding genes (tem, carb, oxa2-like) as well as the tetA, tetB, tetC, tetG, cmlA and floR genes, and integrons type 1 was established by PCR, while the presence of plasmid-mediated DHFR was determined by PCR-RFLP and the CAT activity by a colorimetric assay.
**Results:** Seven different resistance patterns were identified: I. Susceptible (38 strains); II. Amp, Sxt, Gm, A/C (37); III. Amp, Tc, Cm (2); IV. Amp, Tc, Sxt, Cm (2); V. Amp, A/C (4); VI. Amp, Sxt, A/C (1); VII (1). – Sxt. No isolate resistant to nalidixic acid was detected. Resistance to beta-lactam agents was due to the presence of beta-lactamases type TEM-like (pattern V), CARB-2 (III) and TEM-like plus OXA-30 (II, V, VI). Meanwhile resistance to cloranphenicol and tetracycline was associated to CAT activity (III, IV) and floR (III), and tetB (IV) and tetG (III) respectively. No mechanism of cotrimoxazole resistance was detected in the isolates of the patterns II, VI and VII, while dfrA1 was detected in the isolates of the group IV. Resistance to Gm was associated to the presence of the gene aadB, detected in the analysis of integrons type 1. Type 1 integrons were detected in isolates belonging toi the pattern II (750 bp – aadB; 2000 bp – oxa30, aadA1), III (900 bp – carb2, 1100 – aadA1), IV (1500 bp – dfrA1, aadA1), V and VI (2000 bp – oxa30, aadA1).
**Conclusions:** A great diversity of resistance mechanisms has been detected. Those mechanisms might spread among microorganisms resulting in a serious health problem due to the limited number of antibiotic treatments available in the area.

## P1616

## Small outbreaks of VEB-1 ESBL producing *Acinetobacter baumannii* in Belgian nursing homes and hospitals through cross-border transfer of patients from northern France

B. Jans, C. Suetens, Y. De Gheldre, E. Leens, P. Bogaerts, B. Coignard, Y. Glupczynski *(Brussels, Yvoir, BE; Saint-Maurice, FR)*

**Objectives:** Following the national outbreak of multidrug-resistant (MDR) *Acinetobacter baumannii* (Ab) producing a VEB-1 extended-spectrum beta-lactamase (ESBL) which occurred in France in 2003–2004, the French Institut de Veille Sanitaire (InVS) alerted the Belgian sanitary authorities. A national surveillance was set up by the Scientific Institute of Public Health and by the reference laboratory to investigate the possible spread of the French epidemic VEB-1 ESBL-producing Ab in Belgian hospitals.
**Methods:** From 01/04 to 03/05, all Belgian acute hospitals were invited to report cases of nosocomial infections/colonisations due to MDR Ab isolates presenting a resistance profile similar to the French epidemic strain (resistance to all agents except carbapenems and colistin) and to send such isolates to the reference laboratory for phenotypic confirmation and for genotypic characterization (PCR of VEB-1 and class 1 Integron, PFGE typing). Guidelines for detection of the epidemic strain, screening for carriage in patients transferred from hospitals or nursing homes (NH) close to the French border as well as infection control measures were sent to all hospitals.
**Results:** Overall 73 Ab strains from 30 hospitals were sent to the reference laboratory. Only, 26 of these fulfilled the phenotypic resistance patterns and 6 were definitely confirmed as VEB-1 Ab and had a PFGE pattern identical to the French epidemic clone. Two mini-outbreak clusters (each involving 3 cases) were documented in hospitals from two cities (Tournai and Chimay) closed to the French border. Two patients died from their infection. In the first outbreak, all 3 patients were residents who lived in the same NH. Two of them were French citizens who had been hospitalised in different acute care hospitals in the North of France within the last year. In the second outbreak, the index case had also been previously hospitalised in a French hospital. Secondary transmission to two other hospitalised patients occurred in this outbreak.
**Conclusion:** Despite the large extension of the VEB-1 Ab outbreak in France no similar problem occurred in Belgium. However, this national alert allowed to detect two small outbreaks in Belgian institutions located close to the French border. In both outbreaks the epidemic strain was imported from France through patient circuits. This study illustrates that transfers between acute care hospitals and NH may explain cross-border spread of multi-resistant epidemic strains.

## P1617

## Types of extended-spectrum beta-lactamases in *Salmonella* spp. and decreased susceptibility to fluoroquinolones

S. Ercis, Z. Gulay, D. Gur, B. Erdem, G. Hascelik, A. Tunger, S. Gedikoglu, I. Tuncer, B. Sumerkan *(Ankara, Izmir, Bursa, Konya, Kayseri, TR)*

**Objectives:** The aim of this study was to determine the rate of ESBL production in clinical isolates of *Salmonella* spp. and to detect decreased susceptibility to fluoroquinolones in ESBL positive isolates in Turkey.
**Methods:** A total of 620 *Salmonella* spp. isolated from clinical samples from thirteen centres between 2000 and 2002 were included in the study. *In vitro* susceptibility to ampicillin, amoxicillin/clavulanic acid, cefotaxime, gentamicin, chloramphenicol, tetracycline, trimethoprim/sulfamethoxazole and ciprofloxacin were determined using the agar dilution method on Mueller-Hinton agar following the Clinical and Laboratory Standards Institute (CLSI) guidelines. Decreased susceptibility to ciprofloxacin was defined as an MIC of 0.125–1 mg/L. *Salmonella* isolates were screened for ESBL production by double disk synergy method using amoxicillin/clavulanic acid, cefotaxime and ceftazidime disks. Types of ESBL enzymes were analysed by PCR for TEM, CTX-M, SHV and PER-1 genes.
**Results:** In *Salmonella* spp. the highest level of resistance was observed against ampicillin (41.3%) followed by chloramphenicol (36.6%), tetracycline (34.2%) amoxicillin/clavulanic acid (32.4%), trimethoprim/sulfamethoxazole (2.3%), gentamicin (1.6%), and cefotaxime (0.8%). Ciprofloxacin resistance was observed in one isolate (0.2%). Among 620 *Salmonella* isolates, 6 (0.97%) were shown to produce ESBL by double disk synergy testing. These isolates were *Salmonella typhimurium* (n = 2), serogroup C1 (n = 2) and *Salmonella enteritidis* (n = 2). Three isolates were from fecal samples two were from urine and one was from blood. One of the ESBL producing isolates were susceptible to cefotaxime *in vitro*. Two isolates showed decreased susceptibility to ciprofloxacin. All the ESBL producers were resistant to ampicillin, amoxicillin/clavulanic acid, chloramphenicol and harbored CTX-M type enzymes. In three isolates a TEM-type enzyme was also present.
**Conclusion:** Albeit being rare, ESBL production is an important resistance factor among *Salmonella* spp. In order to prevent treatment failures, decreased susceptibility to fluoroquinolones should be investigated routinely in invasive isolates as well as ESBL production.


ISSN: 1470-9465

## P1618

### Incidence of faecal carriage of ESBL-producing *Enterobacteriaceae* in hospital and community patients during two non-outbreak periods of time

F.J. Castillo, M. Pardos, C. Seral, I. Millan, C. Pitart, M.E. Llaneza, M.C. Rubio-Calvo *(Zaragoza, ES)*

**Objectives:** Determine the incidence of faecal carriage of ESBL-producing *Enterobacteriaceae* in the stools of hospitalized patients and in outpatients submitted to our laboratory for stool culture between April 2002–February 2003 and August 2004–July 2005.
**Methods:** A total of 14748 faecal samples were analysed from 9384 patients (26.9% of whom were ambulatory) prospectively collected between April 2002–February 2003 and August 2004–July 2005. Sampling was carried out during two non-outbreak periods from patients submitted to our laboratory for stool culture. The stool samples were cultured by standard methods. *Enterobacteriaceae* growing in modified CCDA (Oxoid) containing cefoperazone 32 mg/L were identified and screened for extended spectrum beta-lactamase (ESBL) production by the double-disk sinergy test. Confirmation was performed by E-test with cefotaxime/cefotaxime-clavulanic acid, ceftazidime/ceftazidime-clavulanic acid and cefepime/cefepime-clavulanic acid.
**Results:** The cefoperazone-containing medium routinely used in our laboratory for the isolation of Campylobacter occasionally (16.9%) yielded strains of the family *Enterobacteriaceae*, some of them ESBL-producing strains. The rates of patient faecal carriage of ESBL-producing enterobacteria increased in our area from 2.2% (103 of 4676 patients) in 2002 to 7.4% (349 of 4708 patients) in 2004 ($p < 0.001$). When hospitalized and ambulatory patients were considered separately, the rates were 2.5% (29 of 1148) and 2.1% (74 of 3528), respectively, in 2002 and 8.1% (112 of 1375) and 7.2% (237 of 3296), respectively, in 2004. The identities of the ESBL-producing isolates recovered during 2002 were: *E. coli* (n = 98), *K. pneumoniae* (n = 2), *P. vulgaris* (n = 2) and *E. cloacae* (n = 1), and isolates recovered during 2004 were: *E. coli* (n = 340), *K. pneumoniae* (n = 3), *K. oxytoca* (n = 1), *P. vulgaris* (n = 1), *P. mirabilis* (n = 2), *E. cloacae* (n = 1) and *E. aerogenes* (n = 1).
**Conclusions:** A dramatic, significant increase in the frequency of faecal carriage of ESBL-producing isolates was demonstrated in 2004 among hospitalized (8.1%) and ambulatory patients (7.2%).The results revealed that the prevalence of faecal carriage among ambulatory patients and hospitalized patients was not significantly different in both periods of time. Outpatients came from the community carrying enterobacteria harbouring ESBL in the intestinal tract, suggesting that the community could be a reservoir for these microorganisms and enzymes.

## P1619

### ESBLs in *Enterobacteriaceae* from university hospitals. APUA-Bulgaria Chapter study

E. Keuleyan, R. Markovska, I. Schneider, M. Sredkova, P. Sotirova, D. Ivanova, A. Bauernfeind *(Sofia, BG; Munich, DE; Pleven, Stara Zagora, BG)*

**Objectives:** To characterize the type of ESBLs and the epidemiology of infections caused by ESBL producing *Enterobacteriaceae* strains, isolated in 3-month period (end 1999/beginning 2000) during APUA Bulgaria Chapter Antimicrobial Resistance Surveillance Study (ARSS).
**Methods:** A total of 71 *K. pneumoniae*, 3 *K. oxytoca*, 11 *E. coli*, 4 *C. freundii*, 3 *S. marcescens*, and 3 *E. cloacae* from 6 university hospitals, isolated from blood, wound, urine, sputum and other clinically significant specimens were proven to produce ESBLs. Antimicrobial susceptibility was determined according to CLSI, 2002; conjugation on a solid medium was performed; isoelectric focusing was followed by bioassay; PCR with beta-lactamase group-specific oligonucleotides was applied, followed by nucleotide sequencing; RAPD with ERIC-1A and ERIC 2 primers was performed.
**Results:** MIC of Ceftazidime varied from 4 to >512 mg/L, MIC of Cefotaxime – 2–512 mg/L; the addition of sulbactam 1: 1 reduced MIC > 3-fold. Transconjugants exhibited resistance both to extended-spectrum cephalosporins and aminoglycosides in 37 of 39 strains. According to their pI, two clusters of beta-lactamase producers could be described: first one – ESBLs focussed at pI 8.2, and the second – pI at 6.3. Results from PCR confirmed the presence of two groups ESBLs: TEM and SHV. Sequencing of representative strains showed the presence of SHV-12 in two participating hospitals and of SHV-5 in only one strain *E. cloacae*, while TEM-3 like enzyme was found in 2 centres and had a clonal dissemination.
**Conclusions:** The data from APUA ARSS revealed that during 1999–2000 ESBLs were emerging in Bulgaria: SHV-12, SHV-5 and TEM-3 like enzymes were identified; SHV-group predominated. These results indicate the spread of ESBLs producing *Enterobacteriaceae* in Bulgaria and the need for stronger attention in microbiological diagnostics, antibiotic therapy and infection control.

## P1620

### Emergence of multidrug-resistant Gram-negative bacteria during selective decontamination of the digestive tract on an intensive care unit

N. al Naiemi, E.R. Heddema, A. Bart, E. de Jonge, C.M. Vandenbroucke-Grauls, P.H.M. Savelkoul, B. Duim *(Amsterdam,NL)*

**Objectives:** During treatment with selective decontamination of the digestive tract (SDD), four strains of multidrug-resistant (MDR) Gram-negative bacteria (three *Escherichia coli* strains and one *Klebsiella pneumoniae*) were isolated at the Intensive Care Unit (ICU) in the Academic Medical Center (AMC) in Amsterdam. These isolates were extended spectrum beta-lactamase (ESBL) positive. We investigated whether this was due to interspecies transfer of resistance genes.
**Methods:** The strains were typed by amplified fragment length polymorphism analysis. The plasmids from these strains were characterized by restriction fragment length polymorphism. Resistance genes of the MDR-strains were characterized by PCR and sequence analysis.
**Results:** AFLP analysis confirmed that the three MDR *E. coli* isolates represented three different strains. The MDR-strains were shown to harbour the same plasmid with identical extended-spectrum â-lactamase (ESBL) genes; CTX-M-15 and SHV-5.
**Conclusions:** Identification of the emergence of such MDR Gram-negative bacteria and recognition of resistance plasmid transfer during SDD treatment is crucial for optimal application of this regimen in ICUs. The use of the third generation cephalosporins in SDD may associate with emergence and increase in the prevalence of ESBLs. Therefore, for optimal screening of resistance to cephalosporins in ICUs, the screening for ESBLs should be included.

## P1621

## Therapy-driven selection of carbapenem resistance in CTX-M-producing *Klebsiella pneumoniae*

D.G. Pillay, P. De, R. Merard, J.W.T. Dallow, M.E. Kaufmann, R.L.R. Hill, D.M. Livermore, N. Woodford *(Sutton Coldfield, London, UK)*

**Objective:** Carbapenems are the drugs of choice for the treatment of serious infections caused by ESBL-producing *Enterobacteriaceae* and the emergence of carbapenem resistance is rarely documented. We investigated pairs of carbapenem-susceptible and resistant *K. pneumoniae* isolates from three patients, collected before and after therapy with carbapenems.

**Methods:** Pre- and post-therapy pairs of ESBL-producing *K. pneumoniae* isolates were from three patients with urinary catheter-associated infections who were treated with ertapenem (ERP, 2 cases) or meropenem (MEM, one case) in a district general hospital with a low incidence of ESBL-producing organisms (0.43/1000 bed days), and meropenem use of 1160 DDD/year. Isolates were compared by PFGE of XbaI-digested genomic DNA. MICs were determined and interpreted by British Society for Antimicrobial Chemotherapy methodology. blaCTX-M alleles were sought by multiplex PCR. Outer membrane proteins (OMPs) were extracted, and analysed by SDS-PAGE.

**Results:** The three patients relapsed following ERP or MEM therapy, and the post-therapy isolates from repeat urine samples were resistant (table), with MICs ERP>MEM>IPM. All six isolates from the three patients belonged to the same PFGE strain, but transmission of the resistant variants is unlikely as the patients were geographically and temporally unrelated and separate selection of resistance in individual patients seems more likely. All isolates had a group 1 CTX-M ESBL; the resistant isolate in each pair had lost a major OMP, consistent with a porin, compared with its susceptible 'parent'. All three patients were successfully treated with amikacin.

| Patient | Age | Antibiotic treatment (days) | Days post-treatment to relapse | Pre-treatment MIC (mg/L) | | | Post treatment MIC (mg/L) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | ERP | MEM | IPM | ERP | MEM | IPM |
| 1 | 85 | ERP (7) | 120 | < =0.125 | < =0.06 | 0.5 | >16 | 8 | 2 |
| 2 | 80 | ERP(5) | 7 | < =0.125 | < =0.06 | 0.5 | 8 | 2 | 1 |
| 3 | 89 | MEM (9) | 12 | < =0.125 | < =0.06 | 1 | >16 | 4 | 2 |

**Conclusion:** The emergence of carbapenem resistance in CTX-M-producing *K. pneumoniae* following therapy severely limits treatment options. Whilst unusual in general, such selection has occurred repeatedly with this strain.

## P1622

## Wide geographic spread of diverse acquired AmpC beta-lactamases in *Escherichia coli* and *Klebsiella* spp. in the UK and Ireland

N. Woodford, E.J. Fagan, R. Hope, K.L. Hopkins, M.E. Kaufmann, J.O. Kistler, M.F.I. Palepou, R. Pike, M.E. Ward, D.M. Livermore *(London, UK)*

**Objective:** To determine the distribution of genes encoding acquired AmpC beta-lactamases in cephalosporin-resistant isolates of *E. coli* and *Klebsiella* spp. submitted to the UK national reference laboratory.

**Methods:** MICs were determined by agar dilution and interpreted according to breakpoints of the British Society for Antimicrobial Chemotherapy. Isolates of *E. coli* or *Klebsiella* spp. resistant to cefotaxime and ceftazidime, irrespective of addition of clavulanic acid, were inferred to have possible AmpC-mediated resistance. Genes encoding six phylogenetic groups of acquired AmpC enzymes were sought with a multiplex PCR assay (Perez-Perez & Hanson. J Clin Microbiol 2002;40:2153–62). Selected isolates were compared by PFGE, and selected blaAmpC amplicons were sequenced.

**Results:** 47 *E. coli* isolates and 8 *Klebsiella* spp. from separate patients yielded PCR amplicons indicating the presence of genes encoding acquired AmpC enzymes. Forty of these *E. coli* isolates (from 20 hospitals) produced CIT-type enzymes, 6 (from 3 Irish hospitals) produced ACC types, and 1 a DHA type. The *Klebsiella* spp. produced ACC (5 isolates from 2 Irish hospitals), FOX (2 isolates from 2 Welsh hospitals) or DHA (1 Irish isolate) enzymes. Genes encoding EBC-/ENT- and MOX-type enzymes were not detected. Twelve *E. coli* isolates from one hospital all produced a CIT-type enzyme; these 12 isolates belonged to an epidemic UK strain, designated strain A; 8 isolates also contained blaCTX-M-15 linked to an upstream copy of IS26, as is characteristic of strain A; 4 isolates lacked blaCTX-M-15. Sequencing of a representative blaAmpC amplicon indicated production of a novel CMY-2 variant in these isolates.

**Conclusions:** Diverse acquired AmpC enzymes are present in *E. coli* and *Klebsiella* spp. in the UK and Ireland, with CIT-types the most common, and ACC types linked to Ireland. The broad resistance profiles of AmpC enzymes compromises patient management. Hence, the acquisition of a CMY-2-like enzyme by epidemic *E. coli* strain A suggests that acquired AmpC enzymes are poised to become an important public health issue in the UK.

## P1623

## Doripenem European surveillance: antimicrobial activity against 6480 contemporary pathogens (2004)

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

**Objective:** To characterize the spectrum of activity and potency of DOR (formerly S-4661) and comparator agents against contemporary wild-type bacterial isolates from medical centres in Europe and the Middle East in 2004. DOR is a novel parenteral 1-B-methyl carbapenem in late stage clinical development whose molecular structure confers stability to B-lactamases and resistance (R) to renal dehydropeptidases.

**Methods:** The collection included 6480 non-duplicate, consecutive clinical isolates from patients in 24 medical centres in Europe (21), Turkey (2) and Israel (1) that were submitted to the DOR surveillance program (2004) for identification confirmation and susceptibility (S) testing. MIC values for >30 antimicrobials were determined using NCCLS broth microdilution methods (2003). A tentative DOR susceptible (S) breakpoint of ≤4 mg/L (≤0.25 mg/L for *S. pneumoniae*) was used for comparative purposes; CLSI (2005) criteria were used for other tested agents.

**Results:** Antimicrobial activities of DOR and other carbapenems vs. selected isolates. DOR consistently displayed activity against staphylococci and streptococci (MIC90, 0.06 and 0.5 mg/L) most similar to that of imipenem, and against *E. coli* and *Klebsiella* spp. (MIC90, 0.06 and 0.5 mg/L, respectively, including 8.7 and 26.9% of strains that met ESBL screening criteria), most similar to that of meropenem. *Enterobacter* spp. isolates, including 35.8% that were ceftazidime-R (indicative of AmpC production), were also highly S to DOR and other carbapenems (0.5 to 1.4% R). DOR also provided slightly enhanced coverage against *P. aeruginosa* (82.7% S) and

*Acinetobacter* spp. (54.4% S) compared to other carbapenems. Carbapenem R among these latter strains is, however, a particularly worrisome development.

$MIC_{90}$ mg/L (% S)

| Organism (no. tested) | DOR | Meropenem | Imipenem |
|---|---|---|---|
| *S. aureus* Oxacillin-S (1,231) | 0.06 (100) | 0.12 (100) | ≤ 0.12 (100) |
| CoNS Oxacillin-S (528) | 0.06 (99.1) | 0.12 (100) | ≤ 0.12 (100) |
| *S. pneumoniae* (603) | 0.5 (82.4) | 0.5 (81.8) | 0.25 (82.9)* |
| *H. influenzae* (459) | 0.25 (100) | 0.06 (100) | 1(100) |
| *Enterobacter* spp. (215) | 0.25 (98.1) | 0.12 (98.1) | 1 (97.2) |
| *P. aeruginosa* (491) | 16 (82.7) | >8 (76.4) | >8 (72.7) |
| *Acinetobacter* spp. (149) | 16 (54.4) | >8 (49.0) | >8 (51.0) |

* Results for 70 isolates only

**Conclusions:** DOR is a new carbapenem with a competitive profile that incorporates both potent Gram-negative and Gram-positive activity, with enhanced activity against the commonly occurring non-fermentative Gram-negative bacilli. Carbapenems are assuming a greater therapeutic role in many nations as multi-drug resistance (including emergence of Ambler class A, C and D B-lactamases) spreads, necessitating their accelerated development.

## P1624

## Phenotypic and genetic characterisations of enterococcal isolates in Tehran sewage, with emphasis on detection of vanA and vanB genes

F. Rahimi, K. Borhani, M. Talebi, J. Vandyousefi, M.R. Pourshafie *(Tehran, IR)*

**Objectives:** Enterococci are members of the normal gut flora of animals and humans and are thus released into the environment directly or via sewage outlets, where they can survive for long time periods. During the last decade the concern has been focused on enterococci that are resistant to the glycopeptide antibiotic vancomycin [vancomycin-resistant enterococci (VRE)]. The aim of the study was to detect and to analyse the biochemical diversity of the Entrococci strains in Tehran sewage and to determine the genetic characterization of VRE.
**Methods:** A total of 410 isolates of Enterococci were selected on mE agar medium. All of the isolates were identified at the species level by the common biochemical tests. Drug susceptibility test of isolates was done by disk diffusion method with 6 antibiotics vancomycin, erythromycin, gentamicin, tetracycline, chloramphenicol and ciprofloxacin. The MIC was also done by macrobroth dilution assay. Analysis of the plasmid profiles and the PCR tests for vanA and vanB genes were done.
**Results:** The results showed that 210, 111, 54, 20, 7, 3, 2, 2 and 1 isolates were *E. faecium*, *E. hirae*, *E. faecalis*, *E. gallinarum*, *E. mundtii*, *E. raffinosus*, *E. dispar*, *E. casseliflavus* and *E. avium*, respectively. The antibiotic resistance to the isolates were as follow: 4, 4, 18, 19, 30, 6% of the isolates were resistant to vancomycin, gentamicin, tetracycline, ciprofloxacin, erythromycin and chloramphenicol, respectively. MIC test showed that 18 of the isolates were highly resistant to vancomycin, 10 isolates were intermediate and 16 isolates were sensitive to vancomycin. The plasmid profiles of the isolates showed 4 strains lack plasmids, 14 strains having four different profiles. PCR analysis showed that from 18 VRE, 83.3% had vanA gene.
**Conclusion:** Enterococcal species, in general, was considered to have a high distribution in Tehran sewage with *E. faecium* as the most predominant strain. Overall, there was a 4% VRE in the Tehran sewage treatment plant. The presence of VRE was limited to *E. faecium*.

## P1625

## Antibiotic susceptibility of vancomycin-resistant enterococci isolated in Greek hospitals

A. Christidou, A. Gikas, E. Scoulica, M. Roumpelaki, A. Georgiladakis, E.I. Kritsotakis, G. Haliotis, S. Levidiotou, J. Kioumis, A. Skoutelis, P. Nikolaidis, Y. Tselentis *(Heraklion, Halkis, Ioanina, Thessaloniki, Patra, GR)*

**Objective:** To investigate the antibiotic susceptibility of vancomycin resistant enterococci (VRE) isolated in 8 Greek hospitals.
**Material and methods:** A total number of 373 VRE were isolated from patients in 8 Greek hospitals Sixty-five isolates were derived from VRE infected and 308 from VRE colonized patients. Species identification and vancomycin resistance characterization were performed by convetional and molecular methods. Susceptibility testing to 14 antibiotics was performed by disk diffusion and E-test methods.
**Results:** Species and type distribution was as follows: *E. faecium* vanA, 118 (31.6%); *E. faecium* vanB, 29 (7.7%); *E. faecalis* vanA, 14 (3.7%); *E. faecalis* vanB, 5(1.3%); *E. avium* vanA, 4(1.1%); *E. hirae* vanA, 2 (0.6%); *E. gallinarum* vanC1, 173(46.4%); *E. gallinarum* vanA, 1 (0.3%); *E. casseliflavus/flavescens* vanC2/C3, 26(7%); *E. casseliflavus* vanA, 1(0.3%) isolates. When all isolates (with VanA, VanB, or VanC phenotype) were considered, antibiotic resistance rates were: Penicillin, 39.1%; Ampicillin, 37.3%; Streptomycin (HLR), 45.3%; Gentamicin (HLR), 23.3%; Tetracycline, 28.7%; Rifampicin, 36.5%; Chloramphenicol, 5.6%; Erythromycin, 75.9%, Norfloxacin, 84.5%; Ciprofloxacin, 70.3%; Imipenem, 39.6%; Vancomycin, 100%; Teicoplanin, 37.5%; and Linezolid 0%. When only the isolates with the VanA, or VanB phenotype were considered as a group, different results were obtained. The antibiotic resistance rates were: Penicillin, 82.2%; Ampicillin, 79.9%; Streptomycin (HLR), 91.4%; Gentamicin (HLR), 50%; Tetracycline, 42.5%; Rifampicin, 50.6%; Chloramphenicol, 12.1%; Erythromycin, 100%, Norfloxacin, 91.4%; Ciprofloxacin, 92%; Imipenem, 83.9%; Vancomycin, 100%; Teicoplanin, 80.5%; and Linezolid, 0%. *E. faecium* presented the highest resistance rates to all antibiotics tested, except gentamicin (HLR) and tetracycline. Sixty-one out of 118 (57.6%) *E. faecium* vanA, 22/29 (75.9%) *E. faecium* vanB, and 1/14 (7.1%) *E. faecalis* vanA isolates exhibited multi-resistance (at least to10 of the antibiotics).
**Conclusion:** VanA phenotype of VRE predominated over the VanB in our hospitals. VRE presented high resistance rates to a wide spectrum of antibiotics. Linezolid and Chloramphenicol were the most active antibiotics against our VRE isolates. Multi-drug resistance was determined in the majority of VanA, and VanB *E. faecium* isolates.

## P1626

## Molecular characterisation of Tn1546 from enterococci isolated from Portuguese human, animal and environmental sources

C. Novais, T.M. Coque, J.C. Sousa, L. Peixe *(Porto, PT; Madrid, ES)*

**Objectives:** To characterise the diversity of Tn1546 among vancomycin resistant enterococcal (VRE) isolates recovered from different sources in different Portuguese regions.

**Methods:** We studied 153 VRE isolates collected in the North and Center of Portugal (1996-2004) from: (i) 81 clinical isolates from 3 hospitals in different cities, (ii) 4 faecal samples from healthy volunteers, (iii) 2 river water samples, (iv) 6 samples collected downstream of hospital sewage water, (v) 2 samples from urban sewage water, (vi) 4 swine faeces (vii) 27 poultry food samples for human consumption. Identification and characterization of vancomycin resistant genes vanA, vanB, vanC1 and vanC2 were determined by a multiplex PCR. The backbone structure of Tn1546 was characterized by a PCR overlapping assay (10 overlapping fragments), and further sequencing.
**Results:** Tn1546 was characterized in 153 vanA isolates that were identified as *E. faecium* (n = 111), *E. faecalis* (n = 37), and *Enterococcus* sp. (n = 5). All isolates were resistant to vancomycin and 93% (142/153) to teicoplanin. Eighty-nine PFGE profiles and 22 types of Tn1546 were detected among the 153 enterococci studied. Some Tn1546 variants were recovered only from specific sources (S, PP-1, PP-11, PP-2, PP-4, PP-9, PP-10, PP-13, PP-23, PP-24, PP-25, PP-27, PP-6, PP-17) while others were detected from isolates of different origins (A, D, X, PP-3, PP-5, PP-15, PP-16, PP-20). Although no polymorphism or size variation in the central regions of Tn1546 (vanRSHA) was seen for most of the enterococci, the remaining parts of the transposon, including the right and left ends, were very heterogeneous involving mutations (in vanS and vanX), deletions (vanY), duplications (vanX, vanZ) or the presence of different insertion sequences (IS1216V, ISEf1, ISEfm1). Tn1546 location was observed both in plasmids and/or in chromosome.
**Conclusions:** A high diversity of Tn1546 was observed among Portuguese VRE, suggesting a high recombination rate of this structure. Variable distribution of Tn1546 variants suggests local availability of specific types in local metagenomes and dissemination of specific genetic elements among different reservoirs.

## P1627

## Trends in prevalence rates and beta-lactamase production of *Haemophilus influenzae* nasopharyngeal carriage strains in children's day-care centres in south-eastern France

B. Dunais, H. Carsenti-Dellamonica, C. Pradier, P. Bruno, P. Touboul, M. Sabah, G. Mancini, P. Dellamonica *(Nice, FR)*

**Objective:** To monitor prevalence rates and beta-lactam susceptibility of *Haemophilus influenzae* (HI) nasopharyngeal carriage strains among children aged 3 to 40 months attending day-care centres in the Alpes Maritimes, France, in the context of antibiotic prescription-reducing campaigns (Alpes Maritimes 2001, 2003, France 2003).
**Method:** Nasopharyngeal aspirates were obtained from a random 2-stage cluster sample of children attending day-care centres in the Alpes Maritimes (AM) areas during 3 consecutive surveys conducted between January and March 1999, 2002 and 2004. HI isolates were characterized using the API-NH© test (BioMérieux). Antibiotic prescriptions over the 3 previous months were recorded.
**Results:** HI was isolated from 53.4%, 50.3% and 55.5% of 298, 294 and 337 children in 1999, 2002 and 2004, respectively. Beta-lactamase-producing strains accounted for 53.1%, 43.9% and 37.9% of all HI isolates in 1999, 2002 and 2004, respectively (chi-square for trend: p = 0.003). Types II, III, I and V made up at least 80% of strains. The proportion of children with a recent history of antibiotic intake declined from 62.7% in 1999 to 52.8% in 2002 and 48.0% in 2004 (p = 0.001).
**Conclusion:** Beta-lactamase production among HI strains has declined significantly since 1999 among children attending day-care centres as antibiotic prescriptions fell among this population.

## P1628

## Susceptibility of *Haemophilus influenzae* in the Netherlands

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

**Background:** Surveys of susceptibility of *Haemophilus influenzae* (HI) by MIC testing are not common. MIC distributions are necessary to determine wildtype cut-off values and clinical breakpoints. We performed a nation wide survey to study susceptibility of *H. influenzae* by E-test and compared these with conventional surveillance results.
**Methods:** 34 laboratories equally distributed through The Netherlands participated in a surveillance study. Each lab was asked to collect 10 strains each of HI from consecutive sputum samples. Strains were identified by participating laboratories using their own standard identification technique. MIC's were determined for ten antimicrobials (Moxifloxacin, Clarithromycin, Azithromycin, Cotrimoxazole (SxT), Doxycyclin, Cefotaxime, Cefuroxim, Amoxicillin (Am), Amoxicillin/Clavulanic acid (AmC)) using the Etest on site following manufacturers instructions; control strain ATCC 49247 was included. Afterwards, strains were collected by the central lab for further analysis if applicable. Results were analysed using WhoNet version 5.3 and compared with the surveillance results until 2002 as published in NETHMAP 2004.
**Results:** The MIC distribution of Am showed 4% of strains (n = 332) with a MIC > 4 mg/L and 7% with a MIC of >2 mg/L, indicating that resistance to Am is still relatively rare and does increase as compared to NETHMAP2004. The lognormal distribution of both Am and AmC (1 strain R) extended to 1 mg/L but showed tailing to 4 mg/L. This may indicate hidden less susceptible strains but could equally well be explained by testing circumstances, since the left part of the MIC distribution showed comparable tailing. All strains were susceptible to moxifloxacin, levofloxacin and cefotaxim. The lognormal distribution of SxT extended to 0.25 mg/L with 10% of strains showing higher values. Doxycyclin resistance was less than 5%. Most of the strains were resistant to clarithromycin and azithromycin with a MIC10 >0.5 mg/L for both.
**Conclusions:** Resistance of HI to common antimicrobials in The Netherlands is still low and does not increase. The results of the MIC distributions form an excellent basis for determining wild type cut-off values and clinical breakpoints for *H. influenzae*.

## P1629

## A current perspective on *Streptococcus pneumoniae* and *Haemophilus influenzae* resistance trends in Europe: GLOBAL Surveillance Study, 2005

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

**Objectives:** *S. pneumoniae* (SP) and *H. influenzae* (HI) are the two most common pathogens associated with community-acquired pneumonia. Changes in the prevalence of resistance or multidrug resistance (MDR) among these pathogens have important therapeutic ramifications. The GLOBAL Surveillance

initiative is a longitudinal study that benchmarks antibacterial resistance among respiratory pathogens.
**Methods:** During 2005, 964 SP and 924 HI were isolated from patient specimens collected at hospital laboratories in France (Fr), Germany (Ger), Italy (It), Spain (Spa), and the United Kingdom (UK). Isolates were centrally tested by broth microdilution against LEV, penicillin (PEN; SP only), azithromycin (AZI), erythromycin (ERY), clindamycin (CLI), ceftriaxone (CTX), cefuroxime (CFX), and trimethoprim-sulfamethoxazole (TMP-SMX) (NCCLS, 2005). Data were analysed according to PEN resistance, MDR, and b-lactamase status. MDR was defined concurrent resistance to ≥2 of the following agents: CTX, CFX, ERY, LEV, PEN, and TMP-SMX.
**Results:** For SP, PEN R was 1.8% in Ger, 2.6% in the UK, 8.5% in It, 15.7% in Spa, and 27.1% in Fr. AZI R was 13.0% in the UK, 22.1% in Ger, 27.8% in Spa, 44.3% in It, and 46.3% in Fr. Overall, LEV R was rare (≤1%) and MIC90s = 1 mg/L in all countries. 62.9% of isolates were susceptible to all of the drugs tested, the most common phenotype encountered. The prevalence (%) of MDR among SP ranged from 4.7 in UK to 32.3 in Fr. Resistance to PEN, ERY, CFX, and TMP-SMX was the most prevalent MDR phenotype found in Europe. Overall 97.4% of MDR SP were susceptible to LEV. For HI, b-lactamase rates varied by country from 4.5% in It to 21.9% in Fr. Based on MIC90 LEV and CTX were the most active agents tested against HI, regardless of b-lactamase status.
**Conclusions:** LEV showed potent activity against SP and HI. For SP, LEV activity was independent of resistance to PEN or MDR phenotype. LEV maintained consistent activity against SP based on MIC90, regardless of country studied. Antimicrobial surveillance data from studies such as the GLOBAL offer guidance to physicians for empiric prescribing.

## P1630

## Eight years of antimicrobial surveillance in *Haemophilus influenzae* isolated in Portugal (1997–2004)

M.P. Bajanca-Lavado, D. Louro, M.M. Caniça, and GEMVSA *(Multicentric Study Group on Antimicrobial Susceptibility)*

**Objectives:** *Haemophilus influenzae* (HI) is responsible for respiratory tract infections as well as for severe invasive infections such as meningitis, sepsis, epiglottitis and arthritis. The aim of this work was to monitor antimicrobial susceptibility of HI isolated in Portugal.
**Methods:** From January 1997 to December 2004, Antibiotic Resistance Unit at the National Institute of Health, in Lisbon, received 4865 HI strains isolated in 26 Hospital Laboratories from different regions of Portugal. Beta-lactamase production (nitrocefin) was determined for all strains. Minimum inhibitory concentrations (MICs, mg/L) were determined, by an agar dilution assay, to ampicillin (3344 strains) and co-amoxiclave (3348 strains). For all beta-lactamase producers (489 strains) a microdilution assay (Dade Behring) was used to perform MICs to other antibiotics.
**Results:** From 1997 to 2004, beta-lactamase production changed as follows: 8.3% (1997), 8.8% (1998), 12.1% (1999), 10.7% (2000), 9.9% (2001), 10.1% (2002), 10% (2003) and 9.3% (2004). Since 1989, the mean value of 10%, in studies performed in our laboratory, has been maintained. Ampicillin resistance in non beta-lactamase producers (ARNBLP) were from 1.5% in 1997 to 5.2% in 2004, with a mean value of 3.5%. Between 2003 and 2004 a small increase was observed in MIC90 to ampicillin (0.25 to 1 mg/L) and to co-amoxiclave (1 to 2 mg/L). Resistance to other antibiotics (considering intermediate and resistant strains) between 1997–2000 (214 bla+) and 2001–2004 (279 bla+) decreased as follows: cloramphenicol, 7.5% to 4.7%; tetracycline, 31.3% to 10%; rifampicin, 2.8% to 0.4%. A high resistance rate of 26.5% to SXT was obtained (2001–2004).
**Conclusions:** Our results suggest that beta-lactamase production does not constitute a threat in HI therapy since values were almost constant. Although with an unregulated fluctuation on ARNBLP percentages, it seems that this mechanism is gaining importance in relation to beta-lactamase production. Thus, we conclude the need to be aware of ARNBLP, as these strains are difficult to detect using the NCCLS (2004) breakpoints. Further molecular studies of the resistance genes responsible of this resistance mechanism are needed. Resistance of beta-lactamase producer strains, to other antibiotics decreased during the period of study, due to the diminished use of these antibiotics. This study shows the importance of monitoring antibiotic resistance in HI in order to detect emerging mechanisms.

## P1631

## Antimicrobial susceptibility of respiratory *Haemophilus influenzae* strains in northern Greece

K. Koraki, P. Karapavlidou, D. Sofianou *(Thessaloniki, GR)*

**Objectives:** To investigate the antimicrobial susceptibility of *Haemophilus influenzae*, one of the most frequent bacterial pathogens of respiratory tract infections. Treatment of these infections is most often empirical and considerable geographical resistance variation has been reported.
**Methods:** Eighty *H. influenzae* strains were collected from respiratory tract specimens (sputum, bronchoalveolar lavages, endotracheal secretions) in a 4-year period (2000–2004). Identification was made by colonial morphology, Gram staining characteristics, X- and V- factor requirements and API NH (bioMerieux, France). Antibiotics were selected to reflect representative current treatment options and susceptibility was determined by Kirby-Bauer disc diffusion method on Haemophilus test medium according to NCCLS guidelines.
**Results:** Out of the 80 *H. influenzae* strains 58 were isolated from children and 22 from adults. 25% of isolates came from children admitted to the intensive care units and 18.7% from cystic fibrosis patients. A seasonal trend was reported for infections since 41.2% of isolates were collected during springtime and 25% during autumn months. Overall ampicillin resistance was 13.3% and resistant strains were isolated exclusively from children. Ampicillin resistance was doubled among cystic fibrosis patients (27.3%). All isolates were susceptible to amoxicillin/clavulanate, chloramphenicol, ciprofloxacin and imipenem. The rank order of cephalosporin activity was cefotaxime and ceftriaxone (100%) followed by cefuroxime and cefaclor (95.8% and 88.9% respectively). Trimethoprim/sulfomethoxazole was active against 95.2% of isolates while erythromycin was the least potent antimicrobial agent with 75% of isolates being susceptible to it. No multiresistant phenotypes were detected.
**Conclusion:** Our results demonstrated that ampicillin resistance among *H. influenzae* in our area is still relatively low and overall antibacterial susceptibility rates are high. Knowledge of antimicrobial resistance among these pathogens is imperative for physicians to choose the most appropriate therapeutic agent.

## P1632

### Current state of Gram-negative hospital-acquired urinary tract infections in Russian intensive care units: pathogens and their resistance phenotypes

E. Khaykina, G. Reshedko, E. Ryabkova *on behalf of the Rosnet Group*

**Objectives:** To reveal the most common gram-negative pathogens of hospital-acquired urinary tract infections (UTIs) and their antimicrobial resistance.
**Methods:** Gram-negative pathogens from ICUs patients with hospital-acquired UTIs were collected in 2002–2004 throughout Russia. Antimicrobial susceptibility testing was performed in accordance with NCCLS. To interpret cefoperazone/sulbactam susceptibility results breakpoints for cefoperazone were used. SFM 2003 breakpoints were applied for polymyxin B.
**Results:** 396 nosocomial gram-negative uropathogens were studied. Most common uropathogens were *P. aeruginosa* (30.8%), *E. coli* (25.8%), *K. pneumoniae* (11.1%), followed by *A. baumannii* (7.3%), *Enterobacter* spp. (5.8%), *S. marcescens* (4.5%), *Proteus* spp. (3.8%) and other gram-negative rods (10.9%). Resistance rates (I+R, %) among *P. aeruginosa* were: gentamicin – 87%, levofloxacin – 84%, ciprofloxacin – 81%, cefoperazone – 79%, cefoperazone/sulbactam – 71%, cefepime – 62%, piperacillin – 55%, amikacin – 48%, ceftazidime – 46%, imipenem – 45%, meropenem – 43%, piperacillin/tazobactam – 43%, polymyxin B – 7%. Resistance rates (I+R, %) among *E. coli* were: piperacillin – 75%, ticarcillin/clavulanic acid – 69%, amoxicillin/clavulanic acid – 62%, ciprofloxacin – 54%, gentamicin – 54%, moxifloxacin – 54%, levofloxacin – 53%, cefoperazone – 48%, ceftriaxone – 45%, cefepime – 40%, ceftazidime – 32%, cefoperazone/sulbactam – 19%, piperacillin/tazobactam – 18%, amikacin – 17%, all strains were susceptible to ertapenem, imipenem, meropenem. Resistance rates (I+R, %) among *K. pneumoniae* were following: piperacillin – 82%, cefoperazone – 75%, ceftriaxone – 71%, gentamicin – 71%, amoxicillin/clavulanic acid – 66%, cefepime – 64%, ceftazidime – 57%, ciprofloxacin – 53%, piperacillin/tazobactam – 46%, moxifloxacin – 43%, cefoperazone/sulbactam – 43%, levofloxacin – 41%, amikacin – 27%, ertapenem – 7%, imipenem and meropenem were active against all isolates.
**Conclusion:** *P. aeruginosa*, *E. coli* and *K. pneumoniae* are the main gram-negative uropathogens in Russian ICUs patients. Imipenem, meropenem, ertapenem showed prominent activity against *E. coli* and *K. pneumoniae*. Cefoperazone/sulbactam, piperacillin/tazobactam, amikacin exhibited considerable activity versus *E. coli*, while *K. pneumoniae* were more resistant to them. *P. aeruginosa* were highly resistant to all tested antimicrobials except polymyxin B, thus leaving virtually no choices for therapy in terms of acceptable patient safety.

## P1633

### Antimicrobial susceptibility of Gram-negative anaerobic bacteria from two hospitals in Smolensk, Russia

D. Galkin, O. Kretchikova, M. Sukhorukova, R. Kozlov *(Smolensk, RU)*

**Objective:** To analyse resistance patterns of gram-negative anaerobic bacteria isolated during the first half of the year 2005 in several hospitals in a Russian city.
**Methods:** Clinical anaerobic isolates from two hospitals in Smolensk (Central region of Russia) were tested using agar dilution method in accordance with CLSI 2005 (M11-A6) guidelines.
**Results:** Overall 69 gram-negative anaerobic bacteria from 41 patients were studied. Isolation sites were represented by intra-abdominal – 25 (60.9%), soft tissue – 7 (17.1%), prostate fluid – 5 (12.2%), bone – 3 (7.4%), and dental – 1 (2.4%) infections. Susceptibility of 31 (44.9%) *Prevotella* spp., 23 (33.3%) *Bacteroides* spp. (predominantly *B. fragilis* group – 20 strains), 7 (10.2%) *Fusobacterium* spp., 4 (5.8%) *Porphyromonas* spp., and 4 (5.8%) *Veillonella* spp. to ampicillin, clindamycin, metronidazole, imipenem, ertapenem, amoxicillin/clavulanic acid and cefoperazone/sulbactam was determined. All species were susceptible to carbapenems. In *Prevotella* spp. there were 64% and 3% strains resistant to ampicillin and clindamycin and 4% of strains with intermediate resistance to metronidazole. Among *Bacteroides* spp. 92% of strains were resistant to ampicillin and 22% to clindamycin. No resistance to metronidazole was detected in *Bacteroides* spp. MIC50/90 of 0.5/2.0 and 4.0/8.0 was achieved for amoxicillin/clavulanic acid and cefoperazone/sulbactam, respectively in this group. All *Fusobacterium* spp. were susceptible to clindamycin, 2/7 strains were resistant to ampicillin and 1/7 strain demonstrated intermediate susceptibility to metronidazole. Amoxicillin/clavulanic acid and cefoperazone/sulbactam showed MIC50/90 of 0.125/2.0 and 1.0/4.0, respectively. Among *Porphyromonas* spp. strains, 1/4 strain was resistant to ampicillin, clindamycin and metronidazole. One *Veillonella* spp. strain was resistant to ampicillin, with no resistance to metronidazole and clindamycin. MIC50/90 of amoxicillin/clavulanic acid and cefoperazone/sulbactam were, respectively 0.125/16.0 and 1.0/32.0 for *Porphyromonas* spp., 0.125/0.5 and 1.0/1.0 to *Prevotella* spp., 0.06/0.5 and 1.0/4.0 to *Veillonella* spp.
**Conclusion:** Metronidazole, carbapenems, and inhibitor-protected beta-lactams are preferred for the therapy of anaerobic infections.

## P1634

### Bloodstream infections in an Estonian university hospital

P. Mitt, K. Lõivukene, V. Adamson, K. Kermes, T. Laeneste, M. Maimets, P. Naaber *(Tartu, EE)*

**Objectives:** The objectives of this study were to: analyse our current blood culture practice; describe the frequency of occurrence and antimicrobial susceptibility of bloodstream infections (BSI) isolates; determine the contamination rate.
**Methods:** We performed a prospective survey of all positive blood cultures received in the Department of Microbiology of Tartu University Hospital (938 beds) in 2004. Blood culture system used was BACTEC 9120. Duplicates within one week were excluded. Isolates were identified using conventional microbiology methods and susceptibility tests were those recommended by NCCLS. To determine extended spectrum beta-lactamase (ESBL) producers an E-test with cefepime and cefepime combined with clavulanic acid was used. Nosocomial infections were defined according to CDC criteria.
**Results:** During study period 7230 blood culture bottles were received, comprising 2840 blood culture sets (10.4 sets per 1000 patient-days). These resulted in 427 (17.2%) positive blood cultures, 127 (29.7%) were considered contaminants and contamination rate was 5.1%. A total of 273 BSI episodes involving 246 patients were identified and 161 (59%) of these were nosocomial. The incidence of nosocomial BSI (N-BSI) and community-acquired BSI (CA-BSI) was 0.6 and 0.4 per 1000 patient-days, respectively. Polymicrobial BSI was detected in 25 patients. Among N-BSI dominated coagulase-negative staphylococci (49/30.4%), *Staphylococcus aureus* (19/11.8%),

*Klebsiella* spp. (21/13%), and *Escherichia coli* (16/9.9%). The most frequent pathogens of CA-BSI were *E. coli* (31/27.7%), *S. aureus* (14/12.5%), *Haemophilus influenzae* (11/9.8%), and *Streptococcus pneumoniae* (10/8.9%). Susceptibility to oxacillin of *S. aureus* and CoNS was 100% and 21.4%, respectively. All *S. pneumoniae* isolates were susceptible to penicillin. 97.8% of *E. coli* strains were susceptible to ciprofloxacin, 67.4% to ampicillin, and 100% to gentamicin. Susceptibility of *Klebsiella* spp. to both ciprofloxacin and gentamicin was 96.7%, and to ampicillin 6.7%. 14.3% of *Klebsiella* spp. and none of *E. coli* isolates were ESBL-producers. The susceptibility patterns of N-BSI and CA-BSI pathogens were similar to each other.

**Conclusion:** Compared to West and North European countries our number of blood culture sets per 1000 patient-days is low. This may explain the relatively low incidence of BSI. The interventions to reduce contamination rate need to be implemented. The susceptibility among BSI isolates was high.

# *Clostridium difficile* revisited

## P1635

### Clinical features of *Clostridium difficile* associated diarrhoea and molecular characterisation of the strains over a 5-year period in a French university hospital

F. Barbut, B. Gariazzo, V. Lalande, R. Luiuz, B. Burghoffer, J.C. Petit *(Paris, FR)*

Recent outbreaks of *C. difficile* associated diarrhoea (CDAD) reported in North America, United Kingdom and the Netherlands have emphasized the importance for an ongoing surveillance of CDAD. The aims of the present study was to determine the epidemiology of CDAD over the past 5 years and the rate of nosocomial transmission in our acute care hospital (750-beds).

**Materials and methods:** All the cases of CDAD diagnosed between January 1st 2000 and December 31st 2004 were retrospectively reviewed. A CDAD case was defined as diarrhoea in hospitalised patients with a positive result for *C. difficile* cytotoxin or with a positive toxigenic culture. CDAD was considered as severe if patient fulfilled at least 2 of the following 3 criteria: fever > 38.5°C abdominal pain or leukocyte count >10,000/mm$^3$ or if the patient had an endoscopically proven pseudomembranous colitis or complications (toxic megacolon, perforation…). CDAD was considered as community acquired if the diarrhoea occurred in patients within 72 h after admission and if the patient had no history of hospitalisation in the previous 2 months, otherwise CDAD was considered as nosocomial. All the strains were serogrouped and characterized by toxinotyping and PCR-ribotyping. Detection of toxin A, toxin B and binary toxin was performed by PCR.

**Results:** 151 cases of CDAD were diagnosed: 147 clinical charts could be reviewed and 131 strains were studied. Global incidence of CDAD was 1.1 per thousand discharges with higher rates in 2003 and 2004. Diarrhoea was community acquired in 19% of patients. For patients with nosocomial CDAD, transmission of the strain from patient to patient (i.e. strain with the same serogroup and PCR-ribotype than the strain from another patient hospitalised in the same ward in the previous 2 months) was demonstrated in 10.1% of cases. Binary toxin was positive in 11% of strains. Binary toxin was associated to a more severe diarrhoea ($p < 0.01$) and to a higher case fatality ($p < 0.01$). A specific clone accounted for 25% of all the strains (serogroup H, PCR-ribotype ''026'') but this clone was found both in nosocomial or community cases. Three strains belonged to toxinotype III but further investigations are needed to know whether these strains correspond to the hypervirulent strains involved in recent outbreaks.

**Conclusion:** Incidence of CDAD is low in our hospital and cross infection is limited. These results also suggest that strains with binary toxin might be more virulent.

## P1636

### The development and application of a new exact typing method for *Clostridium difficile*: multi-locus variable number of tandem repeat analysis

R.J. van den Berg, I. Schaap, K.E. Templeton, C.H.W. Klaassen, E.J. Kuijper *(Leiden, Nijmegen, NL)*

**Objectives:** To study the epidemiology of *Clostridium difficile*, a typing method with a higher discriminatory power, typeability and reproducibility than currently available methods is required. Multi-Locus Variable Number of Tandem Repeat Analysis (MLVA) is a new candidate technique, that has already been tested successfully on a number of bacterial and fungal species. Using the whole genomic sequence, we developed MLVA for *C. difficile* and compared the method to standardized PCR-ribotyping. Additionally, MLVA was tested on a collection of the new emerging hypervirulent PCR-ribotype 027 strains.

**Methods:** Short tandem repeat loci (3 to 9 bp) were identified using Tandem Repeat Finder v3.21 on the genome of *C. difficile* strain 630. Amplification of the repeats was performed using a single PCR-protocol. PCR-fragments were analysed using multi-coloured capillary electrophoresis on an ABI3100, with a ROX500-marker as internal marker for each sample. The number of repeats per fragment was subsequently determined.The discriminatory power of the MLVA was tested on 23 reference strains representing 11 serogroups and 12 toxinotypes. The ability to subtype specific PCR-ribotypes was investigated with 7 subtypes of PCR-ribotype 001 (rep-PCR types 1-7), 6 TcdA-/TcdB+ strains of PCR-ribotype 017, and 11 strains belonging to PCR-ribotype 027. Of these 11 type 027 strains, 9 were isolated from 3 outbreaks and 2 from endemic cases.

**Results:** A total of 7 regions with short tandem repeats were identified. MLVA discriminated all 23 reference strains and the 7 known reference strains of PCR-ribotype 001 (rep-PCR 1-7). Two MLVA-types were recognized among 6 TcdA-/TcdB+ strains; the differences were present in only one of the 7 repeat-regions. Of 11 PCR-ribotype 027 strains, 9 outbreak-related strains were identical to each other. Interestingly, two endemic type 027 strains differed from the other strains in 2 of the 7 regions.

**Conclusion:** MLVA is a highly discriminatory genotyping method for *C. difficile* and is capable to subtype various CR-ribotypes. MLVA is also an important new tool to study the epidemiology of the emerging PCR-ribotype 027 strains.

## P1637

### Comparative study of *Clostridium difficile* diarrhoea in elderly patients treated with moxifloxacin versus amoxycillin for lower respiratory tract infections

L. Mooney, M. Wilcox *(Leeds, UK)*

Fourth generation fluoroquinolones such as moxifloxacin have improved anti-anaerobic activity. Consequently, these new agents could induce *C. difficile* infection (CDI) by inhibition of 'protective' anaerobic flora. Recent reports have suggested such an association. However, further studies are warranted to determine the risk of CDI in elderly in-patients treated with these agents, and notably where exposure to CD is measured/ controlled.

**Methods:** We prospectively investigated the propensity of moxifloxacin (MOX) or amoxycillin/macrolide (AML/MAC) to induce CDI when used to treat lower respiratory tract infections (LRTIs) in elderly in-patients, using a 4-ward, crossover design (18 months total). Patients prescribed MOX or AML/MAC were monitored for gastrointestinal symptoms. Diarrhoea was assessed as due to CD, viral or other cause. Relevant clinical data were collected. Concurrent epidemiological surveillance was also performed to determine environmental exposure to CD.

**Results:** 128 patients were studied, 24 receiving MOX and 104 had AML/MAC. Univariate analysis indicated that there was no significant difference between MOX and AML/MAC patients in gender, age (83.6 vs 86.6 mean years, respectively), or duration of hospitalisation (total, prior to and post diarrhoea). Duration of antibiotic therapy did not differ significantly between MOX and comparator patients (either total days or days before diarrhoea onset). There was a significant association between MOX and overall risk of diarrhoea. However, there was no significance between MOX treatment and CD, viral or other cause of diarrhoea. Risk factor analysis to inform on possible confounders was performed. Initial epidemiological survey results indicate that there was no change in environmental exposure levels to CD on each hospital ward. Molecular typing of all clinical and environmental isolates of CD is ongoing.

| | No. patients | Diarrhoea (%) | CDI (% all diarrhoea) | Viral (% all diarrhoea) | Other (% all diarrhoea) |
|---|---|---|---|---|---|
| Moxifloxacin | 24 | 14 (54%) | 3 (21%) | 3 (21%) | 9 (64%) |
| Amoxycillin/macrolide | 104 | 28 (30%) | 9 (32%) | 4 (14%) | 16 (57%) |
| Significance (P) | | <0.05 | 0.7 | 0.67 | 0.47 |

Evidence of more than 1 cause of diarrhoea was seen in 2 patients (1 MOX, 1 AML/MAC).

**Conclusions:** Although recent reports have highlighted a risk of CDI associated with fluoroquinolones (and increased age), none have specifically studied hospitalised elderly populations prospectively and controlled for exposure to CD. Diarrhoea occurs relatively frequently after antibiotic therapy in the elderly. MOX was associated with an increased rate of diarrhoeal symptoms, but causes other than CDI explained this association. MOX treatment was not significantly associated with CDI when compared with AMOX/MAC treatment for LRTI in elderly in-patients.

## P1638

### Prevalence and association of macrolide-lincosamide-streptogramin B resistance with resistance to moxifloxacin in *Clostridium difficile* strains isolated from symptomatic adults and children hospitalised in two university hospitals in Warsaw

H. Pituch, D. Wultanska, G. Nurzynska, P. Obuch-Woszczatynski, F. Meisel-Mikolajczyk, M. Luczak *(Warsaw, PL)*

**Objectives:** *Clostridium difficile* is the main aetiological agent of nosocomial diarhoea. Clindamycin, penicillins, and cephalosporins have been associated with CDAD. However, several case reports of fluoroquinolone-associated *C. difficile* diarrhea have been published. *C. difficile* strains usually exhibits susceptibility to metronidazole, and vancomycin. We describe prevalence and association of macrolide-lincosamide-streptogramin B (MLSB) type resistance with resistance to moxifloxacin of *C. difficile* strains isolated from adults and children.

**Methods:** Eighty-three *C. difficile* strains recovered from adults and children hospitalised in two university hospitals were investigated (hospital 1: adults n = 38, and children n = 30; hospital 2: adults n = 15). Toxin types were determined by commercial test for toxin A and cytotoxicity test for toxin B. TcdA, tcdB were detected by PCR. MICs of erythromycin, clindamycin, moxifloxacin, vancomycin and metronidazole were determined by E-test (AB Biodisk, Sweden). The ermB gene was detected by PCR.

**Results:** Sixty-seven (81%) *C. difficile* strains were toxigenic. Among these, 44 were A+B+, and 23 were A-B+. All 83 strains were susceptible to vancomycin and metronidazole. High level resistance to erythromycin, clindamycin and moxifloxacin was found in 46%, 42%, 27% of the tested strains, respectively. Twenty-one *C. difficile* strains harboured high level resistance to erythromycin, clindamycin and moxifloxacin, simultaneously. Among these, all were A-B+ and were isolated from adults, only. Twenty-one of the macrolide-lincosamide-streptogramin B (MLSB)-resistant A-B+ strains carried the erythromycin resistance methylase gene (ermB).

**Conclusion:** Resistance against clindamycin, erythromycin and moxifloxacin among Polish A-B+ *C. difficile* strains was very frequent. Fluoroquinolone resistance is associated with resistance to MLSB antimicrobials. We suggest that increasing use of fluoroquinolones is selective pressure for clonal dissemination of A-B+ *C. difficile* strains. Fluoroquinolones use is a strong risk factor for CDAD in our hospitals.

**Acknowledgement:** This work was supported by the Ministry of Scientific Research and Information Technology, Grant no. 2 P05D 074 27.

## P1639

### National surveillance to the incidence of *Clostridium difficile*-associated diarrhoea in the Netherlands

S. Paltansing, R. Guseinova, R. van den Berg, C. Visser, E. van der Vorm, E.J. Kuijper *(Leiden, Amsterdam, NL)*

**Objectives:** The recent outbreaks of *Clostridium difficile*-associated diarrhoea (CDAD) due to the new emerging PCR-ribotype 027, toxinotype III strains has renewed the interest of CDAD as an important nosocomial infection. To determine the incidence of CDAD in the Netherlands, we conducted a prospective surveillance study in 13 hospitals in the Netherlands.

**Methods:** From May 1st to July 1st of 2005, 13 participating hospitals registered all patients diagnosed with CDAD. A standardized questionnaire was devised to obtain patient information. Faeces samples or isolated strains were sent to the Reference Laboratory at the LUMC for culture and the presence of genes for toxins A and B (TcdA and TcdB). PCR-ribotyping was performed according to the method of Bidet and toxinotyping as described by Rupnik et al.
**Results:** Routine methods to diagnose CDAD in 13 laboratories included combinations of cytotoxicity tests (59%), enzyme-immunoassays (56%) and culture of toxinogenic strains (31%). In total, 91 patients with CDAD were reported. The overall incidence (median) of CDAD was 17 for 10,000 patient admissions and varied from 1 to 75. Of 91 patients with CDAD, 41% was community acquired. The median age of 54 patients with nosocomial acquired CDAD was 59 years. Of 54 patients with CDAD, 7 (13.9%) died during the study period. At least 41 different PCR-ribotypes could be recognized among 91 strains. Type 027 was identified in 9 patients from 1 hospital. Toxinotyping revealed the presence of at least 7 different types. Of 91 strains, 87% were TcdA+/TcdB+, 10% TcdA-/TcdB- and 3% TcdA-/TcdB+.
**Conclusions:** The incidence of CDAD in the Netherlands is lower than reported in USA and Canada, but varied considerably per hospital. The new emerging type 027 was found in 9 patients from 1 hospital with a high incidence of CDAD (39 per 10,000 admissions).

## P1640

## Outbreak of *Clostridium difficile* PCR-ribotype 027 toxinotype III in Harderwijk, the Netherlands

S.B. Debast, N. Vaessen, A. Choudry, R. van den Berg, E. Kuijper *(Harderwijk, Leiden, NL)*

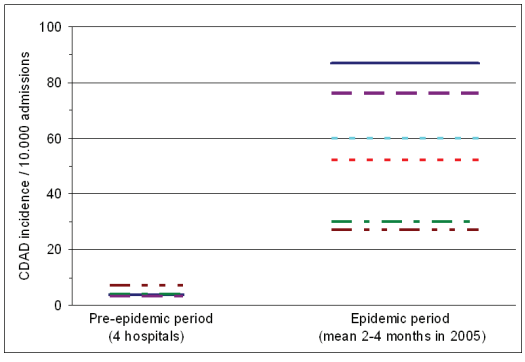
**Objectives:** Since 2002, several epidemics of *Clostridium difficile*-associated diarrhoea (CDAD) caused by *C. difficile* PCR-ribotype 027 toxinotype III have occurred in USA, Canada, and the UK. In April 2005, the first outbreak encompassing 45 patients was observed in a medium large hospital of 350 beds in the Netherlands. The isolated 027 strain was completely resistant to erythromycin and ciprofloxacin. The patient characteristics, predisposing factors and outcome of CDAD were studied.
**Methods:** A case-control study was performed in 45 patients and 90 at random selected controls without diarrhoea who stayed at the same department as the patients when the diagnosis of CDAD was made. Standardized questionnaires were designed to collect data from the patient records and all surviving patients were interviewed 3 months after the diagnosis. Faeces samples were cultured for the presence of *C. difficile* and isolates were typed.
**Results:** The incidence of CDAD increased from 4 per 10,000 patient admissions in 2004 to 81.5 per 10,000 admissions in 2005. Between April and September 2005, 45 patients with CDAD due to type 027 were identified. Of 45 patients, 9 (20%) died of which 3 (7%) as a direct result of CDAD. Eleven (24%) patients experienced one or more relapses. The average age of the cases was 72 yrs, 42.2% of the patients was male. In a multivariate analysis, antibiotic use (OR 15.3, $p < 0.001$), duration of hospital stay (cases 31 days, controls 13 days; $p < 0.001$) and tube feeding (OR 3.5, $p = 0.03$) were found to be significantly associated with CDAD. In particular, the use of ciprofloxacin (OR 11.8, $p < 0.001$) and cephalosporins (OR 5.8, $p < 0.001$) were associated. No association was found between the use of protonpump inhibitors and the risk of CDAD. The use of erytromycin was significantly higher in cases (11.1%) than in controls (2.2%) in a univariate analysis ($p < 0.041$), but this relation was not significant in a multivariate analysis.
**Conclusion:** Antibiotic use (especially ciprofloxacin and cephalosporins), duration of hospital stay and tube feeding were significantly associated with CDAD caused by *C. difficile* type 027, toxinotype III in the Netherlands. We could not confirm the previously described relation between use of protonpump inhibitors and risk of CDAD.

## P1641

## *Clostridium difficile* PCR ribotype 027, toxinotype III in the Netherlands

D.W. Notermans, T. van der Kooi, E.J. Kuijper, R.J. van den Berg, D. Veenendaal, E. van Kregten, S.B. Debast, C.E. Visser, A. Popma, S. van den Hof *(Bilthoven, Leiden, Haarlem, Amersfoort, Harderwijk, Amsterdam, NL)*

**Objectives & Methods:** Shortly after the reports in June 2005 of *Clostridium difficile* PCR ribotype 027, toxinotype III in England, this more virulent type was also detected in The Netherlands. In response, the Dutch Centre for Infectious Disease Control has undertaken measures to monitor and control the outbreak. *C. difficile* 027 guidelines for infection control and treatment were formulated, separately for hospitals and nursing homes. The Leiden University Medical Centre serves as a reference centre for diagnostics and typing of *C. difficile*. Laboratories are encouraged to send in samples for typing in case of a clear rise in the incidence in *C. difficile*, rapid spread, or several clinically suspect cases.Organisation-based surveillance was set up: questionnaires are sent monthly to institutions with *C. difficile* associated diarrhoea (CDAD) outbreaks to obtain information on incidence, *C. difficile* testing strategies, antibiotics use and control measures taken.Measures taken in hospitals dealing with an outbreak of type 027 include: treatment of CDAD with vancomycin in stead of metronidazole, emphasis on frequent and thorough cleaning and disinfection, isolation of all patients with diarrhoea until tested negative for *C. difficile* toxin, cohort isolation of CDAD-cases if individual isolation capacity is exceeded and strong restriction of certain antibiotics, including fluorochinolones.
**Results:** Until November 1st, 2005, 224 samples from 23 institutions have been sent in for typing, resulting in 74 type 027 positives. Epidemic spread of type 027 has been detected in 7 hospitals and one nursing home. Furthermore, in retrospective studies in four hospitals isolated cases of type 027 were detected. It became clear that in one region with three hospitals, the CDAD incidence had already risen in 2002, 2003 and 2004, respectively. Unfortunately, no samples from that period were available for typing. In the hospitals with epidemic spread of type 027, a wide range in the monthly incidence of CDAD was observed, from 50 to 114 per 10,000 admissions during the outbreaks. The incidence in the pre-epidemic period varied from 3 to 7 (see figure).



**Conclusions:** The outbreaks in hospitals are difficult to control: most hospitals continue to have new cases for a long period, although the incidence is decreasing in several hospitals. Fortunately, once a *C. difficile* 027 outbreak in a hospital is recognised, spread to other hospitals has not been observed.

## P1642

## Incidence of *Clostridium difficile* infections in a tertiary Greek hospital

M. Kanellopoulou, M. Martsoukou, N. Skarmoutsou, A. Charalambopoulou, E. Liapi, G. Kaderakis, E. Papafrangas *(Athens, GR)*

**Objectives:** *C. difficile* is a major cause of antibiotic associated diarrhoea (AAD) and colitis (C). The aim of this study was to determine the incidence of these infections in our hospital (450 beds), during a period of 6 months (March–October 2005).
**Methods:** A number of 182 liquid stools from equal adult patients (mean age 61 y, M:110, F: 72) receiving broad spectrum antibiotics (especially cephalosporins) were plated in CCFA (Oxoid) and anaerobic Brucella Agar (BA), after alcohol shock procedure. If the culture was positive, an immunochromatographic test was performed for toxin A (ColorPACTM Toxin A, BD, USA). If the last test was negative, a rapid enzyme immunoassay was performed for toxins A+B (ImmunoCard, Meridian Bioscience Inc. Cincinatti, Ohio).
**Results:** *C. difficile* was isolated in 23/182 (12.6%) samples. Seventeen men (pathological – P, pneumological – Pn, surgical – S, urologic – U, outpatients – O, 7, 6, 2, 1, 1 respectivly) and 6 women (P:4, Pn:1, O:1) harbored *C. difficile* in their intestin. Twelve out of 23 strains (52%) produced toxin A, while the remaining (48%) produced toxin B. Eleven patients had severe diarrhoea (7–10 days). One patient got endoscopic examination, which confirmed colitis findings. The two outpatients received oral cefuroxime in the preceding week of the positive culture.
**Conlusions:** (1) The incidence of *C. difficile* infections in this study is among these reported in international bibliography (12.6%). (2) Since toxigenic B *C. difficile* strains were demonstrated in half cases, the use of the tests detecting both toxins A and B by clinical laboratories is recommended.(3) Molecular technics application (e.g. PFGE and ribotyping) will offer a better knowledge of *C. difficile* spread in our hospital.

## P1643

## Comparison of methods for detection of *Clostridium difficile* toxins

J. Leeming, R. Ferguson, D. Kulpoo, V. Kemp, J. Watts, D. Carrington *(Bristol, UK)*

Assay of the cytotoxicity of stool samples to cells in tissue culture is commonly considered the 'gold standard' for detection of *C. difficile* toxin. However the method is slow and therefore its use can result in delayed patient treatment and implementation of infection control measures. We undertook a comparison of two microtitre plate-based ELISA kits (TechLab *C. difficile* TOX A/B II and Meridian Premier Toxins A & B) and three rapid immunoassay card kits (Remel Xpect *Clostridium difficile* toxin A/B, Meridian ImmunoCard Toxins A & B and TechLab Tox A/B Quik Chek) with an in-house cytotoxin assay. All samples tested had been referred for routine microbiological examination. Toxin tests were done on unformed samples from adult hospital in-patients and bone marrow transplant recipients and on samples where *C. difficile* toxin testing was requested by the referring clinician. All kits were used according to manufacturers' instructions. Three hundred and thirty three specimens were tested using all five kits and cytotoxin assay. Sensitivities and specificities were calculated both (a) assuming the cytotoxin assay to be the 'gold standard' (universally correct) test and (b) taking a concensus view that any sample with at least two tests positive is truly positive. Data are shown in the table below. Overall, the microtitre plate-based ELISA kits were more sensitive than the rapid immunoassay card kits. The cytotoxin assay was negative for seven samples that were positive by at least two other tests. Thus the plate-based ELISA kits were also more sensitive (but less specific) than the cytotoxin assay if consensus data was used to judge true positivity. We conclude that some immunoassay kits offer an acceptable alternative to cytotoxin assays for the detection of *C. difficile* toxin, allowing more rapid diagnosis.

Comparison of *C. difficile* kits (333 samples; PPV & NPV=positive & negative predictive values)

| | TechLab TOX A/B II | Meridian Premier Toxins A & B | Remel Xpect C. difficile toxin A/B | Meridian ImmunoCard Toxins A & B | TechLab Tox A/B Quik Chek | Cytotoxin assay |
|---|---|---|---|---|---|---|
| (a) Gold standard = cytotoxin assay: | | | | | | |
| sensitivity (%) | 97.1 | 98.6 | 68.1 | 87.0 | 81.2 | |
| specificity (%) | 92.0 | 95.5 | 99.2 | 97.0 | 98.5 | |
| PPV (%) | 76.1 | 85.0 | 95.9 | 90.9 | 90.0 | |
| NPV (%) | 99.2 | 99.6 | 92.3 | 96.6 | 95.2 | |
| (b) Consensus - any sample with at least 2 tests positive considered a true positive: | | | | | | |
| sensitivity (%) | 98.7 | 98.7 | 65.3 | 86.7 | 80.0 | 90.7 |
| specificity (%) | 94.6 | 97.7 | 100 | 99.6 | 100 | 99.6 |
| PPV (%) | 84.1 | 92.5 | 100 | 98.5 | 100 | 98.6 |
| NPV (%) | 99.6 | 99.6 | 90.8 | 96.3 | 94.5 | 97.3 |

## P1644

## Location of the enterotoxin gene in strains of *Clostridium perfringens* associated with gastroenteritis

K.A. Grant, S. Kenyon, R. Halford-Maw, E. Pettitt, J. McLauchlin *(London, Chesterfield, UK)*

**Objectives:** *Clostridium perfringens* type A is a common cause of food poisoning and is also associated with non-food borne gastroenteritis including antibiotic associated, infectious and sporadic diarrhoea. The disease symptoms are due to an enterotoxin produced when the organism sporulates in the human small intestine. The *C. perfringens* entertoxin gene (cpe) has been shown to be located either on the chromosome or on one of two large plasmids and it is generally accepted that *C. perfringens* strains associated with food poisoning have a chromosomal cpe gene whilst strains isolated from non-food borne diarrhoea have a plasmid encoded cpe gene. Spores from strains possessing a chromosomal cpe gene have been found to be far more heat resistant than spores from strains with a plasmid encoded cpe gene. Heat resistant spores are more able to survive the cooking process and go on to cause food poisoning, thus explaining why most food poisoning strains have been found to have chromosomally located cpe genes. The purpose of this study was to determine the location of the cpe gene in a range of *C. perfringens* strains from the UK, including those from both food borne and non-food borne illness.
**Method:** A multiplex PCR assay described by Miyamoto et al., (2004) was used to determine the location of the cpe gene in 107 strains of *C. perfringens* isolates associated with food borne illness and 16 strains associated with non-food borne illness.
**Results:** By multiplex PCR assay 35% of *C. perfringens* strains associated with food borne outbreaks in the UK were found to have a plasmid encoded cpe gene. These findings have not been described before. All strains associated with non-food borne illness had the cpe gene located on one of two plasmids, as anticipated.


ISSN: 1470-9465

**Conclusions:** A significant number of food borne outbreaks of *C. perfringens* food poisoning were found to be caused by strains of *C. perfringens* carrying a plasmid encoded cpe gene. Since strains of *C. perfringens* with a chromosomal cpe and plasmid cpe genes have different physiological characteristics this may have a profound impact on their mode of transmission.

**References**

Miyamoto, K., Wen, Q. and McClane, B. A. (2004) Multiplex PCR genotyping assay that distinguishes between isolates of *Clostridium perfringens* type A carrying a chromosomal enterotoxin gene (cpe) locus, a plasmid cpe locus with an IS1470-like sequence, or a plasmid cpe locus with an IS1151 sequence. Journal of Clinical Microbiology 42, 1552–1558.

## P1645

## Novel multiplex-PCR method for simultaneous detection of *Clostridium difficile* toxin A and toxin B and the binary toxin (cdtA/cdtB) genes applied on a Danish cohort

K.E.P. Olsen, S. Persson *(Copenhagen, DK)*

**Objectives:** A new multiplex PCR method was developed for the detection of the *Clostridium difficile* toxin genes: tcdA, tcdB, cdtA and cdtB. This method was applied on 210 *Clostridium difficile* strains isolated from 175 Danish hospitalised patients with diarrhoea in the period from April to October 2005, in order to investigate the present toxin profiles and their correlation to sex and age.

**Method:** A 5-gene multiplex PCR method was developed for the simultaneous amplification of the four *Clostridium difficile* toxin genes tcdA, tcdB, cdtA, cdtB and 16S rDNA as an internal positive control. Template DNA was prepared from plate grown bacterial colonies by a simple boiling procedure, and amplicons were visualized by standard gel electrophoresis.

**Results:** Three different toxin profiles were detected in the Danish cohort: 43 tcdA+, tcdB+, cdtA+/cdtB+; 107 tcdA+, tcdB+, cdtA-/cdtB- and 24 non-toxigenic tcdA-, tcdB-, cdtA-/cdtB-. The prevalence of the binary toxin genes in this study was 25% of the clinical isolates.More than half of the strains (62%) were isolated from the elderly part of the population (>59 years), and 74% of these strains displayed the tcdA+, tcdB+, cdtA+/cdtB+ profile. Of the non-toxigenic strains, 83% of the patients were females. One fourth of the strains isolated from children under 2 years of age were non-toxigenic. In four patients, two different toxin profiles were obtained from independent faecal samples.

**Conclusion:** This method offers a one-step, rapid and specific identification of *Clostridium difficile* toxin genes. This specific toxin profiling allows an evaluation of the pathogenic potential of the isolated *Clostridium difficile* and surveillance of emerging toxin profiles. Further studies of the isolated toxigenic *Clostridium difficile* strains will include gene deletion analyses of the tcdA and the tcdC (toxin regulating gene) which independently have been observed to cause enhanced pathogenicity.

## P1646

## Prevalence of *Clostridium difficile*-associated diarrhoea in hospitalised patients with nosocomial diarrhoea in university of medical sciences hospitals, Tehran, Iran

N. Sadeghifard, M.H. Salari, M.R. Ghasemi, F. Aminharati *(Ilam, Tehran, IR)*

**Objectives:** This study was aimed at determining the prevalence of *Clostridium difficile* associated diarrhoea in hospitalized patients with nosocomial diarrhoea at three University hospitals in Tehran from December 2002 to August 2004.

**Methods:** During the study period, the stool samples of 1500 hospitalized patients with nosocomial diarrhoea were cultured and tested by stool cytotoxin assay, toxigenic culture and also 650 of the samples were examined by enzyme immunoassay.

**Results:** In 159 (10.9%) of samples *C. difficile* grew and 108 stool samples (prevalence: 7.2%) were toxin positive by stool cytotoxin assay, enzyme immunoassay or toxigenic culture. There were no significant relationships between *C. difficile*-associated diarrhoea and sex and age of patients.

**Conclusion:** The results of the present study showed that among requested samples the highest percentage of *C. difficile*-associated diarrhoea was observed from the transplantation department (13.9%), followed by ICU and Paediatric section.

## P1647

## Emergence of toxin A(-)/toxin B(+) variant *Clostridium difficile*

B-M. Shin, E-C. Kim, K. Lee, E-Y. Kwak *(Seoul, KR)*

**Objectives:** The prevalence of toxigenic *Clostridium difficile* (*C. difficile*) has been reported about 70-80% in Korea. Toxin A(-)/toxin B(+) variant *C. difficile* strain is also important in nosocomial *C. difficile* infection. However, characterization of clostridial toxin (toxin A, toxin B) had not been studied.

**Methods:** We used PCR for toxin A and toxin B genes in 260 *C. difficile* isolates from patients admitted in three tertiary hospitals during January to December, 2004. Primers for toxin A genes were NK1-NK2, NK2-NK3 and NK9-NK11 and toxin B gene was NK104-NK105.

**Results:** Toxin A and toxin B positive rates using NK1-NK2, NK3-NK2 and NK9-NK11 were concordant and ranged from 62.1% to 90.9% in 3 hospitals. The proportions of non-toxigenic strains were 10-40%. However, we could differentiate toxin A(-)/toxin B(+) variants using NK9-NK 11 primers. The proportion of toxin A(-)/toxin B(+) *C. difficile* variants were 43.0%, 9.7% and 36.4% in 3 hospitals respectively.

**Conclusion:** The prevalence of toxin A(-)/toxin B(+) variant *C. difficile* were 10–43%. This result revealed the highest prevalent rates of *C. difficile* variant strains in the world. The differences of antibiotic usage pattern and problems of infection control practices might contribute the high prevalence of variant strains. More studies are needed to define the role of variant strains in Korea.

## P1648

## Impact of antimicrobial treatment on dominant faecal flora: the emergence of *Clostridium difficile*

M.F. De La Cochetiere, T. Durand, V. Lalande, P. Lepage, J.C. Petit, J.P. Galmiche, L. Beaugerie *on behalf of the Molecular Bacteriology Group*

**Objective:** Administration of antibiotic drugs has long been known to cause alterations in the gut ecosystem. In some patients, these alterations may create a niche that allows the overgrowth of some pathogens such as *Clostridium difficile*, the main causative agent in nosocomial infectious diarrhoea. A predictive tool to assess the risk of development of *Clostridium difficile*, would be of utmost clinical relevance. It remains to be determined whether specific patterns in pre-existing gut microbiota can predict the risk of onset of *Clostridium difficile*, upon initiation of antibiotic treatment. Using samples from 87

subjects enrolled in a previously published clinical study on antibiotic-associated diarrhoea (AAD), we investigated the potential relationship between their dominant faecal microbiota and the subsequent development of *Clostridium difficile* when subjects received antibiotics.
**Methods:** Temporal Temperature Gradient gel Electrophoresis (TTGE) was used to assess dominant species distribution in gut microbiota. Each electrophoregram was digitised from the migration distances and a regression model [Partial Least Square-discriminant analysis (PLS)] was built to investigate the correlation between pre-treatment dominant faecal microbiota and the acquisition of *Clostridium difficile* during antimicrobial chemotherapy.
**Results:** This PLS model could explain 46% of the subsequent onset of *Clostridium difficile*. This result supports the concept of ''permissive'' flora with preliminary data focusing on *Clostridium coccoides*-phylogenetic group.
**Conclusion:** To our knowledge it is the first time that dominant faecal microbiota is found to heighten susceptibility to the subsequent onset of *Clostridium difficile* upon initiation of antibiotic treatment. These findings insinuate that strategies reinforcing the control of dominant faecal microbiota at homeostasis would be of clinical relevance. This study has been partially financed by Biocodex laboratories.

### P1649

### Quantitative *C. difficile* and anaerobic flora cultures during treatment of *C. difficile*-associated diarrhoea with PAR-101

T.J. Louie, J. Emery, W. Krulicki, D. MacCannell, B. Byrne, M. Mah *(Calgary, CA)*

**Objectives:** Selective therapy of *C. difficile* diarrhea (CDD) requires the reduction of pathogen counts in the colon, but spare the normal flora. To determine if PAR 101 is selective for CDD, serial stool samples were collected at study entry, at day 10, and weekly x5 during the conduct of a phase 2a study of CDD treatment.
**Methods:** Patients (n = 32) were randomized to receive 50, 100 or 200 mg twice daily of PAR 101 for 10 days. No prior therapy was given to 24 patients; 8 receive 1 or 2 doses of standard therapy. As treatment controls, 7 additional patients were treated with vancomycin 125 mg qid for 10 days. Five well persons donated stools as normal flora controls. Fresh stool samples were cultured 10-2, 4, 6, 8 for *C. difficile* vegetative and spore forms; faecal filtrates were tested for cytotoxin B by cell assay. Strains were characterized by tcdA/B, ermB, cdtA/B PCR and by ribotyping. At study entry and day 10, aerobic and anaerobic faecal flora cultures, diluted 10-3, 5, 7, 9, were examined for major floral shifts. Since *Bacteroides* group organisms are ubiquitously present and cultivable, this genera was selected as a indicator of the integrity of the microbial flora.
**Results:** At study entry, mean log 10 CFU + SD vegetative counts of *C. difficile* (all PAR 101 patients) were 6.8 + 3.6, range 2–10.95; at day 10, with the exception of one patient receiving 50 mg, all other patients had *C. difficile* quantitative counts reduced < 2 log10/gm faeces. Vancomycin was similarly effective. At study entry, *Bacteroides* group counts were < 3, 3–8, & 8.5–10 log10 CFU/gm in ~1/3 each of patients. All normal stools showed complex, multi-genera in high counts, with 4–5 *Bacteroides* group species > 11 log10 CFU/g. Mean + SD of log10 CFU of *Bacteroides* group counts/g feces wet weight at study entry and day 10 for 50 mg/day (n = 10) were 6.6 + 2.8/8.2 + 2.6 (p = 0.11, wilcoxon matched pairs signed-ranks test, 2 tailed); for 100 mg/day (n = 8) were 6.6 + 2.8/6.3 + 2.5 (p = 0.44); for 200 mg/day (n = 11) were 7.0 + 2.9/7.3 + 3.1 (p = 0.56); and for vancomycin (n = 7) 7.4 + 2.7/3.6 + 1.9 (p = 0.03).
**Conclusion:** Patients with CDD have variably impaired normal flora. PAR 101 was effective in all dosages in eradicating *C. difficile*. A dose-dependent reduction in *Bacteroides* counts was not observed. Vancomycin significantly reduces *Bacteroides* counts during CDD treatment. PAR 101 is effective against *C. difficile in-vivo*, and is relatively sparing of the normal flora.

# Virology – II

### P1650

### Improved detection of enterovirus in cerebrospinal fluid with a minor groove binder – conjugated fluorogenic TaqMan probe

B. Vincart, F. Brancart, P. Gilot, N. De Schrijver, S. Betermiez, M. Delforge, C. Liesnard *(Brussels, BE)*

**Objectives:** To evaluate PCR primers and probes for the detection of enterovirus (EV) in a real-time (RT) PCR assay and determine their sensitivity and specificity in clinical strains and in patient's cerebrospinal fluid (CSF).
**Methods:** A new set of forward primer and minor groove binder probe (MGB) deduced from the highly conserved 5′ non-coding region of the EV genome was evaluated and compared to two previously published PCR primer-probe sets [Verstrepen et al. (VER) and Rabeneau et al. (RAB)]. Dilutions of cell culture supernatants of various enteroviruses infected MRC5 or Vero cells were prepared to determine the accuracy and the analytical sensitivity of the 3 assays. Sixty-eight CSF samples sent to the laboratory for diagnosis of meningitis were prospectively investigated by the three (RT) PCR assays and viral culture (VC). RNA extraction was performed with Qiagen kit according to the manufacturer's recommendations.
**Results:** The three RT PCR assays were able to detect all enterovirus strains in cell culture supernatants. However the detection limit of the MGB RT PCR was 1 to 2 log10 more sensitive in 14 out of 16 dilutions assays of VC supernatants compared to the RAB and VER RT PCR. All VER and MGB negative CSF were VC negative. Thirty-two CSF specimens from 68 patients suspected of viral meningitis were positive by all RT PCR (47.1%), whereas only 8 were found positive by VC (11.8%). The RAB RT PCR failed to detect 4 CSF confirmed positive by VC (1 Echo 6 and 3 non typable EV). Among samples positive by RT PCR, sensitivity of VER, MGB and RAB was respectively 100%, 100% and 71.9%.
**Conclusion:** In our laboratory, MGB RT PCR has a good correlation with VER RT PCR whereas RAB RT PCR is less sensitive especially for the detection of Echovirus 6. The MGB

RT PCR seems to be the most sensitive of the 3 RT PCR. Further studies, including more EV strains should help to precise the sensitivity of this assay.

## P1651

## Molecular typing of an uncommon enterovirus, coxsackievirus A7, frequently associated with certain diseases in Kuwait

A. Dalwai, S. Ahmad, E. Hussein, A. Pacsa, W. Al-Nakib *(Kuwait, KW)*

**Objectives:** Enteroviruses generally share tissue tropism and present with overlapping disease spectrum, however certain enteroviruses may be over represented in certain diseases than others. Coxsackievirus A7 though has been reported to cause several diseases such as febrile illness, herpangina, aseptic meningitis and acute flaccid paralysis, the frequency was very low. The study aimed to determine the prevalent enteroviruses causing non-specific febrile illness, aseptic meningitis, encephalitis, neonatal disease and myositis, in Kuwait. It also aimed to study the association between a certain enterovirus and a particular disease and its severity.
**Methods:** Diagnosis of enteroviral infection was based on detection of enteroviral RNA by semi-nested RT-PCR of a portion of the 5′UTR of the enteroviral genome followed by Southern hybridization with an enterovirus specific probe to confirm the results. The enterovirus was genotyped by sequencing of the 5′UTR, the VP4 and a portion of the VP2 encoding regions, and the sequence was analysed by BLAST analysis, CLUSTALW alignment and PHYLIP phylogenetic analysis package.
**Results:** Enteroviruses were the only etiological agents detected in 24% (217) of 920 disease cases investigated. Coxsackievirus A7 was identified to be the second most predominant enterovirus (24%; 44 of 187 cases genotyped) associated with disease, after only echovirus 9 (39%; 70/187). Although identified in all the diseases investigated, coxsackievirus A7 occurred less frequently in CNS disease cases (17%; 25/147) than in febrile illness cases (43%; 15/34). In a preliminary study, it was also predominantly detected in 66% (4/6) of myositis cases. The 5′UTR of this virus showed 96% homology with that of coxsackievirus A7 prototype strain (Parker strain) whereas the VP4 and the adjoining region showed greater homology to human enterovirus B genotype sequence.
**Conclusions:** Coxsackievirus A7 was determined to be an emerging enterovirus associated with different diseases in Kuwait. It was frequently represented in mild febrile illness and myositis cases than in CNS disease suggesting that the isolate might be less neurovirulent. Molecular analysis suggests that the isolate might have emerged due to recombination between coding and non-coding segments of coxsackievirus A7 and human enterovirus B group genomes.
**Acknowledgement:** Supported by Research Administration project grants MI 04/01, YM 03/02 and College of Graduate Studies, Kuwait University.

## P1652

## The new proposed enterovirus type 75 is causing meningitis in Spain

A. Avellon, G. Trallero, G. Rubio, N. Rabella, I. Casas, G. Palacios *(Majadahonda, Bilbao, Barcelona, ES; New York, US)*

**Introduction:** Several new proposed enteroviruses (EV) have been recently described, including the named EV75 [1]. A total of 8 isolates of this serotype were identified from 1974 to 2000 in America, Africa or Asia Associated mainly with acute flaccid paralysis or unspecified disease.
**Objective:** To determine if this new serotype circulates in Spain and what type of disease produces.
**Methods:** A total of 300 EV isolates coming in 2005 to the Spanish Enterovirus Reference Laboratory were studied both by micro neutralization assays and by Typing PCR [2]. In the isolates in which EV75 was suspected by the mentioned methods complete VP1 gene was amplified and sequenced with specific designed primers.
**Results:** Four isolates from two different regions of Spain were identified as EV75 (more than 80% of homology with the published sequences). Three of them corresponded to aseptic meningitis in children and were isolated from CSF.
**Discussion:** The present work demonstrates that this new proposed virus circulates also by Europe and is associated to aseptic meningitis. Till the moment it seems that is represented in a minor proportion (4/300 studied), however the possibility of spreading of this viral infection should be considered, as EVs may behave in that way, as previously have been demonstrated [3].
**Reference**
1. Oberste, M.S., et al., Molecular identification and characterization of two proposed new enterovirus serotypes, EV74 and EV75. J Gen Virol, 2004. 85(Pt 11): p. 3205–12.
2. Casas, I., et al., Molecular characterization of human enteroviruses in clinical samples: comparison between VP2, VP1, and RNA polymerase regions using RT nested PCR assays and direct sequencing of products. J Med Virol, 2001. 65(1): p. 138–48.
3. Avellon, A., et al., Molecular analysis of echovirus 13 isolates and aseptic meningitis, Spain. Emerg Infect Dis, 2003. 9(8): p. 934–41.

## P1653

## Evolution of G1 rotavirus strains in Italian children in an 18-year period

S. Arista, G.M. Giammanco, S. De Grazia, S. Ramirez, C. Colomba, A. Cascio *(Palermo, Messina, IT)*

**Objectives:** Rotavirus is the most important cause of severe gastroenteritis in infants and young children through the world and is responsible of 500,000 deaths annually, mostly in developing countries. Therefore, development of rotavirus vaccine is a high priority. Rotavirus strains with G1 types account for the majority of the diarrhoea episodes. Recently, a monovalent G1 attenuated rotavirus vaccine was licensed in Mexico. In view of a hypothetical introduction of such vaccine in Europe, we investigated the variability over time of VP7 antigenic genes of G1 rotavirus strains in our area.
**Methods:** Fifty strains were selected from a total of 780 G1 strains obtained from children of less than 5 years of age hospitalised with acute gastroenteritis at the ''G. Di Cristina'' Children's Hospital of Palermo in the period 1986–2004. The selected strains were genotyped by RT-PCR and 35 of them were submitted to VP7 gene sequence analysis.
**Results:** All but one of the 50 strains were genotyped as G1P(8). The VP7 sequences of 35 of them were distributed into lineages I and II. Lineage I included 14 strains from 7 different years in the range 1986–2004. Lineage II included 21 strains from 9 different years in the range 1989–2004. The degree of similarity among the nucleotide sequences of Italian strains in each lineage were comprised between 94% and 100%. An alignment of the deduced amino acid sequences showed major lineage specific

amino acid changes in the variable antigenic regions with respect to the reference Wa strain.

**Conclusions:** Sequence analysis indicated that in Palermo there was co-circulation of G1 strains belonging to two different lineages. Overall, the G1 strains showed a high degree of similarity inside each lineage and shared specific amino acid modifications. The antigenic differences between circulating strains might permit them to escape neutralization and persist in the infantile population. Our results suggest that rotavirus strains belonging to the two G1 lineages should be both included in a rotavirus vaccine preparation.

## P1654

## Epidemic spread of recombinant noroviruses with four capsid types in Hungary

G. Reuter, H. Vennema, M. Koopmans, G. Szucs *(Pécs, HU; Bilthoven, NL)*

**Objectives:** Noroviruses (''winter vomiting diseases'') are the predominant etiological agent in Hungary and common pathogen worldwide in outbreaks of gastro-enteritis in humans. Noroviruses are genetically diverse group of viruses with multiple genogroups (GG) and genotypes. More recently, naturally occurring recombinant noroviruses were identified. These viruses had a distinct polymerase gene sequence (ORF1, designated GGIIb/Hilversum) and were disseminated through waterborne and food-borne transmission in Europe. Our aim was to characterize these emerging recombinant noroviruses causing outbreaks of gastro-enteritis in Hungary.

**Methods:** Stool and RNA samples – from norovirus outbreaks between January 2001 and May 2005 - containing ''GGIIb/ Hilversum polymerase'' (GGIIb-pol) were selected for analysis of the viral capsid region (ORF2) by reverse transcription-polymerase chain reaction (RT-PCR) followed by sequence- and phylogenetic analysis.

**Results:** Forty (12.6%) of 318 confirmed norovirus outbreaks were caused by the new-variant lineage with the GGIIb-pol. Viral capsid region was successfully characterized in 24 GGIIb-pol outbreaks. Four different recombinants were detected with capsids of Hu/NLV/GGII-3/Mexico/1989 (n = 10, 41.7%), Hu/ NLV/GGII-2/Snow Mountain/1976 (n = 9, 37.5%), Hu/NLV-1/GGII/Hawaii/1971 (n = 4, 16.6%) and Hu/NLV/GGII-4/ Lordsdale/1993 (n = 1, 4.2%). Interestingly, outbreaks caused by recombinant GGIIb-pol strains mostly associated with outbreaks among children (47.5%) and had non-winter seasonality.

**Conclusions:** Epidemic spread of emerging multiple recombinant norovirus strain GGIIb-pol were detected in Hungary that became the second most common norovirus variants – next to the endemic GGII-4/Lordsdale virus – causing epidemics of gastroenteritis in the last 4.5 years.

## P1655

## Human metapneumovirus infection in Cuba: first report

B. Acosta, I. Casas, P. Pérez Breña, C. Savón, A. Goyenechea, S. Oropeza, A. Piñón *(Havana City, CU; Madrid, ES)*

The respiratory infections are the most common diseases in the world being the origin of a great morbidity and mortality especially in infants and elderly. (1) Human Metapneumovirus (hMPV) was first described in Dutch children with acute respiratory tract infections (ARTIs) in June 2001. (2) Very limited studies data are available from tropical and developing countries. We sought to determine the role of hMPV in upper and lower respiratory tract infections in Cuban patients and correlated the presence of virus with clinical characteristics of the disease. Between 1 October 2002 to 30 September 2003, 93 clinical samples received from the National surveillance program of ARTIs at the National Reference Laboratory of Respiratory viruses, for virological study, were used to detect hMPV by RT-nested PCR, amplifying a conserved fragment of 170 nucleotides in the polymerase gene. We found RNA hMPV in 9.6% of samples from the patients with ARTIs. 44.4% of individuals who tested positive for hMPV were under 12 months of age. Patients with evidence of hMPV had symptoms consistent with either upper or lower respiratory tract disease or both. 66.6% of hMPV positive individuals were detected during August-October (Table1). The results of this preliminary study shows that hMPV is present among Cuban patients with ARTI. Constitute the first report of the frequency of hMPV infection in a non-preselected group of Cuban patients with ages ranged from 3 months to 60 years old. It should be noted that this is the first report of hMPV infection in Central America and in the Caribbean region, further confirming the worldwide distribution of the virus (3–5).

**Table 1. Characteristics of nine patients with hMPV-associated respiratory tract infection.**

| N° | Month/Year | Age | Gender | Diagnosis | Hospitalization | Co-infection |
|---|---|---|---|---|---|---|
| 1 | October/02 | 3 months | M | Bronchiolitis | Yes | |
| 2 | February/03 | 9 months | M | Pneumonia | Yes | |
| 3 | April/03 | 19 years | M | Flulike illness | | |
| 4 | June/03 | 5 months | M | Bronchiolitis | Yes | |
| 5 | August/03 | 60 years | M | Pneumonia | Yes | RSV B |
| 6 | August/03 | 55 years | F | Flulike illness | | |
| 7 | September/03 | 33 years | M | Flulike illness | | |
| 8 | September/03 | 10 months | F | Bronchiolitis | Yes | RSV A |
| 9 | October/03 | 18 years | M | Flulike illness | | |

**References**

1. Monto AS. Epidemiology of viral respiratory infections. Am J Med 2002; 112:4S–12S.

2. van der Hoogen, B. G., J. C. de Jong, J. Groen, T. Kuiken, R. de Groot, R. Fouchier; and A. Osterhaus. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat. Med. 2001; 7:719–724.

3. Stockton J, Stephenson I, Fleming D, Zambon M. Human metapneumovirus as a cause of community-acquired respiratory illness. Emerg Infect Dis 2002; 8:897–901.

4. Ebihara T, Endo R, Kikuta H, Ishiguro N, Yoshioka M, Ma X, et al. Seroprevalence of human metapneumovirus in Japan. J Med Virol 2003; 70:281–3.

5. Cuevas L, Abubaker M, Nasser B, Dove W, Gurgel R, Greensill J, et al. Human Metapneumovirus and Respiratory Syncytial virus, Brazil. Emerg Infect Dis 2003;9(12):1626–28.


ISSN: 1470-9465

## P1656

### Detection of human metapneumovirus in paediatric nasopharyngeal aspirates by a TaqMan minor groove binder probe assay: a one-year prospective study in Belgium

W. Verstrepen, P. Bruynseels, A. De Smet, A. Mertens *(Antwerp, BE)*

**Objective:** Human metapneumovirus (hMPV) has a relative high incidence in acute respiratory infections in children but is difficult to isolate in culture. The aim of the study was to decrease the number of undiagnosed viral respiratory infections in our hospital by means of a TaqMan minor groove binder (MGB) probe assay.
**Methods:** From October 2004 to September 2005 a total of 1387 nasopharyngeal aspirates from children presenting at our paediatric facility were analysed. RNA extracts from specimens negative for RSV, parainfluenzavirus and influenzavirus with an (in) direct immunofluorescence assay (IFA) were subjected to a TaqMan MGB probe assay in parallel with a previously published TaqMan assay.
**Results:** Of the 1387 specimens, 371 (27%) were positive by IFA for either RSV (226), parainfluenzavirus (47), influenzavirus A (83) or influenzavirus B (15). HMPV was detected in 83 (8.4%) of the remaining 988 specimens subjected to the newly developed PCR. Of the patients with a positive hMPV assay, 46/52 (88.5%) presented with respiratory symptoms. 72% of the positive specimens were from children less than 2 year as compared to only 6% from children older than 5 years. Viral load was highest in children less than 1 year. A prominent seasonal variation was noted since more than half of the positive specimens occurred during the months March and April. There was no significant difference in the proportion nor viral load of positive specimens from ambulatory patients, patients admitted to a general ward or patients requiring intensive care. As compared to the published Taqman assay, diagnostic sensitivity and specificity were 97.6% and 99.9% respectively, whereas PPV and NPV were 98.8% and 99.8%. Method comparison (NCCLS guideline EP-9A) failed to demonstrate a significant difference between both assays when the threshold cycle (Ct) was between 22 and 41. Strongly positive specimens (Ct < 22) were associated with a lower Ct using the published TaqMan assay. However, the new TaqMan MGB probe assay appeared to be more sensitive for weakly positive specimens (Ct > 41).
**Conclusion:** The number of viral respiratory infections confirmed in our hospital was substantially increased by means of the hMPV TaqMan MGB probe assay. The new assay is a reliable alternative to the previously published TaqMan assay for detection of hMPV in nasopharyngeal aspirates.

## P1657

### Nucleic acid sequence based amplification and molecular beacon detection for the real-time identification of respiratory syncytial virus in paediatric respiratory specimens

R. Manji, F. Zhang, C. Ginocchio *(Lake Success, US)*

**Background:** Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract infection in infants and young children, with bronchiolitis and pneumonia being the major clinical manifestations. The rapid diagnosis of RSV infections is of central importance for individual patient management (rational use of antibiotics and antiviral agents), hospital infection control and monitoring epidemiological disease patterns. This study included a technical validation and a retrospective clinical evaluation of a real time NASBA assay for the detection of RSV A and RSV B in paediatric respiratory samples.
**Methods:** Samples tested included: dilution panels of *in vitro* transcribed RNA, local RSV isolates, isolates of common respiratory pathogens, and frozen respiratory specimens (nasopharyngeal aspirates, washes or swabs) from 231 children (age range: 5 d to 2 yr) who were evaluated in the paediatric emergency department for respiratory disease. Nucleic acid (NA) isolation, amplification and detection were performed using the NucliSens EasyQ Basic Kit and NucliSens EasyQ RSV A+B reagents (bioMérieux). Specimen NAs and a RSV specific internal RNA control (IC) were co-extracted using NucliSens Magnetic Extraction Reagents and the NucliSens miniMAG instrument (bioMérieux) and co-amplified using a single RSV specific primer pair. Included in the reaction were a RSV specific molecular beacon (5′-FAM) and an IC specific molecular beacon (5′-ROX). Target amplification and continuous monitoring of emitted fluorescence were performed using a NucliSens EasyQ analyzer (bioMérieux). Results were compared to direct immunofluorescence (DFA) and/or viral culture using R-Mix cells (Diagnostic Hybrids, OH).
**Results:** The limit of detection for RSV was 2 RNA copies/rxn and the 100% detection rate was 5 copies/rxn. The assay was 100% specific for RSV with no cross reactivity to other respiratory pathogens. The NASBA assay detected 10% more positive specimens than DFA and 44% more positive samples than VC. The NPVs of the assays were: NASBA 94.6%, DFA 90.8% and VC 78.0%.
**Conclusions:** The NucliSens EasyQ RSV assay demonstrated superior sensitivity to both DFA and viral culture for the detection of RSV A and B from respiratory specimens. The assay was easy to use, required minimal hands on time (1 hr) and a faster time to results as compared to rapid culture (4 hr vs. 24–72 hr).

## P1658

### Genetic diversity and molecular epidemiology of respiratory syncytial virus over six consecutive seasons in Cuba

O. Valdes, C. Savon, A. Goyenechea, L. Palerm, Gr. Gonzalez, Gu. Gonzalez, J. Melero, I. Martinez *(Havana City, CU; Madrid, ES)*

Respiratory Syncytial Virus (RSV) is a major cause of acute lower respiratory tract infection in infants and young children. It has previously been shown that HRSV isolates can be divided into two antigens groups A and B. The G protein is the most divergent both between and within the two subgroups and appears to accumulate amino acid changes with time, suggesting evolution under selective pressure. Our knowledge of the molecular epidemiology of RSV has so far been based mainly on studies done in the developed world with e temperate climate. Very limited epidemiological data are available from tropical and developing countries, where RSV infections may follow a different pattern. In this report we examine the molecular epidemiology and evolutionary pattern of the G protein of both subgroups A and B RSV through consecutive epidemics in Cuba. Sixthly four nasopharyngeal swabs were collected from children under 1 years of age with respiratory disease to different hospitals in Cuba between 1994 and 2000, to examine the molecular epidemiology and evolutionary pattern of the G protein of RSV. All samples collected from 1994 to 1999 were RSV subgroup A; however both subgroups co-circulate during

2000. The Cuban isolated from 1994 to 1996 showed a great homogeneity between them and were resemble to an ancient strain (Long) with only five nucleotide differences, this also occur in 1998 and 2000 with two strain. Furthermore was detected different size of G protein (297 or 298 for RSV A and 295 for RSV B) due to change in stop codon used he genetic homogeneity of the Cuban isolates (1994–1996) and their resemble to an ancient strain such as Long was an unusual finding in our country. In both subgroups was observed the predominance of strains with almost similar sequences. Phylogenetic analysis for subgroup A strains showed that strains were cluster in different genotypes with virus isolated in different geographic regions. Both subgroups co-circulated during 2000 and clustered whit South African strains that circulate during at the same time.

## P1659

## Point mutations in respiratory syncytial virus detected by LightCycler PCR and melting curve analysis

U. Germer, L. Nielsen, K. Boye, H. Westh *(Copenhagen, DK)*

**Objective:** The objective was to analyse RSV real-time PCR-positive isolates from clinical samples, which appeared to belong to three different groups according to melting temperature (Tm) of the amplicons. The analysis was done according to genotypic and phenotypic difference and related to geographical distribution.
**Materials and methods:** 322 nasopharyngeal aspirates were collected from children with respiratory distress in the city of Copenhagen. Viral RNA extracted using the MagNAPure LC automated extraction system was amplified in a real-time RT-PCR previously described (1). Five samples from each of the three groups with different Tm's were selected for bidirectional DNA sequencing using the RSV primers. Sequences were analysed using Chromas Lite version 2.3.
**Results:** A total of 322 clinical samples were analysed. 170 (53%) of the 322 samples were positive and 152 (47%) were negative for RSV. Three distinctive groups with different Tm's could be identified from the melting curve analysis. Group 1 (n = 91) had a Tm with a median of 60.9°C, group 2 (n = 59) and 3 (n = 20) had lower Tm's with a median Tm of 58.7°C and 56.8°C respectively. Sequence analysis of amplicons showed that the difference in Tm was due to differences in genotype between the three groups. Genotype 1 and 3 were closely related, differing only in two nucleic acids in position 12525 (C to T) and 12564 (A to T). Both were silent mutations. Only position 12564 is targeted by the probe. Genotype 1 and 3 were both blasted to a complete genome sequence of Respiratory Syncytial Virus subgroup A (GenBank RSU39661) with the highest identity score for genotype 1. Genotype 2 sequences were blasted to Human Respiratory Syncytial Virus mutant cp52 subgroup B (GenBank AF013255). Geographical analysis showed a higher prevalence of the mutant strain (genotype 3) in the northern areas of the greater Copenhagen area compared to central, southern and western areas ($p = 0.01$).
**Conclusion:** We found three genotypes of RSV according to the Tm of the PCR product. Two of the genotypes were closely related with only two point mutations and the same phenotype. Genotype 3 was mainly found in clinical isolates from the northern part of Copenhagen, suggesting a local epidemic spread.
**References**
1. Whiley DM; Syrmis MW; Mackay MJ and Sloots TP.J. Detection of Human Respiratory Syncytial Virus in Respiratory Samples by LightCycler Reverse Transcriptase PCR Clin. Microbiol. 2002;40; 4418–4422.

## P1660

## Evaluation of a real-time NASBA assay for the detection of influenza A and B virus

K. Brengel-Pesce, F. Jacobs, J. Telles, A. Lefeuvre, P. Van de Wiel *(Grenoble, FR; Boxtel, NL)*

**Objectives:** bioMerieux is developing a real-time NASBA assay to detect Influenza A and B RNA in different kind of respiratory clinical samples, by using the NucliSens® EasyQ basic kit in combination with specific primers and molecular beacons.
**Methods:** 89 Nasal/throat swabs in transport medium from hospitalised children (0–16 yrs from Edouard Herriot hospital, Lyon, France) were used for this evaluation. Influenza RNA is isolated using the NucliSens® miniMag extraction. An internal control is added to the sample prior to nucleic acid extraction. The assay is designed to detect in a single tube, using a three-label approach, the internal control and both Influenza A and Influenza B RNA. Amplification reactions were performed in a NucliSens® EasyQ Analyser allowing real-time detection. The results of the clinical samples were compared to cell culture results.
**Results:** Among 89 swabs tested, real-time NASBA detected 10 (11.2%) samples for Influenza A and 2 (2.2%) samples for Influenza B. Comparatively, by cell culture only 5 (5.6%) samples were identified as Influenza A and non as Influenza B. Interestingly, 1 Influenza A positive sample identified by cell culture was found negative in real-time NASBA.
**Conclusions:** The data showed that NucliSens® EasyQ Influenza A/B assay detected 50% more Influenza A virus than cell culture method. Moreover, real-time NASBA detected 2 Influenza positive samples, which were not detected by cell culture. With this assay a qualitative detection of Influenza A and Influenza B viruses in a single reaction can be done within 3 hours. It provides a valuable alternative to cell culture method for the clinical management of patients with Influenza infections.

## P1661

## Clinical manifestations and progression of mumps meningitis in children in Romania

L. Raduta, S-A. Florescu, A.G. Tudor, M. Cotiga, C.P. Popescu, R.F. Botgros, P.I. Calistru, E. Ceausu, L. Paun *(Bucharest, RO)*

**Background:** Mumps meningitis represents one of the major and severe extra salivary manifestations of mumps virus infection and; occasionally occurs mumps encephalitis.
**Methods:** Retrospective clinical study of 2372 mumps cases diagnosed in the last 3 years in our department.
**Results:** 299 patients have developed mumps meningitis and 17 patients were diagnosed with mumps meningoencephalitis. Age limits were from 3 to 17 years and sex ratio M/F was 7/4.Clinical manifestations involved fever (95%), stiff neck (92%), nausea and vomiting (89%), headaches (86%), photophobia (73%) and neurological manifestations such as: equilibrium disorders and drowsiness (6%), convulsions (0.17%), cerebellum syndrome (0.12%). Meningeal symptoms have occurred shortly after parotiditis in 94% of cases and before parotiditis in 3% of cases; the other cases have evolved without parotid swelling. Other localizations of the mumps infection were: parotiditis (97%), pancreatitis (61%), submaxillitis (40%) and orchitis (4%). Lumbar puncture yields CSF containing between 130 and 2830 WBC/mm3. The predominating cells were usually lymphocytes, but 12% of the patients have polymorphonuclear leukocyte predominance at the first puncture. Protein levels are normal to mildly elevated in all cases and hypoglycorrachia was founded in 10% of the patients. Therapy for mumps meningitis was symptom-based (analgesics and antipyretics) in 67% of cases and glucocorticoid therapy in 33% of cases.

**Conclusions:** (1) Neurological involvement in mumps occurred in 13.32% of cases; (2) Men are afflicted two times more often as women, but the age distribution is the same as for uncomplicated mumps; (3) Mumps meningitis was the only localization of the mumps infection in 3% of cases.

## P1662

### Rare manifestation of mumps: anterior uveitis

E. Turk Aribas, I. Erayman, A.C. Inkaya, B. Kandemir, M. Bitirgen *(Konya, TR)*

Mumps is acute generalized infection occurs primarily in school-aged children and adolescents. Most prominent manifestation of mumps is swelling and tenderness of salivary glands especially parotid gland. Uveitis is a rare manifestation of mumps. Here we present a mumps case complicating with uveitis. 26 years old paediatric nurse was admitted to our emergency department because of headache and malaise. On physical examination bilateral parotid enlargement was noticed. Opthalmology consultation revealed anterior uveitis. Local prednisolon and cyclopentholate treatment were prescribed. Lumbar puncture revealed lymphocytic pleocytosis without hypoglychorachea and elevated protein levels. Mumps IgM was found positive. Differential diagnosis made with other viral infections and sarcoidosis. Her headache diminished day after the hospitalisation. Uveitis responded very well to local therapy and patient got well in 2 weeks.

## P1663

### Clinical and epidemiological aspects of a measles epidemic, Bucharest, Romania

S-A. Florescu, M. Cotiga, C.P. Popescu, L. Raduta, G. Scurtu, R.F. Botgros, G. Enache, P.I. Calistru, E. Ceausu *(Bucharest, RO)*

**Objectives:** We studied the clinical and epidemiological aspects of the measels epidemic, still ongoing, in 2005.
**Methods:** Retrospective study of patients with measels hospitalized in Clinical Hospital of Infectious and Tropical Disease ''Dr V. Babes'' Bucharest between 01.04–31.10.2005.
**Results:** There were 108 cases; sex ratio M/F:58/50. The mainly affected age group is under 13 months (38.9%) followed by 13 months–2 years (23.15%), 2–7 years (22.22%), > 16 years (10.18%) and 7–16 years (5.55%). 70.37% cases were hospital-acquired (mostly in paediatric clinics), 3.7% were community-acquired; in 25.9% cases the source was unknown. The most common clinical features were fever (100%), rash (100%), conjunctival hiperemia (78.7%), cough (70.37%), micropoliadenopatia (64.8%), diarrhoea (39.81%). Pulmonary complications were described in 62.3% of cases; 22.38% of them were bacterial pneumonia, 77.62% were viral pneumonia. In 20.37% of cases we diagnosed acute stomatitis, in 12.96% bacterial conjunctivitis; in 14.81% of cases – otitis; in 1.85% of cases – pharingitis, and in one case (0.92%) – urinary tract infection. 7.4% of the patients were previously diagnosed and treated for pulmonary TB. All cases were confirmed serologically through detection of specific IgM antibodies. 25 patients (23.15%) had severe clinical forms of measels. The evolution was good in all cases.
**Conclusion:** 1. This year in the south-east part of the country, evolves a measels epidemic with different features comparing to the previous one (1997–1998).2. The ongoing epidemic is mostly hospital-acquired and it affects mainly infants and small children between 0–2 years old (62%), unvaccinated.3. The severe forms were present in approximately ¼ of patients.4. Clinical evolution was favourable in all cases.

## P1664

### Recombinant measles proteins, as the improved analogue of whole virus based antigen in ELISA to diagnostics reproach genotypes A and D6

M. Kulak, N. Netesova, P. Belavin, G.M. Ignatyev *(Novosibirsk, RU)*

**Objectives:** Whole virus based ELISA test systems have different sensitivity to various genotypes. Antigenic variability between different genotypes of measles virus (MV) has existed. We investigated the recombinant proteins NP and HN to develop new antigen with useful properties for applied in ELISA test systems.
**Methods:** Significant antigenic epitopes of nucleoprotein (NP) and haemagglutinin (HN) measles virus strain Edmonston were generated by computer analysis. Using standard gene-engineering techniques was evaluated two fusion peptides NP and HN consist from only linear T-cell antigenic determinants. The virus-neutralization activity of hyperimmune serum on recombinant proteins was determined by plaque reduction neutralization test (PRN). The level of specific IgG in serum to genotypes A, D4, and D6 of measles virus was determined by enzyme-linked immunosorbent assay (ELISA). We used recombinant proteins NP and HN as antigens for ELISA.
**Results:** Hyperimmune serum was collected from mice after immunization by NP and HN recombinant proteins. The level of neutralize activity was measured in the PRN assay with strain Edmonston. The titre reached up to 1:13.5 and 1:22.9 for NP and HN recombinant proteins, respectively. Interestingly that, hyperimmune serum on recombinant protein NP in ELISA reacted both with NP (titre 1:6400) and with HN (titre 1:3200), and in turn serum on recombinant protein HN reacted only with HN (titre 1:12800). The estimation immunological properties of proteins with use of the panel of serum (50 samples) collected from patients. The diagnosis of measles infection was confirmed in laboratory (by RT-PCR). The nucleotide sequences of RT-PCR products used for genotyping of MV. Selective interaction of antibodies in ELISA with recombinant proteins in relation to various genotypes is revealed. The interaction with genotypes A and D6 was expressed with high level of correlation whereas with genotype D4 any serum did not react authentically (as the control was used recombinant protein N of SARS virus).
**Conclusion:** We have shown that neutralize antibodies formed hot only on superficial proteins such as HN, F and SH but also on core proteins such as NP. Our data demonstrate that the recombinant proteins NP and HN could be a cost-effective alternative to current whole virus based ELISAs for surveillance for immunity to measles and could more efficient in detecting susceptibility to measles in relation to genotypes A and D6.

## P1665

### Intrauterine infection of Crimean-Congo haemorrhagic fever: the courses of two episodes

A. Celikbas, O. Ergonul, U. Yildirim, A. Zenciroglu, D. Erdogan, I. Ziraman, N. Yilmaz, F. Saracoglu, N. Demirel, O. Cakmak, B. Dokuzoguz *(Ankara, TR)*

**Objective:** The clinical courses of two pregnant patients with Crimean-Congo hemorrhagic fever (CCHF) and their children were presented.
**Method:** Two episodes were followed up in Obstetrics and Gynecology and Infectious Diseases clinics of Ankara Numune Education and Research Hospital, and Neonatology and Pediatric Surgery clinics of Dr. Sami Ulus Children's Hospital in Ankara, Turkey.

**Episode 1:** A pregnant woman with thirty-eight week of gestation was hospitalized in obstetrics clinic with the complaints of fever, malaise, and severe vaginal bleeding. On admission, white blood cell count was 6700/mm$^3$, haemoglobin was 11.5 g/L, platelet count was 8 000/mm$^3$. The level of AST was 591 IU, ALT 163 IU, lactate dehydrogenase 1079 IU, and creatinin phosphokinase 2132 IU. The baby was delivered by cesarean section. In serum CCHF IgM was positive by ELISA, and per oral ribavirin was administered after delivery. At the first day of delivery, the clinical and laboratory of findings of the baby were found to be normal. However, on his 5th day, he died because of massive bleeding. His CCHF IgM was found to be negative.
**Episode 2:** A pregnant woman with 19 week of gestation was admitted to the hospital. Her complaints were fever, malaise, headache, myalgia, nausea, vomiting, diarrhoea, and subconjuntival bleeding. In her laboratory investigation, white blood cell count was 3400/mm$^3$, haemoglobin level was 10.4 g/l and platelet count was 53000/mm$^3$. The level of AST was 813 IU, ALT 539 IU, and LDH 741 IU. In her serological analysis CCHF IgM and CCHF virus -PCR was found to be positive. At the twenty six week of gestation in obstetric ultrasound, fetal intraabdominal fluid was visualized and amniocentesis was performed. In serological analysis of amniotic fluid CCHFV-PCR was found to be negative. Intraabdominal fluid had increased and scrotal edema was visualized at thirty eighth weeks of the gestation. After her vaginal delivery, baby was severely ill and was operated with the diagnosis of necrotizing enterocolitis. His laboratory findings were normal except high white blood cell count. On his fifth day, thrombocytopenia occurred and he died because of massive bleeding. His CCHF IgM and PCR were negative.
**Conclusion:** To our knowledge, these are the first episodes of intrauterine CCHF infection. These episodes show that CCHFV can transmit through placenta. Obstetricians in endemic countries should consider CCHF infection among the patients with massive bleeding and thrombocytopenia.

## P1666

## The attack and the infection rate of Crimean-Congo haemorrhagic fever virus infection in an endemic region

O. Ergonul, H. Zeller, S. Menekse, A. Celikbas, S. Eren, N. Baykam, B. Dokuzoguz *(Ankara, TR; Lyon, FR)*

**Objective:** To detect the asymptomatic Crimean Congo Hemorrhagic Fever Virus (CCHFV) infections in an endemic area, and calculate the attack and the infection rate.
**Methods:** The study was performed in a CCHF endemic region. The household members of the index cases were screened for CCHFV IgG and IgM by ELISA. The data related to risk exposure were obtained by a structured form.
**Results:** Eleven index cases were admitted to the clinic, 45 household members of these cases were screened. All the index patients had positive IgM or PCR for CCHFV. Among the household members, three individuals had IgG positivity (%), and only one patient had IgM positivity. None of the screened individuals had symptoms. The mean age was 28 (sd 17), and 52% of the subjects were female. Tick bite was detected a risk factor ($p = 0.040$) for CCHV infection, whereas patient care and contact with body fluids of the patients were not ($p > 0.05$). Eighteen patients had the history of tick bite, and 8 became infected (44%), and five (27%) became ill. Among the infected eight individuals, five became ill (63%).
**Conclusion:** Although we consider that some of the patients do not notice tick bite, we can still suggest that the infection rate of the virus is rather high compared to similar diseases. Tick bite is the major risk factor, in comparison to exposure to blood and body fluids of the infected cases.

## P1667

## Dengue virus infections in children and adolescents

B. Larrú Martínez, E. Quiroz, M. Castrejón de Wong, E. Castaño Guerra, J. Nieto, O. Saldana de Suman, D. Estripeaut Calderon, X. Sáez-Llorens *(Madrid, ES; Panama, PA)*

**Objectives:** Study the prevalence of dengue virus infection on children in Panama and describe their clinical features.
**Methods:** We reviewed all the reports of dengue virus infection from January 2000 to August 2005. Epidemiological and clinical dates were recorded. Diagnosis was made with positive IgM antibody test or increase in serum IgG, 5 days after onset of symptoms or culture of the virus in the first fifth days of illness.
**Results:** 457 children were included in our study. Distribution according sex was: 57.6% female and 42.4% male. Median of age was 13 years (IQR = 6). During the follow-up study we recorded 2 years when the number of cases increased. The distribution of cases among the study was: 8.4% in 2000, 33.9% in 2001, 20.9% in 2002, 6.6% in 2003, 7.0% in 2004 and 23.1% in 2005. The proportion of paediatric patients also varied from; 11.9% in 2000, 9.6% in 2001, 12.4% in 2002, 9.7% in 003, 7.8% in 2004 and 4.4% in 2005. In Panama City we recorded 65.3% of the infants. We detected an increase in the number of patients in the rain season, from May till November. The mean of days between the onset of symptoms and the first blood sample was 6.3 days (DS: 6.7) A second sample was obtained in 23.6% of our infants with an average time of 10.1 days (DS: 7.4). The frequency of classical symptoms related to dengue virus infection was: fever (95.2%), severe headache (74.2%), chill (65.9%) rash (63.5%), myalgia (51.9%), retro-orbital pain (51.6%), arthralgia (43.3%), gastrointestinal symptoms (37.4%), inflamed pharynx (26.7%), cough (26.5%), mild respiratory symptoms (18.6%) and diarrhoea (10.7%). In our infants the symptoms which were detected first were; fever, severe headache, chill, myalgia, retro-orbital pain, arthralgia, mild respiratory symptoms, cough and inflamed pharynx. We did not observed differences on clinical features between girls and boys. However, we detected detected significative differences among symptoms when we compared infants who were ≤5 years old with those who were older ($p < 0.005$). Four of our patients died because of dengue hemorrhagic fever.
**Conclusion:** Dengue is endemic in Panama as in most tropical countries and is one of the worlds major emerging infectious disease. More data about this illness are needed to elaborate sanitary programmes which contribute to control this infection.

## P1668

## Diagnosis of dengue infection by enzyme-linked immunosorbent assay and reverse transcription-polymerase chain reaction from oral specimens

J. Pupaibool, S. Krajiw, K. Arunyingmongkol, A. Nisalak, C. Pancharoen, U. Thisyakorn, W. Kulwichit *(Bangkok, TH)*

**Objectives:** Dengue fever is among one of the major emerging infectious diseases. Polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) using non-blood samples are of diagnostic value for various infections including

dengue. Salivary ELISA has been shown by various investigators to be useful for dengue diagnosis. We sought to perform a pilot evaluation of diagnostic value of ELISA and PCR of oral brushes and saliva for dengue diagnosis in adults.
**Methods:** Adults with acute fever and suspected of dengue infection admitted to our university hospital were enrolled. Dengue diagnosis was made by standard ELISA using serum or plasma. Patients with negative ELISA served as controls. Buccal mucosal cells were collected for RT-PCR and saliva for both RT-PCR and ELISA at least twice, 7–14 days apart. Our ELISA criteria for saliva were single IgM > 20 units or single IgG > 60 units or 2-fold increase in IgG titre with the second titre >40 units for secondary dengue infection. Criteria for primary dengue infection were the same as secondary infection plus IgM:IgG ratio of over 1.8.
**Results:** 23 cases and 16 controls were enrolled. Our country is endemic for dengue and thus there was no primary dengue adult case in this study. As the study was performed in hospitalized patients, most of the first samples were collected one day before or on the day of defervescence. The specificities of either methods and the sensitivity of ELISA method for saliva were 100%. Sensitivities were approximately 32–33% for RT-PCR using buccal cells or saliva specimens. However, a combination of RT-PCR results for both types of oral specimens gave a sensitivity of 52%. The results are summarised in the table.

| Methods / Specimens | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|---|
| RT-PCR using buccal mucosal cells | 7/22 cases 31.82% | 16/16 cases 100.00% | 7/7 cases 100.00% | 16/31 cases 51.61% |
| RT-PCR using saliva | 7/21 cases 33.33% | 14/14 cases 100.00% | 7/7 cases 100.00% | 14/26 cases 53.85% |
| RT-PCR using saliva and buccal cells | 11/21 cases 52.38% | 14/14 cases 100.00% | 11/11 cases 100.00% | 14/24 cases 58.33% |
| ELISA using saliva | 23/23 cases 100.00% | 16/16 100.00% | 23/23 cases 100.00% | 16/16 cases 100.00% |

**Conclusions:** Collection of oral specimens is less invasive and may be more acceptable in certain situations. A single, acute specimen is adequate for diagnosis by RT-PCR. Our specimens, however, were collected late in the course of illness which affected the sensitivity of RT-PCR's. Earlier specimens may give a better yield. A study in paediatric patients is needed to assess the value of these methods for primary dengue infection.

## P1669

## Serologic evidence for Hantaviruses in the northern and eastern Tyrol (Austria) and the southern Tyrol (Italy)

G. Walder, A. Lanthaler, J. Simeoni, G. Morosetti, D. Schönitzer, O. Prinoth, M.P. Dierich, P. Kreidl *(Innsbruck, AT; Meran, Bozen, IT)*

**Objective:** The aim of this study was to assess the proportion of seropositives against Hantaviruses among healthy blood donors.
**Methods:** 1607 volunteer donors were recruited by the institute of transfusion medicine, representing the demographic situation in the Tyrol regarding gender and residence. Sera were tested for IgG with a commercially available ELISA. Positive samples were confirmed by a commercially available dot blot which was also used for identification of the serovar.
**Setting:** The study area comprises North Tyrol (Austria, north of the main ridge of the Alps), South Tyrol (Italy) and East Tyrol (Austria, both south of the main ridge of the Alps). South Tyrol belongs to the catchment area of the Etsch river, which drains into the Adriatic, while North- and East Tyrol are part of the catchment area of the Danube, which drains to the black sea.
**Results:** None of 617 samples from the Italian part of the study area yielded a positive result, wherein 7 of 988 donors of the Austrian part turned out to be seropositive. Two patients were positive for Hantaan, 3 patients were positive for Puumala, one patient was positive for Dobrava and one patient had antibodies against Hantaan and Dobrava. Only one of those patients reported extensive travelling abroad.
**Conclusions:** Evidence was found for the occurrence of Hantaviruses in the Austrian part of the region covering the catchment area of the Danube, but not in the Italian part of the study area covering the catchment area of the Etsch river. Seropositivity to Hantaviruses differs by hydrogeographic areas.

## P1670

## Molecular characterisation of a pantropic variant of canine coronavirus

N. Decaro, V. Martella, G. Elia, M. Campolo, C. Desario, M. Tempesta, C. Buonavoglia *(Valenzano, IT)*

**Objectives:** Canine coronavirus (CCoV) is an enveloped, single-stranded RNA virus, belonging to group I coronaviruses within the family Coronaviridae. Two different CCoV genotypes have been recognised, that are designated CCoVs type I and type II on the basis of their genetic relatedness to feline coronaviruses (FCoVs) type I and type II, respectively. CCoV is usually responsible for mild, self-limiting infections restricted to the enteric tract. We report the molecular characterisation of a pantropic variant of CCoV that caused fatal disease in pups.
**Methods:** CCoV type II strain CB/05 was isolated from an outbreak of fatal disease affecting seven dogs housed in a pet shop in Apulia region, Italy and characterised by fever, lethargy, inappetance, vomiting, haemorrhagic diarrhoea, neurological signs, and severe lesions in the parenchymatous organs. In all tissues, CCoV antigen was detected by immunoistochemistry and CCoV type II RNA was identified by genotype-specific real-time RT-PCR. The 3' end of the genome of strain CB/05 was determined by amplification of seven partially overlapping fragments. The PCR-amplified products were subjected to direct sequencing and the obtained nucleotide (nt) sequences were assembled and analysed using the BioEdit software package and the NCBI's and EMBL's analysis tools. GenBank accession number DQ112226 was assigned to the sequenced 8.7-kb fragment. The inferred amino acid sequences (aa) were compared to the analogous proteins available in the online databases.
**Results:** The structural proteins S, E, M, N of strain CB/05 displayed a high degree of aa identity to the cognate ORFs of CCoV type II, although the S protein showed the highest identity to type II FCoVs. While the nonstructural protein (nsp) 3a had the same length of known CCoVs, the nsp3b was 49-aa shorter than expected due to the presence of a 38-nt deletion at position 4704 and to a frame shift in the sequence downstream the deletion that introduced an early stop codon.
**Conclusions:** Association of strain CB/05 to a severe, fatal disease of dogs, together with virus isolation from organs with remarkable lesions, strongly suggests that this virus has changed the tropism, acquiring the ability to spread from the enteric tract to the internal organs. By sequence analysis of the viral genome, the only striking change was the truncated form of nsp3b, but the role of the deletion in the ORF3b in determining the patho-biological change deserves more in-depth investigation.

## P1671

## SARS coronavirus-like virus in Chinese horseshoe bats in Hong Kong

S.K.P. Lau, P.C.Y. Woo, K.S.M. Li, Y. Huang, H.W. Tsoi, B.H.L. Wong, S.S.Y. Wong, S.Y. Leung, K.H. Chan, K.Y. Yuen *(Hong Kong, HK)*

**Objectives:** To perform a surveillance study for SARS coronavirus (SARS-CoV)-like virus in non-caged wild animals from the wild of Hong Kong Special Administrative Region (HKSAR).
**Methods:** From summer 2004 to spring 2005, 127 bats, 60 rodents and 20 monkeys from 11 locations in HKSAR were captured. Nasopharyngeal and anal swabs and blood samples were collected and tested for SARS-CoV-like virus RNA by RT-PCR using conserved primers targeted to a 440-bp fragment of the RNA-dependent RNA polymerase (pol) gene. The complete genome of the SARS-CoV-like virus from bats (bat-SARS-CoV) was sequenced using RNA extracted from three anal swabs of three bats as template. Phylogenetic tree construction was performed using neighbor-joining method with GrowTree using Jukes-Cantor correction. Prediction of signal peptides and cleavage sites was performed using SignalP, transmembrane domains using TMpred and TMHMM, potential N-glycosylation sites using ScanProsite and protein family analysis using PFAM and InterProScan. Antibodies were detected using a recombinant bat-SARS-CoV nucleocapsid protein enzyme immunoassay and neutralization assay for human SARS-CoV.
**Results:** We identified a coronavirus closely related to SARS-CoV (bat-SARS-CoV) from 23 (39%) of 59 anal swabs of wild Chinese horseshoe bats by RT-PCR. Sequencing and analysis of three bat-SARS-CoV genomes from samples collected at different dates showed that bat-SARS-CoV is closely related to SARS-CoV from humans and civets. Phylogenetic analysis showed that bat-SARS-CoV formed a distinct cluster with SARS-CoV as group 2b coronaviruses, distantly related to known group 2 coronaviruses. Most differences between the bat-SARS-CoV and SARS-CoV genomes were observed in the spike gene, ORF 3 and ORF 8, which are the regions where most variations were also observed between human and civet SARS-CoV genomes. In addition, the presence of a 29-bp insertion in ORF 8 of bat-SARS-CoV genome, not in most human SARS-CoV genomes, suggests that it has a common ancestor with civet SARS-CoV. Antibody against recombinant bat-SARS-CoV nucleocapsid protein was detected in 84% of Chinese horseshoe bats using an enzyme immunoassay. Neutralizing antibody to human SARS-CoV was also detected in those with lower viral loads.
**Conclusion:** Our data support the existence of SARS-CoV-like virus in Chinese horseshoe bats in HKSAR.

## P1672

## Porcine caliciviruses in piglets in Italy

V. Martella, E. Lorusso, M. Camero, A. Lavazza, G. Pezzotti, G. Elia, N. Decaro, C. Buonavoglia *(Valenzano-Bari, Brescia, Perugia, IT)*

**Objectives:** Noroviruses (NoVs) (family Caliciviridae, genus *Norovirus*) cause diarrhoea in humans and animals. The NoV genome is 7.3–7.7 kb long with 3 open reading frames (ORFs). Noroviruses are genetically heterogeneous and form at least 27 genotypes within 5 genogroups, GI, GII, GIII, GIV, and GV, based on the capsid genes. Human NoVs cause an estimated 23 million cases of illness annually in the United States alone and >90% of nonbacterial epidemic gastroenteritis worldwide. Porcine calicivirus have been found to be genetically similar to human GII NoVs or to sapoviruses but calicivirus RNA has been detected at low frequency by RT-PCR in adults or fattening pigs. The close genetic relationships between human and porcine NoVs raise public health concerns regarding their potential for zoonotic transmission and as a potential source of new epidemic human strains.
**Methods:** A total of 46 faecal samples of nursing and weaning piglets with enteritis were collected during 2003–2005 in porcine herds in Italy. An additional 44 samples were include in the analysis, that had been collected during a rotavirus (RV) surveillance study in 2003–2005, all which tested positive to RV by electron microscopy and by RT-PCR. Viral RNA was extracted by the guanidine thiocyanate/glass milk method to eliminate enzyme inhibitors. Primer pair Con2-Con3, targeted to the VP4 outer capsid protein, was used for RV detection. A degenerated version of primer pair 289/290 was used for NV detection, that targets a conserved region in the RNA-polymerase.
**Results:** NoV RNA was detected in 20/46 of the screened samples, while RVs were detected in 24/46 samples. Mixed infections NoV+RV were found in 10 samples. Screening of the 44 RV positive samples allowed detection of 15 mixed infections with NoVs.
**Conclusions:** In previous investigations NoVs were detected in 4 of 1,017 normal slaughtered pigs in Japan, in 2 of 100 pooled pig faecal samples of 3- to 9-month-old fattening pigs in the Netherlands and in 6 out of 274 healthy adult and finisher pigs in the United States. Interestingly, in this study a high rate of positivity to NoVs (35/90) was found in nursing and weaning piglets with diarrhoea, a finding that may suggest a higher frequency of infection by NoV in young pigs or an association between NoV infection and occurrence of enteric disease. Altogether, these findings demonstrate that NoVs are common in porcine herds in Italy and provide new insights into the ecology of NoVs.

## P1673

## Detection of calicivirus genome in calves using Ni/E3 primers

M. Mahzounieh, T. Zahraeisalehi, E. Moghtadaei Khorasgani *(Shahrekord, Tehran, IR)*

Caliciviruses may cause a wide spectrum of disease in animals and are important etiological agent of viral gastroenteritis in humans. Members of the family caliciviridae are small nonenveloped viruses 27 to 35 nm in diameter. They possess a single stranded poly adenylated RNA genome. Caliciviruses have been isolated from mink, dog, cattle and non-human primates. "Norwalk-like viruses" (NLVs) are the most common cause of acute non-bacterial gastroenteritis in humans. Cattle may be a reservoir of NLVs although never bovine NLVs have been found in humans. In this study, we try to detect enteric caliciviruses genome from 50 faecal samples of 6 dairy cattle herds in Shahrekord area using Reverse transcriptase polymerase chain reaction (RT-PCR) assays specific for NLVs found in humans. The primers used for PCR amplification were Ni and E3, which amplify a 113-bp product for the detection of both genogroups I and II SRSV RNA in fecal material. Our results showed that nine specimens (18%) were positive. These findings suggest that calicivirus infection is endemic in dairy herds in Shahrekord, Iran and may be have an important role in calf diarrhea.

## P1674

### Assessment of reovirus epidemiology in dogs

G. Elia, M.S. Lucente, A.L. Bellacicco, M. Camero, N. Cavaliere, N. Decaro, V. Martella *(Valenzano, Foggia, IT)*

**Objectives:** Reoviruses are non-enveloped, 10-segmented dsRNA viruses. In humans and mammalians three distinct serotypes exist, whose prototypes are strains Lang (T1), Jones (T2) and Dearing (T3). Although reoviruses have been isolated both from the enteric and respiratory tract, no diseases has been clearly associated to reovirus infection in humans. The potential association with extra-hepatic biliary atresia, myocarditis, and, above all, neurological and cutaneous diseases require further investigations. Reoviruses are ubiquitous and scarcely species-specific. Reovirus identification is usually based on electronic microscopy or gel electrophoresis and reovirus incidence seems to be very low in humans and most mammalians. In this study, we investigated the presence of reoviruses in dogs by means of molecular methods.
**Methods:** One hundred ninety-two rectal samples from dogs with diarrhoea, 12 ocular swabs, 19 nasal swabs and 27 oropharyngeal swabs from dogs with ocular/nasal discharge were subjected to an RT-PCR assay targeting a conserved region of viral genome segment L1 (primers L1-rv5/L1-rv6). Positive samples were characterised by polyacrylamide gel electrophoresis (PAGE), serotype-specific RT-PCR assays targeting segment S1 and sequence analysis. To increase the sensitivity, a nested PCR using primers L1-rv7/L1-rv8 was performed on samples tested RT-PCR negative.
**Results:** Only 4 faecal swabs (2.1%) were found positive (RT-PCR product of 416 bp). By using a serotype-specific RT-PCR assay and/or sequence analysis, two strains was characterised as type 3 and the other ones as type 1. PAGE of viral dsRNA confirmed the genetic characterisation. Unexpectedly, in second-round PCR 50 faecal samples (26%), 9 ocular swabs and 10 nasal swabs yielded a 344 bp product, while no oropharyngeal swab was positive.
**Conclusions:** These data suggest a wider distribution of reoviruses in dogs than previously thought, even if most reovirus infections were detected only by nested PCR. The ability of reoviruses to induce disease in dogs, alone or in synergism with other pathogens, is still unclear, since attempts to reproduce a specific disease in germ-free dogs have given contradictory results. Due to their poor species-specificity, reoviruses may be easily transmitted from animals to humans (and vice-versa). Further studies are required to understand reovirus ecology and their potential zoonotic impact.

## P1675

### Molecular epidemiology of parvovirus B19 in Slovenia

T. Ursic, M. Petrovec *(Ljubljana, SI)*

**Objectives:** Parvovirus B19 is a member of a family Parvoviridae. On the basis of genetic distances and evolutionary relationships, human parvoviruses are divided into three genotypes: genotype I corresponding to B19-related isolates, genotype II to Lali-related isolates, and genotype III to V9-related isolates. Parvovirus B19 causes a common exanthematous disease in childhood or adult age, arthropathy, hydrops fetalis, various haematological disorders and myocarditis. Up to now, we have had no data of the prevalence of B19 virus in Slovenian population. Consequently, we also lack information on the genotypes of parvovirus B19 that are involved in the patients who suffer from the infection.
**Methods:** To gather information of the genetic variants of B19 virus present in Slovenia, we extracted DNA from 48 serum samples that were sent for serologic diagnostic of parvovirus B19 infection and were positive for specific IgM in the period from January 1997 to June 2005. Nearly half of all 48 patients were children and young adults up to 25 years. The NS1 region of parvovirus B19 was amplified by the nested PCR (primers PB19F1, R1 and PB19F2, R2). All PCR products were directly sequenced.
**Results:** The results of our study show that DNA of parvovirus B19 was present in all 48 samples that tested positive for specific IgM antibodies. After the first round of PCR reaction, 17 samples were positive, and after the second reaction, all 48 samples were positive. Altogether 12 unique genotype variants of parvovirus B19 were identified and all were clustered in the genotype I group of B19-related isolates. Most of the distinct 12 genetic variants differed in 1% to 2% from the sequences deposited in Gene Bank. The majority of sequences obtained from the B19 virus epidemic in 1998 represents a single variant of genotype I with the Gene Bank acc. no. AJ781038. We also found that different genetic variants of parvovirus B19 were circulating in 2002 and were 100% or 99% identical to the genotype I variant with the Gene Bank acc. no. Z68146. In our study, we were not able to identify any variants of other rare genotypes (Lali or V9).
**Conclusion:** Parvovirus B19 DNA was successfully amplified from all IgM positive serum samples of the patients. The genotype I of parvovirus B19 is dominating in infections with parvovirus B19 in Slovenia.

# Bacterial pathogenesis – III

## P1676

### Characterisation of human brucellosis in Great Britain by classical typing and VNTR molecular typing

P. Groussaud, S.D. Brew, A.M. Whatmore, J.A. Stack *(New Haw, UK)*

**Objectives:** Great Britain has been free of animal brucellosis since 1985 (European Commission Decision 93/52). The main source of infection for UK residents is through contact with infected material in foreign countries. The objective of this study is to type human samples received in the UK since 1999 using Variable Number Tandem Repeats (VNTR) molecular typing to confirm results obtained by classical typing and relate these results to the suspected source of infection.
**Methods:** Classical typing is traditionally used and is based on the phenotypic attributes of each strain and biovar. VNTR typing is a recently developed molecular method, which is based on short repeats contained in the DNA that can be amplified to give a banding pattern specific to each strain.
**Results:** Results found using both methods are consistent. The results show geographical differences, consistent with observations of strain genotype distribution found in animal brucellosis.
**Conclusion:** Patient history has been gathered where possible giving information on recent travels. Along with results found

by classical typing and confirmed by VNTR typing we can draw a picture of the sources of infection. These results illustrate the potential of VNTR typing as a tool to aid conventional approaches to epidemiological traceback that, in the presence of a suitably comprehensive database of strain genotypes, could help identify the source of an infection.

## P1677

## IS711-fingerprinting of *Brucella* isolates from humans

E.Stubberfield *(Surrey, UK)*

**Objective:** Brucellosis is a zoonotic disease usually associated with cattle, sheep, goats and pigs. Human infection has been attributed to *B. melitensis*, *B. abortus*, *B. suis*, *B. canis* and *B. maris*. Although the UK is officially bucellosis free there are a number of human cases due to travel and occupation that are submitted to our laboratory for diagnosis. Definitive diagnosis of *Brucella* is by bacteriological culture and microbial tests (classical typing), however these require skilled personnel and the results can be subjective. There are a number of molecular tests that have been developed to assist with diagnosis more rapidly and in some cases to strain level less subjectively. IS711-fingerpinting is a molecular technique that has proved useful for the identification of *Brucella* isolates to species and in some cases stain level. IS711-fingerprinting relies on the variable number and location of the IS711 mobile genetic element found in all *Brucella* isolates.
**Method:** 73 *Brucella* isolates from humans have been tested. Genomic DNA was extracted, digested using restriction endonuclease *Eco*R1, and electrophoresed. Southern blotting was performed, hybridising with a DIG-labelled IS711 probe.
**Results:** The number of *Brucella* IS711 copies range from 5 to more than 20. *Brucella melitensis* remains the most commonly acquired *Brucella* species of travellers, while occupational infections have included *B. abortus* isolated from cattle farmers and *B. suis* associated with pig butchers. Two marine *Brucella* strains have been isolated originating from an occupational perspective (a laboratory worker) and a natural setting from an unknown source. 7 unusual patterns have been observed, 5 of which are unique. One of the new patterns has been observed only in isolates originating in East African countries.
**Conclusion:** Although the diagnosis of *Brucella* to species and strain level is not essential for the treatment of human Brucellosis, it is useful for epidemiological studies. IS711-fingerprinting is able to identify the three biovars of *B. melitensis*, many other techniques do not offer this capability, because of this it may be a useful test in epidemiological studies. This method remains an important diagnostic tool for *Brucella* identification.

## P1678

## Rapid diagnosis of brucellar epididymo-orchitis by real-time PCR assay in urine samples

J.D. Colmenero, M.I. Queipo-Ortuño, J.M. Reguera, N.L. Muñoz, A. Villalobos, A. Plata, P. Morata *(Malaga, ES)*

**Objectives:** To study the diagnostic yield of a real-time PCR assay in urine samples for the rapid diagnosis of brucellar epididymo-orchitis, in comparison with conventional microbiological techniques.
**Methods:** Ten consecutive patients with brucellar epididymo-orchitis were included in the study. The diagnosis of brucellosis was established according to one of the following criteria: first, isolation of *Brucella* spp in blood or any other body fluid or tissue sample or, second, the presence of a compatible clinical picture together with the demonstration of specific antibodies at significant titers or seroconversion. Epididymo-orchitis was diagnosed in patients with scrotal enlargement, swelling and pain not due to other causes.For DNA amplification we used a SYBR Green I LightCycler-based real-time PCR assay. The assay amplifies a 223 bp sequence of a gene that codes for the synthesis of an immunogenetic membrane protein (BCSP31). The pair of 21 nucleotide primers B4 (5′ TGG CTC GGT TGC CAA TAT CAA 3′) and B5 (5′ CGC GCT TGC CTT TCA GGT CTG 3́) were used in the amplification process. After DNA amplication, we performed melting curve analysis to verify the specificity of the PCR products. In order to study the specificity of the technique, all the samples from the patients with brucellosis were paired with an equal number of samples from controls with urinary tract infection. (*E. coli* four cases, *K. pneumoniae* two cases, *P mirabilis* two cases, and *C. freundii* and *P. aeruginosa* one of each).
**Results:** The mean age was 38.4 years (range 21–69). The duration of the symptoms prior to diagnosis was 30.2 ± 16.2 days (range: 5–51). *B. melitensis* was isolated from blood cultures in nine cases (90%). Wright's seroagglutination was negative or inconclusive in 30% of cases. *Brucella* was isolated from urine in only one case whereas real-time PCR assay in urine was positive in nine (90%) cases and the results were available in four hours, whereas the mean time to availability of the final blood culture results was 5.8 days (range 4.5–7 days). Real-time PCR was negative for all the control samples from patients with urinary tract infections.
**Conclusion:** SYBR Green I LightCycler-based real-time PCR assay in urine samples is highly sensitive and specific, easy to perform and could provide the clinician with the results in under five hours. The technique could be a practical and useful tool for the rapid diagnosis of genitourinary complications of human brucellosis.

## P1679

## Molecular diagnosis and characterisation of human isolates

C.E. Dawson *(Weybridge, UK)*

**Objectives:** Although the United Kingdom remains Brucellosis-free, there are more than 500,000 new cases of Human brucellosis reported each year according to the World Health Organisation. UK residents returning from worldwide travel may have encountered exposure through contact with infected animals and animal products such as dairy produce and meat. Phenotypic characterisation or classical methods remain the definitive diagnosis though require skilled personnel and have their limitations. The increasing range of molecular techniques can aid the rapid detection and characterisation of *Brucella* species and their biovars and may have significance in Epidemiological studies.
**Methods:** A study of *Brucella* reference and field strains of mainly human isolates from different geographic locations were analysed for diversity of their genes encoding the outer membrane proteins (omps) 25, 2a and 2b. PCR products of the three genes digested with seven restriction enzymes were analysed for polymorphisms.
**Results:** A re-occurring unique pattern profile seen only in human isolates was observed originating in some European countries and beyond. A growing database of strain types giving a recent overview of *Brucella* infection of humans of many countries.

**Conclusions:** Molecular typing methods may have an advantage over classical typing concerning *Brucella melitensis*, the most common *Brucella* infection of humans. The characterisation of human *Brucella* isolates may be useful in Epidemiological studies for a variety of purposes.

## P1680

## Real-time detection of *Campylobacter jejuni* and *Campylobacter coli* from environmental samples and single nucleotide polymorphism profiling of map A positive strains to determine their clonal complexes

S. Lai, E. Best, S.B. Surman-Lee, R.J. Owen, J.V. Lee *(London, UK)*

**Objectives:** A study to demonstrate the rapid detection and speciation of *Campylobacter jejuni* and of *Campylobacter coli* isolates directly from enrichment broth using a Taqman® assay. Single nucleotide polymorphism analysis of mapA positive strains was used for rapid identification of *C. jejuni* clonal complexes.
**Methods:** Thermotolerant *Campylobacter* species were initially confirmed by culture according to the modified draft ISO 17995 method, where water samples were filtered through 0.2 mm pore size nylon membrane. The filters were transferred to selective enrichment in Preston broth to improve their recovery and therefore detection of any *campylobacter* cells present. DNA was extracted directly from the enrichment broth culture for real-time detection of *C. jejuni* and *C. coli* using the Taqman®. Samples, which were map A positive were, further characterise by single nucleotide polymorphism profiling for rapid recognition of *C. jejuni* clonal complexes.
**Results:** Environmental samples, which were confirmed by culture were also map A positive by Taqman®. SNP profiling of mapA positive isolates identified clonal complexes, which are predominantly contained in isolates of human disease and chicken.
**Conclusions:** This study has demonstrated the feasibility of rapid detection and identification of *C. jejuni* and *C. coli* following short enrichment incubation using a Taqman® assay. A rapid turnaround time of between 3–4 h per batch of 96 samples was achieved. SNP profiling offers important epidemiological grouping at strain level, enabling accurate and phylogenetically valid strain identification for *C. jejuni*, which may have important host associations for tracing sources of infection and consequently improve public health responses.

## P1681

## Species identification of human *Campylobacter* strains

U-M. Nakari, A. Koivumäki, A. Siitonen *(Helsinki, FI)*

**Objectives:** *Campylobacter jejuni* and *C. coli* are recognized as the most common causes of acute bacterial gastroenteritis in humans, *C. jejuni* being the predominant species in most developed countries. The hippurate hydrolysis test is widely used to differentiate *C. jejuni* from other *Campylobacter* species. About 10% of *C. jejuni* isolates fail to hydrolyze hippurate under laboratory conditions. Molecular methods represent an alternative to the phenotype-based methods. We tested two multiplex PCR assays for species identification of human *campylobacter* strains and compared the results with the hippurate hydrolysis test.
**Methods:** *Campylobacter* strains isolated from patients were tested for hippurate hydrolysis with Rosco diagnostic tablets. 50 hippurate-negative and 15 hippurate-positive strains were selected for two multiplex PCR assays. One PCR-method was based on distinctive ceuE-genes of *C. jejuni* and *C. coli*, the other PCR-method detected genes from five major clinically relevant *Campylobacter* species: hipO from *C. jejuni*, glyA from *C. coli*, *C. lari* and *C. upsaliensis*, sapB2 from *C. fetus* subsp. *fetus*, and 23S rRNA gene from *Campylobacter* spp. as an internal validation control.
**Results:** The *C. jejuni* hipO gene was detected in all of the 15 hippurate-positive strains and 10 of the 50 hippurate-negative strains. The *C. coli* glyA was detected in 39 of the hippurate-negative strains. In one hippurate-negative strain, sapB2 from *C. fetus* subsp. *fetus* was detected. Species-specific genes were detected in 53 of the 65 strains with the ceuE-based PCR assay. *C. jejuni* ceuE was detected in 12 hippurate-positive and 5 hippurate-negative strains. *C. coli* ceuE was detected in 36 hippurate-negative strains.
**Conclusion:** All hippurate-positive strains were identified as *C. jejuni*. Of the hippurate-negative strains, 78% were identified as *C. coli*, whereas 20% were identified as *C. jejuni* and one strain as *C. fetus* subsp. *fetus*. The results of the two PCR assays were concordant, although some strains could not be identified with the ceuE-based PCR assay. The results suggest that molecular species identification should be performed on hippurate-negative strains after the hippurate hydrolysis test for accurate species identification. Multiplex-PCR is quick and easy to perform. Using the PCR assay that simultaneously detects five *Campylobacter* species also diminishes the need for further phenotypic testing.

## P1682

## Phenotypic typing of *Cryptosporidium* species isolated from children in Kuwait: a role in unique transmission

J. Iqbal, P. Hira *(Safat, KW)*

**Background:** Cryptosporidiosis is recognized worldwide as a significant cause of diarrhoeal diseases in both adults and children especially in children less than 2 years of age.
**Objective:** *Cryptosporidium* spp. isolated from young children in Kuwait were characterized at the molecular level to understand the transmission of infection. The study was approved by the Ethical Committee, Faculty of Medicine, Kuwait.
**Methodology:** Over a period of 2 years, faecal specimens from 97 Kuwaiti children with persistent diarrhoea found to be positive for *Cryptosporidium* spp. by microscopy were genotyped and sub-typed with a small subunit rRNA-based PCR-restriction fragment length polymorphism analysis. Informed consent was taken from all individuals included in the study.
**Results:** The median age of infected children was 4.9 years, and the majority of the infections (>70%) occurred during the cooler months January–April, indicating a marked seasonal variation. More than 85% of the children with cryptosporidiosis had only *Cryptosporidium* infection. Socio-demographic information did not reveal any particular mode of transmission of infection. Genotyping of the organisms isolated showed that ninety-two (95%) of the children had *C. parvum*, 4 (4%) had *C. hominis*, and 1 (1%) had both *C. parvum* and *C. hominis*. Altogether, 9 subtypes of *C. parvum* and *C. hominis* were observed.
**Conclusion:** Our study revealed a very different distribution of *Cryptosporidium* genotypes in Kuwaiti children as compared to other tropical countries. The genotypes and subtypes isolated are discussed with relation to the seasonality and possible mode of transmission of this infection in Kuwait.
**Acknowledgements:** The financial support for this study was provided by Kuwait University Research Grant 03/03.

## P1683

## *Chlamydophila pneumoniae* in patients undergoing aortic valve replacement

M. Edvinsson, Y. Molin, E. Hjelm, S. Thelin, G. Friman, C. Nyström-Rosander *(Uppsala, SE)*

**Objectives:** The intracellular respiratory pathogen *Chlamydophila pneumoniae* (Cp) might be involved in the pathogenesis of atherosclerosis. Several studies have demonstrated a serological association between Cp and cardiovascular disease and DNA from the bacteria has been found in various atheromatous vessels. After infection in the respiratory tract, Cp is believed to be disseminated systemically within alveolar macrophages. The prevalence of Cp within peripheral blood mononuclear cells (PBMC) has in some studies been shown to be higher in patients suffering from cardiovascular disease than in control patients. We investigated the presence of Cp DNA in aortic heart valves and PMBC in 55 patients (37 men; 18 women; mean age 69 years) undergoing aortic valve replacement because of aortic stenosis. Also, the presence of Cp mRNA was investigated in the sclerotic aortic heart valves as a marker of viable bacteria.
**Methods:** DNA was extracted from aortic valve biopsies and PBMC using the QiaAmp DNA mini kit (Qiagen). mRNA and DNA were extracted from another piece of the same biopsy using Trizol (Invitrogen). Real-time PCR directed against the *chlamydia* MOMP gene was used to detect Cp-specific DNA and mRNA. Patient sera were tested for Cp-specific IgM, IgG and IgA antibodies by the microimmunofluorescence technique.
**Results:** Cp DNA was found in aortic heart valves from 20% (11/55) of the patients and in PBMC from 5% (3/55) of the patients. In one patient Cp DNA was found in both PBMC and heart valve. No patient had Cp-specific IgM antibodies. In patients that were PCR-positive for Cp DNA in the aortic heart valves, 73% had IgG ≥1:64 and 45% had IgA ≥1:32. In patients that were PCR-negative in the aortic heart valves, 59% had IgG ≥1:64 and 23% had IgA ≥1:32. Cp-specific mRNA in aortic heart valves will be presented on the poster.
**Conclusion:** Cp-specific DNA was found in sclerotic aortic heart valves from 20% of patients undergoing aortic valve replacement. This confirms previous investigations supporting a role for Cp in the pathogenesis of aortic valve sclerosis. The prevalence of Cp in PBMC was 5% which is comparable to that reported in healthy blood donors and lower than that recorded in patients suffering from other cardiovascular diseases. If the bacteria are involved in the pathogenesis of aortic sclerosis they have likely been spread to the aortic valve long before the patient is in need of surgery because of the stenotic valve.

## P1684

## Molecular epidemiology of diarrheagenic *E. coli* in children with acute diarrhoea in Tehran, Iran and the antibiotic susceptibility of isolated strains

F.Jafari, S.Salmanzadeh-Ahrabi, M.M.Aslani, F.Baghbani-Arani, M. Azimi-Rad, M.R. Zali *(Tehran, IR)*

**Introduction:** Diarrhoea is one of the most common causes of morbidity and mortality among young children in developing countries. Diarrheagenic *E. coli* strains include several emerging pathogens of worldwide public health. Six important categories are Entero-aggrigative *E. coli* (EAEC), Entropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enterohemorrahgic *E. coli* (EHEC), Entroinvasive (EIEC) and Shigatoxin-producing *E. coli* (STEC). This study investigated the role of different diarrheagenic *E. coli* in Iranian children with acute diarrhoea by molecular methods and the antibiotic susceptibility of isolated strains.
**Methods:** From April 2003 to January 2005, one thousand eighty five children with acute diarrhoea in Tehran hospitals in were enrolled in the study. The fecal samples were cultured on MacConkey for conventional bacterial pathogen and Sorbitol MacConkey agar for Non sorbitol fermenting phenotype, than they were incubated in 37ordm;C. The primary stool cultures were subjected to six different PCR reactions targeting stx1 and stx2 gene, heat-labile enterotoxin (LT) producing gene, heat-stable enterotoxin (ST) producing gene, eae gene and pCVD432 plasmid. The Kirby –Bauer disc diffusion method was used for antibiogram of 110 isolated strains from different diarrheagenic *E. coli* by 12 different antibiotics.
**Results:** Two hundred seventy one diarrheagenic *E. coli* strains were detected. STEC was the most prevalence with 125 (46.1%). The frequency of other strains was 28.7%, 16.3%and 8.8% for ETEC, EAEC and EPEC, respectively. Out of 120 STEC isolated 86 strains (% 68.8) had stx1 or stx2 gene, and 4 strains had stx1 and stx2 gene. The eae gene was found in 13 (10.4) STEC strain. Out of 110 tested strains, 102(92.5%) were resistance to ampicllin and cefalotin, and 99 (90%) to streptomycin.
**Conclusion:** In this study STEC was the most frequent associated with diarrhoea. The strong association between use of antibiotics and colonization with antibiotic resistant *E. coli*, suggest a major role for selection of resistant strains while using antibiotics. The existence of other unknown intestinal adherence factors has been suggested by the isolation of STEC strains that lack the eae gene but are still associated with bloody diarrhoea or hemolytic ureamic syndrome (HUS). Since there is no specific treatment, there is an urgent need for effective preventive measures based on detailed understanding of the epidemiology of STEC infections.

## P1685

## Identification of Shiga toxin-producing *Escherichia coli* in raw beef using DNA hybridization with digoxigenin-labelled probes and multiplex PCRs

M. Weiner, J. Osek *(Pulawy, PL)*

Shiga toxin-producing *Escherichia coli* (STEC) is an important cause of bloody diarrhoea, haemorrhagic colitis, haemolytic uremic syndrome and thrombotic thrombocytopenic purpura. Transmission of STEC occurs through consumption of contaminated food, especially meat, dairy products and water.
**Objectives:** To develop a three-steps procedure based on two multiplex PCRs and DNA hybridization with digoxigenin-labelled probes for identification of STEC in raw beef.
**Methods:** Beef samples inoculated with different number of *E. coli* O157:H7 cells were incubated in TSB medium at 37°C for 6 h. The cultures were then transferred to TSB with mitomycin C and incubated for another 18 h. The resulted cultures were used as a source of DNA template. The mPCR-1 was established to identify Shiga toxins genes (conserved sequence). The positive culture samples were subjected to DNA hybridization with DIG-labelled probes as follows: the culture was diluted and inoculated onto agar plates supplemented with Tergitol® and incubated at 37°C for 18 h. Then, the nylon membranes were put on agar plates, carefully removed and incubated in denaturation, neutralisation and equilibration solutions following incubation with the stx-specific DIG-labelled probes, anti-DIG conjugates and finally developed with enzyme substrates (BCIP and NBT). Dark spots visible on the membranes were

compared with the respective bacterial colonies on the original agar plates. The corresponding bacterial colonies were isolated and characterized using the mPCR-2 test which allows amplification of stx1 (Shiga toxin type 1), stx2 (Shiga toxin type 2), rfbO157 (*E. coli* O157) and fliCH7 gene (H7 antigen). An internal control of amplification (*E. coli* 16S rRNA gene) was also included in both mPCR tests.
**Results:** The first mPCR resulted in two amplification products: 230 bp for stx and 401 bp for 16S rRNA genes. The positive meat samples were further tested with DNA probes and positive colonies were then characterized with the second test (mPCR-2), generating the amplicons either of 348 bp (stx1), 584 bp (stx2), 420 bp (rfbO157), 247 bp (fliCH7) or 798 bp (16S rRNA). The specificity of this procedure was confirmed by testing *E. coli* O157:H7, O157:H-and non-STEC bacteria. The sensitivity of the method was estimated as 4 CFU/g of meat.
**Conclusion:** The obtained results demonstrated the high specificity of the procedure developed and the possibility of using it for identification of Shiga toxin-producing *E. coli* in raw beef.

## P1686

## Correlation between virulence pattern, phylogenetic group and extended spectrum betalactamases genes in *Escherichia coli* strains isolated from blood cultures

M. Damian, C. Usein, D. Tatu-Chitoiu, S. Ciontea, D. Jardan, A. Palade *(Bucharest, RO)*

*E. coli*, heterogeneous species consisting of commensal and pathogenic strains, is causing a broad spectrum of human diseases, including extra intestinal and enteric infections.The strains isolated from invasive infections were documented to be carriers of a large number of genetic structures coding for virulence, as well as for resistance to antimicrobial agents. The aim of this study was to evaluate the virulence of strains in comparison with the presence of ESBL genes and their distribution among the different phylogenetic groups. A total of 51 *E. coli* strains, isolated from blood cultures, in hospitalised patients, adults and children, were screened for virulence factors-encoding genes (pap, sfa/foc, afa, hly, cnf, aer and fimH), for genes encoding resistance to extended spectrum betalactam antibiotics (bla SHV and bla TEM genes) and the appurtenance to one of the main four phylogenetic group based on presence or absence of markers chuA, yjaA and TSPE4.C2 Three strains, negative for all virulence genes, were included in the phylogenetic group A. Ten strains, which were positive for five or six virulence genes, were identified as B2 group. No matter the phylogenetic grouping, the remaining strains possessed at least one virulence gene. No strain was PCR positive for all seven virulence genes targeted. Among the 16 strains which were positive in the double disk test, 12 strains exhibited both bla SHV and bla TEM genes and 4 strains only bla TEM gene. Restriction with Pst I and Dde I and sequencing of the amplicons were performed in order to identify the type of ESBL gene expression product. Taking into account the link between phylogenetic group and virulence, we obtained a good correlation for the bacteremic *E. coli* strains analysed, but there was no relationship with the production of ESBLs.

## P1687

## Isolation of shiga-toxin producing *Escherichia coli* from meat samples, phenotypic and genotypic characterisation of isolated strains

F. Baghbani-Arani, F. Jafari, M.R. Zali, S. Salmanzadeh-Ahrabi *(Tehran, IR)*

**Objective:** Shiga-toxin producing *Escherichia coli* (STEC) is an emerging foodborne pathogen of worldwide public health importance. This bacterium has been reported as an etiological agent of many outbreaks and sporadic cases. Definition of the diversity and antimicrobial susceptibility of (STEC) may be helpful in the management of sporadic cases and outbreaks. Studies in different countries show that food items maybe contaminated by this pathogen. The present study was carried out to determine the frequency of contamination of meat samples by STEC collected in Tehran as well as defining genotypes, serotypes, antibiogram susceptibility patterns and molecular diversity of isolated bacteria.
**Methods:** From July 2004 to June 2005, 250 beef samples were collected from different part of Tehran. A 25 grams of each samples was enriched in EC broth and subculture on Mac-Conkey agar. DNA was extracted from a loop full of bacteria taken from primary first streaking area of Mac-Conkey agar and was subjected to three different PCR reactions targeting stx1, stx2 and eae genes. As much as colonies required were tested for finding the colony responsible for positive results in the first PCR. Antibiogram susceptibility patterns of isolated strains were determined by standard disk diffusing method. The 17 antimicrobial agents were used at this study. All isolates were serotyped by slide agglutination test using standard antisera (MAST Groups) Subtyping of strains was done with RAPD-PCR by 1283primer.
**Results:** Among 250 samples, 47 (19%) samples were positive and their genotypes were as follow: 1(0.4%) stx1+, stx2-, eae-, 35(14%) stx1-, stx2+, eae-, 6(2.4%) stx1-, stx2+ and eae+.4 (1.6%) stx1+, stx2+, eae-, 1(0.4%) stx1+, stx2+, eae+. Among these positive samples 30 strains were isolated. According to the antibiotic susceptibility tests, all isolates were resistance to erythromycin (E) and oleandomycin (OL), and were sensitive to imipenem (I); gentamicin (G) norofloxazin (Nx) enterofloxazin (Ex) ciprofloxazin (Cf) and ceftazidim (ca). In Otyping and Htyping the most frequency were O112ac and H2 serotypes. Analysis of isolates by RAPD-PCR yielded 17different patterns.
**Conclusion:** Our results show that contamination of meat samples by STEC is a life-threatening health problem. Combinational analysis of Antibiogram susceptibility patterns and serotypes with RAPD-PCR patterns can aid to survey the characteristics of STEC strains.

## P1688

## Factors affecting the conjugative transfer of plasmid pIP501 in *Enterococcus faecalis*

A.M. Al-qurashi *(Dammam, SA)*

**Objectives:** Factors which are known to influence plasmid transfer were studies using the conjugative plasmid pI501, which encodes erythromycin resistance, in *Enterococcus faecalis*.
**Methods:** The donors strains *streptococcus a agalactiae* V666 (group B) is resistant to in rifampicin and fusidic acid, non hemolytic and b-lactamase-negative. It contains the broad host range plasmid pI501, which confers resistance to erythromycin and chloramphenicol. The recipient is *Enterococcus faecalis* strain JH2-2 Group D.
**Results:** Transfer of pIP501 occured on a agar, on filters and in broth cultures at relatively high densities (106–108 bacteria/ml).

Transfer frequency was largely unaffected over a wide range of temperatures (23–42°C). The pH of the medium, in the range pH 5–9 had little effect on the transfer frequency. Log phase cultures and donor: recipient ratios of 1:1000-100:1 were required for optimal for plasmid transfer.
**Conclusion:** Factors which modified the transfer efficiency of the conjugative plasmid pIP501 were mating media, solid or liquid environment, mating time, mating temperature, selection temperature, growth temperature of donor and recipient of prior to mating, pH culture age, and donor/recipient cells ratio, to obtain a better understanding of this plasmid and its transfer process will help understand what role they may have in the dissemination resistance among streptococcal and enterococcal populations.

## P1689

## Presence of seven virulence genes in Swedish *Enterococcus faecium* blood-culture isolates collected during a five-year period

H. Billström, Å. Sullivan, B. Lund *(Stockholm, SE)*

**Background:** *Enterococcus faecalis* and *Enterococcus faecium* have during the last years become a significant nosocomial problem. This could be due to the *enterococcus* hardy nature combined with intrinsic and acquired antibiotic resistance. Since most individuals harbour *enterococci* in their normal intestinal microflora there has been a discussion regarding the origin of these isolates. During the last ten years the isolation ratio between *E. faecalis* and *E. faecium* have shifted from 10:1 to 3:1. This could be because of increasing antibiotic resistance among infectious *E. faecium* isolates compared to infectious *E. faecalis* ones. It is possible that this increase also depends upon different virulence genes such as enterococcal surface protein (esp), hyaluronidase variant gene (hylEfm) and *E. faecalis* antigen A variant (efafm).
**Objectives:** The objectives in this study were to determine the presence and frequencies of seven different enterococcal virulence genes in infectious isolates. Further objectives were to see if the number of virulence genes in these isolates vary or increase over time.
**Methods:** A total of 257 strains isolated from bacteraemia patients during year 2000–2005 at the Karolinska University Hospital, Huddinge were used. All isolates were screened for seven different virulence genes using a multiplex PCR. These seven virulence genes were aggregation substance (asa), cytolysin (cyt), collagen binding protein (ace), *E. faecalis* antigen a variant (efafm), enterococcal surface protein (esp), gelatinase (gel) and hyaluronidase (hyl).
**Results:** According to the results about half of all isolates were esp-positive. The prevalence of the other virulence genes asa, efafm, gel and hyl were detected, but in low frequencies (<5%).
**Conclusion:** It seems like the esp gene is the most dominant virulence gene in *E. faecium* isolates. The occurrence of virulence traits in these isolates further indicates that the potential to cause infection is potentiated among this enterococcal population The data from this investigation supports the hypotheses that *enterococci* causing infection in hospitalized patients are probably of nosocomial origin rather than endogenous.

## P1690

## Transcriptomic analysis of the alkaline-tolerance response in *Listeria monocytogenes* 10403S

E. Giotis, A. Muthaiyan, D. McDowell, I. Blair, B. Wilkinson *(Newtonabbey, UK; Normal, US)*

**Objectives:** The ability of *L. monocytogenes* to tolerate alkaline stress is of particular importance, as this pathogen is often exposed to such stress in food processing environments cleaned with alkaline detergents or in the mildly alkaline pH values which prevail within engulfing phagolysosomes. This study aims to investigate the alkaline tolerance response (AlTR) in *Listeria monocytogenes* 10403S using DNA microarray technology. Knowledge of the alkaline-induced stress response will be useful in understanding how this pathogen tolerates alkaline stress.
**Methods:** Transcription profiling of *L. monocytogenes* 10403S was carried out at 15, 30 and 60 min at high pH in order to capture an early, an intermediate and a prolonged expression response to alkaline stress using oligo arrays from the Pathogen Functional Genomic Resource Centre. To verify the microarray results the regulation of some pH stress response genes were confirmed by Real Time Quantitative Polymerase Chain Reaction (RT-PCR).
**Results:** About 1584 genes were upregulated and 1754 genes (of 6347 Open Reading Frames represented on the arrays) were down regulated at least 1.5 fold upon alkaline shock. Many of the repressed genes encode enzymes that are involved in the biosynthesis of amino acids, nucleotides and coenzymes, indicating a metabolic adjustment of the cells to the high pH. Notably, the strongest alkaline-inducible genes were involved in the membrane transport systems.
**Conclusion:** The analysis of the data revealed that cells sense and respond to alkaline stress with an extensive program of changes in gene expression. Interestingly, there is a strong correlation between the AlTR and virulence gene expression. Comparison to various microarray data already in the literature revealed similarity between the response to alkaline stress and the transcriptional response to stresses such as osmotic shock.

## P1691

## Engineering improved listerial stress tolerance "with a twist"!

R. Sleator, C. Hill *(Cork, IE)*

**Objectives:** To engineer *Listeria monocytogenes* strains with a significantly improved ability to tolerate stresses encountered in the external environment and during gastrointestinal transit, thus, improving Listeria's efficacy as a potential vaccine and drug delivery platform.
**Methods and results:** Using a directed evolution approach, based on a random mutagenesis strategy involving the *E. coli* XL1-Red mutator strain, we generated a mutant variant of the listerial betL gene (designated betL*), encoding a secondary betaine uptake system. The mutant betL* promotes a dramatic increase in resistance to a number of biologically relevant stresses when expressed in a variety of different surrogate hosts. Using a luciferase (Lux) reporter system in combination with the IVIS Imager System (Xenogen Corporation, Alameda, CA), we tracked betL* expression, in real time, both *in vitro* under various environmental stresses and *in vivo* in animal models of infection. In each case strains expressing betL* demonstrated a marked improvement over those expressing wild type betL, both in terms of gene expression and bacterial growth. Sequence analysis of the mutated gene revealed a single nucleotide deletion in the spacer region between the -10 and −35 promoter elements upstream of the betL coding region. This deletion presumably introduces a conformational 'twist' in the putative promoter, thereby increasing its transcriptional output. Furthermore, the betL* mutation appears to counter the heretofore unreported 'twisted' cell morphology observed using scanning electron microscopy of *L. monocytogenes* grown at elevated osmolarities.
**Conclusions:** It is possible to selectively improve genes required for bacterial stress survival both inside and outside

the host. Such mutated genes systems may ultimately be used for the construction of more physiologically robust bacterial based vaccine and drug delivery platforms.

## P1693

## Application of fluorescent *in situ* hybridization for detection of *Helicobacter pylori*

A.R. Samarbaf-Zadeh, S. Tajbakhsh, S.M. Moosavian *(Ahwaz, IR)*

**Introduction:** Peptic ulceration following infection of stomach with *H. pylori* is a common disease. Accurate and rapid detection of the bacteria can lead to implementation of appropriate treatment and recovery. This research was undertaken to evaluate the sensivity and specificity of Fluorescent *In-Situ* Hybridization (FISH) in the detection of *H. pylori* in patients who were suffering from dyspepsia.
**Methods:** For this purpose, one hundred gastric biopsy samples taken from antrum and corpus of stomach by endoscopy were tested by FISH and compared with conventional culture method complemented with biochemical tests.
**Results:** FISH detected *H. pylori* in 48 clinical samples while conventional method detected 42 samples. The sensivity and specificity of FISH for detection of *H. pylori* were calculated as 98% and 100% respectively.
**Conclusion:** The findings of this study suggest that FISH is a highly suitable and rapid method for diagnosis of *H. pylori*, especially when the samples are taken from the antrum and the corpus of the stomach this technique potentially can be applied routinely for detection of this bacterium in clinical samples.

## P1694

## Prevalence of *Helicobacter pylori* oral carriage in a children's community

D. De Vito, M. Santantonio, M.F. Coscia, C. Rizzo, R.A. Monno, G. Rizzo *(Bari, IT)*

**Objective:** Numerous studies have demonstrated that *H. pylori* is ubiquitous; approximately 50% of the world's population is infected with the organism. Gastroduodenal diseases associated with *H. pylori* infection are manifested principally in adults. However, it's usually during chilhood that the infection is acquired, and it is possibile that mucosal and humoral responses at this time may determine, at least in part, the course of the natural infection. Our study will describe the prevalence of the *H. pylori* oral carriage in children resident in Bari, south of Italy, using the PCR method.
**Methods:** The evaluation was performed in 404 children, with ages ranging from 6 to 11 years, from primary school district of Local Health Unit of Bari, Italy (AUSL BA/4). The school and the class have been selected using the cluster sampling method. A standardized questionnaire was used to verify socio-economic standard, hygiene and history of previous gastrointestinal disorder. A standard full-mouth examination was made to detect periodontal diseases, then dental plaque and saliva collected from children were placed in PBS and transported in laboratory. *H. pylori* infection status was checked by PCR method. DNA was extracted from oral samples by the boiling method and evaluated for the presence of *H. pylori* CagA and UreA genes using commercial kit (AB Analitica, Padova).
**Results:** A total of 404 children (212 females and 192 males) partecipated to the study. The presence of gene coding for CagA was found in 57 children (14%), but gene UreA was detected only in 22 (5%). The bacteria was detected in saliva, supragingival and subgingival plaque, suggested that these sites may be considered reservoirs for *H. pylori* in ureasi-positive patients. There was statistically significant relationship between who didn't wash their hands frequently and the presence of UreA gene (O.R. 1.71).
**Conclusions:** Current knowledge implies that acquisition of *H. pylori* seems to occur predominantly in childhood and that once acquired the infection persists life-long in most infected subjects. It has been reported at a worldwide level that *H. pylori* infection prevalence in children varies between 10% and 80% and increases with low socio-economic and educational levels and age. The results of this study suggest that oral carriage of *H. pylori* may play a role in the transmission of infection and that the hand may be instrumental in transmission.

## P1695

## The role of *Helicobacter pylori* in otitis media with effusion

T. Yilmaz, M. Ceylan, Y. Akyon, O. Ozcakir, B. Gursel *(Ankara, TR)*

**Objectives:** Otitis media with effusion (OME) is such a common disease of childhood and its pathogenesis still remains unsettled. Pepsinogen and pepsin has been shown in the middle ear fluid of patients with OME, indicating that gastric juice could reach as far as middle ear. If gastric juice could enter the middle ear, Helicobacter pylori, a common inhabitant of gastric juice and mucosa, would also be expected to be found in the middle ear of patients with OME. The objective of this study was to evaluate possible role of *Helicobacter pylori* in pathogenesis of otitis media with effusion.
**Methods:** The study group consisted of 22 children who are to undergo bilateral ventilation tube insertion, adenoidectomy, tonsillectomy with a diagnosis of OME, adenoid hypertrophy and chronic tonsillitis. The control group consisted of 20 children who are to undergo adenoidectomy, tonsillectomy with a diagnosis of adenoid hypertrophy and chronic tonsillitis. For the study group, middle ear fluid was aspirated and a small biopsy was taken from the promontorium mucosa. For the control group, myringotomy was done and a small biopsy was taken from the promontorium mucosa. For both groups, 5 mm deep tissue specimens were obtained from tonsil and adenoid. For all the specimens taken from the patients, culture and a Nested-PCR were performed to show Helicobacter pylori.
**Results:** Middle ear fluid culture was positive for *H. pylori* in 2 patients and mucosa culture was positive in 1 patient only. In the control group middle ear mucosa cultures were always negative. When culture and PCR results were combined together; the middle ear was positive for *H. pylori* in 10 patients in the study group and in 2 patients in the control group. This difference was statistically significant. *H. pylori* presence in the tonsillar and adenoid tissues by culture and PCR was also significantly more frequent in the study group compared to the control group.
**Conclusion:** This study is the first to grow *H. pylori* in the middle ear in OME. Significantly increased colonization by *H. pylori* of the middle ear, tonsillar and adenoid tissue in patients with OME indicates that the bacteria reaching the middle ear through gastroesophageal reflux might be involved in the

pathogenesis of OME. For OME cases resistant to medical treatment it may meaningful to evaluate the patient for gastroesophageal reflux and *H. pylori*.

## P1696

## Distribution of the serine-aspartate repeat protein-encoding sdr genes among nasal carriage and invasive *Staphylococcus aureus* strains

A. Sabat, D. Melles, G. Martirosian, H. Grundmann, A. van Belkum, W. Hryniewicz *(Warsaw, PL; Rotterdam, NL; Katowice, PL; Bilthoven, NL)*

**Objectives:** This study was designed to examine the distribution of the sdr genes among nasal carriage and invasive *Staphylococcus aureus* strains as well as methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S aureus* (MRSA).
**Methods:** The presence or absence of the sdr genes using DNA from 497 *S. aureus* strains was determined by a novel triplex PCR procedure. The two-tailed Fisher's exact test was used to analyse the distribution of the sdr genes among *S. aureus* strains originating from different hosts. P values less than 0.05 were considered a statistically significant difference.
**Results:** The sdr locus was found in all 497 investigated *S. aureus* strains although in 29 strains it contained only the sdrC gene (sdrD – sdrE–). The sdrC + sdrD – sdrE– gene profile was exclusive to MSSA strains (Fisher's exact test; $P = 0.0005$) and was not found in the strains collected from bone infections ($P = 0.0019$). We also found a strong association between the presence of the sdrD gene and MRSA strains ($P < 0.0001$).
**Conclusion:** Our findings suggest that MSSA strains with the newly uncovered sdrC + sdrD – sdrE– gene profile have a substantially decreased potential to establish bone infection.

## P1697

## Sequencing of lukS-PV and lukF-PV in methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* of diverse genetic backgrounds in a Swedish county

C. Berglund, B. Söderquist *(Örebro, SE)*

**Objectives:** Community-aquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) have been reported to carry the loci for Panton-Valentine leukocidin (PVL) in high frequency. The aim of this study was to describe variations within the PVL genes (lukS-PV and lukF-PV) in methicillin-sensitive and methicillin-resistant *S. aureus* of diverse genetic backgrounds.
**Methods:** Twelve PVL-positive *S. aureus* were characterised by multilocus sequence typing (MLST) and MRSA also by staphylococcal cassette chromosome mec (SCCmec) typing. Ten of these were isolated between 1990–2005 in Örebro County, Sweden. Oligonucleotide primers were designed to yield a product size of ~2500 bp including lukS-PV and lukF-PV and flanking regions by PCR amplification. Cyclic sequencing was performed with several sets of primers to overlap the sequences on both strands and was separated on ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). The nucleotide sequences were analysed using ABI PRISM® AutoAssembler™ DNA Sequence Assembly 1.4.0 software and compared using BioEdit 7.0.1.
**Results:** Analysis with MLST differentiated the PVL-positive CA-MRSA into six different sequence types (ST8, 36, 80, 152, 154 and 256) with either SCCmec type IV, IV c, V or unknown types. Six additional STs (ST5, 22, 25, 30, 88 and new) were detected among the PVL-positive methicillin-sensitive *S. aureus*. Sequencing lukS-PV and lukF-PV revealed eight point mutations among these isolates with twelve different origins. Five substitutions had occurred in lukS-PV and three in lukF-PV. Only one substitution was nonsynonymous (histidine → arginine).
**Conclusion:** The PVL-genes were well conserved despite the different genetic origins of the isolates analysed. The PVL is an extracellular product and the genes are not subject to any selective forces and thereby diversify very slowly. Additional nonsynonymous mutations might result in a non-functional toxin.

## P1698

## The first case of *Staphylococcus pseudintermedius* in humans isolated from an ICD lead

L. Van Hoovels, A. Vankeerberghen, K. Van Vaerenbergh, A. Boel, H. De Beenhouwer *(Aalst, BE)*

**Introduction:** *Staphylococcus pseudintermedius* is recently described as a new coagulase-positive species from animals (Devriese et al., 2005). The pathogenic significance of this novel species remains unclear and to our knowledge no human infection due to *S. pseudintermedius* has been reported to date. Here, we present the first isolation of *S. pseudintermedius* in humans with important clinical significance.
**Patient and methods:** A 60-year old male patient was referred to our centre for an ischemic cardiomyopathy and ventricle tachycardia for which he recieved an implantable cardioverter-defibrillator (ICD) in January 2004. In August 2005 he presented with complaints of migration of the ICD device. Clinical examination revealed perforation of the ICD pocket. Infection was suspected and confirmed by the presence of pus in the pocket. The infected ICD was completely removed and several samples (ventricular lead, pus and a tissue sample from the pocket) were sent for culture.Bacteria obtained by routine culture were further characterised by phenotypical identification, Pastorex® Staph-Plus (BioRad), API Staph® (BioMérieux) and Phoenix® (BD). For molecular analysis, PCRs were performed targeting the nuclease (Nuc) and coagulase (Coag) genes of *S. aureus*. Additionally, sequencing of the 16S rRNA gene was performed and further analysed using BLAST.
**Results:** *Staphylococci* with identical phenotypical appearance were isolated from 2 of the 3 ICD samples (lead and pus). Colonies were beta-hemolytic on sheep blood agar, DNase and coagulase positive but clumping factor, mannitol and Pastorex® negative. Biochemical identification by API Staph® and Phoenix® gave a presumptive identification of *S. aureus* with a confidence value of respectively 88,5% and 97%.The PCRs for the Nuc and Coag genes were both negative. 16S rRNA gene sequencing resulted in the identification of *S. pseudintermedius* based on a 100% sequence similarity with a previous reported sequence by Devriese et al.
**Conclusion:** This case report describes the first identification of *S. pseudintermedius* as a significant pathogen in human. Growth characteristics and commercial identification systems misidentify the organism as *S. aureus*. When confronted with an inconsistent phenotypical identification pattern, clinical labs should consider the use of 16S rRNA gene sequencing for final confirmation.

## P1699

### Characterisation of *Staphylococcus aureus* isolates recovered from dairy sheep farms (agr group, adherence, slime, resistance to antibiotics)

E. Vautor, M. Sabah, G. Mancini, M. Pepin, H. Carsenti-Dellamonica *(Sophia-Antipolis, Nice, FR)*

**Objectives:** The purpose of this study was to investigate 46 Staphylococcus aureus natural isolates associated with dairy sheep mastitis for epidemiological key features (agr group, adherence, slime production and antibiotics resistance).
**Methods:** The *S. aureus* isolates (n = 46) were recovered from a field study in the southeast of France in 2001–2004 (28 from subclinical mastitis, 10 from clinical mastitis, 8 from the environment of the dairy sheep farm). A total of thirteen dairy sheep farms, producing cheeses manufactured with raw ewe's milk, were involved. The agr group were determined by multiplex and real-time PCR. The evaluation of adherence and slime production were assessed with methods previously described by Christensen et al. (1982). The susceptibility patterns to 11 antibiotics were determined using the disc-diffusion method on Mueller-Hinton agar plates. Oxacillin susceptibility testing was performed on all the isolates. The 10 others antibiotics susceptibility was only studied on the 28 isolates recovered from subclinical mastitis as they represent the major source of cheese contamination.
**Results:** 80% (37/46) of the isolates belonged to agr group 3, regardless of clinical findings. 39% (18/46) were adherent, strongly adherent or with maximal adherence (biofilm producers). 26% (12/46) were slime producers (moderate or strong producers). All the isolates (n = 46), but seven, were susceptible to all the antibiotics tested. Two isolates recovered from subclinical mastitis were resistant to oxacillin and partly resistant to ampicillin and penicillin-G. The five other isolates were found: partly resistant to erythromycin (n = 1), cefoperazone and penicillin-G (n = 1), erythromycin (n = 1), neomycin (n = 1) or resistant to enrofloxaxin and partly resistant to ampicillin and penicillin (n = 1).
**Conclusions:** *S. aureus* isolates recovered from sheep mastitis in the southeast of France are mainly related to agr group 3 suggesting a role for agr-regulated proteins in the persistence of this bacteria in the sheep udders. Biofilm and slime production may also be an important aspect for intracellular survival of *S. aureus* which could promote the development of persistent intramammary infections. Finally, ewe's milk does not appear to represent a source of resistant *S. aureus* and specially methicillin (oxacillin)-resistant *S. aureus* (MRSA) for human health.

## P1700

### Detection of virulence genes in *Staphylococus aureus* isolates from dairy sheep, goats and cows mastitis, using single-dye DNA microarray

E. Vautor, V. Magnone, G. Rios, M. Pepin, P. Barbry *(Sophia-Antipolis, FR)*

**Objectives:** *Staphylococcus aureus* is a common cause of mastitis in dairy farms animals. Although many putative virulence factors have been identified in *S. aureus* genomes (Kuroda et al., 2001), the differences in pathogenic potential between naturally occurring isolates remain largely unaddressed. The relative importance of host (tissue) factors versus bacterial virulence determinants in disease pathogenesis is not well known, but it is widely accepted that bacterial factors including toxins, cell wall-associated adhesions, and secreted exoproteins are involved in the process. In this study, we use a single-dye DNA microarray assay to investigate the presence or absence of 196 putatives virulence genes in 75 *S. aureus* isolates recovered from cases of ovine, caprine and bovine mastitis.
**Methods:** Mastitis *S. aureus* isolates: sheep (n = 25), goats (n= 22), cows (n = 20).DNA microarray: the arrays were spotted with long oligonucleotides (65-mer) representing 192 known virulence genes and new candidates identified in Mu 50 genome (a human strain) and other *S. aureus* genomes. Each gene were spotted four time. DNA extracted from the strains were labelled with fluorescent Cy5 using the BioPrime® Array CGH (Invitrogen). Control strains with known genetic and phenotypic characteristics were used to normalize the data.
**Results:** (i) The majority of the virulence gene was detected in all the isolates (e.g. coa, ica ADBC operon, htrA, hysA, nuc, sbi, sdrE, ssp, feoB, fnb, sib, spa). (ii) genes were not detected in the majority of the isolates (e.g. cna, edin, lukF-PV, SAV1999,…). (iii) genes were not found in isolates, depending on the herd (e.g. aur or SAV1038 absent in isolates from some dairy sheep farm), on the isolates whatever the species (i.g. bsaP, capH, entK, eta, fnbB, hsdS, lpl2, lukD, Map-ND2C,…). But we found gene mainly related to species (e.g. agrIII, SAV2496,…) Comprehensive results will be given in the poster.
**Conclusions:** The present study indicated that the prevalence of virulence genes among *S. aureus* isolates recovered from dairy farm species depends on the gene. These observations suggest a common occurrence of host-adapted (or tissue-adapted) *S. aureus* strains in which particular virulence genes may play a significant role. When taken with complementary methods such as PCR or/and Southern hybridisation, single-dye DNA microarrays may provide a powerful tool to type *S. aureus* strains for epidemiological and possibly pathogenesis studies.

## P1701

### Detection of DNA sequences distinguishing two closely related genomes of *Staphylococcus aureus* from subclinical versus gangrenous ewe mastitis strains

N. Chevalier, C. Huard, R. Thiery, E. Vautor *(Sophia-Antipolis, FR)*

**Objectives:** *Staphylococcus aureus* is a common cause of mastitis in dairy sheep. The severity of mastitis ranges from subclinical to gangrenous forms. Subclinical mastitis is an inflammation that is not readily detected clinically whereas gangrenous form is an acute necrotizing mastitis. With the ain to find genetic markers or virulence factors that are only present in gangrenous strains a suppression substractive hybridisation (SSH) method was used in the present study to compare two strains of *S. aureus* respectively recovered from subclinical or gangrenous mastitis in the same dairy sheep herd.
**Methods:** 42 ewes were held in the investigated farm. The subclinical strain was recovered in January 2002 from the milk of 6 ewes. The gangrenous strain was recovered in December 2002 from an primipare dairy sheep that subsequently died from this acute mastitis. DNA extracted from the strains were first compared by pulsed field gel electrophoresis (PFGE). Then, SSH was performed by using DNA from the subclinical strain (driver), as described in a commercial kit (Clontech PCR-Select Bacterial Genome Substraction Kit).
**Results:** Using PFGE, four band differences were found between the two strains. Two DNA fragments, presumably specific from the gangrenous strain were detected by SSH and

sequenced: (i) a 262 bp (98% of homology with the sulfide quinone reductase contained in Orf 11 pathogenicity island of the MRSA 252 strain) (ii) 280 bp (98% homology with a gene coding a bacteriophage holine contained in the *S. aureus* N315 genome). Control PCR tests using primers designed from these specific gene candidates confirmed that they were only present in the *S. aureus* gangrenous strain.
**Conclusions:** According to Tenover et al. (1995), a 4 band difference using PFGE indicates that the strains may possibly be related genetically. Although genes classically involved in the virulence of *S. aureus* were not detected in the present study, two putative virulence factors were detected. The sulfide quinone reductase allows *S. aureus* to growth on sulfide (found in animal manure). The holine protein breaks the internal membrane of *S. aureus* to release daughter phages suggesting that a mechanism of horizontal gene transfer could have been mediated by bacteriophages and could explain the acquisition of virulence factors.

# Antimicrobial clinical trials

## P1702

### Outpatient treatment of acute pyelonephritis in pregnancy after 24 weeks. A randomised controlled trial

Z. Ahmadinejad, S. Hantooshzadeh *(Tehran, IR)*

**Objectives:** The purpose of this study was to compare the safety and efficacy of outpatient and inpatient treatment of acute pyelonephritis in pregnancy.
**Methods:** This was a randomized controlled, clinical trial. One hundred twenty eight gravidas past 24 weeks' gestation admitted in IMAM KHOMEINI hospital, Tehran & SAHID Dr BAHONAR hospital, Kerman, divided by random blocks to outpatient or inpatient therapy, received two 1-g doses of intramuscular ceftriaxone at 24-hour intervals while hospitalized, then were discharged and reevaluated within 48–72 hours or remained hospitalized until afebrile for 48 hours. All patients completed a 14-day course of oral cephalexin. We performed urine cultures on admission and 10–14 days after therapy.
**Results:** The two groups were similar with respect to age, parity, temperature, estimated gestational age, initial white blood cell count, and incidence of bacteremia. There were not any significant differences between two groups about the clinical improvement after 48–72 hours, bacteriuria 10–14 days after treatment, relapse of pyelonephritis, requirement to change in antibiotic, date of pregnancy at delivery and preterm labor. The relapse of bacteriuria and preterm labor in inpatients were significantly more than outpatients (PV = 0.0077 and 0.030 respectively). The birth weight of neonate in outpatients were significantly more than inpatients (PV = 0.013).
**Conclusion:** Outpatient antibiotic therapy is effective and safe in selected pregnant women with pyelonephritis. However in this study, the neonatal outcomes were better in outpatients and the maternal outcomes in inpatients.

## P1703

### Experience with daptomycin in patients with renal insufficiency

B.A. Mueller, S. Levy, K. Lamp, L. Friedrich, R. Russo *(Ann Arbor, Lexington, US)*

**Objectives:** To assess the clinical efficacy of daptomycin (DAP) in patients (pts) with renal insufficiency.
**Methods:** The Cubicin Outcomes Registry and Experience (CORE) is a retrospective observational chart review of pts treated with daptomycin in 45 institutions in the United States. Investigators collected demographic, disease state, clinical and microbiological data; outcomes were defined using standard definitions. Patients nonevaluable for outcome were excluded. CORE data were divided and data on cohorts of pts with a creatinine clearance (CrCl) ≥30 or <30 ml/min were examined.
**Results:** Of the 1160 pts enrolled, 770 (66%) had evaluable pt outcomes and either CrCl ≥30 ml/min (NML, n = 598) or CrCl <30 ml/min not yet requiring renal replacement therapy (RI, n = 172). The distribution of males and females was equal in both groups. RI pts were older (45% ≥66 yrs vs 29%, $p < 0.01$). The groups did not differ in the percent coming from the community setting prior to starting DAP (NML 46%, RI 49%). NML had more frequent history of fractures/orthopaedic procedures (11 vs 4%, $p < 0.01$) and haematological cancers (6 vs 1%, $p < 0.02$) while RI had higher rates of any renal disease (4 vs 17%, $p < 0.01$), CHF (6 vs 12%, $p < 0.05$) and other immunologic/ inflammatory disease (2 vs 11%, $p < 0.01$). RI had higher rates of skin infections (60 vs 69%, $p < 0.05$) and endocarditis (3 vs 6%, $p < 0.05$). Infections that were frequently reported for NML and RI were bacteremia, non-catheter-related (10 vs 10%), bacteremia, catheter-related (10 vs 4%), osteomyelitis (14 vs 7%), and foreign body-orthopaedic (6 vs 5%), all $p > 0.05$. Methicillin-resistant Staphylococcus aureus was the most common pathogen; NML 41%, RI 38%. RI had higher rates of coagulase-negative staphylococci (9 vs 15%, $p < 0.02$) and viridans streptococci (0.3 vs 1.7%, $p < 0.05$). There was no difference in the percentage receiving antibiotics prior to DAP; NML 75%, RI 68%. The mean DAP dose and duration were similar; NML 4.7 mg/kg for 18 d, RI 4.6 mg/kg for 16 d. The most frequent dose was 4 mg/kg; NML 58%, RI 39%. RI initial DAP dosing was more frequent than recommended (q 48 h) in 76%. The mean time to clinical response was similar; NML 5.2 d, RI 5.4 d. More pts in NML received concomitant antibiotics with DAP; 53 vs 35%, $p < 0.01$). The clinical success (cure and improved) rates were; NML 95%, RI 96%.
**Conclusion:** DAP shows favourable clinical success rates in pts regardless of the presence of renal insufficiency.

## P1704

### *In vitro* activity of second line antibiotics against *Helicobacter pylori* infection

A. Pérez de Ayala, T. Alarcón, D. Monclús, E. Aznar, J.L. Navarro, N. Arenal, M. López-Brea *(Madrid, ES)*

**Objective:** The aim of our study was to determine the *in vitro* activity of levofloxacin, ciprofloxacin and rifampicin in clinical strains of *H. pylori*.
**Material and methods:** 40 isolates of *H. pylori* from biopsies of dyspeptic patients were obtained following standard methodology. *In vitro* activity of metronidazole, clarithromycin, levofloxacin, ciprofloxacin and rifampicin was determined by E- Test using 5% sheep blood agar and incubated at 37ordm;C during 3–5 days in a CO2 atmosphere. MIC was

determined as the point of complete inhibition of growth. Breakpoint of the NCCLS for other microorganisms were considered for fluorquinolones: resistant if MIC > 4 mg/ l. For rifampicin we considered the strain susceptible if MIC <32 mg/ l, as same studies reported.
**Results:** 42.5% of the strains were resistant to metronidazole and 50% to clarithromycin. MIC50, MIC90 and range (mg/l) was: 0.064, 0.125 and 0.012 → 32 for levofloxacin, 0.064, 0.25 and 0.006 → 32 for ciprofloxacin and 0.75, 1.5, and <0.002–4 for rifampicin. All the strains were susceptible to rifampicin and only 2% of them were resistant to fluorquinolones.
**Conclusions:** The fluorquinolones tested and rifampicin showed an excellent *in vitro* activity against *H. pylori*, despite the high resistance rate to metronidazole and clarithromycin. However, *in vitro* susceptibility test should be done before the use in clinical practice.

## P1705

## *Vibrio* antibodies in serum and breast milk samples of parturient women in Calabar, Nigeria

S. Utsalo *(Calabar, NG)*

**Objectives:** Serum and breast milk samples from 335 parturient women and serum from non–parturient controls were analysed for prevalence and titres of *vibrio* antibodies.
**Methods:** *V. cholerae* agglutinins and vibriocidal antibodies in serum samples were analysed by direct agglutination and immune bacteriolysis techniques respectively, using 96 well microtitre plates. The protective value of breast milk was evaluated by haemagglutination inhibition and rabbit intestinal mucosal attachment of *V. cholerae* cells.
**Results:** *Vibrio* agglutinins were detected in serum samples of 191(57.0%) parturient and 78 (33.9%) non-parturient subjects ($p < 0.05$). High prevalence rates of 68.9% and 39.1% occurred among parturient and control subjects of 21–25 years of age respectively. At 1:160 cut off titre to evaluate *Vibrio cholerae* specific bacteriocidal antibodies, activity was detected in samples of 31 (57.1%) and 9 (56.3%) parturients and controls respectively aged 26–30 years. Breast milk from 67 (20.0%) parturients contained *vibrio* agglutinins with titres ranging between 1:20 and 1:320, while milk samples from 32 subjects showed haemagglutination inhibition (HI) activity titres of $\geqslant$ 1:40. Of the 19 HI positive milk samples 17 (89.5%) showed inhibition of V. cholerae adherence to rabbit intestinal mucosa at titres $\geqslant$ 1:80, and 53–92% reductions in cell attachment.
**Conclusion:** Our study confirms that parturient women in Calabar may benefit from significant serum titres of *V. cholerae* antibodies and provide immune protection for their babies through breast milk secretions.

## P1706

## Moxifloxacin vs clarithromycin for treatment of community-acquired pneumonia associated with common respiratory pathogens: a pooled analysis

R. Grossman, M. Niederman, R. Perroncel, S. Choudhri, D. Haverstock *(Mississauga, CA; Mineola, West Haven, US)*

**Objectives:** *Streptococcus pneumoniae* and *Haemophilus influenzae* are pathogens commonly associated with community-acquired pneumonia (CAP). This study compared the clinical and bacteriologic efficacy of moxifloxacin (MXF) to clarithromycin (CLAR) in CAP patients with these pathogens.
**Patients and methods:** Data were pooled from three double-blind, multicenter, Phase III trials comparing oral MXF 400 mg QD to CLAR 500 mg BID for 10 days. All patients included had mild-to-moderate CAP. Clinical and bacteriologic success rates were identified for *S. pneumoniae* and *H. influenzae* isolated from these studies. Data for the efficacy-valid population was recorded at the test-of-cure (TOC) visit (7–35 days post-therapy).
**Results:** 1437 patients were entered, of which 356 were microbiologically evaluable. Infection with *S. pneumoniae* and/or *H. influenzae* was documented in 149 (42%) of patients (5 MXF and 2 CLAR patients had mixed infection). Within this cohort, the two treatment groups were well balanced based on demographic/baseline medical characteristics (55% male, mean age 50 yrs, 41% smokers, 8% recent antimicrobial therapy). Clinical success and bacteriologic eradication rates (one response per patient) at TOC are presented in the Table.

| Microorganism | | Clinical success | *P*-value | Bacteriologic eradication | *P*-value |
|---|---|---|---|---|---|
| ***H. influenzae*** | MXF | 93% (37/40) | *P*=0.112 | 90% (36/40) | *P*=0.073 |
| | CLAR | 80% (28/35) | | 74% (26/35) | |
| ***S. pneumoniae*** | MXF | 98% (40/41) | *P*=0.157 | 95% (39/41) | *P*=0.379 |
| | CLAR | 90% (36/40) | | 90% (36/40) | |
| ***H. influenzae* + *S. pneumoniae*** **(pooled by patient)** | MXF | 95% (72/76) | *P*=0.047 | 92% (70/76) | *P*=0.070 |
| | CLAR | 85% (62/73) | | 82% (60/73) | |

**Conclusions:** In CAP associated with *S. pneumoniae* and *H. influenzae* there was a trend towards greater bacterial eradication for MXF vs CLAR. Clinical success rates were significantly higher for MXF monotherapy vs CLAR.

## P1707

## Variability of creatinine clearance measurements in inpatients with community-acquired pneumonia

R. Grossman, S. Choudhri, D. Haverstock *(Mississauga, CA; West Haven, US)*

**Objectives:** Moxifloxacin, levofloxacin and gatifloxacin have been recommended as empiric therapies for patients with community-acquired pneumonia (CAP). Levofloxacin and gatifloxacin require dose-adjustment for renal insufficiency while no dose adjustment is required for moxifloxacin. This study was designed to determine the frequency and underlying variability of renal insufficiency in patients with CAP.
**Methods:** A pooled analysis of data from 2211 patients with mild to moderate or severe CAP entered into one of six randomized, controlled clinical trials was undertaken. Renal function (calculated creatinine clearance; CrCl) was assessed in each patient prior to treatment with MXF and then again during and post-treatment.
**Results:** Baseline CrCl levels in this pooled population of patients with CAP were: <25 mL/min in 47 (2.1%) of patients, 25–49.9 mL/min in 436 (19.7%) and ≥50 mL/min in 1728 (78.2%) patients. After the pre-treatment CrCl measurement 88 patients (4%) were lost to follow-up, so there was no during or post treatment value. In patients with CAP the CrCl improved from baseline in many patients during or post-treatment, while some patients experienced a worsening of renal function (see Table).

| Pre-treatment CrCl (mL/min) | No. with second CrCl measurement | CrCl during or post treatment (mL/min) | | |
|---|---|---|---|---|
| | | <25 n (%) | 25–49.9 n (%) | >50 n (%) |
| <25 (n=47) | 45 | 18 (40.0%) | 25 (55.6%) | 2 (4.4%) |
| 25–49.9 (n=436) | 421 | 9 (2.1%) | 307 (72.9%) | 105 (24.9%) |
| ≥50 (n=1728) | 1657 | 0 (0.0%) | 79 (4.8%) | 1578 (95.2%) |

**Conclusions:** Renal function (CrCl) is highly variable in CAP patients with baseline evidence of renal insufficiency. Renal function should be monitored closely to permit appropriate dose adjustments if levofloxacin or gatifloxacin is used in this patient population. Moxifloxacin may be a better empiric choice in this setting as it does not require dose adjustment in patients with renal insufficiency or renal failure.

## P1708

## A prospective, controlled, randomised, non-blind, comparative study of the efficacy and safety of high-dose single daily ceftriaxone plus ciprofloxacin versus thrice-daily ceftazidime plus amikacin in the empirical therapy of febrile neutropenic patients

S. Metallidis, P. Kollaras, A. Tsona, T. Giannakakis, V. Seitanidis, T. Kordosis, A. Hatzitolios, J. Nikolaidis, P. Nikolaidis
*(Thessaloniki, Athens, GR)*

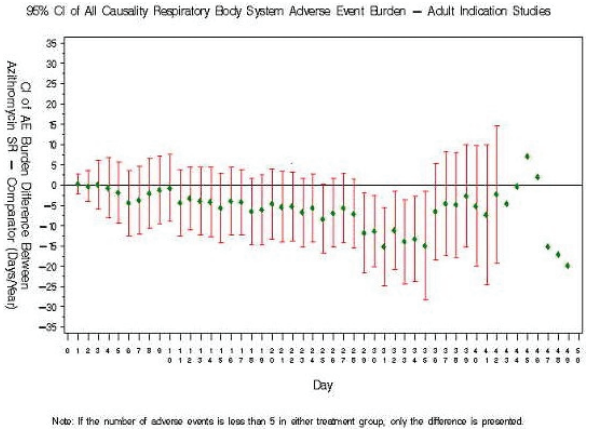
**Objective:** Empirical antibiotic treatment for febrile neutropenia is well established. The best regimen is still controversial. The purpose of this study was to evaluate the efficacy, safety and cost of high-dose single daily ceftriaxone plus ciprofloxacin versus thrice daily ceftazidime plus amikacin in neutropenic febrile patients.
**Patients and methods:** Ninety-five patients with febrile neutropenia were included in a prospective, controlled, randomized, non-blind, comparative study. Patients were randomly assigned to either treatment group (63 in the Ceftriaxone/Ciprofloxacin group and 32 in the Ceftazidime/Amikacin group) and evaluated as successes or failures according to defined criteria. Daily assessments were made on all patients all adverse events were record.
**Results:** The overall incidence of documented infections was 45.9%: 24/47 (51.1%) in the Ceftriaxone/Ciprofloxacin group and 10/27 (37%) in the Ceftazidime/Amikacin group. There was significant difference in clinical efficacy between groups ($p = 0.011$) at the end of therapy. Ceftriaxone/Ciprofloxacin group had an overall incidence of resolution and improvement of 95,7% in comparison to the 75% of the Ceftazidime/Amikacin group. Thirty-nine organisms were isolated, 26 (66.67%) gram-negative and 13 (33,33%) gram-positive. There was low incidence of adverse events in both groups.
**Conclusion:** The combination of high dose single daily ceftriaxone plus ciprofloxacin was more effective than the standard combination of thrice daily ceftazidime plus amikacn with no significant adverse events in either group.

## P1709

## The 'Day 28 phenomenon': single-dose azithromycin microspheres (Zmax) reduce respiratory symptoms in the post-treatment period

D. Jorgensen, C. Roberts, D. Cardinale, J. Breen, M. Dunne, F. Whaley *(New London, New York, Ann Arbor, US)*

**Objective:** In past studies of azithromycin in children, a post-treatment (PT) benefit was observed at Day 28. In 5 recent phase 3 trials in adults, single-dose Zmax was at least as effective as standard comparators for treatment of respiratory tract infections (RTIs), including CAP. Our objective is to demonstrate a PT benefit in this adult population.
**Methods:** Post-hoc analyses, including respiratory adverse event burden (RAEB), were conducted on the All Treated population (N = 2596; 1292 AZ-M, 1304 comparators) in the 5 phase 3 studies. The RAEB is the sum of duration, in days, of all respiratory adverse events, divided by total number of observation days of all patients, normalized to 1 year. The overall and per study day RAEB were calculated for Zmax and the pooled comparators for the 5 studies combined.
**Results:** RAEB, in days/patient year, was 15.5 for AZ-M patients vs 20.2 for comparator patients ($p = 0.08$). The difference in RAEB consistently and progressively favoured Zmax, beginning at Day 11 and achieving statistical significance between Days 29 and 35, when the upper limits of the 95% CIs around the differences were below zero (figure).




**Conclusion:** In 5 phase 3 studies, a single dose of Zmax was safe and effective in the treatment of adult RTIs, and demonstrated an additional benefit in terms of reducing respiratory symptoms in the 2–4 week PT period.

## P1710

## Integrated analysis of efficacy of faropenem medoxomil in the treatment of community-acquired pneumonia

E.E.L. Wang, R. Echols, R. Tosiello *(Louisville, US)*

Faropenem medoxomil (FM) is an oral penem with potent activity against Streptococcus pneumoniae and Haemophilus influenzae. This integrated analysis was conducted to summarize the efficacy of 10 days of 300 mg BID of FM compared with other beta lactams in the management of community acquired pneumonia (CAP).
**Methods:** Efficacy was determined in three multicenter randomized double-blind controlled trials (RCT) and a single uncontrolled study of faropenem medoxomil. Comparators were 14 days of cefpodoxime (C), 10 days of amoxicillin-clavulanate (AC), or 10 days of amoxicillin (A). The analysis allowed examination of treatment effects by age, race, gender and study site subgroups.
**Results:** A total of 2267 subjects were studied. Studies 1 and 4 were conducted in N. America, studies 2 and 3 in Europe, Latin America, Israel, and S. Africa. N. American vs. other studies included subjects at least 12 (vs. at least 18) years of age and only out patient (vs. outpatient and hospitalized)

subjects. The clinical responses for FM in both Per Protocol and Intention-to-Treat populations were non-inferior to comparator for each study and for the three trials combined. No differences were found in treatment effect by age, race, gender, or country. Recovery of an etiologic agent from initial respiratory or blood culture varied between 9.4 and 17.5% of cases in the 4 studies for a total of 236 microbiologically evaluable subjects. S. pneumoniae was eradicated or presumed eradicated in 82/90 (91.1%) and 49/52 (94.2%), *H. influenzae* in 49/61 (80.3%) and 30/32 (93.8%), *S. aureus* in 13/14 (93%) and 8/10 (80%), *H. parainfluenzae* in 6/8 (75%) and 0/2 (0%), and *M. catarrhalis* in 6/7 (85.7%) and 4/4(100%) FM and comparator recipients, respectively. Clinical response for S. pneumoniae bacteremic patients was 18/21 (85.7%) for FM.

| Treatment Group | Clinical Response (%) (PP) | Difference (95% CI) | Clinical Response (%) (ITT) | Difference (95% CI) |
|---|---|---|---|---|
| Study 1 FM | 205/229 (89.5) | 0.9 (-4.8, 6.6) | 223/306 (72.9) | -1.5 (-8.6, 5.5) |
| Study 1 C | 203/229 (88.6) | | 224/301 (74.4) | |
| Study 2 FM | 222/257 (86.4) | -1.7 (-7.5, 4.0) | 242/305 (79.3) | 1.7 (-4.7, 8.3) |
| Study 2 AC | 223/253 (88.1) | | 242/312 (77.6) | |
| Study 3 FM | 260/284 (91.5) | 3.1 (-1.9, 8.1) | 289/329 (87.8) | 3.7 (-1.6, 9.1) |
| Study 3 A | 237/268 (88.4) | | 270/321 (84.1) | |
| All FM- RCT | 687/770 (89.2) | 0.8 (-2.3, 4.0) | 754/940 (80.2) | 1.4 (-2.2, 5.1) |
| Comparator | 663/750 (88.4) | | 736/934 (78.8) | |
| Study 4 | 252/294 (85.7) | | 286/393 (72.8) | |

**Conclusions:** FM efficacy was consistent across studies, within subgroups, and non-inferior to comparators. It is efficacious against the most common bacterial pathogens and in the most severe form (bacteremic) disease. FM is a good option for the treatment of CAP.

## P1711

## *Propionibacterium acnes* strains isolated from acne vulgaris and severe infections

C. Oprica, C.E. Nord *(Stockholm, SE)*

*Propionibacterium acnes* is a member of the resident flora of the skin and is an important factor involved in inflammatory reactions in acne patients. During the last years the prevalence of different severe infections due to P. acnes has increased.
**Objectives:** 1) To detect the prevalence of resistant P. acnes strains isolated from acne patients in Stockholm and different severe infections in Europe; 2) To identify the mechanisms of resistance and the genetic diversity among resistant strains.
**Methods:** *P. acnes* strains isolated from acne vulgaris and severe infections were tested against clindamycin, erythromycin, linezolid and tetracycline and pulsed-field gel electrophoresis was used for further characterization. PCR and sequencing of the genes encoding domain V of 23S rRNA for clindamycin and erythromycin resistant strains and 16S rRNA for tetracycline resistant strains were performed.
**Results:** i) Antibiotic-resistant strains were more often isolated from antibiotic treated patients with moderate to severe acne area than from non-antibiotic treated acne patients. An individual might harbor different pulsotypes of *P. acnes* with various degrees of resistance. ii) Among the 304 clinical isolates from 13 European countries were found resistant strains to tetracycline, clindamycin, and erythromycin. Overall, in the southern Europe a higher prevalence of erythromycin-resistant strains was noticed and in southern and eastern Europe a higher prevalence of resistance to clindamycin. It was noticed a high genomic diversity and the geographical spread of some clones in related areas but also in geographically distant countries. Most clindamycin or erythromycin resistant P. acnes isolates, were found to be members of a single clone that has spread in different geographically countries. iii) *P. acnes* clindamycin and erythromycin resistant strains carrying one of the described mutations within the 23S rRNA were predominantly isolated from Swedish acne patients compared to strains from other infections. Forty-four per cent of tetracycline resistant strains were found to carry a mutation in the 16S rRNA. These strains were isolated from Swedish acne patients, were highly resistant and were clustered in one pulsotype.
**Conclusion:** Surveillance of both the prevalence of resistant *P. acnes* strains and associated resistance mechanisms is important due to the rapid variation in resistance patterns, both in acne patients and other severe infections.

## P1712

## Antimicrobial activity of Unisepta quick and Deconex solarsept on the surface contamination and dental instrument in dental clinics in Iran

F. Shahcheraghi *(Tehran, IR)*

**Objectives:** Quaternary ammonium compounds (QACs) are amphoteric surfactants that are widely used for the control of bacterial growth in clinical and industrial invironment.Unisepta quick and deconex solarsept are new generation of QACs is widely used as adjuncts in Iran to hygine in dental clinics.The aim of present study was to investigate clinical efficiency of these substances on the surface and instruments in dental clinics.
**Material and methods:** The following bacteria and fungi on the base of AOAC standard were used.Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 25923) Bacillus. subtilis ATCC (6051), Mycobacterium bovis ATCC (35443) and wild types of Trichophyton mentagraphit, P. aeruginosa and Salmonella typhimorium (a common fungi in IRAN). A stock solution of deconex solarsept (BORER chemie) and unisepta quick (micro 10 unident) was prepared as recommended by the manufacturer. The concentration of Bacterial suspention was 0.5 MacFarland and the results were reported on the base of decreasing in (CFU) colony forming unit from 107 to 102.
**Results:** The results shows that Both of these disinfectants have bactericidal and fungicidal activity on the standard *P. aeruginosa, S. aureus, S. typhimurium* and *Trichophyton mentagraphit*, The number of bacteria decreased significantly ($P < 0.05$), but no significant difference was seen with *B. subtilis*, wild type of *P. aeruginosa* and *M. bovis*.
**Conclusion:** The results confirm that these QACs are not able to sterilize or disinfect medical and dental instruments, and they can not be used lonely, and it must be used with the other methods for sterilization of surface and dental instruments.

## P1713

## Macrolide as long-term treatment in patients with bronchiectasis colonised by *P. aeruginosa*

M. Schmidt-Ioanas, M. Allewelt, A. Polzin, T. Blum, H. Lode *(Berlin, DE)*

**Background:** A certain efficacy of macrolide against *P. aeruginosa* has been described *in vitro*, mainly through mechanisms such disruption of quorum sensing and suppression of inflammation.
**Aim:** To evaluate the efficacy of macrolide in patients with bronchiectasis colonised by *P. aeruginosa*.
**Methods:** The study prospectively included patients with bronchiectasis and *P. aeruginosa* isolated in sputum in stable

state. All subjects received either azithromycin 250 mg × 3 days/week or clarithromycin 500 mg daily on long term and completed daily diary cards for symptoms and PEF values until the end of therapy. Follow-up period was 1 year.
**Results:** 26 patients with bronchiectasis and *P. aeruginosa* evidence in sputum were included (17 men, mean age 55.0 ± 12.1 yrs.). 12 patients received azithromycin and 14 patients clarithromycin, with a mean duration of 4.5 ± 1.7 months. Five (19.2%) patients discontinued treatment after less than 2 weeks because of adverse events. At the end of therapy, 12 (46.1%) patients showed no evidence of *P. aeruginosa* in sputum while 9 (34.6%) patients still had Ps. aeruginosa in sputum. An improvement in the following parameters could be observed in all patients: sputum volume (75.5 ml/day before therapy versus 31.6 ml/day after therapy, p = 0.04); PEF (311.2±77.1 l/min before therapy versus 476.2 ± 30.2 l/min after therapy, p = 0.03); number of exacerbations/year (2.8 in the previous year versus 1.7 in the follow-up year, p = 0.01).
**Conclusion:** The study shows that macrolide may be an effective therapy in patients with bronchiectasis colonised by *P. aeruginosa*. Independently of the microbial eradication, an improvement of the clinical symptoms and a reduction of exacerbations were observed in all patients.

## P1714

## Fungal pathogens from haematoncology patients and their susceptibility to new and old antifungal drugs

M.A. Petrou *(London, UK)*

The expanding population of immunocompromised hosts has been infected with many established and emerging opportunistic fungi. Most pathogens can be treated empirically whereas for an increasing number of species proper treatment starts once the MIC becomes available. Though invasive Aspergillosis remains the principle life threatening complication in the haematoncology patients (HOP) other pathogens cannot be ignored as selection and resistance during prophylaxis increases the risk of treatment failure.In order to understand the frequency of rare fungal pathogens, selection and emergence of resistance in our trust all fungi from HOP were identified using standard mycological techniques and the MICs to amphotericin B (AMB), flucytosine (5FC), fluconazole (FCZ), itraconazole (ITZ), voriconazole (VCZ) and caspofungin (CFG) were determined using the NCCLS method. 513 specimens were processed, 53% respiratory, 17.5% blood, 12.8% oral, 8.4% other sterile (bile, CSF, drains, lines and tissue biopsies) and 8.3% non-sterile sites. Yeasts accounted for 85% and filamentous fungi (FF) for 15%, representing 12 *Candida* sp, 7 other types of yeast, 6 *Aspergillus* sp and 8 other types of FF. *C. albicans* represented 35.7%, *C. glabrata* 29.3%, *C. krusei* 8.6%, *A. fumigatus* 10% and other *Aspergillus* sp 2% of all isolates. The MIC 90s for all isolates were AMB 0.5, 5FC 2, FCZ >64, ITZ 1, VCZ 1 and CFG 0.06 mg/L. With the exception of *Acremonium* sp, *A. versicolor, A. terreus and Scedosporium apiospermum* all isolates including the 15 isolates of *C. lusitaniae* were sensitive to AMB. Most but not all FF and only one isolate of *C. albicans* from the yeasts were resistant to 5FC. All FF, Rhodotorula sp, *C. albicans* 3%, *C. glabrata* 53% and *C. krusei* 41% were resistant to FCZ. Only Absidia corymbifera, Acremonium sp 25%, *C. albicans* 1%, *C glabrata* 33% and *Saccharomyces cerevisiae* 4% were resistant to ITZ. For VCZ A. *corymbifera*, *Acremonium* sp 25%, *C. albicans* 2%, *C. glabrata* 31%, *C. krusei* 2%, *C. tropicalis* 10%, *Rhodotorula* sp 66.6% and *P. aecilomyces variotii* 50% had an MIC ≥2 mg/L. With CFG the effective concentration was ≥0.5 mg/L for *A. corymbifera, Fusarium solani, Geotrichum capitatum, Sporobolomyces salmonicolor*, *Acremonium* sp 50% and *C. parapsilosis* 25%.The data show that HOP are exposed to many different fungal pathogens some of which are resistant to the old and the new antifungals and that AMB is still the drug with the broader spectrum and less developed resistance for both yeasts and FF.

## P1715

## Faropenem medoxomil in the treatment of acute bacterial sinusitis: an integrated analysis

S. Kowalsky, R. Tosiello, R. Echols *(Milford, US)*

**Background:** Faropenem medoxomil (FM) is an orally absorbed, synthetic, penem antibacterial agent with *in vitro* activity against community-acquired respiratory pathogens.
**Methods:** The efficacy of FM in subjects with acute bacterial sinusitis (ABS) was evaluated in 3 Phase III trials; 100288, 10186, and 100287. Study 100288 was conducted in N. America, Study 10186 was conducted in Europe and Israel and Study 100287 was conducted in the US and Argentina. 100288 and 10186 were prospective, randomized, double-blind, active comparator trials and 100287 was an open-label ''sinus tap'' trial. The dose of FM was 300 mg BID in all 3 studies. The comparator in 100288 and 10186 was cefuroxime axetil (CFX) 250 mg BID. The duration of FM treatment in 100288 was 7 days and 10 days vs CFX for 10 days. In 10186, FM or CFX were given for 7 days. In 100287, FM was administered for 7 days. The primary efficacy variable in all 3 studies was clinical response at the Test-of-Cure (TOC). Microbiologic response at the TOC was a secondary efficacy variable in 10186 (sinus puncture and endoscopic collection) and 100287 (sinus puncture and aspiration). Non-inferiority was defined as the difference in cure rates (FM minus comparator) where the lower boundary of the 95% CI was greater than -10%.
**Results:** The cure rates at the TOC are shown in the table for the valid per protocol (vPP) and the Intent-to-Treat (ITT) populations. The frequency of isolation of key pathogens and the rate of eradication in samples obtained by endoscopically-guided swab and in samples obtained by TAP were consistent across studies. The eradication rates for *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* were 94.8% vs. 96.3% (FM 7/10 d vs. CFX 7/10 d), 82.4% vs. 90.5% (FM vs. CFX) and 96.2% vs. 83.3% (FM vs. CFX), respectively.

| Study | Treatment | Clinical Cure Rates (vPP) | | | Clinical Cure Rates (ITT) | | |
|---|---|---|---|---|---|---|---|
| | | n/N | % | 95% CI for Difference | n/N | % | 95% CI for Difference |
| 100288 | FM 7d | 237/295 | 80.3 | -0.9, 12.7 | 262/370 | 70.8 | -3.2, 10.1 |
| | FM 10d | 229/280 | 81.8 | 0.5, 14.1 | 255/365 | 69.9 | -4.2, 9.2 |
| | CFX 10d | 213/286 | 74.5 | | 250/371 | 67.4 | |
| | | | | | | | |
| 100287 | FM 7d | 246/300 | 82.0 | | 269/354 | 76.0 | |
| | N/A | | | | | | |
| | | | | | | | |
| 10186 | FM 7d | 203/228 | 89.0 | -5.2, 6.5 | 237/275 | 86.2 | -7.0, 4.3 |
| | CFX 7d | 198/224 | 88.4 | | 239/273 | 87.5 | |
| | | | | 95% CI for Point Est. | | | 95% CI for Point Est. |
| Integrated studies | FM 7d | 686/823 | 83.4 | 80.8, 85.9 | 768/999 | 76.9 | 74.3, 79.5 |
| | FM 10d | 229/280 | 81.8 | 77.3, 86.3 | 255/365 | 69.9 | 65.2, 74.6 |
| | FM 7/10d | 915/1103 | 83.0 | 80.7, 85.2 | 1023/1364 | 75.0 | 72.7, 77.3 |
| | CFX 7d | 198/224 | 88.4 | 84.2, 92.6 | 239/273 | 87.5 | 83.6, 91.5 |
| | CFX 10d | 213/286 | 74.5 | 69.4, 79.5 | 250/371 | 67.4 | 62.6, 72.2 |
| | CFX 7/10d | 411/510 | 80.6 | 77.2, 84.0 | 489/644 | 75.9 | 72.6, 79.2 |

**Conclusions:** FM 300 mg BID x 7 days was shown to be non-inferior to CFX in clinical efficacy in two prospective, double-blind, comparative trials. A third, open-label trial, demonstrated similar efficacy in microbiologically documented ABS caused by key pathogens. Longer (10 d treatment) with FM provided no additional efficacy.

## P1716

## Faropenem medoxomil in the treatment of acute exacerbation of chronic bronchitis: an integrated analysis

S. Kowalsky, R. Tosiello, R. Echols *(Milford, US)*

**Background:** Faropenem medoxomil (FM) is an orally absorbed, synthetic, penem antibacterial with *in vitro* activity against community-acquired respiratory pathogens.
**Methods:** The efficacy of FM in subjects with acute exacerbation of chronic bronchitis (AECB) was evaluated in 2 Phase III trials. Study 10187 was conducted in Europe, Israel, Mexico, and South Africa. Study 100291 was conducted in the US and Argentina. Both were prospective, randomized, double-blind, active comparator trials. The dose of FM was 300 mg BID for 5 days in both studies. The comparators were clarithromycin (CLR) 500 mg BID for 7 days and azithromycin (AZI) QD for 5 days (500 mg on Day 1 and 250 mg on Days 2–5). The primary efficacy variable was clinical response at the Test-of-Cure (TOC). Microbiologic response at the TOC, in subjects with a baseline pathogen was a secondary variable. Non-inferiority was defined as the difference in cure rate (FM minus comparator) where the lower boundary of the 95% CI was greater than −10%.
**Results:** The cure rates are shown below for the valid per protocol (vPP), Intent-to-Treat (ITT) and modified ITT populations (all ITT subjects who met inclusion/exclusion criteria). In both the individual studies and the pooled analyses, for all populations, treatment with FM was not less effective than either comparator. 20% of treated subjects in 10187 and 28% of subjects in 100291 were evaluable for microbiological response. In 10187, the eradication rates for the microbiologically evaluable population was higher in the CLR group (77.1%) compared with the FM group (68.6%) (95% CI −23.4, 6.3). In contrast, the eradication rate in 100291 was similar in the FM (80.0%) and AZI (78.9%) groups (95% CI -9.7, 11.8). When the data were pooled across studies, the response rates were similar with FM (75.8%) and combined comparator (78.2%) groups (95% CI -11.0, 6.3). The combined eradication/presumed eradication rates in the pooled FM and comparator groups were 81.8% vs. 86.6%, respectively for *S. pneumoniae* and 76.0% vs. 77.6%, respectively, for *H. influenzae*.
**Conclusions:** FM was shown to be non-inferior to either AZI or CLR in clinical efficacy in two adequate and well-controlled trials. Pooled analysis further strengthened the clinical non-inferiority conclusion. The difference in eradication rates observed in Study 10187 (CLR) was not supported by Study 100291 (AZI).

| Study | Treatment | Clinical Cure Rate (vPP) | | Clinical Cure Rate (ITT) | | Clinical Cure Rate (mITT) | |
|---|---|---|---|---|---|---|---|
| | | n/N | % | n/N | % | n/N | % |
| 100291 | FM 5d | 225/278 | 80.9 | 277/414 | 66.9 | 235/347 | 67.7 |
| 95% CI | | (-9.9, 2.6) | | (-8.5, 4.3) | | (-9.6, 4.2) | |
| | AZI 5d | 236/279 | 84.6 | 283/410 | 69.0 | 241/342 | 70.5 |
| | | | | | | | |
| 10187 | FM 5d | 262/299 | 87.6 | 316/369 | 85.6 | 309/361 | 85.6 |
| 95% CI | | (-7.9, 2.0) | | (-8.1, 1.5) | | (-8.2, 1.5) | |
| | CLR 7d | 288/318 | 90.6 | 337/379 | 88.9 | 330/371 | 88.9 |
| | | | | | | | |
| Integrated studies | FM 5d | 487/577 | 84.4 | 593/783 | 75.7 | 544/708 | **76.8** |
| 95% CI | | (-7.3, 0.6) | | (-7.0, 1.3) | | **(-7.5, 1.0)** | |
| | AZI/CLR 5d/7d | 524/597 | 87.8 | 620/789 | 78.6 | 571/713 | **80.1** |

## P1717

## An integrated safety analysis of faropenem medoxomil: results of 5,023 subjects from phase II/III clinical trials

R. Echols, R. Tosiello *(Milford, US)*

**Objective:** To evaluate the safety profile of faropenem medoxomil (FM), a novel oral penem antibiotic.
**Methods:** 5,023 subjects from 3 phase II and 14 phase III clinical trials received FM, 300 mg BID for 3–10 days for treatment of acute bacterial infections. Randomized controlled trials (RCTs) included 4,223 FM and 3795 comparator treated subjects. Analyses were conducted to identify possible disparate adverse event (AE) reporting based on type of infection, subject age (12–17, 18-45, 4664, 65–75, >75) and gender, duration of treatment (3/5 d v. 7/10 d), geography (NA, EU, ROW), study design (open label v RCT), relationship to treatment. Comparisons were made to control treatment based on antibiotic class ($\beta$-lactam v. Other), and individual antibiotic treatments.
**Results:** FM compared favourably to penicillins, cephalosporins and macrolides. FM was better tolerated than TMP/SMX and co-amoxiclav. Open labeled trials had higher AEs reported v. RCTs. AEs reporting NA = ROW > EU except serious AEs and deaths where ROW = EU>NA. AEs for FM 3/5 d = FM 7/10 d. Underlying infection did influence AE reporting. Female gender had higher AE reporting than male gender. FM was tolerated equally well across age ranges, although deaths and SAEs were more common in >75 age group. Common AEs (>1%/related from RCTs) were diarrhoea, nausea, fungal vaginosis and headache and were generally less frequent with FM than control Rx. No evidence of neuro or cardio toxicity was identified. Laboratory tests identified no hepatic, renal or hematopoietic signals.

| | All Faropenem Medoxomil 300 mg PO BID | | | ----------Randomized Studies---------- | |
|---|---|---|---|---|---|
| | 3d/5d[a] (N=1634) | 7d/10d[a] (N=3389) | Total (N=5023) | Faropenem Medoxomil 5d/7d/10d[a] (N=4223) | Total Comparators (N=3795) |
| Subjects reporting at least one treatment-emergent adverse event | 38.4% | 38.1% | 38.2% | 37.7% | 39.2% |
| Subjects reporting at least one treatment-related[b] adverse event | 18.5% | 18.4% | 18.5% | 18.3% | 20.1% |
| Subjects discontinuing treatment due to adverse event | 2.6% | 4.7% | 4.0% | 3.7% | 3.8% |
| Subjects reporting at least one serious treatment-emergent adverse event[c] | 3.6% | 4.0% | 3.8% | 3.6% | 3.8% |
| Deaths | 0.4% | 0.4% | 0.4% | 0.4% | 0.5% |

**Conclusion:** Faropenem medoxomil, a novel oral penem antibiotic, has the safety profile expected of a $\beta$-lactam but is better tolerated than co-amoxiclav with approximately one-third the GI side effects.

## P1718

### The efficacy of non-surgical and systemic antibiotic treatment regimens in smoking and non-smoking patients

E. Pähkla, K. Lõivukene, P. Naaber, M. Saag *(Tartu, EE)*

Periodontitis is a chronic infectious disease, which leads to the destruction of periodontal ligament fibres and alveolar bone until tooth loss. The objective of this study was to compare the longitudinal effect of combination of non-surgical periodontal therapy with systemic antibiotics in smoking (S) and non-smoking (NS) patients.

**Methods:** There were total of 28 patients with severe generalized chronic periodontitis involved in this study (14 S, 14 NS), who did not respond well to previous mechanical periodontal treatment. The clinical examination included recordings of visible plaque index (VPI), modified gingival index (MGI), bleeding on probing (BOP) and suppuration after probing (SUP), probing pocket depths (PD) and clinical attachment levels (CAL). The non-surgical periodontal therapy was performed within 4 weeks. Clinical parameters were recorded at baseline, 2-3 weeks after the first mechanical treatment and 14 months after combined treatment, during a regular check-up visit. As the patients did not respond to the conventional periodontal therapy, the microbiological analyses were taken and a combination of systemic amoxicillin 500 mg × 3 and metronidazole 250 mg × 2 for 7 days, was prescribed.

**Results:** The results suggested that the combined systemic antibiotic therapy is effective in case of severe generalized chronic periodontitis, as VPI, BOP, SUP, CAL, and MGI improved significantly after the treatment. In the NS group all parameters, except CAL, improved significantly after the treatment. The S showed markedly smaller reduction in SUP, MGI, and CAL.After instrumentation, no periodontal pathogens were isolated in 11 (39%) patients, while 17 patients (61%) were infected with one to three different pathogens. Among the pathogens, Prevotella intermedia/nigrescens (10 patients) and Actinobacillus actinomycetemcomitans (8 patients) were dominating. The total level of microbial load (log10 CFU/ml) as well as the spectrum of pathogens in S and NS patients remained similar.

**Conclusions:** Despite of positive treatment effect in general, there were insignificant improvements in any clinical parameters in the smoking group. Smoking has adverse effect on periodontal therapy; therefore the dentist should cooperate with patients in counselling of smoking cessation to achieve better results in the treatment of periodontitis.

# Diagnostic and laboratory methods for bacteria-II

## P1719

### The usefulness of the measurement of laminin in patients with bacterial infections

H. Lydatakis, I. Hager, T. Miha, V. Babali, N. Tsimberakis, H. Tamvakos, S. Pappas, I. Diamantis *(Piraeus, Athens, GR)*

**Objectives:** Laminin (LN), which is a large multidomain glycoprotein of the extra cellular matrix, has attracted much attention because of its importance in many cellular functions, including induction of cell adhesion, growth promotion and mediation of cell communication. The target of this study was to find out whether there is any relation between the levels of serum LN and the inflammatory activity of a microbial infection.

**Patients/Methods:** From June to October 2005, 48 immunocompetent adults, with confirmed bacterial infection were admitted to our hospital (17 with pneumonia, 24 with pyelonephritis and 8 with cholecystitis) (Group 1). At the same time 50 hospitalised patients for non-infectious causes (stroke, gastrointestinal bleeding, anaemia) were also studied (Group 2). The levels of serum LN and CRP were measured on the day of admission in both groups. The levels of LN were measured using an enzyme immunoassay kit (TaKaRa LAMININ EIA Kit) and 100 healthy volunteers were used to determine its normal limits (130–520 ng/ml). Plasma CRP concentration was assessed by immunoturbidometric method (using RANDOX, UK kits). Normal values were considered those below 10.

**Results:** The mean serum LN levels of patients of Group 1 were 800.90 ± 249.29 (much higher that the normal limits), while the mean CRP value was 117.53 ± 87.78. The mean corresponding values in Group 2 were 475.79 ± 239.25 for LN (within normal limits) and 24.15 ± 32.77 for CRP. There is a statistically significant difference between the mean LN levels of the two groups ($p < 0.001$). Additionally, there is a statistically significant correlation between the levels of LN and CRP (a well studied serum inflammatory marker) in patients with bacterial infection (Group 1) (Pearson correlation coefficient $r = 0.565$, $p = 0.01$).

**Conclusions:** The definition of the LN levels could constitute a new reliable, simple, direct serum marker for the confirmation of an active bacterial infection. Additionally, as the CRP levels are above normal in Group 2 too (patients without infection) while LN lies within normal limits, maybe LN is even more specific than CRP. More studies are required in the future, with more patients included, in order to confirm the outcome of this study.

## P1720

### Performance and clinical significance of a direct tube coagulase test using serum separator tubes for rapid identification of *Staphylococcus aureus* from blood culture broth

D. Kwa, T. Schülin-Casonato, P. Sturm *(Nijmegen, NL)*

**Objective:** Blood cultures are important in the diagnosis of serious infections. Early administration of effective antibiotics is associated with improved patient outcome. The performance of the direct tube coagulase (DTC) using serum separator tubes (SSTs) for rapid identification of *S. aureus* from blood culture broth (BCB) was investigated. The clinical significance of rapid identification was assessed.

**Methods:** Consecutive blood cultures with gram-positive cocci in clusters were tested. BCB was collected in SSTs using a subculture-venting unit. After centrifugation, the supernatant was discarded and 1 ml rabbit plasma was added to the remaining pellet of bacteria. Coagulation was evaluated after 2

and 4 hours incubation at 37°C, and after overnight incubation at room temperature. In parallel, a direct tube coagulase test was performed using a 1:10 saline dilution of BCB as described previously. Isolates were identified by standard microbiology procedures. Clinical significance was measured by comparison of antimicrobial prescription based on Gram stain results, direct coagulase results, and culture results.
**Results:** Over a 6-week period, 90 BCBs from 46 patients were tested. *S. aureus* was present in 22 BCBs. Using the serum separator tube method and the saline dilution method, the sensitivity of the DTC after 2 hours incubation was 91% and 41%, and after 4 hours 100% and 88%, respectively. The specificity of both methods was 100%. Rapid identification of *S. aureus* resulted in initiation (n = 1) or streamlining (n = 4) of antimicrobial therapy in 5 of 13 patients with *S. aureus* bacteremia. Rapid identification of coagulase-negative staphylococci resulted in changes in antimicrobial therapy in 1 of 33 patients.
**Conclusion:** The DTC using SSTs for bacterial enrichment is a very reliable, rapid, cheap and easy to perform method for identification of *S. aureus* from BCB. Implementation of this test can improve antimicrobial therapy.

## P1721

## Evaluation of the results of the Spanish SEIMC External Quality Control Program for the diagnosis of *Enterococcus faecalis* and *Klebsiella pneumoniae* infections

R. Guna, J.L. Pérez, N. Orta, C. Gimeno *on behalf of SEIMC*

**Objectives:** To evaluate the results obtained from four shipments of two different strains by the participants in the SEIMC External Quality Control Program (EQCP). These controls were intended to analyse the percentages of correct species identification and the ability of the participants in detecting some special features of the control strains: vanB phenotype in the case of *E. faecalis*, and the production of extended spectrum betalactamase (ESBL) in *K. pneumoniae*.
**Methods:** The same strain of each microorganism was sent in two different shipments. The vanB *E. faecalis* strain was sent both in a control of year 2002 as well as in other of 2004, while the ESBL-producing *K. pneumoniae* was sent in 1999 and in 2005 to an average of 200 laboratories. The results obtained were compared with those of a reference laboratory that certified both the species identification and the resistance features.
**Results:** In the 2002 control, 92.6% of participants identified correctly *E. faecalis*, while 97.8% did it in 2004. As for the glycopeptide resistance pattern of the enterococcal strain, 39.9% and 53.3% of participants detected the vanB phenotype in 2002 and 2004, respectively. Overall, the *K. pneumoniae* strain was correctly identified in both separate controls by most of the participants (97.1% and 98.6%, respectively). Interestingly, the percentage of laboratories that detected the presence of the ESBL in the *K. pneumoniae* strain sharply increased from 55.0% in 1999 to 89.4% in 2005.
**Conclusions:** The overall percentages of correct species identification were high for the two microorganisms and for both control points. Most important, the ability of the Spanish clinical laboratories in detecting the special resistance features of these strains clearly improved along the study period. These data confirm the importance of implement a continuous surveillance of the diagnostic training in the clinical laboratory, as well as the possible positive intervention of the SEIMC External Quality Control Program in such improvement, since the analysis of results is accompanied of updated reviews on the subject of each control.

## P1722

## Evaluation of the RIDASCREEN® Borrelia IgG and IgM for the serological diagnosis of Lyme borreliosis

A. Bonnet-Pierroz, A. Resenterra, O. Péter *(Sion, CH)*

**Objective:** To evaluate 2 new ELISA RIDASCREEN® Borrelia IgG and IgM for antibody response in patients with confirmed Lyme borreliosis and to compare to the results of VIDAS Lyme (IgG-IgM) and in-house immunoblots (*B. garinii* IgG and IgM for early cases or *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, *B. valaisiana* IgG for late cases).
**Methods:** ELISA RIDASCREEN® Borrelia IgG and IgM was used to screen sera from patients with clinically confirmed Erythema migrans EM (N = 30). Patients with confirmed neuroborreliosis by intrathecal antibody synthesis (N = 45) were evaluated for IgG antibodies to Borrelia. Sera from patients with Acrodermatitis chronica atrophicans ACA (N = 30) and sera and synovial fluids from patients with Lyme arthritis (N = 30) were also evaluated for IgG antibodies. Patients with syphilis (N = 10) and infectious mononucleosis (N = 10) were screened for IgG and IgM antibodies to Borrelia in order to estimate the specificity.
**Results:**

| Clinical symptoms | RIDASCREEN Borrelia IgG and IgM or IgG | VIDAS Lyme IgG-IgM | Immunoblots IgG-IgM or IgG (4 species) |
|---|---|---|---|
| EM | 18/30 | 15/30 | 17/30 |
| Neuroborreliosis | 41/45 (+3 equivocal) | 43/45 (+2 equivocal) | 45/45 |
| ACA | 29/30 (+1 equivocal) | 30/30 | 30/30 |
| Arthritis | 29/30 (+1 equivocal) | 30/30 | 30/30 |
| Syphilis | IgG 0/10 IgM 2/10 | nd | nd |
| EBV IgM pos | IgG 2/10 IgM 9/10 | nd | nd |

**Conclusion:** The ELISA RIDASCREEN® Borrelia IgG and IgM have shown a good sensitivity for the serological diagnosis of Lyme borreliosis. The short evaluation for the specificity of the IgG test revealed a good assay with few false positive reactions, whereas the IgM assay was, as expected more prompt to give false positive results with sera from patients with infectious mononucleosis. So far any equivocal or positive tests should be confirmed by immunoblots.

## P1723

## Is it necessary to incubate the BacT/Alert blood culture bottles more than 3 days?

A. Vilas, D. Fontanals, I. Sanfeliu, I. Pons, D. Mariscal *(Sabadell, ES)*

**Objective:** To assess the incubation time reduction of the aerobic and anaerobic BacT/Alert system bottles from 5 to 3 days.
**Methods:** From 1996 to 2004 we processed 94.303 blood culture sets and detected 9.432 (10%) positive blood cultures with clinical significance. We retrospectively examined the detection time of positive bottles and assessed the clinical significance of the bottles that were positive between the fourth and fifth day.
**Results:** Out of 9432 positive blood cultures with clinical significance, 9238 (97.9%) were detected within the first 3 days

of incubation. Out of the 194 positive blood cultures detected between the fourth and fifth incubation days, 105 were recovered in concurrent cultures within the first 3 days. Chart reviews were conducted from 78 patients with the remaining 89 isolates. Only in 24 patients (0.3% positive blood cultures) changes in antimicrobial therapy based upon the positive blood culture results on day 4 to 5 were made, in the other patients the empirical treatment was adequate. The isolated microorganisms in those 24 patients were: 7 Gram-positive cocci (3 *Staphylococcus* spp. not *S. aureus*, 1 *Staphylococcus aureus*, 2 *Streptococcus viridans* and 1 *Streptococcus pyogenes*), 5 Anaerobes, 3 *Enterobacteriaceae*, 2 *Pseudomonas aeruginosa*, 2 *Campylobacter* spp., 2 *Candida* spp. 1 *Cryptococcus neoformans*, 1 *Brucella* spp. and 1 *Haemophilus influenzae*.

**Conclusions:** Incubation of Bact/Alert blood cultures bottles only for 3 days would have represented a detection loss of 0.3% of the clinically significant isolates, which led to antimicrobial therapy changes. Although we keep employing a 5-day incubation for routine blood cultures, we could reduce the incubation time to 3 days depending on current instrument capacity.

## P1724

## An enzyme immunoassay for anti-diphtheria antibodies: a practical alternative to the Vero cell assay

R. Budd, E. Harley, R. George, A. Efstratiou, K. Broughton, A. Bradwell *(Birmingham, London, UK)*

**Introduction:** In this extended study, results from an anti-Diphtheria toxoid enzyme immunoassay (EIA), specifically designed to detect higher affinity antibodies, were compared with those from a Vero cell assay (VCA).

**Methods and Results:** 154 serum samples with antibody concentrations ranging from 0.008–8.000 IU/mL on the VCA from the Respiratory and Systemic Infectious laboratory (RSIL) were assayed by EIA (The Binding Site Ltd, UK). A further 100 samples from RSIL selected on the basis of being close to the protective level, were assayed to confirm the performance of the EIA. The EIA was calibrated, against the NIBSC reference material 00/496 and the assay measuring range was 0.004–3.0 IU/mL Results were compared using the WHO guidelines of 0.01–0.1 IU/mL as minimum protective level, and > 0.1 IU/mL as protective. Relative agreement, sensitivity and specificity for the first 154 samples were: 91.6%, 98.8% and 83.3% respectively, for the second set of 100 samples performance was: 90.0%, 84.5% and 97.6%, and for the combined 254 samples results were: 91.3%, 92.9% and 89.4% respectively. ROC analysis of the total 254 samples confirmed the highest sensitivity 92.9% and specificity 89.4% occurred at a cut-off of precisely 0.1 IU/mL for the ELISA assay.

**Conclusion:** Of the total 22 discrepant samples, 14 had VCA and EIA values < 0.149 IU/ml, therefore we suggest the possibility of establishing an equivocal zone for the interpretation of the EIA results. If the test is part of a general immune status assessment a grey zone is not required. If undertaken to determine the requirement for immunization, the use of the equivocal zone is recommended. By applying these criteria in the EIA, only one sample would have suggested inappropriate immunization, as indicated by a VCA result > 0.149 IU/mL. Because of the > 90% agreement between the two assays, significant advantages of cost and speed, ease of use and the potential for automation, the EIA could therefore be considered as an alternative to the VCA.

## P1725

## Evaluation of accuracy limits of countable colony-forming units on agar plates

J. Arbique, A. Rendell, K. Forward *(Halifax, CA)*

**Objectives:** Accurate colony counts are an essential component of many microbiology research projects and clinical laboratory processes. The suggested range of accuracy of colony-forming units (CFU) extends from 30 to 300 (Standard Methods for the Examination of Water and Wastewater). This recommendation dates to 1907, and fails to adequately address the numerous sources of inter- and intra-variability. Without more detailed analysis it is difficult to estimate the sample size and number of replicates necessary to ensure accurate results. The purpose of this study was to determine the validity of accuracy limits for quantifying CFUs on agar plates.

**Methods:** *Escherichia coli* (ATCC 25922) and *Staphylococcus epidermidis* (ATCC 12228) were used to prepare series of four organism densities ranging from approximately 40–500 CFU, on three different days. On each day, each of the 4 densities for both organisms was plated on SBA and viable organisms were counted following incubation. An average of the margins of error obtained over the 3 days of testing was used to determine the reproducibility of agar plate counts, and to estimate the optimum number of replicate plates (sample size) required for each organism at each concentration.

**Results:** Margins of error for both organisms were greatest with suspensions yielding approximately 40 CFU, and lowest for suspensions yielding 300 and 500 CFU. Nine replicate plates were required for a suspension of *S. epidermidis* yielding 40 CFU to achieve the same margin of error as obtained with 3 replicate plates at concentrations yielding 100–300 CFU. Seven replicates plates were required for a suspension of *E. coli* yielding 40 and 100 CFU to achieve similar margins of error to those obtained with 4 replicate plates at concentrations yielding 300 CFU, and 3 replicate plates at concentrations yielding 500 CFU.

**Conclusion:** We found that the greater the concentration (300 and 500 CFU), the fewer replicate plates necessary to reliably estimate organism concentrations. The lower the organism density (40 CFU), the more plates necessary to reliably estimate CFUs. Contrary to the recommendations described in Standard Methods for the Examination of Water and Wastewater, CFU of 500 were reliably reproducible. For greatest accuracy, experiments should be conducted so as to assure that colony counts are in the range of 300–500.

## P1726

## Direct microscopy: a valuable instrument for diagnosis and prognosis of periodontal disease

F.S. Alecu, C. Defta, M. Giurgiu, S. Dumitriu *(Bucharest, RO)*

**Objective:** To appreciate the composition of micro flora from periodontal pockets, using light microscopy and to compare it with clinical status.

**Introduction:** It is generally accepted that periodontal disease occurs when anaerobic Gram-negative flora increase in number with the subsequent decrease of facultative anaerobe Gram-positive bacteria. In other words, the switch from Gram-positive to Gram-negative of sub-gingival flora has a pathologic significance and could be observed using direct microscopy.

**Materials and methods:** 30 specimens sampled with sterile paper points from periodontal pockets and 10 samples from clinical healthy persons were included in this study. Each

sample was diluted in 0.5 ml saline solution and, with a calibrated loop, was taken 10 $\mu$L aliquots in order to prepare a smear for microscopic examination and for inoculation on solid media (Columbia with 5% sheep blood). The smears were Gram stained and the culture plates were incubated in anaerobic conditions (72 h, 37° C) and in air (24 h, 37° C).
**Results:** In 95% of samples from patients with periodontal disease, easily notable, high number of Gram-negative bacteria at direct microscopy, associated with abundant growth in anaerobic condition and poor growth in air. In 9 from 10 healthy patients, the Gram-negative flora was almost absent and Gram-positive bacteria were in high number, correlated with the absence of bacterial growth in anaerobiosis and some growth in air.The presence of *Treponema* spp. at direct microscopy was associated with deep and bleeding periodontal pockets. After few days of proper therapy, the good clinical status was well correlated with an increasing number of gram-positive bacteria.
**Conclusions:** 1) Using a diluted sample for microscopic examination, the value of the method increase, offering important information about the composition of sub-gingival flora.2) The good correlation between the clinical status and microscopic finding recommend it as an easy to use diagnostic method in dentistry.

## P1727

## Identification of species and glycopeptide resistance among enterococcal isolates by BD Phoenix

N. Siafakas, M. Pantelaki, E. Kalogeropoulou, S. Chaniotaki, L. Galani, A. Antoniadou, L. Zerva *(Athens, GR)*

**Objectives:** Vancomycin resistant enterococci are emerging in Europe necessitating their fast and accurate identification by the Laboratory. There was an attempt to evaluate the performance of the BD Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, Md.) for the correct identification of species and glycopeptide resistance in comparison to the gold standard of diagnosis, PCR, using a large collection of clinical strains.
**Methods:** A total of 232 enterococcal isolates were tested by the BD Phoenix sytem. These strains were isolated from 145 faecal, 55 urine, 18 pus, 5 blood and 9 samples from other body sites cultures. A multiplex PCR was applied using 8 different pairs of primers, specific for the identification of *E. faecium*, *E. faecalis* and the vanA, vanB, vanC, vanD, vanG, vanE glycopeptide resistance genotypes. Susceptibility to the glycopeptides was also confirmed by the Etest (AB Biodisk, Solna, Sweden).
**Results:** According to the PCR, there were 101 *E. faecium* (including 85 vanA-positive strains), 72 *E. faecalis* (including 1 vanA-positive and 1 vanB-positive strains) and 58 *E. cass/gall* isolates. Two strains were not identified and were excluded from the analysis. Discrepant results between the multiplex PCR and the Phoenix system were obtained for 23/232 isolates (10%) with similar rates amongst faecal (15/145, 10.4%) and the rest of the isolates (8/87, 9.4%). The most common discrepancies were the misidentification of 11 *E. faecium* vanA strains and 7 *E. faecalis* strains as *E. cass/gall* by Phoenix. Two *E. faecalis* strains were incorrectly characterized as vancomycin resistant, two *E. faecium* strains were misidentified as *E. hirae* and *E.cass/gall*, respectively, and one *E.cass/gall* strain was reported as *E. faecium* resistant to both glycopeptides. Thus, the sensitivity and specificity for the identification of *E.cass/gall* by Phoenix were 98.3% (57/58 strains) and 88.4% (145/164 strains), respectively, while 12.8% of vanA strains (11/86 strains) were not recognized by this system.
**Conclusion:** This study demonstrates that the new identification system, Phoenix, similarly to other automated or manual systems, presents with problems regarding correct identification of *enterococcal* species and glycopeptide resistance. Specifically, Laboratories should be aware that clinically significant isolates identified as *E.cass/gall* should be confirmed by another method.

## P1728

## An audit of sputum requisition practices

P. Lal, I. Balakrishnan *(London, UK)*

**Objectives:** To analyse the indications and rationale for the processing of sputum specimens in a London teaching hospital.
**Methods:** Sputum samples received from 01/12/2004 - 28/02/2005 were included in this study. Data were obtained from the patient requisition forms and the Winpath systems and were analysed further as per the objectives.
**Results:** A total of 1309 specimens were received during this period. 1202 (92%) from hospital in-patients and 107 (8%) from general practitioners. Out of the total of 1202 samples received from hospital-in patients 167 (13.9%) had > 25 epithelial cells/LPF. No clinical details were mentioned in 258 (21.4%) and 388 (32.2%) were from patients already on antibiotics. Repeat specimens within one week were sent in 340 (28.2%) cases and 375 (33%) had atypical serology also sent. Out of the 1202 hospital-in patient samples 372 (30.9%) had a significant isolate and 488 (40.5%) had normal respiratory tract flora isolated. Others were reported as: ''gross oral contamination'' [168 (13.9%)], ''no growth'' [29 (2.4%)] and ''no significant growth'' [37 (3.07%)]. There were a few specimens reported as ''inappropriate specimen - two days old'' [35 (2.9%)] and ''leaking'' or ''Saliva only'' [22 (1.8%)]. Out of a total of 107 samples received from GP patients 14 (13.1%) of samples had > 25 epithelial cells/LPF, 25 (23.3%) had no clinical details provided, 5 (4.6%) samples were sent while patients were on antibiotics and 9 (8.4%) samples were repeated within one week. Only 4 (3.9%) had atypical serology also sent.
**Conclusions:** - Less than one-third of specimens yielded a significant pathogen.- Adequate clinical details were lacking in about one-fifth of specimens.- Nearly one-third of specimens were repeated within one week, without a clear indication.- About 16% of specimens were of poor quality.- Atypical serology was only performed in 3.9% of outpatients, as compared with 33% of in-patients.This audit brings forth the fact that the clinical indications for which sputa are being sent for culture need to be clearly defined and an educational campaign instituted amongst relevant healthcare professionals. Sputum collection techniques need to be rigorously applied if good-quality specimens are to be obtained. Indications for performing atypical serology need to be defined and reinforced, particularly in primary care.

## P1729

## A new approach to laboratory diagnostic of infectious gastroenteritis – a follow-up

B. Olesen, D.S. Hansen, H. Tybring, A. Hansen, K.E.P. Olsen, B.G. Bruun *(Hillerød, Copenhagen, DK)*

**Objectives:** In order to optimize use of laboratory facilities and ensure flexibility in relation to current epidemiology, a new approach to laboratory diagnosis of infectious gastroenteritis was applied: From an algorithm the decision of which organisms to test for was defined by the demographic, clinical and epidemiological information submitted to the laboratory on paper/electronic request forms.

**Methods:** From April 1, 2004–June 30, 2005, hospitals and general practitioners submitted a request form with the following information together with the stool sample (s): (1) acute or persistent diarrhoea (duration > 2 weeks); (2) bloody stools; (3) recent history of foreign travel; (4) > 2 patients within same epidemiological setting; and (5) nosocomial infection. Provision of data is mandatory when submitting electronically. Based on these data, analyses were performed according to an algorithm. Examination for *Salmonella, Shigella, Yersinia, Campylobacter*, and *Clostridium difficile* was done by culturing. Verotoxin producing *E. coli* (VTEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and enteroinvasive *E. coli* (EIEC) were identified by PCR for virulence genes and serotyping. Rota and adenovirus were detected by antigen tests and parasites by microscopy.

**Results:** In total we examined 8,628 samples from 4,088 patients. A pathogen was isolated in 19% of patients. In 673 cases (17%) clinical/epidemiological data were missing.

- 1,205 patients had diarrhoea < 2 weeks: 13% with *Campylobacter*, 5% with *Salmonella*, 4% with ETEC, 3% with *Giardia*, and 1% each with EIEC and VTEC
- 1,502 patients had diarrhoea > 2 weeks: 3% with *Campylobacter*, 2% with *Giardia*, 1% each with EPEC and VTEC
- 740 patients had a history of foreign travel: 7% with *Campylobacter*, 6% with ETEC, 4% with *Salmonella*, and 4% with *Giardia*
- 214 patients had bloody stools: 18% with *Campylobacter*, 3% with *Salmonella*, and 2% with VTEC
- 896 patients were < 7 years: 4% with *Campylobacter*, 3% with EPEC, 2% each with *Giardia*, VTEC and *Salmonella*, and 1% with ETEC

**Conclusions:** *Campylobacter* was the most common bacterial pathogens in all groups and rotavirus was the most common pathogen in children < 7 years. The new approach had a number of advantages: more relevant microbiological analysis, collection of data on defined patient groups, and flexibility regarding adaptation to current epidemiological knowledge. Increasing use of electronic submission of request forms will optimize the approach used.

## P1730

## Recognition of *Staphylococcus aureus* isolates as small colony variants applying Fourier-transform infrared spectroscopy

K. Becker, N. Al Laham, W. Fegeler, R.A. Proctor, G. Peters, C. von Eiff *(Munster, DE; Madison, US)*

**Objectives:** Small colony variants (SCVs) are an emerging infectious disease problem, presenting as a naturally occurring, slow-growing subpopulation of *Staphylococcus aureus* that are characterized by tiny colonies on solid media. Studies on SCVs recovered from patients with persistent infections are hampered due to their frequent unstable phenotype. In particular, SCVs are not easily distinguishable from the normal phenotype in broth media and a reversion of SCVs into the normal phenotype is not traceable.

**Methods:** A set of isogenic *S. aureus* isolates comprising the (i) normal and the (ii) SCV phenotype (isogenic to the isolate with normal phenotype) recovered from clinical specimens, as well as (iii) corresponding mutants mimicking the SCV phenotype (knock-out of hemB), and (iv) their complemented mutants were used to investigate the feasibility of Fourier-transform infrared (FTIR) spectroscopy to trace the expressed phenotype in broth media. The respective isolates cultured on solid media served as controls. In addition, all isolates were genotyped by pulsed-field gel electrophoresis and spa typing.

**Results:** Using first-derivative infrared spectra to calculate spectral distances, hierarchical clustering based on spectral information in three different spectral ranges resulted in a dendrogram that showed a clear discrimination between both staphylococcal phenotypes. Distinct clusters comprising the clinical and mutant SCV phenotype on one hand and the normal phenotype (isolate with normal phenotype and complemented mutant) on the other hand were found. Thus, SCVs from different clonal lineages gave spectra that were more similar to one another than to their normal growth parent. FTIR was also shown to be able to trace the switch of the phenotypes in broth when the medium was supplemented.

**Conclusion:** FTIR spectroscopy allows a rapid, reproducible and clear discrimination of different phenotypes of *S. aureus* in fluid media for diagnostic and research purposes. In contrast to genotyping approaches, FTIR staphylococcal fingerprinting is only reliable for typing purposes if the isolates exhibit the same phenotype. In future studies, this technique may also provide an approach for tracing the SCV phenotype in infected tissues.

## P1731

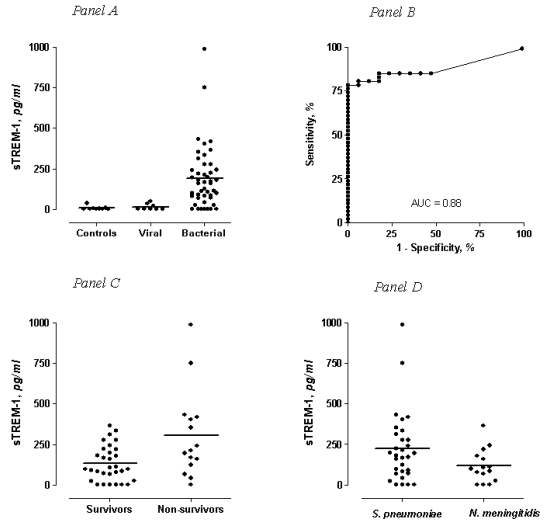
## Levels of sTREM-1 in cerebrospinal fluid as a marker for bacterial meningitis

M. Weisfelt, R. Determann, J. de Gans, A. van der Ende, M.J. Schultz, D. van de Beek *(Amsterdam, NL)*

**Objectives:** Triggering receptor expressed on myeloid cells-1 (TREM-1) is a recently discovered cell surface molecule whose expression on phagocytes is up regulated by exposure to bacteria or fungi. A soluble form of TREM-1 (sTREM-1) can be measured in various body fluids. We studied whether sTREM-1 in cerebrospinal fluid (CSF) could serve as a biomarker for the presence and outcome in patients with bacterial meningitis.

**Methods:** In this retrospective study on diagnostic accuracy we used an ELISA to determine levels of sTREM-1 in CSF from 46 adults with bacterial meningitis, confirmed by CSF culture, who participated in the prospective Dutch Meningitis Cohort Study; 8 patients with viral meningitis, confirmed by polymerase chain reaction of CSF; and 9 healthy control subjects, who underwent lumbar puncture to exclude the diagnosis of subarachnoid haemorrhage. The Mann-Whitney U test and the Chi-square test were used to identify differences between groups. A receiver-operating-characteristic curve (ROC) was constructed to illustrate various cut-off CSF levels of sTREM-1 in differentiating between the presence and absence of bacterial meningitis and diagnostic accuracy was quantified by 95% confidence intervals (95% CI).

**Results:** Levels of sTREM-1 in CSF were higher in patients with bacterial meningitis as compared to those with viral meningitis [median, 159 pg/mL (range, 0 to 988 pg/mL) versus 0.5 pg/mL (range, 0 to 48 pg/mL); P = 0.001] and controls [0 pg/mL (range, 0 to 36 pg/mL); P < 0.001; Fig]. Patients with viral meningitis and controls had similar CSF sTREM-1 levels. The area under the ROC curve for discriminating between patients with and without bacterial meningitis was 0.88 (95% CI, 0.80 to 0.96; P < 0.001). At a cut-off level of 40 pg/mL, sTREM-1 yielded a sensitivity of 0.80 (95% CI, 0.69 to 0.88) and a specificity of 0.94 (95% CI, 0.77 to 0.99). In patients with bacterial meningitis, CSF sTREM-1 levels were associated with mortality [survivors versus nonsurvivors: median 99 pg/mL (range, 0 to 365 pg/mL) versus 214 pg/mL (range, 0 to 988 pg/mL); P = 0.02].




**Conclusions:** Measuring sTREM-1 in CSF may be a valuable new approach to accurately diagnose bacterial meningitis and identify patients at high risk for adverse outcome. Therefore, a prospective study on sTREM-1 as biomarker in bacterial meningitis is needed.

## P1732

## Systematic review of rapid diagnostic tests for enterohaemorrhagic *E. coli*

I. Abubakar, L. Irvine, L. Shepstone, S. Schelenz, C. Aldus, P. Hunter *(Norwich, UK)*

**Objective:** A variety of rapid tests for the detection of Enterohaemorrhagic *Escherichia coli* (EHEC) have recently emerged. Culture on Sorbitol MacConkey (SMAC) agar and biochemical identification, while easy to use and inexpensive, is slow and lacks sensitivity in the detection of non O157:H7 serotypes. This study sought to determine the accuracy of rapid serological or polymerase chain reaction (PCR) assays which have been evaluated for the detection of all EHEC serotypes compared to culture.

**Methods:** A systematic review and meta-analysis of 146 articles, identified via searches of electronic databases, hand searching of selected journals, and through contact with experts and commercial test manufacturers. The majority of these needed to be excluded due to low quality or lack of accuracy data. Sensitivity and specificity of each method was calculated using full biochemical identification as the reference standard. Twenty-one studies met the inclusion criteria, of which 7 used PCR methods and 10 used serological assays and 4 were based on culture. A summary receiver operator curve (SROC) was constructed from these data and the area under the curve (AUC) calculated (using the trapezium rule).

**Results:** Serological tests had individual sensitivities ranging from 0.82 to 1.00 and specificities ranging from 0.67 to 1.00. PCR tests had individual sensitivities ranging from 0.94 to 1.00 and specificities ranging from 0.92 to 1.00. Additional analysis comparing SMAC agar culture with toxin detection methods showed poor sensitivity compared to PCR and serological tests (ranging from 0.24 to 0.38) yet the specificity was very good (1.00 for all 4 studies considered).

**Table 1: Summary of the AUC for each test group**

| Group | N | AUC |
|---|---|---|
| PCR | 7 | 0.951 |
| Serological Tests | 10 | 0.979 |
| Culture Methods | 5 | 0.713 |

**Conclusions:** Our results suggest that both molecular and serological tests may have a potential role in detecting EHEC infection. Whilst there is very little difference in the effectiveness of these techniques, both are faster and have improved sensitivity when compared to traditional culture methods. Fast, reliable diagnosis could lead to more informed treatment choices and improved outbreak control measures. However, given the substantial extra cost of these assays, an assessment of economic feasibility is necessary prior to use in everyday practice.

## P1733

## Antibodies against *Bordetella pertussis* detected by slow agglutination test and ELISA in two age-related groups of vaccinated people suspected of acute pertussis: a comparative study

Z. Medkova, O. Stepanikova *(Brno, CZ)*

**Objective:** The aim of presented study was to describe differences between results of two tests used for detection of antibodies against *Bordetella pertussis* (slow agglutination and ELISA) in two age-related groups of patients suffering from respiratory infection. Each of the people has undergone vaccination against *B. pertussis*.

**Methods:** Paired sera obtained from two age-related groups of patients [(1). age 0–6 years, n = 49; (2). age above 6 years, n = 455] suffering from acute respiratory infection were tested. The first group comprised the children who were vaccinated earlier than one year before testing; the second group was determined by longer interval between the vaccination and the testing. The criterion of positivity of the slow agglutination was based on quadruple increase/decrease of the titer of specific antibodies; the criterion of serodiagnosis of the illness was the same. Each of the patients was tested by ELISA IgA,IgG,IgM (Virotech) during the same period, positive results of each of class of immunoglobulins were evaluated as positive ELISA. The differences of results obtained by the two tests were assessed inside and between the groups.

**Results:** There were found 12.2% (respective 16.9%) concordant positive and 36.7% (respective 19.6%) concordant negative results between the tests in the first (respective second) group. There were found the following discrepancies in the frame of non equal results: agglutination positive/ELISA negative sera were present in 8.2% (respective 13.2%) persons and agglutination negative/ELISA positive samples were present in 42.9% (respective 62.2%) persons.

**Conclusion:** (1). The frequency of serologically confirmed infection based on results of slow agglutination is higher in the group of people older six; the interpretation of the results in the younger group is limited by the influence of actual vaccination. (2). The ELISA evaluated as described above

shows extremely high frequency of positivity in both groups, thus, the usefulness for diagnostics of acute infection seems to be low. (3). The study will be continued to asses relationships between the positive results detected by slow agglutination and the positive ones detected by ELISA in separate classes of specific immunglobulins.

## P1734

## Accuracy of the MicroScan WalkAway system to identify coagulase-negative staphylococci

A. Sáez, B. Ruiz, L. Martínez-Martínez *(Santander, ES)*

**Objective:** To determine the reliability of the identification of coagulase-negative staphylococci (CoNS) with the MicroScan WalkAway 96 (WA, Dade Behring) system at species level when a > = 90% probability is obtained, considering as a reference the results of molecular identification.
**Methods:** One hundred and sixty-eight isolates of CoNS from clinical samples (October 2003-May 2005) for which the identification with the WA system was ≥ 90%, and 9 ATCC type strains were evaluated. Bacteria were identified with the WA system using Pos Combo 1S panels. Absence of coagulase was determined with a latex assay (Pastorex® staph-plus, Bio-Rad). Reference identification was established by sequencing of the 16S rRNA; when identification with WA and 16S rRNA disagree, definitive identification was defined after sequencing of the sodA and tuf genes, as previously described (Drancourt et al. JCM 2000; 38: 3623–30 and Heikens el al. JCM 2005; 43: 2286–90). For identification, the sequences of 16S rRNA, sodA and tuf were compared with those in GeneBank. Homologies values above 97% were considered reliable.
**Results:** All 9 type strains were correctly identified by 16S rRNA sequencing as named by the ATCC. Among the 168 clinical isolates, the molecular method identified the following species (number): *S. hominis* (39), *S. haemolyticus* (35), *S. saprophyticus* (30), *S. epidermidis* (27), *S. lugdunensis* (12), *S. schleiferi* (7), *S. capitis* (7), *S. simulans* (4), *S. pasteuri* (2), *S. warneri* (2), *S. intermedius* (2) and *S. equorum* (1). The WA system correctly identified 8 out of the 9 ATCC strains. *S. pasteuri* is not included in the WA database, and the corresponding ATCC strain was misidentified as *S. warneri*. One hundred and fifty-seven out of the 168 (93.4%) clinical isolates were correctly identified by the WA. Five *S. haemolyticus* were identified by WA as *S. auricularis* (2), *S. simulans* (2) and *S. warneri* (1). Other errors corresponded to: two *S. pasteuri* misidentified as *S. warneri*, one *S. epidermidis* as *S. hominis*, one *S. lugdunensis* as *S. schleiferi*, one *S. hominis* as *S. haemolyticus* and one *S. equorum* as *S. cohnii*. All isolates of *S. saprophyticus*, *S. schleiferi*, *S. capitis*, *S. simulans*, *S. warneri* and *S. intermedius* were correctly identified by the WA system.
**Conclusions:** The MicroScan WalkAway 96 is reliable to identify CoNS at species level when a probability of > = 90% is obtained. *S. pasteuri* should be incorporated to the WA database in order to improve its performance.

## P1735

## The Polish National External Quality Assessment Scheme (POLMICRO 2005) - detection of BLNAR *Haemophilus influenzae*

E. Stefaniuk, B. Chmylak, J. Fiett, E. Zurek, W. Hryniewicz *(Warsaw, PL)*

**Objectives:** The aim of this study was to analyse the results of proficiency testing obtained by Polish microbiology laboratories participating in POLMICRO. *Haemophilus influenzae* is an important pathogen causing a variety of community-acquired respiratory tract infections, acute otitis media and purulent meningitis. Two mechanisms of ampicillin (AMP) resistance in this organism are described. One is mediated by the production of beta-lactamases TEM-1 and ROB-1; these AMP-resistant strains are termed beta-lactamase-producing, AMP-resistant (BLPAR). The second mechanism involves development of altered penicillin-binding proteins (PBP) with decreased affinity to AMP and other beta-lectam agents. Strains with resistance mechanisms mediated by PBP alterations are termed beta-lactamase-nonproducing, AMP-resistant (BLNAR) *H. influenzae*.
**Methods:** Four hundred seventy eight laboratories participated in this part of the scheme. Each participating laboratory received *Haemophilus influenzae* (PM-63)-beta-lactamase negative, ampicillin-resistant strain (BLNAR). The laboratories were asked to provide identification to the species level and of the susceptibility results and interpretation.
**Results:** Correct identification to the species level of this strain was reported by 454 laboratories (97.2%) of the 467 labs involved. Thirteen laboratories reported the analysed strain as *Haemophilus parainfluenzae*. Three hundred ninety eight laboratories (88.1%) of 452 correctly detected the mechanism of resistance to beta-lactams. Only three laboratories incorrectly reported the organism as beta-lactamase producer. The greatest dispersion of inhibition zone was observed in the susceptibility of *H. influenzae* to ampicillin, amoxicillin-clavulanic acid and clarithromycin.
**Conclusions:** Over 90% of the laboratories correctly identified and interpreted beta-lactamase-nonproducing, AMP-resistant (BLNAR) *H. influenzae* strain.

## P1736

## Serological diagnosis of syphilis by a completely automated chemiluminescent immunoassay: Architect syphilis TP

R. Vezzo, L. Vigorè, V. Giuffrè *(Monza, IT)*

Purpose and methods.The Architect Syphilis TP assay is a chemiluminescent EIA that employs three recombinant antigens of *Treponema pallidum* on the solid phase and an anti-human IgM and IgG conjugate. We evaluated this assay in comparison with a conventional EIA (Diesse Enzywell Syphilis Screen Recombinant) on unselected routine serum samples and on repository specimens for whom the results for specific IgG and IgM and of the rapid plasma reagin (RPR) assay were already known. In both instances an immunoblot (IB: Inno-LIATM Syphilis Score, Innogenetics) has been employed on discordant specimens as a confirmatory assay. The precision and robustness of the Architect assay were also evaluated.Results.On 1.165 routine samples (975 from volunteer blood donors and 190 from in and outpatients) the concordance between Architect and EIA was high (1.155 samples, or 99.1%; 7 positives, 1,148 negatives). One of the discordant, positive by Architect and negative by EIA, was confirmed by IB. The specificity of the Architect assay was 99.4% (95% confidence limits: 98.9–99.8). The 177 repository samples assayed belonged to three groups: 1) 9 biological false positives from 6 subjects: all negative by Architect; 2) 134 true positives, all positive by Architect, with a significantly stronger signal (average S/CO: 26.19 vs. 14.53) on the 23 IgM positive samples, all of whom were also positive by RPR; 3) 34 samples positive by RPR and negative by EIA IgG: 32 of them were negative by Architect as well and for IgM, while two specimens

were strongly positive by Architect and positive also for specific IgM and with three specific bands by Inno-LIA, suggesting a pattern of recent infection. The reproducibility of the Architect assay was good, with CVs of 7.78%, 5.71% and 5.51% on 28 replicates over 4 weeks of the assay's negative and positive control and of an internal control; finally, the S/CO distribution of negative specimens confirmed the robustness of the assay, with a mean of 0.09, a median of 0.06, 10 standard deviations between the mean and the cut-off value and the 99th percentile at a S/CO value of 0.53.Conclusion.The automated assay for anti-*Treponema pallidum* antibodies on the Architect system has an excellent sensitivity and a good specificity. The analytical performances, coupled with the elevated throughput and minimal samples handling, make this method a first-choice option for syphilis screening and diagnosis in medium and large volume laboratories.

## P1737

## Usefulness of Sysmex UF-100 and Coral UTI Screen in the diagnosis of urinary tract infection

M. Schinella, P. Rizzonelli, A. Frizzi, L. Collini, P. Gualdi *(Rovereto, IT)*

**Objective:** Quantitative urine culture is the gold standard for defining the diagnosis of Urinary Tract Infection (UTI), because it allows identification of the uropathogenic species. However, this method is time consuming and expensive. Approximately, up to 70% of urine cultures are negative with high cost for unnecessary testing. Thus, we have evaluated the usefulness of two automated analysers for UTI screening to quickly identify the negative samples that can be prompt reported to the clinicians, improving in the quality of patient care and allowing the laboratory to direct more effort into positive samples.
**Methods:** 1.165 of midstream urine samples submitted for microbiological examination were analysed by conventional urine culture plates (McConkey agar + Trypticase Soy agar + Bile Esculine Azide), Sysmex UF-100 (Sysmex, Japan) and Coral UTI Screen (Coral Biotechnology, CA, USA) automated analysers. UTI was defined positive as follows: one or two strains of bacteria with at least $10^5$ UFC/ml for the culture plates, more than 5.000 bacteria/$\mu$l and more than 15 WBC/$\mu$l for the UF-100 and/or an RLU value grater than 2% of the calibrator value for the Coral. When more than two strains of bacteria were found, the culture was classified as contamined.
**Results:** The diagnostic performance of Sysmex UF-100 and Coral UTI Screen are shown in table 1.

*Table 1: Microbiological culture compared with Sysmex UF-100 and Coral UTI Screen*

| True Positive (TP) | True Negative (TN) | False Positive (FP) | False Negative (FN) |
|---|---|---|---|
| 483 (41,5%) | 565 (48,5%) | 96 (8,2%) | 21 (1,8%) |
| **Sensitivity - SE (TP / TP+FN)** | **Specificity - SP (TN / TN+FP)** | **Negative Predict Value - NPV (TN / TN+FN)** | **Positive Predict. Value - PPV (TP / TP+FP)** |
| 95,8% | 85,5% | 96,4% | 83,4% |

**Conclusion:** The Sensitivity (95.8%) and Negative Predictive Value (96.4%) confirm that Sysmex UF-100 and Coral UTI screen are an excellent screening for UTI. After this evaluation, we decide the use of the Sysmex UF-100 and Coral UTI Screen on our routine workflow for UTI screening. The results of both the analysers are sent to a software system (LabFinity Dasit, Italy) connected to the LIS. If the results are lower than the cut-off values, UTI can be excluded and directly reported to the physician. Positive results are submitted to microbiological culture and reported within 24 or 48 hours depending on negative or positive bacterial growth. In our experience, evaluated on further 2.955 samples, this means that 78% of samples are immediately reported within very few hours. Of the 22% of positive samples, 222 (35%) were confirmed by culture and reported within 48 hours, 418 (65%) were not confirmed and reported within 24 hours.

## P1738

## Comparison of the blood and bone marrow culture positivity rates for the diagnosis of brucellosis

S. Iseri, C. Bulut, H. Irmak, M.A. Yetkin, S. Kinikli, N. Tulek, A.P. Demiröz *(Ankara, Samsun, TR)*

**Objectives:** Brucellosis is a common disease, seen worldwide as well as in our country. The diagnosis of brucellosis is made with certainly when brucellae are recovered from blood, bone marrow. In our study, we aimed to compare the blood and bone marrow culture positivity rates in patient with brucellosis.
**Methods:** This study was performed in the Infectious Diseases and Clinical Microbiology Department of Ankara Research and Training Hospital between 2002 and 2004. The diagnosis of brucellosis was made on the history, physical findings, serologic findings and the isolation of the organism. The number of patients with brucellosis included to the study was 102. Blood and bone marrow samples were taken from all of the patients on admission and cultured by using the BACTEC 9050 system.
**Results:** Blood culture positivity for brucellosis was 48% (49/102), while bone marrow culture positivity was 34% (35/102). The difference between those positivity rates was found to be statistically significant ($p < 0.05$). The isolation ratio from blood cultures among acute cases was 66% (40/61) while it was 31% (9/29) among subacute cases. Brucella isolation from blood was not detected in 12 chronic cases. The isolation rates of the microorganism from bone marrow of acute, subacute and chronic cases were 45.9%, 20.7%, 0.9% respectively. Among our patients, 32 had history of medical therapy for brucellosis before admission and 27 of them was treated inadequately. Of those 27 cases, the organism was isolated in 7 (25%) from blood and in 9 (33%) from bone marrow.In the cases with high standard tube agglutination titers, the rate of positivity was also high both in blood and bone marrow cultures. However when compared with low standard tube agglutination titers, that difference was not statistically significant.The mean growing time for the positivity of cultures was 4.2 days for bone marrow and was 5.8 days for blood cultures. The difference between the mean growing times of two culture types was found statistically significant (t-test. $p < 0.05$).
**Conclusion:** Premedication, subacute and especially chronic phases decrease the possibility of isolation of the microorganism from blood culture. Therefore we suggest taking bone marrow culture only for these kinds of patients as it. is a traumatic process.

## P1739

### Serological findings in blood sera of patients with Yersinia-triggered arthritis

E. Golkocheva, R. Stoilov, H. Najdenski *(Sofia, BG)*

**Objectives:** Immunoblot analysis of IgA and IgG antibody response of blood sera from patient with Yersinia triggered reactive arthritis and with undifferentiated arthritis were made.
**Patients and methods:** Serum samples were obtained from patients admitted to Clinic of Rheumatology at Medical University, Sofia, Bulgaria with suspicion of Yersinia triggered reactive arthritis, based on diagnostic criteria. A total of 20 blood serum samples were analysed by immunoblot analysis with specific antigens-Yops (yersinia outermembrane proteins). When *Y. enterocolitica* is cultivated at 37ºC under calcium restriction (0.2 mM $Ca^{2+}$), large amounts of Yops are secreted into medium. These proteins were separated by 2D-SDS-electrophoresis.
**Results:** Immunoblot analysis of IgA and IgG antibody response against Yops in 20 blood sera from patients with arthralgias and polyarthropathies was carried out. *Yersinia enterocolitica*, serotype O: 8, was used as source for Yop. Seven strong bands of the molecular weights 26 kDa-YopE, 33 kDa-YopN, 36 kDa-YopD, 41 kDa -V-ag, 43 kDa-YopB, 46 kDa-YopM and 51 kDa-YopH were visualized. For immunoblot assay the optimal concentration of antigen was established by analytical electrophoresis. Of the 10 blood sera from the patients with Yersinia triggered reactive arthritis IgG antibodies were detected against YopH, YopM, YopB, YopD, YopN and YopE. IgA antibodies were established against YopM, YopB, YopD, YopN and YopE. All 5 sera from the patients with other rheumatic diseases were negative for the presence of anti-yersinia IgA antibodies and two of them were positive for IgG against YopD. Antibodies from two classes were not detected in 5 sera samples from healthy people.
**Conclusions:** Yops are borne by the virulence plasmid, which mean that they are clearly associated with virulence properties of pathogenic strains. Moreover, Yops is not restricted to single serotype and this made them a specific antigen in diagnosis of different Yersinia infections. Conventional techniques such as culture and demonstration of serum agglutinins prove to be insufficient to demonstrate invasive or chronic yersiniosis in contrast with the determination of specific serum IgA and IgG antibodies by immunoblot analysis and antigen detection. The detection of anti-Yops IgG and IgA antibodies by immunoblot can be used for diagnosis of Yersinia triggered arthritis.
**Acknowledgements:** This work was sponsored by NATO-reintegration grant 981256.

## P1740

### Identification and susceptibility results by direct inoculation of Vitek 2 cards from positive Bact/ Alert 3D system

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

**Objectives:** To evaluate the identification and susceptibility results by using suspensions obtained directly from positive blood cultures.
**Methods:** During the period between 1st August and 31st October 2005 we selected all positive cultures grown in Bact/ Alert® SA and SN bottles (bioMérieux) from gram-negative bacilli. Only the first culture positive from each patient was included. We inoculated 5 mL fluid from a positive bottle into a serum separator tube (BD Vacutainer Systems, Plymouth, United Kingdom) and centrifuged at 1500 x g for 10 minutes and the supernatant was carefully aspirated. Using a cotton swab the bacteria were removed from the top of the separator layer to be suspended in 0.45% saline solution to get 0.6 McFarland. The suspension was processed according to standard inoculation procedure for GN and AST-N020 Vitek®2 cards. Positive Bact/Alert 3D bottles were also sub cultured and after an overnight incubation several colonies were used to make a 0.63 McFarland suspension in 0.45% saline. The suspension was processed according to standard Vitek®2 inoculation procedure for GN and AST-N020 cards.
**Results:**
**Identification:** A total 56 gram-negative bacillus from positive blood cultures were investigated. Fifty (89.2%) strains were correctly identified to the species level, four (7.1%) strains were not identified and two (3.6%) strains were misidentified. Antimicrobial susceptibility testing: In all, 1040 MICs were determined for 52 isolated by both methods. The unidentified strains (4) were excluded. The overall MIC agreement between direct and standard inoculation was 93.9%. All individual antimicrobial agents scored > 90%. The overall minor error rate was 2.8% (30 of 1040). The overall major error rate was 1.05% (11 of 1040). The overall very major error rate was 2.1% (22 of 1040). The highest rate of MIC agreement was for amikacin, norfloxacin (100%), meropenem (98%), gentamicin and ofloxacin (96.1%).
**Conclusion:** The direct method from positive Bact/ Alert cultures cannot totally replace the approved methods of identification and susceptibility but in some cases provides earlier information which allows a better patient management and also reduce cost in patient care.

## P1741

### Investigation of *Listeria monocytogenes* "O" antibodies in maternal and cord sera with the agglutination test

E. Us, A.T. Cengiz, O. Gelisen *(Ankara, TR)*

**Objectives:** *Listeria monocytogenes* is a Gram-positive food borne pathogen that is responsible for listeriosis, a human infection with a mortality rate of 30%, which could cause severe mother-to-child infections. This serious pathogen in pregnancy could be treated if diagnosed, but there is no routine screening test for susceptibility to listeriosis during pregnancy. Therefore, we investigate different *L monocytogenes* serotype O antibodies for diagnosis of listeriosis in 275 maternal sera with agglutination test. 69 of them had spontaneous abortion, premature labour or stillbirth (group I), while 206 had no obstetric patology (group II) in their previous pregnancies. Cord bloods were also obtained at the delivery and tested.
**Methods:** All sera were being tested against antigens with the O formulation of serotype 1/2c, 3b, 4ab, 4c and 4d. The antigens were prepared by the method of Osebold, and Larsen et all. The bacterial suspensions were trypsinized for 15 min at 37°C to prevent cross-reactions and contaminations. Sera were diluted by doubling serially in saline followed by addition of an equal volume of antigen. A positive titre of greater than or equal to 1:320 was chosen as positive test result to maximize the sensitivity and specificity.
**Results:** 4.36% of cases have ingested raw milk and diary products, 1.81% ready-to-eat foods, and 5.81% developed non-specific febrile illness (NFI) during their pregnancies. 20% of group I were found positive (28.5% developed NFI) while at group II 22% had positive (26.6% developed NFI) agglutination titres to one ore more serotypes. All the cord blood sera of group I were found negative, whereas two in group II (all 4ab) were positive, with the positive maternal sera of the same serotype. It's evaluated as transmission of the antibody from mother to

foetus. At group I the frequent serotypes were 1/2c = 4ab, at group II 4ab, 1/2c, respectively. The newborns showed no symptoms or signs of listerial foeto-maternal infection.
**Conclusion:** The women encountered the antigens of *L monocytogenes* in any period of their life time (most 21–25 years of age) and produce antibodies against this pathogen. There is a relationship between NFI and positive titres. If the disease is recognized, it is possible to treat the mother and allow the birth of a healthy infant. We propose the less time consuming and easy to perform agglutination test as a routine screening test for susceptibility to listeriosis during pregnancy to prevent bad pregnancy outcomes.

# Molecular detection of microbial ribosomal genes

## P1742

### Prospective evaluation of a real-time PCR assay for direct detection of methicillin-resistant *Staphylococcus aureus* in clinical specimens from hospitalized patients

N. de San, O. Denis, M-F. Gasasira, R. De Mendonça, C. Nonhoff, M.J. Struelens *(Brussels, BE)*

**Objectives:** To evaluate the performance of a real time PCR assay (with a fluorogenic target-specific probe), MRSA-IDI (GeneOhm Sciences) for MRSA detection directly from mucocutaneous swabs in hospitalized patients.
**Methods:** Clinical swabs (1 to 14 samples with a median of 2.1 samples per patient) from nares (n = 330) and skin (n = 326) were prospectively collected for MRSA screening from 321 patients admitted to a 858-bed teaching hospital. Swabs were inoculated onto selective MRSA agar (MRSA-ID, Biomérieux), into the buffer extraction solution for IDI-MRSA PCR assay and into enrichment broth (BHI with 7.5% NaCl). After 24h, BHI broths were subcultured onto MRSA-ID agar. Selective agars were incubated for 48 h at 35ordm;C and examinated daily. Suspected colonies were identified by coagulase testing; oxacillin resistance was tested by cefoxitin disk diffusion according to CLSI recommendations. The PCR assay was performed according to the manufacturer's instructions. PCR results were compared with phenotypic identification test results. In case of discordant results, the assay was repeated, but only results from first testing were considered for calculating test performance.
**Results:** MRSA was detected by culture in 74 specimen (11.3%) from 38 patients. The sensitivity and specificity of the PCR compared with culture was 82.4% and 96.9%, respectively. Positive predictive value and negative predictive value were 77.2% and 97.7%, respectively. The sensitivity of PCR (92%) was higher on nasal swabs than on swabs from other sites (77.5%, $p < 0.001$). The PCR assay detected MRSA in 34 patients (89.5%). The PCR assay provided results in 2 to 12 versus 48 to 72 hours for conventional method.
**Conclusion:** In our hospital, the ID-MRSA PCR assay detected 89.5% MRSA carriers in less than 12 hours when performed on multiple specimen. The assay appeared more sensitive in testing nasal swabs than other clinical specimens. Prospective studies are needed to evaluate the impact of this assay for rapid implementation of infection control procedures and its global costs and benefits.

## P1743

### Rapid and sensitive detection of methicillin-resistant *Staphylococcus aureus* from blood culture bottle by real-time PCR

C. Eroglu, H. Yilmaz, U. Cakmak, D. Turan, H. Leblebicioglu *(Samsun, TR)*

The purpose of this study was to establish a rapid and sensitive real-time polymerase chain reaction (PCR) method for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from blood culture bottle. As a result of over use of broad-spectrum antibiotics after the 1960s in whole the world, an outbreak of MRSA infection has been seen. Severe nosocomial infections with MRSA such as bacteraemia and sepsis may lead to multiple organ failure and high mortality in the hospital. Although standard method took at least 48 hours to identify MRSA by the blood culture method, the presence of mecA and nuc genes which is specific for methicillin resistance and *S. aureus* was determined by real-time PCR method within only 2 hours after blood culture signal positivity. Nineteen *S. aureus* and 33 coagulase negative staphylococci positive blood culture bottles were studied retrospectively for detection of *S. aureus* and methicillin resistance. Staphylococci were identified with classical methods and MICs of oxacillin were determined by Etest (AB Biodisk) on Mueller-Hinton agar supplemented with 2% NaCl. Real-time PCR was performed to all positive blood culture samples for *S. aureus* and methicillin resistance determination. Nineteen (100%) *S. aureus* were determined correctly by real-time PCR method. Forty-four methicillin resistant and 8 methicillin sensitive staphylococci were detected by Etest. Using the real-time PCR method, the mecA gene was detected in 47 Staphylococci except 3. When compared with Etest and real-time PCR method gave sensitivity, specificity, and positive and negative predictive values of 100%, 63%, 94%, 100% for both positive and negative tests, respectively. Agreements between two methods were high (94%); there were 3 discrepant results among the 52 strains were tested. Detection of MRSA bacteraemia and methicillin resistance with real-time PCR definitely is useful for reducing mortality and morbidity of this type infection. In conclusion, this method, as many as sensitive and specific for detection of MRSA bacteraemia and clinically should be beneficial for prevention of unnecessary antibiotic use and determination of appropriate antibiotic treatments of MRSA infection.

## P1744

### PCR detection of Class B, C and D beta-lactamases in environmental and clinical Aeromonas strains

T. Fosse, C. Giraud-Morin, F. La Louze *(Nice, FR)*

**Objectives:** *Aeromonas* spp. strains are waterborne opportunistic pathogens. They are able to produce different types of beta-lactamases (class B, C and D). The determination of beta-lactamase content is not easy by phenotypic methods. We have developed a PCR tool to study diversity and distribution of Class B, C and D beta-lactamases in a set of representative clinical and environmental *Aeromonas* species.
**Method:** A total of 22 references, 20 environmental and 80 clinical strains were tested. Identification was realized by conventional tests and gyrB sequence analysis. Beta-lactam antibiotic susceptibility was determined by diffusion agar and micro broth dilution methods. Three sets of specific primers

were defined for the PCR amplification of the internal region of class B beta-lactamase (MEI1 and MEI2, 297 bp size), class C beta-lactamase (AERCP1 and AERCP2, 840 bp) and class D beta-lactamase (AERD1 and AERD2, 514 bp). All PCR products were sequenced.
**Results:** Class D PCR was positive with most strains except *A. trota*, a ticarcillin susceptible species (3 strains). Class C PCR was positive with most cephalothin resistant strains (MIC >16 mg/l; 61/67 strains, 91%) including *A. hydrophila* and *A. caviae* phenospecies. Class B PCR was positive with most strains of *A. hydrophila* and *A. veronii* phenospecies (33/38; 87%) including three imipenem susceptible strains (MIC < 5 mg/l). beta-lactamase type distribution was species related and was particularly useful to better characterize environmental species such as *A. bestiarum*, *A. popoffii* and *A. allosaccharophila*. Partial beta-lactamase gene sequence analysis allowed phylogenic studies. Some cephalosporinase gene from environmental species was probable progenitor of ampC plasmidic beta-lactamase.
**Conclusion:** PCR with specific primers was a good method to detect class B, C and D beta-lactamase in *Aeromonas* species. Beta-lactamase type distribution and sequence analysis phylogeny were largely species related and could be helpful for molecular diagnostic and taxonomic purpose.

## P1745

## Detection of enterotoxine B producing *Staphylococcus aureus* directly from milk

M. Popa, I. Codita, M. Surdeanu, M. Straut, S. Cretoiu, M. Negut *(Bucharest, RO)*

**Objectives:** The aim of this study was to develop a convenient DNA extraction method and to optimise a PCR reaction in order to detect enterotoxin B producing *S. aureus* strains directly from milk.
**Methods:** We applied a chemical extraction method of bacterial DNA from milk samples artificially inoculated with *S. aureus*. A PCR based method was used for the detection of seb gene (coding for enterotoxin B) and nuc gene (coding for termonuclease). A protocol for the multiplex PCR was developed and optimized. The sensitivity of the reaction was checked by determining the minimum number of organisms·ml$^{-1}$, which can be detected in the multiplex PCR and in each single PCR reaction. Amplification specificity of the seb gene was verified by amplicon digestion with restriction endonucleases.
**Results:** The specific bands for both genes in the multiplex PCR were detected in samples containing a DNA quantity corresponding to 5000 organisms·ml$^{-1}$. In the same reaction, the amplicon for nuc gene was visible for as little as the DNA concentration corresponding to 1000 organisms·ml$^{-1}$. The sensitivity of each single PCR reaction was similar with those of multiplex PCR reaction.
**Conclusion:** The applied DNA extraction method allowed us to obtain a good quality DNA and can be used for a direct milk extraction. Multiplex PCR reaction is a simple, rapid and reliable method for detecting enterotoxin B producing *S. aureus* strains from milk.

## P1746

## Genotypic detection of resistance to fluoroquinolones by PCR-RFLP in *Acinetobacter baumannii* clinical isolates

E. García-peñuela, T. Alarcón, A. Pérez de Ayala, N. Arenal, J.L. Navarro, M. López-Brea *(Madrid, ES)*

**Objective:** To detect the resistance to fluoroquinolones in 30 *Acinetobacter baumannii* strains by a PCR-RFLP assay.

**Methods:** Thirty *A. baumannii* clinical isolates were obtained from different specimens (bronchial aspirates, blood-cultures, catheters, etc.). The MICs (Minimal Inhibitory Concentrations) for ofloxacin were determined by agar dilution following standard methodology.A PCR-RFLP method using one primer pair for amplification of a 344 bp fragment related to gyrA gene (which codifies subunity A of DNA-gyrase) and using one restriction enzyme Hinf I was developed to study the resistance to ofloxacin in the different *A. baumannii* strains. When an *A. baumannii* strain is resistant to fluoroquinolones, a mutation in the position Ser 83 of the DNA-gyrase has been detected, decreasing the affinity for the antimicrobial. Agarosa gel was used to determine the DNA pattern: 2 fragments of 289 bp and 55 bp when there is not mutation and 1 fragment of 344 bp when the Ser 83 to Leu 83 mutation is present.
**Results:** The relationship between the PCR-RFLP pattern and the MIC to ofloxacin is shown in the table 1. The results of PCR-RFLP analysis of most strains were in agreement with the results of MIC. One isolate was susceptible to ofloxacin by agar dilution (MIC = 0.25 mg/l) whereas by PCR-RFLP this isolate seems to be resistant because it presents the mutation in gyrA gene. Two isolates with intermediate MIC (4 mg/l) showed mutation in gyrA.

| DIGESTION WITH *Hinf I* | MUTATION IN *gyrA* | RANGE MIC ofloxacin (mg/l) |
|---|---|---|
| 2 fragments (289 pb + 55 pb) | No mutation | 0,06- 0,5 |
| 1 fragment (344 pb) | Mutation | 0,25- >128 |

**Conclusion:** The genotypic study by PCR-RFLP proved that ofloxacin resistant *A. baumannii* strains showed a punctual mutation in gyrA gene, in the same position inside the sequence of gene.

## P1747

## Evaluation of a rapid amplification-detection assay for the identification of vancomycin-resistant enterococci

J. Fuller, L. Turnbull, S. Shokoples, B. Lui, L. Rosmus, R. Rennie *(Edmonton, CA)*

**Objective:** The routine identification of vancomycin-resistant enterococci (VRE) in clinical laboratories often yields a lengthy turn-around-time that may impede infection control efforts, particularly in an outbreak situation. In search of an improved VRE test, we evaluated the GenoType® Enterococcus assay (Hain Lifescience, Germany), which provides both species and van gene identification for VRE, and compared the results to conventional methods.
**Methods:** Forty clinical enterococcal strains isolated on VRE-screen agar media were selected for study. Lactococcus and Pediococcus were used as negative controls. Conventional testing involved basic culture and identification tests, E-test susceptibility testing for vancomycin and teichoplanin, and PCR for vanA, B, and C genes. The GenoType® Enterococcus assay involved multiplex DNA amplification and reverse hybridization of amplified product on an immobilized DNA strip-blot containing probes for *E. faecium*, *E. faecalis*, *E. casseliflavus*, *E. gallinarum*, vanA, vanB, vanC1, and vanC2/3.

**Results:** The GenoType® Enterococcus assay produced correct species and van gene identification for all 40 (100%) VRE isolates, including 7 *E. faecalis* vanB, 12 *E. faecium* vanA, 12 *E. faecium* vanB, 6 *E. gallinarum* vanC1, 1 *E. gallinarum* vanA-vanC1, and 2 *E. casseliflavus* vanC2/3. The only minor discrepancy was an *E. casseliflavus* that hybridized very weakly with the vanC1 probe in addition to the expected vanC2/C3 probe. The costs per specimen were comparable for each test method. However, the GenoType® Enterococcus assay could be completed within a normal working day in contrast to conventional testing, which required a minimum of two days from the point of isolation on the vancomycin-screen media.
**Conclusion:** From this preliminary evaluation, the GenoType® Enterococcus amplification-detection assay provides VRE species and van genotype identification in a rapid and cost-effective manner, superior to conventional culture methods. Although further study is required, this kit may have clinical utility during a VRE outbreak.

## P1748

## Application of minimal sequence quality values prevents misidentification of blashv type in single bacterial isolates carrying different SHV extended-spectrum beta-lactamase genes

N. Al Naiemi, B. Duim, K. Schipper, C.M. Vandenbroucke-Grauls, A. Bart *(Amsterdam, NL)*

**Background:** Detection of extended spectrum beta-lactamase (ESBL) genes by PCR and sequence analysis is the gold standard for detection of SHV-type beta lactamases. Usually, quality values of sequence analyses are not reported. During a study on ESBL epidemiology, three strains for which the default sequence assembly showed an SHV−2 or SHV-5 gene, showed low quality values at certain positions in individual sequence traces. We investigated the reason for these lower values.
**Methods:** SHV genes were amplified by PCR from three isolates (*Escherichia coli*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*). Individual sequence traces were analysed with the computer programs PHRED and Codon Code. PCR products were ligated in vector pCR2.1 and transformed to *E. coli*. Sequence analysis was performed on eight individual clones from each transformation.
**Results:** Visual inspection of the low quality positions in the sequence traces showed signals for two different nucleotides at three positions in the SHV sequence: A or T at position 92, A or G at position 402 and A or G at position 703. The polymorphisms at positions 92 and 703 lead to aminoacid substitutions, the four different combinations would give SHV types 2, 2a, 5 or 12. The double signals suggested that two or more blaSHV alleles were amplified. PCR amplicons were cloned in *E. coli*, in the sequences of individual clones only two combinations of the three polymorphisms were present: A92G402A703 and T92A402G703. These two combinations correspond to SHV-2 and SHV-12, respectively.
**Conclusions:** (i) In isolates of three different species, two different SHV genes were present: SHV-2 and SHV-12. (ii) Genotypic detection with default sequence assembly parameters may lead to misidentification of the number and type of SHV genes carried by a single strain. (iii) Careful interpretation of sequence data of SHV genes, including analysis of low quality positions, may further improve our understanding of the epidemiology and evolution of these ESBL genes.

## P1749

## Antimicrobial susceptibilities and epidemiological analysis of *Salmonella typhimurium* human isolates in Slovakia by phage typing and pulsed-field gel electrophoresis

V. Majtán, L. Majtánova, M. Szabóová *(Bratislava, SK)*

**Objectives:** *Salmonella Typhimurium* is a common cause of salmonellosis among humans and animals in many countries. In the last few decades the incidence of multidrug-resistant *S. Typhimurium* infections appears to pose a particular health risk. The objectives of this study were analysis by antibiotic susceptibility, phage typing and pulsed-field gel electrophoresis (PFGE) of *S. Typhimurium* human isolates.
**Methods:** A total of 145 strains isolated during 1997–September 2005 were analysed. The susceptibility of isolates to ten antibiotics was evaluated by a disk diffusion method. The phage types were identified according to Anderson et al. (1977) in the National Reference Center for phage typing of Salmonellae. PFGE was used to resolve XbaI macro restriction fragments from all strains.
**Results:** Of human isolates 88 (60.7%) were resistant to more than two antibiotics. Sixty-three of isolates (43.4%) showed a classic DT104 resistance profile to ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline (ACSSuT). Among this resistance type 37.2% were DT104, 7.6% were DT120 and one strain was DT20a. Isolates encompassed 18 phage types. The majority of isolates was found to be definitive phage type DT104, representing 62.8% of all isolates. Other phage types were mainly DT120, DT41 and DT20a. Nine pulsotypes and 18 subpulsotypes were obtained using *Xba*I restriction enzyme, but pattern X1 with its subtypes predominated (86.9%). A major pulsotype X1 was represented by 51.7% of DT104 isolates and was also found among DT120 isolates.
**Conclusion:** Results indicated the spread of different clones of the multidrug-resistant *S. Typhimurium* in the Slovakia, but with predominance of one clone represented mainly by DT104 isolates. The phage typing as well as PFGE may offer an improved level of discrimination for the epidemiological investigation of *S. Typhimurium* human strains.

## P1750

## Novel reverse hybridisation assay to identify CTX-M genotype in cephalosporin-resistant isolates from UK and India

V.M. Ensor, M. Shahid, R. Hope, M. Warner, N. Woodford, D.M. Livermore, P.M. Hawkey *(Birmingham, UK; Aligarh, IN; London, UK)*

**Objectives:** a) To develop a novel reverse hybridisation assay for identification of CTX-M genotypes among large collections of cephalosporin-resistant *Enterobacteriaceae* isolated during surveillance studies in South-East England and North India. b) To validate the assay results by DNA sequencing.
**Methods:** Isolate collection 1: 110 *Enterobacteriaceae* resistant to extended-spectrum cephalosporins, isolated in London and South-East England. These isolates were known to carry phylogenetic group 1 blaCTX-M, but precise genotypes had not been determined. Isolate collection 2: 130 *Enterobacteriaceae* resistant to extended-spectrum cephalosporins, isolated in Aligarh, North India. Resistance determinants had not been investigated previously. A novel multiplex PCR was used to amplify blaCTX-M. Reverse hybridisation was carried out using

biotinylated PCR amplicon and sequence-specific oligonucleotides designed to identify members of CTX-M phylogenetic group 1. Hybridisation results were validated by DNA sequencing for 20 representative isolates from each collection.
**Results:** 109/110 London and SE England isolates known to carry group 1 blaCTX-M gave a consistent profile, corresponding to that for CTX-M-15 and CTX-M-28; 1/110 gave a profile corresponding to CTX-M-3 and CTX-M-22. 82/130 Indian isolates had blaCTX-M genes, all of which belonged to group 1, and all these gave a hybridisation profile corresponding to CTX-M-15 or CTX-M-28. CTX-M-28 and CTX-M-22 are rare variants, suggesting that the enzymes present were more likely to be CTX-M-15 and CTX-M-3, and this was confirmed by DNA sequencing.
**Conclusions:** This is the first reported application of this novel reverse hybridisation assay to the analysis of large numbers of cephalosporin-resistant *Enterobacteriaceae*. Results were validated by DNA sequencing. The assay is cheap and convenient, enables reasonable throughput, provides results within one day and can be used in place of DNA sequencing. We believe it will be valuable for monitoring the prevalence and genotypes of blaCTX-M genes in *Enterobacteriaceae*.

## P1751

## Detection of mexA and mexX efflux genes in *P. aeruginosa*: correlation between QC-RT-PCR and real-time PCR

N. Mesaros, Y. Glupczynski, D. Pierard, A. Dediste, Y. Van Laethem, P. Tulkens, M. Mingeot-Leclercq, F. Van Bambeke *(Brussels, Yvoir, BE)*

**Objectives:** Efflux systems are rarely identified as such in clinical microbiology laboratories. Yet, over expression of transporters such as MexAB-OprM and MexXY-OprM are likely to cause antibiotic multi- and cross-resistance in *Pseudomonas aeruginosa*, leading to potential clinical treatment failures because of their inducible character. We have previously developed and validated with reference strains a QC-RT-PCR method to quantify mexA and mexX expression levels (ECCMID 2005, P1731). In the present study, we have developed a Real-Time-PCR assay and present here the correlation between both methods using control strains and clinical isolates.
**Methods:** Expression levels of mexA and mexX were measured by both techniques in (i) 4 reference strains expressing only one of these efflux mechanisms [mexA (2) or mexX (2)]; and (ii) 8 clinical isolates, in comparison with the wild-type strain PAO1 (basal mexA and mexX expression levels).
**Results:** Real-Time PCR showed an inter-day reproducibility of 95 ± 5.3% (triplicates of 10 strains). Among the clinical strains, 5 over expressed mexA and 3 mexX. The Table shows (i) the mean level of overexpression of mexA and mexX in comparison with the wild type strain PAO1 (set at 1), as detected by Real-time PCR for all strains; (ii) the ratio of these values to those observed by QC-RT-PCR for the corresponding transporters.

| *mexA* (n=7) | | *mexX* (n=5) | |
|---|---|---|---|
| Real-Time-PCR | ratio to QC-RT-PCR | Real-Time-PCR | ratio to QC-RT-PCR |
| 5.99±0.29 | 1.03±0.22 | 6.83±0.31 | 0.98±0.24 |

**Conclusions:** Both QC-RT-PCR and Real-Time-PCR are potentially useful in clinical laboratories as sensitive and rapid diagnostic tools to quantify the expression level of mexA and mexX in *P. aeruginosa*. Combined with phenotypic characterization, this approach may help in a better understanding of the resistance mechanisms and epidemiology of resistance in this difficult-to-treat nosocomial pathogen.

## P1752

## Molecular detection of penicillin resistance in *Streptococcus pneumomiae*

N.G. Rizk, N.A. Abo Khadr, S.M. Abdel Salam, N.M. Gamil, M. Hassan *(Alexandria, EG)*

**Objectives:** The aim of the study was to detect penicillin resistant *Streptococcus pneumoniae* by using seminested polymerase chain reaction (PCR) and to compare it with minimum inhibitory concentration (MIC) of penicillin G.
**Methods:** Fifty clinical isolates of *Streptococcus pneumoniae* where isolated from patients admitted to Alexandria Main University Hospital in Egypt and were recovered from sputum (36 strains), throat swabs (11 strains), and pleural effusion (3 strains). Two species-specific primers 1A-1 and 1A-2, which amplified 1043 bp region of the pbp1A penicillin-binding gene, were used for pneumococcal detection. Two resistance primers, 1A-R1 and 1A-R2, were used to bind to altered areas of pbp1A gene which, together with the down stream primer 1A-2, amplify DNA sequences of 224 bp and 569 bp from isolates with penicillin MIC > 0.1 and ≥ 1 $\mu$g/ml respectively. Results compared with the MIC values obtained by E-test Penicillin G strips.
**Results:** For 98% of the isolates tested, PCR results were in agreement with MIC data. The sensitivity of PCR assay in detection of penicillin resistance was 96.4%, and the specificity was 100%. The positive and negative predictive values of the assay were 100% and 95.6% respectively.
**Conclusion:** The seminested PCR is a rapid specific method in diagnosis of *Streptococcus pneumoniae*, which can be applied directly on clinical specimens. Meanwhile it can detect resistant strains and differentiate between intermediate and high level resistance.

## P1753

## Lipopolysaccharide-binding protein is a marker of severity in patients with bacteraemia

S. Gaïni, S.S. Pedersen, C. Pedersen, O.G. Koldkjær *(Odense, Sønderborg, DK)*

**Objective:** Lipopolysaccharide-binding protein (LBP) is an acute phase protein produced in the liver. The objective of our study was to evaluate LBP as a marker of severity and prognosis in patients with bacteraemia.
**Methods:** 42 adult patients with community-acquired bacteraemia were included in a prospective manner. Daily blood sampling for LBP and Interleukin-6 (IL-6) was performed. The patients were classified according to the Systemic Inflammatory Response Syndrome (SIRS) criteria. Demographic data, co-morbidity, microbiological a*aetiology*, routine biochemical parameters, focus of infection, severity score and mortality on day 28 were recorded. LBP and IL-6 levels were analysed on plasma samples with a chemiluminescent immunometric assay (Immulite-1000®).
**Results:** The median age was 71 yrs. The mortality rate on day 28 was 16.6%. 5 patients had bacteraemia without SIRS, 17 patients had sepsis and 20 patients had severe sepsis. LBP concentrations are presented as medians and range: 32.2 $\mu$g/ml (28.2–34.1) in patients without SIRS, 45.4 $\mu$g/ml (20–85.6) in patients with sepsis and 50.9 $\mu$g/ml (22.9–96.5) in patients with severe sepsis ($P < 0.05$). LBP levels correlated to levels of IL-6 (rs

0.52), C-reactive protein (rs 0.68), leukocytes (rs 0.44) and neutrophils (rs 0.46) ($P < 0.01$). LBP did not predict the outcome of the patients with bacteraemia.
**Conclusion:** LBP levels increased with the severity of sepsis in patients with bacteraemia. LBP correlated to IL-6, C-reactive protein, leukocytes and neutrophils. LBP did not predict the outcome of the patients in this small cohort.

## P1754

## Pyrosequencing of the GRA6 gene to discriminate type I, II and III *Toxoplasma gondii* in clinical samples

B. Edvinsson, B. Evengård *on behalf of the ESGT*

**Objectives:** Infection with *Toxoplasma gondii* in immunocompromised transplant recipients is rare but often fatal. To increase our knowledge about the significance of the genotype of the parasite during infection, methods suitable for routine use need to be developed. Pyrosequencing is a rapid sequencing-by-synthesis method performed in real-time. It is developed for detection of short nucleotide polymorphisms (SNPs), and is suitable for molecular genotyping of microorganisms. We here present a pyrosequencing assay for rapid and reliable discrimination of *Toxoplasma gondii* type I, II and III in clinical samples.
**Methods:** Twenty-two isolates of *T. gondii* were used for pyrosequencing analysis of the GRA6 gene. Real-time PCR was performed using a LightCycler 2.0 instrument to amplify a 176 bp fragment of the GRA6 gene. Pyrosequencing analysis of two different SNPs contained within a 10 bp fragment of the amplified product was preformed to identify *T. gondii* type I, by detection of nucleotides G and A at these respective positions. Type II was G and G, and type III was A and A. To test the assay in a clinical context, blood samples and lung tissue from an immunocompromised patient was analysed.
**Results:** The detection limit of the assay is 10 parasitic genomes in a sample. Reproducibility (R) was calculated as R = Nr/N (Nr = the number of isolates assigned the same type on repeat testing and N = the number of isolates tested). R was determined using three independent runs, and was 1, suggesting clearly interpretable results with little variation. Typeablility (T) of the assay was calculated as T = Nt/N (Nt = the number of typeable strains and N = the number of isolates tested). T was determined using three independent runs, each including four atypical isolates. T was 0.82, suggesting that the assay discriminates correctly between the three main genotypes of *T. gondii*, but does not detect atypical strains. Analysis of the clinical samples revealed type II *T. gondii* in blood samples and lung tissue.
**Conclusion:** When preceded by real-time PCR, pyrosequencing is a rapid process with a high reproducibility and throughput. This makes it a good candidate for routine use. The method does, however, not detect atypical or recombinant strains. More than one gene may have to be analysed for that purpose.
**Acknowledgement:** In particular, we want to thank Marie-Laure Dardé and Hervé Pelloux for provision of the *T. gondii* isolates.

## P1755

## Virulence genes in *Escherichia coli* isolates from calves in Shahrekord area, Iran

T. Zahraeisalehi, M. Mahzounieh, F. Asadian, M. Khosravi, M. Boniadian *(Tehran, Shahrekord, IR)*

Shiga toxin-producing *Escherichia coli* (STEC) strains, also called verotoxin-producing *E. coli* (VTEC) strains, represent the most important recently emerged group of food-borne pathogens around the world. Members of this group are a major cause of gastroenteritis that may be complicated by hemorrhagic colitis (HC) or the hemolytic uremic syndrome (HUS), which is the main cause of acute renal failure in children. Domestic ruminants, mainly cattle, sheep, and goats, have been implicated as the principal reservoir. Transmission occurs through consumption of undercooked meat, unpasteurized dairy products and vegetables, or water contaminated by feces of carriers because STEC strains are found as part of the normal intestinal floras of the animals.We studied the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) in stool specimens of calves with diarrhoea or other gastrointestinal alterations from 10 dairy cattle farms of Shahrekord City (central of Iran). The virulence genes, stx1, stx2, eae, Intimin Hly, enterohemolysin, ST, LT, were detected by multiplex PCR method. STEC strains were detected in 7 (11.5%) of 61 *E. coli* from 180 cases investigated. STEC O157 was isolated in 7 cases (11.5%), whereas non-O157 STEC strains were isolated from 4 animals (6%). STEC strains were the most frequently recovered enteropathogenic bacteria. PCR showed that 5 (8.2%) isolates carried st gene. None of isolates carried an ehxA, eae, and LT (labile toxin) genes. Our results suggest that STEC strains are a significant cause of calf infections in this area and confirm that, infections caused by STEC non-O157 strains are more common than those caused by O157:H7 isolates. The high prevalence of STEC strains (both O157 and non-O157 strains) also found in human patients by other investigators, and their association with serious complications, strongly supports the utilization of protocols for detection of all serotypes of STEC in Spanish clinical microbiology laboratories.

## P1756

## Periplasmic expression of active recombinant shiga toxin (Stx) 1

M. Oloomi, S. Bouzari *(Tehran, IR)*

**Objectives:** Shiga toxins are A-B holotoxin including one enzymatically active A subunit associated non-covalently to five identical receptor binding B subunits. Each subunit can cause different signalling pathways in different cells. To assess the effect of each single subunit the specific clones for expressing the single subunit was designed. Periplasmic expression yielded native AB5 holotoxin or B5 pentamer.
**Methods:** O157 was used as bacterial strain for PCR amplification of shiga toxin gene. Each subunit was amplified by specific primers and the amplified genes were cloned in pBAD expression vector. The expression of the cloned genes was induced and optimized by different concentration of arabinose. The expressed proteins was assessed on SDS-PAGE and detected by ELISA and western blotting. The expressed recombinant AB5 holotoxin and B subunit were purified and assessed for its biological activity on cells. Cell cytotoxicity was shown by the expressed (AB5) holotoxin. Moreover inhibition was observed by B subunit and antibody against it.
**Results:** *E. coli* clones expressing recombinant shiga toxin A and recombinant shiga toxin B subunits were established to release the toxin to periplasmic space. Expressed toxin was examined by SDS-PAGE to visualize two subunits. The whole structure of these expressed subunits was checked in native gel. Active AB5 structure expressed in periplasmic space was extracted by polymyxine B. The biological activity of the constructed recombinant shiga toxin showed both Vero cell Cytotoxicity and inhibition of *in vitro* protein synthesis.

**Conclusion:** In this study it was shown that for B subunit assembly and secretion to periplasmic space as B5 pentamer homologous leader sequence is not needed. Although for biological active holotoxin (AB5) secretion to periplasmic space the presence of homologous leader sequence of gene is essential. These subunits can be used for studying on cell cytotoxicity and also as a vector for antigen presentation in immunotherapeutic approaches.

## P1757

## Characterisation of Gram-positive anaerobic cocci by biochemical tests and partial 16S rRNA sequencing

A. Bryk, A. Kanervo-Nordstrom, M. Hyvonen, E. Kononen *(Helsinki, FI)*

**Objective:** Gram-positive anaerobic cocci, which are common findings in various infections, are difficult to identify in clinical microbiology laboratories, where identification is based only on few phenotypic tests. In recent years, this group of organisms (traditionally known as peptostreptococci) has encountered several taxonomic changes. The aim of the present study was to compare the characterization made by a selection of key phenotypic tests to that by partial sequencing of the 16S rRNA gene.
**Methods:** Fifty-nine clinical isolates sent to our laboratory as gram-positive anaerobic cocci were examined for their colony and cell morphologies and biochemically characterized using spot catalase and indole reaction, 8 enzyme reactions by individual diagnostic tablets (Rosco), sodium polyanethol sulphate susceptibility, glucose fermentation, and determination of metabolic end products. In addition, commercial identification test kit (Rapid ID 32A) patterns were performed. The sequencing of the 16S rRNA gene of the 59 clinical isolates and 4 reference strains comprised of about 470 bp, and the sequences obtained were compared to those in GenBank database by using the multisequence Advanced BLAST comparison software from the National Center of Biotechnology Information.
**Results:** The biochemical characteristics of the isolates were consistent with those of *Peptostreptococcus anaerobius* (n = 8), Peptostreptococcus (Micromonas) micros (n = 6), *Finegoldia magna* (n = 25), *Peptoniphilus asaccharolyticus* (n = 8), *Peptoniphilus* sp. (n = 8) and *Anaerococcus* sp. (n = 2), whereas 2 isolates remained as unidentified gram-positive anaerobic cocci. Biochemical identification correlated with that obtained by partial 16S rRNA sequencing in 56/59 (95%) isolates at genus level and in 40/59 (68%) isolates at species level. The agreement of the biochemical and sequence-based identification was 100% for *P. micros* and *F. magna*. Of 8 isolates biochemically identified as *P. asaccharolyticus*, 2 isolates were identified as *Peptoniphilus harei* and 6 remained as *Peptoniphilus* sp. by sequencing. According to the sequence data, the 2 unidentified isolates were *Peptoniphilus ivorii*.
**Conclusion:** Most isolates from human infections proved to be *F. magna*. A relatively good agreement of identification was obtained using biochemical testing and partial 16S rRNA sequencing.

## P1758

## 23S PCR as a supplementary method for diagnosis of infectious arthritis. A prospective study

C. Moser, K. Andresen, A. Kjerulf, S. Salamon, M. Kemp, J.J. Christensen *(Copenhagen, DK)*

**Objectives:** Molecular methods for identification of infectious agents in patients with clinical infectious disease are increasingly being used. Especially in cases where antibiotics have been given prior to sampling or when fastidious bacteria difficult to grow are the a*aetiology* of the infection. Infectious arthritis is a serious disease where identification of the etiological agent is mandatory for optimal antibiotic treatment as well as indication of the primary focus if not the joint it self.
**Methods:** In the present prospective study, 227 synovial fluids taken from patients in elucidation of affected joints and sent to a Clinical Microbiological laboratory in the Copenhagen area, Denmark, were examined by conventional (culture, phenotypic tests) and molecular methods (PCR/sequencing of 23S ribosomal genes). Conventional methods included Gram-staining and microscopy, aerobic and anaerobic culture and identification. PCR/sequencing included DNA extraction, PCR assay which produced a 700 bp fragment of 23S rDNA, and sequencing of both DNA strands of the amplicons. Sequencing data were edited and a BLAST search in the NCBI database was done.
**Results:** Overall a microorganism was identified in 24 of the 227 synovial fluids (10.6%). In 14 synovial fluids from nine patients bacteria were identified by either methods [*Staphylococcus aureus* (n = 8), *Streptococcus pneumoniae* (n = 3), *Streptococcus dysgalactiae* (n = 2), *Citrobacter freundii* (n = 1)]. Six synovial fluids were only culture positive; in four of those six specimens coagulase negative staphylococci were isolated. In three of the 227 synovial fluids a microorganism was identified by 23S PCR only. In two synovial fluids 23S PCR identified only one microorganism, whereas culturing resulted in two isolates.
**Conclusion:** The present study indicates a significant contribution by molecular methods (PCR/sequencing of 23S ribosomal genes) in recognizing and identification of microorganisms from foci normally considered sterile like synovial fluids. Continued suspicion of infected arthritis despite of negative cultures should result in use of molecular diagnostics.

## P1759

## Direct detection of *Cardiobacterium hominis* by broad-range 16S rRNA PCR and sequencing in the serum of a patient with infective endocarditis

E. Malli, D. Klapsa, A. Vasdeki, M. Morava, M. Pitsitaki, E. Petinaki, A. Maniatis *(Larissa, GR)*

**Objectives:** To describe the detection of *Cardiobacterium hominis* directly in the serum of a patient with infective endocarditis, by employment of broad-range 16S rRNA PCR followed by sequencing.
**Methods:** A series of blood cultures were taken from the patient before starting empirical treatment. In addition, 10 ml whole blood was collected in rubber sealed pyrogen-free tubes for direct detection of bacterial DNA. Bacterial DNA was detected by a broad range PCR reaction and sequencing process allowed identification of bacteria species.
**Results:** *Cardiobacterium hominis* was identified as the causative agent of infective endocarditis, on two days after the serum collection. Blood cultures, simultaneously obtained with the serum sample, remained negative after 5 days of routine incubation; however, after a prolonged incubation of twelve days a gram negative bacterium was isolated from the aerobic bottles, that was identified as *C. hominis* species, by the usual phenotypic studies (catalase, oxidase reaction, indole, nitrate, etc) which are time-consuming.
**Conclusions:** To our knowledge this is the first report of direct detection of *C. hominis* in the serum using molecular methods, emphasizing the need for the establishment of such methods especially for infections caused by fastidious organisms.

## P1760

### Identification of dangerous bacterial pathogens by 16S ribosomal RNA gene sequence analysis

W. Ruppitsch, A. Stoeger, A. Indra, D. Schmid, K. Grif, C. Schabereiter-Gurtner, A. Hirschl, F. Allerberger *(Vienna, AT)*

To assess the usefulness of partial 16S rRNA sequence analysis for identification of dangerous bacterial pathogens, a total of 28 isolates comprising *Bacillus anthracis*, *Brucella melitensis*, *Biovars melitensis*, Suis, Abortus and Bovis, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Francisella tularensis*, *Yersinia pestis*, and 9 genus-related and unrelated control strains were sequenced and analysed using the GenBank database (Blast 2.2.10, National Institute of Health, U.S.A), the MicroSeq500 database (version 1.4.1 and V1.0, Applied Biosystems, Foster City, U.S.A.), The Ribosomal Database Project-II database (RDP-II, release 9, update 26, Michigan State University, U.S.A), and the Ribosomal Differentiation of Medical Microorganisms database (RIDOM, University of Wuerzburg, Germany). On genus level all isolates were identified using GenBank, RDP-II, and MicroSeq V1.0. The older MicroSeq 1.4.1 database identified 92% of the tested samples correctly on genus level. The RIDOM database did not include sequence data of the tested species even on genus level, the RIDOM database none (''there seems to be, at least currently, no close relative available''). Genbank and RDP-II identified all dangerous pathogens correctly. The MicroSeq V 1.0 database identified four of the six species of dangerous pathogens. On species level none of the dangerous pathogens was correctly identified using MicroSeq 1.4.1 or RIDOM. As previously noted by various other authors, the most important reason for failure of databases in identifying a bacterium is a lack of the 16 S rRNA gene sequence of the particular bacterium in the database rather than misidentification because of poor sequence quality. One must also be aware that the following bacterial species or subspecies have the same 16S rRNA gene sequence, which makes differentiation by sequence analysis impossible: *B. anthracis* and *B. cereus*, *Y. pestis* and *Y. pseudotuberculosis*, all *Brucella* subspecies, and *Francisella tularensis* ssp. *holarctica* and *mediasiatica*. In addition to 16S rRNA gene analysis complementary methods are essential to discriminate between these bacteria on species or subspecies level.

## P1761

### Identification of nontuberculous mycobacteria by sequence analysis of the 16S ribosomal RNA, the heat-shock protein 65 and the RNA polymerase beta-subunit genes

S. Shin, J.H. Yoon, E.C. Kim *(Seoul, KR)*

**Objectives:** The diagnosis of diseases caused by nontuberculous mycobacteria (NTM) is difficult because NTM are prevalent in the environment such as soil and water and because they have fastidious properties. In this study, we investigated the distribution pattern of NTM clinical isolates and the identification to the species level.
**Methods:** Among the presumptive NTM clinical isolates, cultured in a third referral hospital from 21-Jan-2003 to 20-Jan-2004 in Seoul, South Korea, which were negative by probe hybridization method for Mycobacterium tuberculosis complex, we selected those of more than 10 colonies or those cultured more than twice in a same patient. A total of 120 isolates were studied for the distribution of NTM including 97 isolates recruited for species identification by direct sequencing of 16S rRNA, hsp65 and rpoB gene segments.
**Results:** Frequently identified NTM species were M. avium 30.8%, M. intracellulare 23.3% and M. abscessus 18.3%. Others were M. gordonae, M. senegalense, M. fortuitum, M. peregrinum, M. kansasii, M. terrae complex, M. lentiflavum, M. chelonae, and M. szulgai. Three M. tuberculosis complex (2.5%) were also identified in the presumptive NTM isolates. The identification rate by sequencing of 16S rRNA, rpoB, and hsp65 were 65%, 82% and 87%, respectively. hsp65 or rpoB gene was more efficient than 16S rRNA in identification of NTM by sequencing.
**Conclusions:** Some NTM are considered to be the causative organisms of clinical diseases even in the countries with intermediate burden of tuberculosis, so accurate identification method by direct sequencing can be adapted to clinical laboratories.

## P1762

### Evaluation of the genotype MTBDR assay for the simultaneous detection of resistance to rifampicin and isoniazid of *Mycobacterium tuberculosis* clinical strains

F. Brossier, C. Truffot-Pernot, N. Veziris, V. Jarlier, W. Sougakoff *(Paris, FR)*

**Objectives:** The rapid determination of drug resistance in *Mycobacterium tuberculosis* is an important challenge to ensure a rapid effective chemotherapy. The Genotype MTBDR test is a commercially available DNA strip assay enabling the molecular genetic identification of the *M. tuberculosis* complex and its resistance to rifampicin (RIF-R) and isoniazid (INH-R) by detecting the most commonly found mutations in the genes rpoB (Asp516Val, His526Tyr, His526Asp, Ser531Leu) and katG (Ser315Thr). Here, we report the evaluation of the Genotype MTBDR assay from a set of 106 clinical isolates of *M. tuberculosis*.
**Methods:** 106 clinical isolates were collected in France over a 2 years period (2003–2004) and were included in the study: 77 were RIF-R, 96 were INH-R (of which 73 were also RIF-R) and 6 were susceptible to both drugs. The susceptibility tests were carried out by the standard proportion method. The mutations involved in RIF-R and INH-R in rpoB, katG, inhA and his promoter region, were characterized by DNA sequencing.
**Results:** The Genotype MTBDR assay identified 100% of the 77 RIF-R strains harbouring mutations in the rpoB gene, of which 37 (48%) showed a Ser531Leu mutation and 14 (18%) a His526Asp or Tyr mutation. 61 of the 96 INH-R strains (63%) harboured a Ser315Thr mutation in katG, all identified by the Genotype MTBDR assay. 58 of this 61 strains displayed a high level of INH-R. Among the other INH-R strains, 3 showed a katG mutation at the level of the 315 regions, which was different from Ser315Thr (2 of which showing a low level of INH-R), and one harboured a deletion in katG (with a high level of INH-R). These 4 mutations were also detected by the strip. Finally, among the 31 remaining INH-R strains not detected by the MTBDR assay, 13 were characterized by a mutation in position -15 of the promoter region for the mabA-inhA regulon (10 with a low level of INH-R), 7 by a Ser94Ala mutation in InhA (all with a low level of resistance) and 11 by other mutations.
**Conclusions:** The MTBDR assay, which can readily be included in a routine laboratory workflow, identified 100% and 68% of the strains resistant to RIF and INH, respectively. Interestingly, 60 of the 68 INH-R strains showing a high level of resistance (88%), but only 5 of the 28 INH-R strains with a low level of resistance (18%), were detected by the MTBDR assay, indicating that complementary tests are necessary for detection of the *M. tuberculosis* strains having a low level of resistance to INH.

## P1763

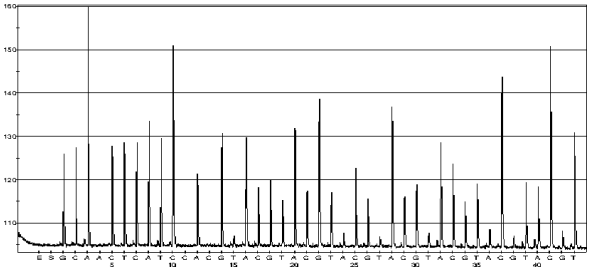
## Variation in the streptococcal 16S rDNA detected by pyrosequencing

M. Haanperä, P. Huovinen, J. Jalava *(Turku, FI)*

Originally the aim of this study was to identify alpha-haemolytic streptococcal isolates to the species level by pyrosequencing the v1 and v2 regions of the 16S rDNA and comparing the results to the sequences of type strains that have been determined earlier. However, the isolates could not be unambiguously identified due to sequence variations detected in the alpha-haemolytic isolates.

**Materials and methods:** Invasive S. pneumoniae isolates (n = 17), alpha-haemolytic streptococcal blood culture isolates (n = 16) and alpha-haemolytic streptococcal isolates from the normal pharyngeal microbiota (n = 34) of six elderly persons were analysed by pyrosequencing the v1 and v2 regions.

**Results:** Varying degree of genetic variation was found in different types of streptococcal isolates. In the pneumococcal isolates, no sequence variation was detected as all the isolates contained the sequence specific for *S. pneumoniae* in both regions. Also the sequences of the alpha-haemolytic blood culture isolates were well in agreement with the sequences of the streptococcal type strains. However, most of these isolates could not be unambiguously identified, as they contained sequences belonging to different species in the v1 and v2 region. Consequently, only five of the isolates could be unequivocally identified as *S. gallolyticus* (n = 2), *S. anginosus* (n = 1), *S. mitis* (n = 1) and *S. sanguinis* (n = 1). The commensal streptococci contained numerous sequences to which an identical type sequence could not be found. Also sequences identical to type strains were found; but similarly to the blood culture isolates, the results enabled the identification of only four isolates: *S. mitis* (n = 2), *S. parasanguinis* (n = 1), and *S. salivarius* or *S. vestibularis* (n = 1). Moreover, the pyrograms of three blood culture isolates and ten pharyngeal isolates indicated heterogeneous 16S rDNA alleles. One such pyrogram of the v1 region is presented in the figure. Interestingly, four of the eight different nonheterogeneous v1 and v2 sequence combinations of the blood culture isolates were also present among the pharyngeal isolates.



**Conclusions:** The results of this study indicate that the variation in commensal streptococci is greater than that of the streptococcal type strains and pathogenic isolates. The presence of identical sequence combinations among the blood culture and pharyngeal isolates supports the assumption that potentially pathogenic isolates are present in the normal microbiota.

## P1764

## Evaluation of partial 16S rRNA gene sequencing for identification of clinical isolates of *Nocardia* species

M. Marín, M. Sánchez, M. del Rosal, E. Cercenado, P. Martín-Rabadán, E. Bouza *(Madrid, ES)*

New species of *Nocardia* are being described. Conventional identification based on biochemical characteristics and PCR-restriction enzyme analysis is frequently unable to distinguish them. Partial sequencing of 16S rRNA gene has proven useful in the identification of bacteria.

**Objective:** To evaluate the utility of 5'end 16S rRNA gene PCR and sequencing in the identification of clinical isolates of *Nocardia* sp. compared with conventional methods and PCR-RFLP of hsp65.

**Methods:** 48 clinical isolates of *Nocardia* sp. were characterized by biochemical reactions and disk diffusion susceptibility testing. Molecular identification was performed by hsp65 PCR-RFLP and PCR of 5'end of 16S rRNA gene followed by sequencing. The sequences obtained were compared with those included in Genebank. Only alignments with similarities higher than 99% were considered. A comparison of sequences of our *Nocardia* isolates with those deposited in Genebank and well characterized phenotypically was performed using Clustal X 1.8 software.

**Results:** Distribution of species after PCR-RFLP of hsp65 was *N. asteroides* VI (22), *N. farcinica* (11), *N. nova* (6), *N. asteroides* I (4), *N. otitidiscaviarum* (4) and *N. asteroides* IV (1). Partial sequence analysis of 16S rRNA revealed a great heterogeneity between the isolates of *N. asteroides* VI, as follows: *N. cyriacigeorgica* (15 isolates), *N. abscessus* (3 isolates) and *N. carnea* (1 isolate). For 3 isolates, no Genebank sequence was found with more than 99% similarity. All *N. farcinica* isolates had the same sequence and showed 100% similarity with those deposited in Genebank. *N. nova, N. asteroides* I and *N. otitidiscaviarum* also showed sequence heterogeneity. Three *N. nova* isolates matched with the recently described *N. veterana* and 3 with N. nova. *N. asteroides* I isolates were identified as *N. abscessus* (1) and *N. beijingensis* (3). All *N. otitidiscaviarum* were identified properly. The isolate of *N. asteroides* IV was identified as *N. transvalensis.*

**Conclusions:** Sequencing of 5'end 16S rRNA gene is a useful and rapid molecular tool for the identification of *Nocardia* clinical isolates. This method could provide more accurate results than the conventional ones used routinely in our laboratory. Sequence analysis of the 5'end 16S rRNA has enabled us to recognize great diversity and new species among our Nocardia isolates. Several species would have gone unnoticed using non-sequencing-based methods.


ISSN: 1470-9465

# Antibacterial susceptibility studies-III

## P1765

### Anaerobic bacteraemia due to *Fusobacterium necrophorum* and *Clostiridium cadaveris*: a case report

M. Panopoulou, E. Alepopoulou, E. Chrisafidou, A. Tsaroucha, C. Simopoulos, S. Kartali *(Alexandroupolis, GR)*

**Introduction:** Anaerobic bacteremia is uncommon accounting 0.5–9% of bacteremias and it is associated with a high mortality rate, which is strongly and independently associated with underlying liver disease.
**Case report:** A 62 year-old man presented to our hospital with a 2-day fever and rigor. He had a history of cancer of the extrahepatic biliary tree, which was found incidentally during an operation for the treatment of echinococcal cyst of the liver. Physical examination reveals high fever (39 C) and tachycardia. Blood tests showed the following results: Hb: 9.9 gr/dl, WBC: 22.500 /ul, PLT: 296.000 /ul, TPROT: 5.9 gr/dl, ALB: 2.4 gr/dl, SGOT: 30 U/l, SGPT: 10 U/l, gGT: 137 U/l, ALP: 554 U/l, TBIL: 1.0 mg/dl, DBIL: 0.5 mg/dl, PT: 12/12.2, INR: 1.42, PTT: 30/34. The ultrasound scan of the abdomen showed a large, cystic lesion of the right lobe of the liver with heterogeneous content and calcified thick walls. The abdominal CT scan confirmed the presence of echinococcal cyst with the presence of intracystic air, suggestive of cyst infection (abcess). Two sets from blood sample cultures inoculated into aerobic and anaerobic bottles and incubated in the BacT/Alert instrument (Bio Merieux-France). Anaerobic bottles were positive after 48 hours of incubation. The Gram stain revealed Gram negative bacilli and Gram positive spore forming bacilli. Cultures on blood agar plates yielded a heavy growth of two different types of colonies. Each colony type subcultured to blood agar plates and incubated aerobically and anaerobically (aerotolerance test). After 24 hours of incubation the two organisms grew only in anaerobic conditions. They identified by the Api 20A system (Bio-Merieux-France) as *Fusobacterium necrophorum* and *Clostiridium cadaveris.* The patient's treatment started with metronidazole, amikacin and ceftriaxone and followed by metronidazole and imipenem. He was discharged after 3 weeks in a good condition.
**Conclusions:** Although anaerobic bacteremia is rare, there is value in performing separate anaerobic blood cultures. The early recognition of anaerobic bacteremia and administration of the appropriate antimicrobial therapy play a major role in preventing mortality especially in patients with underlying disease.

## P1766

### Fluoroquinolone resistance among *Enterobacteriaceae* strains isolated from urinary tract infections

V. Skandami-Epitropaki, P. Fostira, A. Tsiringa, A. Xanthaki, K. Zampitha, M. Toutouza *(Athens, GR)*

**Objectives:** To study the frequency and antibiotic susceptibility of quinolone resistant bacterial stains isolated from patients with community-aquired bacteriuria and compare it with urinary pathogens from hospitalized patients.
**Methods:** During a 12-month period (October 2004–October 2005) a total of 772 bacterial strains were isolated out of 8369 urine samples submitted for culture in our hospital laboratory from the community and from hospitalized patients with urinary tract infection symptoms. Cultures and bacterial identification were obtained by conventional methods. Antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method according NCCLS criteria.
**Results:** Of the 772 bactrial strains studied (*Escherichia coli* 604, *Klebsiella pneumoniae* 97, *Proteus mirabilis* 71), 14.6% of them were found to be quinolone resistant. The percentage of quinolone resistance was 19.9% for hospitalized patients (HP) and 5.7% for community patients (CP). The quinolone resistance for *E. coli* was 11.3% (16.5% for HP and 4.3% for CP), for *K. pneumoniae* 24.7% (28.0% for HP and 6.7% for CP) and for *P. mirabilis* 29.6% (29.6% for HP, 29.4% for CP). Susceptibility pattern of the 113 quinolone resistant isolates to other antimicrobial agents was for hospitalized patients and community patients respectively as following: For *E. coli* ampicillin (AM) 7%–9.1%, amoxicillin-clavulanate (AMC) 45.6%–54.5%, piperacillin-tazobactam (TZP) 69.4%–81.8%, cefuroxime (CXM) 64.9%–72.7%, trimethoprim-sulfamethoxazole (SXT) 15.8%–18.2%, ceftazidime (CAZ) 66.7%–72.7%, cefepime (FEP) 66.7%–81.8%, gentamicin (GM) 80.7%–72.7%. For *K. pneumoniae* AM 0%–0%, AMC 25%–0%, TZP 68.4%–100%, CXM 37.5%–100%, SXT 15.8%–100%, CAZ 37.5%–100%, FEP 41.7%–100%, GM 79.2%–100%. For *P. mirabilis* AM 9.5%–20%, AMC 23.8%–40%, TZP 90.5%–100%, CXM 25%–40%, SXT 4.8%–0%, CAZ 23.8%–40%, FEP 100%–100%, GM 56.3%–60%. Seven strains of *K. pneumoniae* (29.2%) were carbapenem resistant and metallo-beta lactamase producing.
**Conclusions:** High resistance rates to fluoroquinolones were observed in uropathogen bacteria isolated not only from hospitalized patients but also from patients with community-acquired urinary tract infections in Greece. Increasing resistance rates to the rest antibiotic agents make the treatment of urinary tract infections a very difficult problem.

## P1767

### Susceptibility of *Pseudomonas aeruginosa* isolated from the MYSTIC Programme to the carbapenems: meropenem and imipenem

P.J. Turner *(Macclesfield, UK)*

**Objectives:** The Meropenem Yearly Susceptibility Test Information Collection Programme (MYSTIC) was initiated in 1997 in order to track the susceptibility of organisms in centres that were prescribing meropenem. This poster seeks to examine the susceptibility of *Pseudomonas aeruginosa* isolates over this period to the carbapenems; meropenem and imipenem and, in particular, records the susceptibility of imipenem-resistant isolates to meropenem and vice versa.
**Methods:** *Pseudomonas aeruginosa* isolates were speciated by the methods in current use at the participating centres. Minimum Inhibitory Concentrations of meropenem and imipenem were determined using reference methods described by CLSI.
**Results:** A total of 15709 isolates of *Pseudomonas aeruginosa* have been tested globally, of these 78.2% were susceptible to meropenem at the breakpoint of < 4 mg/L and 70.1% to imipenem. Globally, susceptibility to the two carbapenems has remained stable over the period 1997–2005, however when imipenem-resistant isolates were examined (n = 4625) 32.7% proved to be susceptible to meropenem, conversely of the 3359 meropenem-resistant isolates only 7.8% proved to be susceptible to imipenem. A similar pattern was seen when isolates were separated into global regions:USA 425 imipenem-resistant isolates, 25.4% susceptible to meropenemUSA 346 meropenem-

resistant isolates, 8.4% susceptible to imipenemN Europe 1448 imipenem-resistant isolates, 44.9% susceptible to meropenemN Europe 898 meropenem-resistant isolates, 11.7% susceptible to imipenemS. Europe 724 imipenem-resistant isolates, 41.3% susceptible to meropenemS. Europe 465 meropenem-resistant isolates, 9.0% susceptible to imipenemE. Europe 949 imipenem-resistant isolates, 18.6% susceptible to meropenemE. Europe 812 meropenem-resistant isolates, 5.2% susceptible to meropenemS. America 786 imipenem-resistant isolates, 17.4% susceptible to meropenemS. America 668 meropenem-resistant isolates, 3.6% susceptible to imipenem.

**Conclusions:** These results suggest that if a carbapenem is required to be prescribed empirically in a situation where *Pseudomonas aeruginosa* isolates are suspected then meropenem is a logical choice because of its greater *in vitro* potency and activity against some imipenem-resistant isolates.

## P1768

## Third Belgian multicentre survey of antibiotic susceptibility of anaerobic bacteria

I. Wybo, D. Piérard, I. Verschraegen, M. Reynders, K. Vandoorslaer, S Lauwers *for the BVIKM-SBIMC/ GOSPIZ-GDEPIH working group*

**Objectives:** To collect recent data on the susceptibility of anaerobes and to compare with results from previous studies.

**Methods:** 450 anaerobic clinical isolates from various body sites, collected from October 2003 to February 2005 in nine Belgian university hospitals, were tested with E-tests against penicillin (PEN), cefotetan (CTT), cefoxitin (FOX), amoxicillin-clavulanic acid (AMC), piperacillin/tazobactam (TZP), meropenem (MEM), clindamycin (CLI), metronidazole (MTZ), chloramphenicol (CHL), moxifloxacin (MXF) and linezolid (LZD). Minimal inhibitory concentration for tigecyclin (TIG) was determined by NCCLS agar dilution.

**Results:** *Bacteroides fragilis* group (BAFG) accounted for 53% of the isolates, *Fusobacterium* spp. for 7%, other gram negative bacilli (OGNB) for 11%, Clostridia (CLOS) for 14%, nonsporeforming Gram-positive bacilli (NSFGPB) for 7% and cocci for 8%. Beta-lactamases (BL) were detected in 61% of isolates. Most BL + strains belonged to BAFG (98%) and OGNB (70%). At NCCLS-recommended breakpoints, more than 95% of isolates were susceptible to TZP, MTZ, CHL and MEM, 92% to AMC but only 77%, 70%, 62% and 33% to FOX, CTT, CLI and PEN respectively. No NCCLS-breakpoints for anaerobes are available for MXF, LZD and TIG. MIC 50 and MIC 90 for MXF were 1 and 64 mg/L, for LZD 2 and 4 mg/L and for TIG 0.5–8 mg/L. In comparison with similar surveys conducted in 1987 and 1993–1994 susceptibility of BAFG to clindamycin decreased from 83% in 1987, to 66% in 1993–1994 and 48% in 2004. In BAFG 92% of *B. fragilis* and 78% of non-*B. fragilis* were susceptible to AMC in this study; in 1993–1994 susceptibility in these groups was 95% and 89% and in 1987–97% and 94% respectively. All isolates, except 6 BAFG and 1 CLOS, were susceptible to MEM. 98% of the isolates were susceptible to CHL. Susceptibility to MTZ remains stable and is high in all groups except NSFGPB where MTZ is active on merely 35% of the isolates.

**Conclusions:** TZP, MEM and MTZ remain very potent antimicrobial agents in the treatment of anaerobic infections. Although still rare, resistant organisms were detected to each of them. Therefore susceptibility testing of anaerobic isolates is indicated in severe infections to confirm appropriateness of antimicrobial therapy. Further monitoring of background susceptibility is necessary to guide empiric treatment.

## P1769

## Comparative *in vitro* activity of levofloxacin against *Escherichia coli* isolated from acute pyelonephritis in france in 2005

C.J. Soussy, C. Lascols, C. Dib-Smahi *and The Multicenter Group Study.*

**Objectives:** The objective of this study was to evaluate the *in vitro* activity of levofloxacin (LVX) comparatively to other antibiotics against *Escherichia coli* strains isolated from acute pyelonephritis in women consulting Emerging rooms by 23 French hospitals in 2005.

**Methods:** MICs of LVX, ofloxacin (OFX), ciprofloxacin (CIP), nalidixic acid (NAL), amoxicillin-clavulanic acid (AMC), ceftriaxone (CRO), cefixime (CFM), amikacin (AN), gentamicin (GM) and cotrimoxazole (SXT) were determined by agar dilution according to the EUCAST breakpoints approved by 2005 recommendations of the Comité de l'Antibiogramme de la Société Française de Microbiologie. Quality control was performed with *E. coli* strain ATCC 25922.

**Results:** A total of 231 strains were collected. 46.3% of strains were isolated from urinary samples, 10.8 % from blood culture and 42.9% from the two specimens. MICs 50/90 (mg/L), the range of MICs and the percentage of susceptibility (%) are presented in the following table: Concerning the fluoroquinolones, MICs50/90 of LVX were one/two dilution lower than those of OFX and two/one dilution higher than those of CIP. For the other antibiotics, a higher percentage of susceptibility was observed with CRO and AN, when a lower percentage of susceptibility was observed with AMC and SXT.

| | MICs 50/90 (mg/L) | Range (mg/L) | % of Susceptible strains |
|---|---|---|---|
| LVX | 0.06/0.12 | 0.016-32 | 93.1 |
| OFX | 0.12/0.5 | 0.03-64 | 90.5 |
| CIP | 0.016/0.06 | <0.008->64 | 92.7 |
| NAL | 2/8 | 2->128 | 90.5 |
| AMC | 4 :2/32 :2 | 1:2->128:2 | 68.8 |
| CRO | 0.03/0.12 | 0.016-128 | 99.6 |
| CFM | 0.25/1 | 0. 016->64 | 90.5 |
| AN | 4/8 | 2-32 | 94.8 |
| GM | 2/4 | 0.5->128 | 84.8 |
| SXT | 1:19/>64:1216 | 0.25:4.75->64:1216 | 65.4 |

**Conclusions:** Levofloxacin exhibited good *in vitro* activity against *E. coli* strains isolated from acute pyelonephritis with 93.1% of susceptible strains.

## P1770

## *In vitro* activity of double and triple combinations of colistin, imipenem, rifampicin and linezolid against epidemic strains of multidrug-resistant *Acinetobacter baumannii* producing OXA carbapenamases

D.W. Wareham, D.C. Bean *(London, UK)*

**Objectives:** *A. baumannii* has emerged as an important cause of nosocomial infection in critically ill patients worldwide. In the UK three strains in particular exhibiting multi-drug resistance and producing OXA carbapenamases have been responsible for ongoing outbreaks. Treatment options for infection with these organisms are limited as only colistin and tigecycline retaining significant activity *in vitro*. Animal models and *in vitro* studies using other multi-resistant strains suggest that drugs in combination with colistin may be effective. We assessed the

activity of colistin in combinations including imipenem, rifampicin and linezolid against epidemic strains from a recent UK outbreak.
**Methods:** Isolates of *A. baumannii* exhibiting resistance to carbapenems were recovered from patients at Barts and The London NHS. Isolates were referred to the Health Protection Agency and confirmed as belonging to clones producing OXA carbapenemases. Activities of polymyxin, imipenem, rifampicin and linezolid alone and in double and triple combinations were determined using standard chequerboard assays with increasing concentrations of drug 1 on the x axis, drug 2 on the y axis and drug three in multiple replicate plates. After incubation at 24 hours wells were examined for growth and MIC's determined for each combination. Synergy between agents was defined as a fixed inhibitory concentration index (FICI) of < 0.5.
**Results:** The isolates tested belonged to the OXA-23 clone 1, OXA-23 clone 2 and the South East Clone, as confired by the HPA. Colistin was the most active agent alone with MICs from 1–2 mg/L. Imipenem MIC's varied from 4–32 mg/L. The most active combinations were colistin plus rifampicin (FICI = 0.38) and colistin, rifampicin and imipenem (FICI = 0.39). Synergy was not seen with colistin in combination with imipenem alone. Linezolid in combination with colistin (FICI = 0.37), or imipenem (FICI = 0.38) was synergistic but at therapeutically unobtainable linezolid concentrations (64 mg/L).
**Conclusion:** Multidrug resistant strains of *A. baumannii* from the UK producing OXA carbapenemases remain susceptible to polymyxin *in vitro*. Polymyxin exerts its effect on the bacterial cell wall; theoretically assisting other antibiotics to reach their respective targets, and seems a logical choice for inclusion in combination therapy. We have shown that rifampicin is synergistic with polymyxin against these isolates *in vitro* and may be effective in treating severe *A. baumannii* infections in man.

## P1771

## A comparative *in vitro* evaluation of resistance development after exposure to teicoplanin, vancomycin, linezolid and quinupristin/dalfopristin in *Staphylococcus* spp. and *Enterococcus* spp.

E. De Vecchi, L. Nicola, M.R. Gismondo, L. Drago *(Milan, IT)*

**Objectives:** Glycopeptides, linezolid and quinupristin/dalfopristin represent a valid therapeutic choice for treatment of severe gram positive infections, but monitoring of development or increase of resistance is continuously performed. This preliminary study aimed to compare the ability to select for resistance of teicoplanin, vancomycin, linezolid and quinupristin/dalfopristin in clinical strains of staphylococci both susceptible and resistant to methicillin and enterococci by performing one step studies of resistance selection at antimicrobial concentrations achievable *in vivo*.
**Methods:** Methicillin susceptible *Staphylococcus aureus* (MSSA), methicillin resistant *Staphylococcus aureus* (MRSA), methicillin susceptible *Staphylococcus epidermidis* (MSSE), methicillin resistant *Staphylococcus epidermidis* (MRSE), *Enterococcus faecalis* and *Enterococcus faecium* (20 strains for each species) were isolated from different patients at L. Sacco Teaching Hospital of Milan, Italy. Determination of MIC was performed by means of microdilution broth method in accordance to Clinical and Laboratory Standards Institute (CLSI). Frequency of spontaneous single-step mutations after exposure to teicoplanin, vancomycin, linezolid and quinupristin-dalfopristin in MRSA, MSSA, MRSE, MSSE, *E. faecium* and *E. faecalis* strains was determined on agar plates containing each antibiotic at CLSI resistance breakpoints and at peak blood concentrations. After incubation at 37°C for 48 h colonies were counted and compared to the inoculum to calculate frequency of mutation. Colonies grown in plates containing antibiotics were sampled for determination of MIC values.
**Results:** Frequency of mutation was less than 10–9 for all the tested antibiotics at peak blood concentrations. Same results were obtained when breakpoint concentrations for each drug were used.
**Conclusion:** This one-step *in vitro* study demonstrated the ability of teicoplanin, vancomycin, linezolid and quinupristin/dalfopristin to prevent growth of resistant mutants of staphylococci and enterococci, thus suggesting no occurrence of mutational events leading to resistance when bacteria are exposed to blood concentrations of these drugs. In order to establish the development of resistance after *in vitro* serial exposure to the same antibiotics simulating different *in vivo* concentrations, further studies are needed and are now in progress (multi step induction of resistance).

## P1772

## *In vitro* activity of antimicrobial agents against Legionella obtained from hotel water systems in Turkey

H. Erdogan, F. Can, M. Demirbilek, F. Ergin, H. Arslan *(Antalya, Ankara, TR)*

**Objective:** To evaluate *in vitro* activity of antimicrobial agents against Legionella obtained from enviromental sources in Alanya in Turkey.
**Material-Method:** Fifty strains of Legionella obtained from hotel water systems during August 2003–July 2005 years were tested: *Legionella pneumophila* serogroup 6 n = 32, *L. pneumophila* serogroup 1 n = 12, *L. pneumophila* serogroup 3 n = 2, *Legionella* spp. n = 4. The antimicrobial agents tested were levofloxacin, ciprofloxacin, clarithromycin, azithromycin and rifampicin. The MIC of each antimicrobial agent for Legionella was determined by the microdilution method using buffered yeast extract supplemented with alpha ketoglutarate broth. *L. pneumophila* ATCC 33152, *S. aureus* ATCC 29213 and *E.coli* ATCC 25922 were used as controls.
**Results:** MICs were in the following ranges: Claritromycin 0.007–4 mg/L, azithromycin 0.001–2 mg/L, rifampicin 0.001–0.5 mg/L, levofloxacin 0.001–2 mg/L, ciprofloxacin 0.001–4 mg/L. According to MIC50 and MIC90 values of the agents, affectivities were determined as follows: rifampicin > ciprofloxacin > azithromycin > clarithromycin > levofloxacin.
**Conclusion:** This is the first result about *in vitro* activity of antimicrobial agents against Legionella. Rifampicin was found to be the most active agent.

## P1773

## *In vitro* activity of colistin against pan-resistant *Acinetobacter baumannii*

S. Quinteira, H. Ramos, J.M. Amorim, L. Peixe *(Porto, PT)*

**Objectives:** The aim of this study was to evaluate the *in vitro* activity of colistin against endemic pan-resistant *Acinetobacter baumannii* (including resistance to imipenem) isolated during a 4 year period in a university hospital.
**Methods:** 150 imipenem-resistant *Acinetobacter* spp. isolates were collected between January 2001 and October 2004, from a

variety of clinical specimens of different patients attending distinct wards in a university teaching hospital. Isolates were identified by API32GN and by sequencing the 16S rRNA gene. MICs of colistin were determined by agar dilution method, according to NCCLS susceptible breakpoint (≤ 2 mg/L). PFGE (*Apa*I restriction enzyme) was performed.
**Results:** 141 of 150 *A. baumannii* isolates (94%) were susceptible to colistin. Colistin resistance (MIC ≥ 4 mg/L) was observed in 9 isolates (5 isolates with a MIC of ≥ 16 mg/L and 4 isolates with a MIC of 4 mg/L) recovered from different patients in distinct wards. Among these imipenem- and colistin-resistant isolates, 3 distinct PFGE patterns were identified (clones A, B, and C). Resistance to almost all beta-lactams (including carbapenems) and variable susceptibility to aztreonam, amikacin and tobramycin was a common feature of clone A. Isolates belonging to clone B showed resistance to imipenem, amoxicillin and its association with clavulanic acid (AMC), ureidopenicillins and their associations; susceptibility to ceftazidime; and variable behaviour to meropenem, cefepime, cefpirome and aztreonam. The susceptibility profile to aminoglycosides was variable, differing from clone A in its susceptibility to netilmicin and minocycline. Clone C was resistant to imipenem, amoxicillin, AMC, piperacillin, piperacillin + tazobactam, ticarcillin and ticarcillin + clavulanic acid, but remained susceptible to meropenem, aztreonam, cefpirome, ceftazidime and cefepime.
**Conclusion:** Only colistin, one of the few effective drugs available against multi-drug-resistant *Acinetobacter* infections, showed *in vitro* activity against the majority of *Acinetobacter* spp. strains isolated within the sampled hospital. The observed 6% *A. baumannii* resistance to the recently re-introduced colistin seems like the first chapter of a novel repeatedly told for several antibiotics.

## P1774

## Emergence of high-level gentamicin resistance in clinical enterococcal isolates of companion animals in Portugal

C. Pomba, M. Delgado, T. Ribeiro, T. Crespo, M.F. Silva Lopes, J.H. Duarte Correia *(Lisbon, Oeiras, PT)*

**Objectives:** To characterize *in vitro* gentamicin susceptibility among enterococci causing infections in cats and dogs, in order to evaluate the impact of high-level gentamicin resistance in small animal therapeutics.
**Methods:** The samples were collected at the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine and at veterinary private practices in the Lisbon area. From January 1998 until November 2005, a total of 43 enterococci were isolated from dogs and cats with urinary tract infection (UTI), otitis externa (OE) and pioderma. BBL Crystal Gram Positive ID System was used for identification at the species level. Minimal inhibitory concentrations (MIC) were determined by the microdilution method according to NCCLS (1). The bifuntional enzyme gene that confers high-level gentamicin resistance (HLGR) was detected using PCR (2).
**Results:** *Enterococcus faecalis* was the predominant isolate (n = 35), followed in frequency by *Enterococcus faecium* (n = 6). MIC cumulative data analysis showed that MIC50 values were 8 μg/ml and MIC90 1024 μg/ml. Six (14%) HLGR clinical enterococcal isolates were detected, with MIC ranges between 1024–2048 μg/ml. Four of these enterococci were isolated from UTI and 2 from OE. Four of the phenotypically high-level gentamicin resistant isolates carried the aac(6′)-Ie-aph(2′′)-Ia gene.
**Conclusions:** The importance of enterococcal infection in small animal clinical samples has increased over the last years. MIC cumulative data points out low-level gentamicin resistance among clinical enterococci isolates of veterinary origin and the emergence of high-level isolates, as previously detected (2). This fact compromises cell-wall active agents (such as ampicillin or vancomicin) and aminoglicoside *in vivo* synergy. The aac (6′)-Ie-aph(2′′)-Ia gene carriage is of concern because its expression confers resistance also to tobramicin, netilmicin, amikacin and kanamicin. Our findings are of critical importance, as they may have a direct impact in therapeutic decision in the management of companion animal's infections by enterococci. Furthermore, transfer of resistance genes and resistance strains between animals and owners/caretakers by direct contact is a concerning probability.
**References:** (1) NCCLS (2002) – M31 – A2.(2) Silva Lopes M. F. et al. (2003) Gentamicin resistance in dairy and clinical enterococcal isolates and in reference strains Journal of Antimicrobial Chemotherapy 52, 214–9.

## P1775

## Study of antibiotic-resistant *Streptococcus pneumoniae* strains, isolated in Romania, between 2004–2005

M. Pana, M. Ghita, R. Papagheorghe, N. Popescu, I. Nistor, M. Nica, G. Bancescu, M. Andrei, S. Botea, D. Blana, V. Ungureanu *(Bucharest, RO)*

**Objective:** To study the antibiotic resistance in pneumococci isolated last years in Romania.
**Methods:** Four hundred fifty five strains of *Streptococcus pneumoniae* coming from sputum or tracheal aspirate (TA): N = 249,blood (N = 22), CSF (N = 59), others (sinus, eye fluid, middle ear fluid): N = 125,were collected between 2004–2005 at the National Reference Center for *Streptococcus*. The isolates were tested for susceptibility (MICs) to the following antibiotics: penicillin(Pc), erythromycin(Em), cefuroxim(Cxm), trimethoprim/sulfamethoxazole(Sxt), ofloxacin(Ofx), vancomycin(Va) by standard dilution MIC testing.
**Results:** Interpretative criteria were used according to NCCLS 2002.During the study period penicillin resistant strains of *S. pneumoniae* was noted as follows: 67% in sputum or TA,19% in blood,12%in CSF,and 75% in others,against cefuroxim resistant strains:15% in sputum or TA,10% in blood,4% in CSF.Regarding the susceptibility to Ofx,penicillin resistant *S. pneumoniae* strains from sputum or TA revealed 97.9%.The penicillin resistant strains coming from sputum or TA showed resistance as follows; 48% to Em and 78% to Sxt,against strains isolated from others: 58% to Em and 49% to Sxt.No resistant strain to Va was found.
**Conclusion:** The percentage of the penicillin resistant *S. pneumoniae* isolates from the lower respiratory tract, middle ear fluid, eye fluid and sinus was markedly higher than that of the isolates from blood and CSF. The most efficient drugs against penicillin resistant pneumococci were cefuroxim and ofloxacin. These results from Romania also underline the previous observations regarding the higher emerging rates of resistance in *S. pneumoniae* worldwide.

## P1776

## Penicillin resistance in *Streptococcus agalactiae*

F. Cavallini, S. Cagnacci, E. Maioli, R. Bandettini, E. Debbia, A. Marchese *(Genoa, IT)*

**Objectives:** *Streptococcus agalactiae* has become recognized as a cause of serious illness in newborns, pregnant women, and

adults with chronic medical conditions. Heavy colonization of the genital tract with *Streptococcus agalactiae* also increases the risk that a woman will deliver a preterm low-birthweight infant. Early-onset infections (occurring at < 7 days of age) are associated with much lower fatality than when they were first described, and their incidence is finally decreasing as the use of preventive antibiotics during childbirth increases among women at risk. Penicillin or ampicillin remains the drug of choice for intrapartum antibiotic prophylaxis for *Streptococcus agalactiae* colonization in pregnant women. Erythromycin and clindamycin are the drugs of choice for women with serious penicillin allergy who are colonized with *Streptococcus agalactiae*. The objective of this study is to estimate the insorgence of penicillin resistance among *Streptococcus agalactiae*.

**Methods:** All *streptococcus agalactiae* were tested against penicillin by agar dilution method according to Clinical and Laboratory Standards Institute 2005 (CLSI); breakpoints for resistance were those recommended by the CLSI. Antimicrobial agents were obtained from their manufacture as laboratory grade powder.

**Results and discussion:** Four hundred seven (407) clinical isolated were analysed during 2005. *Streptococcus agalactiae* resulted resistant to Penicillin in 1 case; and about 2% resulted borderlines.The present findings indicate a probable evolution in *S. agalactiae* toward penicillin resistance this finding suggest the need a continuous national and international surveillance programs to provide timely data on the evolution of incidence of penicillin resistance in this pathogen.

## P1777

### Ciprofloxacin susceptibility of the most common isolates at bacterial conuctivitis

M. Dimitrova *(Stip, MK)*

**Aim:** Average of the susceptibility of the isolated strains to ciprofloxacin.

**Material and methods:** 6.365 swabs from conuctivity saccus are taken in a period from 01.01.2000 to 31.12.2004 at patients diagnosed with chronic conuctivitis.

**Results:** Positive bacterial findings are 1.793 (28%). From them: *Staphylococcus aureus* 1.471 (82%), *Haemophilus influenzae* 60 (3.3%), *Staphylococcus epidermidis* 110 (6.1%), *Streptococcus pneumoniae* 66 (3.6%), *Escherichia coli* 30 (1.7%), *Pseudomonas* spp. 37 (2.1%), *Enterococcus faecalis* 14 (0.8%). The susceptibility to ciprofloxacin is high and it is: *Staphylococcus aureus* 92%, *Haemophilus influenzae* 100%, *Staphylococcus epidermidis* 94%, *Streptococcus pneumoniae* 88%, *Escherichia coli* 100%, *Pseudomonas* spp. 80%, *Enterococcus faecalis* 100%.

**Conclusion:** According to the average numerals we concluded that all the isolated strains are highly susceptible at ciprofloxacin. Its application in the conuctivial saccus is especially important in curing the conuctivial infections with resistent strains like *Pseudomonas aeruginosa*. We successfully cure the bacteria chronic conuctivitis with the adequately used therapy according to antibiogram.

## P1778

### Antimicrobial resistance patterns of *Acinetobacter baumannii* in clinical isolates

G.T. Tsilika, V.P. Pliatsika, M.T. Tsivitanidou, D.S. Sofianou *(Thessaloniki, GR)*

**Objectives:** *A. baumannii* is a nosocomial pathogen, commonly isolated from critically ill and immunocompromised patients. The aim of the present study was to evaluate the antimicrobial resistance of *A. baumannii* strains isolated in a tertiary care hospital througout a three-year period.

**Methods:** A total of 1311 *A. baumannii* strains were selected from January 2002 to December 2004.The specimens were obtained from inpatients hospitalized in intensive care unit (ICU) and pediatric intensive care unit (PICU) and other departments of our hospital.The identification and the antimicrobial susceptibility testing were performed using the Vitek 2 automated system(bioMerieux,France).

**Results:** The isolates included in the study originated from haemocultures (28.5 %), central venous catheters (10.4 %), urine (10.2 %), bronchial aspirates (25.4 %), wounds (25.5 %). Out of 1311 strains 45.2 % isolated from ICU and PICU and the remaining 54.8 % from the other departments. We determined the occurrence of resistance to 20 common used antimicrobial agents during a three-year period. The majority of isolates were resistant to 17 or more antibiotics tested.Some strains were defined by resistance to all antimicrobial agents exept colistin.The resistance to imipenem rose dramatically from 18.4% in 2002 to 45.9% in 2004.Notable increase detected also to: Trimethoprim/Sulfamethoxazole: from 80.9% in 2002 to 97% in 2004, Amikacin: from 80.5% in 2002 to 96.5% in 2004, Netilmicin: from 79.8% in 2002 to 91% in 2004, Ticarcillin/clavulanic acid: from 76.8% in 2002 to 97.4% in 2004, Piperacillin/tazobactam: from 30.3% in 2002 to 47.5% in 2004. Resistance rates to other antibiotics tested were as following:amikacin 71%, ampicillin 100%, cephalothin 100%, cefotaxime 97.7%, cefoxitin 100%, ceftazidime 93.4%,ciprofloxacine 96.2%, nalidic acid 6.6%, norfloxacin 96.4%,ofloxacin 95.1%, ticarcillin 97%, tobramycin 42.8%. Noteworthy is the decreasing of gentamycin resistance from 38.8% 2002 to 17.2% in 2004.Colistin was the only antimicrobial agent active to all clinical isolates.

**Conclusions:** The emergance and rapid spread of multidrug resistant *A. baumannii* isolates are of a great concern worldwide.Imipenem was one of the most potent agents for treatment of those infections caused by multiresistant strains.The increasing prevalence of imipenem resistance limits therapeutic options and leads to outbreaks of carbapenems resistant strains.

# Tigecycline *in vivo* studies

## P1779

### Ecological impact of tigecycline on the normal oropharyngeal and intestinal microflora

C.E. Nord, E. Sillerström, E. Wahlund, V. Parks, M.L. Odell, J. Vouis *(Stockholm, SE; Paris, FR; Solna, Uppsala, SE)*

**Objectives:** Antibacterial agents disrupt the ecological balance of the normal human microflora. Disturbances may lead to the emergence of antibiotic resistance and/or to infections by potentially pathogenic bacteria. Tigecycline, a member of a new class of antibiotics (glycylcyclines), has been shown to have a potent expanded broad-spectrum activity against most gram-positive and gram-negative aerobic and anaerobic bacteria. The aim of the study was to investigate the ecological effects of tigecycline on the normal oropharyngeal and intestinal microflora in healthy subjects.

**Methods:** Thirteen (13) white subjects (6 women, 7 men) aged 20 to 31 years, received 100 mg of tigecycline in the morning on

day 1 as a 30-minute intravenous (IV) infusion, followed by 50-mg doses of tigecycline given every 12 hours as a 30-minute infusion for 10 days. One (1) subject was withdrawn on day 2 because of an adverse event. Serum, saliva, and faecal samples were collected before, during, and after administration for microbiologic cultivation and for assays of tigecycline. All new colonizing bacteria were tested for susceptibility (resistance >8 mg/L) during the investigation period.

**Results:** The serum concentrations on day 9, 12 hours after dosing, were 0.2 to 2.1 mg/L (mean value 0.4 mg/L, median value 0.2 mg/L, and SD 0.5 mg/L). The faecal concentrations on day 8 were 3.0 to 14.1 mg/kg (mean value 6.0 mg/kg, median value 5.6 mg/kg, and SD 2.9 mg/L). Saliva concentrations were generally low, with highest mean value 0.18 mg/L, median value 0.22 mg/L, on day 10, 3 hours after dosing. A minor effect on the oropharyngeal microflora was observed. The numbers of *Enterococci* and *Escherichia coli* in the intestinal microflora were reduced at day 8, while other enterobacteria and yeasts increased. There was a marked reduction of lactobacilli and bifidobacteria but no impact on bacteroides. No Clostridium difficile strains were isolated. Two (2) *Klebsiella* strains and 5 Enterobacter strains resistant to tigecycline were found.

**Conclusion:** Tigecycline had a minor effect on the oropharyngeal microflora. Tigecycline's effect on the intestinal microflora was due to its spectrum of antibacterial activity and intestinal concentrations.

## P1780

## Single-centre experience of the use of tigecycline in the treatment of deep-seated multidrug-resistant *Acinetobacter* in patients with multiorgan failure

E. Sizer, W. Bernal, G. Auzinger, I. Eltringham, S. Stoll, L. Sawkeng, J. Wendon *(London, UK)*

**Objectives:** To examine and report the use of Tigecycline (Wyeth) in the treatment of multidrug resistant acinetobacter (MDRA) culture positive sepsis in 11 patients requiring mutiorgan support.

**Methods:** All patients were managed within the Liver Intensive Care Unit. Physiological data was collected prospectively and entered onto a specialist database. Patients received standard intensive care management; antibiotic and antifungal therapy administered as indicated by microbiological cultures. Systemic inflammatory response (SIRS) features initiated blood cultures (vascular lines and peripheral), drain fluid culture and broncoalveolar lavage (BAL). Screening swabs were undertaken weekly and samples sent for culture at laparotomy. MDRA positive cultures from blood, BAL, drain fluid or samples taken at laparotomy in the context of SIRS resulted in the initiation of tigecycline treatment.

**Results:** 11 patients received Tigecycline treatment for MDRA infections. The underlying disease states were necrotizing pancreatitis (1), post hepatectomy (1), polytrauma (1), all with postive intra-abdominal cultures. Acute and acute on chronic liver failure (4), MDRA +ive broncho-alveolar lavage ± blood cultures and 4 post liver transplant patients (necrotising pancreatitis in one, 2 with recurrent small bowel perforation and 1 with retroperitoneal haemorrhage) all with positive blood cultures and in 3 positive intra-abdominal tissue/clot. Mean time from admission to treatment for MDRA was 25 days. Mean duration of treatment was 10 days (range 4–15). Mean APACHE II score at initiation of therapy was 18 (range 13–26); 4/11 patients survived to intensive care discharge and 3/11 to hospital discharge. Microbiological clearance of MDRA was observed in 8/11 cases. In those who did not achieve microbiological clearance cause of death was intra-abdominal haemorrhage, recalcitrant organ failure with recurrent small bowel perforation and vasopressor resistant shock. In these patients one remained culture positive for intraabdominal sepsis despite full treatment (small bowel perforation x5). The drug was well tolerated with the only side effect being that of hypercalcaemia observed in 5/12 patients, mean corrected calcium 2.59 mMol/l, range 2.32–2.81. In all cases this resolved on drug discontinuation.

**Conclusion:** Tigecycline appears to be an efficacious agent in the treatment of deep seated MDRA infections.

## P1781

## Pharmacokinetic-pharmacodynamic model for the safety of tigecycline in patients with complicated intra-abdominal infections

A. Meagher, K. Liolios, J. Passarell, B. Cirincione, S. Van Wart, J. Fiedler-Kelly, T. Babinchak, E. Ellis-Grosse *(Buffalo, Collegeville, US)*

**Objectives:** Nausea (N) and vomiting (V) have been reported with tigecycline, a new glycylcycline with expanded broad spectrum activity. Exposure-response relationships and patient covariates predictive of the first N and V occurrence were evaluated in patients with complicated intra-abdominal infections (cIAI).

**Methods:** Data from patients from 3 cIAI trials (one phase 2 and two phase 3), receiving 100 mg loading dose and 50 mg every 12 hours, were pooled for analysis. N and V (definitely, possibly, or probably related to tigecycline) reported from the start of infusion until 24 hours after the last dose were included. Individual exposure measures [AUC0-12 and Cmax] were calculated using a previously developed population PK model. Logistic regression was used to evaluate predictors of first N and V occurrence. Covariates included age, weight, sex, region of treatment, and baseline N and V.

**Results:** The dataset included 928 patients (218 with PK). Mean (SD) age and weight were 46 (18) years and 73 (16) kg. 64% of patients were men and 24%, 37%, and 18% were enrolled in North America, Europe, and Latin America, respectively. Baseline nausea or vomiting was reported in 47% and 35%. Overall, N and V occurred in 18% and 13% of patients receiving tigecycline, however most (62%; 67%) of first N and V events were mild in nature. Women had more N and V (23%; 17%) than men (15%; 11%). N and V were lower in Europe (10%; 6%) than in other regions. AUC0-12 and Cmax were not predictive. The final nausea model included weight, sex, region, baseline nausea, and the interaction of weight/region as predictors of the first nausea occurrence ($p = 0.671$, 0.0006, 0.205, 0.033, & 0.023, respectively). The final vomiting model included weight, sex, region, & 4 interactions (weight/sex, weight/region, sex/region, & weight/sex/region) as predictors of the first vomiting occurrence ($p = 0.054$, 0.819, 0.083, 0.815, 0.02, 0.005, & 0.01, respectively).

**Conclusion:** AUC0-12 and Cmax were not predictors of nausea and vomiting events for tigecycline. The final nausea model would predict: nausea to be less in men, Europeans, and in the absence of baseline nausea. The final vomiting model would predict: heavier men, from all regions except Latin America, and heavier women and to have less vomiting.


ISSN: 1470-9465

## P1782

### Predictors of excess health resource utilisation in treatment of hospitalised patients with community-acquired pneumonia: retrospective analysis of a clinical study comparing intravenous tigecycline and levofloxacin

R. Mallick, S. Sun *(Collegeville, US)*

**Objective:** Because hospitalisation for community-acquired pneumonia (CAP) is associated with substantial morbidity and health resource utilisation, we evaluated the predictors of prolonged hospital length of stay (LOS) and treatment duration.
**Methods:** We conducted a retrospective analysis of data from a double-blind, randomised, multicentre clinical study that compared the efficacy and safety of tigecycline with that of levofloxacin in the treatment of patients with CAP requiring hospitalisation. Patients were stratified by the FINE Pneumonia Severity Index and randomly assigned to receive tigecycline or levofloxacin via IV administration for at least 7 days. Treatment duration and hospital discharge were based on physician assessment of signs and symptoms of infection and patient condition. We used Cox proportional hazards modelling with stepwise selection to identify statistically significant predictors ($p < 0.05$) of treatment duration and hospital LOS.
**Results:** Among 426 patients with CAP in the clinical intent-to-treat population with complete hospitalisation data, mean age was 49.8 years (range 17–92) and 22.8% of patients were aged ≥65 years. Diabetes (11.5%), chronic obstructive pulmonary disease (7.8%), and congestive heart failure (7.0%) were leading co-morbidities. About 37.1% of patients were smokers and 5.6% were characterised by alcohol abuse. Median FINE Pneumonia Severity Index score was 3; 21.1% of patients had a score >4. Among 294 patients with identified microbiology, *Streptococcus pneumoniae* (41.2%) and *Mycoplasma pneumoniae* (26.2%) were leading pathogens. Alcohol abuse [hazard ratio (HR) = 0.53 ($p = 0.006$)] and liver disease [HR = 0.60 ($p = 0.037$)] were significant independent risk factors for prolonged LOS. Among patients with identified microbiology, age ≥65 years [HR = 0.67 ($p = 0.007$)], and the presence of *Haemophilus influenzae* [HR = 0.59 ($p = 0.01$)] or *H. parainfluenzae* [HR = 0.60 ($p = 0.007$)] were additional independent risk factors for longer LOS. Treatment in a European centre was associated with longer treatment duration [HR = 0.30 ($p < 0.0001$)] as well as longer LOS [HR = 0.26 ($p < 0.0001$)]. There were no significant differences between the 2 groups in treatment duration or LOS.
**Conclusions:** Tigecycline, a first-in-class glycylcycline, was associated with treatment duration and LOS similar to that of levofloxacin, adjusting for several identified risk factors.

## P1783

### Tigecycline effective in treating patients with intra-abdominal or skin/skin structure infections who have bacteraemia

E.J. Ellis-Grosse, R. Maroko *(Collegeville, US)*

**Objectives:** The treatment of bacteraemia, which is a potentially fatal complication of infections originating at other body sites, is complicated by increasing resistance. Tigecycline, a first-in-class glycylcycline, has an expanded spectrum of activity against Gram-positive, Gram-negative, anaerobic, and atypical bacteria including resistant strains. Tigecycline is safe and effective in treating complicated skin and skin structure (cSSSI) and intra-abdominal infections (cIAI). This analysis examines tigecycline clinical trial experience in patients with cIAI or cSSSI who had bacteraemia (presence of bacteria in blood) at baseline.
**Methods:** Tigecycline and active comparator data were pooled from 4 Phase 3 studies: cSSSI 300 and 305 studies [vs vancomycin/aztreonam (V/A)] and cIAI 301 and 306 studies [vs imipenem/cilastatin (IMI/CIL)]. A subgroup analysis evaluated the presence of bacteraemia on the primary efficacy endpoint. A generalized linear model was used to examine the interaction effects of treatment and whether or not the subject experienced bacteraemia during the study.
**Results:** At test-of-cure visit in cSSSI subjects [clinically evaluable (CE) population] with bacteraemia, tigecycline cured 19/23 patients (82.6%; 95% CI 61.2, 95.0) and V/A cured 21/24 subjects (87.5%; 95% CI 67.6, 97.3) with an adjusted difference of tigecycline -V/A of −2.1% (95% CI -6.5, 2.4, interaction bacteraemia with treatment arm p-value = 0.780). In cIAI subjects who had bacteraemia, tigecycline cured 33/40 subjects (82.5%; 95% CI 67.2, 92.7) and IMI/CIL cured 40/50 subjects (80.0%; 95% CI 66.3, 90.0) at test-of-cure [microbiological efficacy (ME) population]. The adjusted difference of tigecycline-IMI/CIL was −0.2% (95% CI -4.4, 4.0), interaction bacteraemia with treatment arm p-value = 0.7368. These clinical response results are consistent with overall tigecycline clinical and microbiological efficacy results seen in phase 3 trials that demonstrated the equivalence of tigecycline to its comparators.
**Conclusion:** The baseline status of bacteraemia in patients with cIAI or cSSSI does not appear to influence the efficacy of tigecycline.

## P1784

### Tigecycline vs imipenem/cilastatin for treatment of complicated intra-abdominal infections: European experience

R. Maroko, N. Dartois, Y. Brault, J. Goodrich, E.J. Ellis-Grosse *on behalf of the 301 and 306 cIAI Study Groups*

**Objectives:** Treatment of complicated intra-abdominal infections (cIAI) is challenging due to diverse bacteriology and bacterial resistance. The efficacy and safety of Tigecycline (TGC), a first-in-class glycylcycline approved in Mexico, Brazil, Peru, Colombia and USA for treating cIAI and complicated skin and skin structure infections, was compared with Imipenem/Cilastatin (IMI/CIS) in adult hospitalised patients with cIAI in two double-blind, Phase 3 multinational trials. This analysis evaluated TGC efficacy and safety in the European region of the integrated results of these two trials.
**Methods:** One study was conducted in 96 centres (17 countries) and the other study was conducted in 94 centres (27 countries). Patients were stratified by disease severity (APACHE II score ≤15 vs >15 but ≤30), and randomly assigned to IV TGC (100 mg loading, then 500 mg q12h) or IV IMI/CIS (500/500 mg q6h) for 5–14 days. Clinical response at test-of-cure (TOC, 12–44 days after therapy) for microbiological evaluable (ME) and microbiological modified intent-to-treat (m-mITT) were co-primary efficacy endpoints where cure/failure responses were determined. Safety was assessed by physical examination, laboratory results, and adverse event (AE) reporting.
**Results:** In the European analysis, 703 patients were mITT (received ≥1 dose), 556 m-mITT (283 TGC, 273 IMI/CIS) and 460 ME (237 TGC, 223 IMI/CIS). Treatment groups were balanced with respect to demographics. Patients were mostly white (98.2%) men (58%) with a mean age of 50 years. For ME, clinical cure rates at TOC were 92.4% (219/237) for TGC vs 88.8% (198/223) for IMI/CIS (95% CI = −2.2, 9.4; test for non-inferiority $p < 0.0001$). Clinical cure rates for m-mITT were 87.3% (247/283)

for TGC vs 83.5% (228/273) for IMI/CIS (95% CI = −2.5, 10.0; test for non-inferiority $p < 0.0001$). Most commonly reported treatment emergent AEs (TEAEs, mITT) for TGC and IMI/CIS were nausea (14.7% and 11.8%, $p = 0.267$) and vomiting (10.7% and 7.3%, $p = 0.146$). The IMI/CIS group had significantly higher TEAEs of fever (7.3% IMI/CIS vs 3.2% TGC, $p = 0.017$), hyperglycaemia (1.7% IMI/CIS vs 0 TGC, $p = 0.031$) and dyspnoea (2.8% IMI/CIS vs 0.3% TGC, $p = 0.011$) where TGC had significantly higher amylase increase (3.2% TGC vs 0.6% IMI/CIS, $p = 0.011$) and BUN increase (2.3% TGC vs 0 IMI/CIS, $p = 0.003$).

**Conclusions:** Similar to the overall integrated analysis of the two Phase 3 trails, in the European analysis, TGC was safe and effective in the treatment of hospitalised patients with cIAI in comparison with IMI/CIS.

## P1785

## Tigecycline is safe and effective in the treatment of complicated skin and skin structure infections: European experience of two double-blind phase 3 comparison studies with vancomycin/aztreonam

R. Maroko, N. Dartois, D. Sarkozy, J. Goodrich, E.J. Ellis-Grosse *on behalf of the Tigecycline 300 and 305 Study Groups*

**Objectives:** Tigecycline (TGC) a first-in-class expanded spectrum glycylcycline, has been approved in Mexico, Brazil, Peru, Colombia and USA for treating complicated skin and skin structure infections (cSSSI) and complicated intraabdominal infections. Two phase 3, randomised, double-blind studies were conducted in hospitalised men and women with cSSSI to determine TGC safety and efficacy compared with vancomycin/aztreonam (V/A). The objective of this analysis was to evaluate the efficacy and safety seen in the European population of the integrated analysis of these 2 Phase 3 trials.

**Methods:** One study was conducted in 53 centres in 8 countries while the other study was conducted in 65 centres in 21 countries. Patients were randomly assigned (1:1) to receive either TGC (100 mg, followed by 50 mg IV twice daily) or vancomycin (1 g IV twice daily) plus aztreonam (2 g IV twice daily) for up to 14 days. Clinical response at test-of-cure (TOC, 12–92 days after therapy) for clinically evaluable (CE) and clinical modified intent-to-treat (c-mITT) populations were co-primary efficacy endpoints in which cure/failure responses were determined. Secondary objectives included determination of *in vitro* susceptibility to TGC of a range of bacteria that cause cSSSI and microbiological efficacy. Safety was assessed by physical examination, laboratory results, and adverse event (AE) reporting.

**Results:** In the European analysis, 385 patients comprise mITT (received ≥1 dose of study drug), 326 comprised CE (167 TGC, 159 V/A/CIS) and 376 comprised c-mITT (189 TGC, 187 V/A/CIS). Treatment groups were balanced with respect to demographics. Patients were mostly white (99.7%) men (63.6%) with a mean age of 50 years. In the European region, clinical responses to TGC and V/A at test-of-cure were similar: c-mITT, 84.7% (160/189) versus 86.6% (162/187), difference TGC-V/A was –2.0% (95% CI –9.5, 5.6). Similar results were noted in the CE population with TGC curing 89.8% (150/167) and V/A curing 95.0% (151/159), difference TGC-V/A was –5.1% (95% CI –11.6, 1.2).Most commonly reported treatment emergent AEs (TEAEs, mITT) for TGC and V/A were nausea (17.3% and 3.2%, $p < 0.001$) and vomiting (6.1% and 1.6%, $p = 0.032$). The V/A group had significantly higher TEAEs of SGPT increase (6.9% V/A vs 2.0% TGC, $p = 0.025$) and rash (2.6% V/A vs 0 TGC, $p = 0.028$).

**Conclusion:** In the European analysis of the integrated Phase 3 worldwide clinical studies, TGC monotherapy is as safe and efficacious as the combination of V/A in the treatment of patients with cSSSI.

## P1786

## Safety and tolerability of tigecycline

R. Maroko, N. Dartois, G. Rose, E.J. Ellis-Grosse *(Collegeville, US; Paris, FR)*

**Objectives:** Tigecycline (TGC), a glycylcycline, is a first-in-class, extended, broad-spectrum IV antibiotic that has demonstrated clinical activity in patients with complicated intra-abdominal infections (cIAI) and complicated skin and skin-structure infections (cSSSI). The safety of tigecycline was evaluated in four Phase III trials.

**Methods:** A total of 1415 hospitalized patients from these trials were pooled and evaluable for safety analysis. In the cIAI trial, patients received TGC 50 mg q 12 hrs (following a 100-mg loading dose) or imipenem 500 mg and cilastin 500 mg q 6 hrs. Those in the cSSSI study were treated with either TGC (same dose/schedule) or vancomycin 1 gm with or without aztreonam 2 gm q 12 hrs.

**Results:** The most frequently reported adverse events (AEs) in both TGC-treated groups were nausea (N) and vomiting (V). The incidence of N was 29.5% while V was approximately 19.5%; these were generally mild to moderate in severity. Infection-related serious AEs were slightly more frequent with TGC versus comparators (6.7% vs 4.6%). Discontinuations due to treatment-emergent AEs (including N/V) occurred at similar rates with TGC and comparators (5.0% vs 4.7%). Six patients (0.36%) treated with TGC presented with intestinal perforations and developed sepsis/septic shock compared with 2 (0.12%) for imipenem/cilastatin, with higher baseline Apache II scores in the TGC group; the relationship to treatment could not be determined. In the overall efficacy analysis, subjects with ''perforation of the intestines'' were balanced between the two groups, and overall efficacy was not statistically different. No clinically significant renal, hepatic, cardiac (QTc), bone marrow, or CNS toxicities were noted with TGC.

**Conclusion:** TGC appears to be safe and tolerable for patients with cIAI and cSSSI. N/V were generally mild to moderate in severity, self-limiting, and did not result in increased overall drug discontinuation. There did not appear to be clinically significant renal, hepatic, cardiac, bone marrow, or neurological toxicities related to TGC treatment. All-cause mortality rates did not statistically differ between those treated with TGC and the comparators. Its demonstrated efficacy and favourable toxicity profile make TGC a good monotherapy option for selected serious infections.

## P1787

## Tigecycline as effective as imipenem/cilastatin in the treatment of complicated intra-abdominal infections: experience in India

M.K. Ramesh, R.A. Sastry, A. Rekha, A.C. Ashok, P. Dhar, E.J. Ellis-Grosse *on behalf of Tigecycline 301 Study Group*

**Objective:** Due to diverse bacteriology and bacterial resistance, treatment of complicated intra-abdominal infections (cIAI) is a challenge. In a double-blind, phase 3, multinational trial, the efficacy of tigecycline, a first-in-class glycylcycline, was compared with imipenem/cilastatin (IMI/CIS) in hospitalised patients with cIAI. This subanalysis evaluated tigecycline safety and efficacy from 12 investigational sites in India.

**Methods:** Patients were stratified by disease severity (APACHE II score ≤15 vs >15 but <31), and randomly assigned to IV

tigecycline (100 mg loading, 50 mg q12h) or IV IMI/CIS adjusted for body weight (500/500 mg q6h for ≥70 kg) for 5–14 days. Clinical response at test-of-cure (TOC, 12–44 days after therapy) for microbiological evaluable (ME) and microbiological modified intent-to-treat (m-mITT) populations were co-primary efficacy endpoints where cure/failure responses were determined. Safety evaluations included vital signs, laboratory tests and record of adverse events (AEs).

**Results:** In India, 167 patients received at least 1 dose (mITT, 84 tigecycline, 83 IMI/CIS), 149 patients were clinically evaluable (CE), 85 were ME, 120 were m-mITT. Treatment groups were balanced with respect to demographic/baseline medical characteristics. Primary diagnoses (mITT) were complicated appendicitis (31%), gastric/duodenal perforation (26%), perforation of intestine (18%), cholecystitis (12%), peritonitis (6%), and intraabdominal abscess (5%). Cure rates at TOC in ME in India were 36/41 (87.8%) tigecycline and 40/44 (90.9%) IMI/CIS, which are consistent with overall ME results [80.6% (199/247) tigecycline vs 82.4% (210/255) IMI/CIS (95% CI = −8.4, 5.1; non-inferiority $p < 0.001$)]. In India m-mITT, cure rates at TOC were 47/59 (79.7%) tigecycline and 54/61 (88.5%) IMI/CIS, similar to the overall m-mITT results [73.5% (227/309) tigecycline vs 78.2% (244/312) IMI/CIS (95% CI = −11.0, 2.5; non-inferiority $p < 0.001$)]. Noninferiority of tigecycline among India patients could not be statistically demonstrated because of insufficient sample sizes, however, magnitude of response to study drugs in patients treated in India was comparable to that in overall patients. In India, treatment AEs were similar with significantly higher incidence of dyspnoea in tigecycline (9.5%) vs IMI/CIS (0.0%), $p = 0.007$.

**Conclusions:** Efficacy results in India are consistent with findings from the overall study and results at other centres, suggesting tigecycline is noninferior to comparator in treating cIAI.

# Nosocomial infection: control of environment, viral infections

## P1788

### Bacterial flora contamination of blood pressure cuffs in use on hospital wards

N. Walker, R. Gupta, J. Cheesbrough *(Preston, UK)*

Blood pressure cuffs are a plausbile vehicle for the transmission of nosocomial infection between patients. Despite this, few studies have examined the level of bacterial contamination and tested for the presence of common nosocomial pathogens on their surface. We swabbed 24 cuffs currently in use on hospital wards. Using sterile gloves, a disposable template measuring 10 × 10 cms was placed onto the cuff and a moistened sterile swab was rubbed onto the defined area for 1 minute and then transported in 10 mls of buffer medium. From each sample, 0.5 mls of the buffer was plated onto 6 different media which included a non-selective agar medium for total viable count (TVC) and selective media for *S. aureus*, MRSA, *C. difficile*, coliforms and Vancomycin resistant enterococci (VRE.) Bacterial growth was recovered from all 24 cuffs. Pathogenic organisms were isolated from 14 cuffs (58%). MSSA from 5, MRSA from 1 and *C. difficile* from 5. The remaining three cuffs grew more than one pathogenic organism; MSSA + MRSA + *C. difficile* from one and MSSA + *C. difficile* from 2 cuffs. Colifroms and VRE were not isolated from any of the cuffs. The range of total viable counts recovered per 100 $cm^2$ area of the cuff varied from 1000 > 20000 cfu and the cuffs with the highest counts tended to have more pathogens present. MSSA and *C. difficile* were isolated from 33% of the cuffs sampled and MRSA from 8%. While the actual importance of this potential route of transmission for nosocomial pathogens remains unclear, it can not be dismissed. The impracticality of decontaminating blood pressure cuffs between patients suggests that single patient use cuffs or a barrier between cuff and skin would be a more viable option on a busy general ward.

## P1789

### Needlestick and sharp injuries of health care personnel in a newly founded tertiary hospital: a prospective study

M. Falagas, I. Karydis, G. Georgoulias, P. Hatzopoulou, D. Nikita, I. Kostogiannou *(Athens, GR)*

**Objectives:** Needlestick and sharp injuries of health care workers are a major cause of anxiety and may expose susceptible employees to the risk of infectious diseases. However, the incidence of such injuries has not been examined in a newly founded hospital while preventive programmes are taking place.

**Methods:** We prospectively studied the needlestick and sharp injuries of employees in a newly founded tertiary hospital in Athens, Greece while a vaccination program against hepatitis B virus as well as educational activities for avoidance of injuries were taking place. Serologic studies for hepatitis B and C virus as well as human immunodeficiency virus (HIV) were performed in all injured employees and the source patients (when known).

**Results:** Sixty-eight needlestick, 8 sharp injuries, and 3 splashes were reported during the study period (01/10/2002 to 28/02/2005) in 71 nurses, 2 housekeepers, 3 technicians, and 3 ambulance workers. The overall incidence (percutaneous injuries and splashes) per 100full-time employment-years (100 FTEYs) was 2.4% whereas the incidence of percutaneous injuries alone per 100 FTEYs was 2.3%. A higher incidence of injuries was noted during the first than the second half of the study period (3.8% versus 1.8%, $p = 0.003$). No source patient was found positive for hepatitis C or HIV. The use of high-titre immunoglobulin after adjustment for the incidence of injuries was higher in the first than the second half of the study period (22.6% vs 3.8%, $p = 0.05$).

**Conclusion:** Although we did not adjust for possible confounders, our data show that educational and vaccination preventive programs for needlestick and sharp injuries led to a statistically significant decrease in the incidence of such injuries and use of high-titre immunoglobulin.

## P1790

### Epidemiology of occupational needlestick and sharps injury among healthcare-workers in Turkey

S. Hosoglu *on behalf of the Occupational Infections study group, Turkey*

**Background:** Health care workers (HCWs) are frequently exposed to the danger of infectious agents through needle stick and sharps injury (NSSI) in their occupational efforts. In Turkey, the hepatitis B and C viruses cause an essential threat to the HCWs because of their prevalence rate (2%–4% and 0.5%–1%, respectively).

**Method:** A cross-sectional countrywide survey study was performed on the epidemiology of NSSI among HCWs at 30 hospitals in 19 cities throughout the country. Data relating to the epidemiology of NSSIs were collected using a standard questionnaire in 2004.
**Results:** Totally 5048 HCWs completed the questionnaire forms. Nurses are the leading group (2016 persons) that joined into the study were followed by doctors (1452 persons) andlaboratory technicians (475). Totally 2498 of them (49.5%) declared an occupational exposure or NSSI in the last 12 months related their job. Needle stick injury was reported in 1698 of them (33.6%), splash into the eye in 1132 (22.4%), sharp injury in 737 (14.6%), and the other injuries in 419 (7.0%). The hepatitis positivity was reported in 567 cases (2.27%) in source patients in those exposures. The most frequent exposures were reported among the nurses (58.2%) and doctors (53.4%). Only 1381 of the HCWs (27.4) had specific education for prevention occupational exposures and NSSIs.
**Conclusion:** This survey study showed risky practices and demonstrated employees and locations frequently involved in NSSIs. An education program is needed for all staff at risk of exposure, targeting higher-risk employees.

## P1791

## Microbiological approach to reprocessing of single-use devices for interventional cardiology

I. Caola, F. Tessarolo, M. Disertori, G.M. Guarrera, G. Nollo, P. Caciagli *(Trento, IT)*

**Objectives:** To assess the microbiological status of reprocessed single-use devices for interventional cardiology by testing bioburden, sterility and pyrogenic load.
**Methods:** A total amount of 154 electrophysiology non-lumen catheters (EP) were collected after the first clinical use on patient. 20 devices were contaminated with bacteria spiked human blood and underwent four different pre-sterilization protocols including chlorine, polyphenol, and enzymatic agents. Treated samples were assayed by cultural quantitative methods (CQM) for bactericidal properties and electron micr-oscopy (EM) for biologic residuals. 73 EP were tested for sterility. By the repetition of simulated-use (bacteria spiked blood) and regeneration (enzymatic and chlorine treatment, gas plasma sterilization) we obtained 54, 39, 26, 28, 36, 22 samples respectively reprocessed 1, 2, 3, 4, 5, 6 times. Devices were cultured for 28 days in trypticase soy broth. The pyrogenic status of 61 EP was monitored after clinical use, after decontamination-cleaning treatments and after complete reprocessing by LAL test.
**Results:** High-resolution EM and CQM confirmed the superior properties of chlorine releasing agent added to enzymatic detergent for devices treatment before sterilization. Hypochlorous acid based protocols were more biocide (>3.1 log CFU reduction) than polyphenolic (3.2-1.9 log CFU reduction). Sterility tests showed no positive sample to inoculated strain until the fourth cycle of reprocessing. Catheters showed the growth of the inoculated strain, *Bacillus subtilis* in 1/35 and 1/22 samples after five cycles and six cycles respectively. Every reprocessed device was non-pyrogenic (<20 EU/catheter). In addition, tests conducted on *in-vitro* spiked catheters showed that pyrogenic loads of 200 EU/device were reduced to less than 11 EU/device.
**Conclusions:** Reprocessing procedures following the adopted regeneration protocol were able to satisfy the fundamental microbiological requirements until five in-vitro reuses. Sterility tests showed that devices' sterility was not guaranteed after five reuses. Pre-sterilization treatments including enzymatic solutions and chlorine revealed high cleaning properties with effective bioburden reduction. Storage intervals among reprocessing steps longer than 24 hours should be avoided in order to limit contamination and pyrogenic load. Technical considerations suggest to consider the introduction of reprocessing procedure only in hospitals with a considerable workload.

## P1792

## Room disinfection in the hospital setting using Akacid plus®

C. Kratzer, S. Tobudic, W. Graninger, A. Buxbaum, A. Georgopoulos *(Vienna, AT)*

**Objectives:** Akacid plus®, a novel polymeric guanidine with broad antimicrobial activity also against multi-resistant bacterial strains, was used in the present study as room disinfectant.
**Methods:** Disinfection of closed rooms experimentally contaminated with antibiotic-susceptible and multi-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Escherichia coli* was performed using Akacid plus® at concentrations of 0.1%, 0.25% and 0.5% for 100 minutes. Bacterial suspensions were distributed on stainless steel plates and placed in a test and control room. Recovery of the test bacteria was determinedbefore nebulizing, 60 and 100 minutes after the beginning and 4 hours after the end of room disinfection by a modified simple swab-rinse technique. For the detection of MRSA in isolation units, surface samples were collected by direct swab and enrichment culture.
**Results:** The swab-rinse method demonstrated a dose- and time-dependent effectiveness of Akacid plus® in eradicating *S. aureus*, *E. coli* and *P. aeruginosa* on stainless steel plates. Nebulizing of 0.5% Akacid plus was successful in eliminating all hospital pathogens in 340 min contact time, while MRSA was still detectable after use of 0.25% Akacid plus®. 0.1% Akacid plus® achieved a reduction >100 CFU of *S. aureus* and *P. aeruginosa*, but was only able to eradicate *E. coli* during the observation time.
**Conclusion:** The results suggest that nebulized Akacid plus® at a concentration of 0.5% is a potent substance for eradication of pathogenic organisms in the hospital setting.

## P1793

## Study on the antiviral efficacy of Citrofresh®, a flavonoid based organic acid complex sanitizer

Z. Nack *(North-Geelong, AU)*

**Objective:** Determine the antiviral efficacy of this organic sanitizer against enveloped and non-enveloped viruses using a carrier based method. Seeking registration for Citrofresh® in Australia and in the EU as a Hospital Grade antiviral sanitizer.
**Methods:** The study was performed according to the American Society of Testing and Materials (ASTM) Designation (E 1053–85) recommended by the Australian Therapeutic Goods Administration (TGA) to determine the efficacy of a disinfectant intended to use on inanimate, environmental surfaces. We tested Citrofresh® (diluted in standard hard water) in three different concentrations: 1%, 2% and 4% on adherent cell lines (PK-15, MRC-5, MDCK, A549, L929) in four replicates against five different viruses including: Porcine Parvovirus (non-enveloped, high resistant against sanitizer); Human Rhinovirus-16 (non-enveloped, high resistant against sanitizer); Human Adenovirus-4 (non-enveloped, moderate

resistant against sanitizer); Human Influenza Type A (H3N2) virus (enveloped, moderate resistant against sanitizer); Human Herpes simplex virus Type 1 (enveloped, low resistant against sanitizers). Prior to the viral testings, acute toxicity assay was carried out to determine the adherent cells viability against Citrofresh®.

**Results:** Cell lines exhibited >80% viability after exposure to all three concentration. Herpes simplex Type 1, Human Influenza Type A and Human Adenovirus-4 exhibited the most significant viral log reduction of log10 4 to 5 at 4% concentration of Citrofresh® followed by the Human Rhinovirus-16 and Porcine Parvovirus log10 4 reduction at 4% concentration. The reduction of viable virus load was exhibited after 1 minute exposure time to Citrofresh®, which means no time-dependant activity. Citrofresh® clearly exhibited concentration and pH dependent viral load reduction activity against Influenza Type A and the Human Adenovirus -4 and Human Herpes simplex Type 1 virus. The reduction in viral titre for Porcine Parvovirus and Human Rhinovirus-16 is probably pH dependent (the pH of 1% Citrofresh® is 6.5, 2% is 4.5 and 4% is 3.5).

**Conclusion:** Our investigation shows that Citrofresh® is an effective disinfectant on environmental surfaces, eliminating enveloped and non-enveloped viruses and sufficient to achieve the minimum 4-log reduction with complete viral inactivation which is prerequisite for registration.

## P1794

## Rapid environmental recontamination of an intensive care unit after decontamination with hydrogen peroxide vapour

K.J. Hardy, S. Gossain, P.M. Hawkey, B.A. Oppenheim, N. Henderson, F. Gao *(Birmingham, UK)*

**Objectives:** To evaluate the effectiveness of hydrogen peroxide vapour (HPV) to reduce the levels of total bacterial and methicillin resistant *Staphylococcus aureus* (MRSA) environmental contamination on an Intensive Care Unit (ICU), and to establish the rate of environmental recontamination.

**Methods:** The study took place on a 9 bed open plan ICU. On each environmental screen 3 sites in each bed space (under the bed, the workstation and the monitor) were examined using broth enrichment for the detection of MRSA. In addition total bacterial counts were determined for under the bed and workstation using RODAC plates. Environmental screening was carried out monthly for the 3 months preceding the usage of HPV, increasing to weekly for the 4 weeks prior to usage. Additional sampling was carried out immediately before patients were discharged from ICU, following the subsequent terminal clean and then immediately after HPV use. After readmission of patients sampling was carried out at 24 h, 48 h and then weekly for a period of 8 weeks. Patients were screened for MRSA on admission and then weekly.

**Results:** Sampling of the environment prior to the usage of HPV revealed contamination of the environment with MRSA on 6/7 occasions, with MRSA colonised patients being present on only 3/7 occasions. After discharge of the patients and terminal cleaning of the environment, MRSA was isolated from 5 (13%) environmental sites. After the use of HPV, MRSA was not isolated from any environmental sites upon immediate sampling, but 24 h after patients were readmitted, including 2 patients known to be colonised with MRSA, MRSA was isolated from 5 sites. These sites were not clustered around the colonised patients but were widespread across the ICU. In the 8 weeks post HPV usage MRSA has been isolated every week. The mean total bacterial counts prior to the use of HPV were 22.0/10 $cm^2$ underneath the beds and 3.8/10 $cm^2$ on the workstations, this was reduced after HPV to 0.1/10 $cm^2$ and 0.2/10 $cm^2$ respectively. After patients readmission the counts were 4.1/10 $cm^2$ underneath the beds and 1.2/10 $cm^2$ on the workstations after 48 h and returned to pre-HPV levels of 16.9/10 $cm^2$ and 4.8/10 $cm^2$ at each site respectively after 1 week.

**Conclusion:** Hydrogen peroxide vapour is effective in eliminating bacteria from the environment. The rapid rate of recontamination of the environment suggests that the use of HPV is not an effective means of maintaining low levels of environmental contamination on an open plan ICU.

## P1795

## Survival of some nosocomial infections agents on the surface of different covering materials

H. Yazgi, M.H. Uyanik, M. Ertek, A.E. Aktas, H. Igan, A. Ayyildiz *(Erzurum, TR)*

**Objectives:** The nosocomial infections are more serious and dangerous than community acquired infections since they have high rate of morbidity and mortality as well as they increase the cost of therapy. Recently many precautions have been taken to prevent these infections. One of these applications is that covering of the floor of the wards, clinics, intensive care units and operating rooms of the hospitals with vinyl flooring material, which is believed to be cleaned easily and effectively. In this study it was aimed to determine the duration of survive of the *Staphylococcus aureus*, *Enterococcus feacalis*, *Escherichia coli* and *Pseudomonas aeruginosa*, which were most common encountered as nosocomial infection agents, on the surface of flooring materials such as vinyl flooring, ceramic laminated wood and galvanized sheet at room temperature.

**Methods:** Four kinds of flooring materials were prepared approximately in 4–6 $cm^2$ coupons and sterilized. Separate bacterial suspensions equal to Mc Farland 1 turbidity were swapped to the surface of each flooring materials by sterile cotton swabs. All contaminated test materials were put in sterile petri dishes with cover and kept at room temperature without subjecting to the direct sunlight. On the third day, culture samples were taken from the surface of each material by sterile cotton swaps soaked with sterile saline and streaked on the blood agar surface. Culturing procedure was repeated every other day until no growth detected. In case of three consequently, negative culture results obtained culturing was ended.

**Results:** Overall results of the study were presented on table 1.

Table 1. Survival days of the bacteriae on the surface of covering and flooring materials

| | S. aureus | VRE. faecalis | P. aeruginosa | E. coli |
|---|---|---|---|---|
| Vinyl flooring | 55 | 47 | 11 | 5 |
| Galvanised sheet. | 89 | 43 | 7 | 5 |
| Ceramic | 75 | 69 | 31 | 7 |
| .Laminated wood | 75 | 65 | 25 | 7 |

**Conclusions:** Among the four flooring materials, galvanised sheet seemed to be the most unsuitable one for the bacteria to survive long period. In other words this material should be preferred as to laminated wood for covering benches and laboratory tables. As for the flooring of the floors the vinyl flooring material is better than ceramic.

## P1796

### Evaluation of infection control practices in 31 haematopoietic stem-cell transplant facilities in German-speaking countries: variation of measures reflects lacking evidence

S. Wenzler-Röttele, A. Conrad, W.V. Kern, H. Bertz, F.D. Daschner, M. Dettenkofer *(Freiburg, DE)*

**Objective:** Haematopoietic stem cell transplant (HSCT) recipients are highly immunocompromised during pre- and postengraftment. Thus, they are cared for in specialised facilities and versatile precautions are practised in order to prevent nosocomial infections. However, there is a lack of evidence whether these interventions are effective. Furthermore, most of the measures are cost-intensive and restrict the patients' comfort. For evaluation of precautions, a survey was performed to assess the spectrum of measures commonly practised.
**Methods:** A questionnaire was compiled asking in detail for infection control measures differing according to allogeneic and autologous HSCT recipients. The questionnaire was sent to 61 HSCT facilities in Germany, Austria and Switzerland.
**Results:** 31 questionnaires (51%) were filled in and sent back. Among the 31 centres, 24 were university hospitals, and 4 teaching hospitals. The overall number of transplantations that were performed by the facilities varied considerably and ranged from 3 to 120/y for auto HSCTs and from 3 to 110/y for allo HSCTs. 80% of the institutions performing allo and auto HSCT have implemented different precaution standards for each group. Some measures regarding allo HSCT were routinely adhered to in practically all institutions: accommodation in single rooms (96%), interdiction of plants and opening of windows (100% each) and protection from waterborne bacteria by use of terminal tap water filters (92%). 83% of HSCT facilities perform their allo transplantations in HEPA-filtered rooms and 54% are providing laminar air-flow for this population. There was a broad spectrum of different measures regarding barrier precautions: gowns when entering the room (required in 43% of centres for allo and 24% for auto HSCT) and face masks (83% allo and 66% auto HSCT). Precautions to be followed by the patient varied among centres, e.g. specification of the face mask/respirator to be worn outside the isolation room (for allo HSCT: 41% surgical mask, 5% FFP1, 36% FFP2 and 18% FFP3).
**Conclusion:** The broad variety of different preventive measures performed by the different facilities reflects lacking evidence for many infection control precautions that are commonly practised in the care of HSCT recipients. This survey provides the basis for further studies within the ONKO-KISS project (Hospital Infection Surveillance System for Patients with Haematologic/Oncologic Malignancies).

## P1797

### Microbiological analysis of saline solutions used in wound cleansing

C. Nogueira, M. Alves, A. Magalhães-Sant'Ana, C. Miguéns, J. Gouveia *(Coimbra, Pampilhosa da Serra, PT)*

**Objectives:** In this study it was our aim to evaluate the microbiological contamination of physiological serum flasks in use in medical day center for wound cleaning and to identify the isolated microorganisms.
**Methods:** We have collected 44 saline solutions from 22 health care centres localized in the health sub-region of Coimbra. From each centre we have recovered 2 aleatory flasks in current use. The samples were transported at 4ordm;C and maintained at this temperature until its processing. Saline solutions were seeded by the pour-plate technique in plate count agar and plates incubated at 28 and 37ordm;C for 48 h. The saline solutions were evenly spread over the surface of Blood Agar and Sabouraud Chloramphenicol Agar (SAB CHL-D). The transfers of saline solutions flasks were also tested for microbiological contamination with a sterile cotton swab that was rubbed vigorously, over the transfer surface and directly applied on Blood agar media. Blood Agar plates were incubated at 37° C for 48 h and SAB CHL-D plates were incubated at 28° C and 35° C and examined daily for a period of 30 days before declared as culture negative. Microbial identification was firstly accomplished by employing conventional morphological and biochemical tests. When identification was not possible by these methods, 16S rRNA gene sequence determination and phylogenetic analysis were used for bacterial strains and in the case of moulds we performed the amplification and sequencing of Internal Transcriber Spacers region of 5.8S gene.
**Results:** From the 44 saline solutions analysed, 54.5% were contaminated. A total of 38 strains were isolated, 66% could be identified to species level using morphological and biochemical tests, the remaining 34% were identified by gene amplification and sequencing. About 69.6% of the identified strains were Gram-positive cocci, the second dominant type of strain were Gram-positive bacilli (13%), and the third dominant type of strains were Gram-negative bacilli and moulds, both with 8.7%. The most frequent contaminants belong to human normal flora (64%), supporting the idea that the source of contamination of saline solutions analysed was human, in contrast with 36% of contamination due to the environment.
**Conclusions:** The contamination of the saline solutions is due to inadequate clinical practices. These results claim for more strict hygienic measures and for the replacement of big flasks by single use flasks with an incorporated overture used for wound irrigation.

## P1798

### Frequencies of CMV-IE specific memory T cells are inversely correlated with alloimmune memory and serum creatinine in kidney transplant patients

P. Nickel, G. Bold, F. Presber, C. Schönemann, J. Pratschke, D. Bitti, F. Kern, H.-D. Volk, P. Reinke *(Berlin, DE)*

**Background/Aims:** Cytomegalovirus infection is a significant cause of morbidity in transplant patients and has been associated with allograft rejection. In this study frequencies of IFNg-producing T cells following *ex-vivo* stimulation with protein-spanning peptide pools for CMV proteins pp65 and IE1 as well as donor-reactive T cell frequencies were serially determined during the first 6 months after renal transplantation (Tx) to analyse the relation of CMV specific T cells, virus control and alloimmunity.
**Patients:** 64 kidney transplant recipients were included. Immunosuppression generally consisted of anti-IL-2R mAb, calcineurin inhibitor, MMF and steroids. 2 presensitized patients received an induction by 2x low dose OKT-3, anti-TNF mAb, anti-CD20 mAb and 5x plasmapheresis. 8 Patients received FTY-720, cyclosporine and steroids.
**Methods:** PBMCs from renal transplant recipients were analysed in a computer-assisted ELISPOT assay before and at multiple times (mean 5) post-transplantationfor IFN-y-producing T cells following *in-vitro* stimulation for 24 hrs by irradiated donor cells and pools of overlapping peptides

covering the complete CMV IE-1 and pp65 proteins.Results: CMV seropositive transplant recipients had significantly hightened IE-1 and pp65 specific T cell frequencies compared to seronegative individuals. Patients withevidence of CMV antigenemia or DNAemia could not be discriminated based on CMV- and donor-reactive T cells or serum creatinine. However, recipients of seropositive grafts with low IE1 response showed a tendency towards more frequent CMV infection. CMV disease was observed in only 3/64 individuals. 2 had no detectable IE1- or pp65-T cell response, the third presented with a dominant pp65 response. Interestingly, IE1-specific T cells correlated inversely with early post-Tx donor-reactive T cell frequencies during weeks 1–2 post-Tx. Most importantly, IE1-specific T cell frequencies correlated inversely with serum creatinine at 6 and 12 months at several times post-Tx. In patients without acute rejection, even pre-transplant IE-1 specific T cells correlated inversely with 6 and 12 months creatinine.
**Conclusion:** These data suggest subclinical control of CMV infection by IE-1 specific T cells and subsequently less graft injury by (CMV-induced) alloimmunity.

## P1799

## Universal precautions: knowledge, attitude and practice of healthcare workers regarding HIV, hepatitis B and C

V. Gupta, S. Bhoi, A. Goel, P. Aggarwal *(New Delhi, IN)*

**Objectives:** Increasing incidence of HIV, Hepatitis B (HBV) and Hepatitis C (HCV) in the patients expose the healthcare professionals of acquiring these infections during occupational exposure. We studied the knowledge, attitudes and practices of healthcare workers regarding HIV, HBV, HCV and the risk of occupational transmission of these diseases.
**Methods:** An interview survey was conducted among all the health care workers (HCW) using a standardised questionnaire comprising of 34 items in English and local language, as suitable, by an expert in the Emergency Ward of a tertiary care teaching hospital of a developing nation. Data analysis (Bivariate and multivariate analysis) was done using SPSS version 10.
**Results:** 170 (response rate: 80%) HCW participated in the study. The mean age was 33 ± 6 years, 54 were females. The study population comprised of 40% doctors, 23% nurses, 25% lab technicians and 12% support staff. Respondents had adequate knowledge about causative (65%) usual transmission (63%), symptoms (69%) of AIDS but poor knowledge about HBV and HCV (43%, 41% and 30% respectively). Inadequate knowledge was also revealed about the infectious bodyfluids (31%), disinfection of equipments (32%), pregnancy in HCW as a susceptibility factor (22%), post exposure prophylaxis (34%) and comparative infectivity of HIV and Hepatitis (38%). 70% of HCW became anxious while treating these patients. Poor compliance with Universal Precautions was noticed. High compliance was reported for wearing masks (72%) and wearing gloves (53%). Doctors were more likely to suffer needlestick injury ($p = 0.04$) Occupational exposures was found to be high (48%) with poor declaration rate (10%). Guidelines adherence was influenced by profession ($p < 0.001$), availability or adequacy of protective equipments but not by work experience as HCW ($p = 0.8$). All of the respondents urged for an interactive information session.
**Conclusions:** Results from this study reveal that there is a fair level of knowledge about HIV/AIDS but Hepatitis B and C have not generated adequate concern among the HCW. Incongruity between perceived knowledge and reported practice suggests that there is a need for an interactive awareness course about the universal precautions. The educational programmes need to consider attitudes in conjunction with empirical knowledge.

## P1800

## Molecular characterisation of acute hepatitis A outbreaks in health care workers

H. Choi, S.Y. Jeong, S. Lee, M.A. Lee *(Seoul, KR)*

**Objectives:** The sero-prevalence of hepatitis A (HAV) antibodies are known to be low in young adults in Korea. Recently, seventeen cases of hepatitis A have been reported in health-care workers (HCW) of ICU in a University hospital from May 2005 to July 2005. We performed surveillance, and determined molecular identification of outbreaks.
**Methods:** 1. We checked the HAV IgM from all the patients of SICU with elevated AST/ALT retrospectively and screened AST/ALT level from all the nurses and the doctors in contact with suspicious index case. 2. When we determined the existence of outbreak, the molecular subtypes of HAV from a blood of HCW were determined to provide the data for epidemiologic study. We determined the index case, a transmission route and the intervention for control an outbreak were planned.
**Results:** 1. Seventeen HCW including 13 nurses and 4 doctors who are 22 to 32 years old, suffered from acute HAV over 7 weeks period. 2. The possible transmission of HAV was fecal-oral route from the bed-ridden patients with diarrhea to the exposed HCW. 3. Seventeen HCW were identified with a positive anti-HAV IgM. The eight HCW had a positive HAV RNA. Analysis of the VP1-P2A region of each isolate showed genotype 1A in five strains and co-circulation of 1A and 1B in others.
**Conclusions:** The occurrence of HAV outbreak highlights the importance of standard precaution in a hospital. The HAV vaccination is considered in young aged-HCW. The genotype identification of blood would be useful for the epidemiologic study of suspicious HAV outbreak in a hospital.

## P1801

## Management of a norovirus-associated gastroenteritis outbreak on two psychiatric wards

A. Buehling, U. Arnold *(Magdeburg, DE)*

**Objectives:** We report a norovirus-associated outbreak of gastroenteritis on a closed psychiatric and a gerontopsychiatric wards from December 2004 to February 2005. During this time 46 patients and 25 healthcare workers (HCWs) were affected. Introduction and results of hygiene measures based on published guidelines on psychiatric wards are described.
**Methods:** Effective and adapted measures had to be implemented to stop the outbreak and to prevent the spread of disease to other areas of our hospital. Isolation or cohorting of the psychiatric patients was excluded for therapeutic reasons. Regular hand disinfection in patient rooms was impossible because of the high risk of abuse. The following measures have been introduced: use of gowns, masks and gloves by HCWs during care of infected patients- frequent hand disinfections with alcohol-based disinfectants by HCWs using ''pocket bottles''; recommendation for all persons entering the station to use gowns, gloves and masks and to disinfect their hands frequently, distribution of handouts describing the measures; hand disinfection by all patients after using toilet, before and after taking meals (distribution of disinfectants by HCW);

increased frequency of routine surface disinfection (3 times daily) instead of routine cleaning once daily; routine disinfection of door handles, handrails, wash-basins and –fittings and light switches 3–4 times a shift; avoidance of patient transfer via hospital; visitor restriction during outbreak time; daily evaluation of recommended measures and adaptation to the current situation; exclusion of affected staff from the ward until 48 h symptom free.
**Results:** The hygienic measures have been explained to the local HCWs in daily meetings. They have been fully accepted only after a severe staff shortage in the fifth week of outbreak because of 3 new cases of gastroenteritis during HCWs and 14 newly infected patients. Because of the restrictive application of the adapted guidelines for these special wards the outbreak has been stopped within 4 further weeks.
**Conclusion:** In case of norovirus-based gastroenteritis outbreaks on closed psychiatric wards hygienic measures which are adapted to the concrete situation are necessary. Especially in these cases the compliance with guidelines can be increased by daily meetings and daily evaluation of recommendations. Staff shortage during the outbreak forced the strict compliance with the recommended measures.

# Regional spread of antibiotic resistance

## P1802

### Nosocomial colonisation and infection of ICU patients caused by persistent and multiresistant clones of *Acinetobacter baumannii*

E. Vagiakou, A. Karabinis, D. Mylona-Petropoulou, E. Paramythiotou, P. Tassios, L. Tzouvelekis, C. Mergioti, G. Diamantopoulou, N. Legakis, H. Malamou-Lada *(Athens, GR)*

**Objectives:** To evaluate the nosocomial colonization and infection of ICU patients caused byA. baumannii (Ab) and to investigate the persistent clones over a 2 year period (May 2002-April 2004).
**Methods:** We performed surveillance of patients, healthcare stuff and ICU environment and we registered the infections of Ab during 3 periods of 21 days each one. The interval between 1st–2nd period was 6 months and 2nd–3rd period was 1 year. Rectal, oropharyngeal swabs tracheal aspirates from patients, handswabs from stuff and samples from environment were taken weekly. The identification of Ab was performed using Vitek II system the susceptibility was tested by Kirby-Bauer and MIC methods and the <<DNA fingerprints>>obtained by pulsed field gel electrophoresis (PFGE).
**Results:** During the 1st 2nd and 3rd period, 19 patients (14 men, 5 women), 13 patients (7 men, 6 women) and 12 patients (8 men, 4 women) were hospitalized in ICU respectively. Ab was isolated in 74 from 250 samples (29.6%) at the 1st period, 59 from 336 (17.5%) at the 2nd and 62 from 260 (24%) at the 3rd period. Totally Ab was isolated in 195 from 846 specimens (23%) At the 1st 2nd and 3rd period among the patients carrying Ab, 7/14 (50%), 7/10 (70%) and 5/8 (62%) were infected respectively. The infections observed during the study period were: sepsis (8), urinary tract infection (1), pneumonia (11), meningitis (1), thrombophlebitis (2). All the isolated Ab strains were multiresistant to antimicrobial agents. Molecular analysis of 169 isolated strains by PFGE distinguished the following types: A (64, subtypes A1–A7), B (10) at the 1st period A(6), C(7), D(22), E(2), F(1), G(1), H(2), I(1). J(1) at the 2nd period A(25), B (16), D(1), H(3), K(2). L(5) at the 3rd period. Infections were caused mainly by A and D types while the same types were isolated from the environment and the hands of the ICU stuff.
**Conclusion:** There was a high rate of colonization and infection of ICU patients by multiresistant clones of Ab. The persistence of clone A of *A. baumannii* and the appearance of B type at the 3rd period after its disappearance at the 2nd period despite the application hygiene measures, indicates the need for more strict reinforced infection control in ICU. The transmission via the hands of stuff to patients has become the most important contributor factor in patient colonization and/or infection.

## P1803

### Decrease of antibiotic resistance of group A streptococci in Korea

S. Kim, E.-H. Koh, I.-G. Bae, N.Y. Lee *(Jinju, KR)*

**Objectives:** The antibiotic resistance and its mechanism of group A streptococci (GAS) varies according to nations or study period. We have investigated antibiotic resistance and mechanism of macrolide resistance for the strains isolated from Korean children and compared to the previous (2002) results.
**Methods:** Throat cultures were taken from 2351 elementary school children in Jinju, Korea from October to December, 2004 to isolate GAS. Antibiotic susceptibility test to erythromycin (EM), clindamycin (CC), and tetracycline (TC) was performed by disk diffusion method. Macrolide resistance phenotype and genotype as well as emm genotype were studied.
**Results:** Isolation rate of GAS was 14.0% (328/2351). Resistance rates of EM, CC, and TC were 9.8%, 8.8%, and 18.3% respectively, which were dramatically decreased from 51%, 34%, and 30% in 2002 at the same area. Emm44/61 was prevalent (29%), while emm12 was the most common type (34%) in 2002. cMLSB, M, and iMLSB were observed in 87.5%, 9.4%, and 3.1% respectively, compared to 64%, 34%, and 2% in 2002. The strains with cMLSB and iMLSB had ermB gene and the ones with M phenotype were positive with mefA gene.
**Conclusion:** The resistance rates to EM and CC were dramatically decreased compared to the past (2002). Education to the public and physicians, decreased consumption of antibiotics, acquisition of immunity to the resistant strains, or change of prevalent emm types could be considered to explain the reason of decrease of antibiotic resistance. Although antibiotic resistance rate was decreased, cMLSB type which has high MIC was prevalent suggesting treatment failure for those children carrying these resistant strains in Jinju, Korea.

## P1804

### Analysis of skin and soft tissue infections in European medical centres: report from the SENTRY Antimicrobial Surveillance Program (1998–2005)

G. Moet, P. Strabala, H. Sader, T. Fritsche, R. Jones *(North Liberty, US)*

**Objective:** To analyse the skin and soft tissue infections (SSTI) or wound infections in hospitalized patients in the SENTRY Program for pathogen prevalence and resistance (R) variations

in European (EU) medical centres for the years 1998 to 2005 (8 years). This program also included North America (NA) and Latin America (LA) for the same years, except 2003.
**Methods:** 50 consecutively isolated pathogens/site were collected from each centre per year and varied in number of sites each year in EU: 1998 (23), 1999 (6), 2000 (16), 2002 (3), 2003 (25), 2004 (23), and 2005 (21). Susceptibility testing was determined by CLSI (formerly the NCCLS) broth microdilution methods and interpreted by current (2005) breakpoints.
**Results:** Table of all years total of SSTI pathogens. (See Table) SA was the predominant pathogen in EU ranging from 35.7% of SSTI isolates in 1998 to 47.5% in 2005. The top 5 most prevalent organisms accounted for 80.0% of isolates in all years with PSA and EC ranking second and third, respectively with 22.5% combined, and ENC and ENT ranked fourth and fifth with 10.7% of the total isolates. Compared to the Americas, MRSA and VRE isolation was at a lower occurrence rate in the EU; but between the rates of other monitored continents for CTZ-R PSA, CIPRO-R EC and CTZ-R ENT (AmpC). VRE increased in EU over the 8 year.

| Pathogen | No. tested (% of total) | R marker | EU %R | Americas %R (over all range) |
|---|---|---|---|---|
| *S. aureus* (SA) | 2111(38.9) | oxacillin (MRSA) | 23.0 | 35.9 (29.1-38.1) |
| *P. aeruginosa* (PSA) | 632(11.7) | ceftazidime(CTZ) | 17.4 | 18.4 (10.3-34.3) |
| *E. coli* (EC) | 585(10.8) | ciprofloxacin(CIPRO) | 9.2 | 14.6 (8.3-21.7) |
| *Enterococcus* (ENC) | 309(5.7) | vancomycin (VRE) | 3.6 | 10.1 (3.8-12.0) |
| *Enterobacter* (ENT) | 273(5.0) | CTZ | 23.4 | 22.0 (15.9-33.7) |
| All pathogens | 5420(80.0) | - | - | - |

**Conclusions:** Pathogen prevalence in SSTI for EU has been consistent over the 8 monitored years although SA (with MRSA) appears to be increasing. EU is not a world leader in any key R marker compared to the Americas. However, the R rates are evolving which suggests continued need for surveillance programs at regular intervals to detect mobile genetic R elements.

## P1805

## Mechanisms of carbapenem resistance in multiresistant *Pseudomonas aeruginosa* strains from Germany

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

**Objectives:** Carbapenems play an important role in the therapy of *Pseudomonas aeruginosa* infections. The aim of our study was to characterize the molecular alteration responsible for changed susceptibility towards carbapenems in multiresistant *P. aeruginosa* strains from Germany.
**Methods:** 19 multiresistant *P. aeruginosa* strains from 9 cystic fibrosis and 9 non cystic fibrosis patients were collected in 3 German hospitals in 2004. The strains showed reduced susceptibility (intermediate or resistant; DIN guidelines) to imipenem, piperacillin, ciprofloxacin and gentamicin. Clonality was tested using PFGE. A PCR screening for VIM and IMP was carried out. Effluxpump overexpression was detected using an effluxpump inhibitor (EPI) test. OprD and for strains with positive results in the EPI test the effluxpump repressorgenes mexR and nfxB were sequenced.
**Results:** PFGE patterns revealed no clonal relationship among the multiresistant strains. Neither VIM nor IMP was detected. The geno- and phenotypes found are depicted in table 1. Defective oprD genes caused by premature stopcodons or frameshifts were found in 17 strains. Among those 11 had no mutations in mexR or nfxB and showed the highest MICs found ranging from 8 to >32 and 4 to 32 mg/L of imipenem and meropenem, respectively. 3 additionally had defective mexR genes, but intact nfxB genes, 2 also had modifications in mexR and nfxB, and 1 showed only in nfxB additional alterations. For 2 strains no alterations in oprD but in mexR were proven.
**Conclusions:** The predominating mechanism of carbapenem resistance in multiresistant *P. aeruginosa* strains from Germany was the loss of OprD. Accessory overexpression of MexABOprM due to modifications in mexR did not result in significantly elevated MICs of meropenem. Moreover, the additional overexpression of MexCDOprJ did not lower the MIC of imipenem. In 2 strains with modifications only in mexR only elevated MICs of imipenem indicate a reduced expression of OprD accompanied by overexpression of MexEFOprN as conferred by nfxC-type mutants.

**Table1. Geno- and phenotypes of the tested strains**

| genotype | n | MIC (mg/L) | |
|---|---|---|---|
| | | Imipenem | Meropenem |
| ***oprD* defective** | 11 | 8->32 | 4-32 |
| **oprD defective mexR modified** | 3 | 16 | 8-16 |
| **oprD defective mexR and nfxB modified** | 2 | 16 | 4-16 |
| **oprD defective nfxB modified** | 1 | 16 | 16 |
| **mexR modified** | 2 | 4-16 | 2-16 |

## P1806

## Worldwide antimicrobial susceptibility patterns of inducible *Enterobacteriaceae* isolated from intraabdominal infections: results from SMART 2004

F. Baquero, P-R. Hsueh, D.L. Paterson, F. Rossi, V. Satishchandran, T. Snyder, S. Rawlins, G. Gallagher, M.A. Abramson, M.J. DiNubile, J.W. Chow *(Madrid, ES; Taipei, TW; Pittsburgh, PA, US; São Paulo, BR; West Point, PA, US)*

**Objective:** SMART (Study for Monitoring Antimicrobial Resistance Trends) is an ongoing global antimicrobial surveillance program focused on clinical isolates from intraabdominal infections (IAI). The aim of this analysis was to assess antimicrobial susceptibility patterns among inducible *Enterobacteriaceae* from 5 different regions of the world during 2004.
**Methods:** 81 centres in North America (NA), Latin America (LA), Europe (EU), Middle/East/Africa (ME/A), & Asia-Pacific (A/P) tested the *in vitro* activity of 12 antimicrobial agents commonly used to treat IAI against consecutive unique aerobic and facultative Gram-negative bacilli using microdilution techniques according to CLSI guidelines. All *Enterobacter*, *Serratia*, *Citrobacter*, *Providencia* spp., *Morganella morganii*, *Hafnia alvei*, & *Proteus vulgaris* were considered to have inducible beta-lactamases for the purposes of this study.
**Results:** Inducible *Enterobacteriaceae* were recovered from 1075 of the 5569 patients (19%) in the study worldwide, constituting 1104/6156 (18%) of the total isolates. 359 (33%) of the inducible *Enterobacteriaceae* isolates were recovered within 48 hours of hospitalization. *Enterobacter* spp. (49%), *Citrobacter*

spp. (23%), *M. morganii* (10%) & *Serratia* spp. (10%), were the most commonly isolated inducible *Enterobacteriaceae*. The % susceptible isolates are reported below by region:

| | NA | LA | EU | ME/A | A/P |
|---|---|---|---|---|---|
| | N=172 | N=166 | N=477 | N=86 | N=203 |
| Ertapenem | 97 | 95 | 98 | 98 | 99 |
| Imipenem | 99 | 98 | 99 | 100 | 100 |
| Meropenem | 99 | 99 | >99 | 100 | 100 |
| Cefoxitin | 23 | 25 | 30 | 34 | 28 |
| Ceftriaxone | 68 | 63 | 76 | 76 | 58 |
| Ceftazidime | 68 | 61 | 78 | 78 | 54 |
| Cefepime | 86 | 78 | 93 | 88 | 77 |
| Piperacillin-Tazobactam | 77 | 75 | 86 | 91 | 79 |
| Tobramycin | 91 | 74 | 93 | 87 | 75 |
| Amikacin | 99 | 84 | 98 | 99 | 92 |
| Ciprofloxacin | 87 | 73 | 90 | 83 | 78 |
| Levofloxacin | 89 | 79 | 93 | 87 | 85 |

**Conclusion:** Among inducible *Enterobacteriaceae* causing IAI in this study, carbapenems were the most reliably active drugs *in vitro* across regions.

## P1807

## Worldwide antimicrobial susceptibility patterns of Gram-negative bacilli isolated from intraabdominal infections: results from SMART 2004

F. Rossi, F. Baquero, P-R. Hsueh, D.L. Paterson, V. Satishchandran, T. Snyder, S. Rawlins, G. Gallagher, M.A. Abramson, M.J. DiNubile, J.W. Chow *(São Paulo, BR; Madrid, ES; Taipei, TW; Pittsburgh, West Point, US)*

**Objective:** MART (Study for Monitoring Antimicrobial Resistance Trends) is an ongoing global antimicrobial surveillance program focused on clinical isolates from intraabdominal infections (IAI). The aim of this sub-analysis was to assess antimicrobial susceptibility patterns among Gram-negative bacilli from 5 different regions of the world during 2004.

**Methods:** 6pA total of 81 major medical centres in North America, Latin America, Europe, Middle East/Africa, & Asia/Pacific tested the *in vitro* activity of antimicrobial agents commonly used to treat IAI against consecutive unique aerobic and facultative Gram-negative bacilli from IAI using microdilution techniques according to CLSI guidelines & breakpoints.

**Results:** *Enterobacteriaceae* were recovered from 4905 (88%) & non-*Enterobacteriaceae* were recovered from 826 (15%) of the 5596 patients in the study worldwide, constituting 5317 (86%) & 839 (14%) of the 6156 total isolates, respectively. *E. coli* (n = 2979; 56%) and *Klebsiella* spp. (n = 978; 18%) were the most commonly isolated *Enterobacteriaceae*. *Pseudomonas* spp. (n = 605; 72%) and *Acinetobacter* spp. (n = 110; 13%) were the most commonly isolated non-*Enterobacteriaceae*. Isolates from Asia/Pacific and Latin America were generally more resistant. 2267 (43%) of the *Enterobacteriaceae* & 272 (32%) of the non-*Enterobacteriaceae* were recovered <48 hours after hospitalization. The % susceptible isolates are reported below:

| | Ertapenem | Imipenem | Meropenem | Ceftriaxone | Ceftazidime | Cefoxitin | Cefepime | Piperacillin/ tazobactam | Amikacin | Tobramycin | Ciprofloxacin | Levofloxacin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Enterobacteriaceae; n=5317 | 99 | 99 | >99 | 84 | 84 | 78 | 89 | 91 | 97 | 87 | 80 | 83 |
| Non-Enterobacteriaceae; n=839 | NA | 74 | 76 | 21 | 73 | NA | 69 | 78 | 77 | 73 | 69 | 74 |

**Conclusion:** In this study, *Enterobacteriaceae* were the predominant intraabdominal isolates recovered both <48 h and >48 h after hospitalization. Carbapenems were overall the most active agents against *Enterobacteriaceae* worldwide. Resistance rates varied among geographic regions, with the Asia/Pacific and Latin America regions generally having the most resistance.

## P1808

## Characterisation of streptogramin resistance genes among *Enterococcus faecium* isolates from Austrian animal husbandry

A. Eisner, G. Gorkiewicz, G. Feierl, F. Dieber, E. Marth, J. Köfer *(Graz, AT)*

**Objectives:** The streptogramin virginiamycin has been widely used as a growth promoter in animal husbandry in the European Union but was banned in 1998 because of concerns about evolving cross-resistance to the streptogramin quinupristin-dalfopristin used in human medicine. The aim of the present study was to investigate the prevalence of streptogramin resistance genes of *Enterococcus faecium* recovered from animal faecal specimens collected in Southeast Austria.

**Methods:** We analysed 300 *E. faecium* isolates of cattle (n = 100), pig (n = 100), and poultry (n = 100) for the presence of streptogramin resistance genes. We used selective enterococcal broth for isolation. Species identification was done on basis of Gram stain, catalase and pyrrolidonyl arylamidase activity, motility, Lancefield group D antigen typing, and by the 20 Strep Apitest (bioMérieux). Detection of the resistance genes vat(E), vat(D), and erm(B) was done by PCR.

**Results:** The erm(B) gene encoding macrolide, lincosamine, and streptogramin B (MLSB) resistance was found in each 2 *E. faecium* isolates recovered from pig and cattle, none of the isolates from these animals carried genes coding for streptogramin A resistance. On the contrary, 14 *E. faecium* isolates from broiler specimens contained the vat(E) gene and one isolate contained the vat(D) gene. All of these isolates also contained the erm(B) gene.

**Conclusion:** Our data indicate that the use of the meanwhile banned antimicrobial feed additive virginiamycin has created a reservoir of streptogramin-resistant *E. faecium* in Southeast Austrian poultry.

## P1809

## Characterisation of macrolide-resistant *Streptococcus pneumoniae* isolates from Russia

S.A. Grudinina, O.Y. Filimonova, E.N. Ilina, A. Al-Lahham, M.V. Malakhova, L.G. Stolyarova, S.V. Sidorenko, R.R. Reinert *(Moscow, RU; Aachen, DE)*

**Objectives:** *Streptococcus pneumoniae* (Spn) resistance to macrolide antibiotics continues to be of major concern. The aim of present study was to analyse phenotypic and genotypic characteristics of macrolide resistant Spn isolates.

**Methods:** Eighty one macrolide resistant Spn isolates were collected in Moscow, 2003–2005. The susceptibility testing was performed according to the CLSI guidelines. Macrolide resistance phenotypes were characterized by triple-disk diffusion test, using erythromycin, clindamycin and rokitamycin disks. Detection of genes, coding resistance to macrolides, was done by RT-PCR. Sequencing for QRDR mutations was performed on levofloxacin resistant Spn isolates. Selected isolates were analysed by MLST.

**Results:** By the triple-disk test, 21 isolates were assigned to the M phenotype, 20 of them were carrying mefA gene, and one was

negative. Twenty eight isolates were cMLSB-phenotype, 25 of them were carrying both ermB and mefA genes, in two isolates only ermB was detected, and one isolate was negative for both genes. iMcLSB phenotype was demonstrated by 29 isolates, both ermB and mefA genes were detected in 6 of them, and only ermB in 23. Three isolates didn't demonstrated blunting of zone of inhibition around rokitamycin disk. Associated resistance to penicillin G, tetracycline, chloramphenicol and co-trimoxazole was observed in 67.9%, 86.4%, 42.0% and 75.3% of Spn isolates respectively. Nine multidrug resistant isolates, harbouring both mefA and ermB genes, were subjected to MLST. Among them one isolate was found to share the allelic profile ST 81 (Spain23F-1 clone), and four isolates were single-allele variants of ST 81. In four isolates new allelic profiles were detected. Three isolates were resistant to levofloxacin (MIC ≥4 mg/L), in two of them with levofloxacin MIC > 32 mg/L (ST81 single-allele variants) E85K, S79F and I460V substitutions were detected in GyrA, ParC and ParE, respectively. D83N and I460V substitutions were detected in ParC and ParE of one isolate with new allelic profile.
**Conclusion:** High prevalence of macrolide resistant Spn, harboring both ermB and mefA genes is observed in Moscow, macrolide resistance is associated with resistance to other groups of antibacterials. Some multidrug resistant isolates are highly related to internationally disseminated multiresistant clone Spain23F-1. Strains with fluoroquinolone resistance in Moscow were all single locus variants of the Spain23F-1 clone.

## P1810

## Occurrence of tet(W) gene in a *Clostridium difficile* clinical isolate

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

**Objectives:** To investigate the presence of tet(W), a tetracycline resistance gene recently identified in anaerobic commensal bacteria from animals and humans, in *C. difficile* clinical isolates.
**Methods:** Several *C. difficile* clinical isolates from different Italian hospitals were analysed for the presence of a tet(W) gene by PCR assays. The primers used were designed on the tet(W) sequences available in GenBank. PCR fragments obtained by these amplifications were sequenced. tet(W) DNA flanking regions were also examined with a set of PCRs constructed on the sequence of the conjugative transposon TnB1230 of Butyrivibrio fibrisolvens 1.230, that is the only element carrying a tet(W) partially characterized so far. tet(W) positive isolates were also examined for the tet(M) gene and for the presence of int and tndX, markers for the Tn916 and the Tn5397-like elements, respectively. The Tn916-like elements were further characterized by PCRs designed on *Enteroccus faecalis* Tn916 sequence. Tetracycline MIC values were determined by the E-test method. Tetracycline resistance gene transfer was evaluated by filter mating experiments, using *C. difficile* p881R strain as recipient.
**Results:** A tet(W) gene was found in only one isolate, *C. difficile* cd5, also positive for the tet(M) gene. This isolate was resistant to tetracycline with a MIC of 8 mg/L. Sequence analysis of the tet(W) PCR fragment (about 1860 bp) showed that this gene had an identity of 99% with the genes found in *Clostridium* spp strain K10, Mitsuokella multacida and Butyrivibrio fibrisolvens. No amplifications were obtained with the primers designed on TnB1230, indicating the presence of a different genetic support for tet(W) in *C. difficile*. tet(M) gene of *C. difficile* cd5 was carried by a Tn916-like element that showed nucleotide sequence mutations in the region containing orf 17–20 compared to the element of *E. faecalis*. Conjugative transfer of tet(W) was not observed, whereas the tet(M) gene was transferred to the recipient strain. *C. difficile* transconjugants were resistant to tetracycline with a MIC of 8 mg/L.
**Conclusion:** The results obtained in this study demonstrate for the first time the presence of a tet(W) gene in a clinical isolate of *C. difficile*, providing further evidence of the spread of this resistance determinant among gastrointestinal bacteria.

## P1811

## Macrolide resistance determinants are prevalent and readily selected for in viridans group streptococci among healthy Norwegian adults

P. Littauer, B. Haldorsen, M. Krämer, B. Aasnæs, G.S. Simonsen, A. Sundsfjord *(Tromsø, NO)*

**Background:** Norway has a low prevalence of antimicrobial-resistant bacteria including macrolide resistant (MR) respiratory tract pathogens. We have observed an increase in macrolide consumption in Norway and there is a lack of knowledge on the reservoir of macrolide resistance determinants among viridans group of streptococci (VGS) in the pharyngeal flora.
**Objectives:** Examine the occurrence, selection and persistence of macrolide resistance determinants in VGS pharyngeal flora in healthy Norwegian adults before and after treatment with azithromycin.
**Methods:** Throat samples were collected before (day 1), after treatment (day 7) and after 3 months (day 90) from 20 healthy volunteers. The samples were plated directly as a lawn on PDMII agar plates with 5% defibrinated blood with an erythromycin Etest strip. Photos were used as quantitative comparisons. Up to 10 morphological different colonies with erythromycin Etest MIC ≥1$\mu$g/ml from each specimen were collected; day1 (n = 59), day 7 (n = 157) and day 90 (n = 76). In total 86 representatives MR, VGS-isolates were selected for further studies: (i) MICs of erythromycin, tetracycline and penicillin were determined by Etest. (ii) PCR's for erm(B), erm(TR), and mef(A/E), and subsequent sequence-typing of mef. Species identification was performed by sodA sequencing.
**Results:** A total of 17/20 persons carried a low number (<5) of MR VGS in day1 specimens, while 20/20 had a significant higher number (>100) of MR strains in day2 specimens. In day90 specimens, 20/20 carried a low number of MR, resembling day1. Reduced susceptibility to penicillin was observed in 32/86 (34%) isolates. Tetracycline resistance was found in 43/86 (57%), and mainly in erm(B)-positive strains. mef(A/E)-positive dominated day1 (53%) and erm(B) day2 specimens 52%. Sequence typing revealed mef(E) (n = 41) and mef(A) (n = 3). SodA sequence; *S. mitis* (n = 42), *S. oralis* (n = 9), *S. parasanguinis* (n = 12), *S. salivarius* (n = 22), and *S. sanguinis* (n = 1).
**Conclusion:** There is a pool of VGS carrying macrolide resistance determinants in the normal pharyngeal flora of healthy adults that are readily selected for during azithromycin exposure. The mef(E) and erm(B) were the most prevalent resistance genes and co-resistance to tetracycline was frequently observed, resembling the findings in Norwegian clinical isolates of S. pneumoniae. These VGS may provide a pool of resistant bacteria that may transfer resistance determinants to more pathogenic organisms.

## P1812

### Relationships in genotype, phenotype, T type and PFGE type among macrolide-resistant *Streptococcus pyogenes* strains isolated in the Czech Republic

V. Jakubù, P. Urbášková, L. Straková *(Prague, CZ)*

**Objectives:** To determine relationships between phenotypic and genotypic methods among erythromycin-resistant *S. pyogenes* strains.
**Methods:** A total of 1331 clinical isolates of *S. pyogenes* resistant to erythromycin were collected in 39 microbiology laboratories during 2001–2003. Erythromycin susceptibility was tested by the disk diffusion method. Strains with an inhibition zone <21 mm around the erythromycin disk (15 $\mu$g) were sent to the National Reference Laboratory for Antibiotics (NRL). Presences of MLSB resistance genes (ermTR, ermB and mefA) were tested by PCR. T serotypes were determined in randomly selected representatives of each phenotype (n = 370). PFGE type were determined in strains from year 2003 only (n = 68).
**Results:** The rate of the most prevalent phenotype (constitutive MLSB resistance) was 63%, 55% in the year 2001 and 2003, respectively. The major prevalent T types among the analysed strains were serotype T 28 (58%), T 12 (10%), T 4 (8%) and T B3264 (8%). Gene ermB was the most frequent (54%). The results of PCR method was highly congruent with observed phenotype of resistance. PFGE patterns of strains with constitutive MLSB resistance were highly identical.
**Conclusion:** M phenotypes, constitutive and inducible resistance to MLSB antibiotics were found and ermTR, ermB and mefA genes were detected among the analysed strains. The T serotype 28 was identified the mainly prevalent in our collection. The majority of strains harbouring T serotype 28 were constitutively resistant to macrolides. The study showed close relationships among genotypes, T types, specific resistotypes (phenotype) and PFGE types.

## P1813

### Analysis of trends for susceptibility of the *B. fragilis* group from 1997–2004, a US survey

D.R. Snydman, N.V. Jacobus, L.A. McDermott, R. Ruthazer, Y. Golan, D.W. Hecht, S.M. Finegold, E.J.C. Goldstein, L. Harrell, S. Jenkins, C.L. Pierson, J. Rihs, R. Venezia, V.L. Yu, S.L. Gorbach *(Boston, Chicago, Los Angeles, Santa Monica, Durham, Charlottte, Ann Arbor, Pittsburgh, Baltimore, US)*

**Objectives:** Since recognition of transferable clindamycin and tetracycline resistance in Bacteroides, we have undertaken a US national survey on the susceptibility of *B. fragilis* group to analyse emergence of resistance and trends, since these species are not routinely tested for susceptibility in hospital clinical laboratories.
**Methods:** Agar dilution MICs were determined for 5225 isolates from 1997–2004 for *B. fragilis* and related species from 9 geographically diverse centers in the US. Antibiotics included 3 carbapenems, 3 B-lactam/B-lactamase inhibitors, 2 quinolones, a tetracycline, clindamycin, metronidazole, chloramphenicol, a glycylcycline and linezolid. Isolate identity was confirmed by API 20A.
**Results:** Analysis of resistance trends from 1997–2004 showed a decrease in geometric mean MIC's (geoMIC) for imipenem (0.80 mcg/ml to 0.32 mcg/ml, $p < 0.001$) and meropenem (0.52 mcg/ml–0.42 mcg/ml, $p = 0.003$) for the Bacteroides species. Ertapenem geoMIC remained unchanged (0.99 mcg/ml). For the B-lactamase inhibitors, piperacillin-tazobactam geoMIC declined from 2.56 mcg/ml to 2.22 mcg/ml ($p < 0.001$). Ampicillin-sulbactam geoMIC did not change. Few isolates were resistant to any carbapenem or B-lactamase inhibitor combination. Clindamycin resistance increased, especially for *B. fragilis*, *B. ovatus* and *B. thetaiotaomicron* (all $p < 0.001$). Among quinolones, resistance of Bacteroides to moxifloxacin increased (geoMIC went from 2 mcg/ml to 3.5 mcg/ml, $p < 0.001$). *B. fragilis* remains the most sensitive Bacteroides species to moxifloxacin, although approximately 45% of stains have MIC's ≥to 4 mcg/ml in 2004. Tigecycline susceptibility, tested over 5 years, did not change. The first confirmed metronidazole-resistant isolate (MIC = 64 mcg/ml) obtained in the US was noted in 2002 but none were noted in 2003 or 2004.
**Conclusion:** Improved susceptibility of Bacteroides species to some carbapenems and the B-lactamase inhibitor combinations is unexplained but significant. Clindamycin resistance continues to increase, especially for *B. fragilis*. Moxifloxacin susceptibility for the non fragilis species shows that the majority of strains are resistant. The first metronidazole resistant isolate has been reported from the US. Since resistance trends are associated with species, the differentiation within the species is of extreme importance, since it may impact the choice of antimicrobial agent for the treatment of infections caused by this group of anaerobes.

## P1814

### Observed duration of nasopharyngeal carriage of penicillin-resistant pneumococci: relations to age and serogroup

P. Geli, L. Högberg, H. Ringberg, E. Melander, M. Lipsitch, K. Ekdahl *(Solna, Malmö, Lund, Stockholm, SE; Boston, US)*

**Background and objectives:** Knowledge of how the duration of pneumococcal carriage varies with age and serogroup is essential to understanding how immunity to carriage arises throughout the course of life, and designing appropriate models for the effects of vaccination or other public health initiatives aiming to reduce the pneumococcal transmission in the community. Using data from an ongoing Swedish intervention project, the duration of nasopharyngeal carriage of penicillin-resistant pneumococci (MIC PcG >0.5 mg/L) stratified by both serogroup and age of the carrier were estimated.
**Methods:** The mean duration and corresponding 95% confidence interval was estimated by fitting a gamma distribution to the observed duration of carriage for each serogroup and age stratum.
**Results:** The mean duration of carriage for all cases was 37 days (95% CI 35–38). Children below the age of 5 years carried PRP for significantly longer periods (43 days, 95% CI 41–45) compared with older individuals (25 days, 95% CI 24–27). There were also differences within the group of cases below the age of 5 years, as the duration of carriage became significantly shorter for each year older the cases were. Serogroup 9 and 14 were carried for significantly shorter periods compared with serogroup 6. Serogroup 9 also had significantly shorter carriage duration compared with serogroups 19 and 23 for cases 0–4 years. For cases 5 years or older, no significant difference in carriage duration for different ages or serogroups could be noted.
**Conclusions:** Even though the estimate does not cover any correction for the censored carriage duration and therefore not yield an estimate of the total length of carriage, the results highlight the importance to take both serogroup and age of the


ISSN: 1470-9465

carriers into account when studying the dynamics of pneumococcal transmission and modelling the effect of pneumococcal vaccination in young children.

## P1815

## Erythromycin-resistant *Streptococcus pneumoniae* isolated in Spain: serotypes, clones and mechanisms of resistance

L. Calatayud, J. Linares, C. Ardanuy, E. Cercenado, A. Fenoll, E. Bouza, R. Pallares, R. Martin *for the Spanish Pneumococcal Infection Study Network*

**Objective:** The aims of this study were to characterize the mechanisms of resistance to Erythromycin (Ery) and tetracycline (Tet) in pneumococcal strains collected in a recent Spanish point prevalence study, and to determine the frequency of the International Clones among EryR pneumococcal strains.
**Methods:** ermB, ermA, mefE, mefA, tetM and int genes were detected by PCR in all EryR strains. Serotyping and PFGE (SmaI) were performed in all strains.
**Results:** 15 (35%) of 360 *Pneumococci* isolated during one week (Feb–2004) in 147 Spanish hospitals were EryR. The rate of EryR was higher in children than in adults (39% vs 33% $p = 0.09$) and in Penicillin-resistant than in Penicillin-susceptible strains (63% vs 15%, $p < 0.001$). The most frequent serotypes found among EryR strains were: 19F (25%), 19A (17%), 6B (12%), 14 (10%) and 23F (10%) that accounted for 73% of EryR strains. Among EryR strains 109 (87%) had MLSB phenotype (73% constitutive and 14% inducible) and 16 (13%) had M phenotype. The genes detected in MLSB isolates were: ermB in 102 isolates, and ermB and mefE genes in 7 isolates. All 96 (88%) MLSB isolates with resistance to Tet had tetM gene and 79 of them had int gene (related to Tn916-like). Seven positive ermB strains susceptible to Tet had int gene. No ermA gene was detected among EryR strains. Of the 16 M phenotype strains, 14 had mefE gene (6 of them belonged to Spain9V-3–14 clone, and 8 were unrelated) and 2 had mefA gene and belonged to England14-9 clone. Although MLSB strains belonged to 67 PFGE patterns, 4 clones (Spain23F-1, Spain6B-2, Sweden 15A-25 and ST89-19F) accounted for 44% of these strains. Capsular switching was observed in two clones, Spain23F-1 (serotypes 19F and 19A) and Sweden15A-25 (serotypes 23A, 23F, 19A and 19F).
**Conclusions:** The majority of macrolide-resistant pneumococci isolated in Spain had ermB, tetM and int genes, suggesting the spread of Tn916-like elements. The ermB positive strains, related to Spain23F-1, Spain6B-2, Sweden 15A-25 and ST89-19F clones, were more frequently isolated in adults, whereas mefA/E positive strains related to Spain9V-3-14 and England14–9 clones were more frequently isolated in children.

## P1816

## Changing patterns of antimicrobial resistances among bacterial isolates recovered from European patients hospitalised with pneumonia: report from the SENTRY Antimicrobial Surveillance Programme (1998–2004)

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

**Objective:** To characterize changes in the frequency of occurrence of bacterial pathogens responsible for pneumonia in hospitalized patients in Europe for the years 1998–2004 and examine select antimicrobial susceptibilities (S) for predominant pathogens. The emergence of resistance (R) among pathogens responsible for pneumonia has resulted in changes to empiric therapy, with increasing reliance upon third- and fourth-generation cephalosporins, beta-lactam/beta-lactamase inhibitor combinations, carbapenems and fluoroquinolones.
**Methods:** Participating European medical centres (10–31/year) referred 50 consecutive, non-duplicate pathogens (8419 isolates) from lower respiratory tract sites determined to be significant by local criteria as the probable cause of pneumonia. All identified isolates were tested for S by the broth microdilution method [NCCLS M7-A6, 2003] and results interpreted using CLSI [M100-S15; 2005] breakpoint criteria.
**Results:** Rank order of principle pathogens and changes in select S are in the Table. The composition of the six top-ranked pathogens did not change over the study interval; the rank order did, however, with PA, SA, ESP, KSP and EC being predominant in 1998 and SA, PA, KSP, EC and ESP in 2004. Decreasing S were apparent with most key organism/antimicrobial combinations, including OX and CIP among SA; CRO, CAZ and CIP among *Enterobacteriaceae*; and CAZ, IMI and CIP among PA. PEN and ERY S among SPN decreased until 2000 (66.7 and 49.0%, respectively), increased significantly in 2001 (84.9% S), and returned to near-1998 levels in 2004. ESBL-phenotypes (CRO or CAZ or aztreonam MIC ≥2 mg/L) remained essentially unchanged among EC between 1998 and 2004 (8.2% and 8.3%, respectively), whereas among KSP increases were more substantial (16.7% and 26.9%). Metallo-beta-lactamase-producing PA were identified during the study from Italy (2000–2001; 10 isolates, clonally-related; VIM-1), Germany (2002; one isolate; GIM-1) and Greece (2004; two isolates; VIM).

| Species and Frequency (1998/2004 %) | Antimicrobial Agent | $MIC_{50/90}$ in mg/L (%S) 1998 | 2004 |
|---|---|---|---|
| *P. aeruginosa* (PA; 22.3/20.5) | Ceftazidime (CAZ) | 2/>16(87.8) | 4/>16 (74.2) |
| | Imipenem (IMI) | 2/>8 (74.0) | 1/>8 (70.0) |
| | Ciprofloxacin (CIP) | 0.25/>2 (78.8) | 0.25/>2 (63.1) |
| *S. aureus* (SA; 19.9/23.0) | Oxacillin (OX) | 0.5/>2 (62.3) | 0.5/>2 (59.3) |
| | Gentamicin | 0.5/>8 (75.0) | <=2/>8 (82.3) |
| | Ciprofloxacin | 0.25/>2 (65.0) | 1/>2 (51.4) |
| *Enterobacter* spp. (ESP; 9.2/6.3) | Ceftazidime | 0.5/>16 (73.3) | <=1/>16 (65.7) |
| | Ciprofloxacin | <=0.03/>2 (84.2) | <=0.03/>2 (88.1) |
| *Klebsiella* spp. (KSP; 8.2/9.8) | Ceftriaxone (CRO) | <=0.25/32 (85.2) | <=0.25/>32 (78.8) |
| | Ciprofloxacin | <=0.03/0.25 (96.3) | <=0.03/>2 (79.8) |
| *E. coli* (EC; 7.4/9.7) | Ceftriaxone | <=0.25/0.5 (96.9) | <=0.25/<=0.25 (92.2) |
| | Ciprofloxacin | <=0.03/0.12 (96.9) | <=0.03/>2 (79.6) |
| *S. pneumoniae* (SPN; 5.3/4.9) | Penicillin (PEN) | <=0.03/2 (72.9) | <=0.03/2 (69.2) |
| | Erythromycin (ERY) | <=0.25/>8 (71.4) | <=0.25/>8 (71.2) |

**Conclusions:** Although temporary R declines were seen among some European pneumonia pathogens, all showed increasing R to most class agents during the study period. The increase in ESBL among *Enterobacteriaceae*, and R among PA to most agents except polymyxin B, are especially worrisome. Continued longitudinal comparisons of emerging pathogens and changing susceptibility profiles are critical elements in guiding empiric therapies and epidemiologic interventions.

## P1817

## Distribution of capsular and surface polysaccharide serotypes of methicillin-resistant *Staphylococcus aureus* in Germany

C. von Eiff, K.L. Taylor, A.I. Fattom, G. Peters, K. Becker *(Munster, DE; Rockville, US)*

**Objectives:** *Staphylococcus aureus* is still one of the most feared pathogens because of his ability to cause serious infections, including overwhelming sepsis. Since 1961, the emergence and spread of methicillin-resistant *Staphylococcus aureus* (MRSA) has

been documented in almost every continent and it is associated both with hospital and community-acquired infections. Although *S. aureus* has been found to express 13 capsular types, only two capsular polysaccharides (CPs), type 5 and type 8, comprise ca. 80% of infectious isolates. A renewed interest emerged following the observation that CPs are both essential virulence factors and putative protective antigens.

**Methods:** Four-hundred consecutive MRSA isolates were collected at 10 centres (max. 40 isolates per centre) as part of a multicentre study conducted throughout Germany in 2004. Isolates were collected from various sources, including colonization sites as well as infectious foci. Only one isolate per patient was included and all isolates were spa-genotyped. CPs were determined by slide agglutination with CP-specific antibodies (anti-T5-DT, anti-T8-conjugate, anti-336-rEPA). The serotypes were confirmed by immunodiffusion using lysostaphin-digested cell lysates.

**Results:** In the present study, we serotyped MRSA isolates collected most recently in a German multicentre study. All 400 MRSA isolates evaluated were one of the serotypes tested, with 290 isolates being type 5 (72.5%), 44 isolates being type 8 (11%), and 66 isolates being type 336 (16.5%). The prevalence of capsular and surface polysaccharide serotypes varied greatly from centre to centre, with type 5 ranging from 50–90% (type 8, 2.5–22.5% type 336, 7.5– 35%). While serotype 336 was often associated with spa types 008 and 032 (as observed in different centres), high prevalence of this surface polysaccharide serotype was not due to a major outbreak in a single centre. Altogether, serotype 336 was detected in strains exhibiting 20 different spa types. Sorting by culture specimen source, type 336 was more common in airway-related specimens (e.g. swabs from anterior nares, throat and bronchial secretions).

**Conclusion:** CP serotyping of MRSA isolates from Germany show that the majority of isolates are comprised of serotypes 5. Of interest, type 336 strains are more common than type 8 strains, with one of six strains positive for type 336. The unexpected high prevalence of type 336 positive strains was not due to clonal spread.

## P1818

## Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* isolates collected in a national screening study in hospitals in Luxembourg in 2003

J. Mossong, P. Marques, M. Sertznig, D. Hansen-Koenig, D. Meunier, R. Hemmer, A. Hakim, F. Schneider on behalf of the National Group on Preventing Nosocomial Infections *GNPIN*

**Objectives:** The molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) among inpatients in Luxembourg hospitals in 2003 was studied using pulsed-field gel electrophoresis (PFGE).

**Methods:** A national MRSA carriage screening study was carried out in 2003 in 14 hospitals in Luxembourg. During one week, all participating hospital inpatients were swabbed on three anatomical sites: throat, nose and groin. We investigated the molecular epidemiology of the 46 MRSA isolates collected from 34 patients in 5 hospitals using the PFGE method with the SmaI restriction enzyme. Cluster analysis was carried out using Bionumerics software. Band-based similarity Dice coefficients were used for dendrogram construction, which provides a quantitative assessment of strain similarity. Samples were defined to belong to a cluster using a similarity coefficient of 75% or higher. PFGE profiles were compared with the most similar strains from the Harmony IUMS Global MRSA Database.

**Results:** 21 different restriction profiles were observed among the 46 MRSA isolates and 34 patients. Isolates from the same patient but from different anatomical sites had similar PFGE profiles. 7 clusters of MRSA strains could be identified with the two largest clusters containing 15 (44%) and 10 (29%) patients, respectively. Strains from these 2 major clonal clusters occurred in 5 and 4 out of the 5 hospitals, respectively. Isolates from the cluster with 15 patients were most similar to the well-known Iberian clone: France A (10 strains), Belgium E1 (2 strains), France B, France C and Northern Germany I (1 strain each). Isolates from the next largest cluster of patients correlated with a group of strains previously found in Finland and Belgium: Belgium E3 (2 strains), Finland E1 (7 strains) and Finland E7 (1 strain). The remaining strains were most closely related to Belgium EC2 (3 strains), Berlin IV (1 strain), Southern Germany II (2 strains) and UK E15 (3 strains).

**Conclusion:** Two major clonal clusters of MRSA strains were found to be dominant among hospitals inpatients in Luxembourg. The molecular diversity of circulating strains was fairly diverse and profiles were very similar to previously described patterns in neighbouring countries and Europe. Further sequence-based genotyping is warranted to gain a better understanding of the clonal structure and elucidate transmission patterns.

## P1819

## First detection of vancomycin-resistant enterococci in Russia: genetic background

A. Mironova, E. Cherkashin, G. Kliasova, V. Tishkov, A. Brilliantova, S. Rezvan, S. Sidorenko *(Moscow, RU)*

**Background:** In June 2002 the first vancomycin-resistant *Enterococcus gallinarum*, harbouring vanB gene, was isolated from the patient of haematological centre. The purpose of this study was to determine VRE prevalence and to detect resistance genes.

**Methods:** Screening for VRE was performed among patients of haematological centre from April 2003 to March 2005. *Enterococci* were identified by basic tests and by PCR amplification of ddl genes. Susceptibility testing was performed using the ICLS broth microdilution method. Resistance genes were detected by PCR, selected vanA, vanB and vanC1 amplicons were sequenced. Macrorestriction analysis (SmaI) resolved by pulsed-field gel electrophoresis (PFGE) was performed.

**Results:** During the study 31 VRE isolates with different phenotypes of resistance to glycopeptides were obtained from 3578 specimens. The prevalence of VRE in the gastrointestinal tract was 3.5%. One *E. faecalis* (isolated from patient arrived from US) and 23 *E. faecium* isolates, harbouring vanA genes, demonstrated MIC's of vancomycin (Van) and teicoplanin (Tec) 256–1024 and 16–128 mg/L respectively. Three *E. faecium* and four *E. gallinarum* isolates were vanB-positive, with Van and Tec MIC's >32 and 0.25–1 mg/L respectively. All stains were susceptible to linezolide. Among *E. faecium* isolates with vanA genes one predominant PEGF type was observed, differentiated in nine PEGF sub-types. Each of three other PEGF types detected seemed to be unique. Among six vanA genes sequenced, four demonstrated similarity to vanA gene from *E. faecium* (GeneBank AF516335) and two to - vanA gene from *E. faecalis* (GeneBank AY697425). In two sequenced vanB genes from in *E. gallinarum* nucleotide substitutions, resulting in seven new amino acid substitutions, were detected.

**Conclusions:** Heterogeneity of glycopeptide-resistance genes, circulating in haematological centre, leads to the conclusion that their spread is not a local phenomenon. Spread of VRE is an emerging and, possibly, underestimated problem for Russia.

## P1820

## Study of resistance and clonal relatedness of clinical isolates of *Stenotrophomonas maltophilia* from a hospital in northern Spain

C. Valderrey, E. Sevillano, F. Calvo, L. Gallego *(Bilbao, ES)*

**Objectives:** The aim of this work was to study the antibiotic resistance and genetic relatedness among clinical isolates of *S. maltophilia* isolated from patients with tract respiratory infections.
**Methods:** The study included 36 *S. maltophilia* isolates obtained in a Hospital from Bilbao (Northern Spain) during 2005 (from January to October). Susceptibility to antimicrobial agents was determined by the disk diffusion method following the NCCLS recommendations. The antibiotics tested were imipenem, meropenem, cefotaxime, ceftazidime, cefepime, aztreonam, amikacin, tobramicin, ciprofloxacin, ofloxacin and trimethroprim/sulfamethoxazole. Total DNA was used as target for PCR-fingerprinting experiments with primers RD1, ERIC2, AP3, M13 and RNAr 1 and 2. To detect class 1 integrons, primers 3́CS and 5́CS were used in amplification experiments.
**Results:** Resistance to antibiotics tested was the following: imipenem (100%), meropenem (67%), cefotaxime (92%), ceftazidime (28%), cefepime (64%), aztreonam (86%), amikacin (8%), tobramicin (14%), ciprofloxacin (25%), ofloxacin (25%) and trimethroprim/sulfamethoxazole (0%). PCR-fingerprinting technique was only useful when ERIC2 primer was used identifying 28 distinct genotypes. The other primers were not able to produce reliable band patterns. Patients with several isolates maintained the same clone along time, although there are two patients from which two different genotypes have been isolated, and two clones that have been isolated from more than one patient. Class 1 integrons were detected in 56% of isolates ranging in size from of 1500 to 300 bp (8 isolates bored combinations of two structures).
**Conclusions:** Trimethroprim/sulfamethoxazole and amikacin showed the best activity against the isolates tested. For PCR-fingerprinting experiments the best primer was ERIC2 which produced reliable and reproducible band patterns. There was a high clonal diversity since 28 different genotypes were identified among the 27 patients included in the study. Many isolates bored class 1 integrons with sizes similar to those detected in other non-fermenters bacilli from the same environment.

## P1821

## Invasive pneumococcal disease in adults in North-Rhine Westphalia, Germany, 2001–2004

M. van der Linden, A. Al-Lahham, R. Reinert *(Aachen, DE)*

**Objectives:** Elderly adults are at increased risk for invasive pneumococcal disease (IPD). We conducted a population-based survey of IPD among adults in North-Rhine Westphalia, Germany.
**Methods:** Surveillance for our current study focused on North-Rhine Westphalia, the largest federal state in Germany (18 million inhabitants). 202 (52.2%) acute care hospitals27 microbiological laboratories serving these hospitals agreed to participate. We studied hospitalized patients older than 15 years of age. A case of IPD was identified by the isolation of *S. pneumoniae* from an otherwise normally sterile site. Isolates were verified for species diagnosis by optochin testing and bile solubility, and for serotyping by the Neufeld Quellung reaction. MICs of penicillin G, amoxicillin, cefotaxime, cefpodoxime, cefuroxime, clarithromycin, clindamycin, gatifloxacin, levofloxacin, telithromycin, tetracycline, and trimethoprim/sulfamethoxazole were determined using the microdilution method according to the latest CLSI guidelines.
**Results:** We found that 95.4% of IPD isolates were susceptible to penicillin G and 15.2% were clarithromycin resistant. Serotypes 14 (14.8%), 3 (8.8%), 7F (7.4%), 4 (6.9%) and 1 (5.6%) were the most common serotypes. The serotype coverage of the of the 7-valent pneumococcal conjugate vaccine was 40.1%, the coverage of the 23-valent pneumococcal polysaccharide vaccine was 80.2%. 774 isolates (77%) were obtained from blood, 61 isolates (6%) from CSF. Among the CSF isolates no cefotaxime intermediate or resistant strains were found. Between 2001 and 2004 penicillin resistance increased from 0.7% to 1.5%. Clarithromycin resistance increased from 12.1% to 14.8%.
**Conclusions:** The percentage of isolates from IPD in adults that are resistant to penicillin remains low but is increasing. The level of resistance to clarithromycin is now 14.8%. The coverage of both the the 7-valent pneumococcal conjugate vaccine and the 23-valent pneumococcal polysaccharide vaccine remains high.

## P1822

## Surveillance of carbapenem resistance among *Enterobacteriaceae*: species and geographic distribution

C.A. Wood, J.W. Chow, T.A. Snyder, M.A. Abramson, G. Gallagher, M.J. DiNubile, R.M. Gesser *(West Point, US)*

**Objectives:** Carbapenems are the most reliably active beta-lactam antibiotics against *Enterobacteriaceae* and are often the treatment of choice for infections caused by multi-drug resistant isolates. While carbapenem resistance has occasionally been reported in *Enterobacteriaceae*, there are limited data on its frequency and distribution.
**Methods:** Two large ongoing surveillance databases were searched for imipenem (IMP) and ertapenem (ETP) resistance in *Enterobacteriaceae*: SMART (Study for Monitoring Antimicrobial Resistance Trends), a worldwide program focusing on community- and hospital-acquired intra-abdominal pathogens, and ISS (ICU Surveillance Survey), a US program focusing on ICU isolates from any sterile body site.
**Results:** The overall frequencies of carbapenem-resistant *Enterobacteriaceae* remained <2% in SMART and <4% in ISS throughout the periods of observation (see table). For 2004, resistance (%) in SMART was most common in *E. aerogenes* (7.8), *E cloacae* (2.3), *K. pneumoniae* (2.1), *S. marcescens* (2.0), and E. coli (1.2) and for ISS in *K. pneumoniae* (6.5), *E. cloacae* (5.4), *E. aerogenes* (2.9), *S. marcescens* (2.0), and *E. coli* (1.2). Carbapenem resistance (%) varied by geographic region: Latin America (4.0), Asia (1.6), Middle East/Africa (1.2), North America (1.2), and Europe (0.8). Overall, 5.8% of ESBL producing *K. pneumoniae* and *E. coli* were resistant to ETP or IMP and rates varied by geographic region. All isolates studied to date have exhibited multiple resistance mechanisms.

| Source | Year | # Isolates | ETP Only | IMP Only | ETP & IMP | ETP or IMP |
|---|---|---|---|---|---|---|
| SMART | 2002 | 2570 | 0.5 | 0.1 | 0.3 | 0.9 |
| | 2003 | 4766 | 0.7 | 0.1 | 0.3 | 1.1 |
| | 2004 | 5317 | 1.0 | 0.1 | 0.4 | 1.6 |
| ISS | 1999 | 5455 | 2.5 | 0.7 | 0.4 | 3.6 |
| | 2000 | 4441 | 2.8 | 0.2 | 0.5 | 3.5 |
| | 2001 | 4691 | 2.8 | 0.1 | 0.6 | 3.5 |
| | 2002 | 3354 | 2.0 | 0.4 | 0.4 | 2.8 |
| | 2003 | 3843 | 1.5 | 0.1 | 1.1 | 2.8 |
| | 2004 | 2565 | 1.6 | 0.4 | 1.2 | 3.2 |

**Conclusion:** Carbapenem resistance was uncommon among clinical isolates of *Enterobacteriaceae* in these surveillance studies. Its observed frequency varied by species and geographic region.

## P1823

### Exploring the molecular basis for differences in phenotype of *Salmonella enteritidis* typing phage

N. DeLappe, D. Morris, M. Cormican *(Galway, IE)*

**Objectives:** The *Salmonella enteritidis* phage tying scheme of the Laboratory of Enteric Pathogens, Health Protection Agency, UK, is a widely used method for subtyping this important pathogen. The method is rapid and highly discriminatory. Interpretation of results can be subjective and the typing phage which are central to the method have not been well characterised. Complete sequence data is available for the *Salmonella typhimurium* podovirus phage P22.
**Methods:** The 16 typing phage were propagated on *S. enteritidis* PT1b (PB406). Phage were visualised by electron microscopy. Phage DNA was extracted and digested with *Hin*dIII. Consensus PCR primers were designed based on sequences of P22 and other *S. typhimurium* phage. Additional primers were designed based on the sequence of the S. enterititidis typing phage 3 (a siphovirus). Amplification, sequencing and DNA probe hybridisation of various phage genes were performed using standard techniques.
**Results:** On EM the 16 typing phage comprise 6 podoviridae (phage 1, 8, 10, 14, 15 and 16), 6 siphoviridae (phage 3, 5, 7, 11, 12 and 13) and 4 myoviridae (phage 2, 4, 6 and 9). Digestion with *Hin*dIII subdivided each morphotype into 3 groups. The podoviridae contained genes homologous to P22 while the siphoviridae contained genes homologous to the sequenced *S. enteritidis* typing phage 3. Some sequence variation was detected in podovirus and siphovirus genes however in some cases phage, which differ in their phenotype had no difference detected in *Hin*dIII digestion pattern or partial sequence.
**Conclusions:** The *S. enteritidis* typing phage set comprise 3 distinct phage morphotypes. In some instances distinct phage that contribute to differentiation between *S. enteritidis* phage types had no DNA sequence variation detected. Variations in phage typing reactions may in part be due to epigenetic difference in typing phage, e.g. due to methylation of phage DNA. *Salmonella enteritidis* typing phage biology could provide a model for developing approaches to phage therapy.

# Interesting case reports

## P1824

### Sporadic orofaringeal tularaemia in 2 cases

Y. Tezer, C. Kader, O. Memikoglu, H. Kurt *(Ankara, TR)*

Tularemia is a zoonotic bacterial disease. The causative agent, *Francisella tularensis*, is spread to humans by direct contact with infected rodents, inhalation, ingestion of contaminated water or by arthropod bites. In some endemic regions, outbreaks occur frequently, whereas nearby rural parts may be completely free. We presented two cases of tularemia in non endemic region of the Turkey.
**Case 1:** A 46 year old female patient referred to tertiary hospital due to swollen on the neck for 2 months. Before admission beta lactam antibiotics had been prescribed to her for tonsilopharyngitidis. But her complaints had been continued. So she admitted to our hospital. She had been suffered fever sore throat and neck pain. She had a palpable and painfull cervical lymphadenopathy which was not suppurated. Leukocytosis and elevated C reactive protein were predominant. At screening there were not any lymphadenopathy detected elsewhere. She had been examined about Cytomegalovirus Epstein Barr virus and brucellosis. They were negative. Fine needle aspiration from neck was negative considered as malignancy. Cultures were negative for routine bacteriologic examination. Microagglutination test for tularemia was 1/320 positive. Then we decided to treat her with gentamycin for 10 days. After treatment cervical lymphadenopathy became small. Leukocyte count and C reactive protein levels were reach normal range.
**Case 2:** A 29 year old female patient referred to university hospital due to cervical lymphadenopathy and fever and sore throat. Before admission beta lactam antibiotics were prescribed to her for 2 weeks. But no apparent benefits had been detected. There was a palpable and fistulated cervical lymphdenopathy. Drainage was examined microscopically and cultured for bacteria, mycobacteria and fungi. On routine cultures no microorganisms were grown. Fine needle aspiration was done. It was reported that suppurative granulamatous lympadenitis. So we were examined for tularemia, cat scratch disease. Microaggltunation test for tularemia was 1/320 positive. Then streptomycin had been given for 10 days and excision of lymphadenopathy had been done. No complications or recurrence occur.
**Results:** Both patients were applied to us from non endemic and different regions of the Turkey. They had no known insect bite history. Both of them were diagnosed by serological tests.
**Conclusions:** In the differential diagnosis of tonsillopharyngitidis, tularemia also must be considered in the non endemic regions.

## P1825

### Tularaemia presenting with tonsillopharyngitis and cervical lymphadenitis: two case reports

B. Kandemir, I. Erayman, M. Bitirgen, E. Turk Aribas, A.C. Inkaya, S. Guler *(Konya, TR)*

Tularemia is a zoonotic disease caused by *Francisella tularensis*. *Francisella tularensis* is transmitted to humans by direct contact or ingestion of infected animal tissues, through the bite of infected arthropods, by consumption of contaminated food or water, or from inhalation of aerosolized bacteria. In this report we describe two cases of oropharyngeal tularemia who presented with tonsillopharyngitis and cervical lymphadenitis.
**Case I:** A 43 years old woman with multiple cervical lymphadenitis has been admitted to our clinic. Her complaints started 2 months ago with signs and symptoms of tonsillopharyngitis. She had received non specific treatment (ampicillin+sulbactam) and ten days later cervical lymph nodes appeared. The diagnosis was made serologically. The antimicrobial therapy (streptomycin $1 \times 1$ g im) was given for fourteen days. The patient recovered completely.
**Case II:** A 16 years old girl with multiple cervical lymphadenitis was admitted to hospital. Her complaints started 3 months ago with throat ache after which multiple cervical lymphadenitis appeared. She was admitted to our out patient clinics and diagnosed to have tularemia. Anti-microbial


ISSN: 1470-9465

therapy (streptomycin 1 × 1 g im+doxycyciline 2 × 100 mg) was given for four weeks but no clinical response was achieved. Patient was admitted to the hospital and surgical drainage was performed. Treatment against tularemia was prolonged. Patient was finally recovered at the end of nine weeks of therapy. It can be concluded that early diagnosis and treatment of tularemia are important. Some patients may benefit from surgical drainage and prolonged therapy.

## P1826

## A case of nonclostridial crepitant cellulitis which is due to *Escherichia coli*

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

**Objectives:** This condition is caused by gas forming bacteria that involve the skin, either or as an extension from deeper structures. The origin of infection is an abdominal wound, perianal disease, or operative incisions that have become secondarily infected. Tracking of gas-forming organisms from deeper sites of infection may also present as crepitant cellulitis without a break in the skin. Diabetics are more likely to acquire such infections, especially in the lower extremites. Among the bacteria isolated are anaerobic organisms such as Bacteriodes or anaerobic streptococci, or coliform bacteria, especially *Escherichia coli* and *Klebsiella*. Because of this reason we reported a case of a nonclostridial crepitant cellulitis which is due to *Escherichia coli*.
**Case:** A 40 year old man who was previously healthy, has come with fever, pain, oedema, erythema, crepitant and limitation of movement at the right lower extremity. In his history he had no complaint until 2 weeks ago. Perianal abscess has developed at this time and it has drainged spontaneously 3 days later. Than his complaints has comprised 3 day duration. On physical examination, the temperature was 38.9°C, pulse rate 116/minute, respiratory rate 42/minute and blood pressure was 90/50 mmHg. Laboratory evaluation showed a haemoglobin 8.1 g/dl, leucocyte count of 32700/mm$^3$ (neutrophils 92%). Serum electrolytes, renal and liver function tests were within normal limits. C reactive protein was elevated up to 354 mg/dl, ESR was 77 mm/h. *Escherichia coli* was isolated from wound and blood cultures. He was treated initially with ampicilin-sulbactam (6 g/day) and required attempt. Even with optimal surgical and medical therapy, he dies at the third day of the treatment from septic shock.
**Conclusion:** The onset is generally gradual, and there is usually mild local pain and systemic toxicity, allowing clinical differentiation from the more fulminant clostridial myonecrosis. The surgical approach should be aggressive, but tailored specifically to the underlying cause of infection. Antibiotic therapy is directed at a mixed aerobic-anaerobic flora, until culture reports are available.

## P1827

## A case of iliopsoas abscess which is due to *Pseudomonas aeruginosa*

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

**Objectives:** Pyogenic psoas abscess, a rare but life-threatening infection, results from primary suppuration or is secondary to the spread of infection from an adjacent structure. Primary iliopsoas abscess occurs probably as a result of hematogenous spread of an infectious process from an occult source in the body. Primary iliopsoas abscess can occur in diabetus mellitus, intravenous drug abuse, AIDS, renal failure and immunosupression. Ultrasound is diagnostic in only 60% of the cases. Computed tomography should be done for definitive diagnosis and is considered the gold standard. *Stapylococcus aureus* is the causative organism in patients with primary iliopsoas abscess, but pyogenic psoas abscess caused by *Pseudomonas aeruginosa* is uncommon. Because of this reason we reported this case.
**Case:** A previously well 67 year old woman presented with a month history of right loin to groin pain, limping or limitation of hip movement, fever and nausea. She was a diabetus mellitus patient for 7 years. On her physical examination, the temperature was 38.1°C, pulse rate 104/minute, respiratory rate 28/minute and blood pressure was 140/90 mmHg. Examination of the respiratory system, cardiovascular system and abdomen were found to be normal. Laboratory investigations revealed total leucocyte count of 17800/mm$^3$ (polymorphs 88%), C reactive protein was elevated up to 168 mg/dl, ESR was 77 mm/h. Serum electrolytes, renal and liver function tests were within normal limits, but serum glucose level was elevated to 351 mg/dl. Her blood cultures were sterile, but abscess culture yielded *Pseudomonas aeruginosa* which was taken during the surgery. She was treated imipenem (2 g/day) + amicasin (1.5 g/day) and required surgical drainage. She was treated and followed up 21 days, and discharged at the end of the treatment.
**Conclusion:** In these patients treatment involves the use of appropriate antibiotics along with drainage of the abscess. An adequate knowledge of the causative organisms should guide the initial choice of antibiotics. Depending on the results of the abscess fluid culture and sensitivity, adjustments should be made. Percutaneous drainage or surgical drainage may be done in them. In conclusion early recognition, empiric antimicrobial coverage and aggressive drainage or debriment are indicated in these patients.

## P1828

## Cervical lymphadenitis in a diabetic woman

F. Çokça, A. Azap, S. Göçmen, H. Erdi Sanli, S. Gül *(Ankara, Kirikkale, TR)*

**Objective:** *Rhodococcus equi* infections are commonly seen in immunocompromised patients. Exposure to domestic animals, such as horses and pigs may play a role in some cases. Two thirds of the *R. equi* infections in immunocompromised were reported in HIV infected patients, and the rest divided between transplant recipients, immunosupressive medications and other kinds of immunosupression. The clinical picture presents with pulmonary infection in 80% of patients. Here, we report a rare case of cervical lymphadenitis in a diabetic women due to *R. equi*.
**Case:** A sixty-year-old diabetic woman was admitted with the complaints of fever, right cervical erythematous swelling with tenderness and warmth. On physical examination; inflammation beginning from the right submandibular region and descending to the upper chest was detected. A tender mass of 8 × 5 × cm. was palpated on the right cervical region. Ampicillin/sulbactam 4 g/day was given emprically for a week with no improvement. The CT scan of the neck showed conglomerated lymphadenopathy extending from the submandibular area to the supraclaviculary region with 10.5 × 3 cm in size. The mass began to fluctuate and 500 cc abscess material was drained surgically. Gram's stain of the purulent material showed polymorphonuclear leukocytes with pleomorphic gram positive coccobacilli. The cultures of the material grew *R. equi*. Therapy was changed

to teicoplanin and ciprofloxacin combination and surgical care of the wound with antiseptics was performed. After a month, intrevenous medical therapy was changed to oral route with roxythromycin and ciprofloxacin and was continued to 2 months with complete resolution.
**Conclusion:** Increased awareness and improved laboratory techniques help for the early diagnosis of rhodococcal infections. Timely diagnosis is important because the microorganism is usually resistant to penicillin G, oxacillin, ampicillin, carbenicillin and cefazolin. The use of at least one antibiotic with intracellular activity is necessary in the treatment of *R. equi* infections. Empirical two drug regimens with erythromycin, rifampin and/or ciprofloxacin are recommended.

## P1829

### Bacterial spondylodiscitis: an evaluation of 67 patients

I. Villa Blanco, O. García Regata, J.D. García Palomo, J.L. Peña, J.P. Horcajada, M.C. Fariñas *(Santander, ES)*

**Objectives:** To analysed the features of spondylodiscitis (SD), their clinical presentation, the commonest diagnostic methods and the kind of treatment applied according to the different groups of the study.
**Methods:** A retrospective and descriptive study taking place amongst the patients diagnosed as having SD from 1998 till 2003. In each case we studied the presence of underlying disease, primary infectious sources in the prior 6 months, the way symptoms started, location, diagnostic methods, treatment and evolution, comparing between different aetiologies.
**Results:** 67 patients with SD were studied.53 of them had pyogenic SD,(35 had spontaneous SD and 18 had an SD after spinal surgery) and 14 patients had tuberculous SD. 45 were men (16 to 84 years; mean 51.8). Patients with postoperative SD were the youngest (mean 42.17 y, $p = 0.001$). Underlying diseases were found in 75% of patients, mainly in postoperative SD (50% of cases) ($p = 0.009$). An episode of previous bacteremia or infectious source was found in 40% and 63% respectively of patients with spontaneous pyogenic SD, significantly higher than in surgical SD (6% had bacteremia and 11% other infectious source, $p < 0.01$). The most common presenting symptoms were back pain (98.5%) and neurological deficits (54%). Frank fever occurred in 29% of cases, being more frequent in spontaneous SD (49%) than in postoperative SD (6%) or tuberculous SD (7%), $p \leq 0.002$. Leukocytosis was found only in 28% of patients. Postoperative SD presented the lowest levels of ESR ($p = 0.008$). *S. aureus* was the most frequent bacteria isolated (31%) in pyogenic spontaneous SD, as coagulase negative staphylococci was in surgical SD. Lumbosacral localization was detected in 45% of spontaneous pyogenic SD and in 95% of postoperative SD. Tuberculous SD predominate in dorsolumbar region. Paravertebral abscess formation was observed in 39% of pyogenic SD and in 93% of tuberculous SD ($p = 0.001$). Surgical treatment was required in 46.2% of tuberculous SD and in 9% of pyogenic SD ($p = 0.005$). Outcome of patients with spontaneous SD was worse (sequelae in 62%), than in patients with surgical SD (38.2%) or tuberculous SD (14%) ($p = 0.015$).
**Conclusions:** 1) Spontaneous SD was the most frequent and it occurred mainly in patients suffering from underlying diseases; 2) Nearly all patients had pain but only in 1/3 of them was accompanied by fever; 3) The lumbar zone was the most frequent location; 4) The majority of patients had a complete resolution of their symptoms only with medical treatment.

# Meningitis and endocarditis

## P1830

### Fastidious Gram-negative micro-organisms as causative agents of cerebral abscess. Consequences on medical and surgical treatment

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

**Background:** The ethiopathogenesis of CNS abscess includes a broad spectrum of pathogens and predisposing conditions, so that a polymicrobial flora is a quite frequent event. *Capnocytophaga* spp. includes fastidious Gram-negative organisms, usually underestimated in the common clinical practice, and poorly tested *in vitro* for antimicrobial susceptibility. Surprisingly, also agents usually active on Gram-positive pathogens demonstrated some efficacy against *Capnocytophaga* spp. (i.e. erythromycin, rifampin, tetracyclines, cotrimoxazole, chloramphenicol, and glycopeptides), which is usually responsible of anecdotal episodes of CNS infection (meningitis, brain abscess, and subdural empyema).
**Methods and results:** The fourth case report of *Capnocytophaga* spp. brain abscess is herewith reported. A probable origin from a recent cat bite and a mandibular granuloma is suspected. Due to the lack of clinical and neuroradiological response to neurosurgical debridement and an association therapy including imipenem, amikacin, clindamycin and fluconazole, empiric administration of linezolid (1200 mg/day) was attempted, and a rapidly favorable clinical, microbiological, and neuroradiological response was achieved.
**Conclusions:** Notwithstanding the identification of *Capnocytophaga* spp. as the sole microorganism yielded by purulent drainage of a CNS abscess, patients with multiple risk factors and recent surgery are expected to suffer from a polymicrobial CNS infection. Due to its favourable CNS penetration and its dual mode of administration (both i.v. and oral), linezolid may represent an alternative option in the event of CNS diseases borne by numerous risk factors and a suspected polymicrobial origin, especially when a lack of response to first therapeutic attempts is of concern. In the management of a CNS abscess where the role of microorganisms with an unpredictable sensitivity profile remains of concern, chemotherapy should be directed also against potentially multiresistant organisms. Considering also the relevant limitations given by the often poor CNS penetration, the activity of glycopeptide agents is limited, compared with that of linezolid.

## P1831

### Aetiologies and antimicrobial resistance profiles of purulent meningitis study carried out in a hospital of infectious diseases, Algiers

D. Mohammedi, M. Ouar-korichi, N. Kerrad, D. Ouerdane, F. Mechouet, S. Khaled *(Algiers, DZ)*

**Objectives:** The purpose of our study is to determine the main etiologies of purulent meningitis from the community and their resistance profiles to antibiotics.

**Methods:** -Identification: the strains were identified by colonial morphology, haemolysis on blood agar plates, biochemistral and antigenic identification; antibiotic susceptibility testing: all the strains were tested by disk diffusion according to the National Committee for Clinical Laboratory Standard methods. MICs were determinated by screening test or MIC evaluation in solid media.
**Results:** Our study concerns 252 bacterial strains isolated from January 2000 to June 2005. Among the strains isolated, *Neisseria meningitidis* represented the most number of cases with 43.7 %.These were distributed among all different age groups. Serogroup A was the most predominant and represented 54.5% of total strains while groups B and C represented 15.5% and 14.5% respectively. *Streptococcus. pneumoniae* represents the second causes of purulent meningitis with 32.5% while *Haemophilus. influenzae* b is the third causative bacterial agent with 23.8%.This last agent is most predominant among infants less than 2 years of age in 80% of cases. *Neisseria. meningitis* is susceptible to all types of antibiotics tested. However, *Haemophilus. influenzae* b produced an inactivating enzyme (penicillinase) in 26.7% of cases. The resistance was associated to cotrimoxazole in 68.7% of cases. The results of MIC done on *Streptococcus. pneumoniae* show that 35.4% of strains has an intermediate resistance to penicillin and high level of resistance in 4.9%. The amoxycillin is active in 98.8% of the strains,in the opposite cefotaxim has an intermediate resistance in 6.1% and a high level of resistance in 2.4% of the strains. The resistance to penicillin was associated with resistance to erythromycin, cotrimoxazole or to both in some cases.
**Conclusion:** *Streptococcus. pneumoniae* represents the second causative bacterial agent responsible of purulent meningitis and showed an increasing prevalence of resistance profiles to penicillin and cefotaxim in our hospital. This implicates an effective microbiological and epidemiological control.

## P1832

## Management of meningitis and knowledge of meningitis guidelines amongst medical staff in a large teaching hospital in England

V. Price, M. Pareek, M.J. Wiselka *(Leicester, UK)*

**Objectives:** Bacterial meningitis is a serious clinical and medicolegal consequences if management is incorrect. Meningitis protocols have recently been published by the British Infection Society/Meningitis Research Foundation and are widely disseminated in our institution. Local Guidelines are also available on the hospital intranet and in the Emergency Department and acute Medical Admissions wards. This study investigated the level of understanding about meningitis and knowledge of the guidelines in medical staff of different grades working in the emergency Department and the Acute Medical Admissions Unit in a large teaching and emergency hospital.
**Methods:** 90 medical staff were interviewed faced to face and asked a series of questions on the management of meningitis. Results were stored on a database and responses were analysed.
**Results:** General knowledge about meningitis was variable. Although 96% knew that bacterial meningitis was a notifiable disease only 40% knew the procedure for informing the Health Protection Agency and only 43% would notify viral meningitis. Only 30% of responders were aware that guidelines could be viewed on the hospital intranet. Only 41% correctly identified the indications and cautions for lumbar puncture. Although the majority recognised the need for urgent administration of antibiotics 18% would omit antibiotics until further assessment and lumbar puncture results. Only 30% were aware of the need to consider adding ampicillin to cover *listeria* in patients over 55 years of age and there was uncertainty about the management of patients with penicillin resistance.
**Conclusions:** Although protocols and guidelines for meningitis have been produced and are easily accessible the majority of medical staff were uncertain how to access and utilise this information. The level of knowledge and expertise in managing meningitis amongst medical staff working in A and E and the Acute Medical Unit was poor and there is a need for further education to improve patient management. Guidelines are of no value if they are not disseminated to front-line medical staff.

## P1833

## Prevalence of penicillin-resistant pneumococci in *Streptococcus pneumoniae* meningitis

D. Dimitriu, L. Cochior, A. Vata, O. Filip, V. Luca, C. Luca, D. Mihalache, C. Dorobat, M. Negoita *(Iasi, RO)*

**Objectives:** The aim of this study was to evaluate the prevalence of penicillin resistant and multi-drug resistant pneumococci isolates in *Streptococcus pneumoniae* meningitis.
**Methods:** A retrospective study was carried out on 46 clinical records between January 2001 and October 2005. Among the 46 CSF samples the pneumococcal ethiology was confirmed by 55% positive cultures and 70% Latex agglutination. Antibiotic susceptibility testing was performed by disk diffusion method according to NCCLS standards. Isolates of pneumococci with oxacillin zone sides of >20 mm are susceptible (MIC < 0.06 microg/ml) to penicillin, while at those of <19 mm the MIC has to be determined (by E – test).
**Results:** Isolates from 12 patients (26%) were found with penicillin-resistance (PRP) – of which 67% were multi-drug resistant-and 34 (84%) with penicillin susceptibility – of which 35% were resistant to other drugs. An abrupt onset of disease was found in 58% PRP patients and 79% from non-PRP ones. Chest X ray pulmonary determinations were found in 50% PRP patients and 35% non-PRP ones. Sixty-six per cent of PRP patients and 29% of non-PRP ones had a prior hospitalization. Only 8% of non PRP patients had a positive blood culture. Antibiotic switch was made in 41% cases with PRP isolates and 29% cases with non PRP ones. The overall rate of mortality was 8%, with 16% for PRP patients and 6% for non-PRP ones.
**Conclusions:** Non-PRP isolates were the prevalent ethiology of S. pneumoniae meningitis. 35% of non -PRP strains developed other drug resistance, and 67% PRP strains were multi -drug resistant. PRP meningites evolved more as a hospital-related pathology, with an abrupt onset, frequently associated with pulmonary determinations and higher mortality rate.

## P1834

## Community-acquired bacterial meningitis in the elderly

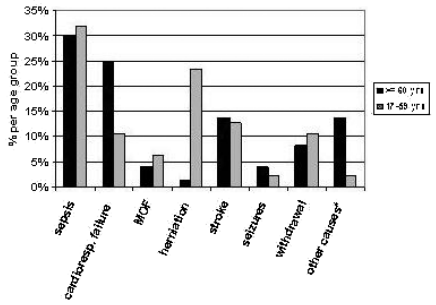
M. Weisfelt, D. van de Beek, L. Spanjaard, J.B. Reitsma, J. de Gans *(Amsterdam, NL)*

**Background:** Although vaccination strategies have shifted the age distribution of meningitis to older age groups, few studies have specifically examined bacterial meningitis in the older adult.
**Methods:** From October 1998 to April 2002, we prospectively included 696 episodes of community-acquired bacterial meningitis, confirmed by culture of cerebrospinal fluid, which occurred in patients aged >16 years. We dichotomized the cohort with respect to age: patients aged ≥60 years were defined

as older adults and patients aged 17–59 years as younger adults. Predictors for an unfavourable outcome (defined as score 1–4 on the Glasgow Outcome Scale) were determined by logistic regression. We tested for statistical interaction between age group and potential prognostic factors by adding multiplicative interaction terms to the model. The Mann–Whitney U test and the Chi-square test were used to identify differences between groups.

**Results:** 257 of 696 episodes (27%) occurred in older adults and 439 episodes in younger adults (69%). *Streptococcus pneumoniae* was the most common pathogen in older adults (69%). Meningitis in younger adults was caused by *Neisseria meningitidis* and *S. pneumoniae* in 50% and 40% of the episodes, respectively. Older adults were more likely to present with the classic triad of bacterial meningitis (fever, neck stiffness and altered mental status) than younger adults (58% versus 36%; $P < 0.001$). The prognostic value of independent risk factors for unfavourable outcome was similar in both age groups. Older adults had more complications during clinical course, resulting in a higher mortality rate than in younger adults (34% versus 13%; $P < 0.001$). Sepsis was the most common cause of death in both age groups (30% in older adults versus 32% in younger adults; Fig). Whereas older adults tended to die more often due to cardiorespiratory failure (25% versus 11%; $P = 0.06$), younger adults more often died due to brain herniation (23% versus 2%; $P = 0.004$).

**Fig. 1. Cause of death in 120 fatal episodes of adulthood bacterial meningitis: comparison of patients aged >=60 years with patients aged 17-59 years**




Cardioresp.failure=cardiorespiratory failure, MOF=multi-organ failure.
Withdrawal denotes withdrawal of care due to poor neurologic prognosis.
*other causes were pulmonary embolism (n=1; only patient aged <60 years in this subgroup), acute cardiac arrest (n=1), dissection of thoracic aorta (n=1), and not otherwise specified (n=8).

**Conclusions:** Bacterial meningitis in older adults is associated with high morbidity and mortality rates. Elderly patients often present with classic symptoms and *S. pneumoniae* is the most common pathogen within this age group. Whereas older adults often die due to cardiorespiratory failure, younger adults more often die due to brain herniation.

## P1835

## Incidence of serogroups and penicillin susceptibility in *Neisseria meningitidis* isolates (1996–2004)

F. Pardo, P.A. Romero, M.L. Pérez del Molino, A. Gómez, L. Moldes, M. Treviño, V. Túñez *(Santiago de Compostela, ES)*

**Objective:** The aim of this study was to analyse the serogroup incidence and penicillin susceptibility in *N. meningitidis* before and after Spanish epidemic outbreak in 1997. In this year the Public Health Service decided a massive vaccination in our sanitary area (Galicia, north-west of Spain, 459.180 inhabitants) and in autum of 2000 the inclusion of vaccine against *N. meningitidis* serogroup C in vaccination programme.

**Methods:** Retrospective study of all cases of meningococcal disease confirmed by culture and/or PCR in the Health Care Area of Santiago de Compostela (Galicia) from 1996 to 2004.

**Results:** In the period 1996–2004 we identified 151 meningococcal disease episodes by microbiologic diagnosis (56.3%, 41.7% and 2.0% to B, C and W135 respectively). In 1996, serogroup C were the 80% of the isolates. In 1997 and 1998 the serogroup incidence was almost the same (B = 45.7%, C = 48.6%). From 1999 an increase in B serogroup cases were detected, in 1999 (66.6%), in 2000 (88.9%) and in 2003 (76.9%). The most frecuent phenotype has been 2b P1:2.5 in C serogroup. During this period an increase in penicillin susceptibility was observed (in B serogroup 0% in 1996 and 44% in 2004 of the isolates were susceptible and in C serogroup 0% in 1996 and 50% in 2004).

**Conclusions:** The B serogroup is the most frecuent isolate during this period except in the years 1996 and 1997. The strain that cause the epidemic outbreak in 1997 (C:2b P1:2.5) was not isolated since 2000. In our Health Care Area, C:2a serotype, was isolated for first time in 2000, and since then, is the unique serotype isolated in C serogroup. Incidence rate in C serogroup has changed from 3.34/100.000 in 1997 to 1.09/100.000 in 2004. This decrease was caused by the drop of incidence rate on the youngest groups (<2 years and 2–4 years). The incidence rate in B serogroup during these years was modified from 1.48/100.000 in 1997 to 2.17/100.000 in 2004.

## P1836

## A four-year retrospective analysis of infective endocarditis in a Belgian university hospital

N. de Visscher, B. Delaere, B. Krug, Y. Glupczynski *(Yvoir, BE)*

**Objectives:** To establish the epidemiology of infective endocarditis (IE) and determine the prognostic factors for adverse outcome in patients admitted to a university hospital with a cardiovascular surgery department.

**Methods:** Between 01/00 and 12/04, the clinical and laboratory features of all consecutive adult patients with a definite diagnosis of IE (Duke criteria) were evaluated retrospectively by two infectious diseases physicians on the basis of clinical data charts and microbiological laboratory.

**Results:** 45 patients (24 men, 21 women) presented with a definite diagnosis of IE. Mean age was 64, 6 yrs; 34 cases (76%) were native valve endocarditis (NVE) and 11 (24%) were prosthetic valve endocarditis (PVE); 30% of patients with NVE had underlying valvular abnormalities (3 bicuspidies, 4 mitral prolapsus or regurgitation, 3 others). Ten out of 11 cases of PVE were late-onset episodes (>1 year after surgery). Global mortality was 42% (18/45 pts), including 15 patients (33%) still under antibiotic therapy. A higher mortality rate was observed in PVE [7/11; (64%)] than in NVE [11/34; (32%)]. Overall, 18 pts (42%) underwent surgery (mean: 18 days following admission). Valvular replacement was contra-indicated in 11 pts because of critical status and/or major co-morbidities. The distribution of isolated pathogens was: streptococci: 20 cases (44%) including 4 cases of *S. bovis*, *S. aureus*: 14 cases (31%, including 2 MRSA), *Enterococci*: 3 cases (7%), miscellaneous: 8 cases. The affected valves were: only aortic: 19 (43%), only mitral: 15 (33%), only tricuspidal: 1, aortic and mitral: 4, mitral and tricuspidal: 2, aortic, mitral and tricuspidal: 2. A high mortality rate was observed in *S. aureus* IE (9/14 [64%]), especially in the subgroup of patients with a PVE (4/5 pts [80%]). The mortality rate in patients with IE episodes caused by *Streptococci* amounted 21% (5/24 pts).

**Conclusion:** As expected in a referral hospital with a cardiac surgery department, the prevalence of *S. aureus* IE was elevated as well as the attributable mortality rate. The high global mortality rate may be explained by the high frequency of severe co-morbidities and by the late referral of patients to hospital. Our data suggest that there is room for improvement in the diagnosis and management of IE in a multidisciplinary collaborative approach.

## P1837

## Prosthetic valve endocarditis in southern Spain. Study of 111 cases

A. Plata, A. Villalobos, J.M. Reguera, A. Alarcon, J. Galvez, J. Ruiz, J.M. Lomas, J. De la Torre, F. Miralles *on behalf of the Andalusian Group for the Study of Cardiovascular Infections*

**Objective:** To determine the clinical, epidemiological, diagnostic, and therapeutic characteristics of a series of 111 cases of prosthetic valve endocarditis.
**Methods:** We undertook a retrospective, descriptive study of 111 cases of prosthetic valve endocarditis obtained from a series of definite or probable 626 left sided infectious endocarditis from six second- or third-level Andalusian hospitals from 1985 to 2005.
**Results:** Of the 111 cases of prosthetic valve endocarditis, 96 (86.5%) were definite and 15 (13.5%) possible. The mean age was 58 ± 13 years, and they were more common in men (61%). Late infection was more common than early involvement (74 vs. 37 cases). The aortic valve was involved in 54 cases (48%) and the mitral valve in 48 cases (43%. Most (66%) of the valves were made of metal and prior handling had taken place in 26 cases (23%). Clinical characteristics were fever 88%, constitutional syndrome 36%, murmur 41%, vascular events 37%, and immune phenomena 17%. Complications included left ventricular failure 54%, kidney failure 22%, peripheral embolism 20%, CNS embolisms 18% and heart block 9%. The etiology was as follows: in early prosthetic valve endocarditis the three most common pathogens were *S. Coagulase*-negative (40%), *S. aureus* (22%) and *Enterococcus* (11%). Late prosthetic valve endocarditis involved *S. viridans* (30%), *S. Coagulase*-negative (17%) and *S. aureus* (13%). Transesophageal echocardiography alone in 14 cases (12%), and transthoracic followed by transesophageal echocardiography in 53 cases (48%). Medical therapy was applied in 54 cases (48.6%) and surgery in 57 (51.3%). A cure was achieved in 71 cases (64%), the other 40 (36%) dying. Of those who underwent surgery, 38.5% died and 31.4% of those who were treated medically died. The death rate from early prosthetic valve endocarditis was greater than that for late prosthetic valve endocarditis (54% vs. 29%).
**Conclusions:** 1) Prosthetic valve endocarditis is a very serious infection which is still associated with an excessively high mortality, despite advances in diagnosis and treatment. 2) Early prosthetic valve endocarditis has a worse prognosis than late prosthetic valve endocarditis, due to its distinguishing pathophysiological features. 3) The greater mortality seen in patients who underwent surgery is probably associated with the fact that they had more complications, such as perivalvular abscesses or persistent infection.

## P1838

## Outcome of infective endocarditis

E.E. Hill, S. Vanderschueren, P. Herijgers, M-C. Herregods, P. Claus, W.E. Peetermans *(Leuven, BE)*

**Objectives:** Despite progress in diagnosis and therapy, almost half of patients with infective endocarditis (IE) has at least one complication and overall mortality remains high. The aim of the present 5-year prospective observational study was to define predictors of outcome in patients with IE.
**Methods:** From June 2000 through December 2004, all first episodes of definite IE by the modified Duke criteria, encountered in a single tertiary-care medical center, were registered and followed-up for 6 months.
**Results:** Overall, 193 patients suffered 203 IE episodes. Sixty-one percentage were males. The median age was 62 years (range 54–73). Fifty-five percentage of episodes were referred from another hospital. At least one complication occurred in 79%. Surgical intervention was performed in 63% and was mainly indicated because of congestive heart failure. The median time from diagnosis to surgery was 6 days (range 0–92). Six-months mortality was 22% (n = 42). In bivariable analyses, factors associated with 6-months mortality were: age, female gender, causative microorganism, nidus of infection and therapeutic policy. Six-months mortality was 18% for native valve IE and 31% for prosthetic valve IE; Twenty-five% for nosocomial IE and 19% for community-acquired IE. Six-months mortality rates for microorganisms were: *Staphylococci* 33% (n = 26) [*S. aureus* 32% (n = 19) and CoNS 35% (n = 7)], *Enterococci* 24% (n = 8), *Streptococci* 8% (n = 4) and other microorganisms 14% (n = 4). The 6-months mortality for patients with a contraindication to surgery was 74% (n = 20), for patients conservatively treated without a contraindication 7% (n = 3) and for combined surgical-medical treatment 16% (n = 19). In multivariable logistic regression predictors of 6-months mortality were age (OR, 1.05; 95% CI, 1.01–1.1; P = 0.03), causative microorganism (OR, 0.74; 95% CI, 0.55–1; P = 0.049) and a contraindication to surgery (OR, 32.26; 95% CI, 7.2–145; P < 0.001).
**Conclusion:** In the present prospective single centre study of 193 patients with definite IE, 6-months mortality rate was 0.22, and was especially high in patients with preestablished contraindications to surgery, in the elderly and in patients with staphylococcal IE. Six-months mortality in patients with combined surgical-medical treatment versus exclusively medical therapy in patients without a contraindication to surgery was not statistically significant. Staphylococcal and enterococcal IE had a worse prognosis compared to streptococcal IE.

## P1839

## Epidemiology and aetiology of infective endocarditis

E.E. Hill, P. Claus, M-C. Herregods, P. Herijgers, S. Vanderschueren, W.E. Peetermans *(Leuven, BE)*

**Objectives:** The epidemiological features of infective endocarditis (IE) have changed. We report the results of a 5-year prospective observational study investigating trends in the epidemiology and etiology of IE.
**Methods:** From June 2000 through December 2004, we registered 203 definite IE episodes according to the modified Duke criteria in 193 patients older than 16 years, hospitalized in a single tertiary-care center.
**Results:** Sixty-one% of episodes involved males. The median age was 62 years (range 54–73).Fifty-five percentage (n = 112) were referred from another hospital. Forty-four percentage (n = 90) were nosocomial. Thirty-four percentage (n = 70) involved prosthetic valves and 17% (n = 12) thereof were of early postoperative onset. The mitral valve was most frequently involved. Exposure to IE risk factors during the previous 6 months was recorded in 67% (n = 136) of the episodes. Twenty-four percentage (n = 49) were intravascular catheter-

related, 20% (n = 40) had previous surgery and 9% (n = 19) were related to urinary or digestive tract procedures. Only 2 patients had illegal substance abuse. The most frequent predisposing acquired cardiac condition for native valve endocarditis was degenerative valvular disease in 55% (71/129). Twelve percentage (n = 24) had prior IE. The most frequent predisposing congenital cardiac condition was a bicuspid aortic valve in 5% (n = 11). In 16% (n = 32), no predisposing heart disease was discernible.

**Causative microorganisms included:** Staphylococci in 43% (n = 87) with *S. aureus* in 31% (n = 62), CoNS in 12% (n = 25), *Streptococci* in 26% (n = 52) with *S. viridans* in 12% (n = 25), *S. bovis* in 8% (n = 16), *Enterococci* in 17% (n = 34) and other pathogens in 3% (n = 7). Culture negative IE was reported in 11% (n = 23). Both in community-acquired and nosocomial IE, *S. aureus* was the most frequent causative agent. Twenty-three percentage (14/62) were methicillin-resistant *S. aureus*. *S. viridans* IE was mainly community-acquired while enterococcal IE was nearly equally distributed between community and nosocomial origin.

**Conclusion:** Compared to older series, we observed a high proportion of nosocomial IE and of prosthetic valve IE. *S. aureus* and *E. faecalis* were the most prevalent causative microorganisms. *Enterococci* were nearly equally distributed between community and nosocomial origin, suggesting that nosocomial enterococcemia should be added as a major criterion, as proposed before for *S. aureus*.

## P1840

## The role of aminoglycosides in combination with a beta-lactam for the treatment of bacterial endocarditis: a meta-analysis of comparative trials

M. Falagas, D. Matthaiou, P. Papastamataki, I. Bliziotis *(Athens, GR)*

**Objectives:** The addition of an aminoglycoside to a beta-lactam for the treatment of patients with infective endocarditis has been supported mainly from data from laboratory and animal studies. We sought to review the evidence from the available comparative clinical trials regarding the role of aminoglycosides in combination with a beta-lactam for the treatment of bacterial endocarditis due to Gram-positive cocci.

**Methods:** The studies for our meta-analysis were retrieved from searches of the PubMed database and from references of relevant articles. Included studies were trials that provided comparative data regarding the effectiveness of the treatment and/or mortality in patients receiving monotherapy with a beta-lactam or beta-lactam/aminoglycoside combination therapy. Two independent reviewers performed the literature search, study selection, and extraction of data from relevant studies published in English during the period 01/1975–08/2005.

**Results:** No clinical trial comparing beta-lactam monotherapy to beta-lactam/aminoglycoside combination therapy for the treatment of enterococcal endocarditis was found. We performed a meta-analysis of 5 available comparative trials (4 randomized controlled trials and 1 comparative prospective trial) that included 261 patients with bacterial endocarditis in native valves due to *Staphylococcus. aureus* (4 studies) or *Streptococcus viridans* (1 study). There was no statistically significant difference between the compared arms regarding mortality (OR 0.59, CI 95% 0.21–1.66), treatment success (OR = 1.25, CI 95% 0.49–3.05), treatment success without surgery (OR = 1.66, CI 95% 0.64–4.30), and relapse of endocarditis (OR = 0.79, CI 95% 0.15–4.29). Nephrotoxicity was less common in the beta-lactam monotherapy arm compared to the beta-lactam/aminoglycoside combination therapy (OR = 0.38, CI 95% 0.16–0.88, p = 0.024).

**Conclusion:** The limited evidence from the available prospective comparative studies does not offer support for the addition of an aminoglycoside to beta-lactam treatment of patients with endocarditis due to Gram-positive cocci. A large multicenter randomized controlled trial may be necessary to reach a definitive conclusion on this issue.

## P1841

## Outpatient antimicrobial therapy for infective endocarditis. Single-centre experience

C. Cervera, A. Del Río, L. García, M. Sala, A. Olivera, M. Almela, A. Moreno, C. Mestres, F. Marco, J.M. Miró *(Barcelona, ES)*

**Objectives:** To evaluate the characteristics and outcome of infective endocarditis (IE) patients included in a Outpatient antimicrobial therapy (OPAT) program.

**Methods:** From January 1997 to May 2005 all patients who received OPAT therapy for an IE were prospectively evaluated. Inclusion in OPAT program require clinical stability and agreement of patients. Active drug addiction was contraindicated for inclusion. Antibiotic treatment was administered in bolus for once-daily antibiotics regimens. We used CADD-Legacy$^{TM}$ PLUS (Deltec, Inc. St Paul. USA) portable infusion system for either continuous or intermittent-programmed bolus infusion.

**Results:** We included 65 patients, 51 male (78%), mean age 60 years old (SD: 19.3 years). The diagnostic of IE was definite in 45 cases (13 with pathologic diagnosis), 15 probable and 5 possible. Mostly of the cases were community-acquired IE (83%). Mitral valve IE was the most frequent anatomical site involved (46%), followed by aortic (32%). Native-valve IE represent the majority of cases (55%), but 32% were prosthetic-valve and 12% were pacemaker lead IE. Viridans group *streptococci* was the most frequent isolate (31 patients, 48%) with 4 cases of *S. bovis* IE. Eleven patients had *S. aureus* IE (17%). At the time of the diagnosis, 10 patients had valve rupture and 4 patients had periannular abscess. A total of 15 patients required some surgical intervention for the IE [9 valvular replacement (2 of them associated with aortic graft), 5 pacemaker extraction and 1 aortic graft]. The majority of the patients received outpatient monotherapy (65%). The most frequent antibiotic used was Ceftriaxone (55% of the cases), followed by cloxacillin 20%, gentamycin 20%, vancomycin 14%, teicoplanin 14%, ampicillin 8% and other antibiotics in 14%. In 60% of the patients the vascular access was a perifierically-inserted venous central catheter and in 26% we used a portable infusion system. Twelve patients (18%) had some complication during OPAT that require hospital readmission, of which 5 could return to OPAT program. Three patients had a fatal outcome (deaths) during admission, not related to IE complications. The mean duration of OPAT was 18.9 days per patient, and globally supposed 1.230 days of hospital admission savings.

**Conclusion:** OPAT for IE can be a good therapeutic option for IE stable patients. This procedure can represent a considerable amount of hospital admissions savings, improving also patients' well-being, and must be take into account for the treatment of this disease.

# Epidemiology and outbreaks

## P1842

### Outbreak of botulism type E associated with eating traditional soup in a family group in Iran

P. Vahdani, D. Yadegarynia, M. Keshtkar Jahromi, O. Eilami, M. Zare Dehabadi *(Tehran, IR)*

**Objectives:** Botulism, a neuroparalytic illness, is caused by toxin produced by clostridium botulinum. Food born botulism, a potentially lethal neuroparalytic disease, is caused by ingestion of preformed toxin. Clinical illness is characterised by cranial nerve paralysis, followed by descending flaccid muscle paralysis. In this article we report a case series including a family group of type *E botulism* after ingestion of an Iranian traditional soup.
**Methods:** In January 2005, 11 patients of a family group developed clinical manifestations of botulism 1–2 hours following ingestion of a traditional soup. Their main clinical presentations were severe weakness (90.9%–10 case) and lethargy (72.8%–8 case). Other signs and symptoms were blurred vision, fixed and dilated pupils, diplopia, dry mouth and decreased gag reflex. Based on clinical finding, all patients received 3 monovalent antitoxins (A, B, C). Stool, gastric fluid and serum samples were sent for toxicological evaluation using the standard mouse bioassay.
**Results:** Type E toxin was detected in the stool and serum sample of only one patient. All patients recovered and discharged one week after admission.
**Conclusion:** This study confirmed that prompt administration of antitoxin can prevent progression of disease based on clinical judgment and also may be life saving. In this case series study, we observed a short incubation period of 1–2 hours only in type *E botulism*.

## P1843

### An outbreak of group G streptococcal pharyngitis among hospital personnel considered to be food-borne

N. Karabiber, A. Gurbuz Ertas, M. Karahan, E. Aykut Arca, Z.C. Karahan, A. Tekeli *(Ankara, TR)*

**Introduction:** Food-born outbreaks of streptococcal pharyngitis are relatively rarely reported,and while Group A *streptococci* are the main causative agents, only a few epidemics caused by Group G streptococci have been published. We describe here an outbreak of Group G streptococcal pharyngitis occurred among the staff of a teaching hospital in Ankara.The outbreak: An explosive outbreak of pharyngitis occured mainly among the staff working in certain departments (i.e. Intensive Care Units, Operation Rooms) of Türkiye Yüksek Ihtisas Teaching Hospital, in 29 January 2004.
**Methods:** A total of 377 (111 and 266; 3 and 47 from catering firm personel) throat cultures were evaluated in 2 days,and 124 BHS strains were isolated, 65 and 59 on the first and the second days, respectively. Presumptive identification by nbacitracin and trimethoprim/sulfametoxazole disk diffusion test showed that 121 strains were non-Group A, 3 strains were Group A streptococci. In definite grouping by *Streptococcus* Grouping Kit (Avipath-Strep,OMEGA),121 strains were found to be Lancefield Group G,3 strains were found to be Group A streptococci (GAS). One of the GAS strains was isolated from a catering staff on the first day, the other two were isolated from two health care personnels on the second day. During the outbreak, 16 of 50 catering firm personel (32%) were found to positive for Group G streptococci. All the BHS tested were found sensitive to penicillin G and erythromycin by agar disc diffusion method.
**Conclusions:** The configuration of the epidemic curve suggested a common source of exposure. Since respiratory spread of streptococci in such a rapid fashion would be highly unlikely and that 16 of 121 positive throat culture were from the staff of the catering firm that provide all the food services for the hospital, and that most of them were working at the departments in which the outbreak occurred, we considered that the outbreak might be food-borne. Prompt treatment with penicillin all the ill personnel and 9-day holiday coming consequently 30 January, terminated the outbreak. All the strains were cryopreserved for further typing studies.We are now typing these strains by Pulsed Field Gel Electophoresis (PFGE) after digestion with *Sma*I restriction endonuclease. Our initial results show that these strains are of the same origin.

## P1844

### Outbreak of acute gastroenteritis in an air force base in Western Greece

E. Jelastopulu, T. Constantinidis, T. Kolokotronis, D. Venieri, G. Komninou, C. Bantias *(Patras, Andravida, GR)*

**Objectives:** On 20 September 2005, an operative training day at the Air Force base in Western Greece, soldiers and staff experienced an outbreak of acute gastroenteritis. The purpose of this study was to determine the causes of the outbreak and develop control measures.
**Methods:** Following the assessment of descriptive epidemiology, a case-control analytic approach was utilized with 100 randomly selected cases and 66 controls. Patients completed a questionnaire pertaining to the presence and severity of gastrointestinal disturbances, date and time of symptoms onset and consumption of food items served in the base on the implied training day. Adequate questionnaire was administered to the controls. Odds ratios were calculated and statistical significance was determined using x2 test. Samples of food items were collected for bacteriological examination.
**Results:** The overall attack rate was at least 50% among the approximately 1050 attendees. The outbreak started abruptly in the late afternoon on 20 September, peaked at midnight and ended about 25 hours later. From the interviews and the analysis it was established that the lunch (beef, macaroni, tomato sauce and grated cheese) consumed several hours prior to onset of symptoms by affected military personnel was the likely source of the outbreak with a strong statistical association. There was only one subject who did not eat lunch. Among the symptoms the most prominent were watery diarrhoea (96%) and abdominal pain (73%). Relatively few indicated vomiting (8%) and nausea (7%). The mean incubation period was 9 h. In the bacteriological examination, *Staphylococcus aureus* was detected in a sample of raw beef and in two samples of grated cheese (rest-cheese from lunch and an unopened package).
**Conclusion:** The short incubation period with abrupt onset, the symptomatology and the short, self-limiting nature of the illness, are suggestive of gastroenteritis caused by an enterotoxin-producing bacterium. *S. aureus* is considered to be the most likely cause. Although mortality and longer-term morbidity are uncommon with food poisoning caused by enterotoxin-producing bacteria, this outbreak highlights its

capacity to cause short term, moderately-severe illness in a young and healthy population. It underscores the need for proper food handling practices and reinforces the importance of appropriate microbiological specimen collection from cases, as well as the public health importance of timely notification of such outbreaks.

## P1845

## Occurrence, characterisation and antimicrobial resistance pattern of *Staphylococcus aureus* strains isolated from dairy products in southern Italy

G. La Salandra, E. Goffredo, C. Pedarra, M.C. Nardella, A. Parisi, A. Dambrosio, N.C. Quaglia, G.V. Celano, G. Normanno *(Foggia, Valenzano, IT)*

**Objectives:** The ingestion of food contaminated by enterotoxins (SEs) synthesized by *Staphylococcus aureus* is responsible of one of the most common foodborne diseases (Staphylococcal Food Poisoning- SFP). Since *S. aureus* is often involved in cases of subclinical mastitis of ruminants, milk may results contaminated. Infact, the dairy products are frequently related to cases of SFP, expecially in areas characterized by a high level of consumption of these products. Consequently an active microbiological surveillance is needed in order to control the risk of SFP and to allow the improvement of the public health standards. *S. aureus* also show a large antimicrobial resistance pattern. In this work are reported the results of a survey conducted on the occurrence of *S. aureus* in dairy products from Apulia region (Southern Italy). Furthermore, the isolated strains were characterized in order to determine their ability in synthesizing SEs and to evaluate their antimicrobial resistance pattern.
**Methods:** 250 samples of dairy products (milk, cheese, mozzarella cheese, ricotta cheeses) were analysed for the detection of *S. aureus*. The isolated strains were tested for the detection of SEs, using the reverse passive latex agglutination test (SEA to SED) and submitted to PCR to detect entA, entB, entC, entD and entE genes. Furthermore, the strains were tested for susceptibility to ampicillin, tetracycline, gentamicin, eritromycin, enrofloxacin, co-trimoxazole, teicoplanin and vancomycin, by the agar diffusion method.
**Results:** Out of 250 samples analysed, 36 (14.4%) resulted contaminated with *S. aureus* and, among these, 19 (52.7%) have been recognized as enterotoxigenic strains (10 samples of milk, 5 samples of mozzarella cheese, 3 samples of cheese from ovine milk and 1 sample of cheese). All the strains tested (one per each positive sample) showed antimicrobial resistance properties but none of these was resistant to teicoplanin and vancomicin.
**Conclusions:** The results obtained from this survey show that milk and dairy products from Southern Italy are frequently contaminated by enterotoxigenic strains of *S. aureus* and highlighted the need to implement strict hygienic control measures along the food chain in order to decrease the risk of SPF. Furthermore, the presence of antimicrobial-resistant strains of *S. aureus* in food may be considered a source of community-acquired infections, with the direct risk of transfer of the antimicrobial-resistance to intestinal human microflora.

## P1846

## Eco-epidemiology of cryptosporidiosis 1999–2003, in HSE West, Ireland

D. McKeown, R. Cloughley *Galway City, IE*

**Objective:** To determine the eco-epidemiology of Cryptosporidiosis in the Health Services Executive – Western Area (formerly the Western Health Board).Concerns about the incidence of Cryptosporidiosis in the Western Area prompted the Department of Public Health to undertake further investigation of potential links between Cryptosporidiosis and environment by focusing on farming activity and water supplies in the first instance.
**Background:** Cryptosporidiosis was not notifiable in the Republic of Ireland prior to 2004, unless cited as a cause of gastroenteritis in a child less than two years old. As a result the incidence of Cryptosporidiosis in the Republic of Ireland at the time was unknown. Nationally it was estimated that up to 8% of cases of gastroenteritis in children less than two years old could be attributed to *Cryptosporidium*. In the Western Area from 1999 to 2003 the proportion of cases of gastroenteritis in children less than two years old attributable to *Cryptosporidium* ranged from 10.8% to 24.5%. This was cause for concern.Many rural locations in the Western Area are served by voluntarily-operated water schemes. Water quality from these schemes is often microbiologically unsatisfactory. The Department of Public Health
**Methods:** Initial research involved analysis of notification records for cases of Cryptosporidiosis received from 1999 to 2003 inclusive. Crude incidence rates for Cryptosporidiosis in the Western Area were compared with crude incidence rates in England & Wales, Northern Ireland, and Scotland for the same time period. Cases of Cryptosporidiosis from the Western Area were geo-coded and mapped to visualize the geographic spread of cases, and are being contrasted with geographic data for farming activity, and also with available data on water supplies.
**Results & conclusions:** The results of the initial phase of this research indicated the incidence of Cryptosporidiosis in the Western Area may be cause for concern. The geographic spread of cases and potential links to farming practices and water supplies will be presented.

## P1847

## Bacterial flora in pharyngeal swabs of family practitioners' patients from Silesian province, Poland

J. Józwiak, I. Szymczyk, W. Lukas, B. Boruta, J. Skorupka *(Zabrze, PL)*

**Objective:** The evaluation of epidemiology and seasonal fluctactions of bacterial flora in pharyngeal swabs taken from family doctors' patients.
**Material and methods:** A total of 7807 of positive pharyngeal swabs ordered by primary care physicians from Silesia were examined during the 2000–2005 period. The microbiological analysis was performed in Silesian Analytic Laboratories. The intake of material, its transport and final identification complied with laboratory standards.
**Results:** The most common pathogens were, in order of prevalence: *Streptococcus viridans* (57.4%), *Moraxella catarrhalis* (45.2%), *Staphylococcus aureus* along with MRSA (31.6%) and MRSA alone (1.6%), *E. coli* (18.8%), *Klebsiella pneumoniae* (6.6%), *Streptococcus B. haemoliticus* (4.7%). *Candida albicans* was identified in 14.2% of positive specimens. Considering seasonal fluctuation, the number of positive swabs in each month tended to gradually increase in spring with its culmination in May (10.4%). As for the most common pathogens *Streptococcus viridans* and *Moraxella catarrhalis* mirrored the general tendency and dominating in spring season (up to 11.7 and 12.1%, respectively) and having less stronger impact in automn (up to 9.3 and 9.4%). The frequency of isolation of the other pathogens revealed seasonal fluctuations confined to either spring, as in the case of *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus* strains (up to 17.7, 12.3

and 11.6%, respectively) or automn season, as stated for *Candida albicans* (up to 11.9% of isolates). No significant seasonal variability in the prevalence of emergence of *Streptococcus B-haemolyticus* strains in swabs was observed.
**Conclusions:** 1. Seasonal fluctuations of pharyngeal, bacterial flora were observed. 2. There was a significant predominance of *Streptococus viridans* and *Moraxella catarrhalis* in analysed samples. 3. Our data suggest low reliability and very strict indications for ordering pharyngeal swabs in everyday management of family practise.

## P1848

## Micro-organisms isolated from corneal and conjunctival samples in a Spanish teaching hospital

N. Arenal, D. Domingo, J.L. Navarro, J.M. Azcona, E. Garcia-Peñuela, M. Lopez-Brea *(Madrid, ES)*

**Aim:** The aim of this study was to identify the microorganisms isolated from corneal and conjuntival samples, isolated from patients attending the ophtalmology department of a Spanish hospital.
**Material and methods:** A total of 69 corneal scrapes and 544 conjunctival swabs were obtained since October of 2002 to October of 2005 in an university hospital of Madrid. Samples were cultured into blood and chocolate agar plates and incubated at 35ordm;C in $O_2$ and $CO_2$ atmospheres, respectively, for two days (conjunctival swabs) and fifteen days (corneal scrapes). Identification and susceptibility tests were performed following standard methodology.
**Results:** Thirty four (49.3%) out of 69 corneal samples and 96 (17.6%) out of 544 conjunctival swabs yielded positive cultures, respectively.
**Results are summarized in the following table:**

| | *CORNEAL SCRAPES (%)* | *CONJUNTIVAL SWABS (%)* |
|---|---|---|
| Staphylococcus aureus | 3 (8.8) | 32 (33.3) |
| Haemophilus sp. | 0 (0) | 16 (16.6) |
| Coagulase negative Staphylococci (CNS) | 11 (32.3) | - |
| Streptococcus pneumoniae | 2 (5.8) | 9 (9.3) |
| Proteus mirabilis | 0 (0) | 5 (5.2) |
| Moraxella catarrhalis | 0 | 4 (4.1) |
| Pseudomonas aeruginosa | 3 (8.8) | 3 (3.1) |
| Serratia marcesces | 3 (8.8) | 3 (3.1) |
| Klebsiella pneumoniae | 0 | 3 (3.1) |
| Stenotrophomonas maltophilia | 3 (8.8) | 0 (0) |
| Morganella morganii | 0 | 3 (3.1) |
| Other Gram negative | 4 (11.7) | 5 (5.2) |
| Other Gram positive | 3 (8.8) | 9 (9.3) |
| Fungi | 2 (5.8) | 4 (4.1) |

**Conclusions :** Corneal scrapes yielded a higher number of positive cultures than conjunctival swabs. Gram-positive microorganisms were more prevalent both from corneal scrapes and conjuntival swabs although the difference was more evident in corneal scrapes. *S. aureus* was the specie most prevalent in conjunctival samples meanwhile CNS were the most prevalent in corneal scrapes.

## P1849

## Spectrum and susceptibilities of bacteria isolated from the vitreous fluid of patients undergoing vitrectomy

D. Mylona-Petropoulou, N. Markomichelakis, K. Ziva, N. Ikonomopoulos, M. Theodoropoulos, C. Koutsandrea, H. Malamou-Lada *(Athens, GR)*

**Objectives:** To determine the spectrum and susceptibility of bacteria in vitreous fluid from patients undergoing vitrectomy for endophthalmitis.
**Methods:** Vitreous fluid samples (n = 177) were obtained from 150 patients (94 male, 56 female) undergoing vitrectomy for endophthalmitis between January 2001 and October 2005. Specimens of undiluted aqueous and vitreous fluid were cultured for aerobic, anaerobic bacteria and fungi by conventional methods. Identification and antibiotic susceptibility were performed by the API system, Vitek II system (BioMerieux) and the agar disk diffusion methods according to CLSI recommendations.
**Results:** Ninety one isolates were recovered from the samples. Gram stain was positive in 133/177 (75.1%), while cultures were positive in 94/177 (53.2%) samples. Gram-positive bacteria were the most common isolates (71/91, 78%), followed by Gram-negative bacteria (11/91, 12%) and fungi (9/91, 10%). *Staphylococci* coagulase-negative were isolated in 41/91 (45%). The next most common species isolated among Gram-positive bacteria were *S. aureus* (6.6%), *Streptococcus* spp (9.9%), *Propionibacterium acnes* (9.9%), *Bacillus* spp (3.3%), *Streptococcus. pneumoniae* (1%) and *Enterococcus faecalis* (1%). Among Gram-negative bacteria eight isolates were *Enterobacteriaceae*, two were non fermenters and one was *Haemophilus inlfuenzae*. Two of the fungal isolates were *Candida albicans*, one *Acremonium* spp and six *Aspergillus fumigatus*. Polymicrobial growth was observed in six patients with two at least isolates. Of staphylococci coagulase-negative 10/41 (24%) were resistant to methicillin. Only one strain of *Staphylococcus aureus* was methicillin resistant. All Gram positive isolates were susceptible to vancomycin. All isolates were sensitive to amikacine and ceftazidime while resistance was observed in 9/177 (5%) isolates to fluoroquinolones.
**Conclusion:** A variety of microorganisms was isolated from the vitreous fluid of patients. The predominant isolates were Gram-positive bacteria, especially staphylococci coagulase-negative with low resistance rate to methicillin. So, therapy should be based on the isolation and identification of the infecting agent and the *in vitro* antibiotic susceptibility to the appropriate antibiotics.

## P1850

## The prevalence of intestinal parasitic infection in the students of primary schools in Nazloo region in Urmia during 2004–2005

K. Hazrati Tappeh *(Urmia, IR)*

**Background:** Intestinal parasitic infections are of the most important hygienic and economical problems of millions of people in all over the world, mostly from developing countries. Understanding their epidemiological situation and relation to environmental and social factors is necessary for struggling with them in every society. This investigation was designed to study the prevalence of parasitic intestinal infections among primary school attending students in Nazloo region of Urmia district in 2004.
**Materials and methods:** 271 students were chosen randomly from 7 schools upon their population. Having their questionnaires filled, two faecal samples were taken from each student and examined with direct wet mount and formalin-ether sedimentation technique. Scotch tape was also applied in order to detect the enterobiasis and taeniasis. 271 students completed the test. All infected persons by *E. vemicularis*, *H. nana* were treated by Mebandazole and *Giardia lamblia* were treated by metronidazole.
**Results:** Overall prevalence of parasitic protozoan infections was 29.5%. *Giardia lamblia* was found in 28 cases (10.3%), *Entamoeba coli* in 27 cases (10%) and *Blastocystis hominis* in 2 cases

(0.7%). 28.4% of students had *Enterobius vermicularis* and just one case (0.4%) showed *Hymenolepis nana* infection. Concomitant infections were found in 17 cases (6.3%) for *Giardia* and *E. coli*, 2 cases (0.7%) for *Giardia* and *B. hominis*, one case (0.4%) for *E. coli* and *B. hominis*, one case (0.4%) for *Giardia* and *E. vermicularis* and one case (0.4%) for *E. coli* and *E. vermicularis*.

**Discussion:** With regard to high prevalences of giardiasis and enterobiasis it increase the prevalence that intestinal parasitic infections. It is suggested to decrease the rate of these parasitic infections in the region by strict programes that help to increase the knowledge of students, their parents and teachers about hygen.

## P1851

### *Legionella pneumophila* as an occupational risk factor for inter-city bus drivers

Y. Polat, Ç. Ergin, I. Kaleli, A. Pinar *(Denizli, Ankara, TR)*

**Objectives:** Legionellaceae are ubiquitous aquatic microorganisms that usually isolated from evaporative condensers. Various man-made sources such as cooling towers, whirlpools and spas are sources for *Legionella pneumophila*. In hot climate, bus air-conditioning and air-circulating systems are possible sources for the organism. In this study, serologic status of bus drivers and their assistants for *Legionella* infections as well as bus air-conditoner moisture exit samples for *Legionella* species were investigated.

**Methods:** Serum samples were collected from bus drivers (n = 63) and their assistants (n = 19). Samples were tested for anti-legionella antibodies by indirect immunofluorescence technique. 1/320 dilution was accepted as a positive result for anti-Legionella pneumophila antibodies. Results were analysed according to risk factors based on hot/cold climate route (Aegean and Mediterranean parts of the Turkey were accepted as hot climate region), immundeficiency, chronic diseases and work hours. According to serologic test results, air-conditioners of buses which has been driven by 1/10 dilution seropositive persons, were investigated. Air-conditioner moisture exit samples were cultured on BCYE-alpha agar supplied with BMPA. Same samples were tested by PCR targeting a 108-bp fragment of the 5S rRNA gene of *Legionella*.

**Results:** Anti-Legionella pneumophila antibodies were positive in 12 (15.2%) bus-persons. Bus drivers' seropositivity was higher than assistants ($p < 0.05$). In hot climate route, seropositivity was higher than cold climate route ($p < 0.05$). No positive PCR result was detected.

**Coclusion:** In conclusion, higher seropositivity rates in bus drivers were pointed out a newer occupational risk factor for legionellosis. Although PCR positivity was not detected for bus air-conditioners, high seropositivity rates show that bus drivers have been somehow exposed to *legionella*. Further legionellosis surveillance studies for bus drivers may help to understand *legionella* exposure during travel.

# Urinary tract infection

## P1852

### The frequency of and risk factors for asymptomatic bacteriuria in type 2 diabetes mellitus

H. Turan, K. Serefhanoglu, A.N. Torun, S. Kulaksizoglu, M. Kulaksizoglu, B. Pamuk, H. Arslan *(Konya, Ankara, TR)*

**Objective:** Asymptomatic bacteriuria is an important risk factor contributing to pyelonephritis and renal disfunction in diabetic patients. In this study, the relationship between microalbuminuria and age, body mass index, duration of the disease, the level of glycohemoglobin, glycosuria and glomerular filtration rate is studied prospectively in diabetic patients who have asymptomatic bacteriuria.

**Methods:** A hundred and twenty-three type 2 diabetic outpatients who were admitted to Baskent University Konya Medical and Research Center between January–October 2005 were included in the study. Ages of the patients were within the range of 26–80 years. The diagnosis of asymptomatic bacteriuria was established according to the CDC criteria. Concurrent samples for urinary culture, glomerular filtration rate, microalbuminuria and glycohemoglobin were obtained.

**Results:** Twenty-two of 123(17.8%) patients had significant bacteriuria. Of these patients 74% were female. Although age, body mass index, creatinine clearence and presence of microalbuminuria were similar, there was a significant difference in glycohemoglobin levels, duration of diabetes and glycosuria between the two groups ($p < 0.05$). *E. coli* was the most common microorganism obtained from urinary samples. Risk factors for asymptomatic bacteriuria were shown in the Table.

**Tablel: Risk factors for asymptomatic bacteriuria in type 2 diabetic patients**

| Risk factors | Presence of bacteriuria | |
|---|---|---|
| | No (n=101) | Yes (n=22) |
| [b] Age (years) | 55.9±10.2 | 60.8±9.5 |
| [b] BMI( kg/m$^2$) | 30.8±4.9 | 32.5±5.8 |
| [a] Glycohemoglobin level (%) | 7.37±2.1 | 8.7±2.0 |
| [b] Glomerular filtration rate (mL/min) | 90.0±37.9 | 77.4±30.5 |
| [a] Duration of diabetes (years) | 6.0±7.0 | 10.9±6.9 |
| [b] Microalbuminuria (0-300 mg/day) | 68.3±247.7 | 34.0±42.9 |
| [a] Glycosuria mg/dL | 62.4±207.5 | 352.2±456.8 |

[a] $p < 0.05$

[b] $p > 0.05$

**Conclusion:** The frequency of asymtomatic bacteriuria was found to be similar with the previous studies. High glycohemoglobin levels and long duration of diabetes were found to be the risk factors contributing to asymptomatic bacteriuria in type 2 diabetic patients.

## P1853

### Descriptive study of complicated pyelonephritis

A. Villalobos, A. Plata, I. Portales, N. Macías, A. Federico, S. Santamaría, D. López, G. Uribarri, J.D. Colmenero *(Malaga, ES)*

**Objective:** To determine the clinical and epidemiological characteristics, etiological agents, and diagnosis of complicated or severe pyelonephritis.

**Methods:** A retrospective, descriptive study of a series of 634 patients with complicated or severe pyelonephritis that fulfilled the IDSA criteria and required hospital admission to the Intensive Care Unit or the Internal Medicine Ward of Carlos Haya Hospital, Malaga, from January 1996 to December 2004.

**Results:** The study included 634 patients, with a mean age of 57.58 ± 19.2. 456 (71.9%) were women. The predisposing factors were: renal lithiasis patients (26.7%), prostatic adenoma (8.2%), vesical structure disease (4.3%), vesical functional disorder (6.6%), chronic kidney failure (4.3%). The underlying diseases included: diabetes mellitus (30.3%), immunosuppression (6.8%), previous urinary tract instrumentation (4.3%), permanent catheter (3.6%). The mean hospital stay was 10.93 ± 8.7 days. The mean duration of symptoms was 5.9 ± 8.2 days, among which were fever (89.1%), chills (79.3%), lumbar pain (77.3%), mictional syndrome (70.5%), flank tenderness (76.8%). Laboratory data included leukocytosis, leukocyturia (90.1%), bacteriuria (23.3%). Blood cultures were positive in 204 patients (41.2% of the blood cultures performed). Urine cultures were positive in 352 patients (59.6% of the urine cultures performed). The most frequent pathogens were: *E. coli* (72.9%), Proteus (7.1%), *Klebsiella*, *Enterobacter*, *Serratia* (5.4%) and *Enterococcus* (4.3%). Abdominal ultrasound was undertaken in 90.9% of the cases, with 46% showing disease. Of note among the complications were sepsis (25.7%), septic shock (8.4%), acute kidney failure (16.1%), and perinephritic abscess (1.6%). 29 patients (4.6%) died; attributable mortality (3.5%).
**Conclusions:** 1) Complicated or severe upper urinary tract infection is a common cause of hospital admission in our setting. 2) The absence of leukocyturia or mictional syndrome does not exclude the presence of complicated upper UTI. 3) The high percentage of bacteriemia necessitates blood cultures, with *E. coli* being the most common pathogen. 4) The associated morbidity and mortality are important in association with sepsis or septic shock.

## P1854

## Urinary tract infections in women suffering from urinary incontinence in individual family practice

E. Mizgala, W. Lukas, H. Trzeciak, W. Drzastwa *(Zabrze, PL)*

**Objective:** The evaluation of prevalence and contributory factors associated with the development of urinary tract diseases among women with urinary incontinence.
**Material and methods:** 124 women aged from 35 to 65 years had their urine culture examination performed. The material was taken from the central stream of first catch urine and transported on Uromedium. Antibiogram was carried out with the use of Becton-Dicinson's discs.
**Results:** In 14 cases the urine culture tested positively which accounted for 11.3% of subject women. The most common pathogens of urinary tract were, in order of prevalence:*E. coli*-64.3%, *Staphylococcus aureus* -14.3%,Citrobacter diversus-7.13% and Klebsiella pneumoniae-7.13%. *Candida albicans* strains were isolated in one patient. *E. coli* had the highest sensitivity to Norfloxacin - 100% and Cefuroxim - 100%, Amoxicillin with clavulonian acid -88.8%, Ampicillin Nitrofurantoin and Trimethiprim – sulfamethoxazole – 77.7% in each case, Cefalothin –66.6%, Tetracycline - 55.5%, and Amikacin - 33.3% but only in 11.1% to Amoxacillin. *Staphylococcus aureus* proved sensitivity only to Gentamicin (100%) and Nitrofurantoin (100%). In the case of Citrobacter diversus 100% sensivity to Norfloxacin, Nitrofurantoin, Tetracycline, Trimethoprim / Sulfamethoxazole, Ceftazidim and Cefotaksym was confirmed.*Klebsiella pneumoniae* also proved sensitivity to Amoxicillin with clavulonian acid, Cefuroksime, Nitrofurantoin, Norfloxacine, Tetracyclin and Trimethoprim / Sulfamethoxazole. When considering the sensitivity of pathogens to antibiotics in the family practise setting of higher reliabilty are Nitrofurantoina, Norfloksacyna.After the administration of guided therapy complete release from symptoms was observed in 10 women (71 %).
**Conclusions:** Women with urinary incontinence relatively seldom suffer from urinary tract infections. The most common pathogen among women with urinary incontinence was *E. coli* sensitive to Floxacins and Cephalosporins but with impaired reaction to Amoxycillin.

## P1855

## Incidence and *in vitro* antibiotic resistance of streptococci in community-acquired urinary tract infections

E. Ikonomopoulou, C. Verra, H. Pavlidou, K. Papadopoulos, N. Baikoussis, E. Stavrianou, I. Katsarou, A. Christopoulos, A. Gioka, A. Regli *(Patras, GR)*

**Objectives:** To estimate the incidence of *streptococci* in community acquired urinary tract infections (UTI) and also to carry out the *in vitro* antibiotic resistance of *streptococci* in urinary tract infections.
**Methods:** During almost a three years period (01/01/2003 – 30/10/ 2005), 301 (10.5%) streptococci strains were isolated from 3010 community acquired urinary tract infections. The identification and the susceptibility test were performed by the Vitek system (Biomerieux). All enterococcal isolates were tested for Vancomycin and Linezolid susceptibility using the E- test (AB Biodisk).
**Results:** The distribution by sex was 69.4% women and 30.6% men. 207 (68.8%) of streptococci strains were *Enterococcus faecalis*, 43 (14.3%) were *Enterococcus faecium*, 7 (2.3%) were *Enterococcus avium*, 6 (2.0%) were *Enterococcus gallinarum* and 38 (12.6%) were streptococci group B. The *in vitro* antibiotic resistance of *Enterococcus faecalis* was: Penicillin G 38.9%, Ampicillin 2.4%, Gentamicin500 29%, Streptomycin2000 52.7%, Nitrofurantoin 0%, Ciprofloxacin 44.4%, Tetracyclines 54.6%, Vancomycin 4.3%, Linezolid 0%. The *in vitro* antibiotic resistance of Enterococcus faecium was: Penicillin G 79%, Ampicillin 69.8%, Gentamicin500 23.3%, Streptomycin2000 53.5%, Nitrofurantoin 0%, Ciprofloxacin 74.4%, Tetracyclines 23.3%, Vancomycin 27.9%, Linezolid 0%. The *in vitro* antibiotic resistance of group B *streptococci* was: Penicillin G 5.3%, Ampicillin 0%, Cefazolin 2.6%, Clindamycin 5.3%, Erythromycin 13.2%, Nitrofurantoin 0%, Ofloxacin 2.6%, Tetracyclines 76%, Vancomycin 0%. 3 strains of Enterococcus gallinarum and 1 of Enterococcus avium were resistant to Vancomycin.
**Conclusions:** Streptococci are responsible for the 10.5% of community acquired urinary tract infections. *Enterococcus faecalis* was the most frequent pathogen (68.8%) and was high resistant to Ciprofloxacin, Tetracyclines, Gentamycin and Ampicillin. *Enterococcus faecium* showed high resistance to the majority of antibiotics including Vancomycin and Ciprofloxacin. Linezolid remains a promising treatment to VRE.

## P1856

## Virulence and resistance of *Escherichia coli* isolates from pregnant and non-pregnant women with community-acquired urinary tract infections in a Kuwait hospital

E. Draghijeva, P. Egbase, S. Mathai *(Kuwait, KW)*

**Objective:** To compare the availability of virulence factors and the resistance of *E. coli* isolates from pregnant women with

uncomplicated community-acquired urinary tract infections (CA-UTIs) and non-pregnant women in London hospital in Kuwait over a period of two years.

**Methods:** Eighty-six pregnant and 90 non-pregnant women with signs of CA-UTIs were enrolled in the study. The strains isolated from the patients who had significant bacteriuria were included in the microbiological analyses. The identification of the strains was performed using the API 20E system (BioMerieux), while their susceptibility was determined by disk diffusion method. The interpretation of the results was realized according to NCCLS guidelines. Quality control was performed using reference strain *E. coli* ATCC 25922. O-serotyping was carried out with polyvalent and monovalent antisera. Hemolysin production was tested on human blood agar plates. Possession of K1 antigen by *E. coli* was tested with agglutination by murine monoclonal antibodies to the group B meningococcal capsule.

**Results:** We found O serogroups O1, O2, O4, O6, O7, O18 and O75 among strains isolated from pregnant and non-pregnant women. Hemolysin was presented in 35% and 40% respective. K1 antigen was presented in 40% of strains in studied groups. There are some statistically significant differences in antimicrobial resistance between both groups. Amoxicillin-clavulanate (AMX-CLV) resistance was higher among UTI haemolytic isolates of *E. coli* in pregnant women (54%) then in non-pregnant women (38%). Similar distinction in Cefuroxime resistance was found – 15% and 0. Amikacin resistance was higher among UTI isolates of *E. coli* in non-pregnant women (47%) then in pregnant women (23%).

**Conclusions:** There are no significant differences in expression of virulence factors of *E. coli* from pregnant and non-pregnant women with CA-UTIs in London hospital, Kuwait. The resistance rates of *E. coli* from pregnant women to AMX-CLV and Cefuroxime are significantly higher than in non-pregnant women.

## P1857

## The penetration of telithromycin in gynaecological tissues and activity in cervicitis patients

H. Mikamo *(Gifu, JP)*

**Objectives:** *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are major causative organisms for sexual transmitted infections in Japan. Although several oral antimicrobial agents are active against *C. trachomatis*, few effective oral antimicrobial agents against *N. gonorrhoeae* exist in Japan. Two studies were conducted: a clinical pharmacology study examining penetration of telithromycin (TEL), an oral ketolide antibiotic, in female genital organ tissues and a clinical study examining TEL 600 mg once daily (qd) in cervicitis patients (pts).

**Methods:** Eleven hysterectomy pts were assessed in the phase II clinical pharmacology study investigating the distribution of TEL in female genital organ tissues by bioassay. Pts took one 600 mg dose of TEL prior to the operation. A Phase III clinical study was also conducted examining the efficacy and safety of TEL 600 mg qd for 5 days in pts with cervicitis caused by *C. trachomatis* or *N. gonorrhoeae*. Clinical efficacy was evaluated on Days 14 and 28.

**Results:** In the pharmacology study (n = 11), mean tissue concentration in female genital organs at 180–435 minutes after one dose of TEL 600 mg was 1.22 $\mu$g/g in portio vaginalis, 1.97 $\mu$g/g in cervix uteri, 2.37 $\mu$g/g in the endometrium, and 2.61 $\mu$g/g in myometrium. Distribution ratios (tissue concentration/plasma concentration) ranged from 2.29 to 4.90. In the clinical study of 58 pts, efficacy on Days 14 and 28 was 81.1% (43/53 pts) and 96.0% (48/50 pts). Bacteriological efficacy on Days 14 and 28 was 81.1% (43/53 pts) and 96.0% (48/50 pts). Eradication of *C. trachomatis* was 80.4% (37/46) on Day 14 and 95.3% (41/43) on Day 28, and of *N. gonorrhoeae* was 90.0% (9/10) on Day 14 and 100.0% (9/9) on Day 28. Safety was evaluated in 58 pts; the incidence of possibly related adverse events in 56, excluding 2 for whom incidence could not be determined, was 26.8% (15/56). Possibly related treatment emergent adverse events were mainly gastorointestinal and of mild or moderate intensity.

**Conclusions:** TEL 600 mg administered once daily for 5 days was clinically active for the treatment of cervicitis caused by *C. trachomatis* or *N. gonorrhoeae*. This activity appears to be supported by the distribution of TEL in female genital organ tissues. Further clinical studies are required to confirm these preliminary results and to study the potential interaction with imidazole derivatives and/or oral contraceptives.

## P1858

## Acute uncomplicated pyelonephritis may be safely treated with 7 days of an oral fluoroquinolone. Analysis of two prospective multicentre studies in the emergency department

D. Elkharrat, P. Veyssier, H. Botto, E. Vicaut, G. Pottel, H. Drugeon, F. Tremolieres *for the French AUP Study Group*

**Background:** Short-course therapy for acute uncomplicated pyelonephritis (AUP) is the newly suggested standard. Talan et al. have demonstrated that oral ciprofloxacin (Cip) for 7 days, was associated with greater cure rates than a 14-day trimethoprim-sulfamethxazole regimen. We assessed efficacy and tolerance of a 7-d. regimen of Cip (Study I), then of levofloxacin (Lvx) in Study II, in patients presenting with AUP to the emergency department (ED).

**Methods:** Methodology and end points were identical in these 2 uncontrolled studies, conducted in 35 (I) and 12 (II) EDs, respectively. Consecutive non-pregnant females with presumed AUP (fever ≥38.5ordm;C, flank pain, positive urine dipstick) were recruited after signing consent. Blood and urine samples were taken for culture before Cip 500 mg bid (I) or Lvx 500 mg once daily (II), was started. AUP was subsequently confirmed if bacteriuria was ≥10 to the 5th CFU/ml of 1 uropathogen and abdomen/pelvis ultrasound was normal. The main end point was bacterial eradication (BE) at 5–9 d. post-treatment (visit 4); others were global cure (GC) [clinical cure + BE] at V4 and at end of study, 28–42 d. post-treatment (V5). Study efficacy was based on Infectious Diseases Society of America (IDSA) guidelines (GC = 80% at V4 and =60% at V5, with <50% of lost to follow up between V4/V5).

**Results:** Of 171 (I) and 82 (II) enrolled pts. 139 aged 31.7 ± 12.9 y., and 69, aged 3.58 ± 15.0 y. were retained for ITT analysis; 16.5% and 16.0% had positive blood cultures. *Escherichia coli* was the uropathogen in 99.2% and 100% of cases. Finally 122 (I) and 60 (II) were retained for per protocol (PP) analysis. At V4 bacterial eradication rates were 93.4% and 98.3 %. Global cure rates were 89.0 and 92.8% at V4 and 77.3% and 75.0% at V5 with only less than 7% of lost to follow-up between V4 and V5 in both cases. Side effects were observed in 12.8% and 27.6% of pts. who received 1 or more FQ doses.

**Conclusions:** AUP treatment with Lvx 500 mg. once daily or Cip 500 mg bid for 7 days is efficacious and well tolerated. *We thank our colleagues for contribution to these studies.

## P1859

### Coryneform bacteria in prostatitis patients: species composition and antibiotic susceptibility

S. Türk, P. Korrovits, K. Ausmees, M. Punab, R. Mändar *(Tartu, EE)*

Chronic prostatitis (CP) is believed to be an infectious disease in most cases. Both aerobic and anaerobic bacteria are involved in the polymicrobial microbiocenosis found in prostate specific specimens. Coryneform bacteria form a remarkable part of this community, yet scarce knowledge exists about their clinical significance, species composition and antibiotic susceptibility.Our aim was to compare the *corynebacteria* of the seminal fluid of CP patients and controls and to evaluate their antibiotic susceptibility.

**Material and methods:** Semen samples from 66 controls and 49 CP patients (NIH IIIa or IV category) were analysed. *Corynebacterium seminale* was identified by beta-glucuronidase activity, the rest of coryneforms using API Coryne (BioMerieux). E-test method was used for susceptibility testing.

**Results:** Coryneforms were found from 78% CP patients and 84% controls ($p > 0.05$). Twelve species and genera were found among 120 strains identified, the most frequent being *C. seminale* (in 60% CP patients and 58% controls). CP patients harboured significantly more Arthrobacter sp. (17% vs 2%, $p = 0.03$) and *Corynebacterium* group G (14% vs 2%, $p = 0.01$), the latter association was especially eminent in case of patients with serious inflammation (>1 WBC/ml): 33% vs 2%, $p = 0.0003$. All tested 65 strains were susceptible to ampicillin-sulbactam, single strains were resistant to doxycycline (5%) and TMP/SMX (5%), however, moderate resistance was common to doxycycline (29%). Resistance to clindamycin (38%), benzylpenicillin (28%), nitrofurantoin (17%), erythromycin (16%) and norfloxacin (11%) was observed as well. Half of CP-related Corynebacterium group G strains showed resistance to nitrofurantoin and benzylpenicillin. In addition, they were often moderately resistant to clindamycin, erythromycin and, finally, norfloxacin frequently used to treat CP.

**Conclusions:** Most of men have coryneforms in their semen, more than half harbour *C. seminale*. *Corynebacterium* group G and *Arthrobacter* sp are more frequently found in CP patients than the controls. In the treatment of CP of unknown etiology it is useful to take into consideration the susceptibility profile of *Corynebacterium* group G.

## P1860

### Serologic evaluation of cytomegalovirus and *Listeria monocytogenes* in women with abortion

M. Jamshidi Makiani, M. Amirian, A. Sotode, M. Zangane *(Bandar Abbas, IR)*

**Objective:** To evaluate the role of CMV and *listeria monocytogenes* in abortion.

**Methods:** This descriptive prospective study was done on 450 women, 250 women with spontaneous abortion before 20th weeks of pregnancy as a case group and 200 healthy woman with full term delivery as a control group. Serum samples were taken from all patients. ELISA test was done for evaluation of CMV (IgG and IgM) and *Listeria* antibodies in both groups. Prevalence of seropositivity was determined. Data were analysed by x2 and chi-square test.

**Results:** 450 seologic tests were done on samples. Average age in case group was $25.6 \pm 7.6$ and in control group was $25.3 \pm 6.5$ years. In cases with abortion 89 (35.6%) and in control group 35 (17.5%) were seropositive for *Listeria monocytogenes*. Difference in seropositivity between 2 groups is statistically significant ($p = 0.0001$). CMV IgG antibodies were positive in 235 (94%) of case group and in 150 (75%) of control group; the difference is significant statistically ($p = 0.0001$). CMV IgM antibody was positive in 13 (5.2%) of case group and none in control group. Difference is significant ($p < 0.0001$) there was no correlation number of previous abortion and seropositivity for *Listeria* and CMV.

**Conclusion:** The present study showed an important role of *Listeria monocytogenes* and CMV infection in abortion.

## P1861

### Serum and prostatic tissue concentrations of moxifloxacin (400 mg) after a single intravenous infusion in patients with benign prostatic hyperplasia undergoing transurethral resection of the prostate

F.M.E. Wagenlehner, C. Lunz, F. Kees, W.F. Wieland, K.G. Naber *(Straubing, Regensburg, DE)*

**Background:** The spectrum of bacterial prostatitis comprises Gram-negative, Gram-positive and atypical pathogens. Because of its broad spectrum of activity, moxifloxacin might be a suitable antibiotic for the treatment of bacterial prostatitis.

**Aim:** In this study the penetration of moxifloxacin into prostatic tissue after intravenous application of 400 mg as single dose was investigated.

**Methods:** In a prospective, multicentric study patients with benign prostatic hyperplasia received a single dose of moxifloxacin 400 mg in an 1 hour lasting infusion (250 ml) for perioperative prophylaxis before undergoing transurethral resection of the prostate (TUR-P). Serum concentrations were determined in all patients before infusion, at the end of infusion (time point 0), 0.5, 1 and 2 h after the end of infusion. Patients were randomized for tissue sampling either 0, 0.5, 1 or 2 h after the end of infusion. At the beginning of TUR-P approximately 1 g of tissue was sampled for analysis. Concentrations of moxifloxacin in serum and tissue were determined by HPLC.

**Results:** 39 patients were evaluated in the study. The concentrations (mean, SD, median, 25/75% quantile) are shown in the table. The prostatic tissue concentrations of moxifloxacin were approximately twice as high as in serum. At the end of infusion the tissue and serum concentrations were already equilibrated, because the tissue-serum ratios did not differ significantly from the end of infusion until 2 h after the end of infusion.

| | Serum concentrations (mg/L) | | | | | | Prostatic tissue concentrations (mg/kg) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| time | n | mean | SD | median | 25 % | 75 % | n | mean | SD | median | 25 % | 75 % |
| 0 h | 39 | 5.28 | 2.16 | 4.94 | 4.01 | 6.35 | 10 | 9.54 | 3.36 | 8.50 | 7.85 | 9.80 |
| 0.5 h | 39 | 3.26 | 0.40 | 3.22 | 2.90 | 3.58 | 13 | 5.98 | 1.83 | 5.97 | 5.22 | 7.04 |
| 1.0 h | 39 | 2.89 | 0.37 | 2.94 | 2.58 | 3.13 | 9 | 5.63 | 1.70 | 5.32 | 4.34 | 6.00 |
| 2.0 h | 39 | 2.48 | 0.32 | 2.46 | 2.26 | 2.72 | 7 | 4.37 | 0.99 | 3.88 | 3.81 | 4.96 |

**Conclusion:** After an intravenous infusion of 400 mg the serum and prostatic tissue concentrations of moxifloxacin were well above the MIC values of the most important prostatic pathogens until 2 h after the end of infusion. Therefore, moxifloxacin might be a good alternative for the treatment of bacterial prostatitis and/ or perioperative prophylaxis for TUR-P.

# FUO, soft tissue and miscellaneous infections

## P1862

### A prospective multi-centre study of the value of F-18-fluorodeoxyglucose positron emission tomography as part of a structured diagnostic protocol in patients with fever of unknown origin

C.P. Bleeker-Rovers, F.J. Vos, A.H. Mudde, A.S.M. Dofferhoff, L.F. de Geus-Oei, A.J. Rijnders, P.F.M. Krabbe, F.H.M. Corstens, W.J.G. Oyen, J.W.M. van der Meer\ *(Nijmegen, Doetinchem, Arnhem, NL)*

**Objectives:** Infection accounts for about one-third of cases of fever of unknown origin (FUO), which remains a major diagnostic challenge. Recently, F-18-fluorodeoxyglucose (FDG) positron emission tomography (PET) has entered the field of clinical infectious diseases. FDG accumulates in tissues with a high rate of glycolysis, which is present in malignant cells and in all activated leukocytes. The aim of this prospective multi-centre study was to validate the use of FDG-PET as part of a structured diagnostic protocol in the general patient population with FUO.
**Methods:** From December 2003 to July 2005, 70 patients with FUO, defined according to the revised Petersdorf criteria, were recruited from one university hospital and five community hospitals. A structured diagnostic protocol was used. FDG-PET was performed after certain obligatory laboratory tests, chest X-ray and abdominal ultrasound. The final clinical diagnosis was used for comparison with the FDG-PET results.
**Results:** A final diagnosis was established in 35 patients (50%): 12 infections, 5 malignancies, 16 non-infectious inflammatory disorders and 2 miscellaneous causes. Of the total number of FDG-PET-scans, 33% were helpful. Positive predictive value of FDG-PET was 70% and negative predictive value was 95%. FDG-PET was helpful in all patients diagnosed with an infection except for one case of pyelonephritis. Contribution of FDG-PET to the final diagnosis did not differ significantly between the university hospital and the community hospitals. FDG-PET was not helpful in any of the patients with normal erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).
**Conclusion:** In addition to the apparent value of FDG-PET in diagnosing different infectious diseases as described in several case series, FDG-PET is a valuable imaging technique as part of a diagnostic protocol in the general patient population with FUO and a raised ESR or CRP. Based on previous studies comparing Gallium-67-citrate or labelled leukocyte scintigraphy and FDG-PET in patients with FUO and resulting from favourable characteristics of FDG-PET, conventional scintigraphic techniques may be replaced by FDG-PET in institutions where PET is available.

## P1863

### Emergence of clindamycin-resistant *Streptococcus pyogenes* causing cellulitis

C. Gudiol, J. Carratala, N. Fernandez-Sabe, R. Alfonso, L. Calatayud, F. Tubau, C. Ardanuy, F. Gudiol *(Barcelona, ES)*

**Objective:** Clindamycin is recomended in combination with penicillin for invasive infections due *Streptococcus pyogenes* such as streptococcal toxic shock syndrome (STSS). The aim of our study was to assess antimicrobial susceptibilities of *S. pyogenes* isolated from patients with cellulitis.
**Methods:** Review of medical records of consecutive adults hospitalized for community-acquired cellulitis from January 1995 to December 2004. HIV-infected patients and drug addicts were excluded. Antimicrobial susceptibilities of all *S. pyogenes* isolates were studied by microdilution method, and macrolide resistance phenotype by double disc test. Macrolide resistance genes were detected by PCR.
**Results:** Over the 10-year study period, there were 523 episodes of cellulitis. The infection was microbiologically documented in 191 cases of whom 39 (7.5%) were caused by *S. pyogenes* (blood cultures 21, needle aspiration culture 18, surgical sample culture 4). Antimicrobial susceptibilities of *S. pyogenes* isolates were: penicillin 100%, erythromycin 85%, clindamycin 90%, and ciprofloxacin 95%. Six (15%) *S. pyogenes* strains were erythromycin-resistant; 2 had M phenotype (mefA+), 3 had constitutive MLSB phenotype (ermB+) and 1 had inducible MLSB phenotype (ermA+). Rates of *S. pyogenes* cellulitis due to erythromycin-resistant strains increased significantly, from 0 of 19 episodes from 1995 to 1999 to 6 of 20 (30%) episodes from 2000 to 2004 ($p = 0.020$). Of note, all 4 cases of cellulitis due to clindamycin-resistant strains occurred during the last 3 years of the study. Five (13%) patients presented with STSS and died (1 due to an erythromycin-resistant strain). Overall mortality (<30 days) was 33% in patients with infections due to erythromycin-resistant strains and 12% in the remaining patients ($p = 0.224$).
**Conclusion:** Our study suggests that clindamycin-resistance among *S. pyogenes* causing cellulitis may be increasing. This resistance might become a problem when treating *S. pyogenes* infections, especially STSS cases.

## P1864

### Risk factors for community-acquired bacteraemic Gram-negative cellulitis

G. Peralta, E. Padrón, M.P. Roiz, M.J. Rodriguez-Lera, I. De Benito, L. García-Mauriño, J.C. Garrido, L. Ansorena, M.J. Grande, M.B. Sánchez *(Torrelavega, Santander, ES)*

**Objectives:** Empirical therapy in patients with cellulitis frequently doesn't cover Gram-negative bacteria so the detection of these microorganisms in the blood cultures has special therapeutic implications. However risk factors of community adquired bacteremic Gram-negative cellulitis (BGNC) are not well defined in the literature. We explore risk factors for BGNC.
**Methods:** To identify all cases of limb cellulitis attended at our hospital emergency service between January 1997 and December 2004, an administrative database was used. Then we selected the patients with blood cultures obtained at the time of the cellulitis episode using the microbiology laboratory database. Nosocomial cellulitis were excluded. A standardized data collection form was used to review the hospital records. In statistical analyses, Student's t test was used for the comparison of mean values and chi square test and Fisher's exact test for the comparison of categorical data (two tailed).
**Results:** Of the 2251 patients with limb cellulitis identified in the study period, 301 patients had blood cultures and were selected for the analysis. Bacteremia was detected in 59 of the 301 patients (19.6%), 15 of them due to Gram-negative microorganisms. These microorganisms were: *P. aeruginosa* (n = 3) (one of them mixed with *S. aureus*), *P. multocida* (n = 2), *Moraxella* spp (n = 2), *H. influenzae* (n = 2), *A. xylososidans* (n = 1), *Acinetobacter* spp (n = 1), *E. coli* (n = 1), *K. pneumoniae* (n=1), *E. aerogenes* (n = 1), *Leclercia adecarboxylata* (n = 1).


ISSN: 1470-9465

Statistical significant differences were detected between patients with and without BGNC in the proportion of patients older than 65 years (81.3% vs 42.5%), the antecedent of recent animal bite (18.8% vs 0.7%), the presence of immunosuppression (12.5% vs 1.8%), the presence of haematological illness (12.5% vs 0.7%), and the degree of leukocytosis at admission (8989 ± 3736 vs 12690 ± 6028 cel/$\mu$L).
**Conclusions:** BGNC is frequently detected in our patients. Age older than 65 years, the existence of immunosuppression, the existence of haematological illness, and the antecedent of animal bite are more frequent among patients with BGNC. Patients with BGNC had a lower degree of leukocytosis at admission. These factors should be borne in mind to select empiric therapy for patients with cellulitis.

## P1865

## Is erysipelas-associated *Tinea pedis* a site of streptococcal colonisation?

L. Hocqueloux, L. Bret, M. Niang, D.M. Poisson *(Orleans, FR)*

**Objectives:** *Tinea pedis* is considered the most frequent portal of entry of erysipelas of legs (SEL) but whether it is the site of streptococcal colonisation is unknown.
**Methods:** From June to October 2005 we prospectively searched for clinical *Tinea pedis* in patients hospitalised in our infectious diseases ward for SEL (acute and unilateral feature with fever were only retained). All patients had bacteriological samples on inter-digital spaces of both feet (SEL side and contra lateral side).
**Results:** Fifteen patients were included. All but one were treated by intra-venous Penicillin-G followed by oral amoxicillin. On SEL side: *Tinea pedis* was found in 10/15 (67%) and, when present, streptococcal colonisation (C or G *streptococcal* groups) was found in 7/10 (70%), although streptococcal colonisation was never found (0/5) in its absence. On contra lateral sides : no streptococcal colonisation was found without *Tinea pedis*, which was observed in 7/15, with streptococcal colonisation in 6/7. Then there is a strength statistical association between streptococcal colonisation and *Tinea pedis*, on SEL side ($p = 0.025$) as well as on contra lateral side ($p = 0.003$). In one patient blood-cultures yielded with the same Streptococcus than found in foot samples.
**Discussion:** Streptococcal colonisation of *Tinea pedis* is a common finding on both feet of patients hospitalised for SEL. Whether inter-digital colonisation is a primary stage of invasive disease remains unproved. In our experience, a strain of Streptococcus that colonised inter-digital space was isolated in patient's blood, suggesting this hypothesis may be true in some cases. If confirmed, this concept could lead to a new strategy for secondary prophylaxis of recurrent SEL by decontaminating streptococcal colonisation of *Tinea pedis*.
**Conclusion:** Patients hospitalised for SEL with *Tinea pedis* often exhibit streptococcal colonisation of feet's inter-digital spaces. Curiously C and G groups, but no A group, are over-represented in this setting.

## P1866

## Bacteriology of acute erysipelas

T. Siljander, S. Vähäkuopus, M. Karppelin, J. Syrjänen, M. Toropainen, J. Kere, J. Vuopio-Varkila *(Helsinki, Tampere, FI; Huddinge, SE)*

**Objectives:** This study aims at identifying individuals and families suffering from acute erysipelas and streptococcal infections and focuses on the molecular properties of *streptococci* involved in infections and carriage.
**Methods:** From March 2004 to March 2005, 90 patients with 98 disease episodes presenting with acute erysipelas and 89 age and sex matched control subjects were recruited, interviewed and clinically examined at Tampere University Hospital. In addition, 48 family members and other relatives were recruited. Bacterial swabs were taken from throat and from the affected skin of index patients (blisters, wounds or abrasions). From the samples, *betahemolytic streptococci* and *S. aureus* were isolated and identified. The *streptococci* were classified for their group antigens (A, C, F and G), the species was determined and the isolates were emm sequenced and T-serotyped when possible.
**Results:** From the 235 throat samples, *betahemolytic streptococci* or *S. aureus* grew in 19% (19/98) of index patients, 31% (12/39) of their household members, 11% (1/9) of other relatives and 16% (14/89) of control subjects. *Streptococcus* group G (GGS, 6/19), A (GAS, 2/19) and F (2/19) were more frequently found in the throat samples of index patients. Similarly, in the household members' samples GGS (5/12) was found more commonly. In contrast, in controls' samples GGS was missing. Of the 73 skin samples from index patients, 48% were culture positive and of these groups G (18/35, 51%), A (5/35, 14%) and G + A (1/35, 3%) were found either alone or in combination with *S. aureus*. Among GGS, 11 different emm types were found; stG6, stG480 and stG643 predominated. Among GAS, 8 types were found, emm81 predominated. One patient had the same GGS isolate in throat and skin. Six patients had recurrent infections during the study; two of them with 3 disease episodes. Of the 35 culture positive skin samples, 12 were taken from the erysipelas infection focus (42% positive for GGS) and 23 from another site (61% positive for GGS), e.g. wound, intertrigo, between toes or an unknown site.
**Conclusion:** A predominance of GGS was seen in the throat of erysipelas patients and their families whereas GGS was not present in control subjects. GGS, instead of GAS, also seems to predominate in erysipelas skin lesions. Several emm types were present in both groups and there was no clear predominance of a distinct type. The recurrent nature of erysipelas became evident also during this study.

## P1867

## The evaluation of Fournier's gangrene severity index score in 11 patients

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

**Objectives:** Fournier's gangrene is synergistic necrotizing fasciitis of the perineum and abdominal wall along with the scrotum and penis in men and the vulva in women. It is rare but life-threatening process. In this study we identify effective factors in the survival of patients with fournier's gangrene and to determine the accuracy of the fournier's gangrene severity index score (FGSIS).
**Methods:** We evaluate 11 patients with fournier's gangrene who were threated and followed up from us between January 2005 and September 2005 in the department of General Surgery prospectively.
**Results:** The results were evaluated in two groups: those who died (n: 3) and those who survived (n: 8). No statiscally significant difference was found between the age of the survivors and those who died. The admission and final laboratory parameters that correlated statiscally signinificant with outcome includes leucocyte count, hematocrit, urea, creatinine, lactate dehydrogenase, bicarbonate and albumin.

The mean FGSIS for survivors was 2.1 ± 1.3 compared with 14.3 ± 4.6 for nonsurvivors. There was a strong correlation between the FGSIS and the death rate (p¡Ü 0.0001). *Pseudomonas aeruginosa* was isolated from wound cultures in nonsurvivors.
**Conclusion:** Fournier's gangrene is an infectious disease affecting on ever aging population of patients. Patients metabolic status and the extent of disease at presentation ia an important factor in the prognosis of Fournier's gangrene. Because of this reason the FGSIS be used clinically to evaluate the therapeutic options and asses the results.

## P1868

## Clinical outcomes of *Staphylococcus aureus* infections in the community

S. Davis, M. Perri, A. Singh, K. Spiers, M. Hayden, R. Muder, M. Zervos *(Detroit, Royal Oak, Chicago, Pittsburgh, US)*

**Background:** The purpose of this study is to gain a better understanding of the clinical outcomes of community associated MRSA (CA-MRSA).
**Methods:** Over a 2 year period (October, 2003 – 2005) patients with *S. aureus* infections originating in the community were identified. Data was extracted from medical records and patient interviews. Outcomes of patients with CA-MRSA, health care associated MRSA (HA-MRSA) and community associated MSSA (CA-MSSA) were compared.
**Results:** Patients with CA-MRSA (n = 102), HA-MRSA (n = 102) and CA-MSSA (n = 102) had a median age of 46, 62 and 52 respectively (p < 0.001). Sites of culture were skin/soft tissue (75, 74 and 92%), respiratory tract (3, 4, and 3%), blood (4, 7 and 1%), urine (3, 11 and 0%), and other (3, 2 and 2%). 30-day mortality was 0% in this population. 50% of patients received antibiotic therapy alone, 5% surgery alone, 30% antibiotics + surgery, 3% other therapy, and 12% no treatment. The most common antimicrobial classes received were vancomycin (27%), beta-lactams (23), fluoroquinolones (11), and cotrimoxazole (10) with 14% of patients receiving multiple agents. Median duration of antibiotic therapy was 12, 15, and 10 days, in the CA-MRSA, HA-MRSA and CA-MSSA groups respectively. 47, 82, and 57% received adequate antimicrobial therapy (p < 0.001). Hospital admission was required in 46, 57, and 17% of patients (p < 0.001). Clinical success rates of initial therapy were 61, 63, and 84% (p < 001), and recurrences were more common in the CA-MRSA group, (18, 0, and 6%, p < 001). Characteristics associated with outcome are listed in Table 1. In multivariate analysis, presence of MRSA and diabetes were predictive of clinical failure.

Table 1. Univariate analysis of risk factors for clinical outcome

| Characteristics | | Success (n=212) n (% in predictor) | Failure (n=94) n (% in predictor) | *P* |
|---|---|---|---|---|
| Group | CAMRSA | 62 (61) | 40 (39) | <.001 |
| | HAMRSA | 64 (63) | 38 (37) | |
| | CAMSSA | 86 (84) | 16 (16) | |
| Infection Site | Blood | 9 (75) | 3 (25) | NS |
| | Skin | 166 (68) | 79 (32) | |
| | Respiratory | 9 (90) | 1 (10) | |
| | Urine | 10 (71) | 4 (29) | |
| | Other | 18 (72) | 7 (28) | |
| *Comorbid conditions* | | | | |
| Diabetes mellitus | | 48 (61) | 31 (39) | .040 |
| Non-diabetic | | 164 (72) | 63 (28) | |
| Renal disease | | 11 (52) | 10 (48) | .071 |
| Normal renal function | | 201 (71) | 84 (29) | |
| Treatment | none | 26 (70) | 11 (30) | NS |
| | Antibiotics alone | 110 (71) | 44 (29) | |
| | Surgery alone | 11 (73) | 4 (27) | |
| | Antibiotics + Surgery | 57 (63) | 34 (37) | |
| | Other | 8 (99) | 1 (1) | |

**Conclusion:** In the community setting, MRSA infections are associated with an adverse impact on outcome compared to MSSA infections and patients with CA-MRSA are significantly less likely to receive adequate antibiotic therapy.

## P1869

## Microbiological analysis of root canals associated with periapical abscesses and the antimicrobial susceptibility of isolated bacteria

S. Ozbek, A. Ozbek, M. Koseoglu, S. Evcil, A. Erdogan, A. Ayyildiz *(Erzurum, TR)*

**Objective:** The periapical abscess is a collection of pus in the pulp or around the root of teeth. Many odontogenic infections can be managed without antimicrobial therapy or bacteriologic investigation. However, when an acute bacterial infection has progressed or antimicrobial therapy might be of benefit to patients, antibiotics are prescribed. We aimed to identify microorganisms in root canals with periapical abscess and the antimicrobial susceptibility profile of them and to revise antimicrobial treatment protocols when antimicrobials is used empirically.
**Methods:** 30 patients with odontogenic infections included in this study. The microbiologic investigation was performed under strict aseptic conditions. A standardize routine of root canal therapy was instituted, and in each case a single root canal was sampled. In multirooted teeth only the largest canal was sampled to preserve the identity of a single endodontic/ microbiologic ecosystem. For microbial sampling, two sequential paper points were introduced into the full length of the canal, and kept in place for 1 min. One of the paper points was used for aerobic culture and the other one for anaerobic culture. To identify isolated bacteria, whole bacterial fatty acid profiles were evaluated by using Microbial Identification System. Antimicrobial susceptibility results were obtained by disc diffusion test for aerobics, and E-test for anaerobics.
**Results:** Totally 156 bacterial strains were isolated. 86 of them were aerobic and 70 of them were anaerobic. 18 or 60% of cultured specimens yielded mixed (aerobic and anaerobic) species. The most prevalent bacteria were *Staphylococcus* spp. as aerobic, *Peptostreptococcus prevotii* and *Streptococcus morbillorum* as anaerobic.
**Conclusion:** Beta-lactam antibiotics combined with beta-lactam inhibitor (amoxicillin-clavulanic acid) had a quite effect on Gram (+) and (–) aerobics. When we take into consideration that beta-lactam antibiotics stimulate production of beta-lactamase, amoxicillin-clavulanic acid combination appears a good first step antimicrobial. Clindamycin may be second alternative for that purpose. For anaerobics, cefoxitin and metronidazol had well effect. Although imipenem and piperasilin-tazobactam are perfect, they should not be first step of therapy. Due to the frequency of mixed infections, a combination of amoxicillin-clavulanic acid and metronidazol or a combination of clindamycin and metronidazol considered to have well effect for mixed infections.

## P1870

## Human infections due to *Pasteurella multocida* in a tertiary care hospital: a 12-year review

A. Christidou, S. Maraki, Z. Gitti, K. Magurean-Zervaki, Y. Tselentis *(Heraklion, GR)*

**Objective:** *P. multocida* infections in humans are well known as animal derived infections. The purpose of this retrospective

study is to review the spectrum of *P. multocida* infections in our centre.
**Methods:** We studied the medical records of all patients who had positive cultures for *P. multocida* between 1993 and 2004. Demographic, epidemiological, clinical and microbiological data including age, sex, animal exposure, site of infection, underlying diseases, type of therapy and outcome were evaluated. All isolates were identified by standard conventional microbiological methods. Antibiotic susceptibility testing was performed by the disk diffusion method onto Muller-Hinton agar supplemented with 5% sheep blood and the MICs of the antibiotics tested were determined by the E-test method.
**Results:** Thirteen cases of *P. multocida* infections were diagnosed during this period. The male to female ratio was 10:3 and most patients (62%) were >70 years of age. Respiratory tract infections were most commonly encountered (61.5%), followed by soft-tissue infections (30.8%) and septicemia (7.7%). Underlying disease was present in 8 (61.5%) patients. Among them, 4 presented a kind of malignancy. Bullous pemphigoid, mitral valve stenosis, coronary disease, chronic obstructive pulmonary disease, and intracranial haemorrhage served also as predisponding factors. A traumatic animal exposure was reported in only 3 patients and non-traumatic in 2 cases. All isolates were susceptible to beta-lactams (penicillin, amoxicillin, amoxicillin/clavulanic acid, cefepime, cefuroxime, ceftriaxone, imipenem, and meropenem), quinolones (ciprofloxacin, norfloxacin, levofloxacin, and sparfloxacin), chloramphenicol, tetracycline, trimethoprim/sulfamethoxazole and 54% were intermediately resistant to aminoglycosides (gentamicin). Appropriate antibiotic therapy was administered to all patients and a clinical response was observed in 10 (77%) of them. Mortality rate was 23%.
**Conclusions:** *Pasteurella multocida* must be considered as a possible etiology for a variety of infections, even without an obvious animal exposure. Although this organism is susceptible to a large spectrum of antibiotics, a failure to treatment may be recorded especially in severe infections and in compromised patients.

## P1871

## Infections caused by *Nocardia cyriacigeorgici* in Zaragoza, Spain: identification and antibiotic susceptibility

C. Villuendas, B. Moles, V. Rodriguez-Nava, A. Couble, F. Laurent, M. Revillo, P. Boiron *(Zaragoza, ES; Lyon, FR)*

**Objectives:** Nocardia species known to date differ in their clinical presentation, antibiotic resistance patterns and geographic distribution. *Nocardia cyriacigeorgici* is a recently described species.The aim of this study is to analyse the identification results, antimicrobial susceptibility together with the clinical data, of 13 *N. cyriacigeorgici* clinical isolates, recovered from 1998 to 2004 in our laboratory.
**Methods:** Identification of *Nocardia* spp. isolates was achieved in our laboratory on the basis of the following: visualization of the colony, Gram stain and parcial acid-fast positivity by modified acid-fast staining, casein, xanthine and tyrosine hydrolysis, opacification of Middlebrook 7H10 agar, production of arylsulphatase after 14 days incubation and antimicrobial susceptibility pattern.Identification at species level was achieved by 16S rDNA gene sequencing (Laboratoire de Mycologie. Faculté de Pharmacie. Lyon. France)Antimicrobial susceptibility tests included commercial broth microdilution (EMIZA 9 EF Sensititre®) and gradient strip agar dilution (E-test AB Biodisk®). Interpretation of results was done according to NCCLS standard guidelines.
**Results:** In the six years of study, 129 isolates of *Nocardia* spp. were recovered, 13 of them belonging to *N. cyriacigeorgici* species (10%). *N. cyriacigeorgici* represents the third species in frecuency in our serie, after *N. abscessus* and *N. farcinica*. The 13 strains were recovered from 13 patients, 12 from respiratory specimens and one from blood-culture.Pneumonia was the most frequent clinical manifestation, being COPD and previous corticosteroid therapy the most common predisposing conditions. All *N. cyriacigeorgici* isolates showed susceptibility to: amikacin, tobramycin, cefotaxime, imipenem, trimethoprim-sulfamethoxazole and linezolid, and resistance to: amoxicillin-clavulanic acid and ciprofloxacine.
**Conclusion:** *N. cyriacigeorgici* is not an infrequent cause of nocardiosis in our geographical area. The uniformity showed in the antimicrobial profile can be useful for iťs identification. In our hospital, patients with COPD and receiving corticoid therapy is the most important group of risk for adquiring *N. cyriacigeorgici* infection. Whit the technics available in our laboratory the isolates were identified as *Nocardia* spp. and identification at species level was only possible by phylogenetic analysis using 16 rDNA sequencing.

## P1872

## High frequency of single-step resistance mutations in *Nocardia farcinica* exposed to quinolones

U.S. Jensen, J.D. Knudsen, K. Schønning *(Hvidovre, DK)*

**Objectives:** *Nocardia farcinica* infections often require prolonged antibiotic therapy and perorally administered agents are desirable. Isolates commonly display *in vitro* susceptibility to quinolones when tested by disc diffusion methodology. In the present study, we investigated the activity of three different quinolones (ciprofloxacin, levofloxacin and moxifloxacin) against *N. farcinica* and assessed the robustness of their activity by determining the frequency of single step resistant mutants when exposed to inhibitory concentrations of quinolones.
**Methods:** 10 isolates of *N. farcinica* were used in the study; correct identification to the species level was verified by 16S rDNA sequencing. MICs of ciprofloxacin, levofloxacin and moxifloxacin against *N. farcinica* as well as *S. aureus* ATCC 25923 and *E. coli* CCUG 17620 were determined by the agar dilution method using inocula of approximately 10.000 CFU and 48 h of incubation. Single step mutation frequencies were determined by heavily inoculating selective agar plates containing quinolone at a concentration of 4x MIC and counting resistant colonies after 5 days incubation. Inoculum was quantified by seeding a dilution series of the inoculum employed on unselective plates and counting colonies after 48 h of incubation and frequencies were calculated by dividing the number of resistant colonies by the number of CFU present in inoculum.
**Results:** When MICs were determined by agar dilution method all quinolones displayed roughly the same potency against *N. farcinica* isolates (MICs between 0.25 and 4). As expected moxifloxacin were the most potent quinolone against *S. aureus*. However, all three quinolones selected for single step resistant mutants, the frequency of which was higher for ciprofloxacin ($\sim 10^{-6}$) than for levofloxacin ($10^{-7}$–$10^{-8}$), which again was higher than for moxifloxacin ($10^{-8}$–$10^{-9}$). However, even for moxifloxacin the frequency against *N. farcinica* was comparable to the single step mutation frequency of ciprofloxacin against *S. aureus* ($10^{-9}$).

**Conclusions:** Although quinolones may exhibit activity against *N. farcinica*, *N. farcinica* is capable of rapid development of resistance. Therefore, quinolones should probably be avoided, at least as single agents, in the treatment of *Nocardia* infections.

### P1873

### Correlation between clinico-laboratory findings and a positive IgM ELISA test for leptospira: a retrospective study

E. Mendrinou, P. Goudas, A. Regli *(Patra, GR)*

**Objective:** To correlate a positive Elisa test for IgM antibodies against *Leptospira* with the clinical and laboratory findings in patients with suspected Leptospirosis.
**Method:** We retrospectively analysed the history, clinical course and laboratory findings in a total of 420 patients, with suspected Leptospirosis. All patients fulfilled the criteria for clinical diagnosis of Leptospirosis. From the 420 patients, 21 had to be transferred to the dialysis unit for haemodialysis and 6 patients had to be admitted to the Intensive Care Unit (ICU) due to severe pulmonary haemorrhage. Serum samples from all patients were tested for IgM antibodies against *Leptospira*.
**Results:** From the total of 420 patients death occurred to only four, due to respiratory failure from severe pulmonary haemorrhage. The rest of the patients recovered completely. From the total of 420 patients 25 had a positive Elisa IgM test for Leptospirosis (5.95%). However, from the 21 patients that were transferred to the dialysis unit, 8 had a positive Leptospirosis test (25%) and from the six patients admitted to the ICU, three had a positive test (50%). Among other laboratory findings there was a stronger correlation between very low platelet levels (<30.000 mm3) and very high blood bilirubin levels (>20 mg/dl) with a positive test for Leptospirosis. All 25 patients with a positive test had less than 30.000 platelets per mm3 and 18 had blood bilirubin over 20 mg/dl. The differential diagnosis of icterohemorrhagic fevers includes a vast number of pathogens, some of which are untraceable with the common laboratory methods. In our study, from the total of 420 patients, only 5.95% had a positive test for Leptospirosis. In 92 of the rest 395 patients, many different pathogens were traced, most of them being several kinds of viruses (CMV, EBV), *Brucella* and *Coxiella*. In 303 of the patients no pathogen was traced.
**Conclusions:** Taking into consideration the high sensitivity of the Elisa test we conclude that: 1. Icterohemorrhagic Leptospirosis comprises only a small subtotal of icterohemorrhagic fevers; 2. There is a correlation between higher levels of bilirubin and/or very low platelet levels with Leptospira infection; 3. There seems to be a correlation between Leptospira infection and severity of icterohemorrhagic fevers.

### P1874

### Evaluation of continuous ambulatory peritoneal dialysis-related peritonitis attacks in Ankara

S. Tekin Koruk, M.A. Yetkin, I. Koruk, F.S. Erdinc, S. Sahan, N. Tulek, M. Duranay, A.P. Demiröz *(Ankara, Konya, Samsun, TR)*

**Objectives:** Peritonitis is a common clinical problem that occurs in patients with end stage renal disease treated by peritoneal dialysis. The aims of this study were to assess demographic aspects, rates of peritonitis, causative organisms, clinical outcomes and treatment approach for continuous ambulatory peritoneal dialysis (CAPD) -related peritonitis cases.
**Methods:** Seventy cases of peritonitis occurred in 55 patients treated in Infectious Diseases and Clinical Microbiology Department between May 2003 and April 2004 were enrolled into this study. The mean age of the patients was 48.4 years (range 16–77 years). Cloudiness of the peritoneal dialysis fluid and/or abdominal pain were considered suggestive of peritonitis and were confirmed by cell count and culture. Baseline cell count, Gram stain, and cultures were obtained, and repeated with periodic follow-up.
**Results:** The overall incidence of peritonitis was 2.5 ± 2.5 episodes/patient-year. In 52.7% of patients there were only one peritonitis attack, where as in 47.3% of them had two or more attacks. Age, gender, education and profession of the patients have not been found as a risk factor in peritonitis attacks.The most common presenting symptoms of the patients were abdominal pain, cloudiness of the peritoneal dialysis fluid, nausea and vomiting. Peritoneal dialysate fluid white blood cell count was 1773 ± 1224/mm3 in 70 episodes. Cultures were positive in 51 (72.9%) peritonitis episodes; coagulase-negative staphylococci was the most common organism (%22.8), followed by *Staphylococcus aureus* (%21.4), 19 episodes (%27.2) had negative culture results. There was a statistically significant decrease in serum CRP and ESR levels and at the end of the treatment when compared with the levels on admission.At the end of the study, 61 episodes of 70 peritonitis cases were treated with IP cefazolin and gentamicin protocol. Seven of the patients did not respond initiate therapy and the therapy was converted to IV protocol. Seven episodes were treated with IV antibiotics on admission for medical reasons (systemic infection and/or concurrent exit-side or tunnel infection). There were two deaths. Two catheters were removed and the patients were transferred to haemodialysis programme.
**Conclusion:** Despite all technical improvements during recent decades peritonitis is still the major complication of CAPD. For the accurate treatment of complications, causative organisms and their antimicrobial susceptibilities must be known.

# Paediatric viral infections

### P1875

### Particularities of measles outbreak in Constanta, Romania

C.S. Cambrea, S. Rugina, M. Ilie, C. Marcas, D. Tanase, I. Cretu, C.N. Rugina *(Constanta, RO)*

**Background:** In Romania Measles vaccination is included in National Program of Vaccination, but in nomad collectivities (gipsy) the program of vaccination it's very difficult to apply. In the last 6 years in Constanta County we didn't noticed any case of measles, but starting June 2005 appeared an increasing number of children with measles.
**Objective:** To present some particular aspects of measles outbreak which starts 6 months ago.
**Material and method:** We performed a prospective and retrospective study about children hospitalised in Children Infectious Diseases Department of Constanta Infectious Diseases Clinical Hospital.
**Results:** Over a period of 5th and half months we hospitalised in our department 628 cases of children with measles. The

evolution on months was: 26 cases in June, 85 cases in July, 123 cases on September, 209 cases on October and 146 cases in the first 15 days of November. The age of children was between 2 months and 11 years, but the majority (91.56%) was less than 2 years old. The majority (95.38%) were gipsy children. Children from family foci were also majority (82.48%). Clinical diagnosis was confirmed by serological diagnosis in all cases. The most frequent complication was: pneumonia and bronchopneumonia (96.97%), hepatic injury (62.73%), and laryngitis (34.23%). Hemorrhagic rash was present in 4.14% cases. Koplick spot was found in 74.56% cases. Measles was associated with Streptococcal tonsillitis in 47.92% cases, with oral candidiasis in 20.22% cases and with pulmonary tuberculosis in 8.12% cases. Severe forms of evolution were observed in complicated cases with: encephalitis (0.79%) or bronchopneumonia (5.89%), which required intensive care unit survey. We registered only one deceased, in a case of measles encephalitis; this child presented also pulmonary tuberculosis.
**Conclusions:** The majority of cases were among gipsy children and presented slight and medium forms of evolution (93.31%). In gipsy collectivities even it's very difficult, it's necessary to perform a better epidemiological survey for correct implementation of vaccination program.

## P1877

## Human metapneumovirus infection in children presenting to hospital in Belfast

E. McHenry, P.V. Coyle, P. Jackson, M. Shields *(Belfast, UK)*

**Objectives:** Acute respiratory tract infections are a major cause of morbidity and mortality worldwide, however 15–35% of cases of bronchiolitis and pneumonia in children are of uncertain aetiology. hMPV is a recently described respiratory pathogen belonging to the same Paramyxoviridae family as respiratory syncytial virus (RSV). We aimed to describe the local epidemiology of paediatric hMPV infections and assess the impact of the routine use of a multiplexed reverse-transcriptase polymerase chain reaction method for virus detection from respiratory samples, which was introduced in July 2003.
**Methods:** A retrospective computer and chart audit of all children attending the Royal Belfast Hospital for Sick Children who tested positive for hMPV from July 2003 to July 2004 was performed. Respiratory samples were tested routinely for twelve common respiratory pathogens.
**Results:** Over the study period, of 587 samples processed, 40 patients tested positive for hMPV with 39 case notes available for inspection. Median age was 6 months (range 3 weeks to 13 years). Thirty-six cases were community-acquired and 23 (60%) patients had significant co-morbidities. Cough was the predominant reported symptom. Chest X-Ray was performed in 31 cases, of which 28 showed abnormalities. Bronchiolitis (22/39) was the commonest initial clinical diagnosis. The majority (69%) of patients received antibiotic therapy, but a convincing bacterial pathogen was isolated in less than half of these cases. Thirty patients were admitted for management. More than one virus was identified from 14 patients, with rhinovirus being the predominant co-infection. Overall, the average length of stay was 6.6 days. However, where hMPV was the sole pathogen identified, average length of stay was 3.1 days.
**Conclusion:** Our data suggests that hMPV infections are more common in children with underlying co-morbidities. The rate of radiological imaging was higher than expected and perhaps is a reflection of the patient population or the degree of severity of illness. Nosocomial acquisition occurred in 3 cases, which has implications for patient cohorting. Effective diagnosis of viral respiratory pathogens is necessary to elucidate their epidemiology and assess clinical impact. Moreover, such data is crucial for effective infection control in hospitals and may ultimately allow more rational prescribing of antibiotics.

## P1878

## Prevalence of parainfluenza and influenza viruses amongst children with upper respiratory tract infection

U. Arslan, D. Findik, I. Tuncer *(Konya, TR)*

**Objective:** Viruses are a frequent cause of upper respiratory tract infections in children. Among the respiratory viruses, influenza viruses are known to cause outbreaks globally. The present study was carried out to identify the influenza virus serotypes causing acute respiratory infection in children attending univesity hospital in Konya in Turkey.
**Methods:** Thorat swabs were collected from 117 acute viral upper respiratory infection suspected children attending the out patient clinic of Meram Medical Faculty Hospital. Two swabs were taken fron each chidren and one of the swabs was used for bacteriological cultures and if these were negative the other one was used for viral diagnosis. Totally 100 bacteriological cultures negative swabs were investigated by Real-time PCR for the presence of Parainfluenza 1, 2 and 3, Influenza A and B.
**Results:** One or more viral pathogens were detected in 24 children, with Parainfluenza 1 9% being the most commonly identified virus. Parainfluenza 2 in 7% and Parainfluenza 3 in 5%, Influenza A were identified in 5% and Influenza B in 1%. From the specimens of 3 children more than one virus detected.
**Conclusion:** The influenza viruses cause morbidity and mortality among children and elderly. This study analysed the occurrence of influenza and paranfluenza respiratory ifections due to influenza and paranfluenza viruses. Molecular methods used directly on clinical material have an important role in the rapid diagnosis and surveillance of influenza viruses and can be applied in clinical practice for correct diagnosis and administration of effective treatment.

## P1879

## Clinical features of children with adenovirus serotype 3 infection

C-H. Lin, Y-C. Huang, C-G. Huang, T-Y. Lin, T-Y. Lin
*(Kweishan, Taoyuan, TW)*

**Purpose:** To define clinical manifestations and laboratory findings of children with adenovirus serotype 3 infection.
**Materials and Methods:** A total of 499 children with a diagnosis of adenovirus infection based on throat virus culture were treated in Chang Gung Children's Hospital from January 2004 to May 2005. Serotypes were determined in 197 strains. One hundred and forty-seven of them were serotype 3. The other serotypes included serotypes 1, 2, 5, 6, 7, 8 and 35. The demographics, clinical presentations and laboratory findings of the 147 patients with serotype 3 were presented.
**Results:** The mean age was 4 y7 m, ranging from 5 months to 12 y2 m. Seventy percents of children with serotype 3 infection clustered between October 2004 and January 2005. The mean duration of a positive culture result was 8.5 days. The mean duration of fever was 6.0 days, with 4 days before admission. Forty (27%) children were treated as outpatients. The mean length of hospital stay was 5.4 days. The 3 most common diagnoses were exudative tonsillitis (25%), pneumonia or bronchopneumonia

(24%) and pharyngoconjunctival fever (12%). The 3 most common symptoms and signs were fever (100%), cough (88%) and coryza (74%). Neurologic complications were noted in 2 children. Eighteen children had documented coinfection (including virus, bacteria and Mycoplasma pneumoniae). Leukopenia (WBC < 5000/microliter) was noted in two of 111 cases while leukocytosis (WBC > 15000/microliter) in 32 (29%). Six (5.5%) of 110 cases had a normal serum C-reactive protein (CRP) level (<10 mg/L), while 80% of 110 children had a serum CRP greater than 40 mg/L. Seventy (47.6%) of 147 children ever received antibiotics therapy. The outcomes were excellent in these cases.
**Conclusion:** Recognizing that children with adenoviral serotype 3 infection may present with prolonged high fever, leukocytosis and elevated CRP, which mimics bacterial infection, the clinician may not prescribe unnecessary antibiotics for these children.

## P1880

## Infectious mononucleosis-like syndrome in a children's hospital in Greece

I. Varzakakos, K. Papavsileiou, H. Papavasileiou, A. Makri, D. Sbokou, Z. Karakatsani, A. Voyatzi *(Athens, GR)*

The Infectious Mononucleosis like syndrome (IM) is an acute febrile disease of older children and young adults, and is characterized by lymphadenopathy, tonsillitis, splenomegaly, liver dysfunction and by the presence of peripheral lymphocytosis with >10% atypical lymphocytes. Epstein –Barr virus (EBV) is responsible for over 90% of the cases, Cytomegalovirus (CMV) for 5%–7% and Toxoplasma gondii <1%.Herpes simplex, rubella and adenovirus are rare. The infection is usually characterized by mild symptoms. However in some cases the clinical manifestations may be rather atypical and severe.
**Objective:** To determine the prevalence of IM like syndrome among patients in a children's hospital and its possible association with etiologic factors, age, major symptoms and atypical manifestations.
**Material and methods:** During a one-year period (January to December 2004) a total of 700 samples were examined in our laboratory. The study population was children between 1–15 years old, which either examined in the outpatient's clinic or hospitalized. All serum specimens were examined by1. Indirect Immunofluorescence for the presence of IgG and IgM antibodies against the viral capsid antigen (VCA) EBV, 2.Immuno Chemistry luminescence for the detection of IgG and IgM antibodies to CMV and 3.EIA for the detection of IgG,IgM abs of Herpes simplex I and II and Toxoplasma gondii.
**Results:** Of the 700 children examined 43 (6.1%) were found positive for IgG and IgM VCA antibodies and 28(4%) showed positive specific IgG and IgM antibodies for CMV. These patients had one or more of the primary following symptoms: fever (87%), lymphadenopathy (80%), pharyngalgia (50%), cough (15%), skin eruption (9%). Atypical manifestations as meningoencephalitis were found in two children one aged 20 months (caused by EBV) and the other of 7 years old (caused by HSV I) confirmed by PCR. The laboratory data showed positive serology for EBV and CMV infection, the existence of atypical lymphocyte (76%), LDH, ASAT and ALAT were moderately elevated (44 %) and CRP increased (18%).
**Conclusion:** The frequency of IM like syndrome in Greece, though it's relatively low, it's not rare. The above results suggested that EBV, CMV, HSV should be considered in any young patient with IM and acute neurological illness of uncertain etiology.

## P1881

## Detection of enterovirus, parvovirus B19 and human herpes virus type 6 by real-time PCR in blood samples of infants with febrile syndrome

M. García-Álvarez, M.D. Folgueira, C. Prieto, M.J. Babiano, P. Rojo, J.R. Otero *(Madrid, ES)*

**Objectives:** Enterovirus, parvovirus B19 and human herpes virus type 6 (HHV-6) are a common cause of infection in young infants. The objective of this study was to determine what portion of the infants who received a clinical diagnosis of febrile syndrome have a viral etiology by these three genera of viruses.
**Methods:** Ninety-six patients were included in the study, all of them were admitted to the pediatric casualty of a tertiary care hospital, and all of them presented a febrile syndrome without a clear focus of infection (urinary tract, lung and meningeal infections were discarded). The assay was carried out in 96 blood samples by real-time PCR. DNA was isolated from 200 $\mu$l of blood by semi-automated system MagNA Pure LC Total Nucleic Acid Isolation kit (Roche Diagnostics, Nederland BV). PCR was performed in a LightCycler instrument (Roche Molecular Biochemicals) by a uniform cycling parameters: 10 min at 95°C for polymerase activation, and 50 cycles of 15 s at 95°C and 60 s at 60°C for amplification of the specific target sequence (5 UTR gene for enterovirus, VP2 gene for parvovirus B19 and DNA polymerase gene for HHV-6). PCR product formation was detected continuously by the use of TaqMan probes.
**Results:** A viral amplification was detected in 52 (54%) of the 96 patients included in this study. Enterovirus was detected in 12 (12.5%) of the patients, parvovirus B19 in 10 (10.4%) and HHV-6 in 35 (36.4%). In five cases two viral amplifications were detected at the same time: 3 parvovirus B19/HHV-6 and 2 enterovirus/HHV-6. The mean age of the patients was 2 years old (range from 24 days to 14 years). In group of infants <6 months old (n = 27) there were 7 enterovirus and 6 HHV-6. In the infants from 7 months to 3 years old (n = 53) there were 3 enterovirus, 3 parvovirus and 24 HHV-6. In the last group of infants >3 years old (n = 16) there were 2 enterovirus and 7 parvovirus B19.
**Conclusions:** Viral infections are an important cause of sepsis in infants admitted to hospital. Enterovirus was the most frequent virus detected in infants <6 months, parvovirus B19 the most frequent in children >3 years old, and the HHV-6 was detected in all age groups. Qualitative real-time PCR in blood is a rapid and sensitive method for diagnosis of enterovirus and parvovirus. However, is not the better method for diagnosis of HHV-6, a latent virus, in which this technique is not capable of distinguish between recent and acute infection.

## P1882

## Molecular epidemiology and clinical features of rotavirus infection in Iranian children

M. Taremi, F. Farahtaj, L. Gachkar, E. Mazaheri Tehrani, H. Edalatkhah, A. Fayaz, M. Zali *(Tehran, IR)*

**Objectives:** Group A rotaviruses are a major cause of acute gastroenteritis in infant and young children worldwide. In this study, the molecular epidemiology and clinical features of rotavirus infection in Iranian children was investigated.
**Methods:** Between February 2004 to January 2005, thirty hundred and seventy two diarrhoea stools from children under 5-years-old with acute diarrhoea that attended the biggest paediatric hospital in Tehran (Iran), were analysed

using ELISA, electropherotyping and reverse transcription-polymerase chain reaction (RT-PCR).
**Results:** Ninety-four samples (25.3%) were positive for the presence of rotavirus either by PAGE, ELISA, or both. According to PAGE, the predominant electrophoretic pattern detected was the long profile 62 of 67 (92.5%) followed by the short electropherotype five of 67 (7.5%). Out of the positive samples, 49 were further characterized by RT-PCR typing assay for identification of G types, resulting in 29 strains of G1 genotype while 20 samples could not be assigned a G type. All of G1 genotypes had a long RNA electropherotype. Among the patients with rotavirus infection, 23 (24.5%) required hospitalization. Watery diarrhoea (92.6%), vomiting (73.4%) and fever (64.9%) were significantly more frequent in children suffering from rotavirus gastroenteritis. Seven out of 94 rotavirus-positive patients had severe dehydration ($P < 0.0001$). Rotavirus infection mostly affected children under 2 years of age with a peak incidence of 40% in children 1–2 years of age and it occurs year round with a seasonal pattern: more frequently during winter (46.2%).
**Conclusion:** This study revealed that rotavirus is an important etiological agent of acute gastroenteritis in Tehran. We found that a major proportion of the specimens were untypeable. Improved detection and characterization of incompletely typed strains will help to develop comprehensive strain information that may be required for tailoring effective rotavirus vaccines.

## P1883

## Serological study of prevalent rotaviruses in Tehran

E. Habibi, S. Ghorbani, A. Jarollahei, Z. Habibi *(Tehran, Zanjan, IR)*

**Objectives:** Rotaviruses are icosohedral and non-enveloped viruses that belong to reoviridae family which consist of three layers of protein surrounding 11 segments of dsRNA. Rotavirus is one of the most important agents of acute gastroenteritis in children. In this survey, the most prevalent serotypes in Tehran and seasonal distribution in a year were detected.
**Methods:** In this study, a total number of 180 specimens of faecal samples of children and infants with acute gastroenteritis were collected from two children hospitals in Tehran. The samples were tested by ELISA procedure. Serotyping investigation of Iranian rotavirus isolates, using 7 serotypes monoclonal antibodies (G1-G2-G3-G4-G6-G8-G9) in ELISA tests and Immunosorbent Electron microscopical studies using trapping and decoration techniques were performed.
**Results:** Rotavirus type A infection was identified in 66 samples (37%). Serotyping investigation in ELISA tests proved that serotypes G1 and G4 were the most common serotypes circulating among infected children and infants in Tehran. By electron microscopic studies the characteristic of rotavirus particles were observed in the faecal samples of infected children. The maximum incidence of infection was determined to occur among the cold months of the year.
**Conclusion:** It was approved that G1 and G4 serotypes are the main rotavirus serotypes present among children in Tehran. It was detected that rotavirus diarrhoea was most prevalent among children of under 3 years of age.

## P1884

## Neurological manifestations in varicella

C.P. Popescu, S-A. Florescu, M. Cotiga, G. Enache, R.F. Botgros, L. Raduta, P.I. Calistru, E. Ceausu *(Bucharest, RO)*

**Objectives:** Clinical,paraclinical and terapeutical evaluation of neurological manifestations in varicella.
**Methods:** We analysed patients with varicella and neurological manifestations admitted in Clinical Hospital of Infectious and Tropical Disease ''Dr.V.Babes'' Bucharest from January 2001 to October 2005.
**Results:** From 447 patients with varicella 19 presented neurological manifestations (sex ratio M/F:10/9). 18 had acut cerebellar ataxia and one had encephalitis. We estabilished the diagnosis on the basis of clinical aspects (including neurological examination), cerebrospinal fluid examination and electroencephalogram. The age interval was between 7 months and 30 years. Most cases were diagnosed in children and teenager (17); one case toddlers, and 2 cases in adults. Neurological manifestations appeared in most cases among 7 and 10 days after the onset of rash (15 cases). In the order of frequency: gait disorders (15), cerebellar ataxia (11), fever (7), vomiting (5), nistagmus (3), seizures and coma (1). CSF showed limphocytic pleiocytosis and elevated levels of protein (10 cases); in 9 cases CSF had normal aspects. Electroencephalogram had dominant theta wave with totally or partially suppression of alpha activity in all patients. All cases showed clinical and EEG improvement at the end of the treatment.
**Conclusions:** The most frequent neurological manifestation was cerebellar. The evolution was good under treatment, with no sequelae at 1 month of follow up.