7 clonal types and two of them were prevalent. B type was prevalent among the strains isolated up to September 2006, and A type among the strains isolated after this period. 16 out of the 17 B type strains were MDR and 50% of them harboured class 1 integrons. All the 30 A type strains harboured class 2 integrons and 29, out of them, were considered MDR isolates.
Conclusions: The incidence of MDR phenotype was high among the studied strains. Class 2 integrons were more prevalent than class 1 ones in *Acinetobacter* spp. isolates. Although both classes of integrons seem to be associated with MDR isolates, this phenotype was statistically related with A and B clonal types and it might be consequence of accumulation of different resistance mechanisms in specifics clonal types.

P795 Aminoglycoside resistance in a clinical isolate of *Acinetobacter* genomic species 13TU is associated with the up-regulation of its AdeABC-like efflux system

A. Nemec, P.G. Higgins, P. Jezek, L. Krizova (Prague, CZ; Cologne, DE; Pribram, CZ)*

Objectives: *Acinetobacter* genomic species (GS) 13TU is a member of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex. Although commonly isolated from hospitalized patients, this species, unlike *A. baumannii*, is usually well susceptible to antibiotics. In a Czech hospital, two isolates of a GS 13TU strain differing in their susceptibilities to aminoglycosides were obtained from the same patient. The aim of this study was to assess whether the difference is associated with the up-regulation of the GS 13TU efflux system related to the AdeABC system in *A. baumannii*.
Methods: The two isolates, NIPH 952 and NIPH 953, were obtained, respectively, from the sputum and gastric juice of an ICU patient. Compared to NIPH 952, NIPH 953 showed elevated MICs to aminoglycosides, in particular to gentamicin (1 versus 8 mg/l) and netilmicin (2 versus 16 mg/l). The presence of the genes encoding putative efflux components was tested by PCR using primers derived from the AdeABC genes. The ability of NIPH 952 to produce aminoglycoside-resistant variants was assessed by challenging it with 4 or 8 mg/l of gentamicin. The adeB gene was partially sequenced and its expression level was examined by real-time reverse transcription PCR (RT-PCR).
Results: PCR amplicons of expected sizes were obtained with primers targeting adeA, adeB and adeS in both NIPH 952 and NIPH 953. The sequences of the adeB-like amplicons were identical in both isolates and were 84–89% identical to the known adeB sequences in *A. baumannii*. Variants with gentamicin MICs of more than 4 mg/l were obtained from NIPH 952 at frequencies of $\sim 5 \times 10^{-9}$. Two of these variants were further investigated, i.e. NIPH 952-I (gentamicin MIC 8 mg/l) and NIPH 952-IV (gentamicin MIC 24 mg/l). Compared to NIPH 952, the susceptibility patterns of NIPH 952-I, NIPH 952-IV and NIPH 953 shared elevated MICs to aminoglycosides, tetracycline, tigecycline and ciprofloxacin, whereas no changes in MICs were observed for piperacillin, cefotaxime, sulphonamides or polymyxins. Consistently, RT-PCR identified 27-fold, 214-fold and 38-fold increases in mRNA transcripts for adeB in NIPH 952-I, NIPH 952-IV and NIPH 953, respectively, as compared to NIPH 952.
Conclusion: The aminoglycoside resistance of the gastric GS 13TU isolate is likely to result from the up-regulation of its efflux system homologous to the AdeABC system in *A. baumannii*.
Supported by grant 310/08/1747 of the Grant Agency of the Czech Republic.

P796 Involvement of pmrA/B in colistin resistance in clinical isolates of *Acinetobacter baumannii*

A. Beceiro, M. Doumith, M. Hornsey, H. Dhanji, G. Bou, D. Livermore, N. Woodford (London, UK; A Coruna, ES)*

Objectives: Colistin (Col) resistance is rare in *A. baumannii* and little is known about its mechanism, although the pmrA/B genes which encode a two-component regulatory system have been implicated in laboratory mutants. We investigated the role of pmrA/B using a small panel of international clinical *A. baumannii* strains and laboratory mutants.
Methods: Clinical isolates included 5 that were Col-R (MIC 8–64 mg/L) and 5 Col-S (MIC $\leqslant$1 mg/L). We raised Col-R mutants of both ATCC 19606 and clinical isolate ABRIM (MICs for parent/mutant: 1 vs. 64 and 1 vs. 16, respectively), and also used a pair of clinical isolates, Ab133 and Ab132, belonging to a single strain and isolated from the same patient (MICs, 2 and 64 mg/L). Isolates were identified by phenotype and species-specific PCR. PFGE was used to determine relatedness. MICs were determined by agar dilution and Etest. Expression of pmrA/B was monitored by RT-PCR. Nucleotide sequences of pmrA/B were determined.
Results: Nucleotide sequences of pmrA/B were identical to reference sequences (e.g. CP000521) in all Col-S clinical isolates, whereas 4/5 Col-R clinical isolates harboured single mutations in PmrB (either S14L, L87F, M145K or P233S); the fifth had both F387Y and S403F. No mutations were found in PmrA. Single mutations in PmrB were also found in Col-R mutants of strains ATCC 19606 (A227V), ABRIM (N353Y) and in the Col-R clinical isolate Ab132 (L87F). RT-PCR identified mean 20.6 and 7.4-fold increases in pmrA and pmrB expression, respectively, in Col-R vs. Col-S isolates. Likewise, expression of pmrA was higher in the Col-R mutants of ATCC 19606, ABRIM and in isolate Ab132 (6.4, 19.5 and 4.2 fold, respectively), compared with their parents. Expression of pmrB was not elevated in the Col-R mutant of ATCC 19606 or in Ab132, but there was a 10.5 fold increase in the mutant of strain ABRIM.
Conclusions: Colistin is one of the few options for treating multi-resistant *A. baumannii* infections. Resistance requires at least two distinct genetic events: isolates must acquire at least one point mutation in PmrB (which are not localized to a specific domain) and up-regulation (at least) of pmrA. The functional significance of the individual PmrB mutations and the precise genetic events causing pmrAB up-regulation remain to be defined. Prompt detection and effective infection control measures are critical to prevent spread of resistant strains.

P797 Molecular characterization and outbreak analysis of multidrug-resistant *Acinetobacter baumannii* from German hospitals

Y. Pfeifer, S.H. Cho, P.G. Higgins, A.M. Fahr, T.A. Wichelhaus, K.P. Hunfeld, M. Martin, W. Witte (Wernigerode, DE; Seoul, KR; Cologne, Heidelberg, Frankfurt/M., Lubeck, DE)*

Objectives: Emergence and dissemination of multidrug-resistant *Acinetobacter baumannii* are of special concern because of limited therapeutic options and increased mortality. In many cases colistin is the only antimicrobial substance for an adequate treatment. In Germany *A. baumannii* outbreaks were observed mainly in the summer months. Here we report on molecular-epidemiological analysis of *A. baumannii* from different German hospitals.
Methods: The strain collection includes 22 isolates from two outbreaks in one hospital (A) in 2007 as well as 42 outbreak isolates collected in a surgical ICU (hospital B) in 2006–2008. Furthermore 32 isolates (2005–2009) from two diagnostic laboratories were analysed. Molecular typing by PFGE and sequence-based multiplex PCR to identify isolates belonging to members of the European clonal complexes I-III were performed. Relevant resistance genes (blaOXA, blaVIM and blaIMP) were identified by PCR and sequencing.
Results: All above mentioned isolates were resistant to fluoroquinolones, aminoglycosides, sulfonamides and β-lactams including carbapenems. PCR and sequencing of resistance genes revealed the presence of OXA-β-lactamases in all isolates, with OXA-23 (n = 45) and OXA-58 (n = 47) as the most prevalent types. In several outbreak-independent isolates the genes blaOXA-72 (n = 1), blaOXA-58+blaOXA-23 (n = 1) or blaOXA-66 + insertion sequence ISAba1 were identified. In one single carbapenem-resistant isolate no carbapenemase gene was found.
Multiplex-PCR analysis for identification of clonal lineages revealed that nearly all isolates are related to the European clones I (n = 19), II (n = 51)