Resistance genomic islands related to AbaR1 are common in Acinetobacter baumannii strains belonging to European clone I

**Objective:** Acinetobacter baumannii strains belonging to European (EU) clone I are commonly resistant to multiple antimicrobial agents. A number of resistance genes were recently detected on an 86-kb genomic resistance island (AbaR1) inserted in the ATPase gene of EU clone I strain AYE. The aim of this study was to assess the presence of AbaR1-related structures in epidemiologically unrelated strains of EU clone I.

**Methods:** The study set included 25 multi-drug resistant (MDR) strains of EU clone I collected in 19 European countries in 1978–2004 and 10 genomes (and partially in ATCC17978) that were absent in SDF. DNA analysis by PFGE and RAPD revealed an outbreak caused by a unique clone. Detection of clonal related isolates among different services may be because of the treatment of these patients at the same services before and this may explain the spread of PER-1 positive strains.

**Results:** Of the 100 A. baumannii isolates, 78 (78%) were determined as ceftazidime-resistant by E-test. Among the 78 ceftazidime-resistant A. baumannii isolates the PER-1 gene was identified in 18 (23%). The similarity of the bands were calculated according to “dice similarity coefficients” and all PER-1 positive isolates were found as clonally related.

**Conclusion:** In our study the prevalence of PER-1 was lower than the previous studies. But the presence of high ceftazidime resistance rates among these isolates may indicate the presence of other beta-lactamases. DNA analysis by PFGE and RAPD revealed an outbreak caused by a unique clone. Detection of clonal related isolates among different services may be because of the treatment of these patients at the same services before and this may explain the spread of PER-1 positive strains.

**[O442]** Resistance genomic islands related to AbaR1 are common in Acinetobacter baumannii strains belonging to European clone I

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**Objective:** Acinetobacter baumannii strains belonging to European (EU) clone I are commonly resistant to multiple antimicrobial agents. A number of resistance genes were recently detected on an 86-kb genomic resistance island (AbaR1) inserted in the ATPase gene of EU clone I strain AYE. The aim of this study was to assess the presence of AbaR1-related structures in epidemiologically unrelated strains of EU clone I.

**Methods:** The study set included 25 multi-drug resistant (MDR) strains of EU clone I collected in 19 European countries in 1978–2004 and 10 genotypically unique, fully susceptible strains. Using PCR, all strains were investigated for the presence of the ATPase gene and for nine genes found to be associated with AbaR1. Furthermore, the strains were tested for the disruption of the ATPase gene using PCR primers directed against the 3’ and 5’ ends of this gene. Strains with the disrupted gene were investigated for the presence and structure of the ATPase gene-AbaR1 connecting regions using PCR mapping and RFLP. PCR primers were derived from the known sequence of strain AYE.

**Results:** All strains were positive for the ATPase gene. The 10 susceptible strains had an intact ATPase gene whereas all MDR strains failed to produce the expected amplicon in the ATPase disruption test. All EU clone I strains yielded positive results for the ATPase gene-AbaR1 connecting regions, the structure of which corresponded to those of AYE. These findings suggest the presence of ATPase integrated elements in clone I strains, the integration of which had invariably taken place at the same locus site. None of the AbaR1-associated resistance genes were found in any of the susceptible strains. In contrast, the MDR strains harboured the following AbaR1-associated genes (% positive strains): aacC1 (21), aadA1 (21), aphA1 (21) strA (3), mexA (20), tetA (18), cat (23), the gene encoding heavy metal detoxification protein (25). Individual MDR strains carried from one to nine AbaR1-associated genes in 11 different combinations. There was a good correlation between the content of resistance genes and resistance phenotypes.

**Conclusion:** Genetic structures related to AbaR1 are common in strains belonging to EU clone I. The heterogeneity of resistance patterns in this clone is likely to result from the variations in the content of AbaR1-related structures. Supported by grant 310/08/1747 of the Grant Agency of the Czech Republic.

**[O443]** Mutation frequency of rifampicin resistance in Acinetobacter baumannii clinical isolates

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**Objectives:** To study the differences in mutation frequency and evaluate the possible correlations between drug resistance development and mutation rate in Acinetobacter baumannii (AB).

**Methods:** The mutation frequency (MF) of rifampicin (RIF) resistance was used as a surrogate measure of differences in mutation rate and for detection of the presence of mutator phenotype. The MF of RIF resistance was measured in 100 clinical isolates collected from different AB infections over a 20 year period in Europe and USA and two reference strains (AB ATCC 19606 and 19798). MF of RIF in AB isolates was compared with E. coli control strains with altered mutation rates. 10–20 cultures of each isolate were grown over night and plated onto RIF containing Mueller-Hinton agar plates and non RIF containing plates to determine the MF of rifampicin resistance. After 24 hour incubation colonies were counted and the frequency was calculated. The MF was compared to each individual strain’s resistance profile (resistant vs multidrug resistant, MDR) and infection specific history (sporadic vs outbreak isolate).

**Results:** The MF of RIF resistance ranged between 2.2·10⁻¹⁰ to 7.8·10⁻⁸. None of the isolates displayed any MF near the mismatch- repair (mutS, 3.0·10⁻⁸) or dam-mutant strain (mutL, 6.3·10⁻⁸). There were no significant mean differences between sporadic (1.3·10⁻⁸) and outbreak (1.2·10⁻⁸) isolates. In the comparison of resistance profile and MF, the MIC values of 5 different antibiotic classes were used. The isolates were classified as resistant (resistant to <2 antibiotics) and MDR (resistant to ≥3) by the MIC values according to EUCAST breakpoints. The mean MF of resistant versus MDR isolates were 8.8·10⁻⁹ and 1.2·10⁻⁸, respectively.

**Conclusion:** Although no A. baumannii isolates displayed a strong mutator phenotype, the diverse mutation frequencies reflect the past evolutionary history of these isolates. The existence of a significant number of possible ‘weak-mutators’ (<10 times increased MF) among clinical isolates presents a hidden potential for the rapid evolution of resistant isolates. It was previously shown in E. coli that a slightly increased mutation rate (4–20 fold) can drive the evolution of antibiotic resistance. Our finding that MDR A. baumannii isolates had increased mutation rates can have an impact on future development of resistance in these isolates.

**[O444]** Genome comparison of Acinetobacter baumannii: evaluation of drug resistance, adhesion, haemolysis and other potential virulence factors

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**Objectives:** Despite the importance of Acinetobacter baumannii as an emerging pathogen worldwide, studies on the mechanisms underlying its pathogenesis are lacking. Multidrug resistant strains are emerging throughout many geographic areas and two pan-epidemic European clones (I and II) have been documented, endowed with a broad range of antibiotic resistance. In this study, the genome sequences of the multidrug resistant A. baumannii ACICU (European clone II; Iacono et al. AAC 2008) and AYE (European clone I, Vallenet et al. PLOS One 2008) strains were compared with the genome of the antibiotic susceptible, not pathogenic SDF strain, and with that of one of the reference ATCC19798 strain (Smith et al. Genes Dev 2007) with the aim of identifying genomic regions implied in pathogenesis and drug resistance.

**Methods:** protein similarities were searched by the BLASTp algorithm. Pathogenicity studies were performed using the Galleria mellonella model of infection. Haemolysis was evaluated on defibrinated horse blood.

**Results:** In this study, we used larvae of the insect G. mellonella (greater wax moth) as infection model to compare the virulence of the A. baumannii strains ATCC17978, AYE, ACICU and SDF. The 50% lethal dose (LD50) was comparable for AYE and ACICU, while it was ca. 10- and 100-fold higher when larvae were infected with ATCC17978 and SDF, respectively. Thus, the SDF genome was used as reference genome to identify functions acquired by pathogenic strains with a possible role in antibiotic resistance and pathogenicity. Sixty-nine clusters, corresponding to almost 870 CDSs, were identified in the ACICU and AYE genomes (and partially in ATCC17978) that were absent in SDF.