Diversity of antibiotic resistance determinants among the recent population of *Acinetobacter baumannii* strains belonging to European clone II

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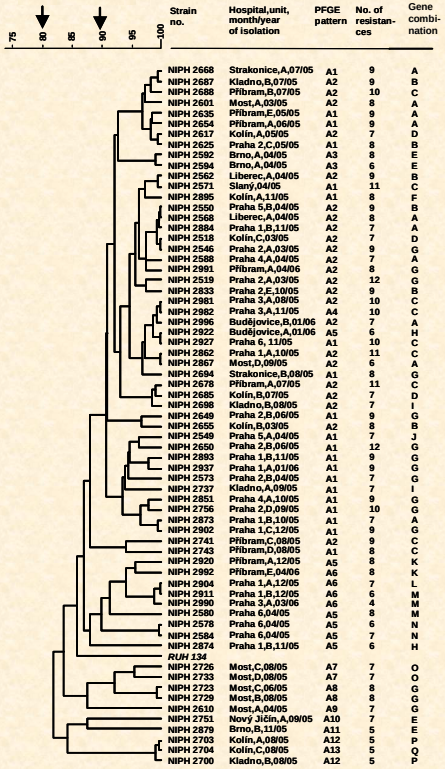


Fig. 1. Dendrogram of cluster analysis of AFLP fingerprints of 66 *A. baumannii* isolates belonging to EU clone II.
Cut-offs of 80% and 90 % correspond to the approximate grouping levels of isolates of the same clone and the same strain, respectively. Numbers following the city name indicate different hospitals in the city; capitals denote different ICUs in the same hospital. Capitals in the last column (A-Q) denote different combinations of detected genes (Table 1).

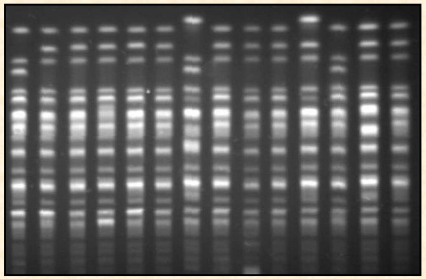


Fig. 2. *Apa*I macrorestriction patterns of the EU clone II isolates.

Objective

- **The recent increase in *Acinetobacter baumannii* resistance to carbapenems in the Czech Republic has been associated with the spread of strains belonging to European (EU) clone II. Although multidrug resistance was a common property of these strains, they differed in resistance to particular agents.**
- **The aim of this study was to assess the genetic basis of this variation.**

Strains

- **Sixty-six isolates of EU clone II included in the present study were obtained during a recent prospective study aimed to analyse the emergence of carbapenem resistance in *Acinetobacter* in the Czech Republic (see abstract O 303 for details).**
- **The 66 isolates were from clinical specimens of patients hospitalised at 36 ICUs of 17 hospitals in 15 cities in the period 2005-6.**

Results

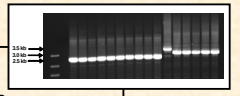
- **All isolates were positive for the genes encoding OXA-51, AmpC and the AdeABC efflux system while no strain tested positive for those encoding metallo-β-lactamases[4], OXA-23 and OXA-24 carbapenemases, or aminoglycoside-modifying enzymes AAC(3)-II, AAC(6')-I, and ANT(2'')-I.**
- **The strains varied with respect to the presence of the genes encoding the following proteins (% positive strains): TEM-1 (80), Tet(B) (92), Tet(A) (5), AAC(3)-I (83), APH(3')-I (80), APH(3')-VI (30), OXA-58 (3) and a class 1 integrase (83). IS*Aba1* was found in 95% strains and three integron variable regions were identified, differing only in the number of copies of the *orfX* cassette (Fig. 3).**
- **The presence of genes and the corresponding resistant phenotype were in good agreement (Fig. 4-6).**
- **Individual strains carried from 4 to 12 resistance genes in 17combinations (Table 1). Different combinations were also found in isolates from the same ward and having identical PFGE patterns (Fig. 2).**

Conclusions

- **The variation in antibiotic resistance in the strains results from the differences in the presence of acquired resistance genes and, possibly from the effect of IS*Aba1* on the expression of intrinsic genes.**
- **The high genetic versatility of EU clone II might contribute to its ability to develop resistance to nearly all clinically relevant antibiotics.**

Table 1. Diversity of resistance genes among 66 EU clone II isolates.

gene combination	*adeB*	*adeS*	Integron VR	*int1*	*aacC1*	*aphA1*	*aphA6*	*tet*(B)	*tet*(A)	*bla* TEM-1	*bla* OXA-58	ISAba1-*bla* OXA-51	ISAba1-*ampC*	ISAba1	number of isolates
A	+	+	3.0	+	+	+	-	+	-	+	-	-	+	+	10
B	+	+	3.0	+	+	+	+	+	-	+	-	-	+	+	7
C	+	+	3.0	+	+	+	+	+	-	+	-	+	+	+	9
D	+	+	3.0	+	+	+	-	+	-	+	-	+	+	+	3
E	+	+	3.0	+	+	+	-	+	-	-	-	-	+	+	4
F	+	+	2.5	+	+	+	-	+	-	-	-	-	+	+	1
G	+	+	-	-	-	-	+	-	+	-	-	-	+	+	14
H	+	+	-	-	-	-	-	+	-	-	-	-	+	+	2
I	+	+	2.5	+	+	+	-	+	-	-	-	-	+	+	2
J	+	+	3.0	+	+	-	-	+	-	-	-	-	+	+	1
K	+	+	-	-	-	-	+	+	-	+	-	+	+	+	2
L	+	+	-	-	-	-	-	+	-	+	-	+	+	+	1
M	+	+	-	-	-	-	-	+	-	+	-	-	+	+	3
N	+	+	-	-	-	-	+		-	+	+	+	+	+	2
O	+	+	3.0	+	+	-	-	+	-	+	-	+	+	+	2
P	+	+	2.5	+	+	+	-	-	+	-	-	-	-	-	2
Q	+	+	3.5	+	+	+	-	-	+	-	-	-	-	-	1



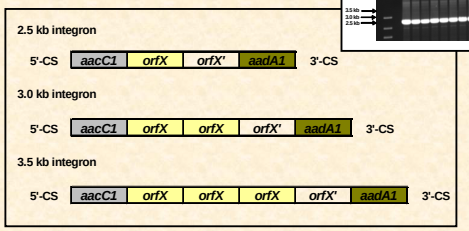


Fig. 3. Structure of three class 1 integron variable regions found in the EU clone II isolates. 5'CS and 3'CS are conserved segments of an integron structure; *aacC1* and *aadA1* are genes encoding aminoglycoside-modifying enzymes; *ofrX* and *orfX'* indicate different casettes of unknown function. Results of PCR detection of the variable regions are shown in the right upper corner.

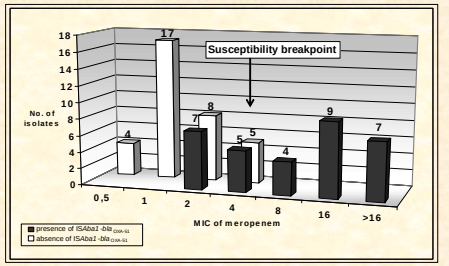


Fig. 4. Relationship between meropenem MIC (mg/L) and the presence of IS*Aba1* upstream of the bla_{OXA-51} gene.

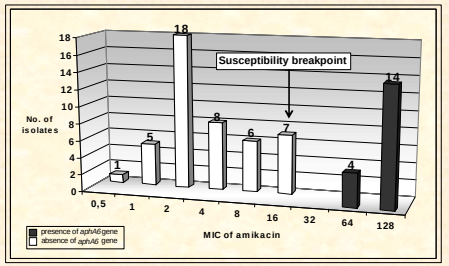


Fig. 5. Relationship between amikacin MIC (mg/L) and the presence of the *aphA6* gene encoding phosphotransferase (APH(3')-VIa).

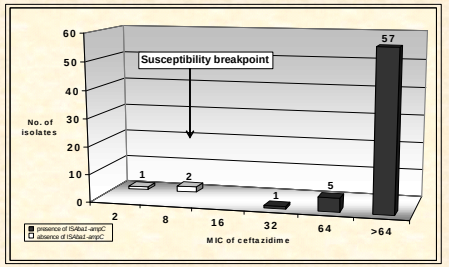


Fig. 6. Relationship between ceftazidime MIC (mg/L) and the presence of IS*Aba1* upstream of the *ampC* gene.

Methods

- **AFLP fingerprinting [5]**
- **Macrorestriction analysis (PFGE)**
- **MIC determination (agar dilution test)**
- **PCR detection of resistance genes (Table 1)**
- **Determination of class 1 integron structures by PCR mapping[1]**
- **Detection of IS*Aba1* in the upstream region of bla_{OXA-51}[2] and *ampC* [3].**

References: [1]Nemec et al, *J Med Microbiol* 2004;53:1233-40; [2]Turton et al., *FEMS Microbiol Lett* 2006; 258: 72-7; [3]Ruiz et al., *FEMS Microbiol Lett* 2007; 274: 63-6; [4]Ellington et al., *J Antimicrob Chemother* 2007; 59, 321–322; [5]Nemec et al., *Int J Syst Evol Microbiol* 2001; 51, 1891-1899.
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