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

Alexandr Nemec,[1] Paul Higgins,[2] Petr Ježek[3] & Lenka Křížová[1]

[1]Laboratory of Bacterial Genetics, National Institute of Public Health, Prague, Czech Republic; [2]Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany; [3]Department of Clinical Microbiology, Příbram Hospital, Příbram, Czech Republic

Objective

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 susceptible to antibiotics.

In a Czech hospital, two isolates of the same strain of GS 13TU differing in their susceptibilities to aminoglycosides were obtained from one patient.

The aim of this study was to assess whether this difference is associated with the up-regulation of the GS 13TU efflux system related to the AdeABC system in *A. baumannii*.

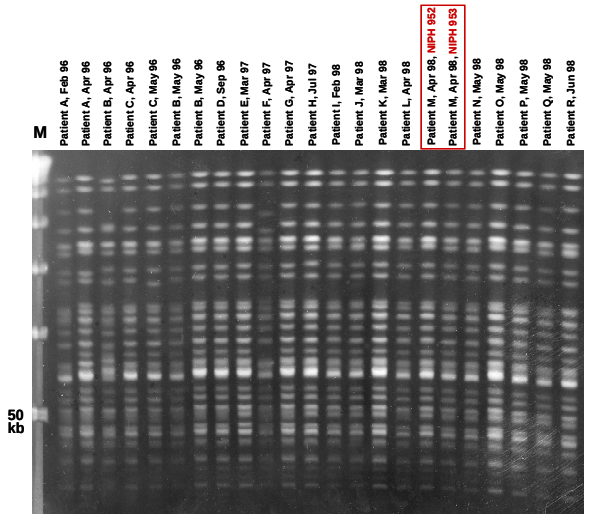

Fig. 1. *Apa*I-macrorestriction (PFGE) patterns of 23 isolates obtained from 18 patients in the ICU of the Příbram hospital in 1996–8. Except for NIPH 953, all isolates were from sputum and were susceptible to ceftazidime, sulbactam, ticarcillin, piperacillin, co-trimoxazole, quinolones and aminoglycosides, and resistant to tetracycline.

Conclusion

The aminoglycoside resistance and decreased susceptibility to some other agents (e.g. fluoroquinolones and tigecycline) of the gastric GS 13TU isolate is likely to result from the up-regulation of its efflux system homologous to the AdeABC system of *A. baumannii*.

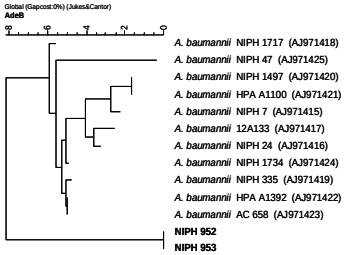

Fig. 2. Neighbor-Joining dendrogram of the *adeB* sequences of *A. baumannii* and the two isolates of GS 13TU.

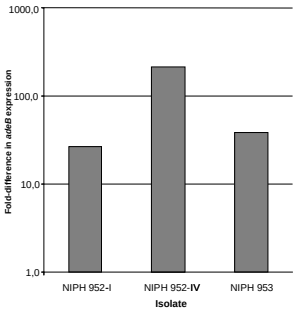

Fig. 4. Fold-difference in *adeB* expression compared to isolate NIPH 952.

Isolates

- NIPH 952 and NIPH 953 were selected from isolates of a strain endemic in the ICU of the Příbram hospital, Czech Republic (Fig. 1).
- NIPH 952 and NIPH 953 were isolated, respectively, from the sputum (26th April 1998) and gastric juice (24th April) of the same 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* 32 mg/l).
- Both isolates were identified as GS 13TU by the *rpoB* gene sequence analysis [1] and amplified rDNA restriction analysis [2].

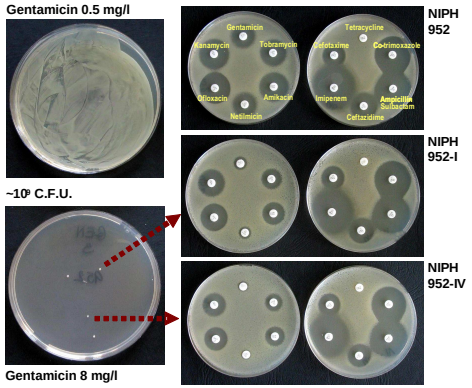

Fig. 3. Challenging NIPH 952 with gentamicin.

Table 1. Antimicrobial susceptibilities of NIPH 952, its variants and NIPH 953

Isolate	MIC (mg/l)*															
	GM	NC	KM	TM	AK	OF	CI	AM	PP	TZ	CT	MR	DC	TGC	SX	CO
952	1	2	2	1	4	0.5	0.125	64	8	4	16	0.5	4	1	8	0.5
952-I	**8**	**32**	4	2	8	0.5	0.5	64	8	4	16	1	8	**4**	8	0.5
952-IV	**32**	**128**	**8**	**4**	**32**	**2**	**1**	64	8	8	16	1	**16**	**16**	8	0.5
953	**8**	**32**	4	2	8	0.5	0.25	64	8	4	16	1	8	**4**	8	0.5

* AM, ampicillin; AK, amikacin; CI, ciprofloxacin; CO, colistin; CT, cefotaxime; DC, doxycycline; GM, gentamicin; KM, kanamycin; MR, meropenem; NC, netilmicin; OF, ofloxacin; PP, piperacillin; SX, sulfomethoxazole; TGC, tigecycline; TM, tobramycin; TZ, ceftazidime. The MIC values that are at least four fold higher than those of NIPH 952 are indicated in bold.

Results

- **Detection of the AdeB gene.** 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* amplicons were identical in both isolates and were 84-89 % identical to the known *adeB* sequences in *A. baumannii* (Fig. 2).
- **Challenging NIPH 952 with gentamicin.** Variants with gentamicin MICs of more than 4 mg/l were obtained from NIPH 952 at frequencies of ~5×10^{-9} (Fig. 3). Two of these variants were further investigated, i.e. NIPH 952-I (gentamicin MIC 8 mg/l) and NIPH 952-IV (gentamicin MIC 32 mg/l).
- **PFGE.** NIPH 952, NIPH 952-I, NIPH 952-IV and NIPH 953 had the same *Apa*I macrorestriction profile.
- **Antimicrobial susceptibilities.** Compared to NIPH 952, the susceptibility patterns of NIPH 952-I, NIPH 952-IV and NIPH 953 shared elevated MICs to some antibiotics (Table 1).
- **RT-PCR.** 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 (Fig. 4) There was no significant difference in *adeJ* expression between the isolates.

Methods

- PCR using primers derived from the AdeABC genes
- Challenging NIPH 952 with 4 or 8 mg/l of gentamicin
- Macrorestriction analysis (PFGE)
- Partial sequencing
- Real-time reverse transcription PCR (RT-PCR) [3]

References

1. Gundi et al. *Microbiology* 2009; 155: 2333–41.
2. Dijkshoorn et al. *Syst Appl Microbiol* 1998; 21: 33–9.
3. Higgins et al. J Antimicrob Chemother 2004; 54: 821-3.

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Correspondence. Alexandr Nemec, National Institute of Public Health, Šrobárova 48, 10042 Praha, Czech Republic. anemec@szu.cz

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