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Diversity of mutations in regulatory genes of resistance-nodulation-cell division efflux pumps in association with tigecycline resistance in Acinetobacter baumannii

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Background: The increasing frequency of *Acinetobacter baumannii* strains resistant to tigecycline (TGC), one of the last-resort antibiotics, is of great concern. This study aimed to investigate the mechanisms of TGC resistance in *A. baumannii* isolates obtained from patients in Greece, Italy and Spain between 2012 and 2014 during the EU-funded MagicBullet clinical trial.

Material/methods: In total, 64 non-duplicate *A. baumannii* isolates were investigated. TGC MICs were determined by Etest. TGC non-susceptibility was interpreted as MIC ≥2mg/L (EUCAST breakpoint for *Enterobacteriaceae*). Isolates were subjected to whole-genome sequencing by MiSeq and assembled using Velvet. Further analysis was done using IS-Mapper to search for insertion sequences. The nucleotide sequences of resistance-nodulation-cell division (RND)-type efflux pump regulatory genes *adeRS*, *adeN* and *adeL* were compared to the reference strain *A. baumannii* ACICU.

Results: In total, 58 isolates were TGC non-susceptible and 6 isolates TGC susceptible. The regulatory genes *adeRS* and *adeN* were undisrupted in the susceptible isolates. In TGC non-susceptible isolates *adeS* was disrupted by IS*Aba1* (n=2) and *adeN* was disrupted by IS*Aba1* (n=19), IS*Aba27* (n=1) or IS*Aba125* (n=1). Additionally *adeRSABC* was missing or truncated in four isolates. *AdeL* was undisrupted in all tested isolates. Besides disruption, several mutations were found in the regulatory genes; amino acid substitution N58T in AdeN was detected in 28 isolates (susceptible and non-susceptible). Additional mutations within *adeN* include frameshifts due to deletions (n=6) or insertions (n=5), a 87 nucleotide deletion (n=1), premature stop codons (n=4) as well as the amino acid substitutions H170Y (n=1), D181N (n=1) and G215V (n=2). In *adeL* the amino acid substitutions

I37L (n=7), YT339RV (n=1), Q262R (n=1) were detected. Only the substitution I37L was found exclusively in TGC non-susceptible isolates. Multiple different amino acid substitutions were detected in *adeRS* (Table 1).

Table 1: Amino acid substitutions in *adeR* and *adeS* in TGC non-susceptible *A. baumannii* isolates.

	adeR	number of isolates	*adeS*	number of isolates
amino acid substitutions	I120V, V136A, V243I	2	D167N, V186G, H268N, I348V, S357P	2
	G25S, D26N	1	A325T	2
	V119I	1	I62M	1
	D26N	2	A325T	1
	D21V, D26N	14	V27I, V32I, A94V, V186G, F214L, H268N, SQ280AD, Q299R, Q339K, I348V	3
	GD25SN, E147K	3	V27I, V32I, A94V, V137F, V186G, F214L, H268N, SQ280AD, Q299R, Q339K, I348V	1
	I120V, V136A	4		
	N115K	1		

Conclusions: Besides disruption of RND-type efflux pump regulatory genes by insertion sequences, a great diversity of mutations in *adeRS*, *adeN* and *adeL* was detected in association with tigecycline resistance. Deletions, premature stop codons or frameshifts impair the repressor function of *adeN* and thus are associated with increased TGC MICs. The detected amino acid substitutions may affect the regulatory function of *adeRS*, *adeN* and *adeL,* but only substitutions found exclusively in TGC non-susceptible isolates may contribute to TGC resistance.