Supplementary information for:

A novel genome editing platform for drug resistant *Acinetobacter baumannii* revealed an AdeR-unrelated tigecycline resistance mechanism

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Table of content:

Figure S1: Development of artificial tigecycline resistance in A. baumannii using serial passages. *Strain ATCC-17978 and its ΔadeR mutant were grown for 12 days in medium containing indicated tigecycline concentrations. The cells were passaged every 48h into fresh medium containing a duplication in the tigecycline concentration. The artificially evolved tigecycline resistant strains derived from strain A. baumannii ATCC-17978 and its ΔadeR mutant were designated ATCC-17978(TGC) and ATCC-17978$_{\Delta adeR}$(TGC), respectively. The figure was produced, in part, by using Servier Medical Art.*

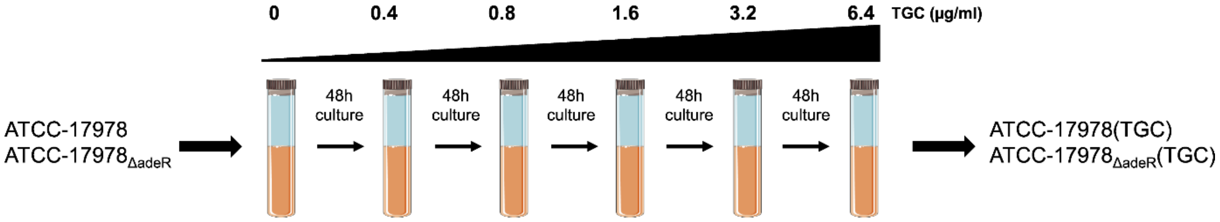

Table S1: Oligonuclotides used in this study.

Oligo name	Sequence (5’-3’)
oVT01	GGCGCGCCCTGTCAGACCAAGTTTACTC
oVT02	GGCCGGCCTATCAGCTCACTCAAAGG
oVT05	ACTGACTTTAGCCGGTGGTG
oVT08	GTTTTCCCAGTCACGACGC
oVT93	GCAGCTTGTAGGCGTTCATAC
oVT94	AACTTGCTCAATACGACGGC
oVT106	TTTTAATATTATCCCGGGAGAAAATCTGGCTATAGAAAGTG
oVT107	GCCAGATTTTCTCCCGGGATAATATTAAAAAATAGCTAGGGAATATTTTATG
oVT113	CCGCTCGAGCCTAGGGAATTCAAAGAGGAGAAAATGGCACAGCTATATTTCTACTATTCCGC
oVT114	AACTGCAGAAGCTTGGCGCGCCAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGTTAATCGTGGCGATGCCTTTC
oVT134	TTTTTGCGGCCATTGGTGAG
oVT141	CATTTTCTCCTCTTTGTATTCGCGCGGCCGCG
oVT142	CGCGGCCGCGCGAATACAAAGAGGAGAAAATG
oVT149	AATTGAGGCCTCTCGAGGAATTCGAGCTCGGTA*CCCGGG*GATCCTCTAGAGCCTGCAGGAAGCTTCG
oVT150	GGCCCGAAGCTTCCTGCAGGCTCTAGAGGATC*CCCGGG*TACCGAGCTCGAATTCCTCGAGAGGCCTC
oVT151	AAAAGAGAGTGAGGGGGCAC
oVT154	GGAGAATTGAGGCCTCTCGAGTGCACCTTCAGTGACAAAAG
oVT155	AGCTTCCTGCAGGCTCTAGATTTAAGAACTTTTTCAACTACCG
oVT160	ACTGCCATAACCACACCAGC
oVT161	CCCATTCATCTTCGGGCTCA
oVT312	AAGGAGAGTTGACACAGCTG
oVT412	AGAATTGAGGCCTCTCGAGGAATTCGCACCTGCACCTAAAAGC
oVT413	CTGGTCTTGCCCGGGGGAGAAAACCGACACTGATATAC
oVT414	GGTTTTCTCCCCCGGGCAAGACCAGTTGCCGATC
oVT415	CCGCAAGCTTCCTGCAGGCTCTAGACGCTGTGCCCGTTATAATG
oVT416	GAGGAATTCGAGCTCGGTACCTTACAAAAACGGCAAGCC
oVT417	CCGCAAGCTTCCTGCAGGCTCTAGAAGCAATCATCACGTCCAG
oVT423	GGCTACTACAGGAGCAGCAG
oVT424	CTACTTTGATGGCGGCGTTG
adeB-qRT-F	GGATTATGGCGACAGAAGGA
adeB-qRT-R	AATACTGCCGCCAATACCAG
rpoD-qRT-F	GAGTCTAATGGCGGTGGTTC
rpoD-qRT-R	ATTGCTTCATCTGCTGGTTG

Table S2: Antibiotic resistance profile of the in vitro evolved tigecycline resistant strains and their parental strains.

MIC (µg/ml)	**Tigecycline**	**Tetracycline**	**Meropenem**	**Ciprofloxacin**	**Gentamycin**
ATCC-17978	0.4	2	0.5	0.5	2
ATCC-17978(TGC)	25	32	0.5	2	0.5
ATCC-17978$_{\Delta adeR}$	0.4	2	0.25	0.25	1
ATCC-17978$_{\Delta adeR}$(TGC)	6.3	64	0.5	4	0.5

Table S3: Important mutations identified by whole genome sequencing of in vitro evolved tigecycline resistant strains.

Mutated gene or region	**Mutations**			
	ATCC-17978(TGC)	**ATCC-17978$_{\Delta adeR}$(TGC)**	**Protein function**	**References**
trm (A1S_2858)	IS-17 like transposon inserted in the coding sequence	Adenine in position 311 of *trm* deleted leading to early stop codon	Methyltransferase potentially involved in rRNAs methylation	(1)
RNase E (A1S_0403)	58 bp deletion in the coding sequence	-	Ribonuclease involved in rRNAs processing and RNA decay	(2)
Intergenic region between *RNase E* (A1S_0403) and *23S rRNA pseudouridylate synthase* (A1S_0404)	IS*Aba11* element inserted at position 439,336 in the intergenic region	-	RNase E see above. 23S rRNA pseudouridylate synthase involved in modification of 23S rRNA	(3)
adeN (A1S_1979)	-	IS*Aba11* transposon inserted in the coding sequence	Transcriptional repressor of the *adeIJK* efflux pump	(4)
abeM (A1S_0395)	-	Cytosine to thymidine mutation (position 429,259) in the promoter region of *abeM*	MATE family efflux pump	(5)
rpsJ (A1S_3081)	-	S12F substitution in the coding sequence	30S ribosomal protein S10 part of tigecycline binding site in the ribosome	(6, 7)

Table S4: Tigecycline resistance profile of the trm knockout and trm restored strains.

	***trm* knockout**		***trm* wildtype recovery**					
	ATCC-17978		**BV185**		**BV186**		**BV191**	
Tigecycline MIC (µg/ml)	**WT**	***Δtrm***	**WT**	***trm+***	**WT**	***trm+***	**WT**	***trm+***
	0.4	0.8	3.1	1.6	3.1	1.6	12.5	6.3

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