

Comparative transcriptomics of multidrug-resistant *Acinetobacter baumannii* in response to antibiotic treatments

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Supplementary Tables

Table S1. Minimum inhibitory concentrations (MICs) of strains used in this study.

Strains	Minimum inhibitory concentration (MIC) µg/ml										
	AMK	FEP	CFP-SUL	CAZ	CIP	GEN	IPM	LVX	MEM	PIP	TZP
R1	256 (R)	128 (R)	32	128 (R)	64 (R)	>128 (R)	32 (R)	4 (I)	64 (R)	512 (R)	>128 (R)
R2	4 (S)	128 (R)	32	128 (R)	64 (R)	128 (R)	16 (R)	4 (I)	4 (S)	512 (R)	>128 (R)
R3	2 (S)	>128 (R)	32	128 (R)	64 (R)	64 (R)	32 (R)	4 (I)	4 (S)	512 (R)	128 (R)
R4	>256 (R)	64 (R)	32	64 (R)	64 (R)	>128 (R)	2 (S)	8 (R)	2 (S)	512 (R)	128 (R)
R5	>256 (R)	64 (R)	64	128 (R)	32 (R)	>128 (R)	>32 (R)	4 (I)	>64 (R)	>512 (R)	>128 (R)
R6	>256 (R)	>128 (R)	64	>128 (R)	64 (R)	>128 (R)	>32 (R)	4 (I)	64 (R)	>512 (R)	>128 (R)
R7	>256 (R)	128 (R)	32	128 (R)	64 (R)	>128 (R)	>32 (R)	8 (R)	>64 (R)	>512 (R)	>128 (R)
R8	>256 (R)	64 (R)	32	64 (R)	32 (R)	>128 (R)	32 (R)	4 (I)	32 (R)	>512 (R)	>128 (R)
R9	2 (S)	128 (R)	32	16 (I)	16 (R)	2 (S)	>32 (R)	8 (R)	32 (R)	512 (R)	>128 (R)
S1	2 (S)	4 (S)	2	4 (S)	0.25 (S)	0.5 (S)	0.25 (S)	0.12 (S)	0.25 (S)	16 (S)	16 (S)
S2	2 (S)	4 (S)	32	2 (S)	0.12 (S)	0.5 (S)	0.12 (S)	0.06 (S)	0.25 (S)	16 (S)	2 (S)
S3	2 (S)	8 (S)	2	4 (S)	0.12 (S)	0.5 (S)	0.12 (S)	≤0.03 (S)	0.12 (S)	8 (S)	8 (S)

Antibiotics abbreviations: AMK: Amikacin; FEP: Cefepime; CFP-SUL: Cefoperazone-sulbactam; CAZ: Ceftazidime; CIP: Ciprofloxacin; GEN: Gentamicin; IPM: Imipenem; LVX: Levofloxacin; MEM: Meropenem; PIP: Piperacillin; TZP: Piperacillin/tazobactam.

(R): Resistant; (I): Intermediate resistant; (S): Sensitive.

Table S2. Strain information.

Strain name	Isolation Site	PFGE Pattern	MLST (Oxford)							
			gltA	gyrB	gdhB	recA	cpn60	gpi	rpoD	ST
R1	MSU	Ia	1	3	3	2	2	97	3	208
R2	Bag Urine	Ia	1	3	3	2	2	97	3	208
R3	PCN Urine	Ia	1	3	3	2	2	97	3	208
R4	Sputum	Ia	1	3	3	2	2	97	3	208
R5	Blood Culture	Ia	1	3	3	2	2	102	3	218
R6	Wound Swab	Ia	1	3	3	2	2	96	3	195
R7	Blood Culture	Ia	1	3	3	2	2	102	3	218
R8	Wound Swab	Ib	21	15	3	2	35	202	4	806
R9	Bag Urine	II	1	43	50	31	1	94	26	940
S1	Blood Culture	V	21	12	2	28	1	157	4	new
S2	Blood Culture	III	1	96	11	48	18	114	43	new
S3	Blood Culture	II	1	15	56	1	1	187	45	new

Table S3. Plasmid constructions for transformation.

Gene name	Plasmid construct	Plasmid construction method	Tag
Mel	pET101-TOPO-Mel	Champion™ pET101 Directional TOPO® Expression Kit	V5 isotope, 6x His tag
Mph	pET15b-Mph	Gene synthesis	Flag tag
Positive control AacBI	pET101-TOPO-AacBI	Champion™ pET101 Directional TOPO® Expression Kit	V5 isotope, 6x His tag
Negative control	pET101-TOPO-negative	Champion™ pET101 Directional TOPO® Expression Kit	NA

Table S4. Antibiotic concentrations of applied for the transcriptome study.

Strain	Antibiotics	Concentration ($\mu\text{g/ml}$)	Proportion of MIC
R3	Amikacin	0.03125	1/64
	Imipenem	1	1/32
	Meropenem	0.5	1/8
R4	Amikacin	4	1/64
	Imipenem	0.0625	1/32
	Meropenem	0.25	1/8
R5	Amikacin	4	1/64
	Imipenem	1	1/32
	Meropenem	8	1/8

Table S5. Published genomes used in this study.

Strain name	NCBI Accession ID
ATCC 17978	NC_009085.1
1656-2	NC_017162.1
AB0057	NC_011586.1
AB307-0294	NC_011595.1
ACICU	NC_010611.1
AYE	NC_010410.1
BJAB07104	NC_021726.1
BJAB0715	NC_021733.1
BJAB0868	NC_021729.1
D1279779	NC_020547.1
MDR-TJ	NC_017847.1
MDR-ZJ06	NC_017171.1
SDF	NC_010400.1
TCDC-AB0715	NC_017387.1
TYTH-1	NC_018706.1

Table S6. Primers used in this study.

Gene id	Gene	Annealing temperature	Amplicon size	Forward primer	Reverse primer
PCR					
G2819, G2828, G2826	Amikacin-resistant candidate region amplicon 1	57 °C	3951 nt	TCCTCACCTTTGTCACGCC	CCCAACTGAGCTTTTGCTCC
G2819, G2826	Amikacin-resistant candidate region amplicon 2	57 °C	795 nt	TGCAAAGAACCCAAACAGCG	ACATTGGAAACACCAAGGCG
G2828, G2826	Amikacin-resistant candidate region amplicon 3	57 °C	453 nt	GAGTGCGAGTACACAGTTGC	TTGTCCGTAAGCAACAAGCC
qPCR					
G2819	Transposase tnpD	60 °C	141 nt	CAATGCCGATACTGCCAAGC	AGCTGCTTTAAAAACGCCCC
G2828	Macrolide 2'-phosphotransferase	60 °C	134 nt	ATCACTTGCTGAAGCACACG	CATCACGACGAGGAATACGC
G2826	Macrolide efflux protein	60 °C	82 nt	AAACGCCAGCAAGCTAATCG	CAAGTCGTCCAGCACTTTTCG
Cloning					
G2826	Macrolide efflux protein (Mel)	55 °C	1473 nt	CACCATGAGTTTAATTATTAAAGCGAGAAACATACG	ACAATCTCTCTCAAAGTCTTGATGA
G1418	Aminoglycoside 6'-N-acetyl transferase type Ib (AacBI)	62 °C	698 nt	CACCGCCTCGGGCATCCAAGCAG	GGCAACACTGCGTGTTTCGCT

Table S7. Transposon-resistance gene combinations upregulated under antibiotic treatment

Antibiotic resistant gene		Associated transposon	
G2966	beta-lactamase OXA-23	G2278	transposition helper
G3868	streptomycin 3"-kinase	G3891	hypothetical protein
		G3584	transposase 1
		G3585	transposase
		G3902	transposase
G4338	Gentamicin acetyltransferase I (AAC(3)-I)	G4252	transposase
		G4274	transposase
G4241	beta-lactamase TEM	G4252	transposase
		G4274	transposase
		G4285	Site-specific recombinase, DNA invertase Pin-like protein
G2792	Chloramphenicol acetyltransferase	G4252	transposase
		G4274	transposase
		G4285	recombinase, DNA invertase Pin-like protein
G3879	Streptomycin 6-kinase	G3891	hypothetical protein
		G3902	transposase
		G3584	transposase 1
		G3585	transposase
G4342	Aminoglycoside adenylyltransferase	G4252	transposase
		G4274	transposase
		G4285	recombinase, DNA invertase Pin-like protein
G1418	aminoglycoside 6'-N-acetyl transferase type Ib	G4252	transposase
		G4274	transposase
		G4285	recombinase, DNA invertase Pin-like protein
G2790	aminoglycoside 3'-phosphotransferase aphA1	G4252	transposase
		G4274	transposase
		G4285	recombinase, DNA invertase Pin-like protein