



**Supplementary Information for
Mutational background influences *P. aeruginosa* ciprofloxacin
resistance evolution but preserves collateral sensitivity
robustness**

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Table S1. MIC values ($\mu\text{g/mL}$) of antibiotics belonging to different structural families in the final populations of *P. aeruginosa* after ALE on tobramycin.

		TOB	CAZ	CIP	ATM	FOF	IPM
Parental	PA14	1	1	0.064	2	64	0.75
	<i>parR87</i>	1.5	1	0.125	3	48	1
	<i>orfN50</i>	3	3	0.19	6	16	1
	<i>nfxB177</i>	1	1	2	2	48	0.75
	<i>mexZ43</i>	1	1.5	0.125	2	96	1
	MDR6	1.5	1.5	0.125	1	32	0.5
	MDR12	32	1	0.38	3	96	0.5
TOB ALE	PA14 1	4	3	0.064	3	32	0.75
	PA14 2	4	3	0.094	6	32	0.75
	PA14 3	3	1	0.064	3	32	0.75
	PA14 4	3	3	0.064	6	48	0.75
	<i>parR87</i> 1	24	2	0.38	3	24	1.5
	<i>parR87</i> 2	8	1.5	0.19	3	32	1.5
	<i>parR87</i> 3	16	4	0.38	3	64	1
	<i>parR87</i> 4	3	1.5	0.19	3	48	2
	<i>orfN50</i> 1	12	2	0.25	4	8	1
	<i>orfN50</i> 2	8	2	0.19	4	8	1
	<i>orfN50</i> 3	6	3	0.25	3	8	1
	<i>orfN50</i> 4	24	3	0.25	4	8	1
	<i>nfxB177</i> 1	8	3	0.19	4	16	0.75
	<i>nfxB177</i> 2	2	2	2	3	32	0.75
	<i>nfxB177</i> 3	3	4	0.125	4	32	1
	<i>nfxB177</i> 4	2	1	1.5	0.5	24	0.75
	<i>mexZ43</i> 1	12	2	0.25	4	32	1.5
	<i>mexZ43</i> 2	12	2	0.38	6	24	2
	<i>mexZ43</i> 3	12	3	0.5	4	16	1.5
	<i>mexZ43</i> 4	12	3	0.38	6	24	1
MDR6 1	6	0.5	0.125	1	32	0.38	

MDR6 2	8	0.5	0.25	1.5	32	0.38
MDR6 3	6	1.5	0.25	3	12	0.5
MDR6 4	8	2	0.19	6	24	0.5
MDR12 1	96	1.5	0.5	4	32	0.75
MDR12 2	64	2	0.5	2	16	0.75
MDR12 3	64	1.5	0.5	3	64	0.75
MDR12 4	96	1.5	0.5	2	32	0.75

TOB: tobramycin, CAZ: ceftazidime, CIP: ciprofloxacin, ATM: aztreonam, FOF: fosfomicin, IPM: imipenem.

Table S2. MIC values ($\mu\text{g/mL}$) of antibiotics belonging to different structural families in the final populations of *P. aeruginosa* after ALE on aztreonam.

		TOB	CAZ	CIP	ATM	FOF	IPM
Parental	PA14	1	1	0.064	2	64	0.75
	<i>parR87</i>	1.5	1	0.125	3	48	1
	<i>orfN50</i>	3	3	0.19	6	16	1
	<i>nfxB177</i>	1	1	2	2	48	0.75
	<i>mexZ43</i>	1	1.5	0.125	2	96	1
	MDR6	1.5	1.5	0.125	1	32	0.5
	MDR12	32	1	0.38	3	96	0.5
	ATM ALE	PA14 1	1	4	0.19	12	64
PA14 2		1	3	0.25	16	64	0.5
PA14 3		2	4	0.19	16	64	0.38
PA14 4		1	3	0.25	12	64	0.75
<i>parR87</i> 1		1.5	3	0.5	8	96	0.5
<i>parR87</i> 2		2	3	0.5	8	96	0.75
<i>parR87</i> 3		2	3	0.5	6	128	1.5
<i>parR87</i> 4		1	4	0.75	8	64	1
<i>orfN50</i> 1		3	8	0.094	16	8	0.75
<i>orfN50</i> 2		2	6	0.094	16	96	0.5
<i>orfN50</i> 3		3	6	0.094	12	12	0.5
<i>orfN50</i> 4		1	8	0.38	24	96	1
<i>nfxB177</i> 1		1	8	0.25	48	64	0.5
<i>nfxB177</i> 2		1.5	4	0.38	8	64	0.38
<i>nfxB177</i> 3		1	6	0.5	8	64	0.5
<i>nfxB177</i> 4		1.5	6	0.5	12	96	1
<i>mexZ43</i> 1		3	2	0.5	8	96	0.5
<i>mexZ43</i> 2		3	3	0.19	12	32	3
<i>mexZ43</i> 3		4	8	0.19	24	16	0.5
<i>mexZ43</i> 4		1.5	3	0.5	12	96	0.5
MDR6 1		1	4	0.125	8	4	0.5

MDR6 2	1	3	0.094	6	6	0.125
MDR6 3	1	4	0.125	12	12	0.5
MDR6 4	0.75	6	0.094	12	12	0.5
MDR12 1	32	2	0.5	12	>1024	0.75
MDR12 2	32	3	0.5	12	384	0.75
MDR12 3	32	3	0.5	8	256	0.75
MDR12 4	16	4	0.5	8	256	0.75

TOB: tobramycin, CAZ: ceftazidime, CIP: ciprofloxacin, ATM: aztreonam, FOF: fosfomicin, IPM: imipenem.

Table S3. MIC values ($\mu\text{g/mL}$) of antibiotics belonging to different structural families in the final populations of *P. aeruginosa* after ALE on ciprofloxacin.

		TOB	CAZ	CIP	ATM	FOF	IPM
Parental	PA14	1	1	0.064	2	64	0.75
	<i>parR87</i>	1.5	1	0.125	3	48	1
	<i>orfN50</i>	3	3	0.19	6	16	1
	<i>nfxB177</i>	1	1	2	2	48	0.75
	<i>mexZ43</i>	1	1.5	0.125	2	96	1
	MDR6	1.5	1.5	0.125	1	32	0.5
	MDR12	32	1	0.38	3	96	0.5
	CIP ALE	PA14 1	0.75	1	1	2	64
PA14 2		0.38	1.5	1.5	1.5	64	0.75
PA14 3		0.38	1.5	1.5	1.5	48	0.75
PA14 4		0.5	1.5	3	1	64	0.75
<i>parR87</i> 1		1	2	3	1.5	64	0.5
<i>parR87</i> 2		0.75	1.5	2	1.5	48	0.75
<i>parR87</i> 3		0.5	1.5	4	1.5	64	0.75
<i>parR87</i> 4		0.75	1	16	1	48	0.75
<i>orfN50</i> 1		0.75	1.5	1.5	1	8	0.5
<i>orfN50</i> 2		0.5	2	1.5	1.5	12	0.5
<i>orfN50</i> 3		0.75	1.5	2	1.5	16	0.38
<i>orfN50</i> 4		1	1.5	3	1.5	16	0.75
<i>nfxB177</i> 1		0.5	0.75	24	1	64	0.125
<i>nfxB177</i> 2		0.5	0.5	16	1	64	0.094
<i>nfxB177</i> 3		0.5	0.75	>32	1	48	0.19
<i>nfxB177</i> 4		0.5	0.38	8	1	32	0.19
<i>mexZ43</i> 1		0.5	1.5	1.5	1.5	48	0.75
<i>mexZ43</i> 2		1.5	1.5	6	1.5	32	1
<i>mexZ43</i> 3		1	0.75	6	4	24	0.75
<i>mexZ43</i> 4		0.75	1	2	1.5	48	0.5
MDR6 1		0.5	1	3	0.5	24	0.38

MDR6 2	0.5	0.5	2	0.5	24	0.38
MDR6 3	0.5	0.5	1.5	0.5	24	0.38
MDR6 4	0.5	0.5	2	0.75	24	0.38
MDR12 1	16	1.5	1.5	1	32	0.38
MDR12 2	24	1.5	4	3	64	0.5
MDR12 3	24	2	3	1.5	48	0.38
MDR12 4	16	1.5	2	1.5	64	0.5

TOB: tobramycin, CAZ: ceftazidime, CIP: ciprofloxacin, ATM: aztreonam, FOF: fosfomicin, IPM: imipenem.

Table S4. MIC values ($\mu\text{g/mL}$) of antibiotics belonging to different structural families in the final control populations of *P. aeruginosa* after ALE with no antibiotic.

		TOB	CAZ	CIP	ATM	FOF	IPM
Parental	PA14	1	1	0.064	2	64	0.75
	<i>parR87</i>	1.5	1	0.125	3	48	1
	<i>orfN50</i>	3	3	0.19	6	16	1
	<i>nfxB177</i>	1	1	2	2	48	0.75
	<i>mexZ43</i>	1	1.5	0.125	2	96	1
	MDR6	1.5	1.5	0.125	1	32	0.5
	MDR12	32	1	0.38	3	96	0.5
Control ALE	PA14 1	1	1	0.064	2	64	0.75
	PA14 2	1	1	0.064	2	64	0.75
	PA14 3	1	1	0.064	2	64	1
	PA14 4	1	1	0.064	3	48	1
	<i>parR87</i> 1	1.5	1	0.19	2	48	1
	<i>parR87</i> 2	1.5	1	0.125	2	48	1
	<i>parR87</i> 3	1.5	1	0.125	2	48	1.5
	<i>parR87</i> 4	1.5	1	0.19	2	48	1
	<i>orfN50</i> 1	2	3	0.125	6	16	0.75
	<i>orfN50</i> 2	2	2	0.125	6	16	0.75
	<i>orfN50</i> 3	2	3	0.125	6	12	0.75
	<i>orfN50</i> 4	3	2	0.125	6	16	0.75
	<i>nfxB177</i> 1	0.75	1	2	2	48	0.75
	<i>nfxB177</i> 2	0.75	1	2	2	48	0.75
	<i>nfxB177</i> 3	0.75	1	2	2	48	0.75
	<i>nfxB177</i> 4	1	1	2	2	48	0.5
	<i>mexZ43</i> 1	1	1	0.19	2	96	0.75
	<i>mexZ43</i> 2	0.75	1	0.125	2	64	1
	<i>mexZ43</i> 3	1	1	0.19	1.5	64	0.75
	<i>mexZ43</i> 4	1	1	0.125	2	64	0.75
MDR6 1	1.5	1	0.125	0.75	32	0.38	

MDR6 2	1.5	1	0.125	1	24	0.38
MDR6 3	1.5	1	0.125	1.5	32	0.5
MDR6 4	1.5	1	0.125	1	32	0.38
MDR12 1	32	1	0.38	2	96	0.5
MDR12 2	32	1	0.38	2	64	0.5
MDR12 3	32	1	0.38	2	64	0.75
MDR12 4	32	1	0.38	2	96	0.5

TOB: tobramycin, CAZ: ceftazidime, CIP: ciprofloxacin, ATM: aztreonam, FOF: fosfomycin, IPM: imipenem.

Table S5. FIC index of Ciprofloxacin-Tobramycin and Ciprofloxacin-Aztreonam combinations for parental strains used within this work.

	FIC index _{CIP-TOB}	FIC index _{CIP-ATM}
PA14	0.75	0.75
<i>parR87</i>	0.63	0.57
<i>orfN50</i>	0.75	1
<i>nfxB177</i>	1.5	0.75
<i>mexZ43</i>	0.5	0.75
MDR6	0.75	1
MDR12	1	0.75

TOB: tobramycin, CIP: ciprofloxacin, ATM: aztreonam.

Table S6. Primers used in this study

Primer	Sequence (5'-3')	Description
<i>gyrA.fw</i>	AGTCCTATCTCGACTACGCGAT	To amplify
<i>gyrA.rv</i>	AGTCGACGGTTTCCTTTTCCAG	QRDR* of <i>gyrA</i>
<i>gyrB.fw</i>	CGCGGTGGAACAGGAGATGGGCAAGTAC	To amplify
<i>gyrB.rv</i>	CTGGCGGAAGAAGAAGGTCAACAGCAGGGT	QRDR of <i>gyrB</i>
<i>nfxB.fw</i>	AAACCAACCGGGACCCATCG	To amplify
<i>nfxB.rv</i>	GTGCCATGCGGCGACGAGAG	<i>nfxB</i>
<i>mexS.fw</i>	TGAGGTTATTCACCCCGTGA	To amplify
<i>mexS.rv</i>	TTCGCTGCGGGTCGTCCTCAAT	<i>mexS</i>

*QRDR: Quinolone Resistance Determining Region.