

Supplementary Table S1 | Genetic modifications detected in ceftazidime and ceftazidime/avibactam evolved *P. aeruginosa* PA14 populations.

Gene	Replicate	Mutation	Localization	Aa Change
BOTH TREATMENTS				
<i>nalD</i>	4, 6, 8	G-->T	32	Thr11Asn
<i>mexB</i>	1	C-->G	1126	Leu376Val
	3	T-->G	2300	Val767Gly
	6	C-->T	1345	Leu449Phe
	8	C-->T	1693	Pro565Ser
<i>ftsI</i>	1	G-->A	1510	Arg504Cys
	5	C-->T	1567	Val523Met
	5	C-->T	1511	Arg504His
	8	C-->T	851	Arg284Gln
<i>clpA</i>	1	Del-A	515	His172fs
	5	A-->G	1634	Tyr545Cys
CEFTAZIDIME TREATMENT				
<i>mexR</i>	1	Ins-A	80-81	Glu27fs
	3	Del-AAT	126-128	Leu43del
<i>mpl</i>	2	Del-CGGCGGCTT	1305-1313	Phe438_Gly440del
	2	T-->G	371	Val124Gly
	4	T-->G	416	Val139Gly
<i>orfN</i>	2	Ins-G	138-139	Val50fs
<i>infB</i>	2	C-->T	2407	Val803Ile
<i>pitA</i>	2	A-->C	133	Thr45Pro
	4	A-->C	367	Thr123Pro
<i>grpE</i>	3	Del-G	39	Glu14fs
<i>clpP</i>	3	Del-C	365	Gly122fs
<i>dnaK</i>	3	G-->A	1115	Ala372Val
<i>dacB</i>	4	C-->T	343	Gly115Ser
CEFTAZIDIME/AVIBACTAM TREATMENT				
<i>dnaJ</i>	5	G-->A	1081	Pro361Ser
<i>pepA</i>	6	C-->T	1439	Gly480Asp
<i>ctpA</i>	6	G-->A	971	Ser324Asn
<i>glnD</i>	6	Ins-G	2466-2467	Asp823fs
<i>flgF</i>	6	C-->A	676	Glu226*
<i>pcm</i>	8	Ins-G	530-531	Arg180fs
<i>spoT</i>	8	T-->C	931	Phe311Leu

<i>PA14_45870</i>	5	Del-21 pb	712-732	Glu238fs
<i>PA14_45880</i>	7	C-->A	159	Met53Ile
	7	G-->A	1001	Ser334Leu
<i>PA14_45890</i>	7	G-->A	836	Pro279Leu
	5	G-->A	830	Ala277Val
INTERGENIC MUTATIONS				
Between				
<i>PA14_45410</i> and <i>PA14_45430</i>	10	C-->T	4044801	-
Between 16S				
ribosomal RNA and <i>PA14_70920</i>	12	T-->C	6317713	-
Between <i>grpE</i> and				
<i>recN</i>	1	T-->C	5621761	-
Between <i>mexR</i> and				
<i>mexA</i>	2	C-->T	486683	-

Nucleotide location of the mutations and associated amino acid changes. Genetic modifications in intergenic regions are also included, and their locations referred to the nucleotide position in *P. aeruginosa* UCBPP-PA14 reference chromosome (NC_008463.1). Fs: frameshift. *: stop codon. Del: deletion. Ins: insertion.

Supplementary Table S2 | Primers used to verify nucleotide modifications detected in whole-genome sequencing.

Gene	Genetic change	Localization	Primer fw (5'-3')	Primer rv (5'-3')
SNVs/MNVs				
<i>nalD</i>	C-->A	32	AATACTTCGAGTCCGCCC	TTGAGCATCTCGTTGAACAG
<i>mexB</i>	C-->G	1126	ATCCTCCTCGTGTTCCCTG	CAGGTATTCGCGCATC
	C-->T	1345		
	C-->T	1693		
	T-->G	2300		
<i>ftsI</i>	C-->T	1510	AACAAGTGGTCGAGGCCCCAG	TGCTGTTCGGTGGCCGT
	G-->A	1567		
	G-->A	1511		
	G-->A	851		
<i>clpA</i>	Del-A	515	TCAAGCAGCAGAGCATCGC	CTACCAGCAGCGGGTTGTT
	A-->G	1634	TCGTTCTCTTCGCCGGT	CGTTGTTGTCGGTCAGGGT
<i>mexR</i>	Ins-A	80-81	ATGAACTACCCCGTGAAT	AAGCTGCGCTGGTCGCT
	Del-AAT	126-128		
<i>mpl</i>	T-->G	371	GAGCATGTCCTGAACAAGGG	CGGTAATGGACGAACTTCGA
	T-->G	416		

<i>mpl</i>	Del-CGGCGGCTT	1305-1313	AACCTGGGCTGGGACCT	TCACTCGGCCAGCGCCGC
<i>orfN</i>	Ins-G	138-139	ATGGACGTTCCCAATGCCCG	CCGCCAGAATCAGCAAAACC
<i>infB</i>	C-->T	2407	ATCTGCGGGAGAACATCCT	CCTTCTCGAACACTTCGATC
<i>pitA</i>	A-->C	133	ATGTTTCGATCTTTTCAGCGG	CGCGATGTCGATCGCCTT
<i>grpE</i>	Del-G	39	GAACCGTGGAAAGCCGTCCC	AACTTGTGCGCCTTCTCGAC
<i>clpP</i>	Del-C	365	TTGCTGTTCCCTGGAGGCTGA	TTCAGACGTCCTTGATGAA
<i>dnaK</i>	G-->A	1115	TGAAGGATGCCGGCCTGGAT	TCTTGTGGACGCTGAAGA
<i>dacB</i>	C-->T	343	ACCTATGCCGCCCTGGAAA	CGTCGTTGAATACCGCAA
<i>dnaJ</i>	G-->A	1081	AAGCTGTTCCGCCTGCGCG	ATAAAACCACTCCACGCG
<i>PA14_45870</i>	Del-21 pb	712-732	TCGAGAACAAGCTGCTGCAT	CGGTACTGGTCAGCATCT
<i>pepA</i>	C-->T	1439	CTGTTCGACGAATACCAGGA	TCACTTGGCCCCGTTCCAG
<i>ctpA</i>	G-->A	971	AGGAAGTGGTCAAGGCGCT	TGGATGGAGCGCCCGTT
<i>glnD</i>	Ins-G	2466-2467	ACTCCATCGGCAACAACCC	ACAGCTGCTCGACCAGGGC
<i>flgF</i>	C-->A	676	TGAAGCAGATGGAGAAGGG	TTAGCTGATTTGCAAAACCC
<i>PA14_45880</i>	C-->A	159	TCGAGGACGAGGCGAAGA	CGAGGAAGGAGAACGGCTGA
<i>pcm</i>	Ins-G	530-531	TTCGTTGGGGCGATGGCTG	TACCGAGTCGAGTACCTGG
<i>spoT</i>	T-->C	931	TCAACGAGATCATGGACGTC	CTCGTCGTTGGACTTGTACA

<i>PA14_45890</i>	G-->A	1001	GCCAACGACCAGTTGTTCAA	ACCGTAGCGACGATCAGGGT
	G-->A	836		
	G-->A	830		
LARGE DELETIONS				
Del 1	Del 55009 bp	3387426-3442435	ATGCAACACAGCCAAGTGT	TCAGAACGGTTTGGTCGGCA
			CTGTTCGAGATCGTCGAAGG	TTCGAACTCTTTCTCCTGTA
Del 2	Del 220701 bp	3288650-3509351	ATGCAACACAGCCAAGTGT	TCAGAACGGTTTGGTCGGCA
			CTGTTCGAGATCGTCGAAGG	TTCGAACTCTTTCTCCTGTA
Del 3	Del 258271 bp	3210297-3468568	GCTTCACCGGTTTCGCTGAAG	TCAGTCAATCGCCGCCCG
			CTGTTCGAGATCGTCGAAGG	TTCGAACTCTTTCTCCTGTA
Del 4	Del 299648 bp	3200274-3499932	GCTTCACCGGTTTCGCTGAAG	TCAGTCAATCGCCGCCCG
			CTGTTCGAGATCGTCGAAGG	TTCGAACTCTTTCTCCTGTA
Del 5	Del 442741 bp	3203830-3646571	GCTTCACCGGTTTCGCTGAAG	TCAGTCAATCGCCGCCCG
			GTGGGCAGCATCTACCTGATT	TGATGAGTTCCGGCGCCTT

The locations of SNVs/MNVs are referred to the specific gene, whereas the large deletions locations remit to the nucleotide position in *P. aeruginosa* UCBPP-PA14 reference chromosome (NC_008463.1).

Supplementary Table S3 | MIC values ($\mu\text{g/ml}$) obtained by antibiotic dilution for the population replicates during selective pressure with either ceftazidime or ceftazidime/avibactam.

Treatment	Replicate	5 d	10 d	15 d	20 d	25 d	30 d
CEFTAZIDIME	1	20	40	75	100	300	300
	2	20	60	250	300	400	400
	3	20	40	75	100	150	150
	4	25	40	100	400	300	400
CEFTAZIDIME + AVIBACTAM	5	40	50	150	250	300	400
	6	15	30	75	250	400	200
	7	20	80	200	400	300	400
	8	15	40	50	75	150	150
CONTROLS	9	4	4	4	4	4	4
	10	4	4	4	4	4	4
	11	4	4	4	4	4	4
	12	4	4	4	4	4	4

The table shows the liquid MIC values against ceftazidime for each replicate population every five days (the antibiotic concentration was doubled every 5 days). The grey boxes indicate the steps in which these particular replicates were unable to grow. In these situations, the previous ceftazidime concentrations (8MIC in populations 6, 8; and 16MIC in populations 3, 7) were maintained for the rest of the experiment.

Supplementary Table S4 | Disk diffusion tests with antibiotics of distinct structural families in the ceftazidime and ceftazidime/avibactam final evolved populations.

Sample	TGC	TET	ATM	IPM	MEM	CAZ	CIP	LVX	NOR	TOB	STR	AMK	GEN	CST	PMB	CHL	ERY	FOF
	15*	30	30	10	10	30	5	5	10	10	25	30	120	50	38.1	50	30	50
PA14 wt	14	16	26	30	21	27	36	31	32	26	22	25	31	17	14	18	13	14
CONTROLS																		
9	12	14	28	30	19	28	37	28	32	22	14/21	23	28	17	14	19	13	13
10	13	15	27	31	17	28	37	29	32	23	20	24	30	17	14	19	12	14
11	13	15	28	32	18	28	38	30	34	24	21	25	29	17	14	18	12	15
12	14	15	28	32	23	28	36	27	32	24	20	25	30	17	14	17	12	16
CEFTAZIDIME																		
1	13	12	0	19	0	0	30	21	26	26	26	29	32	19	15	0	0	27
2	15	12	0	19	0	0	33	26	28	29	29	31	35	19	16	0	0	33
3	15	10	0	25	0	0	31	24	28	25	24	28	32	19	16	0	0	27
4	15	12	0	22	0	0	33	24	28	27	28	31	36	19	16	0	0	31
CEFTAZIDIME + AVIBACTAM																		
5	19	19	0	10	0	0	33	30	31	24	23	28	31	17	15	0	6	27
6	20	22	7	24	0	0	34	30	33	25	22	29	34	18	16	0	0	26
7	23	22	0	9	0	0	38	32	34	23	22	26	33	19	16	0	0	25
8	21	19	0	24	0	0	33	29	28	26	24	30	34	19	16	0	0	27

The table presents the diameters of halo (mm) in the disk diffusion assays for each replicate population at the end of the experimental evolution. The antibiotics in which halos differed were selected for an E-test assay (Table 1). Indeed, in some cases we chose for the E-test only one or two representative cases of a particular structural family, in order to avoid redundancy. * The load of each antibiotic disk is indicated in μg .

TGC: tigecycline, TET: tetracycline, ATM: aztreonam, IPM: imipenem, MEM: meropenem, CAZ: ceftazidime, CIP: ciprofloxacin, LVX: levofloxacin, NOR: norfloxacin, TOB: tobramycin, STR: streptomycin, AMK: amikacin, GEN: gentamycin, CST: colistin, PMB: polymyxin B, CHL: chloramphenicol, ERY: erythromycin, FOF: fosfomicin.