Table S1. Characterization of *in vitro* and *in vivo* ceftazidime resistant mutants**.**

Strain	Description[a]	CAZ MIC (μg/ml)	Comp. pUCPAD/ pUCPADE[b]	*ampD* mutations [c] [effect]	*ampR* mutations [effect]	*dacB* mutations [effect]
PAO1	Reference strain	1	-/-	None	None	None
PAOΔ*mutS*	PAO1 *mutS* knockout mutant	1	-/-	None	None	None
1A1	PAO1-*in vitro*-CAZ4	32	-/+	None	None	G-A nt 819 [W273X]
1A2	PAO1-*in vitro*-CAZ4	24	-/+	None	None	G-T nt 1102 [G368X]
1A3	PAO1-*in vitro*-CAZ4	32	-/+	None	None	Ins 1 bp (C) nt 336 [frameshift]
1A4	PAO1-*in vitro*-CAZ4	24	-/+	None	None	A-G nt 965 [Q322R]
1A5	PAO1-*in vitro*-CAZ4	24	-/+	None	None	Del. nts 1149-1231 [Deletion/frameshift]
1D4	PAO1-*in vitro*-CAZ16	32	-/+	None	None	A-C nt 235 [T79P]
1D5	PAO1-*in vitro*-CAZ16	32	-/+	None	None	Del. nts 592-593 [frameshift]
1D6	PAO1-*in vitro*-CAZ16	32	-/+	None	None	Del. nts 983-993 [deletion/frameshift]
1D7	PAO1-*in vitro*-CAZ16	32	-/+	None	None	Del. nts 1069-1138 [Del. T357-E379]
1D8	PAO1-*in vitro*-CAZ16	32	-/+	None	None	Duplication nts 800-856 [Insertion]
1B2	PAOΔ*mutS* -*in vitro*-CAZ4	96	-/+	None	None	C-T nt 109 [Q37X]
1B3	PAOΔ*mutS* -*in vitro*-CAZ4	32	-/+	None	None	C-T nt 215 [S72L]

1B4	PAOΔ*mutS* -*in vitro*-CAZ4	24	-/+	None	None	C-T nt 1318 [R440C]
1B5	PAOΔ*mutS* -*in vitro*-CAZ4	32	-/+	None	None	G-A nt 1310 [G437D]
1B6	PAOΔ*mutS* -*in vitro*-CAZ4	64	-/+	None	None	T-C nt 971 [F324S]
1C3	PAOΔ*mutS* -*in vitro*-CAZ16	64	-/+	None	None	T-C nt 1340 [W447R]
1C4	PAOΔ*mutS* -*in vitro*-CAZ16	48	-/+	None	None	T-C nt 911 [L304P]
1C5	PAOΔ*mutS* -*in vitro*-CAZ16	64	-/+	None	None	T-C nt 611 [L204P]
1C6	PAOΔ*mutS* -*in vitro*-CAZ16	64	-/+	None	None	G-A nt 1310 [G437D]
1C7	PAOΔ*mutS* -*in vitro*-CAZ16	64	-/+	None	None	C-T nt 1109 [S370L]
2A2	PAO1-*in vivo*-CAZ4	48	-/+	None	None	Del nts 1074-1078 [Deletion/frameshift]
2A4	PAO1-*in vivo*-CAZ4	48	-/+	None	None	G-A nt 1339 [W459X]
2D3	PAO1-*in vivo*-CAZ4	48	-/+	None	None	A-C nt 1282 [T428P]
2D4	PAO1-*in vivo*-CAZ4	32	-/+	None	None	None
2D5	PAO1-*in vivo*-CAZ4	48	-/+	None	None	Del nts 1351-1385 [Del. A451-S461]
2A3	PAO1-*in vivo*-CAZ16	24	-/+	None	None	Del nts 342-792 [Del K114-Y264]
2A7	PAOΔ*mutS* -*in vivo*-CAZ4	48	+/+	C-T nt 130 [Q44X]	None	None
2A9	PAOΔ*mutS* -*in vivo*-CAZ4	64	+/+	T-C nt -23 [mutation in promoter (-10 box)]	None	None
2B2	PAOΔ*mutS* -*in vivo*-CAZ4	64	+/+	C-T nt 503 [P168L]	None	None

2C7	PAOΔ*mutS* -*in vivo*-CAZ4	96	-/+	None	None	G-A nt 1050 [W350X]
2C8	PAOΔ*mutS* -*in vivo*-CAZ4	32	+/+	C-T nt 341 [S114F]	None	None
2A8	PAOΔ*mutS* -*in vivo*-CAZ16	96	-/+	None	None	T-G nt 978 [N316X]
2B1	PAOΔ*mutS* -*in vivo*-CAZ16	96	-/+	None	None	A-G nt 224 [K75R]
2B3	PAOΔ*mutS* -*in vivo*-CAZ16	96	-/+	None	None	G-A nt 1164 [W388X]
2C9	PAOΔ*mutS* -*in vivo*-CAZ16	64	-/+	None	None	G-A nt 1050 [W350X]
2D1	PAOΔ*mutS* -*in vivo*-CAZ16	96	-/+	None	None	None

a The mutants were obtained (Plasencia *et al* 2007), *in vitro* (one-step spontaneous mutants) or *in vivo* (after 3 days of treatment with humanized ceftazidime regimen in mouse model of lung infection), at two ceftazidime (CAZ) concentrations (4 and 16 μg/ml), and from the wild-type strain PAO1 (normal mutation rate supply) or its *mutS* deficient derivative PAOΔ*mutS* (high mutation rate supply).

[b] For complementation experiments, plasmids pUCPAD (harbouring the wild-type *ampD* gene), pUCPADE (harbouring the complete wild-type *ampDE* operon) or pUCP24 (cloning vector) were electroporated into the different β-lactam resistant strains or PAO1 (as control). Complementation was considered positive when the MICs of ceftazidime for the transformants were at least 3 two-fold dilutions lower than those of the parent strains. Additionally, plasmids pUCPADE2A1, pUCPADE1C5 or pUCPADE2C2 containing a non functional *ampD* and a wild-type *ampE* were electroporated in selected mutants, failing in all cases to complement the resistance phenotype.

[c] Mutations or a modified expression of *ampE*, *ampDh2*, or *ampDh3* was not detected in any of the mutants.

References

Plasencia V, Borrell N, Maciá MD, Moya B, Pérez JL, et al. (2007) Influence of high mutation rates on the mechanisms and dynamics of in vitro and in vivo resistance development to single or combined antipseudomonal agents. Antimicrob Agents Chemother 51: 2574-2581.