

## Supplementary Material

**Table S1.** *P. aeruginosa* PBP3 variants within the transpeptidase domain<sup>a</sup>

Variants	N242S	A244S/T	A246V	V254M	G257R	T267A	R284H	P298S	Y367C	S368L	Q372P	H394R	A419G	N427K/S	L434V	A454D/V	M460V	L461V	V464A	V465A/G	E466L	Q468L	V471L/G	Q475R	A482V	K490M	Y503H	R504C/H/L	S505P	F507L/V	A513T	V523L/A/M	I524T/S	P527S/T	G531D/E	F533L	V537L	S538L/P	A539T	V541M	S543G	Study
<b>Clinical Isolates</b>																																										
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<b>Experimentally evolved</b>																														3												
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<sup>a</sup>Sequence variants in the transpeptidase domain of PBP3 in clinical isolates and experimentally evolved -lactam-resistant mutants are listed. Variants predicted using the bioinformatics tool Provean<sup>1, 17, 18</sup> to affect protein function are shown in red, and those predicted to not significantly affect protein function are in blue. The table is derived from a figure from<sup>1</sup>.

**Table S2.** *P. aeruginosa* PBP3 variants outside the transpeptidase domain<sup>a</sup>

PBP3 variants	A9T	W13R	R14L	A28T	D40V	G46S	A60V	G63S/D/C/R	I65T	R153S	R175H	G188A	G191E/D	N212T	P215L	G216S	R226C	Study
Clinical Isolates																		
																		3
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																		5
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																		11
																		12
																		14

<sup>a</sup>Sequence variants in the cytoplasmic (yellow), membrane spanning alpha-helix (green) and domain likely involved in protein-protein interactions (Brown) of PBP3 in clinical isolates and experimentally evolved β-lactam-resistant mutants are listed. Variants predicted using the bioinformatics tool Provean<sup>1, 17, 18</sup> to affect protein function are shown in red, and those predicted to not significantly affect protein function in blue. Figure is derived from a figure from<sup>1</sup>.

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