Identifying and exploiting genes that potentiate the evolution of antibiotic resistance

Danna Gifford[§], Victoria Furió[§], Andrei Papkou, Tom Vogwill, Antonio Oliver and R. Craig MacLean

Table S1: Mutation rate estimates for the 8 strain used. Mutation rates were estimated by Luria-Delbrück fluctuation test using the p_0 method.

	Mean density	Cultures	No. without mutants	Proportion (p_0)	$-\ln(p_0)$	Mutation rate (95% upper bound)
P. aeruginosa PAO1	3.65×10^{6}	480	367	0.765	0.268	7.36×10^{-8}
P. fluorescens Pf0-1	5.20×10^{6}	480	421	0.877	0.131	2.52×10^{-8}
P. fluorescens SBW25	6.20×10^{6}	480	465	0.969	0.032	5.12×10^{-9}
P. fulva CCUG 12573	4.10×10^{7}	480	168	0.350	1.050	2.56×10^{-8}
P. mendocina CCUG 1781	1.30×10^{7}	480	186	0.388	0.948	7.29×10^{-8}
P. protegens Pf-5	2.80×10^{6}	480	368	0.767	0.266	9.49×10^{-8}
P. putida KT2440	1.70×10^{6}	480	380	0.792	0.234	1.37×10^{-7}
P. stutzeri ATCC 17588	8.50×10^{6}	480	49	0.102	2.282	2.68×10^{-7}

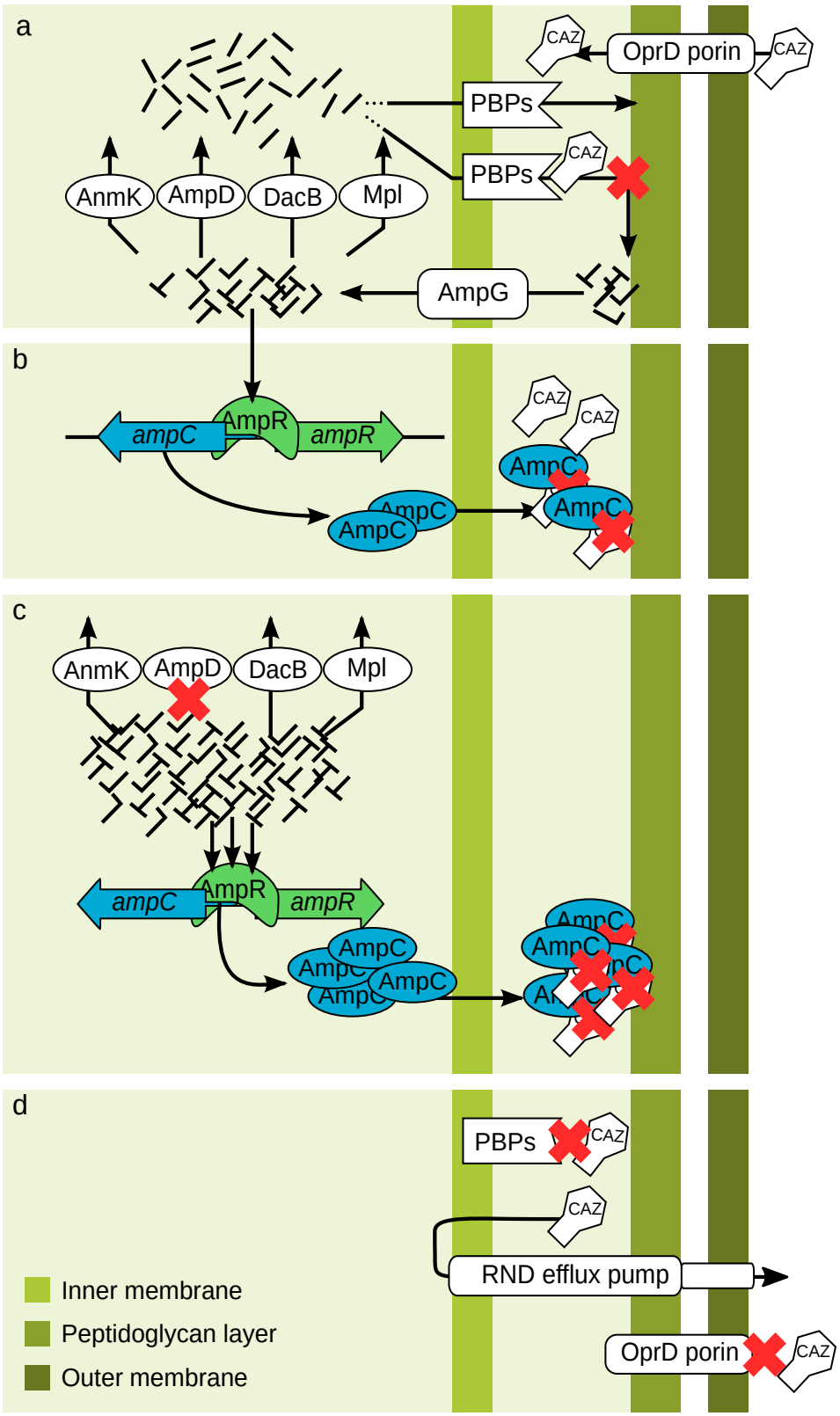

Figure S1: Mechanisms of ceftazidime action and resistance a Ceftazidime (CAZ) enters into the periplasm by diffusion through outer membrane porins. CAZ inhibits penicillin binding proteins (PBPs), resulting in an incrased accumulation of peptidoglycan catabolites. **b** The accumulation of peptidoglycan catabolites in the cytoplasm converst the AmpR transcription factor into an activator of *ampC* expression. AmpC is secreted into the periplasm, where it hydrolyses CAZ. **c** Loss of function mutations in peptidoglycan recycling genes lead to increased resistance by increasing *ampC* expression. **d** Resistance can also be caused by structural alterations to PBPs, elevated expression of RND efflux pumps, or mutations in porin genes.

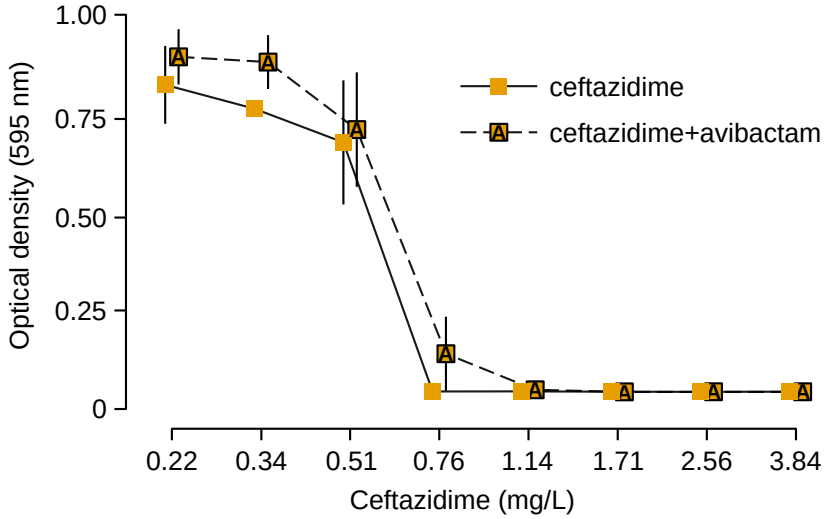

Figure S2: Ceftazidime and ceftazidime+avibactam MIC in *Pseudomonas aeruginosa* PAO1. The addition of avibactam increased the MIC of ceftazidime from 0.76 mg/L to 1.14 mg/L.

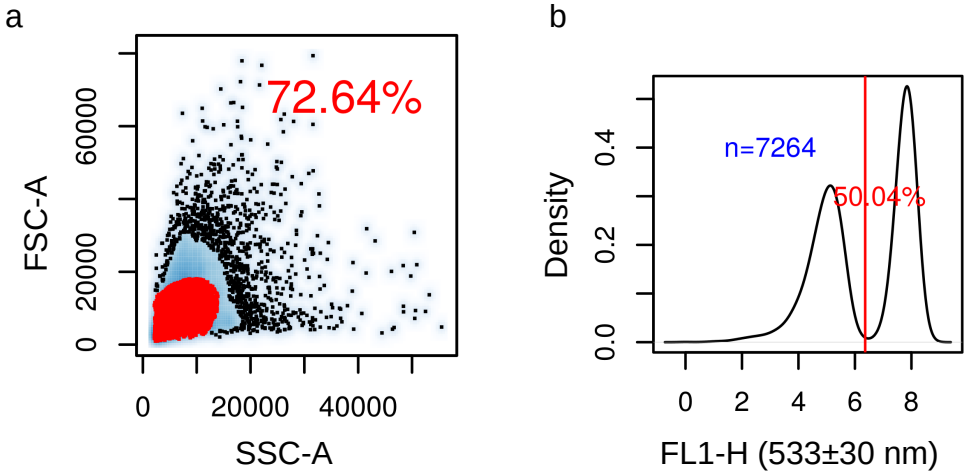

Figure S3: Flow cytometry gating strategy. a Around 10000 events were acquired per sample. The gating procedure described below would typically preserve 7000-8000 events for subsequent analysis. During data acquisition, a lower cut off was set at 10,000 for FSC-H and at 8000 for SSC-H. In the gating pipeline, the events were automatically gated on size/shape by retaining the cells within 2 standard deviations around the median in the bivariate normal distribution of FSC-A and SSC-A ("norm2Filter" from flowCore package, red area). **b** A k-means clustering algorithm was applied on fluorescence intensity FL1-H to differentiate YFP-expressing versus non-fluorescent cells ("kmeansFilter" from flowCore package, red horizontal bar). For each antibiotic concentration, we ensured that YFP-expressing strain could be well separated from non-fluorescent strains by overlaying non-mixed controls (overlap is usually less than 2% of the cells).