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Supplementary information

Bottleneck size and selection level reproducibly impact evolution of antibiotic resistance

In the format provided by the authors and unedited

Supplementary Table 1: Statistical results of Welch t-tests for area under curve (AUC) of harmonic mean Population sizes from the GEN and CIP experiments.

	GEN experiment			CIP experiment		
Comparison	**t**	**Df**	**P**	**t**	**Df**	**P**
IC80 k50-IC20 k50	0.2012	13.279	0.8436	14.654	11.373	**<0.0001**
IC80 M5-IC20 M5	5.6754	7.4104	**0.0006**	5.9505	8.5935	**0.0003**

The test compares the difference in AUC (harmonic mean population size over transfer) between the individual treatment groups.

Supplementary Table 2: Statistical results of a general linear mixed model for yield over time for the GEN and CIP experiments.

	GEN experiment			CIP experiment		
Predictor	χ^2	**Df**	***P***	χ^2	**Df**	***P***
IC	31.34	3	**<0.0001**	16.05	3	**<0.0001**
BN	17.19	2	**<0.0001**	33.35	2	**<0.0001**
Transfer	21.46	1	**<0.0001**	48.44	1	**<0.0001**
IC*BN	4.47	6	**0.0345**	1.54	6	0.2151
IC*Transfer	15.40	3	**<0.0001**	5.45	3	**0.0195**
BN*Transfer	5.97	2	**0.0145**	2.98	2	0.0842
IC*BN*Transfer	0.73	6	0.3919	2.01	6	0.1565

The model tests the influence of drug concentration (IC), bottleneck size (BN) and time of transfer, as well as their individual interactions, on population yield. Formula: Yield ~ IC * BN * Transfer * (1 | Well on plate).

Supplementary Table 3: Statistical results of a Tukey's HSD test for difference in yield over time for the GEN and CIP experiments.

	GEN experiment		CIP experiment	
Comparison	**diff**	***P***	**diff**	***P***
IC20 M5-IC20 k50	0.19	**<0.0001**	0.43	**<0.0001**
IC80 k50-IC20 k50	-0.11	**0.0058**	- 0.56	**0.0003**
IC80 M5-IC20 k50	-0.05	0.4816	0.15	0.0974
IC80 k50-IC20 M5	-0.31	**<0.0001**	- 0.99	**<0.0001**
IC80 M5-IC20 M5	-0.24	**<0.0001**	- 0.28	**0.0001**
IC80 M5-IC80 k50	0.06	0.2979	0.71	**<0.0001**

The test compares the difference in mean yield between the individual treatment groups. Formula: Yield ~ Treatment.

Supplementary Table 4: Statistical results of a Tukey's HSD test for area under curve (AUC) of growth rate from evolved populations from the GEN and CIP experiments.

	GEN experiment		CIP experiment	
Comparison	**diff**	***P***	**diff**	***P***
IC20 M5-IC20 k50	-0.0086	**0.0069**	0.0334	**<0.0001**
IC80 k50-IC20 k50	-0.0004	0.9976	-0.0916	**<0.0001**
IC80 M5-IC20 k50	-0.0041	0.3691	0.0013	0.9956
IC80 k50-IC20 M5	0.0082	**0.0107**	-0.1250	**<0.0001**
IC80 M5-IC20 M5	0.0045	0.3199	-0.0321	**<0.0001**
IC80 M5-IC80 k50	-0.0037	0.4653	0.0929	**<0.0001**

The test compares the difference in AUC (growth rate over transfer) between the individual treatment groups. Formula: AUC ~ Treatment.

Supplementary Table 5: Drug concentrations used to test for evolved resistance in the GEN and CIP experiments (all concentrations given in ng/ml).

	IC20	**IC80**	**MIC**	**2*MIC**	**4*MIC**	**8*MIC**	**16*MIC**
GEN	380	500	600	1200	2400	4800	9600
CIP	15	40	60	120	240	480	960

Supplementary Table 6: Linear mixed effect model results for evolved resistance for the GEN and CIP experiments.

		GEN experiment			CIP experiment		
Predictor	**Df**	**Sum Sq**	**F**	***P***	**Sum Sq**	**F**	***P***
IC	3	0.22	0.71	0.4084	6.32	32.70	**<0.0001**
BN	2	0.24	0.76	0.3903	0.64	3.33	0.0823
IC*BN	6	3.53	11.43	**0.0022**	3.84	19.87	**0.0002**

The model tests the influence of drug concentration (IC), bottleneck size (BN) and their interaction on evolved resistance (AUC) to the treatment drug. Formula: AUC ~ IC * BN.

Supplementary Table 7: Statistical results of Tukey's HSD for evolved resistance for the GEN and CIP experiments.

	GEN experiment		CIP experiment	
Comparison	**diff**	***P***	**diff**	***P***
IC20 M5-IC20 k50	-0.83	**0.0292**	- 0.78	**0.0094**
IC80 k50-IC20 k50	-0.49	0.3110	- 0.69	0.4646
IC80 M5-IC20 k50	0.03	0.9994	0.83	**0.0059**
IC80 k50-IC20 M5	0.34	0.6257	0.09	0.9975
IC80 M5-IC20 M5	0.86	**0.0280**	1.61	**<0.0001**
IC80 M5-IC80 k50	0.53	0.2841	1.52	**0.0184**

The test compares the difference in mean resistance (AUC) between the individual treatment groups. Formula: AUC ~ Treatment.

Supplementary Table 8: Statistical results of a Tukey's HSD test for minimal inhibitory concentrations (MIC) of evolved populations from the GEN and CIP experiments.

	GEN experiment		CIP experiment	
Comparison	**diff**	***P***	**diff**	***P***
IC20 M5-IC20 k50	-6832.5	**0.0199**	-682.5	**0.0086**
IC80 k50-IC20 k50	-4350	0.3089	-540	0.7728
IC80 M5-IC20 k50	-2603.57	0.8545	900	**0.0003**
IC80 k50-IC20 M5	2475	0.8635	143	0.9997
IC80 M5-IC20 M5	4221.43	0.3834	1582.5	**<0.0001**
IC80 M5-IC80 k50	1764	0.9760	1440	**0.0091**

The test compares the difference in MIC between the individual treatment groups. Formula: MIC ~ Treatment.

Supplementary Table 9: Number of mutations in most frequently affected genes per treatment group at the final transfer of the GEN experiment.

Gene	IC20-k50	IC20-M5	IC80-k50	IC80-M5	Total
ptsP	1	13	2	4	20
pmrB	5	0	1	7	13
parS	3	0	0	1	4
parR	1	0	3	1	5
phoQ	1	0	2	0	3
rbsR	0	2	0	0	2
HPr	0	2	0	1	3

Supplementary Table 10: Number of mutations in most frequently affected genes per treatment group at the final transfer of CIP experiment.

Gene	IC20-k50	IC20-M5	IC80-k50	IC80-M5	Total
mexS	4	0	0	10	14
mexZ	7	0	0	0	7
nfxB	1	0	0	6	7
copZ	4	0	0	0	4

Supplementary Table 11: Shannon's diversity indices H and Hmax for each treatment group for the GEN and CIP experiments.

	GEN experiment		CIP experiment	
Treatment	**Shannon's H**	**Hmax**	**Shannon's H**	**Hmax**
IC20-k50	3.27	3.46	2.44	2.81
IC20-M5	2.04	2.58	0.97	1
IC80-k50	2.85	3	1	1
IC80-M5	2.05	2.32	1.58	1.58

Supplementary Table 12: General linear model results for evolved resistance in populations carrying either *pmrB* or *ptsP* mutations.

Predictor	Df	Sum Sq	F	*P*
Treatment	3	3.56	12.25	**0.0002**
Gene	1	3.12	32.24	**<0.0001**

The model tests the influence of treatment regimen and gene variant on evolved resistance (AUC). Formula: AUC ~ Treatment + Gene.

Supplementary Table 13: Clones used in the three competition experiments.

Competition Experiment	Treatment during evolution experiment	*pmrB* mutant from population	*ptsP* mutant from population	control
1	IC80-k50	D12	A12	PA14
2	IC20	A6 (k50)	B11 (M5)	PA14
3	IC80-M5	A5	E7	PA14

Supplementary Table 14: Tukey HSD results for multiple pairwise-comparison of frequency of *ptsP* mutants in competition against *pmrB* mutants and treatment group.

Comparison	diff	p adj.
IC20 M5-IC20 k50	- 0.19	**0.0138**
IC80k50-IC20 k50	- 0.32	**<0.0001**
IC80 M5-IC20 k50	- 0.06	0.8671
IC80 k50-IC20 M5	- 0.17	**0.0352**
IC80 M5-IC20 M5	- 0.14	0.1457
IC80 M5-IC80 k50	- 0.10	0.3983

The test compares the difference in mean frequency of *ptsP* clones in competition between the individual treatments. Formula: Frequency ~ Treatment

Supplementary Table 15: Sequence-specific primers for amplicon sequencing (first PCR step).

Name	Populations	Sequence
pmrB-5636922-forward	A5, D12, A6	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGACCTTGCCACCGAAGACC
pmrB-5637204-forward	A5, G8, A6	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACCATGAACCTGCTGCTGTT
ptsP-394249-forward	E7	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTCATGATCAACGACCGCTTC
ptsP-393899-forward	E7	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCGGCTCGGGCAACTC
ptsP-393042-forward	A12	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACAAGCGTTCCATCGGCAA
ptsP-394724-forward	B11	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGTCGACTTCCTTTCGGTC
pmrB-5636922-reverse	A5, D12, A6	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACAGGGCGTAGCCGAG
pmrB-5637204-reverse	A5, G8, A6	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACCTCGGCCTGCACTTC
ptsP-394249-reverse	E7	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGGTTGTCTTCCTTGATCGG
ptsP-393899-reverse	E7	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTAGCCATCGACGATCAGG
ptsP-393042-reverse	A12	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATCACCCGCCTATGGTG
ptsP-394724-reverse	B11	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATCGTCGACCACCTTCTTCA

Names consist of the target gene, the position of the SNP in the genome and F or R for forward or reverse primer. The population(s) for which the primer amplifies the SNP region are listed. Overhangs of the primers are labelled in black. Sequence-specific parts of primers are in labelled in red.

Supplementary Table 16: Sequencing primers for amplicon sequencing (second PCR step).

2nd PCR	Name	Sequence
forward	F-1	AATGATACGGCGACCACCGAGATCTACACAACCGCATACACTCTTTCCCTACACG
	F-2	AATGATACGGCGACCACCGAGATCTACACAAGGCCTTACACTCTTTCCCTACACG
	F-3	AATGATACGGCGACCACCGAGATCTACACAGAGTGTGACACTCTTTCCCTACACG
	F-4	AATGATACGGCGACCACCGAGATCTACACCACAAGTCACACTCTTTCCCTACACG
	F-5	AATGATACGGCGACCACCGAGATCTACACCGTTCCTAACACTCTTTCCCTACACG
	F-6	AATGATACGGCGACCACCGAGATCTACACGCTTGGATACACTCTTTCCCTACACG
	F-7	AATGATACGGCGACCACCGAGATCTACACGTCAACACACACTCTTTCCCTACACG
	F-8	AATGATACGGCGACCACCGAGATCTACACGTCACTGAACACTCTTTCCCTACACG
	F-9	AATGATACGGCGACCACCGAGATCTACACTCTCGTCAACACTCTTTCCCTACACG
	F-10	AATGATACGGCGACCACCGAGATCTACACTTGGTACGACACTCTTTCCCTACACG
reverse	R-A	CAAGCAGAAGACGGCATACGAGATAACCGGAAGTGACTGGAGTTCAGACG
	R-B	CAAGCAGAAGACGGCATACGAGATAGAGTGACGTGACTGGAGTTCAGACG
	R-C	CAAGCAGAAGACGGCATACGAGATCAACTGGTGTGACTGGAGTTCAGACG
	R-D	CAAGCAGAAGACGGCATACGAGATCGTTCGTTGTGACTGGAGTTCAGACG
	R-E	CAAGCAGAAGACGGCATACGAGATCTGTTCACGTGACTGGAGTTCAGACG
	R-F	CAAGCAGAAGACGGCATACGAGATGCTTGCAAGTGACTGGAGTTCAGACG
	R-G	CAAGCAGAAGACGGCATACGAGATGTCAACTGGTGACTGGAGTTCAGACG
	R-H	CAAGCAGAAGACGGCATACGAGATTCCTCATGGTGACTGGAGTTCAGACG
	R-I	CAAGCAGAAGACGGCATACGAGATTCGACTAGGTGACTGGAGTTCAGACG
	R-J	CAAGCAGAAGACGGCATACGAGATTTGCAAGCGTGACTGGAGTTCAGACG

Barcodes are labelled in blue. Illumina adapters are labelled in black.