Supplementary Information

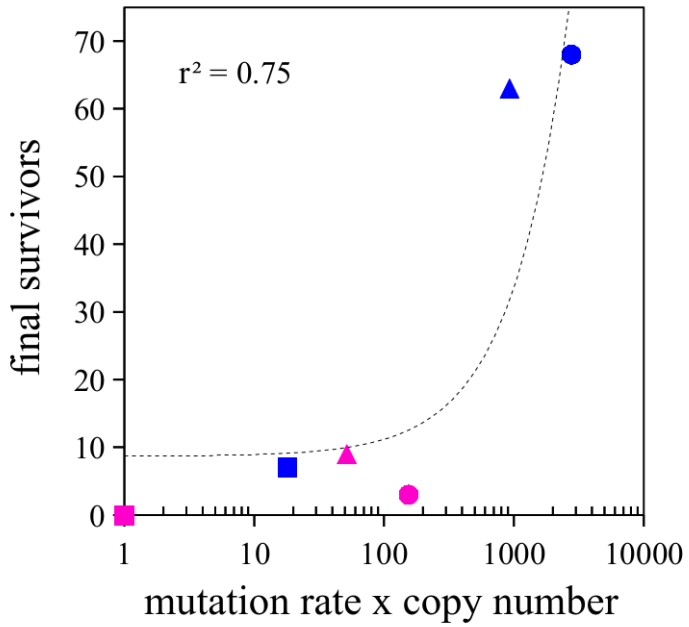

Figure S1. **Correlation between the final number of survivors and the estimated product of mutation rate and *bla*$_{TEM-1}$ copy number.** Mutation rate was estimated by fluctuation test to rifampicin resistance (n=12, Jones median estimator); copy number was considered 1 for the single-copy and 18 for the multiple-copy setting. Dots correspond to wild-type (squares), *ΔmutH* (circles) and *ΔmutT* (triangles) lineages; carrying either one (pink) or multiple (blue) copies of the gene coding for the beta-lactamase TEM-1. The linear regression fit (dashed line) and its coefficient of determination are also shown.

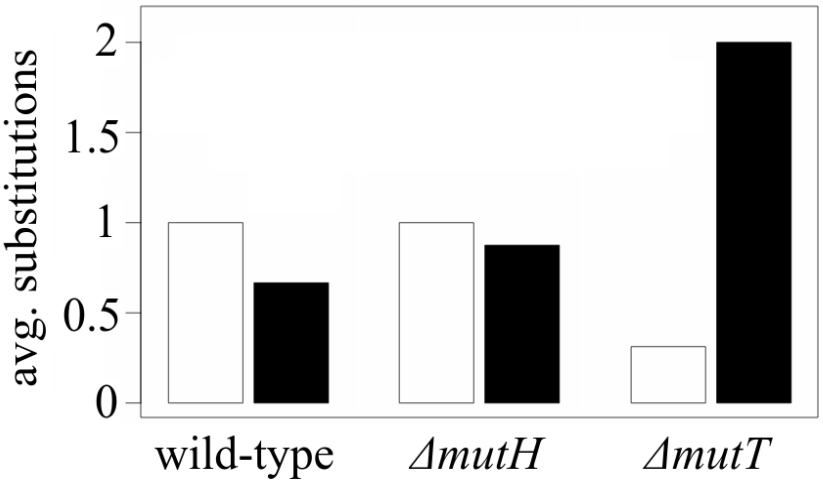

Figure S2. **Genotypic profiles of high-resistance isolates from wild-type and mutator populations.** Bars represent the average number of substitutions per clone observed in TEM-1 (white) and PBP3 (black) among end-point isolates from plasmid-carrying lineages that survived until the end of the experiment (MIC ≥ 64mg/L). The figure shows how the *ΔmutT* background compensated for its inability to elevate adaptive substitutions in TEM-1 by exploiting adaptive pathways within PBP3.

Strain	Plasmid	Level[1]	POR[2]	PUM[2]	EryS[2]	*acrA*[3]	*acrB*	*acrR*	*envZ*	*ompR*	*ompF*	*ompC*
ΔmutT	no	0.5	1	1	0	-	W634G	E67S	H333P	-	-	L361R
ΔmutH	no	64	1	1	0	-	W634G	F52C	-	Y5D	-	-

1: CT concentration (in mg/mL) at which the clone was isolated

2: Labels denote absence (0) or presence (1) of altered porins (POR), increased efflux (PUM) and erythromycin hypersusceptibility (EryS). See Material and Methods for details.

3: Observed aminoacid substitution at the specified locus

Table S1. Non-synonymous mutations in loci involved in β-lactam efflux and permeability in two independent strains with positive phenotypes

Table S2. Non-synonymous mutations at loci *bla* and *ftsI* from a collection of end-point isolates

Isolate[1]	Plasmid	Level[2]	POR[3]	PUM[3]	EryS[3]	CT[4]	bla[5]	E104[6]	H153	R164	M182	G238	ftsI[5]	R167	N255	A257	G270	G306	V309	P311	V313	V314	M315	A317	L318	I336	L350	V355	N361	S365	K366	A368	L369	A370	N390	L393	A413	V423	V467	A475	G478	A492	A498	I532
W1	no	0.5	1	1	0	2	0						0																														
W2	no	0.5	1	0	0	2	0						1							A																								
W3	no	4	1	0	0	4	0						1																												Δ			
W4	no	4	1	1	0	8	0						0																															
W5	yes	0.5	1	0	0	0.5	0						0																															
W6	yes	0.5	1	0	0	1	0						0																															
W7	yes	0.5	1	0	0	0.5	0						0																															
W8	yes	2	1	0	0	4	0						1			V																												
W9	yes	2	0	0	0	2	0						1																															L
W10	yes	2	1	0	0	2	0						1			V																											
W11	yes	64	1	0	0	16	1					S	0																															
W12	yes	64	1	0	0	8	1			S			1																				E										
W13	yes	64	1	0	0	>128	1					S	0																															
W14	yes	64	0	0	0	>128	1					S	2				R	V																										
W15	yes	64	0	0	0	>128	1					S	1					V																										
W16	yes	64	0	0	0	128	1					S	0																															
H1	no	16	1	1	1	32	0						2																	F													V	
H2	no	16	1	0	1	2	0						1							S																							
H3	no	16	1	0	1	16	0						2	C																												V		
H4	no	16	1	0	0	16	0						2																				E	V									
H5	no	16	1	0	1	32	0						1											V																			
H6	no	16	1	1	0	4	0						1								M																						
H7	no	64	1	1	0	64	0						3											V						E												V	
H8	no	64	1	1	0	64	0						2											V					S													
H9	no	64	1	0	0	64	0						2						A														F									
H10	no	64	1	0	0	64	0						1																															L
H11	yes	16	1	1	0	32	0						2		S						M																						
H12	yes	16	0	0	0	4	1					S	0																															
H13	yes	16	1	0	0	16	0						2	C																														L
H14	yes	16	1	0	1	32	0						1																S													
H15	yes	64	0	0	1	4	1					S	1																											V			
H16	yes	64	0	0	0	64	2	K				S	0																															
H17	yes	64	0	1	0	16	0						1											V																			
H18	yes	64	0	0	0	>128	1					S	0																															
H19	yes	64	1	1	1	>128	0						1																														V	
H20	yes	64	1	1	1	>128	0						2			V																	F										
H21	yes	64	1	1	0	>128	1					S	1																	F													
H22	yes	64	0	1	0	>128	3	K			T	S	0																															
H23	yes	64	1	1	0	>128	2		R			S	1																	F													
H24	yes	64	1	0	0	>128	1					S	1																													T		
H25	yes	64	1	0	1	>128	1					S	1																S													
H26	yes	64	1	1	0	>128	3	K			T	S	0																															
T1	no	16	1	0	0	8	0						2																	F			F										
T2	no	16	1	1	0	4	0						1									G																					
T3	no	16	1	1	0	32	0						1														G																
T4	no	16	1	0	0	32	0						2									G																					L
T5	no	16	1	1	0	32	0						2													S																	L
T6	no	64	1	1	0	64	0						2					V															F									
T7	no	64	1	0	0	64	0						2									G																					L
T8	no	64	1	1	0	64	0						3																				F				G					L
T9	no	64	1	0	0	64	0						3								G												F									L
T10	no	64	1	0	0	64	0						2													S																	L
T11	yes	16	0	0	0	4	0						1																															L
T12	yes	16	1	0	1	32	0						2													S																	L
T13	yes	16	1	0	0	8	1					S	1																															L
T14	yes	16	1	0	1	32	0						3								G												F									L
T15	yes	64	0	0	0	8	1					S	1																															L
T16	yes	64	1	0	0	32	1					S	2														V							F									
T17	yes	64	1	1	0	64	0						3								G												F									L
T18	yes	64	1	0	1	>128	0						3													S							F									L
T19	yes	64	1	1	1	64	0						2									G																					L
T20	yes	64	1	1	1	128	0						3																				F				G					L
T21	yes	64	1	0	0	128	1					S	0																															
T22	yes	64	1	1	0	>128	0						2												W																	L
T23	yes	64	1	1	0	>128	0						3										L										F									L
T24	yes	64	1	1	1	>128	0						2														F								L							
T25	yes	64	1	1	0	>128	0						3																				F				V					L
T26	yes	64	1	0	1	>128	1					S	1																															L

1: First letter denotes wild-type (W), ΔmutH (H) and ΔmutT (T)
2: CT concentration (in mg/mL) at which the clone was isolated
3: Labels denote absence (0) or presence (1) of altered porins (POR), increased efflux (PUM) and erythromycin hypersusceptibility (EryS). See Material and Methods for details.
4: CT MIC (in mg/mL)
5: Number of substitutions observed in the corresponding locus
6: Black background indicate mutations corresponding to the mutational spectrum of each mutator.

Table S3. Alleles of proteins TEM-1 and PBP3 from the collection of end-point isolates

allele	substitutions	wild-type	*ΔmutH*	*ΔmutT*	total
FtsI-1	R167C, A475V		1		1
FtsI-2	R167C, I532L		1		1
FtsI-3	N255S, V313M		1		1
FtsI-4	A257V		1		1
FtsI-5	A257V, L369F		1		1
FtsI-6	G270R, G306V	1			1
FtsI-7	G306V	1			1
FtsI-8	G306V, L369F			1	1
FtsI-9	V309A, L369F		1		1
FtsI-10	P311A	1			1
FtsI-11	P311S		1		1
FtsI-12	V313M		1		1
FtsI-13	V313G, L369F, I532L			3	3
FtsI-14	V314G			1	1
FtsI-15	V314G, I532L			3	3
FtsI-16	M315L, L369F, I532L			1	1
FtsI-17	A317V		2		2
FtsI-18	A317V, N361S		1		1
FtsI-19	A317V, K366E, A498V		1		1
FtsI-20	L318W, I532L			1	1
FtsI-21	I336S, I532L			3	3
FtsI-22	I336S, L369F, I532L			1	1
FtsI-23	L350V, L369F			1	1
FtsI-24	L350F,N390L			1	1
FtsI-25	V355G			1	1
FtsI-26	N361S		2		2
FtsI-27	S365F		2		2
FtsI-28	S365F, A498V		1		1
FtsI-29	S365F, L369F			1	1
FtsI-30	A368E	1			1
FtsI-31	A368E, A370V		1		1
FtsI-32	L369F, V423G, I532L			1	1
FtsI-33	L369F, V467G, I532L			1	1
FtsI-34	L369F, L393V, I532L			1	1
FtsI-35	A413V		1		1
FtsI-36	G478Δ	1			1
FtsI-37	A492T		1		1
FtsI-38	A498V		1		1
FtsI-39	I532	1	1	4	6
TEM-15	E104K, G238S		1		1
TEM-52	E104K, M182T, G238S		2		2
TEM-112	H153R, G238S		1		1
TEM-12	R164S	1			1
TEM-19	G238S	5	6	5	16