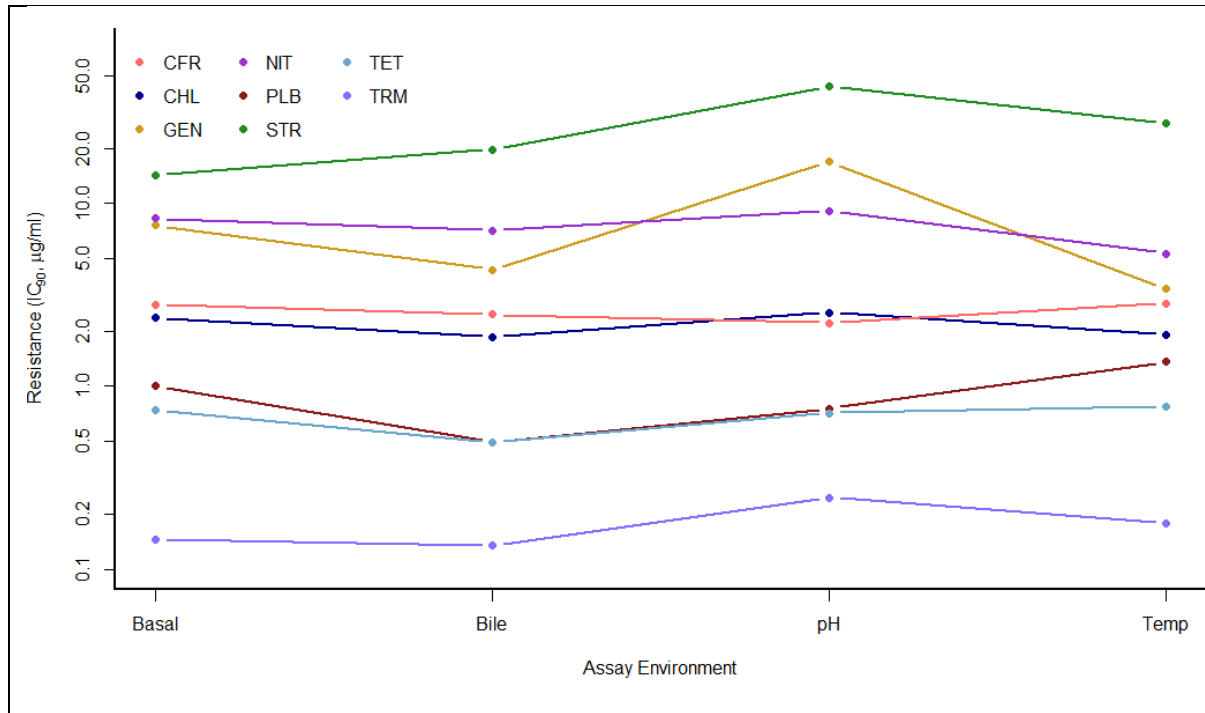
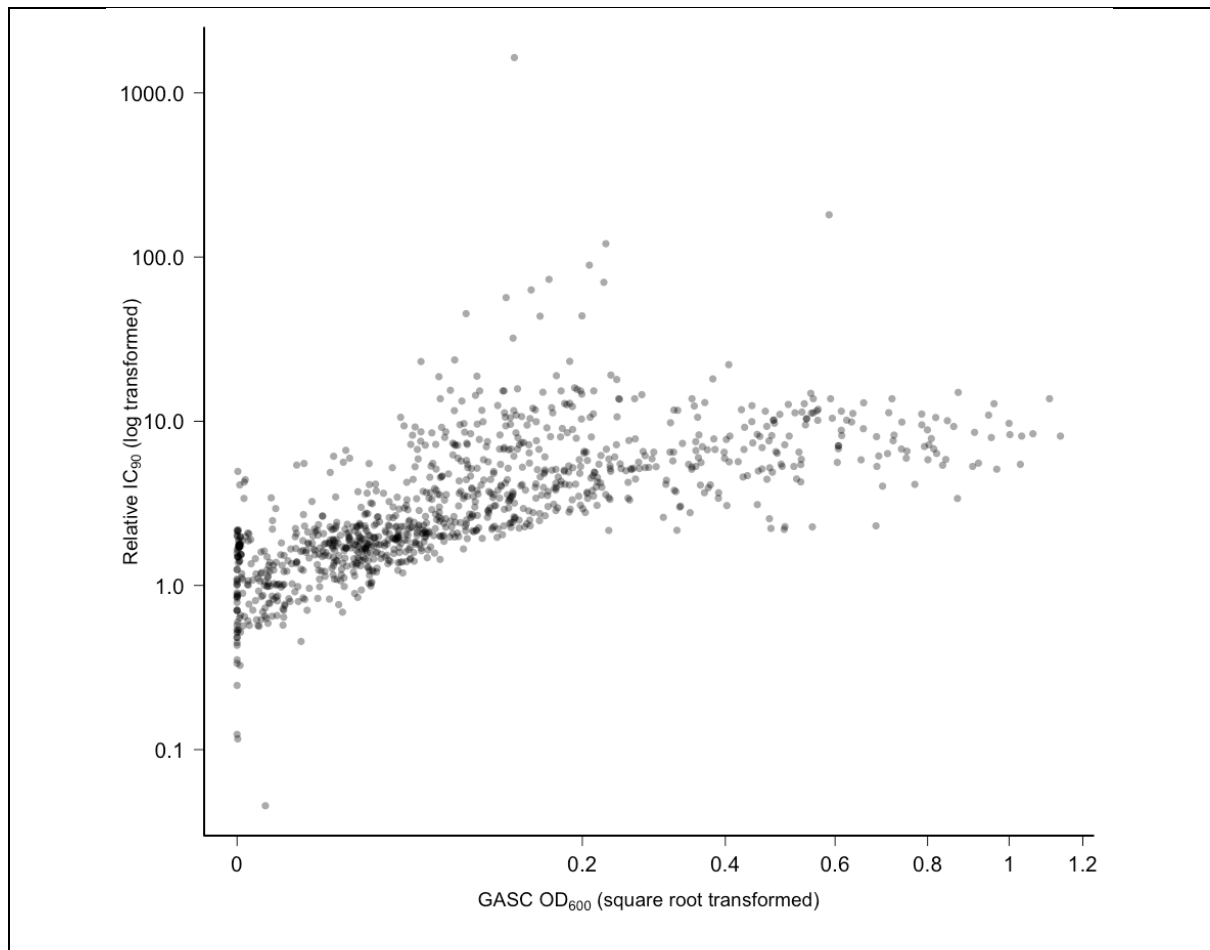


1 Supplementary information for: Collateral sensitivity  
2 interactions between antibiotics depend on local abiotic  
3 conditions

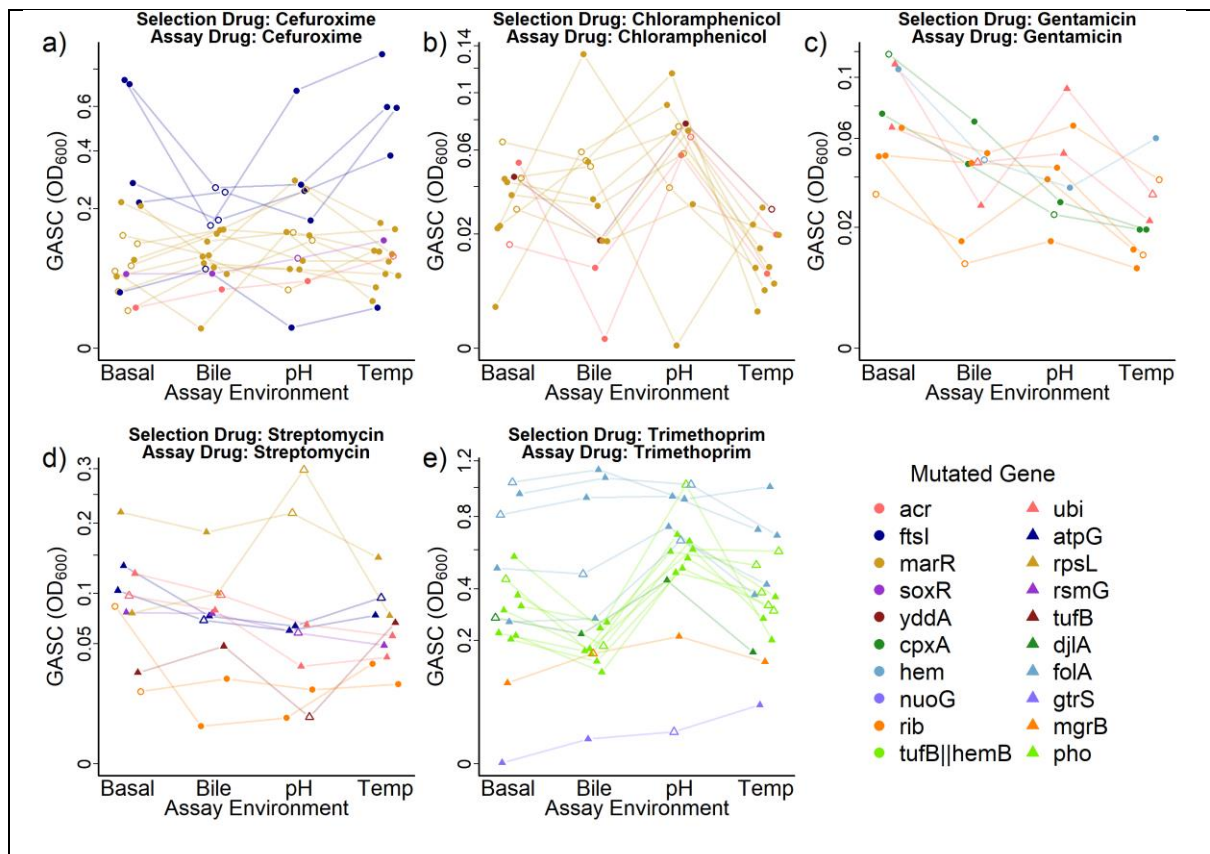
4 Supplementary Figures  
5



**Fig. S1:** IC<sub>90</sub> for the ancestral *E. coli* strain to each of the antibiotics used in this study, assayed in each of the different environments. Points are the mean of 2-4 replicates and the y axis is log transformed. Assay environment had a predictive effect on the level of resistance to gentamicin ( $\chi^2_3 = 29.5$ ,  $p < 0.0001$ ), polymyxin ( $\chi^2_3 = 10.8$ ,  $p < 0.05$ ) and streptomycin ( $\chi^2_3 = 11.8$ ,  $p < 0.01$ ). All IC<sub>90</sub> comparisons reported in the text are relative to the ancestral IC<sub>90</sub> in the same abiotic environment. This could in theory influence the main effect of assay environment on collateral effects as reported in the main text (seen for the following selection/paired drug combinations of cefuroxime/gentamicin, chloramphenicol/polymyxin B and trimethoprim/nitrofurantoin). However, we see the same main effect of assay environment in two of these three combinations when we use absolute IC<sub>90</sub> instead of IC<sub>90</sub> relative to the ancestor (cefuroxime/gentamicin,  $\chi^2_3 = 141$ ,  $p < 0.0001$ ; chloramphenicol/polymyxin B  $\chi^2_3 = 69.4$ ,  $p < 0.0001$ ). We also see a significant effect for streptomycin selected mutants (streptomycin / tetracycline  $\chi^2_3 = 20.1$ ,  $p < 0.001$ ), which was not significant using IC<sub>90</sub> relative to the ancestor (see main text).



**Figure S2: Correlation between growth at selection concentration (GASC) and IC<sub>90</sub> for the ancestor and 66 mutants, each measured against their relevant selection drug in each of the four assay environments. Each point is from an independent dose response assay, with IC<sub>90</sub> and GASC calculated from the same data. The different measures are transformed according to the way that the variables are input into the relevant models. These measures are highly correlated ( $\tau=0.61$ ,  $p<0.0001$ ).**



**Fig. S3: Population growth for resistant mutants selected with each selection drug (panels a-e) when grown with that antibiotic at selection concentration (GASC), measured in four different assay environments. Each set of four connected points shows a single resistant mutant, coloured according the gene or gene family that was mutated. Growth was measured in the presence of the selection drug at the selection concentration (see methods) using optical density (OD600) after 20h. Hollow points indicate sympatric combinations (selection environment = assay environment). Points are means of 2-4 independent replicates (mean  $n = 3.86$ ). The y axis is square-root transformed and varies between panels. GASC varied depending on mutated gene (main effect of genotype: cefuroxime,  $F_{4,20} = 21.7$ ,  $p < 0.0001$ ; chloramphenicol,  $F_{3,79} = 24.7$ ,  $p < 0.0001$ ; gentamicin,  $\chi^2_{23} = 9.07$ ,  $p < 0.05$ ; streptomycin,  $F_{11,134} = 181$ ,  $p < 0.0001$  and trimethoprim,  $F_{5,19} = 83.6$ ,  $p < 0.0001$ ), and the interaction between mutated gene and assay environment (genotype by assay environment interaction effect on GASC: cefuroxime,  $\chi^2_{29} = 21.0$ ,  $p < 0.05$ ; chloramphenicol,  $\chi^2_{26} = 13.1$ ,  $p < 0.05$ ; streptomycin,  $\chi^2_{215} = 26.3$ ,  $p < 0.05$ ; and trimethoprim,  $\chi^2_{212} = 47.6$ ,  $p < 0.0001$ ).**

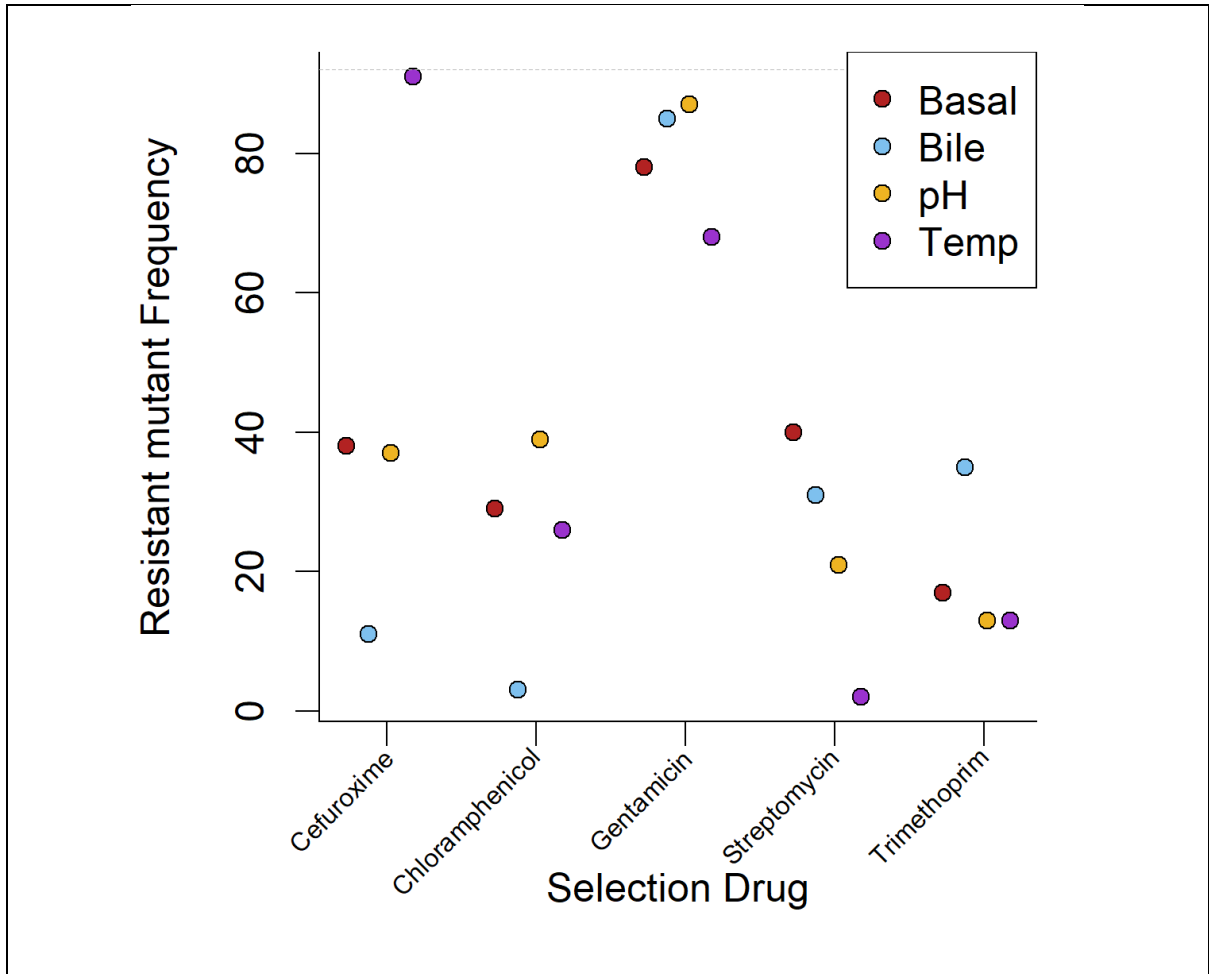
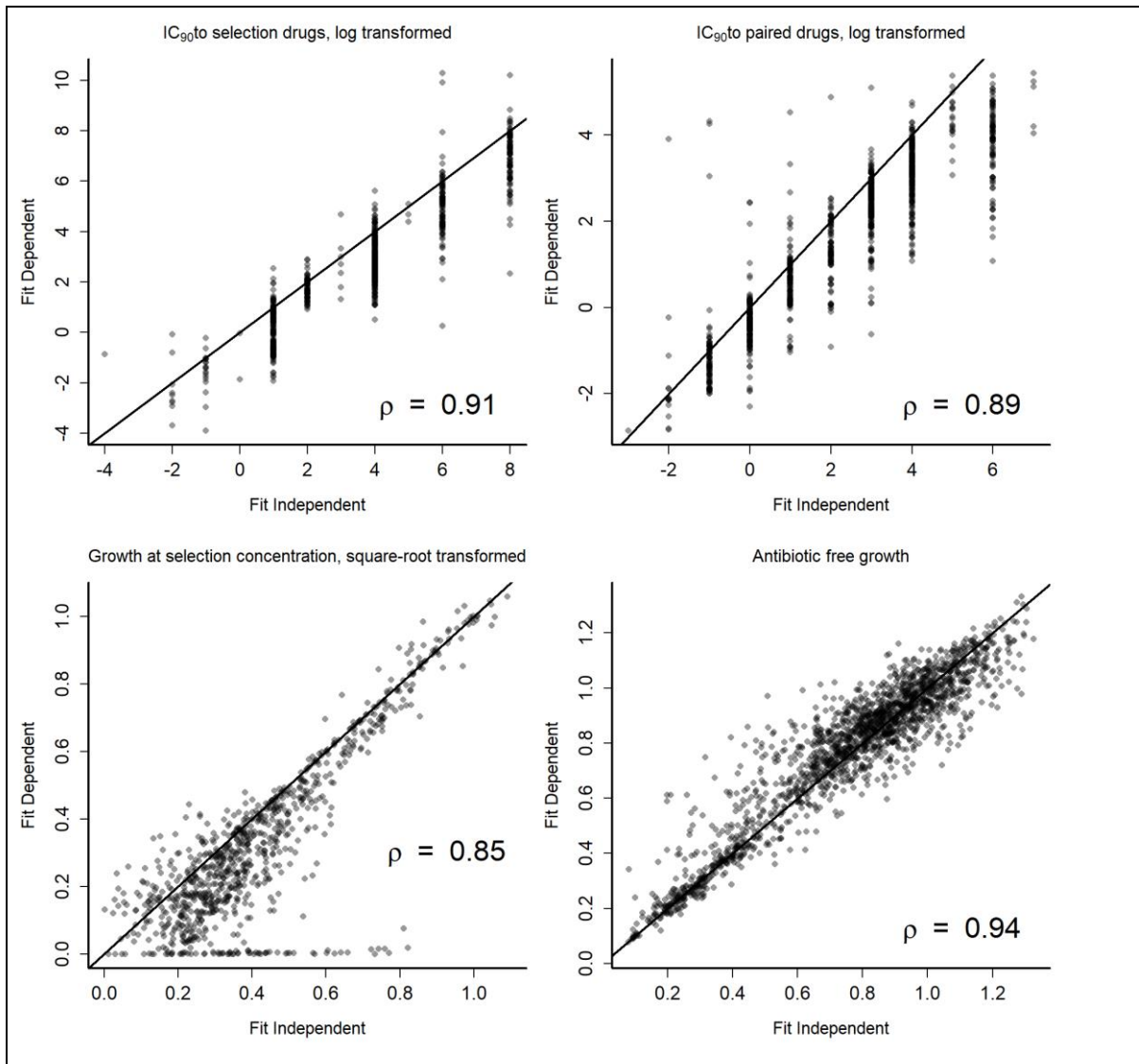


Figure S4: Number of independent wells (out of 92) which had resistant colonies after plating *E. coli* with different drugs in different environments. The number of populations in which resistance was seen (one or more resistant colonies) varied with the combination of antibiotic and selection environment (binomial glm of number of independent wells with resistance: Antibiotic : environment interaction,  $\chi^2_{12} = 160$   $p < 0.0001$ ).



**Fig S5:** Correlation between the 4 phenotypes measured when calculated using a method that requires a fitted Hill function (fit dependent) and a method that does not require a fitted Hill function (fit independent). Points represent values calculated from individual replicate dose response measurements. Only points where both fit dependent and fit independent methods were calculated are plotted. The correlation measured by Pearson's correlation coefficient is given at the bottom-right of each panel.

## 12 Supplementary Tables

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Selection Drug	Selection Environment	Replicate Mutant	Genotype		Included in phenotype assays	Included in model				Notes
			Full	Family		Selection drug IC <sub>90</sub>	GASC	Paired drug IC <sub>90</sub>	Antibiotic Free growth	
Cefuroxime	Basal	1	marR	mar	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	Basal	2	marR	mar	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	Basal	3	marR	mar	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	Basal	4	marR	mar	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	Basal	5	marR    yegH/asmA	mar	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	Basal	6	marC/marR	mar	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	Bile	1	marR	mar	Yes	No	No	No	No	
Cefuroxime	Bile	2	ftsI	ftsI	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	Bile	3	ftsI	ftsI	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	Bile	4	ftsI	ftsI	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	Bile	5	ftsI    yraR	ftsI	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	Bile	6	ftsI	ftsI	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	pH	1	marR	mar	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	pH	2	marR	mar	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	pH	3	soxR	soxR	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	pH	4			No	No	No	No	No	No mutation identified
Cefuroxime	pH	5	marR	mar	Yes	Yes	Yes	Yes	Yes	Enriched
Cefuroxime	pH	6	pykF    marR	mar	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	Temperature	1			No	No	No	No	No	No mutation identified
Cefuroxime	Temperature	2			No	No	No	No	No	No mutation identified

Cefuroxime	Temperature	3			No	No	No	No	No	No mutation identified
Cefuroxime	Temperature	4			No	No	No	No	No	No mutation identified
Cefuroxime	Temperature	5	acrR	acr	Yes	Yes	Yes	Yes	Yes	
Cefuroxime	Temperature	6			No	No	No	No	No	No mutation identified
Chloramphenicol	Basal	1	marR	mar	No	No	No	No	No	Duplicated with CHL3Bile
Chloramphenicol	Basal	2			No	No	No	No	No	No mutation identified
Chloramphenicol	Basal	3	marR	mar	Yes	Yes	Yes	Yes	Yes	Enriched
Chloramphenicol	Basal	4	acrR	acr	Yes	Yes	Yes	Yes	Yes	Enriched
Chloramphenicol	Basal	5	marR	mar	Yes	Yes	Yes	Yes	Yes	Enriched
Chloramphenicol	Basal	6	marC/marR	mar	Yes	Yes	Yes	Yes	Yes	Enriched
Chloramphenicol	Bile	1	marR	mar	Yes	Yes	Yes	Yes	Yes	
Chloramphenicol	Bile	2	marR	mar	Yes	Yes	Yes	Yes	Yes	
Chloramphenicol	Bile	3	marR	mar	Yes	Yes	Yes	Yes	Yes	
Chloramphenicol	pH	1	marR    wzxB	mar	Yes	Yes	Yes	Yes	Yes	
Chloramphenicol	pH	2			No	No	No	No	No	No mutation identified
Chloramphenicol	pH	3	marR	mar	Yes	Yes	Yes	Yes	Yes	
Chloramphenicol	pH	4	marR	mar	Yes	Yes	Yes	Yes	Yes	Enriched
Chloramphenicol	pH	5	acrA/acrR	acr	Yes	Yes	Yes	Yes	Yes	
Chloramphenicol	pH	6			No	No	No	No	No	No mutation identified
Chloramphenicol	Temperature	1			No	No	No	No	No	No mutation identified
Chloramphenicol	Temperature	2			No	No	No	No	No	No mutation identified

Chloramphenicol	Temperature	3			No	No	No	No	No	No mutation identified
Chloramphenicol	Temperature	4	yddA	yddA	Yes	Yes	Yes	Yes	Yes	
Chloramphenicol	Temperature	5			No	No	No	No	No	No mutation identified
Chloramphenicol	Temperature	6			No	No	No	No	No	No mutation identified
Gentamicin	Basal	1	ribB/yqiC	rib	Yes	Yes	Yes	Yes	Yes	
Gentamicin	Basal	2	cpxA	cpxA	Yes	No	No	No	No	
Gentamicin	Basal	3	cpxA	cpxA	Yes	Yes	Yes	Yes	Yes	
Gentamicin	Basal	4	cysS    fusA	fusA	Yes	No	No	No	No	
Gentamicin	Basal	5	metQ    ygfZ	ygfZ	Yes	No	No	No	No	
Gentamicin	Basal	6	fusA	fusA	Yes	No	No	No	No	
Gentamicin	Bile	1	ribB/yqiC    ribB/yqiC	rib	Yes	Yes	Yes	Yes	Yes	
Gentamicin	Bile	2			No	No	No	No	No	No mutation identified
Gentamicin	Bile	3	dxs    tfaP	dxs	Yes	No	No	No	No	
Gentamicin	Bile	4	ubiH	ubi	Yes	Yes	Yes	Yes	Yes	
Gentamicin	Bile	5	hemC	hem	Yes	Yes	Yes	Yes	Yes	
Gentamicin	Bile	6	ubiJ	ubi	Yes	No	No	No	Yes	
Gentamicin	pH	1	cpxA	cpxA	Yes	Yes	Yes	Yes	Yes	
Gentamicin	pH	2	ribB/yqiC	rib	No	No	No	No	No	Duplicated with GEN1Basal
Gentamicin	pH	3	ubiH	ubi	Yes	No	No	No	No	
Gentamicin	pH	4	ptsl    nuoG	nuoG	Yes	No	No	No	Yes	
Gentamicin	pH	5	cpxA	cpxA	No	No	No	No	No	Duplicated with GEN2Basal
Gentamicin	pH	6	tufB    hemB	tufB    hem	Yes	No	No	Yes	Yes	Ambiguous family, grouped separately



Gentamicin	Temperature	1	ribB/yqiC    yncE/ansP	rib	Yes	Yes	Yes	Yes	Yes	
Gentamicin	Temperature	2	sbmA    ribB/yqiC    ribB/yqiC	rib	No	No	No	No	No	Duplicated with GEN1Basal
Gentamicin	Temperature	3			No	No	No	No	No	Not genotyped (Unable to culture)
Gentamicin	Temperature	4	ubiH	ubi	Yes	Yes	Yes	Yes	Yes	
Gentamicin	Temperature	5	hemL	hem	Yes	No	No	No	No	
Gentamicin	Temperature	6	ribE	rib	Yes	Yes	Yes	Yes	Yes	
Streptomycin	Basal	1	ribF	rib	Yes	Yes	Yes	Yes	Yes	
Streptomycin	Basal	2	dxs	dxs	Yes	No	No	No	No	
Streptomycin	Basal	3	ubiF	ubi	Yes	Yes	Yes	Yes	Yes	
Streptomycin	Basal	4	ribB/yqiC    ribB/yqiC    gcl	rib	No	No	No	No	No	Duplicated with STR5Basal
Streptomycin	Basal	5	yebT    ribB/yqiC    ribB/yqiC	rib	Yes	No	No	No	No	
Streptomycin	Basal	6	ribB/yqiC    ribB/yqiC	rib	Yes	Yes	Yes	Yes	Yes	
Streptomycin	Bile	1	atpG	atpG	Yes	Yes	Yes	Yes	Yes	
Streptomycin	Bile	2	mngB/cydA	cydA	Yes	No	No	No	No	
Streptomycin	Bile	3	ubiF	ubi	Yes	No	No	No	No	
Streptomycin	Bile	4	ribD	rib	Yes	No	No	No	No	
Streptomycin	Bile	5	ubiB	ubi	Yes	Yes	Yes	Yes	Yes	
Streptomycin	Bile	6	ribE	rib	Yes	No	No	No	No	
Streptomycin	pH	1	rpsL    arcB	rpsL	Yes	Yes	Yes	Yes	Yes	
Streptomycin	pH	2	rsmG	rsmG	Yes	Yes	Yes	Yes	Yes	
Streptomycin	pH	3	rpsL	rpsL	Yes	No	No	No	No	

Streptomycin	pH	4	arcB    rpsL	rpsL	Yes	Yes	Yes	Yes	Yes	
Streptomycin	pH	5	tufB	tufB	Yes	Yes	Yes	Yes	Yes	
Streptomycin	pH	6	ykg-ddlA deletion	hem	Yes	No	No	No	No	Δ83kb (78 genes) including hemB
Streptomycin	Temperature	1	atpG	atpG	Yes	Yes	Yes	Yes	Yes	
Streptomycin	Temperature	2			No	No	No	No	No	Not genotyped (Unable to culture)
Trimethoprim	Basal	1	djlA/yabP	djlA/yabP	Yes	Yes	Yes	Yes	Yes	
Trimethoprim	Basal	2	folA	folA	Yes	No	Yes	Yes	Yes	
Trimethoprim	Basal	3	phoP	pho	Yes	Yes	Yes	Yes	Yes	
Trimethoprim	Basal	4	folA	folA	No	No	No	No	No	Duplicated with TRM2Basal
Trimethoprim	Basal	5	kefC/folA	folA	Yes	No	Yes	Yes	Yes	
Trimethoprim	Basal	6	folA	folA	No	No	No	No	No	Duplicated with TRM6pH
Trimethoprim	Bile	1	folA	folA	Yes	Yes	Yes	Yes	Yes	
Trimethoprim	Bile	2	phoP	pho	No	No	No	No	No	Duplicated with TRM3Basal
Trimethoprim	Bile	3	mgrB	mgrB	Yes	Yes	Yes	Yes	Yes	
Trimethoprim	Bile	4	phoQ	pho	Yes	No	No	Yes	Yes	
Trimethoprim	Bile	5	mgrB/yobH	mgrB	Yes	No	No	No	No	
Trimethoprim	Bile	6	ybhH    phoP	pho	Yes	Yes	Yes	Yes	Yes	
Trimethoprim	pH	1			No	No	No	No	No	Not genotyped (Unable to culture)
Trimethoprim	pH	2	gtrS	gtrS	Yes	Yes	Yes	Yes	Yes	
Trimethoprim	pH	3	folA	folA	Yes	Yes	Yes	Yes	Yes	
Trimethoprim	pH	4	phoQ	pho	No	No	No	No	No	Duplicated with TRM1Temp
Trimethoprim	pH	5	phoP    fucP	pho	Yes	Yes	Yes	Yes	Yes	

Trimethoprim	pH	6	folA	folA	Yes	No	Yes	Yes	Yes	
Trimethoprim	Temperature	1	phoQ	pho	Yes	Yes	Yes	Yes	Yes	
Trimethoprim	Temperature	2	phoQ	pho	Yes	Yes	Yes	Yes	Yes	
Trimethoprim	Temperature	3	phoQ    alaV/rrlH	pho	Yes	Yes	Yes	Yes	Yes	
Trimethoprim	Temperature	4	folA	folA	No	No	No	No	No	Duplicated with TRM2Basal
Trimethoprim	Temperature	5	phoQ    uacT	pho	Yes	Yes	Yes	Yes	Yes	
Trimethoprim	Temperature	6	phoP	pho	Yes	Yes	Yes	Yes	Yes	
Totals		113	95		85	61	64	66	68	

**Table S1: Mutants used in this study.** This table shows all 113 mutants isolated in the resistance selection screen. The first three columns show the antibiotic used for selection, the environment where selection occurred, which replicate isolate (for the treatment) the mutant corresponds to, giving 113 independent mutants. After genotyping we identified mutations at high frequency (see methods) for 95 mutants with full genotype giving all genes with where mutations were at high frequency where a forward slash (/) between two genes means that the mutation occurred between the two genes and horizontal bars (||) separate different mutations that were found at high frequency in a mutant. We then grouped these full genotypes into mutants where genes in the same family were mutated, in order to better model the effects of genotype. When assigning a gene family for these mutants we used genes mutated in multiple independent lines (e.g. all genotypes with where rib mutations are grouped together rather than multiple different unrelated groups according other mutated genes). Of the genotyped mutants we measured resistance phenotypes for 85 mutants, excluding those where other mutants had very similar genotypes. We were able to use between 61-68 of these isolates for models of IC<sub>90</sub> to selection drug, growth at selection concentration (GASC), IC<sub>90</sub> to the paired drugs and antibiotic free growth (having to exclude strains with poor replication, see methods). The rightmost column gives additional information, particularly why mutants were not phenotyped. Here shorthand is used to describe mutants (e.g. TRM1Temp is the first replicate strain selected with trimethoprim at high temperature).

Selection Drug	Assay Drug	Phenotype	Model A : Genotype					Model B: Selection Conditions				
			Random Effects		Fixed Effects			Random Effects		Fixed Effects		
			Strain	Strain : Block	Assay Environment	Genotype	Interaction	Strain	Strain : Block	Assay Environment	Selection Environment	Sympatry
Cefuroxime	Cefuroxime	IC <sub>90</sub>										
Chloramphenicol	Chloramphenicol	IC <sub>90</sub>										
Gentamicin	Gentamicin	IC <sub>90</sub>										
Streptomycin	Streptomycin	IC <sub>90</sub>										
Trimethoprim	Trimethoprim	IC <sub>90</sub>										
Cefuroxime	Cefuroxime	GASC										
Chloramphenicol	Chloramphenicol	GASC										
Gentamicin	Gentamicin	GASC										
Streptomycin	Streptomycin	GASC										
Trimethoprim	Trimethoprim	GASC										
Cefuroxime	Gentamicin	IC <sub>90</sub>										
Chloramphenicol	Polymyxin B	IC <sub>90</sub>										
Gentamicin	Cefuroxime	IC <sub>90</sub>										
Streptomycin	Tetracycline	IC <sub>90</sub>										
Trimethoprim	Nitrofurantoin	IC <sub>90</sub>										
Cefuroxime	None	Cost										
Chloramphenicol	None	Cost										
Gentamicin	None	Cost										
Streptomycin	None	Cost										
Trimethoprim	None	Cost										

**Table S2: Terms included in the minimal models reported in the text (black cells).** Models A and B were separately fitted to the different phenotypes. The models were independently simplified to the minimal models by dropping non-significant terms (not-included in higher order interactions), as described in more detail in the methods.