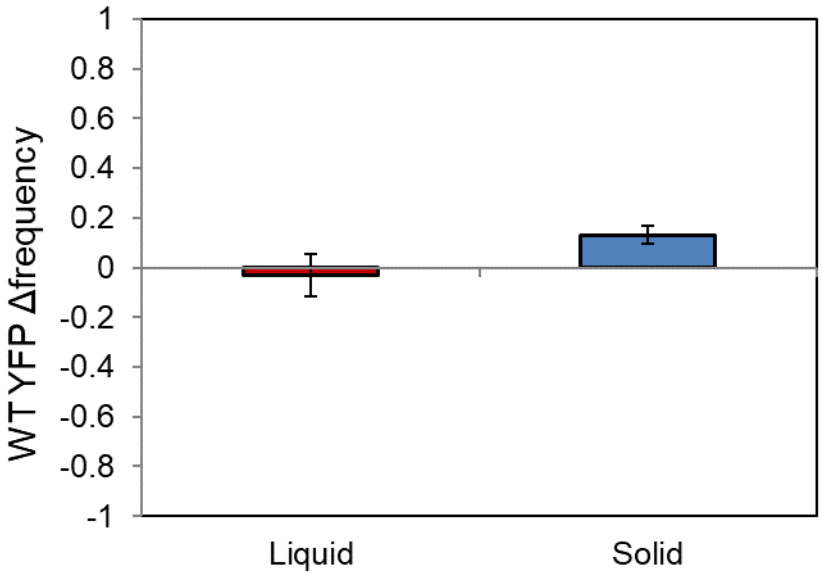
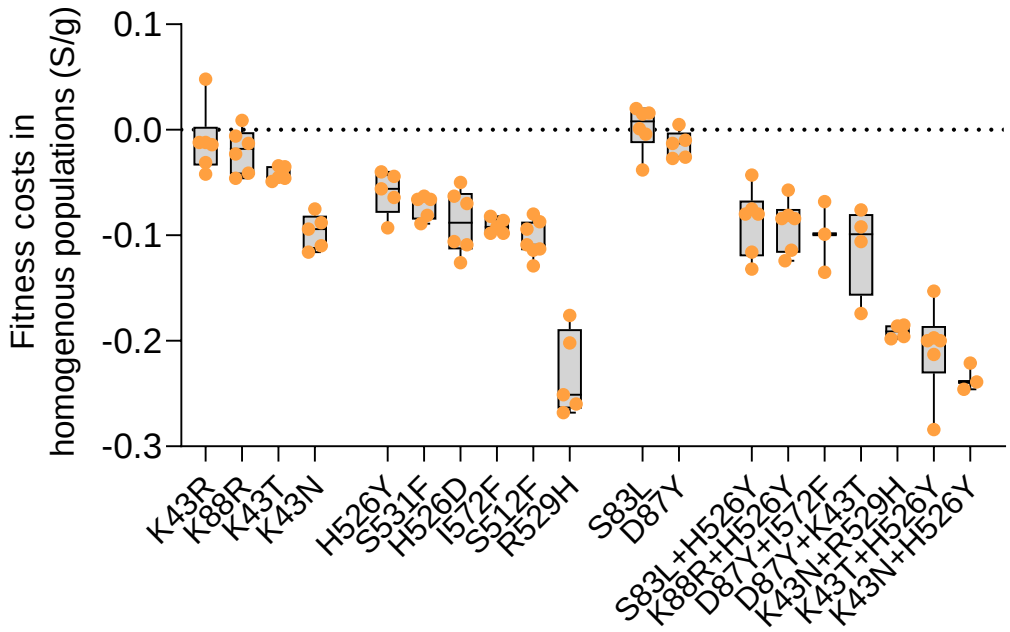
**SUPPLEMENTAL MATERIAL**

**Supplemental Table 1 – Frequency change of antibiotic resistance of resistant mutants after 24h in homogenous and structured populations.** Frequency change of resistance (ΔAR) was calculated as $\Delta AR = [(AR_{final\ frequency} - AR_{initial\ frequency})/ AR_{initial\ frequency}]$. The values on the table represent the average and 2x standard error of at least 3 biological replicates.

| Gene and mutation | Resistance to antibiotic | Homogenous populations | Structured populations |
|---|---|---|---|
| *rpsL*$^{K43N}$ | streptomycin | -0.54 ± 0.07 | -0.56 ± 0.07 |
| *rpsL*$^{K43T}$ | streptomycin | -0.31 ± 0.03 | -0.32 ± 0.05 |
| *rpsL*$^{K43R}$ | streptomycin | -0.07 ± 0.17 | -0.06 ± 0.08 |
| *rpsL*$^{K88R}$ | streptomycin | -0.14 ± 0.12 | -0.11 ± 0.14 |
| *rpoB*$^{S512F}$ | rifampicin | -0.54 ± 0.09 | -0.43 ± 0.12 |
| *rpoB*$^{H526D}$ | rifampicin | -0.49 ± 0.11 | -0.44 ± 0.03 |
| *rpoB*$^{H526Y}$ | rifampicin | -0.36 ± 0.09 | -0.32 ± 0.10 |
| *rpoB*$^{R529H}$ | rifampicin | -0.92 ± 0.04 | -0.84 ± 0.05 |
| *rpoB*$^{S531F}$ | rifampicin | -0.45 ± 0.05 | -0.50 ± 0.06 |
| *rpoB*$^{I572F}$ | rifampicin | -0.55 ± 0.02 | -0.51 ± 0.05 |
| *gyrA*$^{S83L}$ | fluroquinolone | 0.02 ± 0.11 | 0.08 ± 0.09 |
| *gyrA*$^{D87Y}$ | fluroquinolone | -0.09 ± 0.09 | 0.05 ± 0.06 |
| *rpoB*$^{H526Y}$*rpsL*$^{K43T}$ | Rif + Strep | -0.88 ± 0.04 | -0.50 ± 0.05 |
| *rpoB*$^{H526Y}$*rpsL*$^{K88R}$ | Rif + Strep | -0.54 ± 0.10 | -0.18 ± 0.05 |
| *rpoB*$^{R529H}$*rpsL*$^{K43N}$ | Rif + Strep | -0.89 ± 0.01 | -0.71 ± 0.05 |
| *rpoB*$^{H526Y}$*rpsL*$^{K43N}$ | Rif + Strep | -0.92 ± 0.02 | -0.62 ± 0.04 |
| *gyrA*$^{D87Y}$*rpoB*$^{I572F}$ | Fluor + Rif | -0.55 ± 0.22 | -0.14 ± 0.06 |
| *gyrA*$^{S83L}$*rpoB*$^{H526Y}$ | Fluor + Rif | -0.63 ± 0.11 | -0.36 ± 0.05 |
| *gyrA*$^{D87Y}$*rpsL*$^{K43T}$ | Fluor + Strep | -0.52 ± 0.11 | -0.30 ± 0.15 |




**Supplemental Fig. 1 – Fluorescence markers are neutral in both homogenous and spatially structured populations.** Two strains susceptible to antibiotics completely isogenic apart from the YFP gene (conferring yellow fluorescence) or the CFP gene (conferring cyan fluorescence) were competed against each other in LB liquid with shaking (homogenous populations) and in LB agar (spatially structured populations) for 24h. The bars represent the average of at least 3 competition experiments and the error bars represent a 2x standard error (2SE). There was no significant effect of the fluorescence on the frequency allele of the strains (one sample two tailed t-test).




**Supplemental Fig. 2** – **Fitness costs of the AR mutations measured in homogenous populations**. All the mutants competed with the susceptible background in pair-wise competitions in homogeneous populations (liquid LB with shaking*)*. The fitness costs per generation (S/g) of each mutant strain in homogeneous populations was estimated as the per generation difference in Malthusian parameters: S = ln(Rf/Ri)/t, where t is the number of generations after 24h growth for the susceptible strain and Rf and Ri are the final and initial ratios between resistant and reference strains, respectively.