

Supplementary Table 1. Frequencies of *S. enterica*-resistant mutants observed in lineages evolving in monoculture and co-culture conditions at different time points (generations 440 and 640) during the evolution experiment.

Generation	Community type	Replicate	Frequency
440	Co-culture	1	7.56358E-08
440	Co-culture	2	6.35032E-09
440	Co-culture	3	8.45773E-09
440	Co-culture	4	8.92559E-09
440	Co-culture	5	7.02941E-09
440	Co-culture	6	0
440	Co-culture	7	0
440	Co-culture	8	0
440	Co-culture	9	0
440	Co-culture	10	3.38069E-09
440	Co-culture	11	7.42093E-09
440	Co-culture	12	0
440	Co-culture	13	2.28258E-09
440	Co-culture	14	6.01955E-09
440	Co-culture	15	0
440	Co-culture	16	0
440	Co-culture	17	7.19829E-09
440	Co-culture	18	1.32852E-08
440	Co-culture	19	2.68613E-09
440	Co-culture	20	1.11664E-08
440	Co-culture	21	2.40187E-09
440	Co-culture	22	6.11861E-09
440	Co-culture	23	0
440	Co-culture	24	1.3347E-08
440	Monoculture	1	1.91865E-09
440	Monoculture	2	4.3244E-09
440	Monoculture	3	2.82926E-09
440	Monoculture	4	1.50267E-09
440	Monoculture	5	0
440	Monoculture	6	1.53345E-09
440	Monoculture	7	1.39636E-09
440	Monoculture	8	2.97129E-09
440	Monoculture	9	1.82052E-09
440	Monoculture	10	2.83783E-09
440	Monoculture	11	3.08771E-09
440	Monoculture	12	3.28941E-09
440	Monoculture	13	1.61703E-09
440	Monoculture	14	4.33089E-09
440	Monoculture	15	4.33212E-09
440	Monoculture	16	4.75247E-09

440	Monoculture	17	2.99558E-09
440	Monoculture	18	4.24494E-09
440	Monoculture	19	2.59563E-09
440	Monoculture	20	1.38442E-09
440	Monoculture	21	8.10497E-09
440	Monoculture	22	0
440	Monoculture	23	5.41171E-09
440	Monoculture	24	2.82748E-09
640	Co-culture	1	8.42425E-06
640	Co-culture	2	1.3162E-05
640	Co-culture	3	3.04341E-07
640	Co-culture	4	7.6325E-07
640	Co-culture	5	1.20188E-06
640	Co-culture	6	0
640	Co-culture	7	6.73532E-07
640	Co-culture	8	1.76161E-07
640	Co-culture	9	1.87893E-06
640	Co-culture	10	0.000394355
640	Co-culture	11	3.11384E-06
640	Co-culture	12	0
640	Co-culture	13	3.29869E-07
640	Co-culture	14	4.18347E-07
640	Co-culture	15	1.09046E-06
640	Co-culture	16	0
640	Co-culture	17	9.6363E-07
640	Co-culture	18	1.77222E-06
640	Co-culture	19	7.6063E-07
640	Co-culture	20	0
640	Co-culture	21	0
640	Co-culture	22	3.43787E-07
640	Co-culture	23	2.82249E-07
640	Co-culture	24	1.52222E-07
640	Monoculture	1	1.44099E-06
640	Monoculture	2	1.47931E-06
640	Monoculture	3	3.6846E-06
640	Monoculture	4	1.32827E-06
640	Monoculture	5	8.34311E-07
640	Monoculture	6	8.25121E-07
640	Monoculture	7	0
640	Monoculture	8	1.18075E-06
640	Monoculture	9	1.06568E-06
640	Monoculture	10	9.18279E-07
640	Monoculture	11	0
640	Monoculture	12	0
640	Monoculture	13	3.01045E-06
640	Monoculture	14	1.17204E-06

640	Monoculture	15	2.9705E-06
640	Monoculture	16	1.24775E-06
640	Monoculture	17	1.02956E-06
640	Monoculture	18	1.4811E-06
640	Monoculture	19	6.10617E-06
640	Monoculture	20	1.42072E-06
640	Monoculture	21	2.81688E-06
640	Monoculture	22	2.24242E-06
640	Monoculture	23	1.32102E-06
640	Monoculture	24	1.75963E-07

Supplementary Table 2. Minimum inhibitory concentrations (MIC) of streptomycin for *S. enterica*-resistant mutants isolated from lineages evolving in monoculture and co-culture. For each clone, MIC was tested for two biological replicates.

<i>S. enterica</i> strain ID	Community type	Replicate 1	Replicate 2
Ancestral <i>S. enterica</i>	NA	16	16
DA81227	Co-culture	768	768
DA81228	Co-culture	192	192
DA81229	Co-culture	192-256	192-256
DA81230	Co-culture	16-24	24-32
DA81231	Co-culture	256	256
DA81232	Co-culture	192-256	96-128
DA81233	Co-culture	384	192
DA81234	Co-culture	192	192
DA81235	Co-culture	96	96
DA81236	Co-culture	512	512
DA81237	Co-culture	192-256	256
DA81238	Co-culture	64	48-64
DA81239	Co-culture	16	16-24
DA81240	Co-culture	128-192	192-256
DA81241	Co-culture	192	128-192
DA81242	Co-culture	96	96
DA81243	Co-culture	96	96-128
DA81244	Co-culture	128	128
DA81245	Co-culture	16	12-16
DA81246	Co-culture	128	128-192
DA81247	Co-culture	192	192
DA81248	Co-culture	192	192-256
DA81249	Monoculture	48	32-48
DA81250	Monoculture	96	96
DA81251	Monoculture	24	16-24
DA81252	Monoculture	96	128
DA81253	Monoculture	128	96-128
DA81254	Monoculture	16	16

DA81255	Monoculture	96	96
DA81256	Monoculture	96-128	96-128
DA81257	Monoculture	16-24	16
DA81258	Monoculture	12	12
DA81259	Monoculture	96	96-128
DA81260	Monoculture	96-128	128-192
DA81261	Monoculture	8	8
DA81262	Monoculture	96	96-128
DA81263	Monoculture	128	128-192
DA81264	Monoculture	16-12	12
DA81265	Monoculture	12	12
DA81266	Monoculture	96	96
DA81267	Monoculture	256-384	256-384
DA81268	Monoculture	128	128
DA81269	Monoculture	48-64	48-64
DA81270	Monoculture	64-96	96

Supplementary Table 3. Fitness measurements of *S. enterica*-resistant mutants and their *aadA* knockouts at sub-MIC of streptomycin (1 µg/ml). Fitness was determined by measuring selection coefficients (s) for competition experiments between appropriate mutants and the $\Delta aadA$ for *S. enterica* mutant. The selection coefficient measured for the resistant mutant, for the resistant mutant's $\Delta aadA$ knockouts, and for *aadA* are shown under column headings $S_{res+aadA}$, S_{res} and S_{aadA} , respectively. The predicted values are calculated using an additive model.

Community type	<i>S. enterica</i> Strain_ID	$S_{res+aadA}$ (gen ⁻¹)	S_{res} (gen ⁻¹)	S_{aadA} (gen ⁻¹)	Pred. $S_{res+aadA}$
Co-culture	DA81227	0.23 ± 0.04	0.18 ± 0.01	0.2 ± 0.011	0.38 ± 0.01
Co-culture	DA81228	0.26 ± 0.01	0.21 ± 0.01	0.2 ± 0.011	0.41 ± 0.01
Co-culture	DA81229	0.12 ± 0.01	0.08 ± 0.01	0.2 ± 0.011	0.28 ± 0.01
Co-culture	DA81230	0.31 ± 0.02	0.04 ± 0.00	0.2 ± 0.011	0.24 ± 0.00
Co-culture	DA81231	0.31 ± 0.01	0.03 ± 0.00	0.08 ± 0.011	0.31 ± 0.00
Co-culture	DA81233	-0.03 ± 0.04	-0.19 ± 0.01	0.08 ± 0.011	-0.12 ± 0.01
Co-culture	DA81235	-0.16 ± 0.01	-0.15 ± 0.02	0.08 ± 0.011	-0.07 ± 0.02
Co-culture	DA81236	0.20 ± 0.05	0.05 ± 0.01	0.08 ± 0.011	0.12 ± 0.01
Co-culture	DA81239	0.25 ± 0.01	0.22 ± 0.02	0.08 ± 0.011	0.29 ± 0.02
Co-culture	DA81240	0.14 ± 0.0	-0.09 ± 0.00	0.08 ± 0.011	-0.02 ± 0.00
Monoculture	DA81252	0.15 ± 0.02	0.01 ± 0.01	0.08 ± 0.011	0.09 ± 0.01
Monoculture	DA81253	0.22 ± 0.02	0.11 ± 0.01	0.08 ± 0.011	0.18 ± 0.01
Monoculture	DA81256	0.25 ± 0.03	-0.11 ± 0.00	0.08 ± 0.011	-0.03 ± 0.00
Monoculture	DA81260	0.14 ± 0.01	-0.05 ± 0.04	0.08 ± 0.011	0.03 ± 0.04
Monoculture	DA81262	0.23 ± 0.01	0.13 ± 0.01	0.08 ± 0.011	0.20 ± 0.01

Monoculture	DA81263	0.25 ± 0.02	0.04 ± 0.01	0.08 ± 0.011	0.12 ± 0.01
Monoculture	DA81266	0.30 ± 0.01	0.03 ± 0.02	0.08 ± 0.011	0.11 ± 0.02
Monoculture	DA81267	0.28 ± 0.02	0.09 ± 0.02	0.08 ± 0.011	0.16 ± 0.02
Monoculture	DA81268	0.26 ± 0.03	0.16 ± 0.01	0.08 ± 0.011	0.24 ± 0.01
Monoculture	DA81270	0.39 ± 0.01	-0.02 ± 0.01	0.08 ± 0.011	0.05 ± 0.01

Supplementary Table 4. Minimum inhibitory concentrations (MIC) of streptomycin for *S. enterica*-resistant mutants and their respective *aadA* knockouts. MIC was determined using Etests.

<i>S. enterica</i> Strain_ID	Minimum Inhibitory Concentration (MIC)			
	original resistant mutant		<i>aadA</i> knockout	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
DA81227	768	768	12	12
DA81228	192	192	16	16
DA81229	192-256	192-256	12	12
DA81230	16-24	24-32	12	12
DA81231	256	256	8	8
DA81233	384	192	16	16
DA81235	96	96	32	32
DA81236	512	512	24	24
DA81239	16	16-24	12	12
DA81240	128-192	192-256	12	12
DA81252	96	128	12	12
DA81253	128	96-128	12	12
DA81256	96-128	96-128	48	48
DA81260	96-128	128-192	16	16
DA81262	96	96-128	12	12
DA81263	128	128-192	16	16
DA81266	96	96	64	64
DA81267	256-384	256-384	64	64
DA81268	128	128	16	16
DA81270	64-96	96	16	16

Supplementary Table 5. *S. enterica* population densities of lineages evolving at sub-MIC of streptomycin in monoculture and co-culture conditions. Population densities were measured using a flow cytometer.

Community type	Lineage	<i>S. enterica</i> Pop. Density (cfu/μl) at generation 640
Co-culture	1	6291362.89
Co-culture	2	3722845.704
Co-culture	3	3285791.796

Co-culture	4	3930561.328
Co-culture	5	4160160.546
Co-culture	7	1484709.472
Co-culture	8	5676613.672
Co-culture	9	3193306.836
Co-culture	10	2992224.61
Co-culture	11	2890320.704
Co-culture	12	2265431.054
Co-culture	13	3031503.124
Co-culture	14	2390360.742
Co-culture	15	1834088.282
Co-culture	17	6226453.124
Co-culture	18	2821322.852
Co-culture	19	2629398.828
Co-culture	20	1132805.664
Co-culture	21	3013306.836
Co-culture	22	2908777.734
Co-culture	23	3542966.016
Co-culture	24	6569346.094
Monoculture	1	5551743.75
Monoculture	2	4731944.14
Monoculture	3	4071002.344
Monoculture	4	3011442.968
Monoculture	5	5992969.14
Monoculture	6	4847775.782
Monoculture	7	5417615.624
Monoculture	8	5928453.516
Monoculture	9	4691823.828
Monoculture	10	4355972.266
Monoculture	11	5009719.532
Monoculture	12	4472325
Monoculture	13	4650461.328
Monoculture	14	5119258.594
Monoculture	15	5722946.094
Monoculture	16	6411523.438
Monoculture	17	5827735.546
Monoculture	18	5401403.124
Monoculture	19	5731903.906
Monoculture	20	4927094.532
Monoculture	21	4970040.234
Monoculture	22	5351362.89
Monoculture	23	5298917.968
Monoculture	24	5683006.25

Supplementary Table 6. Mutations observed in the resistant clones of *S. enterica* isolated after 640 generations from lineages evolving under co-culture and monoculture conditions. Mutations are shown as the type of mutation (i.e intergenic changes, base-pair insertions (+), deletions (Δ), or non-synonymous changes), and the position on the genes where the mutation is observed.

Strain_ID	Community Type	gene	annotation
DA81227	Co-culture	<i>fis</i>	T75P (ACC→CCC)
		<i>ubiF</i>	Δ 83bp
DA81228	Co-culture	<i>fimW</i>	L141R (CTG→CGG)
		<i>gidB</i>	P79L (CCG→CTG)
		<i>ydiV</i>	Q123* (CAG→TAG)
DA81229	Co-culture	<i>barA</i>	+1 bp
		<i>cheR</i>	T126A (ACA→GCA)
		<i>dxs</i>	G287C (GGC→TGC)
		<i>pncB</i>	K398T (AAA→ACA)
		<i>rfbJ</i>	S119G (AGT→GGT)
		<i>rfbP</i>	I94N (ATC→AAC)
DA81230	Co-culture	<i>cydA</i>	Δ 52bp
		<i>galR</i>	G67D (GGC→GAC)
		<i>hflB / ftsJ</i>	intergenic (-2/+102)
		<i>rfbF</i>	P158L (CCA→CTA)
		<i>uvrY</i>	L200Q (CTG→CAG)
DA81231	Co-culture	<i>aceE</i>	S42A (TCT→GCT)
		<i>fimZ</i>	H12Y (CAC→TAC)
		<i>rpoC</i>	L283P (CTG→CCG)
DA81232	Co-culture	<i>fepD</i>	M1M (ATG→ATA) †
		<i>rfbP</i>	+1 bp
		<i>rfbV</i>	S172P (TCT→CCT)
DA81233	Co-culture	<i>cydD</i>	W427* (TGG→TGA)
		<i>rfbJ</i>	S119G (AGT→GGT)
		<i>rfbP</i>	E86G (GAG→GGG)
		<i>uvrY / STM1948</i>	intergenic (-54/-258)
DA81234	Co-culture	<i>fis</i>	Y38* (TAT→TAG)
		<i>STM1263 / aadA</i>	intergenic (+38/-71)
		<i>ydeW</i>	V46A (GTT→GCT)
DA81235	Co-culture	<i>glpT</i>	F35L (TTC→TTA)
		<i>ubiB</i>	+1 bp
DA81236	Co-culture	<i>cydB</i>	+2 bp
		<i>motA</i>	T13A (ACA→GCA)
		<i>uvrY</i>	H10R (CAC→CGC)
DA81237	Co-culture	<i>barA</i>	+19 bp
		<i>clpB</i>	A390T (GCT→ACT)
		<i>cydB</i>	+2 bp
		<i>motA</i>	T13A (ACA→GCA)

DA81238	Co-culture	<i>aceE</i>	S42A (TCT→GCT)
		<i>fepD</i>	T253P (ACG→CCG)
		<i>fimW</i> / <i>argU</i>	intergenic (-14/-251)
		<i>rpoC</i>	L283P (CTG→CCG)
		<i>ugtL</i>	H63R (CAT→CGT)
DA81239	Co-culture	<i>cydC</i>	Δ18bp
		<i>ftsA</i> / <i>ftsZ</i>	intergenic (+44/-17)
		<i>glpT</i>	W275* (TGG→TAG)
		<i>rfbF</i>	R89H (CGT→CAT)
		<i>STM4103</i>	C77F (TGC→TTC)
		<i>uvrY</i>	+2 bp
DA81240	Co-culture	<i>barA</i>	L319* (TTA→TGA)
		<i>cydD</i>	Δ1bp
		<i>cytR</i>	+2 bp
		<i>rfbI</i>	+1 bp
DA81241	Co-culture	<i>cydB</i>	+2 bp
		<i>fimW</i>	R97Q (CGG→CAG)
		<i>fold</i> / <i>fimA</i>	intergenic (-281/-266)
DA81242	Co-culture	<i>barA</i>	G455C (GGC→TGC)
		<i>dxs</i>	A191E (GCG→GAG)
		<i>motA</i>	K236R (AAG→AGG)
		<i>rfbJ</i>	coding (357/900 nt)
		<i>yneJ</i>	S237S (AGT→AGC)
DA81243	Co-culture	<i>motA</i>	T13A (ACA→GCA)
		<i>uvrY</i>	H10R (CAC→CGC)
		<i>yigC</i>	P423L (CCT→CTT)
DA81244	Co-culture	<i>arcA</i>	Δ1bp
		<i>rfbP</i>	I94N (ATC→AAC)
DA81249	Monoculture	<i>cydC</i>	Δ5bp
		<i>fimZ</i> / <i>fimY</i>	intergenic (-386/+218)
		<i>flgA</i>	A12D (GCT→GAT)
		<i>rfbC</i>	S52L (TCA→TTA)
		<i>treC</i> / <i>treR</i>	intergenic (-930/+675)
DA81250	Monoculture	<i>fliA</i>	D87E (GAT→GAG)
		<i>hemL</i>	+1 bp
		<i>lrhA</i>	I103S (ATC→AGC)
		<i>motA</i>	H154Y (CAT→TAT)
		<i>rfbF</i>	P158L (CCA→CTA)
		<i>sufD</i>	A71A (GCC→GCT)
		<i>treC</i> / <i>treR</i>	intergenic (-930/+675)
		<i>uvrY</i>	Δ11bp
DA81251	Monoculture	<i>fimW</i>	+1 bp
		<i>fimZ</i> / <i>fimY</i>	intergenic (-337/+267)
		<i>ubiH</i>	Δ84bp
		<i>ydcG</i>	Q55R (CAG→CGG)
DA81252	Monoculture	<i>fepC</i>	G45S (GGC→AGC)

		<i>fimZ</i>	I19F (ATC→TTC)
		<i>fimZ</i> / <i>fimY</i>	intergenic (-18/+551)
		<i>ftsL</i>	Δ5bp
		<i>treC</i> / <i>treR</i>	intergenic (-930/+675)
		<i>ybbP</i> / <i>STM0509</i>	intergenic (+99/-87)
DA81253	Monoculture	<i>cheB</i>	L97R (CTG→CGG)
		<i>motA</i>	Y252C (TAT→TGT)
		<i>rfbH</i>	T223I (ACA→ATA)
		<i>ubiF</i>	+1bp
DA81254	Monoculture	<i>cheZ</i>	D30Y (GAC→TAC)
		<i>cydA</i>	W131* (TGG→TAG)
		<i>ilvB</i>	E344A (GAA→GCA)
		<i>motA</i>	Y252C (TAT→TGT)
		<i>rfbH</i>	T223I (ACA→ATA)
DA81255	Monoculture	<i>hemL</i>	T297P (ACC→CCC)
		<i>motA</i>	Y252C (TAT→TGT)
		<i>rfbH</i>	T223I (ACA→ATA)
		<i>ydiV</i>	Δ1bp
DA81256	Monoculture	<i>cydD</i>	E563* (GAA→TAA)
		<i>fimZ</i>	I19F (ATC→TTC)
		<i>fold</i> / <i>fimA</i>	intergenic (-281/-266)
		<i>motA</i>	Y252C (TAT→TGT)
		<i>rfbH</i>	T223I (ACA→ATA)
		<i>treC</i> / <i>treR</i>	intergenic (-930/+675)
DA81257	Monoculture	<i>fimW</i> / <i>argU</i>	intergenic (-114/-151)
		<i>flgA</i>	A12D (GCT→GAT)
		<i>rfbC</i>	S52L (TCA→TTA)
		<i>yceF</i>	N190K (AAC→AAG)
DA81258	Monoculture	<i>cydD</i>	W43* (TGG→TAG)
		<i>fliA</i>	V89G (GTG→GGG)
		<i>lrhA</i>	I103S (ATC→AGC)
		<i>motA</i>	H154Y (CAT→TAT)
		<i>rfbF</i>	P158L (CCA→CTA)
		<i>uvrY</i>	D38N (GAT→AAT)
DA81259	Monoculture	<i>fimW</i>	coding (515/597 nt)
		<i>hemL</i>	T297P (ACC→CCC)
		<i>rfbV</i>	Δ1bp
		<i>STM0081</i> / <i>STM0082</i>	intergenic (+158/+60)
		<i>treC</i> / <i>treR</i>	intergenic (-930/+675)
DA81260	Monoculture	<i>fepG</i>	A173E (GCG→GAG)
		<i>fimZ</i>	I19F (ATC→TTC)
		<i>rfbV</i>	Δ1bp
		<i>STM0081</i> / <i>STM0082</i>	intergenic (+158/+60)
DA81261	Monoculture	<i>fliA</i>	V89G (GTG→GGG)
		<i>hema</i>	D301Y (GAT→TAT)
		<i>rfbV</i>	Δ1bp

		<i>STM0081 / STM0082</i>	intergenic (+158/+60)
DA81262	Monoculture	<i>fliA</i>	V89G (GTG→GGG)
		<i>hemL</i>	G270R (GGG→AGG)
		<i>rfbF</i>	P158L (CCA→CTA)
DA81263	Monoculture	<i>fepD</i>	A157E (GCG→GAG)
		<i>fimZ</i>	I19F (ATC→TTC)
		<i>fimZ / fimY</i>	intergenic (-322/+282)
		<i>treC / treR</i>	intergenic (-930/+675)
DA81264	Monoculture	<i>barA</i>	T268P (ACC→CCC)
		<i>cheA</i>	A331D (GCC→GAC)
		<i>cydD</i>	+1bp
		<i>motA</i>	K236R (AAG→AGG)
		<i>rfbJ</i>	E162A (GAG→GCG)
		<i>rfbP</i>	V209L (GTC→CTC)
		<i>STM0020 / bcfA</i>	intergenic (+170/-260)
DA81265	Monoculture	<i>fimZ / fimY</i>	intergenic (-246/+358)
		<i>flgA</i>	A12D (GCT→GAT)
		<i>rfbC</i>	S52L (TCA→TTA)
		<i>rpoC</i>	L1109P (CTG→CCG)
		<i>ubiF</i>	+1bp
DA81266	Monoculture	<i>fliA</i>	V89G (GTG→GGG)
		<i>hemL</i>	Δ12bp
		<i>lrhA</i>	I103S (ATC→AGC)
		<i>motA</i>	H154Y (CAT→TAT)
		<i>rfbF</i>	P158L (CCA→CTA)
		<i>ydiV</i>	Q123* (CAG→TAG)