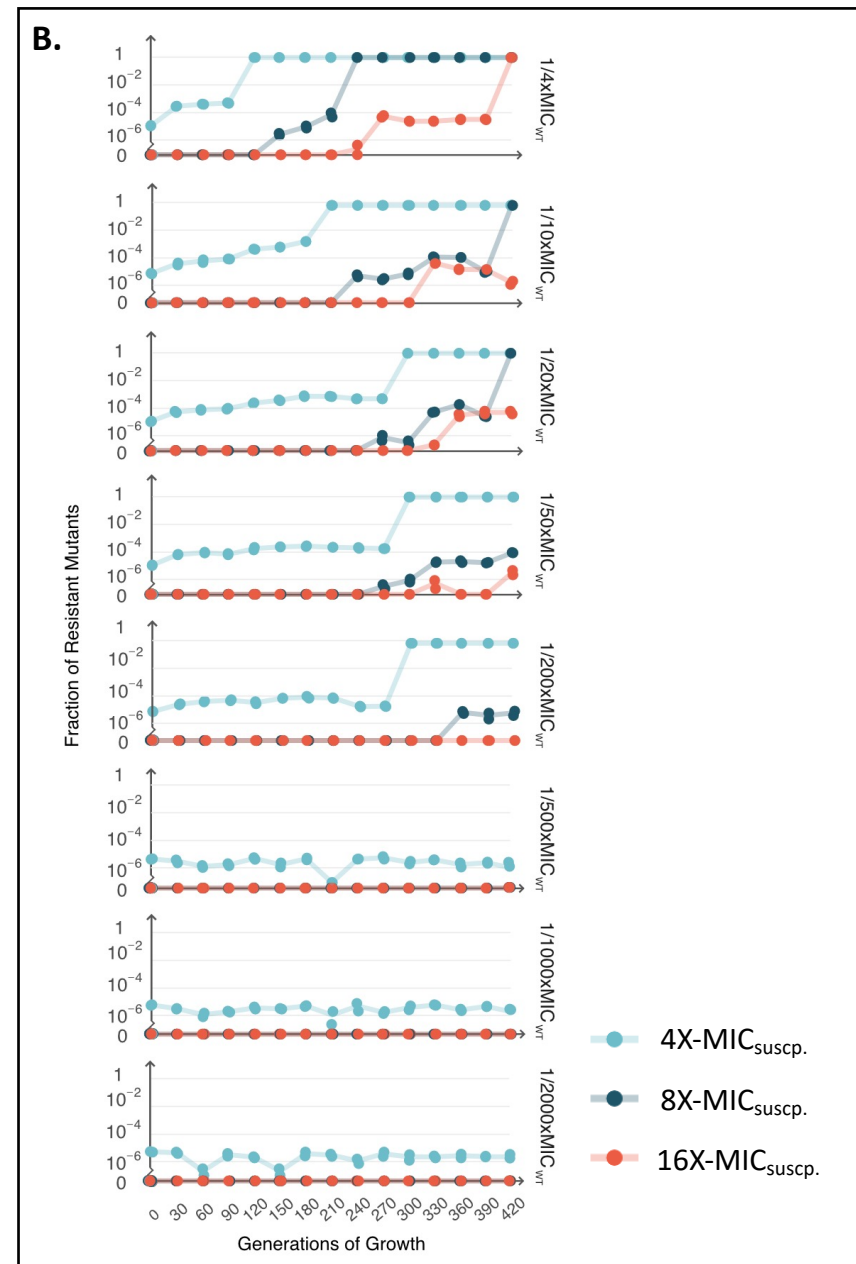
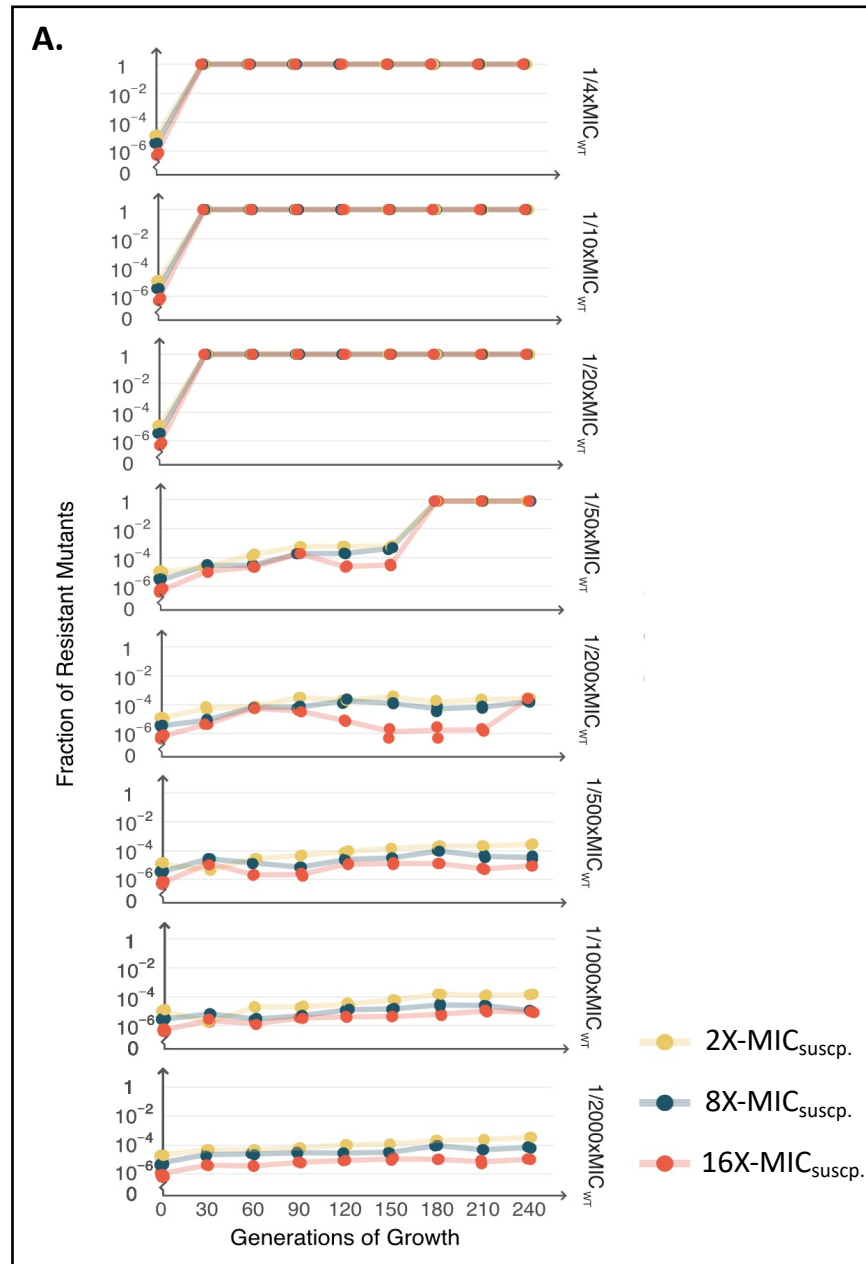
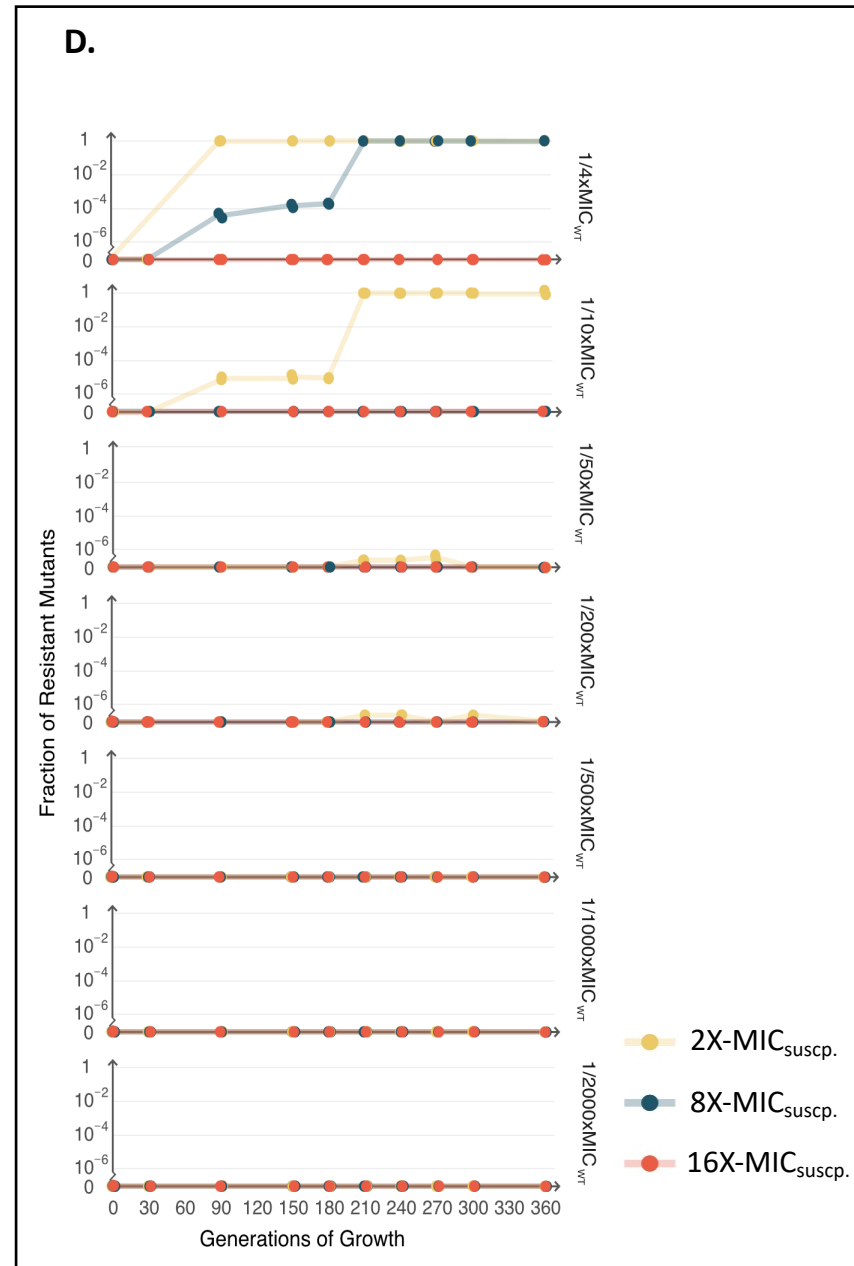
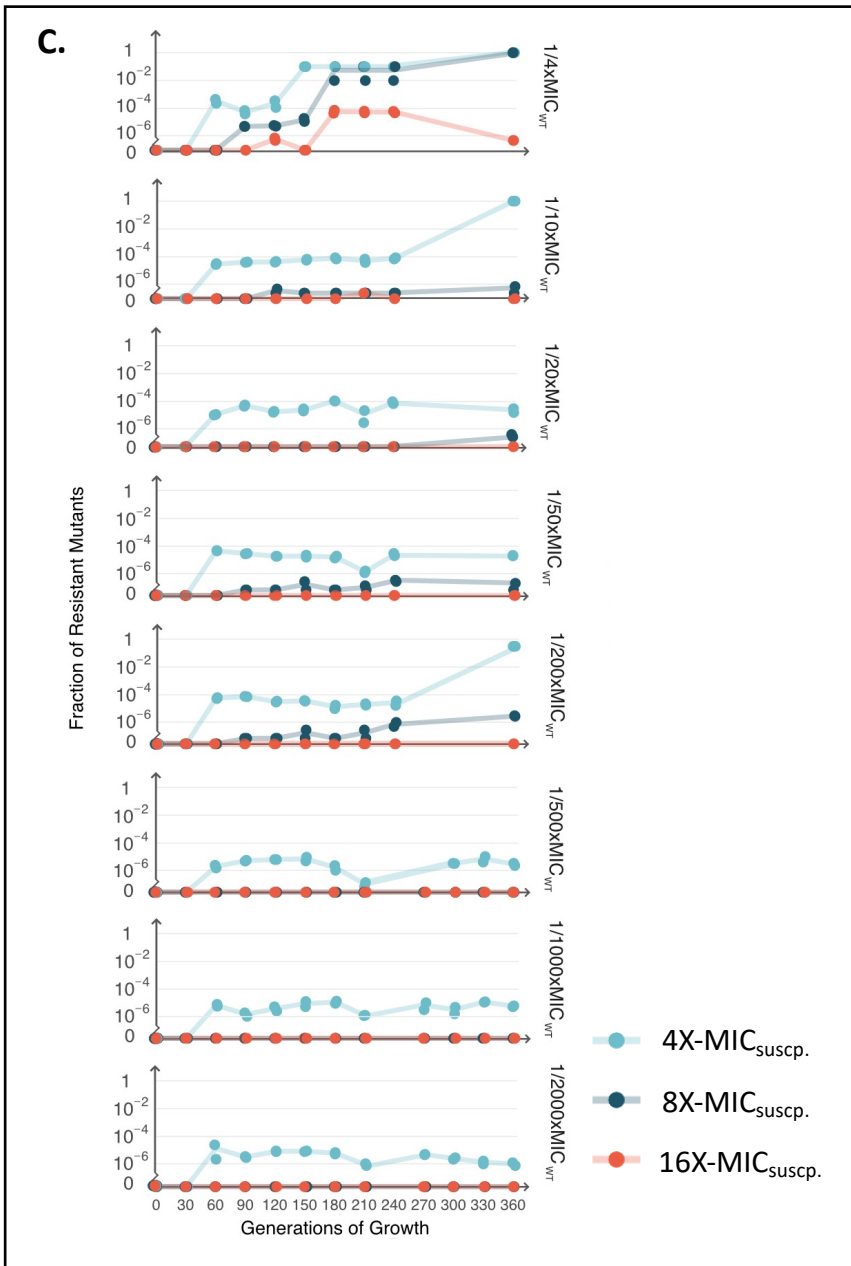


**Supplementary Fig. 1.** Increase in frequency of resistant mutants with different levels of resistance in populations evolving at sub-MICs of antibiotics. A) Fosfomycin B) Tetracycline C) Nitrofurantoin and D) Ciprofloxacin. Different levels of resistance was determined by plating the evolving populations at different antibiotic concentrations, which are shown in different colors in the figures



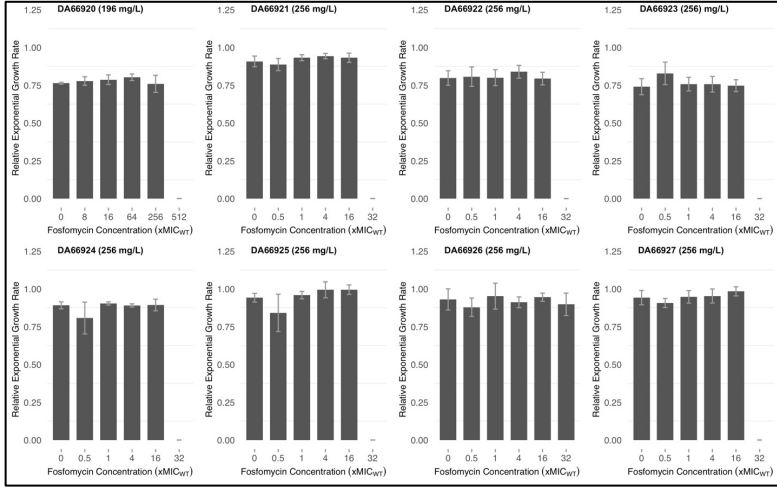
Supplementary Fig. 1.



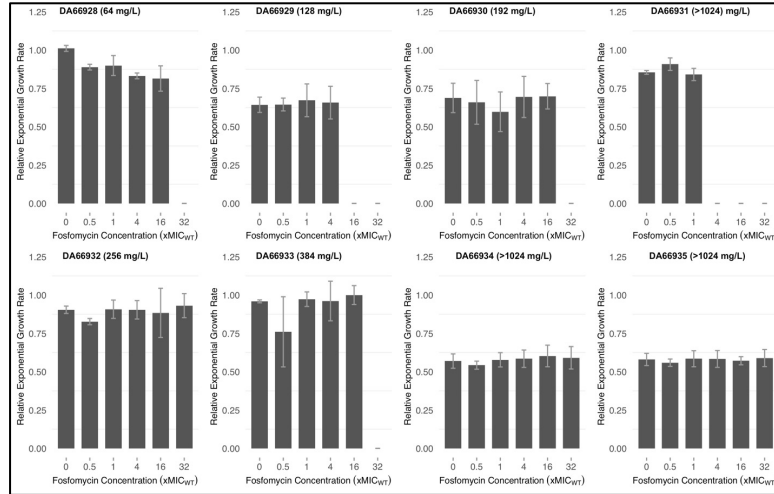
**Supplementary Fig. 2.** Exponential growth-rates at different antibiotic concentrations for resistant mutants isolated on A) Fosfomycin B) Tetracycline C) Nitrofurantoin and D) Ciprofloxacin

**A.**

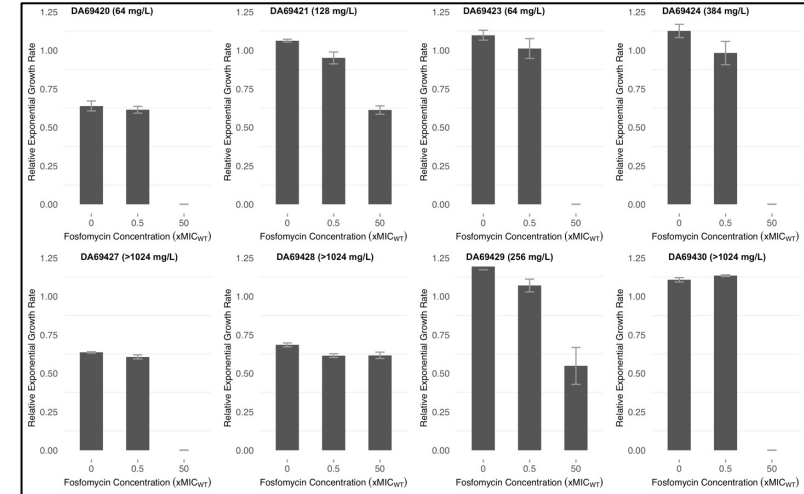
Mutants isolated at 1/4<sup>th</sup> MIC<sub>susceptible</sub> of fosfomycin



Mutants isolated at 1/200<sup>th</sup> MIC<sub>susceptible</sub> of fosfomycin

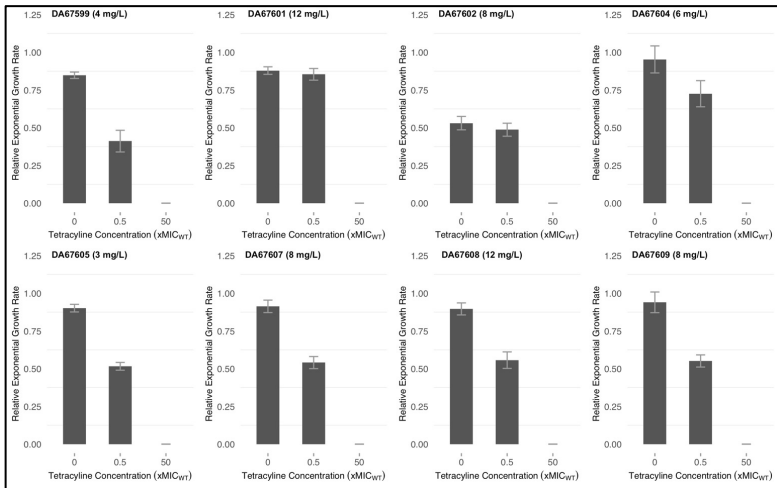


Mutants isolated at 1/2000<sup>th</sup> MIC<sub>susceptible</sub> of fosfomycin

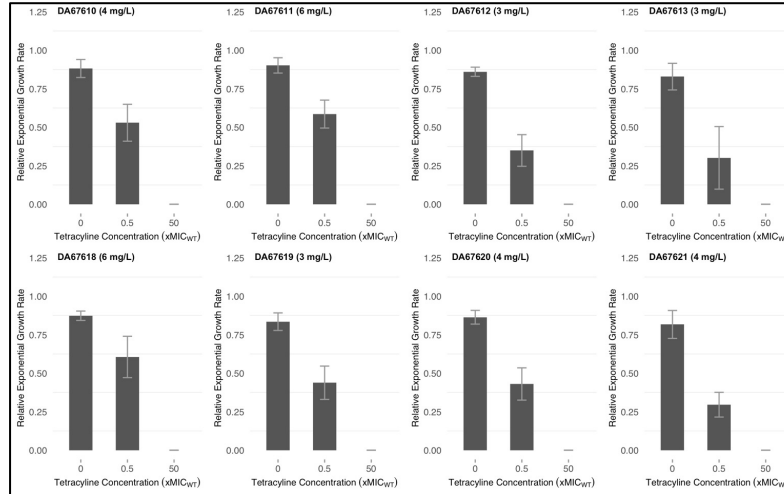


**B.**

Mutants isolated at 1/4<sup>th</sup> MIC<sub>susceptible</sub> of tetracycline

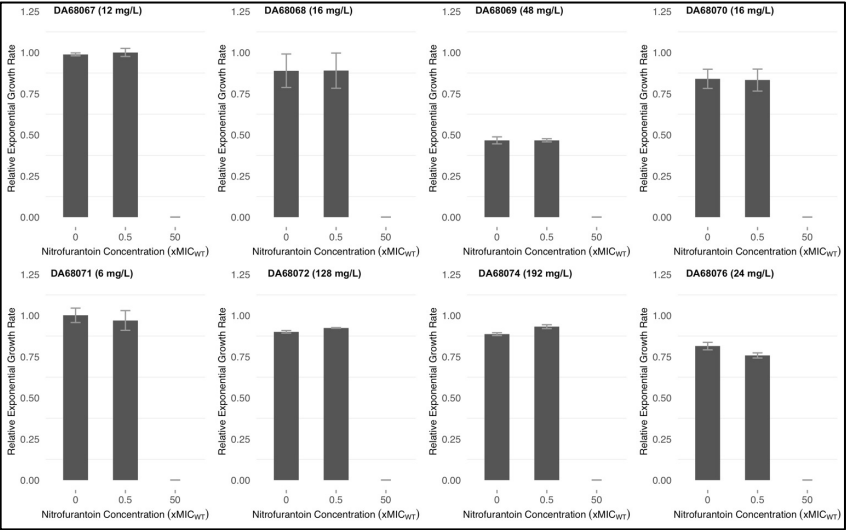


Mutants isolated at 1/200<sup>th</sup> MIC<sub>susceptible</sub> of tetracycline

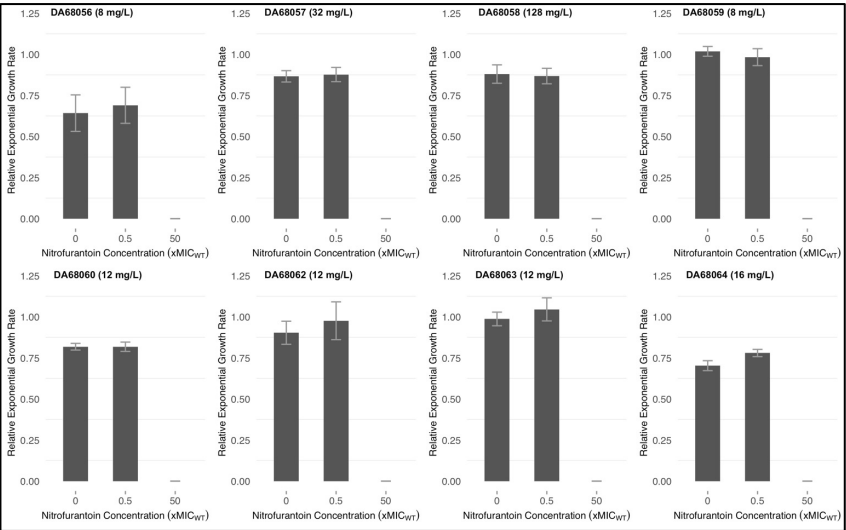


**Supplementary Fig. 2.** Exponential growth-rates at different antibiotic concentrations for resistant mutants isolated on A) Fosfomycin B) Tetracycline C) Nitrofurantoin and D) Ciprofloxacin

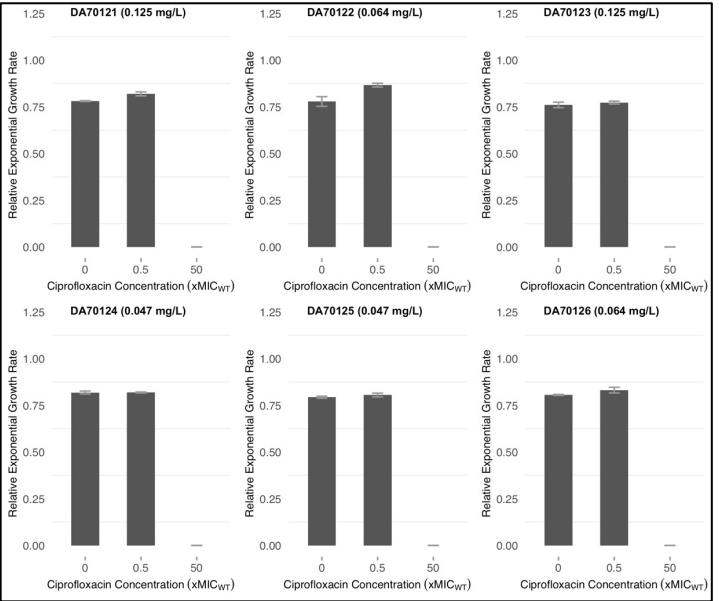
**C.** Mutants isolated at 1/4<sup>th</sup> MIC<sub>susceptible</sub> of nitrofurantoin



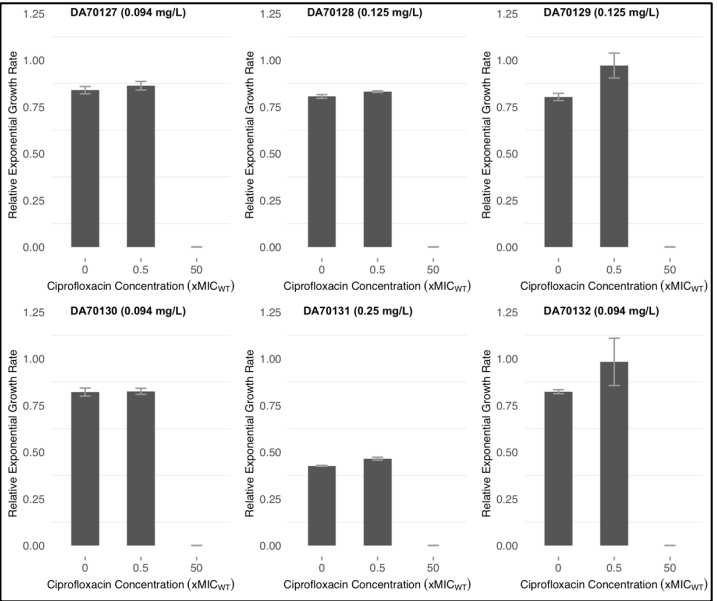
Mutants isolated at 1/200<sup>th</sup> MIC<sub>susceptible</sub> of nitrofurantoin



**D.** Mutants isolated at 1/4<sup>th</sup> MIC<sub>susceptible</sub> of ciprofloxacin



Mutants isolated at 1/10<sup>th</sup> MIC<sub>susceptible</sub> of ciprofloxacin





**Supplementary Fig. 3.** Mutations identified through whole-genome sequencing of resistant mutants isolated at the end-point of the experiment for populations evolving at sub-MICs of A) Fosfomycin B) Tetracycline C) Nitrofurantoin and D) Ciprofloxacin. Genes marked green are known resistance genes for the antibiotic on which selection was performed, those marked in yellow are known resistance genes but for other antibiotics and those marked red have been shown to confer media adaptation.

**B.**

		<i>envZ</i>	<i>fbaA</i>	<i>malT</i>	<i>yahL</i>	<i>gatY</i>	<i>yeaR</i>	<i>mdtF</i>	<i>rho</i>	<i>rpoC</i>	<i>fimE</i>	<i>gatC</i>	<i>elaD</i>	<i>dhaK</i>	<i>plsB</i>	<i>trkA</i>	<i>ptsP</i>	<i>aat</i>	<i>ymdA</i>	<i>kdpD</i>	<i>suhB</i>	<i>mutL</i>	<i>mutS</i>	Intergenic insertions	
Sel.: 1/4xMIC	DA67599	c.66C>A L22F	c.461T>C N154S	c.658A>C T220P	c.527:: IS5	c.644:: IS5	c.115:: IS186	c.1402:: IS1	c.259C>T R87C															<i>malP</i> (460)/ <i>malT</i> (148)::IS5	
	DA67601	c.40G>A R14C								c.2176G>A A726T c.3149A>G T1050A														c.653G>A G218E c.2444::IS1	
	DA67602	c.40G>A R14C	c.704A>G V235A									c.501:: IS1													c.2444::IS1
	DA67604	c.442G>A P148S								c.250A>T S84C		c.168:: IS1													
	DA67605											c.272:: IS5	c.85:: IS5												
	DA67607													c.963C>A A321=											
	DA67608	c.1189G>A R397C										c.272:: IS5	c.85:: IS5		c.858G>C G268=	c.794C>A R265L	c.1078A>C S360R								<i>ompF</i> (220)/ <i>ansS</i> (-383):A>G
DA67609	c.1189G>A R397C										c.272:: IS5	c.85:: IS5			c.794C>A R265L										
Sel.: 1/200xMIC	DA67610	c.146A>G V49A						c.2297T>C V766A			c.125:: IS5	c.826:: IS5						c.2002A>G C668R	c.571T>C N191D	c.303::IS5	c.823A>G S275P		c.275T>C L92P		
	DA67611							c.1402:: IS1													c.48delT				
	DA67612										c.125:: IS5	c.826:: IS5						c.2002A>G C668R		c.309::IS5			c.275T>C L92P		
	DA67613							c.1402:: IS1											c.1063A>G S355P		c.309::IS5	c.986A>G V329A			c.1966C>A R656S
	DA67618										c.125:: IS5										c.309::IS5		c.699 G233=		
	DA67619	c.146A>G V49A	c.551T>C D184G									c.125:: IS5	c.826:: IS5						c.2002A>G C668R	c.571T>C N191D	c.309::IS5			c.275T>C L92P	
	DA67620	c.146A>G V49A	c.551T>C D184G									c.125:: IS5	c.826:: IS5						c.2002A>G C668R	c.571T>C N191D	c.309::IS5	c.823A>G S275P		c.275T>C L92P	
	DA67621											c.125:: IS5	c.826:: IS5												c.275T>C L92P

Positions for mutations outside of coding sequences are given relative to the first nucleotide in the start codon of the downstream gene (negative numbers).

\* stop codon; Δ deletion; fs frame shift; del deletion; ins insertion; dup duplication; c. coding region

Genes mutated in our study that earlier have been described to confer resistance to tetracycline

Genes mutated in our study that earlier have been described as genetic adaptations to growth in laboratory conditions (Knöppel et al., 2018)

Genes mutated in our study that earlier have been described to confer antimicrobial resistance

Strains in our study that were found to be hypermutators

**Supplementary Fig. 3.** Mutations identified through whole-genome sequencing of resistant mutants isolated at the end-point of the experiment for populations evolving at sub-MICs of A) Fosfomycin B) Tetracycline C) Nitrofurantoin and D) Ciprofloxacin. Genes marked green are known resistance genes for the antibiotic on which selection was performed, those marked in yellow are known resistance genes but for other antibiotics and those marked red have been shown to confer media adaptation.

C.

	<i>nfsA</i>	<i>yeaR</i>	<i>uhpT</i>	<i>relA</i>	<i>yfaS</i>	<i>nfsB</i>	<i>ptwF</i>	<i>ycgB</i>	<i>menC</i>	<i>leuV</i>	<i>rpsK</i>	<i>fimE</i>	<i>kdpD</i>	<i>rph</i>	<i>mpaA</i>	<i>ydcl</i>	<i>yhaC</i>	<i>mutT</i>	<i>yfdE</i>	<i>cheA</i>	<i>ptsP</i>	<i>yobF</i>	<i>putP</i>	<i>aat</i>	<i>mdtH</i>	<i>degQ</i>	<i>rho</i>	Intergenic insertions	Deletions		
Sel.: 1/4xMIC	DA68067	c.674::IS30	c.115::IS186	c.1003_1281del Δ A335-G427	c.2172_2184del Δ A274-G727																								<i>dut(-84)/slmA(23):T&gt;C</i>		
	DA68068	c.674::IS30	c.115::IS186	c.1003_1281del Δ A335-G427	c.2172_2184del Δ A274-G727																								<i>dut(-84)/slmA(23):T&gt;C</i>		
	DA68069	c.380::IS186																												<i>lhrA(137)/yrbQ(780)::IS5</i>	
	DA68070	c.674::IS30	c.115::IS186	c.1003_1281del Δ A335-G427	c.2172_2184del Δ A274-G727																									<i>dut(-84)/slmA(23):T&gt;C</i>	
	DA68071	c.674::IS30		c.1003_1281del Δ A335-G427	c.2172_2184del Δ A274-G727																									<i>dut(-84)/slmA(23):T&gt;C</i>	
	DA68072	c.380::IS186																													
	DA68074	c.380::IS186	c.115::IS186																												
	DA68076	c.674::IS30	c.115::IS186	c.1003_1281del Δ A335-G427	c.2172_2184del Δ A274-G727																									<i>dut(-84)/slmA(23):T&gt;C</i>	
Sel.: 1/200xMIC	DA68056	c.289del L98 fs																											<i>csgC(-54)/ymdA(63)::IS2</i>		
	DA68057	c.434T>G L145 *																													
	DA68058	c.635G>A W212*																												<i>Δ ymdE-[ymdC](12.112kbp)</i> <i>IS3-mediated</i> <i>pyrE(42)/rph(-53)::Δ1bp</i>	
	DA68059																												<i>fimB(-461)/fimE(14)::IS5</i>		
	DA68060																												<i>ompC(128)/rcsD(608)::IS5</i>	<i>Δ yrdA-yrdD(562bp)</i> <i>IS1-mediated</i>	
	DA68062	c.380::IS186																												<i>Δ ymdE-[ymdC](12.112kbp)</i> <i>IS3-mediated</i> <i>pyrE(42)/rph(-53)::Δ1bp</i>	
	DA68063	c.380::IS186																													
DA68064	c.380::IS186																												<i>c.304C&gt;A R102S</i>	<i>fimE(-198)/fimA(281)::IS5</i>	<i>Δ marA-marB(94bp)</i>

\* stop codon; Δ deletion; fs frame shift; del deletion; ins insertion; dup duplication; c. coding region

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**D.**

		<i>ydcl</i>	<i>gyrB</i>	<i>ptsP</i>	<i>icd</i>	<i>emrA</i>	<i>yphG</i>	<i>ssuD</i>	<i>rph</i>	<i>yhal</i>	<i>trkH</i>	<i>gyrA</i>	<i>ymdA</i>	<i>caiD</i>	<i>fimE</i>	<i>brnQ</i>	<i>ydH</i>	<i>tap</i>	<i>proB</i>	<i>kdpD</i>	<i>ycdT</i>	<i>cho</i>	<i>ydeO</i>	<i>oxyR</i>	<i>ssuA</i>	<i>rpsF</i>	Intergenic insertions	Deletions						
Sel.: 1/4xMIC	DA70121	c.859_894del Δ D286-I298	c.1389_1394del A65 fs	c.1545_1555del R119 fs	c.1098C>T H366= c.1123T>C L375=	c.591A>T P197=	c.2250T>C L750=																											
	DA70122		c.1389_1394del A65 fs		c.1098C>T H366= c.1100C>T T370= c.1123T>C L375= c.1155C>T N385= c.1158G>C A386= c.1161A>G K387=		c.2250T>C L750=	c.703T>C E243=	c.559_560del S186 fs	c.68:: IS1	c.1040C>A A347D																yai[S1547]tauA (66)::IS5 m[E(-13)]m[E(81)]G>C	m[E(-27)]m[E(67)]Tdel						
	DA70123			c.1545_1555del R119 fs																												pyr[E(46)]rph(-49);C>T glt(T(-171)]m[B(23)]A>T		
	DA70124	c.735_744del Δ E79-Y248		c.1545_1555del R119 fs																													mipA(358)yeeG (74)::IS2 ykgD(-427)ykgE(97)::IS5 cirA(230)lysP(-64)G>T	Δ (uspC otaA [otsB]) (2.153bp)
	DA70129			c.328_330dup L111 fs																													pyr[E(46)]rph(-49);C>T glt(T(-171)]m[B(23)]A>T	
	DA70130			c.328_330dup L111 fs																													pyr[E(46)]rph(-49);C>T glt(T(-171)]m[B(23)]A>T yjlL310)yjM(-50)insTARGGCAC	
	DA70132			c.328_330dup L111 fs																													pyr[E(46)]rph(-49);C>T glt(T(-171)]m[B(23)]A>T yjlL310)yjM(-50)insTARGGCAC	
Sel.: 1/10xMIC	DA70133		c.1389_1394del A65 fs		c.1098C>T H366= c.1100C>T T370= c.1123T>C L375=		c.2250T>C L750=																									pyr[E(40)]rph(-55)delA pflA(188)]pflB(-4);C>T		
	DA70134		c.1389_1394del A65 fs		c.1098C>T H366= c.1100C>T T370= c.1123T>C L375=		c.2250T>C L750=																										fimE(-363)]fimA(119)insA	
	DA70135			c.1678_1681del T560 fs																													pspR(96)]pspA (71): A>G Δ (ydcJ [ydcI]) (1566bp)	
	DA70137																																c.710T>C D337G	c.349_350del A116 fs

\* stop codon; Δ deletion; fs frame shift; del deletion; ins insertion; dup duplication; c. coding region

Genes mutated in our study that earlier have been described to confer resistance to ciprofloxacin

Genes mutated in our study that earlier have been described as genetic adaptations to growth in laboratory conditions (Knöppel et al., 2018)

Genes mutated in our study that earlier have been described to confer antimicrobial resistance







DA70125	This study	<i>E. coli</i> mutant isolated from evolution in presence of ciprofloxacin at concentration of 1/4 the MIC of the WT strain.
DA70126	This study	<i>E. coli</i> mutant isolated from evolution in presence of ciprofloxacin at concentration of 1/4 the MIC of the WT strain.
DA70127	This study	<i>E. coli</i> mutant isolated from evolution in presence of ciprofloxacin at concentration of 1/4 the MIC of the WT strain.
DA70128	This study	<i>E. coli</i> mutant isolated from evolution in presence of ciprofloxacin at concentration of 1/4 the MIC of the WT strain.
DA70129	This study	<i>E. coli</i> mutant isolated from evolution in presence of ciprofloxacin at concentration of 1/4 the MIC of the WT strain.
DA70130	This study	<i>E. coli</i> mutant isolated from evolution in presence of ciprofloxacin at concentration of 1/4 the MIC of the WT strain.
DA70132	This study	<i>E. coli</i> mutant isolated from evolution in presence of ciprofloxacin at concentration of 1/4 the MIC of the WT strain.
DA70133	This study	<i>E. coli</i> mutant isolated from evolution in presence of ciprofloxacin at concentration of 1/10 the MIC of the WT strain.
DA70134	This study	<i>E. coli</i> mutant isolated from evolution in presence of ciprofloxacin at concentration of 1/10 the MIC of the WT strain.
DA70135	This study	<i>E. coli</i> mutant isolated from evolution in presence of ciprofloxacin at concentration of 1/10 the MIC of the WT strain.
DA70136	This study	<i>E. coli</i> mutant isolated from evolution in presence of ciprofloxacin at concentration of 1/10 the MIC of the WT strain.
DA70137	This study	<i>E. coli</i> mutant isolated from evolution in presence of ciprofloxacin at concentration of 1/10 the MIC of the WT strain.
DA70138	This study	<i>E. coli</i> mutant isolated from evolution in presence of ciprofloxacin at concentration of 1/10 the MIC of the WT strain.

**Supplementary Table 2.** Frequency of resistant mutants observed at different sub-MICs

<b>Fosfomycin</b>	<b>Selective Concentration</b>	<b>2xMIC</b>	<b>8xMIC</b>	<b>16xMIC</b>
	1/4	1	1	1
	1/10	1	1	1
	1/20	1	1	1
	1/50	1	1	1
	1/200	2.90E-04	1.60E-04	2.80E-05
	1/500	2.80E-04	3.40E-05	8.90E-06
	1/1000	1.80E-04	1.30E-05	1.10E-05
	1/2000	2.00E-04	4.00E-05	6.20E-06
	control evolved populations	1.19E-05	4.39E-06	1.76E-06

<b>Tetracycline</b>	<b>Selective Concentration</b>	<b>4X</b>	<b>8X</b>	<b>16X</b>
	1/4	1	1	1
	1/10	1	1	2.80E-06
	1/20	1	1	5.50E-05
	1/50	1	9.90E-05	3.90E-06
	1/200	1	9.70E-06	0
	1/500	4.90E-06	0	0
	1/1000	6.50E-06	0	0
	1/2000	6.50E-06	0	0
	control evolved populations	1.6E-05	0	0

<b>Nitrofurantoin</b>	<b>Selective Concentration</b>	<b>4xMIC</b>	<b>8xMIC</b>	<b>16xMIC</b>
	1/4	1	1.8 x 10 <sup>-6</sup>	5.0 x 10 <sup>-7</sup>
	1/10	1	5.0 x 10 <sup>-7</sup>	0
	1/20	4.10E-05	6.2 x 10 <sup>-7</sup>	0
	1/50	7.20E-05	5.0 x 10 <sup>-7</sup>	0
	1/200	1	1.0 x 10 <sup>-5</sup>	0
	1/500	1.00E-05	0	0
	1/1000	2.00E-05	0	0
	1/2000	3.80E-06	0	0
	control evolved populations	3.21E-07	0	0

<b>Ciprofloxacin</b>	<b>Selective Concentration</b>	<b>2xMIC</b>	<b>8xMIC</b>	<b>16xMIC</b>
	1/4	1	1	0
	1/10	1	0	0
	1/20	0	0	0
	1/50	0	0	0
	1/200	0	0	0
	1/500	0	0	0
	1/1000	0	0	0
	1/2000	0	0	0
	control evolved populations	0	0	0

**Supplementary Table 3.** Description of genes that contain mutations as identified by whole-genome sequencing of resistant mutants.

Gene	Description	Clones with mutations (Mutation)	Potential fitness benefit
<i>aat</i>	leucyl/phenylalanyl-tRNA-protein transferase	DA66928 (G146D); DA67610 (N191D); DA67619 (N191D); DA67620 (N191D); DA68060 (c.125::IS1); DA68063 (c.125::IS1)	Undetermined
<i>allB</i>	allantoinase	DA66935 (S45R)	Undetermined
<i>bglG</i>	transcriptional anti terminator	DA66931 (L82*)	Media adaptation <sup>1</sup>
<i>brnQ</i>	membrane protein	DA70124 ( $\Delta$ M419-L422)	Undetermined
<i>caiD</i>	crotonobetainyl-CoA hydratase	DA70123 (E167A)	Undetermined
<i>cheA</i>	histidine protein kinase	DA68058 (A42V); DA68062 (A42V)	Undetermined
<i>cho</i>	endonuclease of nucleotide excision repair	DA70135 (A131T)	Undetermined
<i>degQ</i>	periplasmic serine endoprotease	DA68062 (I119T)	Antibiotic resistance <sup>2</sup>
<i>dhaK</i>	dihydroxyacetone kinase subunit K	DA67608 (G268=)	Undetermined
<i>elaD</i>	deubiquitinating protease	DA67607 (A321=)	Undetermined
<i>emrA</i>	multidrug efflux pump membrane fusion protein	DA70121 (P197=)	Antibiotic resistance <sup>3</sup>
<i>envZ</i>	sensor histidine kinase	DA67599 (L22F); DA67601 (R14C); DA67602 (R14C); DA67604 (P148S); DA67608 (R397C); DA67609 (R397C); DA67610 (V49A); DA67619 (V49A); DA67620 (V49A)	Antibiotic resistance <sup>4,5</sup>
<i>fbaA</i>	fructose-bisphosphate aldolase	DA67599 (N154S); DA67602 (V235A)	Antibiotic resistance <sup>2</sup>
<i>fimE</i>	fimbral protein	DA66920 (c.214::IS1); DA66922 (c.125::IS5); DA66923 (c.125::IS5); DA66925 (c.272::IS5); DA66927 (c.272::IS5); DA66929 (c.125::IS5); DA66930 (c.125::IS5); DA66935 (c.201::IS1); DA67602 (c.501::IS1); DA67604 (c.168::IS1); DA67605 (c.272::IS5); DA67608 (c.272::IS5); DA67609 (c.272::IS5); DA67610 (c.272::IS5); DA67612 (c.125::IS5); DA67618 (c.125::IS5); DA67619 (c.125::IS5); DA67620 (c.125::IS5); DA67621 (c.125::IS5); DA68057 (c.272::IS5); DA68058 (T87P); DA68060 (H73P); DA68062 (T87P); DA68063 (H73P); DA68074 (c.125::IS5); DA69423 (c.125::IS5); DA69424 (c.6::IS1); DA69427 (G163 fs); DA69428 (G163 fs); DA69429 (c.519::IS5); DA69430 (c.519::IS5); DA70123 (c.125::IS5); DA70129 (c.125::IS5); DA70130 (c.125::IS5); DA70132 (c.125::IS5)	Media adaptation <sup>6</sup>
<i>gatA</i>	galactitol-specific PTS enzyme	DA69427 (c.203::IS5)	Undetermined
<i>gatB</i>	galactitol-specific PTS enzyme	DA69428 (c.68::IS5)	Undetermined
<i>gatC</i>	galactitol-specific PTS enzyme	DA67605 (c.85::IS5); DA67608 (c.85::IS5); DA67609 (c.85::IS5); DA67610 (c.626::IS5); DA67612 (c.626::IS5); DA67619 (c.626::IS5); DA67620 (c.626::IS5); DA67621 (c.626::IS5)	Undetermined
<i>gatY</i>	tagatose-1,6-bisphosphate aldolase 2	DA67599 (c.644::IS5)	Media adaptation <sup>6</sup>
<i>glpT</i>	<i>sn</i> -glycerol 3-phosphate: phosphate antiporter	DA66920 (D88E); DA66921 (c.862::IS5); DA66922 (c.862::IS5); DA66923 (c.862::IS5); DA66924 (V86 fs); DA66925 (D88E); DA66926 (L255R); DA66927 (D88E); DA66933 (P212 fs)	Antibiotic resistance <sup>7</sup>

		DA69421 (c.909::IS5); DA69429 ( $\Delta$ I443-R456)	
<i>gyrA</i>	DNA gyrase subunit A	DA70123 (G81D); DA70124 (S96 fs); DA70129 (G81D) DA70130 (G81D); DA70132 (G81D); DA70135 (D40 fs) DA70137 (D87G)	Antibiotic resistance <sup>8</sup>
<i>gyrB</i>	DNA gyrase subunit B	DA70121 (A65 fs), DA70122 (A65 fs) DA70133 (A65 fs); DA70133 (A65 fs)	Antibiotic resistance <sup>9,10</sup>
<i>hchA</i>	protein/nucleic acid deglycase 1	DA66929 (c.635::IS1); DA66930 (c.635::IS1)	Undetermined
<i>icd</i>	isocitrate dehydrogenase	DA70121 (H366=; L375=) DA70122 (H336=; T370=; L375=; N385=; A366=; K387=) DA70133 (H366=; L375=, T370=); DA70133 (H366=; L375=, T370=)	Undetermined
<i>iscR</i>	DNA-binding transcriptional dual regulator	DA666924 (P61L)	Undetermined
<i>kdpD</i>	sensor histidine kinase	DA66921 (R508H); DA66922 (D774G); DA66923 (D774G) DA66924 (G836E); DA66926 (R620L); DA66929 (R511L) DA66930 (R511L); DA66931 (E651A); DA67610 (S275P) DA67613 (V329A); DA67620 (S275); DA68056 (V845G) DA68059 (S541P); DA68060 (A426V); DA68063 (A426V) DA68064 (T633M); DA69427 (R508H); DA69428 (R508H) DA70133 (R508H); DA70134 (R508H)	Media adaptation (this study)
<i>leuV</i>	tRNA-Leu (CAG)	DA68069 (c.65::IS186)	Undetermined
<i>malT</i>	DNA-binding transcriptional activator	DA67599 (T220P)	Media adaptation <sup>6</sup>
<i>mdtF</i>	multidrug efflux pump permease	DA66921 (c.1402::IS1) DA66929 (c.1402::IS1); DA66930 (c.1402::IS1) DA66935 (c.1402::IS1); DA67599 (c.402::IS1) DADA67610 (V766A); DA67611 (c.1402::IS1) DA67613 (c.1402::IS1)	Antibiotic resistance <sup>11</sup>
<i>mdtH</i>	multidrug efflux pump	DA68062 (P99Q)	Antibiotic resistance <sup>12</sup>
<i>menC</i>	o-succinylbenzoate synthase	DA68069 (c.132::IS196)	Undetermined
<i>mpaA</i>	murein tripeptide amidase A	DA68056 (D185E)	Undetermined
<i>mutL</i>	DNA mismatch repair protein	DA67610 (L92P); DA67612 (L92P); DA67618 (L92P) DA67619 (L92P); DA67620 (L92P)	Undetermined
<i>mutS</i>	DNA mismatch repair protein	DA67601 (G218E; c.2444::IS1); DA67602 (c.2444::IS1) DA67613 (R656S)	Undetermined
<i>mutT</i>	8-oxo-dGTP diphosphatase	DA68057 (R23L)	Undetermined
<i>nfsA</i>	NADPH-dependent nitroreductase/ NADPH-dependent quinone reductase	DA68056 (L98 fs); DA68057(L145*); DA68058 (W212*) DA68062 (c.380::IS186); DA68063 (c.380::IS186) DA68064 (c.380::IS186); DA68067 (c.674::IS30) DA68068 (c.674::IS30); DA68069 (c.380::IS186) DA68070 (c.674::IS30); DA68071 (c.674::IS30) DA68072 (c.380::IS186); DA68074 (c.380::IS186) DA68076 (c.674::IS30)	Antibiotic resistance <sup>13</sup>
<i>nfsB</i>	NAD(P)H-dependent nitroreductase	DA68058 (c.38::IS5); DA68069 (c.38::IS5) DA68072 (c.38::IS5); DA68074 (c.38::IS5)	Antibiotic resistance <sup>13</sup>
<i>nhaR</i>	DNA-binding transcriptional activator	DA69424 ( $\Delta$ I124-C360 IS1-mediated)	Undetermined
<i>nmpC</i>	DLP12 prophase; putative outer membrane porin	DA66926 ( $\Delta$ L1-A337 IS5-mediated)	Undetermined
<i>oxyR</i>	DNA-binding transcriptional dual regulator	DA70135 (L191 fs)	Undetermined
<i>plsB</i>	glycerol-3-phosphate 1-O-acyltransferase	DA67608 (R265L); DA67609 (R265L)	Undetermined
<i>proB</i>	glutamate 5-kinase	DA70130 (R118 fs); DA70131 (R118 fs)	Undetermined
<i>ptsI</i>	phosphoenolpyruvate-protein phosphotransferase PtsI	DA66934 (H439 fs)	Antibiotic resistance <sup>14</sup>

<i>ptsP</i>	phosphoenolpyruvate-protein phosphotransferase PtsP	DA66920 (Q340*); DA66932 (A239 fs); DA66933 (A239 fs) DA66934 (A239 fs); DA67610 (C668R); DA67612 (C668R) DA67613 (S355P); DA67619 (C668R); DA67620 (C668R) DA68057 (L425*); DA68058 (N23 fs); DA68062 (N23 fs) DA70121 (R119 fs), DA70123 (R119 fs); DA70124 (R119 fs) DA70129 (L111 fs); DA70130 (L111 fs); DA70132 (L111 fs) DA70135 (T560 fs)	Media adaptation
<i>ptwF</i>		DA68060 (R1=); DA68063 (R1=); DA68069 (R1=)	Undetermined
<i>putP</i>	proline:Na <sup>+</sup> symporter	DA66935 (L60Q); DA68060 (V338L); DA68063 (V338L)	Undetermined
<i>puuB</i>	$\gamma$ -glutamylputrescine oxidase	DA69424 (G254=)	Undetermined
<i>rbsR</i>	DNA-binding transcriptional dual regulator	DA66928 (A181T); DA66932 (c.360::IS2); DA66933 (c.360::IS2) DA66934 (c.360::IS2); DA69429 (T169M); DA69430 (T169M)	Undetermined
<i>relA</i>	GDP/GTP pyrophosphokinase	DA68067 ( $\Delta$ A274-G727); DA68068 ( $\Delta$ A274-G727) DA68070 ( $\Delta$ A274-G727); DA68071 ( $\Delta$ A274-G727) DA68076 ( $\Delta$ A274-G727)	Media adaptation <sup>6</sup>
<i>rho</i>	transcription termination factor	DA67599 (R87C); DA67604 (S84C); DA68064 (R102S)	Antibiotic resistance <sup>15</sup>
<i>rph</i>	truncated RNAase PH	DA66921 (E507*); DA66928 ( $\Delta$ A203-R230); DA68056 ( $\Delta$ A203-R230) DA68059 ( $\Delta$ A203-R230); DA68060 ( $\Delta$ A203-R230) DA68063 ( $\Delta$ A203-R230); DA69421 ( $\Delta$ A203-R230) DA69423 ( $\Delta$ A203-R230); DA69424 ( $\Delta$ A203-R230) DA69429 ( $\Delta$ A203-R230); DA69430 ( $\Delta$ A203-R230) DA70122 (S186 fs); DA70135 ( $\Delta$ A203-R230)	Media adaptation <sup>6</sup>
<i>rpoC</i>	RNA polymerase subunit $\beta'$	DA67601 (A726T; T1050A)	Media adaptation <sup>6</sup>
<i>rpsF</i>	30S ribosomal subunit protein S6	DA70137 (A116 fs)	Undetermined
<i>rpsK</i>	30S ribosomal subunit protein S11 30S ribosomal subunit protein S11	DA68069 (G39D)	Undetermined
<i>sapC</i>	putrescine ABC exporter membrane protein SapC	DA69424 (P65T)	Undetermined
<i>ssuA</i>	aliphatic sulfonate ABC transporter periplasmic binding protein	DA70137 (D337G)	Undetermined
<i>ssuD</i>	FMNH <sub>2</sub> -dependent alkanesulfonate monooxygenase	DA70122 (E243=)	Undetermined
<i>suhB</i>	Nus factor SuhB	DA67618 (G233=)	Undetermined
<i>tap</i>	methyl-accepting chemotaxis protein-dipeptide-sensing protein	DA70129 (c.822_832dup)	Undetermined
<i>trkA</i>	NAD-binding component of Trk potassium transporters	DA67608 (S360R)	Media adaptation <sup>6</sup>
<i>trkH</i>	K <sup>+</sup> transporter TrkH	DA66925 (L80K); DA66927 (L80K) DA66935 (Y130H); DA70122 (A347D)	Media adaptation and Antibiotic resistance <sup>6,16</sup>
<i>ubiF</i>	2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol hydroxylase	DA66922 (R235=); DA66923 (R235=)	Antibiotic resistance <sup>17</sup>
<i>uhpA</i>	DNA-binding transcriptional activator	DA66924 ( $\Delta$ L43-G45); DA69421 ( $\Delta$ A41-L43) DA69429 ( $\Delta$ A41-L43); DA69430 ( $\Delta$ A41-L43)	Antibiotic resistance <sup>14</sup>
<i>uhpB</i>	sensory histidine kinase	DA66931 (V33 fs); DA66932 (W132*) DA66933 (G469R); DA66935 (c.1012::IS1)	Antibiotic resistance <sup>18</sup>
<i>uhpC</i>	inner membrane protein sensing glucose-6-phosphate	DA66922 (T72P); DA6923 (T72P) DA66929 (c.235::IS5); DA66930 (A129T); DA66934 (Q34*)	Antibiotic resistance <sup>18</sup>

		DA69423 (c.760::IS5); DA69427 (N164K); DA69428 (ΔQ131-S143)	
<i>uhpT</i>	hexose-6-phosphate:phosphate antiporter	DA66920 (c.214::IS1); DA66921 (F365L); DA66925 (c.948::IS186) DA66927 (c.948::IS186); DA66928 (P423 fs) DA68067 (ΔA335-G427); DA68068 (ΔA335-G427) DA68070 (ΔA335-G427); DA68071 (ΔA335-G427) DA68076 (ΔA335-G427); DA69420 (c.382::IS2) DA69424 (c.118::IS1)	Antibiotic resistance <sup>7,18</sup>
<i>yahL</i>	uncharacterized protein YahL	DA67599 (c.527::IS5)	Undetermined
<i>ycgB</i>	PF04293 family protein	DA68069 (c.192::IS2)	Undetermined
<i>ydcl</i>	DNA-binding transcriptional regulator	DA66921 (c.21::IS1); DA66922 (K258*); DA66923 (c.85::IS2) DA66924 (H77R); DA66927 (T233M); DA66929 (Q58 fs) DA66930 (Q58 fs); DA66931 (F2 fs); DA66933 (c.241::IS2); DA68056 (N4 fs); DA68064 (c.278::IS2) DA69421 (R14L); DA69423 (L123S); DA69428 (S92 fs) DA70121 (ΔD286-I298); DA70124 (ΔE79-Y248)	Antibiotic resistance <sup>19</sup>
<i>ydcT</i>	putative ABC transporter ATP-binding protein	DA70133 (S11=)	Undetermined
<i>ydeJ</i>	PF02464 family protein	DA66933 (c.29::IS2)	Undetermined
<i>ydeO</i>	DNA-binding transcriptional dual regulator	DA70135 (S2=)	Undetermined
<i>ydhL</i>	DUF1289 domain-containing protein	DA70124 (R63H)	Undetermined
<i>yeal</i>	putative c-di-GMP binding protein	DA66924 (F343L)	Undetermined
<i>yeaR</i>	DU1971 domain-containing protein	DA67599 (c.115::IS186); DA68067 (c.115::IS186) DA68068 (c.115::IS186); DA68070 (c.115::IS186) DA68074 (c.115::IS186); DA68076 (c.115::IS186)	Undetermined
<i>yegX</i>	putative glycosyl hydrolase	DA69420 (P192Q)	Undetermined
<i>yehH</i>	pseudogene	DA66923 (V254A)	Undetermined
<i>yfaS</i>	putative macroglobulin family protein, N-terminal family	DA68068 (T30I)	Undetermined
<i>yfdE</i>	acetyl-CoA:oxalate CoA-transferase	DA68058 (c.457::IS2)	Undetermined
<i>yffS</i>	CPZ-55 prophage; uncharacterized protein YffS	DA66928 (A148T)	Undetermined
<i>yhaC</i>	uncharacterized protein	DA68056 (c.1101::IS5)	Undetermined
<i>yhal</i>	putative inner membrane protein	DA70122 (c.68::IS1)	Undetermined
<i>ymdA</i>	uncharacterized protein	DA66930 (G59 fs); DA66933 (c.289::IS2); DA67610 (c.303::IS5); DA67611 (c.48delT); DA67612 (c.309::IS5); DA67613 (c.309::IS5) DA67618 (c.309::IS5); DA67619 (c.309::IS5); DA67620 (c.309::IS5) DA67621 (c.309::IS5); DA70123 (c.308::IS5); DA70129 (F18 fs); DA70130 (c.309::IS5); DA70132 (c.309::IS5)	Undetermined
<i>yobF</i>	DUF2527 domain-containing protein	DA68059 (c.106::IS1); DA69427 (R19P)	Undetermined
<i>yphG</i>	UF5107 domain-containing protein	DA69427 (L750=); DA69428 (L750=) DA70121 (L750=); DA70122 (L750=) DA70133 (L750=); DA70134 (L750=)	Undetermined



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**Supplementary table 4:** Cross-resistance tested for subset of mutants selected on tetracycline and nitrofurantoin

Isolate	Genotype	Selection	Cross-resistance tested on			
			FOS	TET	NIT	CIP
DA5438	-	-	0.38	1.5	4	0.012
DA67604	<i>envZ</i> (P148S); <i>rho</i> (S84C); <i>fimE</i> (c.501::IS1)	TET (1/4x MIC)	1		8	0.38
DA68067	<i>uhpT</i> (A335-G427del); <i>relA</i> (A274-G727); <i>nfsA</i> (c.674::IS30); <i>yeaR</i> (c.115::IS186)	NIT (1/4x MIC)	128	1.5		0.008
DA68068	<i>uhpT</i> (A335-G427del); <i>relA</i> (A274-G727); <i>nfsA</i> (c.674::IS30); <i>yeaR</i> (c.115::IS186); <i>yfaS</i> (R30I)	NIT (1/4x MIC)	64	1		0.016
DA68070	<i>uhpT</i> (A335-G427del); <i>relA</i> (A274-G727); <i>nfsA</i> (c.674::IS30); <i>yeaR</i> (c.115::IS186)	NIT (1/4x MIC)	64	1.5		0.012
DA68071	<i>uhpT</i> (A335-G427del); <i>relA</i> (A274-G727); <i>nfsA</i> (c.674::IS30)	NIT (1/4x MIC)	48	0.75		0.012
DA68076	<i>uhpT</i> (A335-G427del); <i>relA</i> (A274-G727); <i>nfsA</i> (c.674::IS30); <i>yeaR</i> (c.115::IS186)	NIT (1/4x MIC)	32-64	1		0.012
DA68060	<i>ptwF</i> (R1=); <i>putP</i> (V338L); <i>aat</i> (c.125::IS1); $\Delta$ <i>yrdA-yrdD</i> (562bp); <i>kdpD</i> (A426V); <i>rpH</i> (A203-R230del); <i>fimE</i> (H73P)	NIT (1/200x MIC)	0.75	1		0.006
DA68063	<i>nfsA</i> (c.380::IS186); <i>ptwF</i> (R1=); <i>putP</i> (V338L); <i>aat</i> (c.125::IS1); <i>kdpD</i> (A426V); <i>rpH</i> (A203-R230del); <i>fimE</i> (H73P)	NIT (1/200x MIC)	1.5	1.5		0.008