Supplemental Information
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Sublethal Antibiotic Treatment Leads to Multidrug Resistance via Radical-Induced Mutagenesis

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Supplemental Results

Bactericidal Antibiotics Lead to Low-Level Increases in MIC for a Range of Antibiotics

Treatment with 25 ng/ml norfloxacin led to significant increases in the MIC for norfloxacin and kanamycin as well as modest, low-level increases in the MIC for ampicillin, tetracycline and chloramphenicol (Figure S1A). This increase in MIC for norfloxacin was concentration dependent. Treatment with 50 ng/ml norfloxacin led to a 6-fold increase in the MIC for norfloxacin (Figure S1B); however, we were unable to observe an increase in the MIC for ampicillin, kanamycin, tetracycline, or chloramphenicol following treatment with 50 ng/ml norfloxacin for 5 days (Figure S1B). Interestingly, selection of drug-resistant mutants following quinolone treatment is concentration dependent, with higher concentrations of quinolone selecting only quinolone-resistant strains and lower levels of quinolone selecting broadly for drug-resistant mutants with mutations in a wide array of targets in *E. coli* (Drlica, 2003) and *Mycobacterium tuberculosis* (Zhou et al., 2000). It is possible that treatment with 50 ng/ml norfloxacin selects for naturally occurring quinolone-resistant mutants before the drug-induced mutagenesis has a chance to create mutants resistant to other drugs.

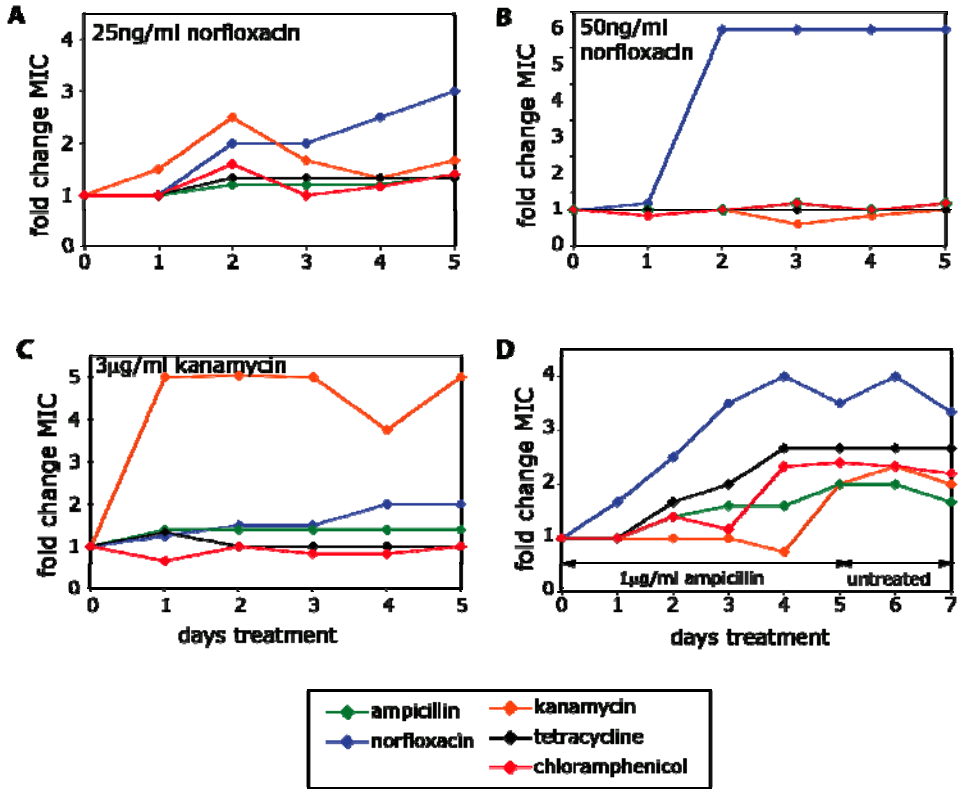

Figure S1. Bactericidal Antibiotics Can Lead to Broad-Spectrum Increases in MIC
(A-C) Fold change in MIC relative to a no-drug control for ampicillin, norfloxacin, kanamycin, tetracycline and chloramphenicol, following 5 days of growth in the presence of (A) 25ng/ml norfloxacin, (B) 50ng/ml norfloxacin, or (C) 1µg/ml kanamycin.
(D) Ampicillin-mediated increases in MIC are stable. Fold change in MIC relative to a no-drug control for ampicillin, norfloxacin, kanamycin, tetracycline and chloramphenicol, following 5 days of growth in the presence of 1µg/ml ampicillin and an additional 2 days of growth in the absence of drug.

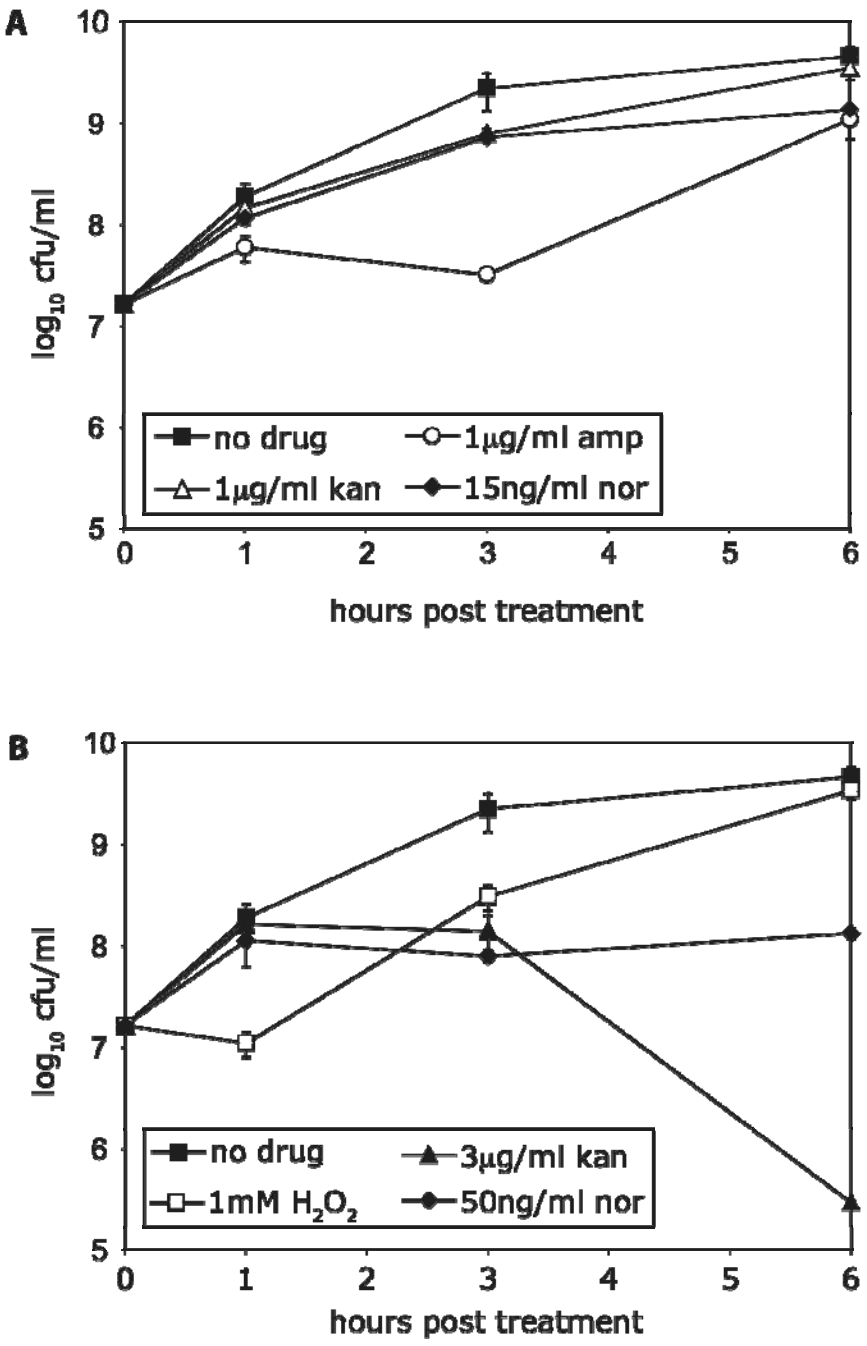

Figure S2. Survival of *E. coli* Following Treatment with Near-MIC Levels of Antibiotics

(A) Survival of MG1655 following treatment with no drug (filled squares), 1μg/ml ampicillin (amp, open circles), 1μg/ml kanamycin (kan, open triangles), and 15ng/ml norfloxacin (nor, filled diamonds), respectively. (B) Survival of MG1655 following treatment with no drug (filled squares), 3μg/ml kanamycin (filled triangles), 1mM hydrogen peroxide (H_2O_2, open squares), and 50ng/ml norfloxacin (filled diamonds), respectively.

Table S1. PCR Primers and Sequencing Primers

PCR Primers	
Primer Name	**Sequence (5`-3`)**
gyrA-F	CCA GAC TTT GCA GCC TGG ACT T
gyrA-R	AAC TCA CCT TCC AGA TCC CAC CA
gyrB-F	TGA ACG CCT TAT CCG GCC TAC AA
gyrB-R	CTC TGA GCT TGA TGA TGA GCG TCG
tolC-F	TGA CTG CCG TTT GAG CAG TCA TGT G
tolC-R	TTA CGT TGC CTT ACG TTC AGA CGG
marRA-F	TAG CTA ACG GCA GCA ACA CCA C
marRA-R	CAA TGT ATT TGG CTT GCG GTG GC
acrAB-F	TCG TAT GAG ATC CTG AGT TGG TGG TTC
acrAB-R	AAT GCC AGT AGA TTG CAC CGC
acrAB-F2	ACT TAT TAC TAC GCG ATC GCC TGC T
acrAB-R2	GCA GTG AAC CAG AAT AGC AAC GAC GA
sdhB-F	CTG CCA ACT TCC GTA CCG AAA G
sdhB-R	AGC TCT TGT CTA CGT AGT GGC TC
icdA-F	CTG GTA GAA CGT TGC GAG CT
icdA-R	GAC TAG TAG TAG AAC TAC CAC CTG ACC G
iscR-F	GTT ACC AAA GGT TCC GTC CAT CGT
iscR-R	CGT CTT ATC AGG CCT ACA GTG TAC AG
cpxR-F	CGA CAT GCT GCT CAA TCA TCA GC
cpxR-R	GCT TAA TGA ACT GAC TGC CAG CGT TGA
arcA-F	GAC TGC TCA ACT CTG CCG ATA G
arcA-R	TGC TGT TAA AAT GGT TAG GAT GAC AGC CGT
ampC-F	AGG CAA CGA CCA GAA ATG CAG CT
ampC-R	TAT GCA CCA CGC GAT GCA CGA T
Sequencing Primers	
Primer Name	**Sequence (5`-3`)**
gyrA1	CAG GCA TTG GAT GTG AAT AAA GCG TAT AGG
gyrA2	ATC ATT AAC GGT CGT CGC GGT ATT G
gyrA3	TGC GTG ATG GTC TGT ACT ACC TGA
gyrA4	TCC TCA CCG AGT TCA ACC GTC T
gyrB1	TCA GTG CTG AAC ACG TTA TAG ACA TGT CGG
gyrB2	GAC GGC AAA GAA GAC CAC TTC CAC T
gyrB3	AAG CGC GCT TCG ATA AGA TGC T
gyrB4	GTT TGA TGT TCA CAC CAA TGC TGA GC
tolC1	TAT GGC ACG TAA CGC CAA CCT
tolC2	TAA CCT TGA TAA CGC GGT AGA GCA GC
tolC3	GCT CAA GCG TGC CTG TAA CA
marRA1	AGC TAG CCT TGC ATC GCA TTG A

marRA2	CGG ACG AAG TGG CAA CAC TTG AGT AT
marRA-M1	AGG TAT GAC GAT GTC CAG ACG CA
marRA-M2	TGC GTC TGG ACA TCG TCA TAC CT
acrA1	CAG CTG CTT TTG CAA TCT CGC
acrA2	CTG CTC GGT ACT CAG TAC ATC AGT AAG C
acrA3	TGC AGA AAG TGC GTC CTG GTG T
acrA4	ATT ACC GCC ATC AAA GCG CAG
acrA8	CTC CAT CAA TAA TCG ACG CCG TTC T
acrA9	TGT AAG CCA GAT TGA TCC GCG CA
acrA-M1	GTT CTG TAC CAA TGC GCC TTC CGT
acrA-M2	ACG GAA GGC GCA TTG GTA CAG AAC
icdA1	TAG CCT AAT AAC GCG CAT CTT TCA TGA CG
icdA2	ATT CGC TTC CCG GAA CAT TGT GGT A
icdA3	CTA CCC CAA AAC TAC CGA GGG GTT
icdA4	CCA GTC TTT AAA CGC TCC TTC GGT
icdA-R5	GGA GCG TTA CGC TCC CGT TAA TA
icdA-M1	GGT ATC GAA TGG AAA GCA GAC TCT GC
sdhB1	TCG ACT TCC CGG ATC GTG ATG ATG A
sdhB2	TTC TTT GTT ACG CCT GAT GCG CT
iscR1	TGG GTT GCG GAG TAG TCG AGA TAA
iscR2	ATA TGG CGT TCA CGC CGC AT
cpxR1	ACG CTG TTC GCT ATC CAG AAG CTC
cpxR2	GCA GCG GTA ACT ATG CGC ATC ATT
arcA1	GTG ACC CGT AAT ATC GAC TGG TAT GC
arcA2	GTA CCC ACG ACC AAG CTA ATG ATG
ampC1	TGG CTG CTA TCC TGA CAG TTG TCA
ampC2	GTC TGT ATG CCA ACT CCA GTA TCG GT

Supplemental References

Drlica, K. (2003). The mutant selection window and antimicrobial resistance. J Antimicrob Chemother *52*, 11-17.

Zhou, J., Dong, Y., Zhao, X., Lee, S., Amin, A., Ramaswamy, S., Domagala, J., Musser, J.M., and Drlica, K. (2000). Selection of antibiotic-resistant bacterial mutants: allelic diversity among fluoroquinolone-resistant mutations. J Infect Dis *182*, 517-525.