Supporting Information

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SI Materials and Methods

Bacterial Strains and Strain Construction. All experiments were performed using laboratory strains of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. Ancestral wild-type *E. coli* K-12 EMG2 +fertility factor plasmid (F+) obtained from Yale *E. coli* Genetic Stock Center (ECGC 4401) was the reference wild-type *E. coli* strain used in all experiments, and *S. typhimurium* LT2 [American Type Culture Collection (ATCC) 700720] was the reference strain of nontyphoidal *Salmonella* (1–4). Single-gene knockout mutants were constructed from an *E. coli* Kan^R knockout library (5) via lambda Red transduction (6) into wild type. All *Salmonella* knockouts and any *E. coli* mutants not available in the knockout library were constructed using the Datsenko-Wanner PCR products method (6). PCR primers for the Datsenko-Wanner method were designed using Oligocalc (7) and obtained from Integrated DNA Technologies. Strains and primers used in this study are presented in Table S1.

Rescue plasmids were constructed using the pZ system (8). Purification of restriction digest products and PCR products was performed using commercially available kits (Qiagen). Cloning primers contained an ∼8-bp GC-rich leader sequence, a restriction site for plasmid insertion, and a ∼26-bp homology sequence overlying the START or STOP codon of the gene of interest (Table S1). Genes for complementation were cloned from *E. coli* EMG2 using hot-start Phusion PCR (Sigma-Aldrich). PCR products and the parent plasmid pZA21-GFP were digested using KpnI/BamIII and ligated using T4 DNA ligase (Sigma-Aldrich), replacing the GFP cassette with the gene of interest.

E. coli strains were transfected with the spectinomycin-resistant Pro cassette to enable activation from the $P_{Ltet0\text{-}1}$ promoter. Strains were then made electrocompetent by washing twice in ice-cold sterile water and twice in ice-cold sterile 10% glycerol. Electrocompetent strains were incubated 20 min with plasmid in cold 10% (vol/vol) glycerol before electroporation at 2,400 V. Plasmid-transformed cells were inoculated in super optimal broth with catabolite repression (SOC) media and incubated 1 h at 37 °C to allow expression of plasmid-based resistance genes before addition of the appropriate antibiotic for selection.

Antibiotics and Chemicals. The following concentrations of antibiotics were used in this study: 100 μg/mL ampicillin or carbenicillin, 10–60 μg/mL kanamycin, 0.5–2 μg/mL ciprofloxacin, and 1–5 μg/mL ofloxacin. Strains containing kanamycin-resistance plasmids were grown with 30–60 μg/mL kanamycin for selection, and strains containing spectinomycin-resistance cassettes were grown with 50 μg/mL spectinomycin. Otherwise, antibiotic treatments were ≥10× minimum inhibitory concentration (MIC) to ensure killing of sensitive cells (9). All antibiotics were purchased from Sigma-Aldrich. L-tryptophan, indole [≥99%, Food Chemicals Codex (FCC)], and hydrogen peroxide [American Chemical Society (ACS) reagent, stock strength 30%] were purchased from Sigma-Aldrich.

In all experiments, cultures were grown in media containing antibiotics for plasmid selection (35 μg/mL kanamycin), and 25–50 ng/mL anhydrotetracycline (aTc) was added after 2–4 h growth for induction of plasmid-borne genes.

Growth and Tolerance Assays. All experiments were performed in accordance with standard protocols unless otherwise stated. Briefly, bacterial cultures were grown in light-insulated shakers at 37 °C with shaking at 300 rpm (14-mL Falcon tubes, Fisher Scientific) or 900 rpm (96-well, clear, flat-bottom culture plates, Fisher Scientific; with Breathe-Easy adhesive gas-permeable membrane, USA Scientific). Cultures were grown in tryptophan-free minimal medium [M9 + 0.2% Casamino acids + 0.4% (wt/vol) glucose, M9CG, pH >7.2] or in rich media (LB).

MIC was determined via growth assay in 96-well plates, using $\log_2$ dilution series of antibiotics (10).

Antibiotic tolerance was assessed by incubating cultures for at least 3 h with antibiotic to allow full killing of sensitive cells (11, 12). For stationary-phase cultures, ciprofloxacin treatment was used for killing of sensitive cells, as quinolones have bactericidal activity against stationary-phase bacteria (11, 13, 14). Ten microliters of culture medium was removed before addition of antibiotic and after antibiotic challenge for serial dilution in 1× PBS, and serial dilutions were spot-plated onto LB agar (10-μL spots). Serial dilution plates were allowed to grow overnight at 37 °C, and colony counts were used to calculate cfu/mL. The cfu measurements before and after antibiotic treatment were compared to determine survival.

The *S. typhimurium* oxidative stress response (Δ*oxyR*) mutant, despite lacking a homolog to the *E. coli* flocculation gene (*flu*) (15), strongly self-aggregates, making dilution plating difficult. In tolerance assays where this strain was used (Fig. 2), all samples were collected by spinning 2 min at ∼7,600 × *g* in a tabletop centrifuge, washed twice in 10 volumes sterile 1× PBS, and resuspended in one volume sterile 1× phosphosaline (pH 4) + 0.5% Tween-20 by pipetting and vortexing. All strains used in these assays were treated identically to ensure consistency. Serial dilution was performed in resuspension buffer, and dilutions were immediately plated onto LB agar. As we were unable to clear the antibiotic-resistance cassette from Δ*oxyR* and phage shock response (Δ*pspBC*) strains using pCP20 (6), tolerance assays using these knockouts were performed with *S. typhimurium* LT2 nitrate reductase 2 mutant bearing a chloramphenicol resistance cassette (Δ*narV*:*CmR*) (described in the next section) as the reference strain.

Coculture of *E. coli* and *S. typhimurium*. *E. coli* Pro was inoculated 1:200 from overnight LB cultures into M9CG containing 0–2 mM tryptophan, then dispensed in 150-μL aliquots into a flat-bottomed clear 96-well culture plate, covered with Breathe-Easy gas-permeable membrane, and incubated at 37 °C as previously described for 16 h (stationary phase). *S. typhimurium* was inoculated 1:500 from overnight LB cultures into M9CG, aliquoted into a 96-well plate, and grown under identical conditions for 3.5 h (exponential phase) before coculture. Twenty percent of stationary-phase *E. coli* cultures was removed and replaced with an equal volume of exponential-phase *S. typhimurium* culture, a fresh gas-permeable membrane was applied, and cocultures were returned to incubation for 1 h before treatment with 2 μg/mL ciprofloxacin. Cultures were incubated 6 h with ciprofloxacin before dilution plating onto selective media (25 μg/mL spectinomycin for *E. coli* or 25 μg/mL chloramphenicol for *S. typhimurium*).

The *S. typhimurium* LT2 Δ*narV:CmR* knockout was generated to allow separation of bacterial strains. The *narV* gene was selected for deletion because the activity encoded by this gene (nitrate reductase) is functionally redundant in *S. typhimurium* LT2 (16).

Growth of *S. typhimurium* on Conditioned Media. Conditioned media was prepared by inoculating 1 mL M9CG + 1 mM tryp-

tophan 1:200 from overnight cultures of LT2, EMG2 wild type, and EMG2 Δ*tnaA* and growing to stationary phase. *S. typhimurium*-conditioned media was used as the control condition. Cultures were pelleted in a tabletop centrifuge (2 min at 6,000 × *g*), and supernatant was sterilized by syringe filtration with a 0.22-μm filter. Sterility of conditioned media was confirmed by incubating filtered media overnight at 37 °C under standard culture conditions. *S. typhimurium* was inoculated 1:200 from overnight culture into 150 μL M9CG in 96-well plate and covered with a gas-permeable membrane before incubation. After 1 h, these cultures were mixed with conditioned media (30% exponential-phase culture in 70% conditioned media, final volume 150 μL); cultures were incubated 3.5 h further before treatment with ciprofloxacin (0.5 μg/mL) to assess tolerance. All cultures were incubated at 37 °C with shaking.

Indole Quantification. Indole quantification was performed via HPLC using a protocol based on (17), (18), and (19). Cell-free medium for quantification of extracellular indole was obtained by centrifugation of whole culture (6,000 × *g*, tabletop centrifuge) and purification of the resulting supernatant on Spin-X filter columns. HPLC was performed using a Waters C_{18} (5 μm, 4.6 × 150) column with H_20 + 0.1% (vol/vol) formic acid/acetonitrile (1:1) as the isocratic mobile phase. Under these conditions, in M9 + 0.2% casamino acids + 0.4% glucose, indole eluted at ∼4.8 min as a single peak at 271 nm. A series of indole standards (12.5–600 μM) in sterile growth medium was used to estimate indole concentration as a linear function of peak height.

Quantitative PCR. RNA was collected from indole-treated and untreated cultures of *S. typhimurium* LT2. Cultures were inoculated 1:500 from overnight LB cultures into 1 mL of M9CG in 14-mL Falcon tubes and incubated 3.5 h before treatment with indole. After 30-min incubation with indole (0, 50, or 125 μM), cultures were stabilized with RNAprotect Bacteria Reagent (Qiagen) according to the manufacturer's protocol, and the resulting cell pellets were stored overnight at −80 °C. RNA extraction was performed using RNeasy Mini Kit (Qiagen), and DNA contamination was eliminated using TURBO DNA-Free (Ambion) according to the manufacturers' protocols. Sample concentration was estimated using the ND-1000 NanoDrop spectrophotometer. Standard PCR using Taq polymerase and qPCR primers (see below for primer information) was used to test for DNA contamination in RNA samples after TURBO DNA-Free digestion (4% RNA suspension by volume, 30–35 cycles). RNA was stored at −80 °C.

cDNA for qPCR was synthesized from RNA using the SuperScript III First Strand Synthesis kit (Invitrogen) and stored at −20 °C. Guanylate kinase (*gmk*), DNA gyrase subunit B (*gyrB*), and 16S ribosomal RNA (*rrsH*) were used as reference genes for *S. typhimurium*. Quantitative PCR primers for each transcript of interest and the reference transcripts were designed based on the NCBI *E. coli* K-12 MG1655 genomic sequence (Refseq NC_000913) or the NCBI *S. typhimurium* LT2 genomic sequence (Refseq NC_003197) using Primer3Plus software (20) (Table S1). Primers were designed under the following constraints: amplicon size was 130–160 bp, the calculated primer melting temperature was ∼55 °C, GC content was 45–55%, and probabilities of primer-dimer/hairpin formations were minimized. Primer specificity was confirmed via standard Taq polymerase PCR on genomic DNA. qPCR using LightCycler 480 SYBR Green I Master Kit (Roche Applied Science) were prepared manually according to the manufacturer's instructions. qPCR were performed with a total volume of 20 μL, containing 0.5 μM of forward primers and 0.5 μM of reverse primers and 10 μL 2× 480 SYBR Green Master Mix. Reactions were carried out in white LightCycler 480 96-well plates (Roche). One negative control (replacing cDNA with PCR H_2O) was performed for each primer set in all qPCR runs. PCR parameters were denaturation (95 °C for 10 min) and 30–35 cycles of three-segment amplification (95 °C for 10 s, 50–52 °C for 10 s, and 72 °C for 10 s). The thermal-cycling program was concluded with a dissociation curve (65 °C ramped to 95 °C, 10 s at each 1 °C interval) to detect nonspecific amplification or primer-dimer formation. C_t values were obtained using standard methods (21). Alkyl hydroperoxide reductase (*ahpF*) was excluded from final analyses because induction of this gene could not be detected after treatment with 60 μM hydrogen peroxide, possibly due to error in the primers used here (Fig. S3).

Induction of OxyR. Hydrogen peroxide treatment was performed as follows. Cultures were inoculated 1:200 (*E. coli*) or 1:500 (*S. typhimurium*) from overnight LB cultures into M9CG (1 mL in 14-mL Falcon tubes or 150 μL in 96-well plates) and allowed to grow to exponential phase (3.5 h) or stationary phase (24 h) at 37 °C. Cultures were incubated 1 h with the indicated concentration of hydrogen peroxide before treatment with antibiotics. Serial dilution plating was performed before and after 1 h of hydrogen peroxide incubation and after 3 h of ofloxacin treatment to determine survival at each stage.

C. *elegans* Intestinal Model for *S. typhimurium* Infection. The temperature-sensitive germ line proliferation (*glp*) mitogen-activated protein kinase kinase (*sek*) mutant strain *AU37* [*glp*-4 (*bn2*) I; *sek*-1 (*km4*) X] of *C. elegans* (Caenorhabditis Genetics Center) was used as a model organism for *Salmonella* pathogenesis in the host intestine. *C. elegans* cultures were synchronized from 10–14 100-cm agar plates using a standard protocol (22). Synchronized L1 larvae were washed to remove dauer pheromone and grown 3–4 d in S-medium (22) + concentrated *E. coli* OP50 at 25 °C with shaking at 200 rpm to allow the larvae to reach adulthood. Synchronized cultures of adult *C. elegans* were then sucrose-washed to remove OP50 and other debris (23) and resuspended in 1 mL S-medium with *E. coli* EMG2 Δ*tnaA* Pro as a food source (50× concentrated from overnight stationary-phase cultures in LB, 100 μL food per mL worm culture). Indole (0 or 125 μM) was added with bacteria, and worm cultures were incubated 24 h at 25 °C to acclimatize worms. *S. typhimurium* Δ*narV:*CmR was then grown to late exponential/early stationary phase in LB, then spun down and resuspended at 50× concentration; 25 μL of concentrated *S. typhimurium* cultures were introduced to 1 mL worm culture, and cultures were incubated for 12 h at 25 °C to allow establishment of the initial infection, after which cultures were washed 4× in M9 worm buffer (22) to remove external pathogens and incubated 24 h further on *E. coli* (100 μL 50× food per mL worm culture as previously described) in S-medium ± indole to allow *Salmonella* infection to progress. Cultures were then treated with 2 μg/mL ciprofloxacin; higher concentrations were used here than in in vitro experiments because the worm represents a barrier to diffusion of the drug.

At 0 and 24 h antibiotic treatment, samples of *C. elegans* cultures were washed 6× in M9 worm buffer to remove external bacteria, and internal bacteria were harvested by mechanical disruption of worms in M9 worm buffer + 0.5% (vol/vol) Triton X-100 + 5% (wt/vol) SDS. Internal bacteria were pelleted by centrifugation (2 min at 13,500 × *g* in a tabletop centrifuge), washed 3× in M9 worm buffer to remove Triton X-100 and SDS, and dilution-plated on selective media to determine cfu/mL. Subsamples (25–100 μL) of undisrupted worm culture were paralyzed with 10% sodium azide, dropped on LB agar, and counted under a dissection microscope to determine worms/mL; this number was used to calculate average cfu/worm.

These experiments were repeated using *E. coli* EMG2 wild type or Δ*tnaA* pZS4-mCherry as a food source and *S. typhimurium* pZA21-GFP as the infectious agent. After 24–48 h development

of *Salmonella* infection, samples of worm culture were washed and mounted on 2% agar slides for fluorescent microscopy. Images were collected at 10× magnification [Plan apochromat (Apo) VC 100×] using an Eclipse Ti epifluorescence inverted microscope (Nikon Instruments, Inc.) equipped with the Perfect Focus System, a ProScan II three-dimensional (XYZ)-motorized stage (Prior Scientific, Inc.), and a CoolSNAP HQ^2 charge-coupled device (CCD) camera (Photometrics) and controlled by the Nikon Instruments software (NIS)-Elements Advanced Research software (Nikon Instruments, Inc.). Images were acquired in the differential interference contrast (DIC) configuration and in the GFP (FITC) and mCherry (TRITC) fluorescent channels, using a motorized fluorescence excitation and emission filter turret with a fluorescent channel filter set (Chroma Technology Corp.), a Lambda SC SmartShutter (Sutter Instrument), and an Intensilight C-Mercury Fiber Illuminator, motorized (HGFIE) mercury fluorescent lamp (Nikon Instruments, Inc.). Images were autoscaled after acquisition to reduce background in the fluorescence channels. Quantitation of relative bacterial load per worm from the fluorescence microscopy channels was deemed intractable due to the ubiquitous presence of saturated pixels and the inadequacy of single 2D epifluorescence images to convey infection intensity across the *z* axis in the nematode intestine.

To determine whether indole production by *E. coli* could affect *Salmonella* persistence in the worm model, synchronized cultures of adult *C. elegans* were incubated in modified S-medium (pH ~7, adjusted with 10N NaOH) with 0–1 mM tryptophan using *E. coli* EMG2 Pro or Δ*tnaA* Pro as a food source. When prepared according to standard protocols, fresh S-medium has pH ~5.5, and indole production in *E. coli* is inefficient under acidic conditions (24, 25). Cultures were incubated as previously described for 24 h to allow production of indole before infection. After 24 h, *S. typhimurium* Δ*narV:*CmR (50× concentrated culture prepared as previously described) was introduced, and infection was permitted to develop for 48 h; cultures were not washed to remove external pathogens during this step, as this would remove any *E. coli*-produced indole from the medium. Antibiotic treatment, worm disruption, and cfu/worm counts were performed as described above. *T* tests conducted for these data were one-sided, as data from batch culture experiments indicated that the presence of indole could be expected to increase survival of *Salmonella*, and this expectation could be tested against a null hypothesis of no difference.

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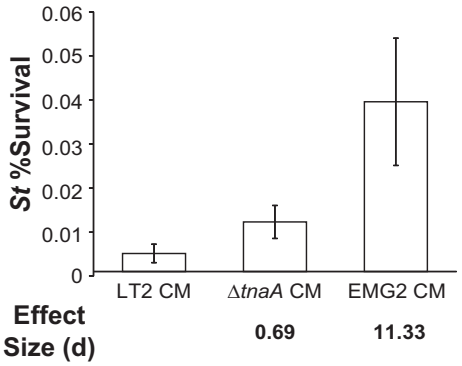

Fig. S1. *E. coli*-produced indole in conditioned media induces antibiotic tolerance in *Salmonella typhimurium*. Conditioned media was prepared by inoculating M9CG + 1 mM tryptophan with *S. typhimurium* LT2, EMG2 wild type, and EMG2 Δ*tnaA* and growing to stationary phase; *S. typhimurium* was grown to exponential phase in 30% fresh/70% conditioned media and treated with ciprofloxacin (0.5 μg/mL). *S. typhimurium*-conditioned media was used as the control condition, and Cohen's effect sizes (*d*) were calculated with respect to this condition. Bars represent mean ± SD of seven biological replicates. Exposure to Δ*tnaA*-conditioned media did not have a large effect on *Salmonella* tolerance compared with exposure to *S. typhimurium*-conditioned medium ($d = 0.69$), whereas exposure to media conditioned by wild-type *E. coli* did have a large effect on tolerance relative to the control ($d = 11.33$), indicating that most if not all of the increase in tolerance upon exposure to *E. coli* is due to the effects of indole signaling.

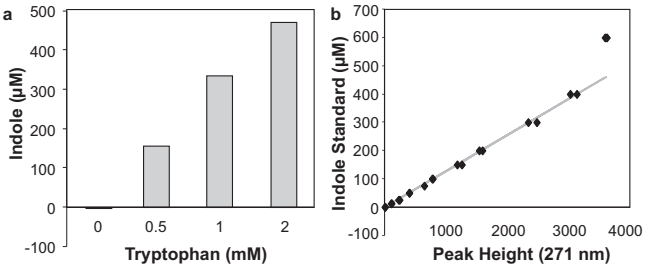

Fig. S2. Indole production as determined by HPLC. (*A*) Indole concentrations in conditioned *E. coli* medium. Wild-type *E. coli* was grown to stationary phase (16 h) in M9CG + indicated concentrations of tryptophan. (*B*) Linear regression against a series of indole concentration standards in M9CG was used to calculate indole concentration in conditioned media (calculated regression line: $m = -1.64$, $b = 0.127$).

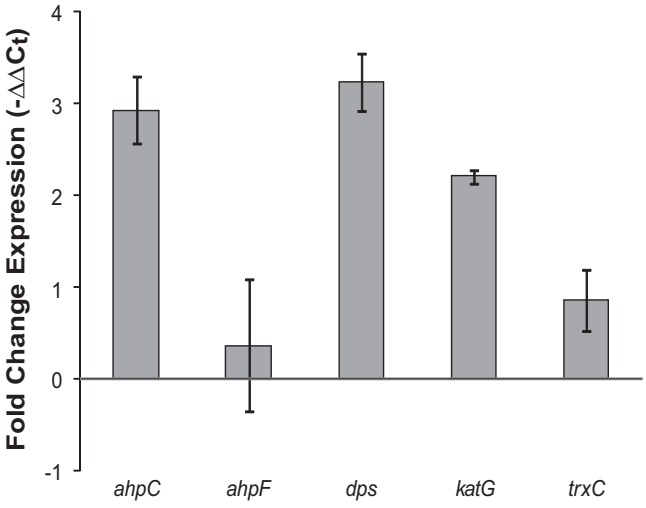

Fig. S3. qPCR of OxyR regulon genes after treatment of *S. typhimurium* with hydrogen peroxide. Exponential-phase (3.5 h) cultures of *S. typhimurium* in M9CG were treated with 60 μM H_2O_2 for 15 min. Bars represent mean ± SD of three biological replicates.

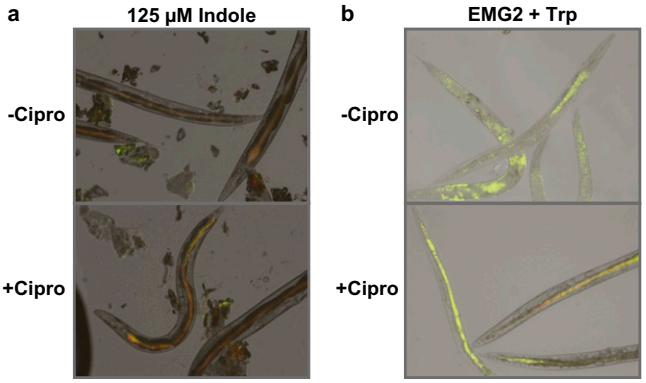

Fig. S4. Ciprofloxacin treatment does not produce visually obvious changes in bacterial content of the *C. elegans* intestine after 24 h. All experiments were performed in S-Medium (*SI Materials and Methods*) at 25 °C to ensure sterility and survival of *C. elegans* AU37. Images were taken from ciprofloxacin-treated and untreated cultures 24 h after the addition of antibiotic and were selected to represent the variation observable in each experimental condition. DIC images are shown overlaid with red and green fluorescent channels. The lack of a clear difference in fluorescence between ciprofloxacin-treated and untreated cultures may be the result of the high stability of the fluorescence proteins and the fact that ciprofloxacin does not cause lysis or significant permeabilization of bacteria cells. For these reasons, the fluorescence of cells killed by ciprofloxacin should resemble the fluorescence of untreated cells. (*A*) *C. elegans* fed on *E. coli* (mCherry) and infected with *S. typhimurium* (GFP) with 125 μM indole added to media. (*B*) *C. elegans* fed on *E. coli* EMG2 (mCherry) and infected with *S. typhimurium* (GFP) with 0.5 mM tryptophan added to media.

Table S1. Bacterial strains, plasmids, and primers used in this study

Strains and primers	Genotype or sequence
E. coli K12 strains	
EMG2	Wild type
EMG2 *tnaA*	K12 EMG2 Δ*tnaA*
EMG2 Pro	K12 EMG2 Pro, Sp^R, lacR, tetR
EMG2 *tnaA* Pro	K12 EMG2 Δ*tnaA* Pro, Sp^R, lacR, tetR
Salmonella typhimurium strains	
S. typhimurium LT2	Wild type
LT2 *narV*	*S. typhimurium* Δ*narV* Ω Cm^R
LT2 *oxyR*	*S. typhimurium* Δ*oxyR* Ω Cm^R
LT2 *pspBC*	*S. typhimurium* Δ*pspBC* Ω Cm^R
Knockout confirmation primers	
tnaA Forward	CCAGAGCCAAACCGATTAGA
tnaA Reverse	ACCATAACACCCCAAAATGC
narV Forward	CTGGGAAGAAGAACAGGTGAAG
narV Reverse	GAAGGCGTCAACATGGTAAATC
mCherry F internal	TGTACGGTTCCAAGGCCTAC
mCherry R internal	CCCATGGTCTTCTTCTGCAT
Sp^R F internal	GGTCACCGTAACCAGCAAAT
Sp^R R internal	GCAGTCGCCCTAAAACAAAG
CmR F internal	GGGCGAAGAAGTTGTCCATA
CmR R internal	TCCGGCCTTTATTCACATTC
KmR F internal	AATATCACGGGTAGCCAACG
KmR R internal	TGCTCCTGCCGAGAAAGTAT
oxyR detect F LT2	TAAGTGCGGTCGCGTACAAGG
oxyR detect R LT2	CCGCGGTATGGATGTGAAAGTG
pspBC detect F LT2	TCCTGTTGAGCCTGAAGAGAAG
pspBC detect R LT2	CGACATCGTGAACGCCAATATC
Primers for phage-mediated PCR product knockouts	
LT2 *narV* Forward	TGAACGGGAAGAGCAGGAAAATAGTCATGTGTAGGCTGGAGCTGCTTCG
LT2 *narV* Forward extend	TACTCAAACGGCGCGCTCCAGACATGAACCAGACGGGTGAACGGGAAGAGCAGG
LT2 *narV* Reverse	TGCGCTATGACTACGGACAATATACCTGCGCGAATATCCTCCTTAGTTCCTATTCC
LT2 *narV* Reverse extend	TGCGCCACGGTATTCTTCCTCGGCAGTTGGCTGCGCTATGACTACGGAC
LT2 *oxyR* Upstream F	GGATCCACTAGTTGGCGTCATCATGCACCTG
LT2 *oxyR* Upstream R	GCCTACACGCATGCTGCGTAGCCGTTATGAGCAACTG
LT2 *oxyR* AbR F	GCTACGCAGCATGCGTGTAGGCTGGAGCTGCTTCG
LT2 *oxyR* AbR R	CGGGCGGTGTCCTCGAGCATATGAATATCCTCCTTAGTTCC
LT2 *oxyR* Downstream F	CATATGCTCGAGGACACCGCCCGTTCAGAGGG
LT2 *oxyR* Downstream R	GCGGTGGCGGCCGCATACCTGGCTTGACTGCCTGCATATGG
LT2 *oxyR* Insert F	AAACGCCCACGTTGTCAG
LT2 *oxyR* Insert R	CTATGCTAATGGCCGTACCG
LT2 *pspBC* Upstream F	GGATCCACTAGTCCATATTCATCGCGTGCGTATAC
LT2 *pspBC* Upstream R	GCCTACACGCATGCCTGTAAGTGATATATCGGGAAGG
LT2 *pspBC* AbR F	CTTACAGGCATGCGTGTAGGCTGGAGCTGCTTCG
LT2 *pspBC* AbR R	CATGAGCTCGAGCATATGAATATCCTCCTTAGTTCCTATTC
LT2 *pspBC* Downstream F	CATATGCTCGAGCTCATGCGTTCTCCTTACAC
LT2 *pspBC* Downstream R	GCGGTGGCGGCCGCGTTATCCCGTCGTATTGAAC
LT2 *pspBC* Insert F	ATTCATCGCGTGCGTATACCC
LT2 *pspBC* Insert R	ATTGAACAGGCTACGGCTCAG
Rescue plasmid construction	
tnaA cloning F	CCGCGCCGTGCGGTACCATGGAAAACTTTAAACATCTCCCTG
tnaA cloning R	CGCGGCGTCGTCTAGAGCTAACATCCTTATAGCCACTCTG
Rescue plasmids	
pZA21G	Kan^R; $P_{ltetO-1}$-GFP
pZA21-*tnaA*	derivative of pZA21G; *tnaA* gene cassette replaces GFP (KpnI/HindIII)
qPCR primers	
LT2 pspA F	AGATCGAGTGGCAGGAAAAAG
LT2 pspA Reverse	GTGACTTCCTGTTCAAGCGTAG
LT2 pspE F	CAGACAGGAACGATACGGTAAA
LT2 pspE Reverse	ACTGATACCGCCCATATTCATC
LT2 ahpC F	CACAGCAGCTCTGAAACTATCG
LT2 ahpC Reverse	CTTCATCTTCACGCATGTTGTC
LT2 ahpF F	GAACGAAATCACCGAACGTAAC
LT2 ahpF Reverse	CCAGTATCCACTTTAGCGACAA

Table S1. Cont.

Strains and primers	Genotype or sequence
LT2 trxC F	ATCTTCCAGTGGTGATCGACTT
LT2 trxC Reverse	CTTTGACGAAACGGACTTTACC
LT2 dps F	CACTGACCGATCATCTGGATAC
LT2 dps Reverse	CGGATAGCTTTTCAGTGGAGTT
LT2 katG F	GATCCGGAGTTCGAGAAGATTT
LT2 katG Reverse	GCTTTTGGTCCCATATCTCTGT
LT2 napF F	GGATCTGGTTTTTACGCTCAC
LT2 napF Reverse	GATAACGTGGGACGAAAGGTAA
LT2 invF F	GAATGCTGGGAGAAGACTATGG
LT2 invF Reverse	ACGCCAGTTTCGTAATTCACTC
LT2 ramA F	CACGATTGTCGAGTGGATTG
LT2 ramA Reverse	CCAGACTCTCCCCTTTGTACTG
LT2 gmk F	CACGGTGAGCACTATTTCTTTG
LT2 gmk Reverse	AGTGCCGTAGTAATTGCCAAAC
LT2 gyrB F	AGCGATGGATCAGTACCAGATT
LT2 gyrB Reverse	TGGCGTTATATTCAGAGACCAG

AbR, antibiotic resistance; F/R, forward/reverse; inv, invasion protein; KmR, kanamycin resistance; lacR, lactose repressor; mtr, tryptophan/indole:H+ symporter; nap, nitrate reductase; ramA, resistance antibiotic multiple; Sp^R, spectinomycin resistance; tetR, tetracycline repressor; trx, thioredoxin.