Supporting Information

Blair et al. 10.1073/pnas.1419939112

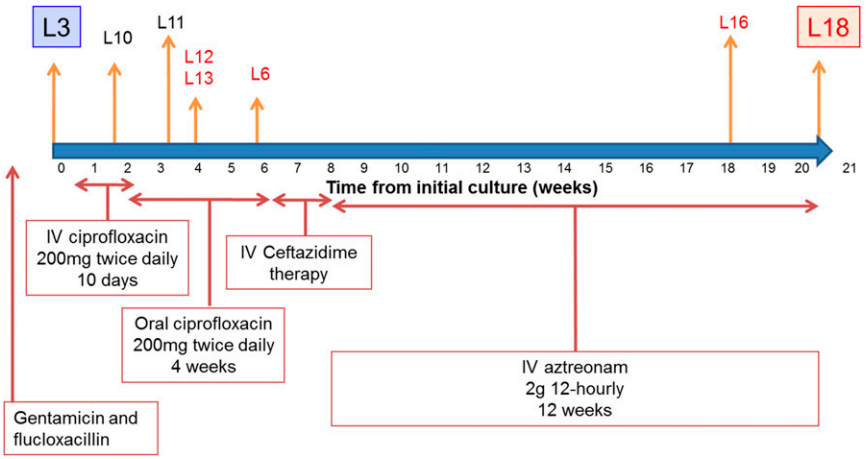

Fig. S1. Clinical timeline showing time of isolation. Orange arrows indicate time of isolation. Treatment regimen and additional diagnoses shown below the timeline. Isolate numbers in black have G288 and those in red have the G288D substitution. The ciprofloxacin doses shown were those recommended at the time of isolation (15). However, the British National Formulary now recommends 500 mg rising to 750 mg orally or 400 mg over 60 min, every 8–12 h if administered intravenously.

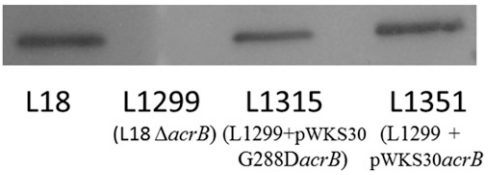

Fig. S2. Western Blot for AcrB showing that the strains L1315 and L1351 carrying wild-type and mutant *acrB* respectively, produce similar amounts of the AcrB protein.

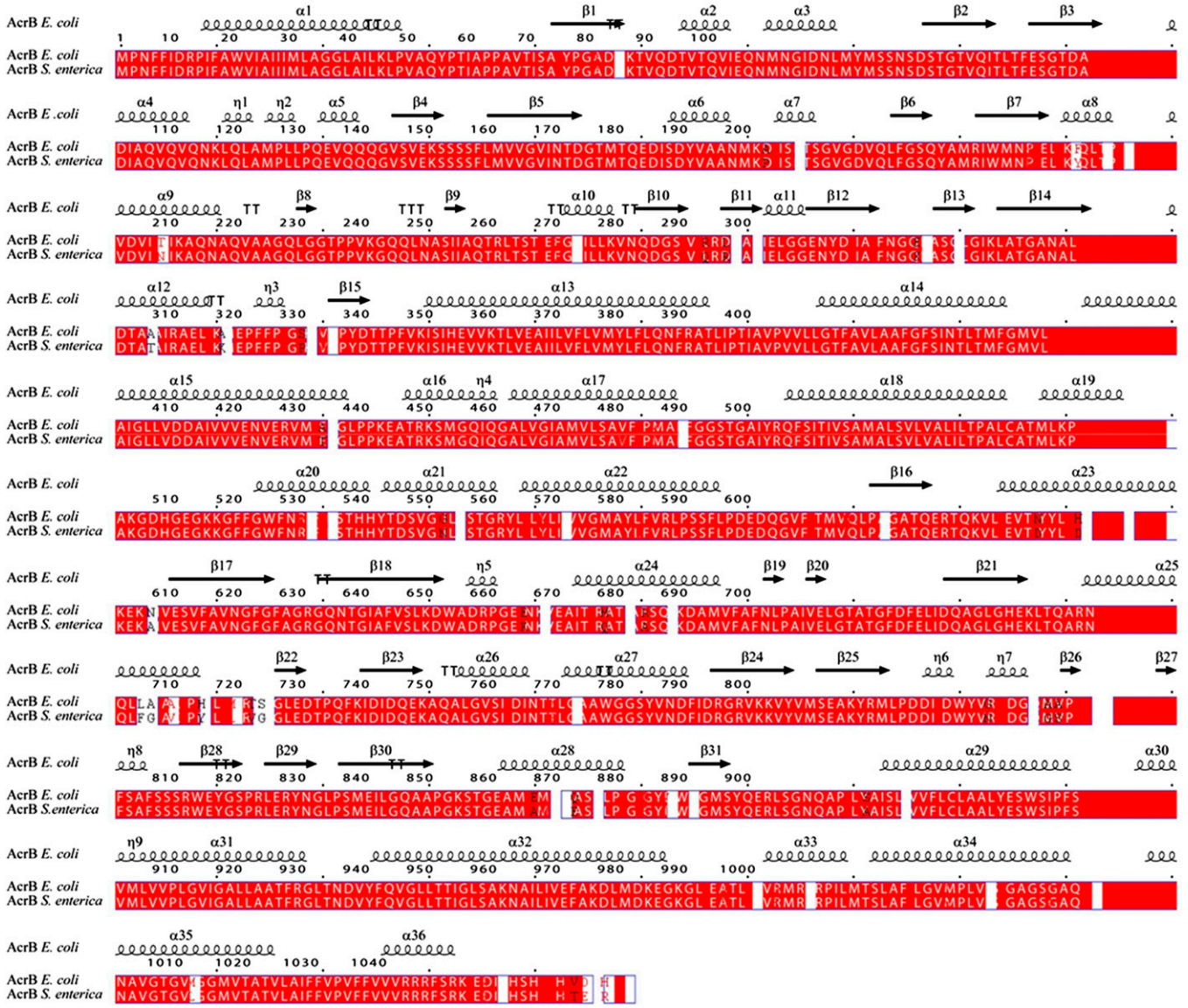

Fig. S3. Structural alignment of AcrB from *Salmonella enterica* and *Escherichia coli* based on the *E. coli* structure as in PDB ID code 1IWG demonstrates the high level of sequence conservation between the two species and the lack of gaps in the alignment, allowing for unambiguous assignment of the corresponding residues in the drug-binding pocket.

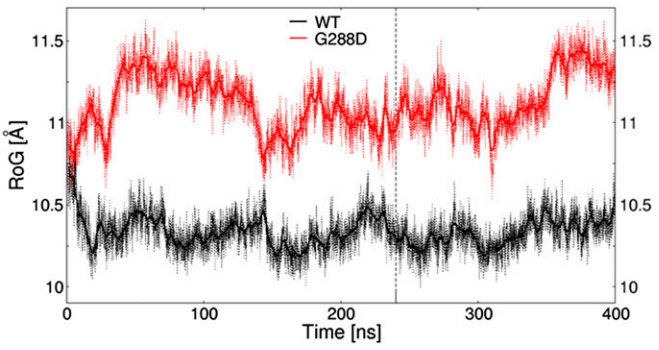

Fig. S4. Evolution of the distal pocket's radius of gyration along the 400-ns-long MD simulations of the wild-type (dotted black line) and G288D (dotted red line) variant of AcrB. Running averages over 100 frames are shown as bolded lines.

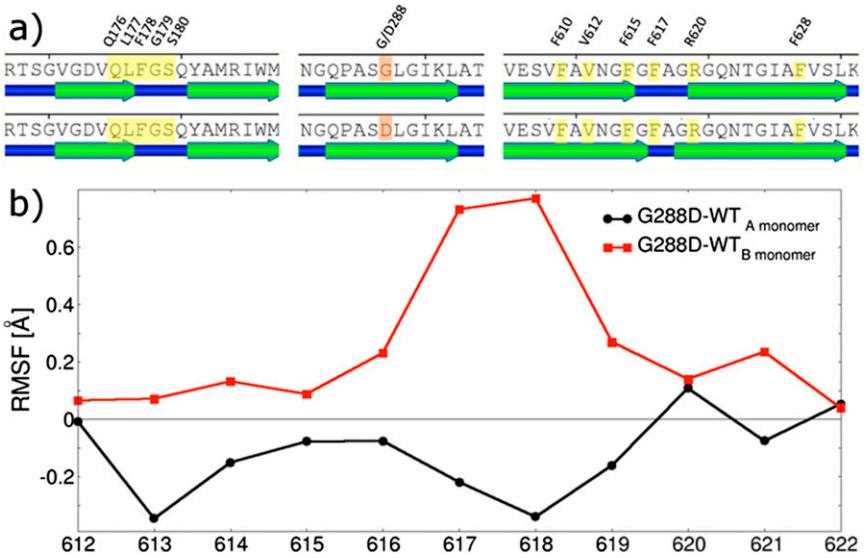

Fig. S5. (*A*) Schematic diagrams of the secondary structure of the distal drug-binding pocket (calculated on the closest-to-average structure extracted from MD simulations) in wild-type (upper diagram) and G288D mutant (lower diagram) of AcrB. Secondary structure elements are numbered according to Murakami et al. (1). (*B*) The difference between the RMSFs of the switch loop in G288D mutant and wild-type AcrB for monomers in the access (A, black curve) and binding (B, red curve) configurations. A and B refer to the configurations that the monomers are supposed to assume during the proposed functional rotation.

1. Murakami S, Nakashima R, Yamashita E, Matsumoto T, Yamaguchi A (2006) Crystal structures of a multidrug transporter reveal a functionally rotating mechanism. *Nature* 443(7108): 173–179.

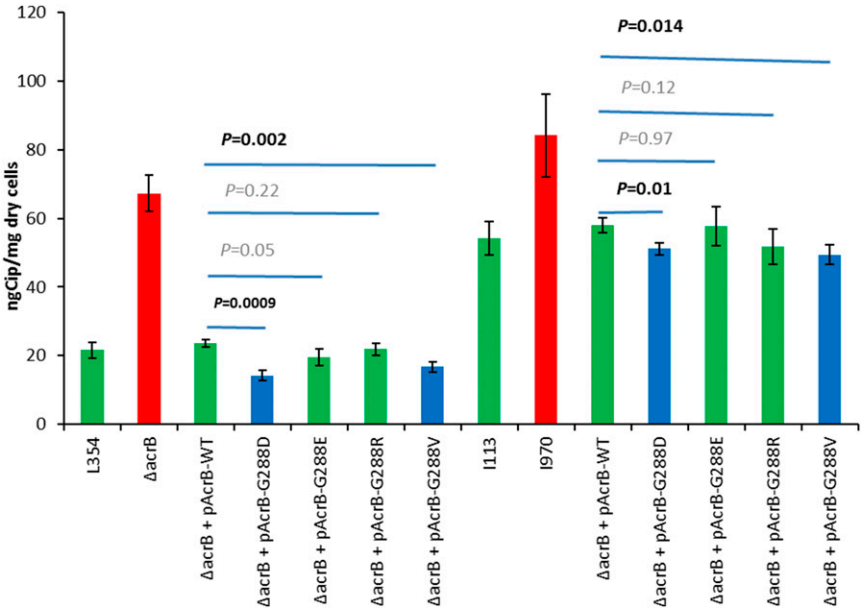

Fig. S6. Accumulation of 10 μg/mL of ciprofloxacin by *Salmonella* Typhimurium SL1344, *E. coli* MG1655, and respective *acrB* mutants. Data presented are the mean of the steady-state values for three biological replicates ± SD.

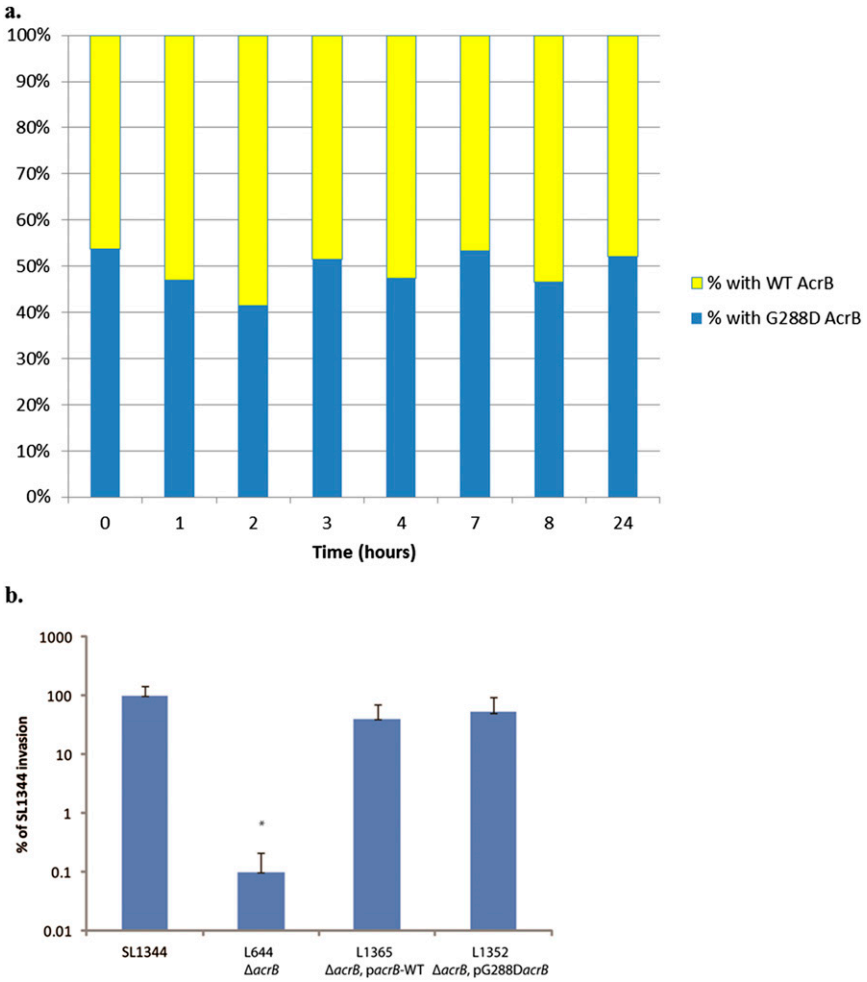

Fig. S7. (*A*) Growth of *Salmonella* with G288D in competition with *Salmonella* with wild-type AcrB. Strains were grown in LB broth at 37 °C with aeration for 24 h. Data presented as the percentage of the population being represented by each genotype at the given time point. (*B*) Invasion of *Salmonella* strains into human intestinal cells (INT-407). Data shown is the mean of at least three independent experiments. Student's *t* tests were used to compare values for each strain with that of the wild type, SL1344. *P* values of ≤0.05 were considered significant and are indicated by asterisks.

Table S1. SNPs identified in L18 that are not in L3

Mutations found in L18 vs. LT2, not found in L3 vs. LT2

Position (LT2 genome coordinates)	LT2/L3 sequence	L18 sequence	Quality score	Read depth covering position	Mutation type	Gene affected	Amino acid change
531534	C	T	222	157	Nonsynonymous	*acrB*	Gly288Asp
1935663	G	A	222	98	Nonsynonymous	*cspC*	His32Tyr
2670530	G	A	222	165	Nonsynonymous	STM2532	Thr723Met
3440083	C	A	222	148	Intergenic	—	—
4039879	G	T	222	187	Nonsynonymous	*gyrB*	Ser464Tyr

Table S2. MICs of 10 additional antimicrobials for the clinical isolates

Strain	Weeks posttherapy	MIC, μg/mL										
		Nor*	Enx*	Ofx*	Fox*	Amc†	Ery†	Rif†	Nov†	PmB	Bile	*acrB* expression
L3	0	0.06	0.5	<0.06	1	1	2	16	32	4	2,048	1
L10	1	0.5	1	0.5	16	4	512	32	512	4	2,048	2.07
L11*	3	0.25	4	0.5	4	—	—	—	—	4	2,048	2.07
L12	3	2	4	2	16	4	512	16	256	4	2,048	2.10
L13*	3	0.25	1	0.06	1	—	—	—	—	4	2,048	1.77
L6	5	1	4	1	16	4	512	16	256	4	2,048	1.75
L16	17	1	1	0.12	1	—	—	—	—	4	2,048	2.20
L18†	19	2	4	2	32	16	32	16	32	4	2,048	2.57

Expression of *acrB* was determined using a GFP reporter fused to the promoter of *acrAB*. Amc, ampicillin; Bile, bile salts; Enx, enoxacin; Ery, erythromycin; Fox, cefoxitin; Nor, norfloxacin; Nov, novobiocin; Ofx, ofloxacin; Rif, rifampin; PmB, polymyxin B.
*From ref. 1.
†From ref. 2.

1. Piddock LJ, Griggs DJ, Hall MC, Jin YF (1993) Ciprofloxacin resistance in clinical isolates of *Salmonella typhimurium* obtained from two patients. *Antimicrob Agents Chemother* 37(4): 662–666.
2. Griggs DJ, Gensberg K, Piddock LJ (1996) Mutations in *gyrA* gene of quinolone-resistant *Salmonella* serotypes isolated from humans and animals. *Antimicrob Agents Chemother* 40(4): 1009–1013.

Table S3. Strains used in this study

Strain	Genotype	Source
SL1344		1
L3	Pretherapy isolate	2
L10	1 wk posttherapy	2
L11	3 wk posttherapy	2
L12	3 wk posttherapy	2
L13	3 wk posttherapy	2
L6	5 wk posttherapy	2
L16	17 wk posttherapy	2
L18	19 wk posttherapy	2
L644	Δ*acrB*	3
L1365	L644 + p*acrB*-WT	This study
L1352	L644 + pG288D*acrB*	This study
L1299	L18 *acrB::aph*	This study
L1351	L1299 + p*acrB*-WT	This study
L1315	L1299 + pG288D*acrB*	This study
MG1655	*E. coli*	
I970	MG1655 Δ*acrB*	This study
I971	I970 + p*acrB*-WT	This study
I972	I970 + pG288D*acrB*	This study

1. Wray C, Sojka WJ (1978) Experimental *Salmonella typhimurium* infection in calves. *Res Vet Sci* 25(2):139–143.
2. Piddock LJV, Whale K, Wise R (1990) Quinolone resistance in *salmonella*: Clinical experience. *Lancet* 335(8703):1459.
3. Eaves DJ, Ricci V, Piddock LJV (2004) Expression of *acrB, acrF, acrD, marA,* and *soxS* in *Salmonella enterica* serovar Typhimurium: Role in multiple antibiotic resistance. *Antimicrob Agents Chemother* 48(4):1145–1150.

Table S4. Expression of *acrB* measured by RT-PCR

Strain	Genotype	Relative *acrB* expression
SL1344		1.00
L644	Δ*acrB::aph*	0.00
L1352	L644 + p*acrB*-WT	1.59
L1365	L644 + pG288D*acrB*	1.15
L3	Pretherapy isolate	1
L18	Posttherapy isolate	27.23
L1299	L18 *acrB::aph*	0.00
L1351	L1299 + p*acrB*-WT	10.12
L1315	L1299 + pG288D*acrB*	12.86

Table S5. Residence times of waters within the distal pocket of monomer B in wild-type and G288D variant of AcrB

AcrB	n_s	τ_s	n_m	τ_m	n_l	τ_l
Wild type	26.7	72.8	7.3	392	1.1	4,278
G288D	30.9	92.4	14.9	469	3.9	2,546

Number and average residence times (in picoseconds) of short- (ν_s, τ_s), medium- (ν_m, τ_m), and long- (ν_l, τ_l) residence-time waters within the distal pocket of monomer B. The values are computed by fitting the reduced survival probability $\Delta N_w(t)$ with three exponentials (1, 2).

1. Case DA, et al. (2014) *Amber 14* (Univ of California, San Francisco).
2. Gensberg K, Jin YF, Piddock LJV (1996) Corrigendum to "A novel *gyrB* mutation in a fluoroquinolone-resistant clinical isolate of *Salmonella typhimurium*". *FEMS Microbiol Lett* 137(2–3):293.

Table S6. MICS of seven antimicrobials for L18 containing either wild-type or G288D AcrB

		MIC, μg/mL						
Strain	Genotype	Cip	Lvx	Fle	Dfx	Doxy	Cef	ETBr
SL1344		0.015	0.03	0.12	0.12	4	4	>1,024
L3	Pretherapy isolate	0.008	0.008	0.03	0.03	1	0.5	256
L18	Posttherapy isolate	0.5	0.5	1	2	8	8	512
L1299	L18 *acrB::aph*	*0.015*	*0.06*	*0.25*	*0.12*	*1*	*0.5*	*64*
L1351	L1299 + p*acrB*-WT	*0.06*	*0.06*	*0.25*	*0.12*	*1*	128	256
L1315	L1299 + pG288D*acrB*	**0.25**	***0.25***	***0.5***	***0.5***	*1*	**256**	***64***

Experiments were carried out on at least three separate occasions and the mode value is presented. MIC values indicated in italic are two or more dilutions lower than for L18. MIC values indicated in bold indicate a difference between the MIC for L18 in which *acrB* has been inactivated and replaced with mutant (G288D) AcrB or wild-type AcrB. Cef, cefalothin; Dfx, difloxacin; Doxy, doxycycline; ETBr, ethidium bromide; Fle, fleroxacin; Lvx, levofloxacin.

Table S7. Selected properties of the antibiotics considered in this study

Compound	Log P*	Solubility*, mmol/l at pH 7.4	Major tautomer at pH 7.4†	MPA*, Å^2
Tet	−1.47	2.86	Zwitterionic	60.07
Cip	0.65	0.67	Zwitterionic	42.80
Chl	1.02	8.92	Zwitterionic	39.36
Nal	1.19	23.70	Charged −1	34.30
Doxo	2.82	7.99	Charged +1	67.05
Mino	−0.65	13.3	Zwitterionic	66.16

Chl, chloramphenicol; Cip, ciprofloxacin; Doxo, doxorubicin; Mino, minocycline; Nal, nalidixic acid; Tet, tetracycline.

*Calculated with the package ACD/Percepta, Physchem Suite, version 2014 (Advanced Chemistry Development, Inc; www.acdlabs.com).

†Calculated with the package Marvin 6.2.0, version 2014 (ChemAxon; www.chemaxon.com).