Supporting Information

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NANG



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.

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L18	L1299	L1315	L1351
	(L18 ∆ <i>acrB</i>)	(L1299+pWKS3 G288DacrB)	0 (L1299 + pWKS30acrE

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.

AcrB E. coli	مع مومومومومو	000000000	β1 Τ	a2 a	3 1000 - I	32 β3 Q
AcrB E. coli AcrB S. enterica	MPNFFIDRPIFAWVIAIIIMLA MPNFFIDRPIFAWVIAIIIMLA	GGLAILKLPVAQYPTIAPPA	AVTISA YPGAD KTVQ AVTISA YPGAD KTVQ	DTVTQVIEQNMNG DTVTQVIEQNMNG	IDNLMYMSSNSDSTG IDNLMYMSSNSDSTG	TVQITLTFESGTDA TVQITLTFESGTDA
AcrB E .coli	α4 ηl η2 0000000 <u>000 000</u>	α5 β4	β5	α6 α7 000000 000	ο β6	β7 α8
AcrB E. coli AcrB S. enterica	110 120 130 DIAQVQVQNKLQLAMPLLPQ	140 150 160 EVQQQGVSVEKSSSSFLM	170 180 1 VVGVINTDGTMTQEDI	90 200 SDYVAANMKD IS	ISGVGDVQLFGSQYA	
AcrB E coli	α9 β8	μ. β9		β11 α11	β12 β1	3 β14
AcrB E. coli	210 220 230	240 250 260 GTPPVKGQQLNASIIAQTRI	270 280 2	SV TRD A IELGO	ENYD IA FNGQ ASC	GIKLATGANAL
AcrB S. enterica	<u>VDVI</u> Ν ΙΚΑQΝΑQVAAGQLG α12 η3	GTPPVKGQQLNASIIAQTRL β15	LTST EFG ILLKVNQDO α13	SS V LRD A TELGO	<u>εννό ia fngo</u> aso α14	LGIKLATGANAL
AcrB E. coli AcrB E. coli	000000000TT 000 310 320 330	340 350 360	370 380 3	0000 90 400 NERATLIPTIAVPVV		
AcrB S. enterica	DTATAIRAEL K EPFFP G	V PYDTTPFVKISIHEVVKT	LVEAIILVFLVMYLFLQ	NFRATLIPTIAVPVV	LLGTFAVLAAFGFSIN	TLTMFGMVL
AcrB E. coli	α15 000000000000000000000000 410 420 430	α16 η4 000 00000000 440 450 460	<u>470</u> 480 4	و وو 190 500	α18	
AcrB E. coli AcrB S. enterica	AIGLLVDDAIVVVENVERVM AIGLLVDDAIVVVENVERVM	E GLPPKEATRKSMGQIQG E GLPPKEATRKSMGQIQG	ALVGIAMVLSAVF PM ALVGIAMVLSAVF PM	A FGGSTGAIYRQF A FGGSTGAIYRQF	SITIVSAMALSVLVALI SITIVSAMALSVLVALI	LTPALCATMLKP LTPALCATMLKP
AcrB E. coli	α2(000000 510 520 530	α21 00000 000000000 540 550 560	α22 00000000000000 570 580 5	00000 90 600	β16	α23 202020202020202020
AcrB E. coli AcrB S. enterica	AKGDHGEGKKGFFGWFNR AKGDHGEGKKGFFGWFNR	STHHYTDSVG IL STGR	YL LYLI /VGMAYLFVR YL LYLI /VGMAYLFVR	RLPSSFLPDEDQGVF	F TMVQLP, GATQERT F TMVQLP, GATQERT	QKVL EVT YYL E QKVL EVT YYL
AcrB E. coli	β17	β18 η5 000	α24 202020200	<u>00000</u> β19	β20	β21 α25
AcrB E. coli AcrB S. enterica	KEKN VESVFAVNGFGFAGRO	SQNTGIAFVSLKDWADRPG SQNTGIAFVSLKDWADRPG	ENH PEAIT RAT LESO	KDAMVFAFNLPAI KDAMVFAFNLPAI	VELGTATGFDFELIDO	AGLGHEKLTQARN AGLGHEKLTQARN
AcrB E. coli	<u> </u>	β23 α26 ΤΤ <u>00000</u>	α27	α β24	β25 η6	$\eta^7 \qquad \beta 26 \qquad \beta 27$
AcrB E. coli AcrB S. enterica	QLLA A PH L FRS GLED QLFG A PY L RTG GLED	740 750 760 PQFKIDIDQEKAQALGVSI PQFKIDIDQEKAQALGVSI	770 780 7 DINTTLC AAWGGSYVI DINTTLC AAWGGSYVI	90 800 NDFIDRGRVKKVYV NDFIDRGRVKKVYV	MSEAKYRMLPDDI DV MSEAKYRMLPDDI DV	WYVR DG MVP WYVR DG MVP
AcrB E. coli	η8 β28 β29	β30	α28	β31	0000000	α29 α30 00000000000000000000000000000000000
AcrB E. coli	810 820 830 FSAFSSSRWEYGSPRLERYNO	840 850 860	870 880 8	90 900 W GMSYQERLSG	NOAP LYAISL VVFLC	
AarP E coli	η9 α31	SLPSMEILGQAAPGRSTGEA	α32		33 C	x34
AcrB E. coli	910 920 930 VMLVVPLGVIGALLAATFRG	940 950 960 TNDVYFQVGLLTTIGLSAK	970 980 9 NAILIVEFAKDLMDKE	90 1000 SKGL EATL VRMR	RPILMTSLAF LGVM	PLV GAGSGAQ
AcrB S. enterica	VMLVVPLGVIGALLAATFRGI	TNDVYFQVGLLTTIGLSAK	NAILIVEFAKDLMDKEC	GKGL EATL VRMR	RPILMTSLAF LGVM	PLVSGAGSGAQ
AcrB E. coli	000000000000000000000000000000000000000	. 1040				
AcrB E. coli AcrB S. enterica	NAVGTGVI 5GMVTATVLAIFI NAVGTGVI 5GMVTATVLAIFI	FVPVFFVVVRRRFSRK EDI FVPVFFVVVRRRFSRK EDI				

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.

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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 μ g/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 \pm SD.





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

DN A C

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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	С	Т	222	157	Nonsynonymous	acrB	Gly288Asp
1935663	G	А	222	98	Nonsynonymous	cspC	His32Tyr
2670530	G	А	222	165	Nonsynonymous	STM2532	Thr723Met
3440083	С	А	222	148	Intergenic	_	_
4039879	G	Т	222	187	Nonsynonymous	gyrB	Ser464Tyr

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

MIC,	μg/mL
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Strain	Weeks posttherapy	Nor*	Enx*	Ofx*	Fox*	Amc^{\dagger}	$\operatorname{Ery}^{\dagger}$	Rif^\dagger	Nov^{\dagger}	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
$L18^{\dagger}$	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.

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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	∆acrB	3	
L1365	L644 + p <i>acrB</i> -WT	This study	
L1352	L644 + pG288D <i>acrB</i>	This study	
L1299	L18 acrB::aph	This study	
L1351	L1299 + p <i>acrB</i> -WT	This study	
L1315	L1299 + pG288D <i>acrB</i>	This study	
MG1655	E. coli		
1970	MG1655 ∆acrB	This study	
1971	1970 + p <i>acrB</i> -WT	This study	
1972	l970 + pG288D <i>acrB</i>	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.

Strain	Genotype	Relative <i>acrB</i> expression
SL1344		1.00
L644	∆acrB::aph	0.00
L1352	L644 + p <i>acrB</i> -WT	1.59
L1365	L644 + pG288DacrB	1.15
L3	Pretherapy isolate	1
L18	Posttherapy isolate	27.23
L1299	L18 acrB::aph	0.00
L1351	L1299 + p <i>acrB-</i> WT	10.12
L1315	L1299 + pG288D <i>acrB</i>	12.86

Table S4. Expression of acrB measured by RT-PCR

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

AcrB	ns	τ_{s}	n _m	τ_{m}	nı	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).

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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 <i>acrB</i> -WT	0.06	0.06	0.25	0.12	1	128	256
L1315	L1299 + pG288D <i>acrB</i>	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 †				

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).

MPA*, Å²