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Chromosomal Mutations that Accompany *qnr* in Clinical Isolates of *Escherichia coli*

Laura Vinué[a,*], **David C. Hooper**[a], and **George A. Jacoby**[b]

[a]Massachusetts General Hospital, Boston, Massachusetts, USA

[b]Lahey Hospital and Medical Center, Burlington, Massachusetts, USA

Abstract

We examined 13 *qnr*-positive and 14 *qnr*-negative clinical isolates of *Escherichia coli* for mutations previously seen in a *qnr*-containing laboratory strain exposed to supra MIC concentrations of ciprofloxacin. Among the *qnr*-positive strains, those with ciprofloxacin MICs of ≥ 2 μg/ml had at least one mutation in *gyrA*. Mutations in *parC* were present in strains with a ciprofloxacin MIC of ≥128 μg/ml. The 6 most ciprofloxacin-resistant strains contained additional plasmid-mediated quinolone resistance determinants. *aac(6')-Ib-cr* was found in 5 of the 6 strains. Eleven of the 13 strains had alterations in MarR, 9 in SoxR, and 5 had mutations in AcrR, All had elevated expression of at least one efflux pump gene, predominantly *acrA* (92% of the strains), followed by *mdtE* (54%) and *ydhE* (46%). Nine had functionally silent alterations in *rfa*, 2 had mutations in *gmhB*, and one of these also a mutation in *surA*. An *E. coli* with ciprofloxacin MIC of 1024 μg/ml contained 4 different plasmid-mediated quinolone resistance determinants as well as *gyrA*, *parC*, *parE* and pump overexpression mutations. Nine of the 14 *qnr*-negative strains had mutations in topoisomerase genes with the ciprofloxacin MIC starting at 0.25 μg/ml and reaching 256 μg/ml. The three most resistant strains also had mutations in *parE*. Twelve had alterations in MarR, 10 in SoxR and 5 in AcrR. Ten of the 14 strains had elevated expression of efflux pumps with *acrA* (71.4%), followed by *ydhE* (50%) and *mdtE* (14.3%). A diversity of resistance mechanisms occurs in clinical isolates with and without *qnr* genes.

Keywords

E. coli; quinolones; *qnr*; mutations; clinical isolates

[*]Corresponding author. Division of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts 02114, USA, Phone: +1 (617) 724-9066, lvinue-santolalla@mgh.harvard.edu.

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1. Introduction

Plasmid-mediated *qnr* genes are often found in clinical isolates with mutations in the quinolone resistance determining region of type II topoisomerase genes [[1], [2], [3], [4]], providing an additive effect on resistance [[5]]. Hence, it was unexpected that *gyrA* mutations were absent or very rare when mutants with higher levels of ciprofloxacin resistance were selected from a *qnr*-containing strain of *E. coli* [[6], [7], [8]]. Selected instead are mutations in *marR* and *soxS* regulator genes that increase expression of the AcrAB efflux pump, increased expression of MdtEF and YdhE efflux pumps, and mutations in genes for inner core lipopolysacharide (LPS) synthesis. The LPS defects also cause reduced stability of the outer cell membrane and hypersensitivity to hydrophobic antibiotics such as novobiocin. How commonly such mutations accompany *qnr* in clinical isolates is not yet known. We examined a set of clinical *E. coli* strains containing *qnrA, qnrB*, and *qnrS* alleles for these associated mutations in comparison to their occurrence in control strains lacking *qnr* genes.

2. Materials and methods

2.1 Bacterial strains and susceptibility testing

Twenty-seven clinical *E. coli* isolates previously characterized for plasmid-mediated quinolone resistance genes were evaluated. Thirteen were *qnr*-positive and 14 were *qnr*-negative [[9], [9], [11], [12]]. The *qnr*-negative strains were collected at Seoul National University Hospital (South Korea) in the same time periods, in the intervals from 1998 to 2006, as *qnr*-positive strains (6–42, 6–75, 3–41, 3–48, 6–74). Other *qnr*-positive strains (4, 7, 10, 12, 29 and 76) came from a hospital in Shanghai (China) between March 2000 and March 2001. Each strain was from a different patient and had a unique plasmid profile [[9]]. *E.* coli J53 Azi^R [[13]] was used as a recipient in outcrosses with 100 μg/ml of sodium azide for counterselection plus either ciprofloxacin (0.5 μg/ml), ampicillin (100 μg/ml), kanamycin (25 μg/ml) or chloramphenicol (32 μg/ml). Plasmid DNAs from the resulting outcrosses were isolated with the Qiagen Plasmid MIDI kit (Qiagen, Valencia, CA).

MICs of ciprofloxacin and novobiocin (Sigma-Aldrich) were determined by agar dilution on Mueller-Hinton agar at 37°C with an inoculum of ~10^4 CFU following CLSI guidelines [[14]]. Susceptibility testing to 13 antimicrobial agents (amikacin, ampicillin, cefepime, cefotaxime, cefotetan, ceftazidime, chloramphenicol, gentamicin, kanamycin, streptomycin, tetracycline, tobramycin and trimethoprim-sulfamethoxazole (BD diagnostics) was performed for all strains by disk diffusion [[14]].

2.2 PCR and DNA sequencing

We amplified by PCR and sequenced the quinolone-resistance determining regions (QRDRs) of *gyrA, gyrB, parC* and *parE* genes, other plasmid-mediated quinolone resistance genes not previously studied in these strains, such as *aac(6')Ib-cr*, *oxqAB*, *qepA1*, regulator genes such as *acrR, marR, soxR,* lipopolysaccharide biosynthesis genes *rfaD*, *rfaE, rfaF* and *gmhB,* and other genes *surA* and *rpoB*. DNA templates were prepared by boiling, and the primers used were described previously [[8], [12], [15], [16], [17]]. We used the Maxima

Hot Start PCR Master Mix (Thermo Scientific, Waltham, MA) in a final volume of 50 μl. PCR products were purified using a PCR purification kit (Qiagen, Valencia, CA) and sent for sequencing by the Tufts University Core Facility, Boston, MA.

2.3 Relative expression of genes encoding efflux pumps and regulators

Reverse transcription followed by real-time quantitative PCR (RT-qPCR) was used to determine the expression levels of selected efflux pump genes, including *acrA, mdtE, ydhE,* and regulators such as *marA* and *soxS.* Comparison was made to expression of the housekeeping gene *mdh*. Primers used and specifications of RNA extraction and generation of cDNA were as previously described [[8]]. At least three different assays with three different RNA extractions were performed for each gene tested. *E. coli* J53 Azi^R was used as a reference to calculate relative expression.

2.4 Site-directed point mutations in RfaF in the *E. coli* chromosome

To evaluate the significance of alterations found in RfaF, we introduced single point mutations in the chromosomal *rfaF* gene of *E. coli* HS996 by site-directed mutagenesis using the Red®/ET® recombination system (Gene Bridges, Heidelberg). For verification of the correct *rfaF* mutations, PCR amplification and sequencing was employed as described previously [[8]].

3. Results

Thirteen *qnr*-positive and 14 *qnr*-negative clinical *E. coli* isolates were studied to determine if they contained gene mutations found in an earlier study evaluating events after selection of J53 pMG252 (*qnrA1*) mutants with increased ciprofloxacin resistance [[8]].

3.1 Susceptibility

Overall *qnr*-positive strains were more resistant to antimicrobial agents (besides ciprofloxacin and novobiocin) than *qnr*-negative strains (Supplementary table). Twelve of 13 *qnr*-positive strains were multiresistant (resistant to three or more classes of antimicrobials), while only 7 of 14 of the *qnr*-negative strains were multiresistant, and two *qnr*-negative strains were pansusceptible.

3.2 Quinolone resistance determinants

By themselves *qnr* genes provide a modest loss of ciprofloxacin susceptibility with MICs in *E. coli* of 0.25 – 0.5 μg/ml compared to an MIC of ~0.010 μg/ml for a fully susceptible strain. The ciprofloxacin MIC in the clinical strains containing *qnr* genes ranged from 0.125 to 1024 μg/ml (Table 1). Strains with ciprofloxacin MICs of 2 μg/ml and above had at least one mutation in *gyrA* as did one strain with an MIC of 0.5 μg/ml. Mutations in *parC* were present in strains with a ciprofloxacin MIC of at least 128 μg/ml. The six most ciprofloxacin-resistant strains contained additional plasmid-mediated quinolone resistance (PMQR) determinants. In particular, *aac(6')-Ib-cr* was found in 5 of the 6 strains. Strain 76 was remarkable for a ciprofloxacin MIC of 1024 μg/ml. It contained *qnrA1, aac(6')-Ib-cr, oqxAB,* and *qepA1* in addition to mutations in *gyrA, parC* and *parE* genes. On outcross from this strain to *E. coli* J53 single transconjugants contained only *oqxAB* or *qepA1,* while

transconjugants with all four PMQR genes located in a single plasmid had a ciprofloxacin MIC of 3–4 μg/ml. Nine of the 14 *qnr*-negative strains had mutations in topoisomerase genes, with the ciprofloxacin MIC reaching 256 μg/ml in a strain with *qepA*1. Another *qnr*-negative strain contained *oqxAB*. Interestingly, the 3 most resistant strains showed different mutations in ParE (D420N, S458A, and S458T). Mutations in the QRDR of GyrB were not detected in any strain.

3.3 Expression of efflux pumps and mutations in regulators

Eleven of the 13 *qnr*-positive strains had alterations in MarR, 9 had alterations in SoxR, and 5 had mutations in AcrR, using the *E. coli* K-12 strain MG1655 sequence for comparison (GenBank accession number U00096.3).

Among the MarR alterations, the S3N, G103S, Y137H amino acid changes are known not to affect repressor activity [[18]]. The remaining K62R mutation has been identified before in clinical isolates but showed no evidence of contributing to organic solvent tolerance, suggesting that it also is a silent mutation with regard to resistance [[19]]. Four *qnr*-positive strains had 2.4- to 8.5-fold increased *marA* expression without evident MarR alterations, suggesting a role for additional regulators. The T38S and G74R changes in SoxR have been previously identified in *E. coli* clinical isolates from Spain that had increased basal *soxS* expression [[20]]. However, no consistent increase in *soxS* expression was seen in our strains with these mutations. The SoxR A111T alteration observed in strain 76 also did not elevate *soxS* transcript levels, confirming earlier observations [[20]]. Five of the 10 mutations detected in AcrR caused frameshifts and were associated with 2.1- to 11.9 -fold increased expression of *acrA*. All the *qnr*-positive strains had elevated expression of at least one efflux pump gene, predominantly *acrA* (92% of the strains) followed by *mdtE* (54%) and *ydhE* (46%).

Similar mutations were found in the *qnr*-negative clinical *E. coli* isolates. Twelve had alterations in MarR, 10 in SoxR, and 5 in AcrR. One of the amino acid changes in MarR (D76G), one in SoxR (I140V), and 4 in AcrR (I113V, T213I, N214T and T32P) were not seen in the *qnr*-positive strains. Ten of the 14 strains had elevated expression of efflux pump genes with *acrA* (71.4%) predominating, followed by *ydhE* (50%) and *mdtE* (14.3%).

Recently, Pietsch *et al.*, [[15]] identified mutations in *rpoB*, the gene coding for the β-subunit of RNA polymerase, as novel contributors to ciprofloxacin resistance via increased expression of the *ydhE* (also known as *mdtK*) efflux pump gene. We sequenced the entire *rpoB* gene for all strains with and without increase *ydhE* expression, but detected no *rpoB* mutations.

3.4. LPS defects and novobiocin susceptibility

E. coli mutants lacking heptose in the LPS core display a variety of phenotypes due to the reduced stability of the outer membrane, including hypersensitivity to hydrophobic antibiotics such as novobiocin. In our strains the novobiocin MICs ranged from 40 to 320 μg/ml with somewhat lower values than previously seen in laboratory strains [[8]]. In an attempt to find out if the strains had defects on the LPS pathway we sequenced *rfaD, rfaE, rfaF* and *gmhB* genes and using the *E. coli* K-12 strain MG1655 sequence for comparison.

Two *qnr*-negative strains had a RfaE (A245T) mutation, 9 of 13 *qnr*-positive strains had alterations in Rfa as did all the *qnr*-negative strains, and 2 *qnr*-positive strains had 2 mutations in GmhB. (Table 3).

Defects in these genes are known to cause novobiocin susceptibility, but the specific mutations we observed have not been published. We created point mutations (I136V and A141S) in the *rfaF* gene of *E. coli* HS996 by site-directed mutagenesis. The MICs for ciprofloxacin (0.008 μg/ml) and novobiocin (640 μg/ml) were unchanged in the mutants indicating that the I36V and A141S changes are functionally silent.

Two *qnr*-positive strains had frameshift mutations, along with point mutations, in the *gmhB* gene, and one of these strains also had a mutation in *surA*, but novobiocin hypersensitivity was not observed (Table 3). The reason to sequence the *surA* gene was that SurA is the primary periplasmic molecular chaperone that facilitates the folding and assembling of outer membrane proteins in Gram-negative bacteria, and SurA-deficient cells are more susceptible to hydrophobic drugs [[21]].

4. Discussion

Many studies have evaluated the *gyrA* and *parC* mutations that accompany *qnr* in clinical isolates [[1], [2], [3], [4], [22], [23], [24], [25]] and a few investigations have examined expression of efflux genes in quinolone-resistant strains without *qnr* [[26], [27], [28], [29]], but this study is the first to evaluate both topoisomerase mutations and pump expression in *qnr*-containing clinical isolates.

The strains were selected by the presence or absence of *qnr* genes, but other PMQR genes were also evaluated, with the finding that *aac(6')Ib-cr* was present in 5 of 13 *qnr*-positive but none of the *qnr*-negative strains. In both groups non-*qnr* PMQR determinants were found in the most ciprofloxacin-resistant isolates and were associated with mutations in GyrA and ParC. Interestingly, three different mutations in ParE were also detected in this study, only one of which (D420N) was in a known QRDR region [[17]]. The D420N mutation has been described to increase the ciprofloxacin MIC in *Vibrio cholerae,* [[30]] but to our knowledge, this is the first time that it has been found in *E. coli*. The ParE S458A and S458T mutations have been described before, and the S458A alteration has been associated with high levels of resistance to fluoroquinolones in *E. coli* [[31], [32], [33]]. Such topoisomerase mutations were the main contributors to the high ciprofloxacin MICs of the clinical strains. Outcross of the plasmid from *E. coli* strain 76 with a ciprofloxacin MIC of 1024 μg/ml produced a transconjugant with a ciprofloxacin MIC of only 4 μg/ml, indicating the contribution of chromosomal mutations to the high level ciprofloxacin resistance observed. The plasmid carried *qnrA1*, *aac(6')-Ib-cr, oqxAB*, and *qepA1* and thus demonstrated that a strain with the combination of four PMQR genes can reach the current CLSI breakpoint for ciprofloxacin resistance (≥ 4μg/ml) in the absence of topoisomerase mutations or efflux pump overexpression.

Eleven of the *qnr*-negative strains had elevated *marA* expression, but only 4 of the *qnr*-positive strains; in both sets of strains, AcrA was the predominant overexpressed efflux

pump. Increased MarA levels not only increase ciprofloxacin MICs but also protect bacteria from the bactericidal effect of the fluoroquinolones [[34]]. Most of the mutations found in *marR* in this study were already published as not contributing to resistance. Marcusson *et al.*, [[35]] reported that mutations in *acrR* and *marR* were associated with a significant fitness cost. Therefore, increased AcrA levels might have arisen to take advantage of MarA–regulated bactericidal protection by mechanisms other than alteration in MarR or AcrR because of their associated cost. Existing data also support a model in which AcrA plays a role in quorum sensing by emitting quorum-sensing signals, so it is also possible that clinical strains increase levels of AcrA because doing so confers a fitness advantage not directly related to drug efflux [[26]].

There were only 3 *qnr*-negative strains that did not overexpress any efflux pump studied (their ciprofloxacin MICs ranged from 0.06 to 1 μg/ml). The rest of the *qnr*-negative and *qnr*-positive strains overexpressed *acrA*, *mdtE*, and *ydhE* separately or in combination. It has been seen before that overexpression of each of the three pumps separately resulted in roughly similar levels of quinolone resistance, whereas simultaneous overexpression of *mdtE* or *ydhE* in combination with *acrA* gave synergistic increases in quinolone resistance [[28], [29]]. In our data, there was only one *qnr*-positive strain that overexpressed *mdtE* by itself (ciprofloxacin MIC of 0.5 μg/ml) and only one *qnr*-negative strain that overexpressed the single pump *ydhE* but had a borderline increase in expression of *acrA* (a 1.9-fold change). This finding suggests that all the combinations of expression of the three efflux pumps contribute to the different levels of resistance detected.

Regarding the LPS defects and novobiocin susceptibility, we identified several new mutations in *rfaE*, *rfaF* and *gmhB* genes. Heptose biosynthesis in *E. coli* is a process which involves several enzymes (GmhA, RfaE, GmhB and RfaD) that act in different steps in the pathway. Interruption of biosynthesis or transport of heptose causes a heptose-less phenotype called “deep-rough” that is unstable and leads to increase susceptibility to hydrophobic compounds like novobiocin [[36]]. We recreated the most frequent point mutations detected (I136V and A141S in the *rfaF* gene) but found no associated phenotype. A stop codon was also detected in *gmhB* in two strains that nonetheless lacked novobiocin hypersensitivity. In a laboratory strain of *E. coli* deletion of *gmhB* encoding D-α,β-D-heptose 1,7-bisphosphate phosphatase did not confer a complete heptose-less LPS core phenotype, suggesting the presence of another as yet unidentified phosphatase activity that can partially compensate in the synthesis of a complete core [[37]].

5. Conclusions

Many *qnr*-containing clinical isolates of *E. coli* had in addition to topoisomerase mutations increased expression of efflux pump genes, especially *acrA*. Similar efflux gene overexpression was also seen in control strains lacking *qnr*. Disabling mutations in core LPS synthesis, such as were detected on selecting more ciprofloxacin-resistant derivatives from a *qnr*-containing laboratory strain, were not found in the clinical isolates. Other PMQR genes often accompanied *qnr* in clinical strains. In one such *E. coli* strain with *gyrA*, *parC* and *parE* topoisomerase mutations, overexpression of AcrA, and four PMQR genes, the ciprofloxacin MIC reached 1024 μg/ml. Bacteria evidently have many ways to achieve

protection from a lethal agent such as fluoroquinolone, and a diversity of resistance mechanisms occurs in clinical isolates with and without *qnr* genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Elevated expression of efflux pumps accompanies topoisomerase mutations.
- AcrA is the predominant overexpressed efflux pump.
- Other PMQR genes often accompanied *qnr* in clinical strains.
- In one *E. coli* clinical strain the ciprofloxacin MIC reached 1024 μg/ml.
- A diversity of resistance mechanisms occurs in clinical isolates with and without *qnr* genes.

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Table 1

Ciprofloxacin and novobiocin MICs of *qnr*-positive and *qnr*-negative strains with the quinolone resistance determinants found.

Strains	MIC (μg/ml)		PMQR	Topoisomerase mutations[a]			Other PMQR	Reference
	Ciprofloxacin	Novobiocin		GyrA	ParC	ParE		
6–42	0.125	40	*qnrB1*	WT[b]	WT	WT	-	[12]
EC35	0.25	80	*qnrB6*	WT	WT	WT	-	[11]
UAB4	0.5	40	*qnrA1*	WT	WT	WT	-	[9]
6–75	0.5	80	*qnrB19*	WT	WT	WT	-	[12]
3–41	0.5	80	*qnrB4*	S83L	WT	WT	-	[12]
3–48	1	80	*qnrS1*	WT	WT	WT	-	[12]
6–74	2	80	*qnrS1*	S83L	WT	WT	-	[12]
4	128	160	*qnrA1*	S83L, D87N	S80I	WT	*aac(6')-Ib-cr*	[10]
29	128	160	*qnrA1*	S83L, D87N	S80I	WT	*aac(6')-Ib-cr*	[10]
7	256	80	*qnrA1*	S83L, D87N	S80I, E84G	WT	-	[10]
10	256	320	*qnrA1*	S83L, D87N	S80I	WT	*aac(6')-Ib-cr*	[10]
12	128	320	*qnrA1*	S83L, D87N	S80I	WT	*aac(6')-Ib-cr*	[10]
76	1024	40	*qnrA1*	S83L, D87N	S80I	S458A	*aac(6')-Ib-cr, qepA1, oqxAB*	[10]
3–62	0.016	320	-	WT	WT	WT	-	[12]
6–66	0.016	40	-	WT	WT	WT	-	[12]
3–25	0.03	80	-	WT	WT	WT	-	[12]
3–7	0.06	80	-	WT	WT	WT	-	[12]
3–33	0.06	80	-	WT	WT	WT	-	[12]
6–52	0.25	80	-	S83L	WT	WT	-	[12]
4–76	0.5	80	-	S83L	WT	WT	-	[12]
5–58	0.5	80	-	S83L	E84G	WT	-	[12]
5–66	1	80	-	S83L	S80I	WT	-	[12]
5–65	4	40	-	S83L	S80I	WT	-	[12]
5–81	32	80	-	S83L, D87N	S80I	WT	-	[12]
6–13	64	160	-	S83L, D87N	S80I	S458A	*oqxAB*	[12]
5–33	64	160	-	S83L, D87N	S80I	D420N	-	[12]
5–59	256	160	-	S83L, D87N	S80I	S458T	*qepA1*	[12]

[a] Mutations in *gyrB* were not detected.

[b] Wild type.

Table 2

Mutations in MarR, SoxR and AcrR and relative expression of regulators and efflux pumps in clinical strains.

Strains	Mutated protein (mutation detected)	Mean fold change in expression relative to J53 strain (SEM)[b]				
		marA	*soxS*	*acrA*	*ydhE*	*mdtE*
6–42	MarR (S3N, G103S, Y137H); SoxR (G74R)	**4.18** (1.89)	**10.30** (1.95)	**15.55** (4.18)	**5.73** (1.80)	**5.49** (1.75)
EC35	MarR (G103S, Y137H); SoxR (G74R)	1.03 (0.17)	1.26 (0.34)	**2.61** (0.47)	1.69 (0.47)	1.39 (0.43)
UAB4	MarR (S3N, G103S, Y137H); SoxR (G74R)	**2.35** (0.15)	1.47 (0.13)	1.89 (0.005)	1.44 (0.49)	**2.70** (1.01)
6–75	MarR (G103S, Y137H)	**4.08** (2.28)	**2.63** (0.82)	**3.87** (0.94)	**2.69** (0.65)	**2.35** (0.71)
3–41	-	0.86 (0.16)	1.41 (0.41)	**3.61** (0.63)	1.45 (0.54)	**2.19** (0.53)
3–48	MarR (G103S, Y137H)	1.77 (0.35)	**2.47** (0.77)	**4.20** (0.68)	**2.61** (0.63)	**2.29** (0.65)
6–74	MarR (G103S, Y137H)	**8.53** (3.65)	**4.50** (0.46)	**6.82** (1.70)	**3.30** (0.85)	**2.04** (0.47)
4	MarR (K62R, G103S, Y137H); SoxR (T38S, G74R); AcrR (L109fs[a])	0.89 (0.31)	1.67 (0.63)	**5.14** (1.55)	0.65 (0.12)	0.38 (0.13)
29	MarR (K62R, G103S, Y137H); SoxR (T38S, G74R); AcrR (L109fs)	0.63 (0.11)	**2.83** (0.50)	**9.59** (2.59)	**4.05** (0.24)	0.75 (0.09)
7	MarR (K62R, G103S, Y137H); SoxR (T38S, G74R); AcrR (E190fs)	0.53 (0.15)	1.92 (0.53)	**2.10** (0.18)	0.30 (0.18)	0.40 (0.10)
10	MarR (K62R, G103S, Y137H); SoxR (T38S, G74R); AcrR (L109fs)	0.50 (0.13)	1.83 (0.44)	**5.52** (1.14)	1.15 (0.32)	1.19 (0.53)
12	MarR (K62R, G103S, Y137H); SoxR (T38S, G74R); AcrR (L109fs)	1.75 (0.65)	**4.15** (0.14)	**11.92** (1.08)	**3.08** (0.84)	**4.38** (1.06)
76	SoxR (A111T)	0.79 (0.36)	1.01 (0.28)	**2.01** (0.50)	1.00 (0.39)	0.64 (0.15)
3–62	-	**2.59** (0.37)	**3.11** (1.10)	**8.15** (4.43)	**6.76** (3.15)	**3.62** (1.62)
6–66	MarR (3SN, G103S, Y137H); SoxR (G74R)	**19.56** (8.09)	**8.95** (3.73)	**27.20** (0.85)	**21.43** (6.05)	**2.99** (1.56)
3–25	MarR (G103S, Y137H); SoxR (T38S, G74R); AcrR (T213I, N214T)	**2.96** (0.64)	**2.88** (0.65)	**6.21** (1.93)	**6.67** (1.19)	1.57 (0.73)
3–7	MarR (K62R, G103S, Y137H); SoxR (T38S, G74R); AcrR (I113V, T213I, N214T)	**2.72** (0.06)	1.40 (0.28)	1.27 (0.70)	1.03 (0.86)	0.79 (0.15)
3–33	MarR (G103S, Y137H); SoxR (I40V, G74R)	**6.65** (1.63)	**3.31** (1.76)	1.68 (1.37)	0.68 (0.51)	1.17 (0.11)
6–52	MarR (K62R, G103S, Y137H); SoxR (T38S, G74R)	**6.18** (2.70)	**2.71** (0.75)	**6.53** (2.57)	0.91 (0.16)	0.70 (0.28)
4–76	MarR (G103S, Y137H); SoxR (T38S, G74R); AcrR (T213I, N214T)	**5.95** (0.66)	**4.74** (0.75)	**6.37** (1.09)	**3.66** (0.15)	1.06 (0.50)
5–58	MarR (G103S, Y137H); SoxR (T38S, G74R); AcrR (T213I, N214T)	**2.78** (0.99)	1.55 (0.560)	**6.95** (2.63)	1.56 (1.35)	0.65 (0.18)
5–66	MarR (K62R, G103S, Y137H); SoxR (T38S, G74R)	**3.25** (0.57)	1.96 (0.30)	1.92 (0.67)	0.88 (0.08)	0.35 (0.06)
5–65	MarR (K62R, G103S, Y137H); SoxR (T38S, G74R)	**9.06** (4.21)	**4.09** (0.73)	**6.43** (3.33)	**6.97** (4.52)	1.31 (0.72)
5–81	MarR (D76G, G103S, Y137H)	**2.30** (0.67)	1.09 (0.19)	**2.05** (0.85)	**3.72** (2.16)	1.612 (0.52)
6–13	MarR (G103S, Y137H)	1.17 (0.84)	**3.70** (1.04)	**2.52** (1.42)	1.36 (0.31)	1.47 (1.13)
5–33	SoxR (A111T); AcrR (T32P)	0.96 (0.60)	**2.39** (0.32)	**3.11** (0.70)	1.34 (0.43)	0.95 (0.48)

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Strains	Mutated protein (mutation detected)	Mean fold change in expression relative to J53 strain (SEM)[b]				
		marA	***soxS***	***acrA***	***ydhE***	***mdtE***
5–59	MarR (G103S, Y137H)	0.73 (0.51)	1.25 (0.36)	1.98 (1.05)	**2.26** (1.42)	1.12 (0.24)

[a] fs, frameshift

[b] Values in boldface type differ from values for the parent by at least 2-fold.

SEM= error standard of the mean.

Table 3

LPS core biosynthesis mutations detected.

Protein	Mutation detected	Strains
RfaE	A245T	3–25, 5–58
RfaF	I136V	6–42, 4, 29, 7, 10, 12, 6–66, 3–7, 3–33, 6–52, 5–66, 5–65, 5–81, 5–59
	I136V, I144V, A348V	6–75, 6–74
	I136V, A141S	76, 3–62, 5–33
	I136V, V335A	3–25, 4–76, 5–58
	I136V, Y287F	6–13
GmhB	V145A, I161V, Q191Stop	UAB4
	V145A, Q191Stop	3–48
SurA	P346A	3–48