ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2000, p. 3118–3121 Vol. 44, No. 11
0066-4804/00/$04.00+0

Evidence for an Efflux Pump Mediating Multiple Antibiotic Resistance in *Salmonella enterica* Serovar Typhimurium

LAURA J. V. PIDDOCK,[1]* DAVID G. WHITE,[2] KARL GENSBERG,[1] LILIAN PUMBWE,[1] AND DEBORAH J. GRIGGS[1]

Antimicrobial Agents Research Group, Division of Infection and Immunity, University of Birmingham, Birmingham, B15 2TT, United Kingdom,[1] and Center for Veterinary Medicine, Food and Drug Administration, Laurel, Maryland 20708[2]

Received 6 April 2000/Returned for modification 25 May 2000/Accepted 9 August 2000

The mechanism of multiple antibiotic resistance in six isolates of *Salmonella enterica* serovar Typhimurium recovered from a patient treated with ciprofloxacin was studied to investigate the role of efflux in the resistance phenotype. Compared to the patient's pretherapy isolate (L3), five of six isolates accumulated less ciprofloxacin, three of six isolates accumulated less chloramphenicol, and all six accumulated less tetracycline. The accumulation of one or more antibiotics was increased by carbonyl cyanide *m*-chlorophenylhydrazone to concentrations similar to those accumulated by L3 for all isolates except one, in which accumulation of all three agents remained approximately half that of L3. All isolates had the published wild-type sequences of *marO* and *marR*. No increased expression of *marA*, *tolC*, or *soxS* was observed by Northern blotting; however, three isolates showed increased expression of *acrB*, which was confirmed by quantitative competitive reverse transcription-PCR. However, there were no mutations within *acrR* or the promoter region of *acrAB* in any of the isolates.

In *Escherichia coli*, expression of the multiple antibiotic resistance (MAR) phenotype is mediated by the decreased expression of the porin OmpF and overexpression of the *acrAB* locus encoding the multidrug efflux pump AcrB, controlled by the *marRAB* operon, although it may also involve other efflux systems yet to be identified (1). MarA is a transcriptional activator for *marRAB* and binds to the marbox located within the operator *marO*. Homologues of MarA, such as SoxS and Rob, have been shown to bind to the marbox and also regulate expression of the *mar* locus (15, 24). The expression of the *E. coli* AcrAB efflux system is increased in MAR mutants, and overexpression of *acrAB* confers organic-solvent tolerance as well as MAR (25, 32). The outer membrane protein TolC, proposed to act as an efflux channel for AcrAB, is essential for the maintenance of organic-solvent tolerance. Like *marRAB*, *acrAB* and *tolC* are positively regulated by MarA, SoxS, and Rob (2, 7, 25, 32).

Less is known about the control of MAR in salmonella. Studies have shown that some MAR mutants of salmonella have reduced expression of OmpF (14, 26, 27), while other MAR isolates have no porin changes (10, 11, 26). The *marRAB* locus in *Salmonella enterica* serovar Typhimurium has been shown to be structurally and functionally similar to that in *E. coli* (29), and there is close homology between the *soxRS* genes of *Salmonella* serovar Typhimurium and *E. coli* (28).

Lacroix et al. constructed an *acrB*-disrupted mutant of *Salmonella* serovar Typhimurium which lost the ability to grow in the presence of bile salts and chemical detergents and showed increased susceptibility to antibiotics (16, 17). An AcrAB-overproducing mutant of *Salmonella* serovar Typhimurium with reduced susceptibility to multiple antibiotics compared to its parent strain has also been described (22). Recently, mutants of *Salmonella* serovar Typhimurium with reduced accumulation of ciprofloxacin have been shown to overexpress AcrA, suggesting the involvement of the AcrAB efflux pump in multiple drug resistance (11).

The clinical isolates of *Salmonella* serovar Typhimurium used in the present study have been described previously (26, 27). Strain L3 was isolated from a hematoma in a patient prior to intravenous ciprofloxacin therapy, and 11 quinolone-resistant posttherapy isolates were obtained from wound drainage fluid over a period of 19 weeks. Several of the posttherapy strains were subsequently shown to harbor mutations in *gyrA* (12, 26) or *gyrB* (9). No mutations were detected in *parC* (unpublished data). Six posttherapy isolates were MAR and had reduced accumulation of ciprofloxacin and norfloxacin which did not correlate with lack of OmpF. In the present study, we sought to further characterize the mechanism of MAR and reduced accumulation in these isolates and to ascertain the role of efflux in the resistance phenotype.

MATERIALS AND METHODS

Bacterial strains and susceptibility to antibiotics, dyes, detergents, and organic solvents. *Salmonella* serovar Typhimurium L3 was isolated from a patient prior to a course of ciprofloxacin. Six posttherapy strains were MAR and exhibited reduced accumulation of quinolones, and in addition, L5 possessed a mutation in *gyrA* (Ala119→Glu) and L18 possessed a mutation in *gyrB* (Ser463→Tyr) which contributed to quinolone resistance (9, 12, 26, 27). The remaining isolates had no mutations in *gyrA* or *gyrB*. *Salmonella* serovar Typhimurium NCTC 74 (L19) was obtained from the Public Health Laboratory Service (Colindale, United Kingdom), and *Salmonella* serovar Typhimurium T39 (parent strain; spontaneous streptomycin-resistant mutant) and LX1054 (*acrB* mutant) were supplied by F. Lacroix (16). Susceptibility to antimicrobial agents, detergents, and dyes and tolerance to hexane and cyclohexane were determined as described previously (4, 13, 32).

Antibiotic accumulation. The accumulation of ciprofloxacin, [^{3}H]chloramphenicol, and [^{3}H]tetracycline by all isolates, with and without the presence of 100 μM carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), was measured as described previously (5, 18).

DNA isolation, PCR, and DNA sequencing. Genomic DNA was prepared using cetyltrimethylammonium bromide (CTAB)-chloroform extraction (6). The *marR* and *marO* regions were amplified from genomic DNA using primers derived from the *Salmonella* serovar Typhimurium *marRAB* sequence (29) as described previously (12). A 1,536-nucleotide *acrB* gene fragment was amplified using the primers derived from the partial sequence of *Salmonella* serovar Typhimurium LX1054 *acrB* (17). Gene fragments of *acrR* and the putative promoter region of *acrAB* were amplified by PCR using primers derived from the

* Corresponding author. Mailing address: Antimicrobial Agents Research Group, Division of Infection and Immunity, University of Birmingham, Birmingham, B15 2TT, United Kingdom. Phone: 0121-414-6969. Fax: 0121-414-6966. E-mail: l.j.v.piddock@bham.ac.uk.

TABLE 1. Susceptibilities of *Salmonella* serovar Typhimurium isolates to antibiotics, detergents, and dyes

Isolate[a]	MIC[b]																
	Antibiotics (μg/ml)									Dyes (μg/ml)				Detergents (mg/ml)			
	NAL	CIP	TET	CHL	FOX	AMC	ERY	RIF	NOV	ACF	MB	EtBr	AO	DHCO	CTAB	SDS	NP-40
L3	8	0.03	4	2	1	1	2	16	32	64	128	32	128	1	16	0.25	2
L5	16	0.25	8	64	64	2	4	32	32	64	128	32	128	1	16	0.25	2
L6	64	0.25	8	32	16	4	512	16	256	>512	128	>512	>512	>16	>16	16	>16
L10	32	0.12	16	32	16	4	512	32	512	>512	>512	>512	>512	2	>16	2	16
L12	32	0.5	16	32	16	4	512	16	256	>512	>512	>512	>512	4	>16	16	>16
L15	32	0.25	8	32	8	2	4	16	256	>512	>512	>512	>512	2	>16	16	>16
L18	32	0.5	16	32	32	16	32	16	32	256	128	32	128	1	4	0.25	2
L19	2	0.03	2	2	1	1	16	128	16	512	>512	>512	>512	1	>16	0.25	2
T39	16	1	8	32	32	4	512	128	256	>512	>512	>512	>512	2	16	1	4
LX1054	16	0.25	2	2	2	2	4	16	16	64	32	32	128	1	4	0.5	2

[a] L3, pretherapy parent isolate; L5, L6, L10, L12, L15, and L18, posttherapy isolates; L19, *Salmonella* serovar Typhimurium NCTC 74. T39 (parent strain) and LX1054 (*acrB* mutant) were from Lacroix et al. (16).
[b] NAL, nalidixic acid; CIP, ciprofloxacin; TET, tetracycline; CHL, chloramphenicol; FOX, cefoxitin; AMC, ampicillin; ERY, erythromycin; RIF, rifampin; NOV, novobiocin; ACF, acriflavine; MB, methylene blue; EtBr, ethidium bromide; AO, acridine orange; DHCO, dehydrocholic acid; SDS, sodium dodecyl sulfate; NP-40, Nonidet P-40.

sequences of *E. coli acrRAB* (20). DNA sequencing was performed by MWG-Biotech AG (Ebersberg, Germany).

Northern blot analysis for *acrB*, *tolC*, *marA*, and *soxS*. For Northern blot analysis of *marA* and *soxS*, total RNA was extracted with an RNeasy Midi kit (Qiagen). The probes for *marA* and *soxS* were prepared by PCR, [^{32}P]dCTP labeled with the High Prime DNA labeling kit (Boehringer Mannheim Corporation), and hybridized to the RNA in ULTRAhyb ultrasensitive hybridization solution (Ambion) according to the manufacturer's protocol. Northern blotting of *acrB* (1,067 nucleotides; analogous to nucleotides 3550 to 4616 of *E. coli acrAB*) and *tolC* was performed with RNA extracted with TRIZOL (Life Technologies Ltd.) and as described in the Gene Images kit (Amersham Pharmacia Biotech). Probes for *acrB* and *tolC* were prepared by PCR and labeled with fluorescein using the Gene Images kit. Sample-to-sample RNA uniformity was determined by examining 16S rRNA expression in parallel. DNA sequencing of all probes confirmed their identities.

QCRT-PCR of *acrB*. Differential expression of *acrB* mRNA was compared by quantitative competitive reverse transcription-PCR (QCRT-PCR) as described by Freeman et al. (8). An 894-bp internal competitor DNA standard for *acrB* was generated by PCR amplification of genomic DNA using primer STACRB1 (CGAGAACGTCGAACGTGTTA) and the 40-mer reverse primer ACRBi (TCACACGACCGCGATCGATAGCCGAACAACTGATTACGTG). Total RNA was extracted with TRIZOL reagent. Reverse transcriptase PCR was performed on the RNA template to generate cDNA of *acrB*. Competitor DNA was added at concentrations from 0.01 to 2 pg to replicate tubes containing identical aliquots of cDNA. PCR was performed on the competitor DNA-cDNA mixture using primers STACRB1 and ACRBR1 (TCACACGACCGCGATCGATA). The two products were separated by polyacrylamide gel electrophoresis, the gel was silver stained (31), and the bands were quantified by densitometry. The concentration of competitor DNA at which both bands were of equal density was taken to be the concentration of the target cDNA.

SSCP analysis of *acrR* and the putative promoter region of *acrAB*. Genomic DNA was prepared using CTAB-chloroform extraction (6), and *acrR* and the putative *acrAB* promoter region were amplified by PCR using three pairs of primers based on the *acrRAB* sequence from *E. coli* (20). The PCR amplimers were analyzed by single-strand conformational polymorphism (SSCP) as described previously (31).

Nucleotide sequence accession numbers. The DNA sequences from wild-type *Salmonella* serovar Typhimurium NCTC 74 were submitted to GenBank under accession numbers U78314 (*acrB*-like gene) and AF209869 to AF209870 (*acrR* and the putative promoter region of *acrAB*).

RESULTS AND DISCUSSION

All six posttherapy isolates were less susceptible to nalidixic acid, ciprofloxacin, and other antibiotics than the pretherapy isolate, L3. L6, L10, L12, and L15 were also more resistant to dyes and detergents (Table 1). L5 was as susceptible to dyes and detergents as L3, while L18 was fourfold more resistant to acriflavine only (Table 1). Isolates L6, L10, and L12 were hexane tolerant. For all strains, the MIC of ciprofloxacin was unaltered by the presence of 100 μM CCCP, and none of the strains grew in the presence of cyclohexane.

Isolates L5, L6, L10, L12, and L15 accumulated approxi-

TABLE 2. Accumulation of antibiotics by *Salmonella* serovar Typhimurium isolates

Isolate[a]	Resistance phenotype or genotype[b]	Accumulation (as ng of antibiotic/mg)[c]								
		Ciprofloxacin			Chloramphenicol			Tetracycline		
		−CCCP	SD	+CCCP	−CCCP	SD	+CCCP	−CCCP	SD	+CCCP
L3	Wild type	102.2	16.8	112.4	42.8	6.8	35.9	15.1	1.4	17.7
L5	MAR; *gyrA*	27.6	7.4	94.8	36.6	5.6	46.7	9.4	0.8	13.8
L6	MAR; dyes, detergents, hexane	61.3	8.3	102.7	23.9	1.6	45.2	7.3	1.9	13.3
L10	MAR; dyes, detergents, hexane	23.3	3.8	105.8	14.2	1.2	31.8	3.3	0.6	6.9
L12	MAR; dyes, detergents, hexane	47.4	7.8	44.3	27.4	6.9	26.5	4.9	1.5	8.8
L15	MAR; dyes, detergents	22.4	5.6	39.3	28.7	1.6	42.5	6.3	1.6	13.4
L18	MAR; acriflavine; *gyrB*	89.9	2.9	96.3	34.2	4.9	55.5	7.7	0.3	13.5
L19	Type strain (NCTC 74)	88.9		ND	38.1		39.3	9.5		9.9
T39	Parent strain	ND		ND	15.8		25.7	4.0		11.7
LX1054	*acrB* mutant	ND		ND	28.4		34.0	8.8		10.4

[a] L3, pretherapy parent isolate; L5, L6, L10, L12, L15, and L18, posttherapy isolates. T39 (parent strain) and LX1054 (*acrB* mutant) were from Lacroix et al. (16).
[b] *gyrA*, Piddock et al. (26); *gyrB*, Gensberg et al. (9); MAR, 2- to 256-fold more resistant to quinolones and six or seven unrelated agents than L3.
[c] −CCCP, accumulation without CCCP; +CCCP, accumulation in the presence of CCCP; SD, standard deviation; ND, not done.

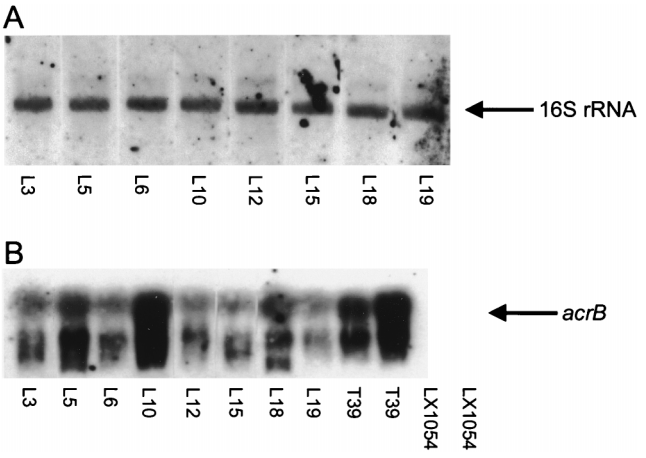

FIG. 1. Northern blot analysis of *acrB* transcript levels in clinical isolates of *Salmonella* serovar Typhimurium, *Salmonella* serovar Typhimurium L19 (NCTC 74), and strains T39 (wild type) and LX1054 (T39 carrying a Tn*phoA* insert disrupting *acrB* [17]).

mately two- to fourfold less ciprofloxacin than L3 (Table 2). In the presence of CCCP, the concentrations of ciprofloxacin accumulated by L5, L6, and L10 increased to that accumulated by L3. The concentration of ciprofloxacin accumulated by L15 was almost doubled in the presence of CCCP, but it remained less than half that observed in L3. CCCP had no effect upon ciprofloxacin accumulation for isolates L12 and L18. L6, L12, and L15 accumulated approximately three-quarters and L10 accumulated one-third the concentration of chloramphenicol accumulated by L3. The concentrations of chloramphenicol accumulated by all isolates except L12 were increased in the presence of CCCP (Table 2). All six posttherapy isolates accumulated approximately two- to fourfold less tetracycline than L3 (Table 2). In the presence of CCCP, the accumulation of tetracycline by L5, L6, L15, and L18 was increased to concentrations similar to that accumulated by L3. Although the concentrations of tetracycline accumulated by L10 and L12 approximately doubled in the presence of CCCP, they still remained less than half that of L3.

Northern blot analysis indicated that none of the clinical strains overexpressed *marA*, *soxS*, or *tolC*; *tolC* was expressed at low levels. The posttherapy isolates L5, L10, and L18 showed consistently higher transcript levels of *acrB* than the pretherapy isolate, L3 (Fig. 1). No *acrB* transcripts were detected in the *acrB* mutant LX1054. QCRT-PCR demonstrated that L10 expressed four times and L5 and L18 expressed two and a half times more *acrB* mRNA than L3. SSCP analysis of the entire *acrR* gene and the putative promoter region of *acrAB* identified mutations within these regions in control strains of *E. coli* (data not shown); however, all posttherapy isolates of *Salmonella* serovar Typhimurium showed patterns identical to those of the pretherapy isolate, L3, and the control, L19. The SSCP data were corroborated by DNA sequencing, which confirmed that there were no mutations within the region.

Recent work has suggested that the AcrAB efflux pump of *E. coli* plays a major role in MAR, as fluoroquinolone resistance was lost on inactivation of the *acrAB* locus in strains with mutations in *gyrA* (23). The same may not be true for salmonella, as CCCP (which should inactivate AcrB) made no difference in susceptibility to ciprofloxacin for any of the isolates in the present study, including both isolates with gyrase mutations (L5 *gyrA* and L18 *gyrB*; data not shown).

To confirm that MAR in the isolates L5, L10, and L18 is mediated by AcrB efflux, we propose to inactivate *acrB* in these strains and examine whether the phenotype is reversed by disruption of the gene. This work is currently under way. It is unlikely that sequencing of the *acrAB* gene cluster in these isolates would reveal any mutations, as changes in this gene have been shown to increase susceptibility in *Salmonella* serovar Typhimurium (17, 22). As SSCP analysis and DNA sequencing of *acrR* and the promoter region of *acrAB* failed to show any differences between the MAR isolates and the pretherapy parent strain, any mutation mediating resistance is more likely to be found in a regulatory gene. The data presented here also suggest that *marOR*, *soxS*, and *tolC* are not overexpressed. However, in *E. coli*, TolC has been shown to be essential for the function of the AcrB efflux pump (7) and for the maintenance of organic-solvent tolerance (2). It may be that the low level of TolC was sufficient to act as an outer membrane channel for AcrAB; however, an alternative explanation for the data is that another pump which affects the expression of *acrB* may be involved in multiple antibiotic resistance in *Salmonella* serovar Typhimurium and may explain the apparent lack of involvement of TolC in the resistance phenotype of these isolates. Only three of the six strains in the present study exhibited any degree of organic-solvent tolerance (L6, L10, and L12 to hexane only), which may be consistent with the lack of increased expression of *tolC* in these isolates. It would be interesting to examine whether the expression of Rob is increased in these isolates, as it is known that this protein affects the function of the AcrAB efflux pump in *E. coli* (3, 30). Unfortunately, despite employing a variety of strategies, PCR amplification of *rob* using primers based on the DNA sequence of *E. coli rob* failed to amplify its homologue from chromosomal or plasmid DNA preparations from any of the control or clinical isolates of *Salmonella* serovar Typhimurium (unpublished data).

The accumulation of ciprofloxacin by L15 and tetracycline by L12 was increased by CCCP but remained well below the concentrations accumulated by L3. Similarly, reduced accumulation of ciprofloxacin and chloramphenicol by L12 was unaffected by CCCP. This suggests that accumulation was reduced in these two strains by an additional mechanism resistant to CCCP inhibition. Previous work has shown that reduced outer membrane permeability did not contribute to resistance in L12 and L15 (26). It is probable that another efflux pump with a substrate profile similar to that of AcrB but which is resistant to inhibition by CCCP, perhaps similar to EmrAB of *E. coli*, is contributing to resistance in L12 and L15 (21). The use of other efflux inhibitors may help elucidate the nature of other putative efflux pumps in salmonella. It is evident that isolates with quite different phenotypes arose in this patient, and it is likely that individual isolates may possess a complex mixture of multiple mutations.

The regulation of AcrB-mediated efflux in *Salmonella* serovar Typhimurium remains to be established and may differ from that described for *E. coli* (1). Ma et al. (19) found increased transcription of *acrAB* in *E. coli* strains which lacked *acrR* when exposed to general stress conditions. They concluded that *acrR* is a secondary modulator of *acrRAB* expression and suggested that up-regulation under general stress conditions is controlled by an unidentified regulator, possibly a homologue of the known global regulator MarA, SoxS, or Rob. It is feasible that this alternative regulatory mechanism, independent of *mar-sox-rob*, may be responsible for the control of AcrB expression in *Salmonella* serovar Typhimurium, and this requires further investigation. When the sequencing of the *Salmonella* serovar Typhimurium LT2 genome is completed

(33), identification of homologues of the known regulators of the *mar* regulon of *E. coli* will help to elucidate the regulation of efflux-mediated fluoroquinolone resistance in *Salmonella* serovar Typhimurium.

ACKNOWLEDGMENTS

We are grateful to Julie Sherwood, Craig Munday, Nirinder Singh, and Mark Webber for technical assistance.

This study was supported in part by grants from the Leverhulme Trust (number F94AT) (K.G.) and USDA-CSREES-NRICGP (number 9635208) (D.G.W.).

REFERENCES

1. **Alekshun, M. N., and S. B. Levy.** 1997. Regulation of chromosomally mediated multiple antibiotic resistance: the *mar* regulon. Antimicrob. Agents Chemother. **41:**2067–2075.
2. **Aono, R., N. Tsukagoshi, and M. Yamamoto.** 1998. Involvement of outer membrane protein TolC, a possible member of the *mar-sox* regulon, in maintenance and improvement of organic solvent tolerance of *Escherichia coli* K-12. J. Bacteriol. **180:**938–944.
3. **Ariza, R. R., A. Li, N. Ringstad, and B. Demple.** 1995. Activation of multiple antibiotic resistance and binding of stress-inducible promoters by *Escherichia coli* Rob protein. J. Bacteriol. **177:**1655–1661.
4. **Asako, H., H. Nakajima, K. Kobayshi, M. Kobayshi, and R. Aono.** 1997. Organic solvent tolerance and antibiotic resistance increased by overexpression of *marA* in *Escherichia coli*. Appl. Environ. Microbiol. **63:**1428–1433.
5. **Asuquo, A. E., and L. J. V. Piddock.** 1993. Accumulation and killing kinetics of fifteen quinolones for *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **31:**865–880.
6. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1995. Current protocols in molecular biology. John Wiley and Sons, New York, N.Y.
7. **Fralick, J. A.** 1996. Evidence that TolC is required for functioning of the Mar/AcrAB efflux pump of *Escherichia coli*. J. Bacteriol. **178:**5803–5805.
8. **Freeman, W. M., S. J. Walker, and K. E. Vrana.** 1999. Quantitative RT-PCR: pitfalls and potential. BioTechniques **26:**112–125.
9. **Gensberg, K., Y. F. Jin, and L. J. V. Piddock.** 1995. A novel *gyrB* mutation in a fluoroquinolone-resistant clinical isolate of *Salmonella typhimurium*. FEMS Microbiol. Lett. **132:**57–60.
10. **Gibb, A. P., C. S. Lewis, and O. J. Garden.** 1991. Development of quinolone resistance and multiple antibiotic resistance in *Salmonella bovismorbificans* in a pancreatic abscess. J. Antimicrob. Chemother. **28:**318–321.
11. **Giraud, E., A. Cloeckaert, D. Kerboeuf, and E. Chaslus-Dancla.** 2000. Evidence for active efflux as the primary mechanism of resistance to ciprofloxacin in *Salmonella enterica* serovar Typhimurium. Antimicrob. Agents Chemother. **44:**1223–1228.
12. **Griggs, D. J., K. Gensberg, and L. J. V. Piddock.** 1996. Mutations in *gyrA* gene of quinolone-resistant salmonella serotypes isolated from humans and animals. Antimicrob. Agents Chemother. **40:**1009–1013.
13. **Griggs, D. J., M. C. Hall, Y. F. Jin, and L. J. V. Piddock.** 1994. Quinolone resistance in veterinary isolates of salmonella. J. Antimicrob. Chemother. **33:**1173–1189.
14. **Howard, A. J., T. D. Joseph, L. L. Bloodworth, J. A. Frost, H. Chart, and B. Rowe.** 1990. The emergence of ciprofloxacin resistance in *Salmonella typhimurium*. J. Antimicrob. Chemother. **26:**296–298.
15. **Jair, K. W., X. Yu, K. Skarstad, B. Thony, N. Fujita, A. Ishihama, and R. E. Wolf, Jr.** 1996. Transcriptional activation of promoters of the superoxide and multiple antibiotic resistance regulons by Rob, a binding protein of the *Escherichia coli* origin of chromosomal replication. J. Bacteriol. **178:**2507–2513.
16. **Lacroix, F. J. C., C. Avoyne, C. Pinault, M. Y. Popoff, and P. Pardon.** 1995. *Salmonella typhimurium* Tn*phoA* mutants with increased sensitivity to biological and chemical detergents. Res. Microbiol. **146:**659–670.
17. **Lacroix, F. J. C., A. Cloeckaert, O. Grepinet, C. Pinault, M. Y. Popoff, H. Waxin, and P. Pardon.** 1996. *Salmonella typhimurium acrB*-like gene: identification and role in resistance to biliary salts and detergents and in murine infection. FEMS Microbiol. Lett. **135:**161–167.
18. **Li, X. Z., D. M. Livermore, and H. Nikaido.** 1994. Role of efflux pump(s) in intrinsic resistance of *Pseudomonas aeruginosa*: resistance to tetracycline, chloramphenicol, and norfloxacin. Antimicrob. Agents Chemother. **38:**1732–1741.
19. **Ma, D., M. Alberti, C. Lynch, H. Nikaido, and J. E. Hearst.** 1996. The local repressor AcrR plays a modulating role in the regulation of *acrAB* genes of *Escherichia coli* by global stress signals. Mol. Microbiol. **19:**101–112.
20. **Ma, D., D. N. Cook, M. Alberti, N. G. Pon, H. Nikaido, and J. E. Hearst.** 1993. Molecular cloning and characterization of *acrA* and *acrE* genes of *Escherichia coli*. J. Bacteriol. **175:**6299–6313.
21. **Nikaido, H.** 1996. Multidrug efflux pumps of gram-negative bacteria. J. Bacteriol. **178:**5853–5859.
22. **Nikaido, H., M. Basina, V. Nguyen, and E. Y. Rosenburg.** 1998. Multidrug efflux pump AcrAB of *Salmonella typhimurium* excretes only those beta-lactam antibiotics containing lipophilic side chains. J. Bacteriol. **180:**4686–4692.
23. **Oethinger, M., W. V. Kern, A. S. Jellen-Ritter, L. M. McMurry, and S. B. Levy.** 2000. Ineffectiveness of topoisomerase mutations in mediating clinically significant fluoroquinolone resistance in *Escherichia coli* in the absence of the AcrAB efflux pump. Antimicrob. Agents Chemother. **44:**10–13.
24. **Oethinger, M., I. Podglajen, W. V. Kern, and S. B. Levy.** 1998. Overexpression of the *marA* or *soxS* regulatory gene in clinical topoisomerase mutants of *Escherichia coli*. Antimicrob. Agents Chemother. **42:**2089–2094.
25. **Okusu, H., D. Ma, and H. Nikaido.** 1996. AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of *Escherichia coli* multiple antibiotic resistance (Mar) mutants. J. Bacteriol. **178:**306–308.
26. **Piddock, L. J. V., D. J. Griggs, M. C. Hall, and Y. F. Jin.** 1993. Ciprofloxacin resistance in clinical isolates of *Salmonella typhimurium* obtained from two patients. Antimicrob. Agents Chemother. **37:**662–666.
27. **Piddock, L. J. V., K. Whale, and R. Wise.** 1990. Quinolone resistance in salmonella: clinical experience. Lancet **335:**1459.
28. **Pomposiello, P. J., and B. Demple.** 2000. Identification of SoxS-regulated genes in *Salmonella enterica* serovar Typhimurium. J. Bacteriol. **182:**23–29.
29. **Sulavik, M. C., M. Dazer, and P. F. Miller.** 1997. The *Salmonella typhimurium mar* locus: molecular and genetic analyses and assessment of its role in virulence. J. Bacteriol. **179:**1857–1866.
30. **Tanaka, T., T. Horii, K. Shibayama, K. Sato, S. Ohsuka, Y. Arakawa, K. I. Yamaki, K. Takagi, and M. Ohta.** 1997. RobA-induced multiple antibiotic resistance largely depends on activation of the AcrAB efflux. Microbiol. Immunol. **41:**697–702.
31. **Wain, J., N. T. T. Hoa, N. T. Chinh, H. Vinh, M. J. Everett, T. S. Diep, N. P. Day, T. Solomon, N. J. White, L. J. V. Piddock, and C. M. Parry.** 1997. Quinolone-resistant *Salmonella typhi* in Vietnam: molecular basis of resistance and clinical response to treatment. Clin. Infect. Dis. **25:**1404–1410.
32. **White, D. G., J. D. Goldman, B. Demple, and S. B. Levy.** 1997. Role of the *acrAB* locus in organic solvent tolerance mediated by overexpression of *marA*, *soxS*, or *robA* in *Escherichia coli*. J. Bacteriol. **179:**6122–6126.
33. **Wong, R. M., K. K. Wong, N. R. Benson, and M. McClelland.** 1999. Sample sequencing of a *Salmonella typhimurium* LT2 lambda library: comparison to the *Escherichia coli* K12 genome. FEMS Microbiol. Lett. **173:**411–423.