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Mechanisms of Quinolone Resistance in Clinical Isolates of *Shigella dysenteriae*

In gram-negative bacteria, gyrase and topoisomerase IV are primary and secondary targets, respectively, of the fluoroquinolones. In addition to the mutations in the genes encoding the target enzymes (1, 4), quinolone resistance may also be associated with increased efflux of the drugs (2, 5). Possible mechanisms of quinolone resistance were investigated in clinical isolates of *Shigella dysenteriae* obtained from the International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh (AK) and the National Institute of Cholera and Enteric Diseases, Calcutta, India (CI, DS, IPB, and IMC). The quinolone resistance-determining regions (QRDR) of *gyrA* and *parC* were amplified with the primer pairs 5′TACACCGGTCAACATTGAGG3′-5′TTAATGATTGCCGCCGTCGG3′ and 5′GTATGCGATGTCTGAACTGGGCCTG3′-5′CGACAACCGGGATTCGGTG3′, respectively. The Ser83→Leu substitution appeared sufficient to confer high-level nalidixic acid resistance (MIC > 250 μg/ml) as determined by standard methods (3) (Table 1). Four strains—DS-1, DS-2, CI-1, and CI-2—for which the norfloxacin MICs were 2 μg/ml and the ciprofloxacin MICs were between 0.5 and 1 μg/ml harbored the mutation Asp87→Gly in GyrA. None of the isolates examined had any mutations in the QRDR-encoding part of the *parC* gene.

Accumulation of norfloxacin was studied as described by Ghosh et al. (2) by using carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (100 μM) as proton motive force (PMF) uncoupler. The DS, CI, and AK series (with the exception of AK 19520) showed steady-state levels of norfloxacin accumulation (both before and after addition of CCCP) similar to those for the susceptible strain AK19520 (Table 1)—evidence against involvement of a PMF-dependent efflux pump in resistance in these strains. Considering the DS and CI series, the mutation corresponding to Asp87→Gly therefore appeared sufficient to confer approximately 10-fold resistance to the fluoroquinolones in comparison with AK19520. On the other hand, the accumulation of norfloxacin at steady state (before addition of CCCP) was less in the IPB and IMC series in comparison with AK19520. Addition of CCCP increased the level of accumulation in these resistant strains to a level comparable to that of AK19520, suggesting a role of a PMF-dependent efflux pump in the development of resistance. Since these strains lacked *gyrA* or *parC* mutations in the QRDR, increased efflux pump activity appears likely to be sufficient to confer 20- to 80-fold resistance to the fluoroquinolones compared to AK19520. We have previously shown, using isogenic strains (2), that increased efflux of the fluoroquinolones may be a mechanism of development of fluoroquinolone resistance. The data obtained in this study with clinical isolates supports this notion. However, the possible involvement of *gyrB* or *parE* mutations in the decreased susceptibilities of the isolates to quinolones cannot be excluded.

TABLE 1. Quinolone susceptibility, alterations in GyrA, and norfloxacin accumulation in clinical isolates of *S. dysenteriae*[a]

Strain(s)	MIC (μg/ml)				Codon change at position:		Accumulation of NFLX[b]	
	NAL	NFLX	OFLX	CFLX	83	87	Before addition of CCCP	After addition of CCCP
AK19520	16	0.25	0.25	0.25	—[c]	—	0.23 ± 0.01	0.34 ± 0.013
AK21104	>250	2	1	2	Ser (TTG)→Leu (TCG)	—	0.22 ± 0.005	0.33 ± 0.012
AK27228	>250	2	2	2	Ser (TTG)→Leu (TCG)	—	0.22 ± 0.005	0.33 ± 0.011
AK24467	>250	2	1	2	Ser (TTG)→Leu (TCG)	—	0.22 ± 0.005	0.32 ± 0.013
AK21809	>250	2	1	4	Ser (TTG)→Leu (TCG)	—	0.23 ± 0.010	0.33 ± 0.014
IPB32, IPB34, IMC118, IMC119	32	16	4	4	—	—	0.10 ± 0.001	0.30 ± 0.012
IPB38	16	16	4	4	—	—	ND[d]	ND
IMC67	16	16	4	4	—	—	0.10 ± 0.001	0.30 ± 0.013
DS-1, DS-2	64	2	2	0.5	—	Asp (GAC)→Gly (GGC)	0.25 ± 0.010	0.32 ± 0.014
CI-1, CI-2	64	2	2	1	—	Asp (GAC)→Gly (GGC)	0.23 ± 0.010	0.33 ± 0.012

[a] NAL, nalidixic acid; NFLX, norfloxacin; OFLX, ofloxacin; CFLX, ciprofloxacin.
[b] The data are means of three determinations ± standard deviations, expressed in micrograms per milligram (dry weight) of cells.
[c] —, identical to the codon in *E. coli*.
[d] ND, not determined.

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