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Novel Combination of Mutations in the DNA Gyrase and Topoisomerase IV Genes in Laboratory-Grown Fluoroquinolone-Resistant *Shigella flexneri* Mutants

A ciprofloxacin-susceptible strain (wild type [WT]) of *Shigella flexneri* (MIC, 0.015 μg/ml), isolated from a stool sample in 1996 was passaged (about 10^{10} cells) onto ciprofloxacin-containing (0.5 to 32 μg/ml) Isosensitest medium (Oxoid) to obtain single colonies after 36-h incubation. Two resistant mutants (A and B) were thus obtained. Table 1 shows MICs of nine antimicrobial agents, determined by the agar dilution method (14).

The quinolone resistance determining regions (QRDR) of *gyrA* (648 bp), *gyrB* (184 bp), *parC* (531 bp), and *parE* (265 bp) for WT and its two mutants were amplified with the following primer pairs: 5′-TACACCGGTCAACATTGAGG-3′ and 5′-TTAATGATTGCCGCCGTCGG-3′, 5′-CAGACTGCCAGG AACGCGAT-3′ and 5′-AGCCAAGTGCGGTGATAAGA-3′, 5′-GTACGTGATCATGGACCGTG-3′ and 5′-TTCGGCTG GTCGATTAATGC-3′, and 5′-TACCGAGCTGTTCCTTG TGG-3′ and 5′-GGCAATGTGCAGACCATCAG-3′. Single-strand conformational polymorphism analysis was applied to all PCR products (CleanGel S48 kit; Pharmacia) and revealed mutations in the QRDR of *gyrA* and *parC*, but not *gyrB* and *parE*, in mutants A and B. Cycle sequencing subsequently identified these mutations with the nested primers 5′-ACGC CAGACAACCGTTGA-3′ and 5′-ATGCGGTGGAATATC GGTCG-3′ for *gyrA* and *parC*, respectively. Changes in amino acids at the mutation sites are shown in Table 1.

The genetic basis of resistance to quinolones in gram-negative bacteria has been defined mainly in *Escherichia coli*. Substitution of the highly conserved residue Ser-83 in GyrA is the most common alteration in both in vivo- and in vitro-resistant isolates (5, 15). The mutation altering the residue Asp-87 is usually found to augment the Ser-83 mutation for further resistance increase (5, 12). Replacement of the residues Ser-80 and Glu-84 in ParC is commonly associated with the *gyrA* mutations to procure high fluoroquinolone resistance (6, 8, 13). Mutations in the QRDR of *gyrB* and *parE* of *E. coli* appear to be uncommon (2). Mutation in the codon for Ser-83 has been reported to be present in isolates of *Shigella dysenteriae* and *S. flexneri* resistant to nalidixic acid (1, 11). We report here the association of ciprofloxacin resistance with a novel combination of alterations at Asp-87 (to Tyr) in GyrA and Asp-79 (to Ala) in ParC in two laboratory-grown mutants. Higher resistance was associated with an additional mutation at Glu-84 (to Ala) in ParC in mutant B. Although the amino acid changes in GyrA and ParC identified have not been proven to cause the resistance phenotype, these findings suggest that they may be associated with the development of higher-level fluoroquinolone resistance. A single alteration at Asp-87 (to Tyr) or its homologs in gram-negative bacteria is uncommon and only found in isolates with low level of resistance, e.g., *Serratia marcescens* (7), *Haemophilus influenzae* (3), *Citrobacter freundii* (9), and *Salmonella* (4). Construction of hybrid plasmids and transformation demonstrated that changes at Asp-87 alone led to an increase in the ciprofloxacin MIC from 0.02 to 0.16 mg/ml for *E. coli* (10). Alteration at Asp-79 (to Ala) in ParC has so far not been reported in association with fluoroquinolone resistance. The increases in MICs of tetracycline, tobramycin, chloramphenicol, and acriflavin suggest that another resistance mechanism(s) may also be involved, such as Mar as described for *Pseudomonas aeruginosa* after serial exposure to fluoroquinolones (16).

Nucleotide sequence accession numbers. The partial sequence data of the *S. flexneri gyrA* and *parC* genes reported here appear in the GenBank database under the accession no. AF065131 and AF065132, respectively.

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TABLE 1. Mutations in the *gyrA* and *parC* QRDR in the WT and mutants A and B and MICs of nine antimicrobial agents

Strain	MIC (μg/ml)[a]									*gyrA* codon 87	*parC*	
	CIP	NAL	NOR	GEN	TOB	ACR	TET	CHL	IMP		Codon 79	Codon 84
WT	0.015	1	0.12	0.5	0.25	16	128	64	0.25	GAC (Asp)	GAT (Asp)	GAA (Glu)
A	16	>1,024	32	0.5	1	32	512	512	0.25	TAC (Tyr)	GCT (Ala)	GAA (Glu)
B	64	>1,024	64	0.5	1	64	512	512	0.12	TAC (Tyr)	GCT (Ala)	GCA (Ala)

[a] CIP, ciprofloxacin; NAL, nalidixic acid; NOR, norfloxacin; GEN, gentamicin; TOB, tobramycin; ACR, acriflavin; TET, tetracycline; CHL, chloramphenicol; IMP, impenem.

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