Letters to the Editor

Alteration in the GyrA Subunit of DNA Gyrase and the ParC Subunit of Topoisomerase IV in Quinolone-Resistant Shigella dysenteriae Serotype 1 Clinical Isolates from Kolkata, India

After the emergence of multidrug-resistant Shigella strains (8, 11), fluoroquinolones, such as ciprofloxacin and norfloxacin, were used in India to treat shigellosis. However, recently, genetically clonal ciprofloxacin-resistant Shigella dysenteriae serotype 1 strains have been isolated from sporadic and epidemic cases of dysentery in southern Asia, including eastern India (1, 9, 12; S. Dutta, A. Ghosh, K. Ghosh, D. Dutta, S. K. Bhatta-charya, G. B. Nair, and S.-I. Yoshiida, Lett. J. Clin. Microbiol. 41:5833-5834, 2003).

Quinolone resistance is linked mainly to mutations located in the quinolone resistance-determining regions (QRDRs) of DNA gyrase (GyrA and GyrB) and topoisomerase IV (ParC and ParE) (3, 4, 5). Replacement of residues Ser 80 and Glu 84 in the QRDRs of DNA gyrase (GyrA and GyrB) and topoisomerase IV (ParC) commonly supplements gyrA mutations at residues Ser 83 and Asp 87 in members of the Enterobacteriaceae family to develop high fluoroquinolone resistance (13, 14). Besides topoisomerase mutations, overexpression of the energy-dependent multidrug efflux pump AcrAB due to mutations within repressor (AcrR) and multiple target and nontarget gene changes also contribute to fluoroquinolone resistance phenotypes in bacteria (2, 7, 15).

In this report, we demonstrate the mutations in the QRDRs of gyrA, gyrB, parC, and parE genes of fluoroquinolone-resistant S. dysenteriae serotype 1 strains isolated in Kolkata, India. The type strain of S. dysenteriae serotype 1 (GTC 786) was procured from the culture collection of the Graduate School of Medicine, Gifu University, Gifu, Japan. Among 12 wild strains of S. dysenteriae serotype 1 included in this study, eight DS strains from sporadic cases (1) and one SKN outbreak strain (9) of dysentery showed resistance to fluoroquinolones, such as nalidixic acid, ciprofloxacin, and norfloxacin. The other three IBM strains were susceptible to all fluoroquinolones (8). These two groups of strains were compared to the type strain to determine any mutations.

The QRDRs of the gyrA (648-bp), gyrB (309-bp), parC (249-bp), and parE (290-bp) genes for all study strains were amplified with primer pairs designed from the genome sequence of S. flexneri 2457T, which is available at the DBGET database. For gyrA, the forward primer 5′ TAC ACC GGT CAA CAT TGA GG 3′ (nucleotide [nt] 24 to 43) and the reverse primer 5′ TTA ATG ATT GCC GCC GTC GG 3′ (nt 652 to 671) were used. For gyrB, the forward primer 5′ TGA AAT GAC CCG CCG TAA AGG 3′ (nt 1170 to 1190) and the reverse primer 5′ TGC TTG ATA CAG CAG TTT GTC CGG G 3′ (nt 1455 to 1479) were used. For parC, the forward primer 5′ TGC TGA ACT GGG CCT GAA TGC 3′ (nt 147 to 167) and the reverse primer 5′ AGC AGC TCG GAA TAT TTC GAC TGG 3′ (nt 373 to 395) were used. For parE, the forward primer 5′ TTA ATG ATT GCC GCC GTC GG 3′ (nt 652 to 671) and the reverse primer 5′ TGC TTG ATA CAG CAG TTT GTC CGG G 3′ (nt 1455 to 1479) were used. For parE, the forward primer 5′ TGC TGA ACT GGG CCT GAA TGC 3′ (nt 147 to 167) and the reverse primer 5′ AGC AGC TCG GAA TAT TTC GAC AA 3′ (nt 373 to 395) were used. The amplification was performed in a DNA thermal cycler as follows: (i) 30 cycles,
with 1 cycle consisting of 1 min at 92°C, 1 min at 64°C, and 2 min at 74°C; and (ii) a final extension step of 10 min at 74°C. The purified PCR products were used directly as templates for sequencing, which was performed with CEQ dye terminator cycle sequencing using the Quickstart kit (Beckman Coulter, Inc.) in an automated sequencer (CEQ 2000 XL DNA analysis system; Beckman Coulter). The amino acid sequences of QDRs were determined, and the homology of sequences was performed using the DNASTAR program (Hitachi Software, Tokyo, Japan).

The MICs of nalidixic acid, ciprofloxacin, and norfloxacin were determined for each strain by using the Etest kit (AB Biodisk, Solna, Sweden), and the readings were interpreted using the National Committee for Clinical Laboratory Standards (NCCLS) breakpoint criteria (6).

In Table 1, two groups (groups A and B) were discerned on the basis of their fluoroquinolone resistance profiles. None of the strains possessed any mutation in the gyrB or parE gene. Mutations in amino acid sequences were detected in GyrA and ParC regions of all (100%) fluoroquinolone-resistant strains (group A). However, no single parC mutation was found without the concomitant presence of a gyrA mutation. All fluoroquinolone-resistant strains showed two mutations, one mutation in gyrA at codon 83 (C→T transition), resulting in the replacement of serine (TCG) by leucine (TTG), and one mutation at codon 87 (G→A or A→G transition), resulting in the replacement of aspartic acid (GAC) by asparagine (AAC) or glycine (GGC). Another mutation at codon 80 (G→T transition) of parC resulted in the replacement of serine (AGC) by glutamic acid (GAC); however, no change was found at codon 84 (GAA; glutamic acid) of the gene. Since only the QDRDs of the genes were sequenced and the wild-type susceptibility was not knocked in, we do not rule out the possibility of mutations, although unlikely, present outside the region sequenced.

Our results corroborated earlier findings of mutations in the gyrA gene of quinolone-resistant S. dysenteriae serotype 1 strains (10, 12). A difference in the amino acid substitution at codon 87 of the GyrA subunit of the sporadic strain (this study) and outbreak strain (12) is noteworthy. However, in addition to the gyrA mutation, the present study also reports another substitution at codon 80 of the parC gene of fluoroquinolone-resistant S. dysenteriae serotype 1 strains.

**Nucleotide sequence accession numbers.** The partial sequences of gyrA, gyrB, parC, and parE genes containing QDRDs of fluoroquinolone-susceptible S. dysenteriae serotype 1 strain (IBM 1) reported in this article have been deposited in the GenBank database under accession no. AY648051, AY648052, AY648053, and AY648054, respectively.

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**REFERENCES**


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