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NOTE

Mutations in the GyrA Subunit of DNA Gyrase and the ParC Subunit of Topoisomerase IV in Clinical Strains of Fluoroquinolone-Resistant *Shigella* in Anhui, China

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In this research 26 *Shigella* isolates were examined by PCR and direct nucleotide sequencing for genetic alterations in the quinolone-resistance determining regions (QRDRs). We tested for the presence of *qnr* genes by PCR in 91 strains, but no *qnr* genes were found. The results did show, however, some novel mutations at codon 83 of *gyrA* (Ser→Ile) and codon 64 of *parC* (Ala64→Cys, Ala64→Asp), which were related to fluroquinolone resistance.

Keywords: Shigellosis, DNA gyrase, topoisomerase IV, *qnr* gene

The resistance to multiple antibiotics is common among clinical isolates of *Shigella*. In China, fluoroquinolones have been the primary choice of treatment for shigellosis since multidrug-resistant strains first appeard, however, ciprofloxacin-resistant *Shigella* isolates have also emerged (Bhattacharya *et al.*, 2003; Naheed *et al.*, 2004). Fluoroquinolones resistance appears to be due mainly to alterations in the GyrA subunit of DNA gyrase, or in the ParC subunit of topoisomerase IV; *qnr* genes present in *Shigella* isolates are also responsible for fluoroquinolones resistance.

A total of 91 *Shigella* isolates were collected in 31 hospitals in Anhui, China from September 1 to September 30, 2005. Among the 91 isolates, there were 57 isolates of *S. flexnery*, 31 strains of *S. sonnei*, and 3 strains of *S. boydii*. The susceptibility of the 91 isolates to fluoroquinolones was tested by the agar dilution method in accordance with the 2005 recommendations of the Clinical Laboratory Standards Institute (CLSI, 2005).

The resistant rates of the isolates to nalidixic acid, ciprofloxacin, and levofloxacin reached 97.80%, 27.47%, and 23.08%, respectively. The *gyrA* and *gyrB* genes of DNA gyrase and the *parC* and *parE* genes of topoisomerase IV in 26 *Shigella* isolates (20 isolates showing ciprofloxacin resistance at ≥4 ug/ml, and 6 isolates selected showing ciprofloxacin resistance at <4 μg/ml) were amplified by PCR in a thermal cycler (Biometra, Germany). The *qnrA* genes of the 91 strains were also identified by PCR using the following primers: 5′-GCA AGAGGATTTCTCACGCCAGGAT-3′ and 5′-TCGGCAA AGGTCAGGTCACAGC-3′ (476 bp). In addition, other *qnr* genes were identified according to previously described (Robicsek *et al.*, 2006a) using the primers 5′-GATCGTGA AAGCCAGAAAGG-3′ and 5′- ACGATGCCTGGTAGTTGT CC-3′ for *qnrB* (469 bp), 5′-ACGACATTCGTCAACTGCA A-3′ and 5′-TAAATTGGCACCCTGTAGGC-3′ for *qnrS* (417 bp). The amplifications of the *gyrA, gyrB, parC,* and *parE* genes were performed under previously described conditions (Dutta *et al.*, 2005) using the following primers: 5′-TACACC GGTCAACATTGAGG-3′ and 5′-TTAATGATTGCCGCCG TCGG-3′ for *gyrA* (648 bp), 5′-TGAAATGACCCGCCGTA AAGG-3′ and 5′-GCTGTGATAACGCAGTTTGTCCGGG-3′ for *gyrB* (309 bp), 5′-GTACGTGATCATGGACCGTG-3′ and 5′-TTCGGCTGGTCGATTAATGC-3′ for *parC* (531 bp), and 5′-ATGCGTGCGGCTAAAAAAGTG-3′ and 5′-TCGTC GCTGTCAGGATCGATAC-3′ for *parE* (290 bp). All the purified PCR products were sequenced with an ABI PRISM 377 type DNA sequencer and BigDye Terminator v3.1 cycle sequencing kit. An ABI PRISM 3730 type DNA Analyzer was also used for the sequencing.

There was no *qnr* gene for quinolone resistance contained within the multiresistance-encoding plasmid, and all the fluoroquinolone-resistant strains showed mutations in *gyrA* and/or *parC*. Also, no mutations in the *gyrB* or *parE* gene was found. However, we did find a total of 42 mutations in *gyrA* and *parC* in the 26 isolates. Three of the ciprofloxacin-resistance isolates had a novel mutation at codon 64 of *parC* (Ala64→Cys for two *S. sonnei* isolates and Ala64→Asp for one *S. flexnery* isolate). The mutation at codon 83 of *gyrA* (TCG→ATC) is the first to be reported in *Shigella* isolates. The other mutations found in this study have been reported previously (Ahamed *et al.*, 1999; Dutta *et al.*, 2005). The most common mutation found in 20 of the isolates (47.62% of mutations) was at codon 83 of *gyrA* (TCG→ATC and TCG→TTG transition), resulting in the replacement of serine by isoleucine and leucine. Of these,

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Table 1. Alterations in *gyrA* and *parC* in clinical *shigella* isolates

No. of strains	MICs (μg/ml)[a]			Nucleotide and amino acid change[b]			
				GyrA position		*ParC* position	
	NAL	CIP	LVX	Ser83 (TCG)	Asp87 (GAC)	Ala64 (GCC)	Ser80 (AGC)
5	> 256	0.06-0.25	0.0154-0.25	—	—	—	—
1	> 256	0.5	0.5	Ile (ATC)	—	—	—
5	> 256	1-8	2-4	Leu (TTG)	—	—	—
1	> 256	4	4	—	—	—	Ile (ATC)
2	> 256	4	4	Leu (TTG)	—	—	Ile (ATC)
2	> 256	4-8	4-8	Ile (ATC)	—	—	Ile (ATC)
1	> 256	8	4	Leu (TTG)	Gly (GGC)	—	Ile (ATC)
3	> 256	4-16	8-16	Leu (TTG)	Asn (AAC)	—	—
1	> 256	8	8	Leu (TTG)	Asn (AAC)	—	Ile (ATC)
1	> 256	16	16	Thr (ACC)	Asn (AAC)	—	—
1	> 256	16	16	Leu (TTG)	Asn (AAC)	—	Ile (ATC)
2	> 256	16	16	Leu (TTG)	—	Cys (TGC)	—
1	> 256	16	32	Leu (TTG)	—	Asp (GAC)	Ile (ATC)

[a]NAL=nalidixic acid; CIP=ciprofloxacin; LVX= levofloxacin.
[b]No change in the amino acid sequence.

eight isolates had an additional mutation, and six isolates had two additional mutations.

The ciprofloxacin MICs for all isolates without mutations and the one with a single *gyrA* mutation (Ser83→Ile) were ≤0.5 μg/ml. Previously in *Shigella* isolates, strains where the MIC of ciprofloxacin was ≥0.125 μg/ml and ≤2 μg/ml were considered to have reduced susceptibility to ciprofloxacin (Hirose *et al.*, 2005), and it has been reported that decreased susceptibility to ciprofloxacin (MIC: 0.125 to 2 μg/ml) was associated with a mutation at codon 83 of *gyrA* in *S. sonnei* strains (Hirose *et al.*, 2005). The MICs for isolates with a single *gyrA* mutation (Ser83→Leu) were higher than the MIC of the isolate with a single *gyrA* mutation (Ser83→Ile) (Table 1). The most common mutations of *parC* in this report were at codon 80. However, the mutation at codon 80, along with one other *gyrA* mutation, caused only slight resistance to fluoroquinolones. It has been suggested that a mutation at Glu84 of *parC* appears to have more deleterious effects than one at Ser80 (Hiasa, 2002). The novel mutation at codon 64 of *parC*, plus one other *gyrA* mutation, conferred a higher level of resistance to fluoroquinolones than the mutation at codon 80 of *parC* together with the other *gyrA* mutation. The presence of a *parC* mutation (Ala64→Asp) plus a *gyrA* mutation at codon 83 and a *parC* mutation at codon 80 was associated with the highest level of resistance in the study.

The strains in this study may present an additional quinolone-resistance mechanism such as the simultaneous overexpression of efflux pump systems and the alteration of outer membrane proteins, which can decrease intracellular drug accumulation (Mark *et al.*, 2001). They may also contain the *aac(6')-Ib-cr* gene, which can modify ciprofloxacin to N-acetylate ciprofloxacin, increasing the MIC of CIP in *Shigella* clinical isolates (Robicsek *et al.*, 2006b). Therefore, we will focus on these areas in further studies.

Nucleotide sequence accession numbers

The partial sequences of the *gyrA* and *parC* genes reported in this article have been deposited in the GenBank database and assigned accession numbers DQ898178, DQ898179, DQ898180, and DQ898305.

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