Characterization of fluoroquinolone-resistant *Shigella flexneri* in Hangzhou area of China

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**Objectives:** The aim of this study was to characterize fluoroquinolone-resistant *Shigella* and determine whether the *qnr* and *aac(6')-Ib-cr* genes could contribute to sporadic shigellosis in the clinic in the Hangzhou area of China.

**Methods:** A total of 202 strains of *Shigella* (79 *Shigella sonnei* and 123 *Shigella flexneri*) isolated from sporadic cases of shigellosis from 1998 to 2007 were analysed for their antimicrobial susceptibility. The *gyrA*, *gyrB*, *parC*, *parE*, *qnr* and *aac(6')-Ib-cr* genes and the profiles and incompatibility of plasmids were characterized. Chromosomal DNA fingerprinting was determined by *Xba*I-based digestion and PFGE.

**Results:** All strains of *S. sonnei* were susceptible to fluoroquinolones (ciprofloxacin and levofloxacin) while 15 out of 123 strains of *S. flexneri* were resistant. All of the 15 resistant strains displayed common mutations in the *gyrA* and *parC* genes and formed eight distinct groups with unique molecular characteristics. Notably, 10 isolates showed mutations at codon 87 of *gyrA*, and the other 5 were *qnrS*-positive. Two strains were positive for the *aac(6')-Ib-cr* gene. Importantly, this is the first report of *qnrS*- and *aac(6')-Ib-cr*-positive *Shigella* in China, the *qnrS*-positive *S. flexneri* serotypes 1a, 2a and 4c and the *aac(6')-Ib-cr*-positive *S. flexneri* serotypes 2a and 4c worldwide.

**Conclusions:** The common mutations at position 83 of *gyrA* and position 80 of *parC* were crucial for resistance to nalidixic acid in *S. flexneri*. The mutation at position 87 of *gyrA* or the presence of the *qnrS* gene is necessary for high-level resistance to fluoroquinolones in *Shigella* isolates from China.

**Keywords:** antibiotic resistance, *qnrS*, *aac(6')-Ib-cr*, plasmid replicon

Introduction

Diarrhoea caused by *Shigella* species is a major public health problem in developing countries because many cases are caused by drug-resistant *Shigella*.1,2 Cases infected with fluoroquinolone-resistant *Shigella* have been reported in some areas of China, but not in Hangzhou.1 Fluoroquinolone resistance is mainly mediated by mutations in the genes encoding DNA gyrase and topoisomerase IV, i.e. the *gyrA*, *gyrB*, *parC* and *parE* genes, in the quinolone resistance-determining regions (QRDRs).3 It is also caused by the plasmid-borne genes *qnrA*, *qnrB* and *qnrS* in many strains of Enterobacteriaceae, including *Shigella*, worldwide.4–7 However, since the *qnrS* gene was first identified in isolates of *Shigella flexneri* 2b in Japan in 2005,8 no new case related to *Shigella* has been reported worldwide. The *aac(6')-Ib-cr* gene is carried by a plasmid, leading to transferable fluoroquinolone resistance in Enterobacteriaceae,9 and was recently identified in isolates of *Shigella boydii* serotype 1 and *S. flexneri* 3b.2 However, *aac(6')-Ib-cr*-mediated fluoroquinolone resistance in *Shigella* has not been reported in China. Here, we present novel strains of fluoroquinolone-resistant *Shigella* from the Hangzhou area of China.

Materials and methods

**Bacterial strains**

A total of 202 strains of *Shigella* species (79 *Shigella sonnei* and 123 *S. flexneri*), which included 8 of serotype 1a, 22 of 2a, 1 of 3a,
13 of 4a, 21 of 2b, 1 of 3b, 1 of 4b, 43 of 4c, 2 of 6f, 7 of X variant and 4 of Y variant) were isolated from hospitalized patients with sporadic cases of shigellosis in Hangzhou, Zhejiang Province, China, from 1998 to 2007. Individual isolates were analysed by standard biochemical tests and serotyped using antisera (DENKA SEIKEN, Oakthorpe, Swadlincote, Derbyshire, UK).

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility was determined by the disc diffusion method, in accordance with CLSI (formerly NCCLS) guidelines (M7-A5) for nalidixic acid (30 µg disc), ciprofloxacin (5 µg disc) and levofloxacin (10 µg disc). *Escherichia coli* ATCC 25922 was used for quality control.

**Isolation of plasmids**

The plasmids from fluoroquinolone-resistant strains were extracted using the plasmid Miniprep kit (QIAGEN), according to the manufacturer’s instructions. Plasmid profiles were characterized by electrophoresis using a 1% agarose gel.

**Analysis of mutations in the QRDRs**

A total of 15 strains of fluoroquinolone-resistant *S. flexneri*, together with 8 strains of *S. flexneri* that were susceptible to fluoroquinolones (ciprofloxacin and levofloxacin), 4 susceptible to nalidixic acid and the other 4 resistant, were grown in Luria–Bertani (LB) medium.

**Characterization of the qnr and aac(6’)-Ib-cr genes**

The qnrA, qnrB and qnrS genes were characterized by multiplex PCR. The sequences of the primers specific for the qnrS gene were forward 5’-TGGAAACCTACAATCATATCG-3’ and reverse 5’-TTAGTCAGGATAAACAACAATACCC-3’, which produced a 656 bp fragment. The primers for the aac(6’)-Ib-cr gene were forward 5’-GCAAACGCAAATAACAAGTTAGG-3’ and reverse 5’-GTGTTTGAACCATGTACA-3’, which generated a 560 bp product. All positive PCR products were further sequenced.

**PCR-based plasmid replicon typing**

All fluoroquinolone-resistant isolates carried the common mutations in the QRDRs

**PFGE and plasmid analysis**

The DNA fingerprinting of 15 isolates showed eight distinct *XbaI* restriction patterns by PFGE and the similarity among them was from 75% to 92%, determined by Dice’s coefficient analysis (data not shown). Interestingly, two isolates (11 and 12) that were negative for *qnr* and *aac(6’)-Ib-cr* genes were identical, which was similar to isolate 13 carrying both the *qnr* and *aac(6’)-Ib-cr* genes. Furthermore, the profiles of plasmids from the fluoroquinolone-resistant isolates showed no association with the presence of the *qnr* and *aac(6’)-Ib-cr* genes (Figure 1). Thus, it is unlikely that those strains of fluoroquinolone-resistant *S. flexneri* originated from a progenitor.
Table 1. Characteristics of the fluoroquinolone-resistant *S. flexneri*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>Year</th>
<th>MIC (mg/L)</th>
<th>Nucleotide and amino change</th>
<th>ParC position</th>
<th>PFGE</th>
<th>qnrS</th>
<th>aac(6')-Ib-cr</th>
<th>Inc</th>
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<tr>
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<td>LEV</td>
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<td>[GAC(Asp)]</td>
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<td>GGC (Gly)</td>
<td>ATC (Ile)</td>
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aNAL, nalidixic acid; CIP, ciprofloxacin; LEV, levofloxacin.
We found that no isolate of *S. sonnei* showed fluoroquinolone resistance while 15 strains of *S. flexneri* had different levels of resistance to fluoroquinolones. The percentage of fluoroquinolone-resistant *S. flexneri* was similar to that in other areas of China. Notably, one strain of fluoroquinolone-resistant *S. flexneri* 2a was isolated from the Hangzhou area in 1998. However, the incidence of shigellosis with fluoroquinolone-resistant *S. flexneri* in this area remains at a stable level, which differs from a report from India. The low incidence may be caused by the changes in socioeconomic conditions and education in this area during the past 10 years. This also suggests that fluoroquinolone antibiotics may still be effective for many incidences of shigelloses in this area.

Genetic characterization of fluoroquinolone resistance revealed that all strains of quinolone-resistant *S. flexneri* had mutations at position 83 of gyrA and at position 80 of parC of the QRDRs, similar to that in isolates from other areas. These mutations are responsible for mediating quinolone resistance. Notably, these 15 strains of *S. flexneri* either showed mutation at position 87 of gyrA or carried the *qnrS* gene. However, four strains of *S. flexneri* that were resistant to nalidixic acid, but susceptible to ciprofloxacin and levofloxacin, displayed neither the mutation at position 87 (Asp) of the *gyrA* gene nor the *qnrS* gene.

Interestingly, the *qnrS* gene was first detected in a *S. flexneri* serotype 1a isolate in 2002, earlier than the first report in Japan in 2005. Importantly, the *qnrS*-positive strains of *S. flexneri* showed high-level resistance to fluoroquinolones. Therefore, the plasmid-mediated *qnrS* gene may have been present in different serotypes of *S. flexneri* in China for a while. To our knowledge, this is not only the first report of *S. flexneri* serotypes 1a, 2a and 4c carrying the *qnrS* gene, but also the first report of *qnr*-positive *Shigella* isolated from China.

The *aac(6′)-Ib-cr* gene was recently identified in many Enterobacteriaceae and is responsible for high-level resistance to fluoroquinolones. We found that two strains of *S. flexneri* (serotypes 2a and 4c) carried the *aac(6′)-Ib-cr* gene and expressed high levels of fluoroquinolone resistance, particularly to ciprofloxacin. This is the first report of *aac(6′)-Ib-cr*-positive *S. flexneri* 2a and 4c worldwide. The *aac(6′)-Ib-cr*-positive *S. flexneri* 2a was isolated in 1998, suggesting that the *aac(6′)-Ib-cr* gene had been present in China for many years. Interestingly, we found that isolate 13 carried both the *qnrS* and *aac(6′)-Ib-cr* genes and had high-level resistance to fluoroquinolones, similar to *E. coli* isolates that carry the *aac(6′)-Ib-cr* and *qnrS* genes. Therefore, surveillance for plasmid-mediated fluoroquinolone resistance genes may be an ideal strategy for the effective control of shigellosis.

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**Transparency declarations**

None to declare.

**References**


