Prevalence of plasmid-mediated quinoline resistance determinants in association with β-lactamases, 16S rRNA methylase genes and integrons amongst clinical isolates of Shigella flexneri

Shigellae remain a public health concern throughout the world (Kotloff et al., 1999). However, the emergence of multidrug resistance (MDR) amongst clinical isolates has made the selection of effective antimicrobial therapy more difficult (Niyogi, 2007). Quinolones are among the most important antibacterial agents used extensively for the treatment of shigellosis. Recently, quinolone resistance has been rising among Shigella isolates (Xiong et al., 2010). Quinolone resistance mainly results from chromosomal mutations in the quinolone resistance-determining regions (QRDRs) of DNA gyrase and topoisomerase IV (Dutta et al., 2005). However, since 1998, three kinds of plasmid-mediated quinolone resistance (PMQR) determinants have been described: Qnr, Aac{6’}-Ib-cr and QepA. These mechanisms are prevalent amongst common clinical isolates and have been detected conferring low-level resistance to quinolones (Cattoir & Nordmann, 2009). Co-existence of resistance genes, such as β-lactamases genes, 16S rRNA methylase genes and integrons, on the same plasmid could, in part, explain the appearance of MDR strains. These responsible genes are primarily located on transferable plasmids and could enhance the acquisition and dissemination of antimicrobial resistance genes by horizontal transfer (Luo et al., 2011; Hu et al., 2011). We investigated the prevalence of PMQR determinants in clinical isolates of Shigella flexneri, and assessed the relatedness of β-lactamase genes, 16S rRNA methylase genes and integrons with PMQR determinants within the same strain.

A total of 125 S. flexneri isolates were collected from six hospitals in Anhui, China, from September 2007 to October 2010. The susceptibility of the 125 isolates to 16 antimicrobial agents was determined by the agar dilution method, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2010). All clinical Shigella isolates were screened for the presence of qnr, aac(6’)-Ib-cr and qepA genes using the methods described previously (Xiong et al., 2010; Yamane et al., 2007). Different β-lactamases genes, plasmid-borne bla{AmpC}, 16S rRNA methylase genes, integrons and mutations in the QRDRs were analysed by PCR for the PMQR-positive isolates (Dallenne et al., 2010; Doi & Arakawa, 2007; Hu et al., 2011; Dutta et al., 2005). All purified PCR products were directly sequenced by the dideoxy chain-termination method using an ABI 3130 DNA Sequencer (Applied Biosystems). Conjugation experiments were carried out in Luria–Bertani broth with sodium azide-resistant Escherichia coli 153 as the recipient using the methods described previously (Wang et al., 2003). Bacterial plasmid DNA was extracted from the 12 PMQR-positive clinical isolates and transconjugants by using the rapid alkaline lysis protocol (Kado & Liu, 1981). All transconjugants were confirmed by PCR as carrying plasmid-mediated resistance genes. DNA fingerprinting profiles were analysed by pulsed-field gel electrophoresis (PFGE) according to the procedures developed by the US Centers for Disease Control and Prevention (CDC) Pulse Net program.

In total, 12 (9.6%) of the 125 isolates carried at least one PMQR gene. The dominant PMQR gene amongst S. flexneri isolates was aac(6’)-Ib-cr (6/125, 4.8%). One of the aac(6’)-Ib-cr-positive isolates co-harboured the qnrB6 gene (Table 1). This agrees with a previous report that qnr alleles were frequently co-expressed with aac(6’)-Ib-cr on the same plasmid (Luo et al., 2011). qnrS was the most frequent allele (4/125, 3.2%) of the qnr genes, followed by qnrB, which was identified in two (1.6%) of the 125 S. flexneri isolates. However, qnrA, which was reported to be the most common qnr allele among the S. flexneri isolates in China (Xiong et al., 2010), was not found. In this study, all three qnrS-positive isolates belonged to PFGE cluster B, indicating that clonal spread was responsible for the dissemination of the qnrS2 gene amongst the S. flexneri isolates. Five (41.7%) PMQR-positive isolates with high-level resistance to ciprofloxacin (MICs ≥16 μg ml⁻¹) had two mutations in the gyrA (S83L or D87N or D87Y) and parC (S80I) genes (Table 1). However, over one-half (58.3%) of the 12 PMQR-positive isolates exhibited decreased susceptibility to ciprofloxacin (MICs 0.5–2 μg ml⁻¹) or low-level resistance to ciprofloxacin (MICs 4–8 μg ml⁻¹), most (85.7%, 6/7) of which had no QRDR mutations or only one mutation in the gyrA or parC genes. This suggests that most of the PMQR determinants provide only decreased susceptibility to fluoroquinolones or low-level resistance to fluoroquinolones (Cattoir & Nordmann, 2009).

All the 12 PMQR-positive isolates that carried three or more resistance determinants on the same strain were multiresistant to at least five antimicrobial agents. For example, the isolate S121, which harboured at least seven resistance genes, was resistant to 15 antimicrobial agents (Table 1). Of the PMQR-positive isolates, 91.7% co-harboured β-lactamase genes. The dominant β-lactamase gene in this study was bla{CTX-M-1} (9/12, 75%), which encoded resistance to ampicillin. Interestingly, all qnrS-positive isolates that were resistant to at least 12 antimicrobial agents co-harboured bla{CTX-M-1} and class 2 integrons, suggesting that these determinants exhibited a significant correlation amongst the S. flexneri isolates. The bla{CTX-M} genes (especially bla{CTX-M-14}).
Table 1. Characteristics of the 12 multidrug-resistant isolates carrying plasmid-mediated quinolone resistance (PMQR) determinants

QRDRs, quinolone resistance-determining regions; AMP, ampicillin; CTX, cefotaxime; CIP, ciprofloxacin; CAZ, ceftazidime; FEP, cefepime; FOX, cefoxitin; NAL, nalidixic acid; CIP, ciprofloxacin; LEV, levofloxacin; NOR, norfloxacin; GAT, gatifloxaclin; GM, gentamicin; AMK, amikacin; CHL, chloramphenicol; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>PFGE type</th>
<th>PMQR determinant(s)</th>
<th>Mutation in QRDRs</th>
<th>β-Lactamases</th>
<th>Methylase</th>
<th>Integron genes</th>
<th>Resistance profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>S12</td>
<td>A</td>
<td>aac(6′)-Ib-cr</td>
<td>S83L</td>
<td>CTX-M-14</td>
<td>OXA-1</td>
<td>intI1, intI2</td>
<td>AMP, CTX, NAL, LEV, GAT, GM, TET, CHL, SXT</td>
</tr>
<tr>
<td>S21</td>
<td>G</td>
<td>qepA</td>
<td>D87Y S80I</td>
<td>OXA-1</td>
<td>RmtB</td>
<td>intI1, intI2</td>
<td>AMP, NAL, CIP, LEV, NOR, GM, AMK, TET, CHL, SXT</td>
</tr>
<tr>
<td>S36</td>
<td>A</td>
<td>aac(6′)-Ib-cr</td>
<td>S83L</td>
<td>OXA-1</td>
<td></td>
<td>intI1, intI2</td>
<td>AMP, CTX, FEP, NAL, CIP, GM, TET, CHL, SXT</td>
</tr>
<tr>
<td>S43</td>
<td>E</td>
<td>aac(6′)-Ib-cr</td>
<td>S83L</td>
<td>CTX-M-14</td>
<td>OXA-1</td>
<td>intI1</td>
<td>AMP, NAL, CIP, LEV, GAT, NOR, GM, TET, CHL, SXT</td>
</tr>
<tr>
<td>S55</td>
<td>A</td>
<td>qnrB4</td>
<td>D87N S80I</td>
<td></td>
<td></td>
<td>intI1</td>
<td>AMP, CTX, CAZ, NAL, CIP, LEV, GAT, NOR, GM, TET, CHL, SXT</td>
</tr>
<tr>
<td>S69</td>
<td>H</td>
<td>qnrS2</td>
<td>S83L S80I</td>
<td>CTX-M-14</td>
<td>DHA-1</td>
<td>ArmA</td>
<td>intI2</td>
</tr>
<tr>
<td>S104</td>
<td>B</td>
<td>qnrS2</td>
<td>S83L S80I</td>
<td>CTX-M-14</td>
<td>OXA-1</td>
<td>intI1, intI2</td>
<td>AMP, CTX, FEP, NAL, CIP, LEV, GAT, NOR, GM, TET, CHL, SXT</td>
</tr>
<tr>
<td>S120</td>
<td>B</td>
<td>qnrS2</td>
<td>S83L S80I</td>
<td>CTX-M-14</td>
<td>DHA-1</td>
<td>RmtB</td>
<td>intI1, intI2</td>
</tr>
<tr>
<td>S121</td>
<td>B</td>
<td>qnrS2</td>
<td>S83L S80I</td>
<td>CTX-M-14</td>
<td>OXA-1</td>
<td>intI1, intI2</td>
<td>AMP, NAL, CIP, NOR, TET, CHL, SXT</td>
</tr>
<tr>
<td>S123</td>
<td>D</td>
<td>aac(6′)-Ib-cr, qnrB6</td>
<td>S83L</td>
<td></td>
<td></td>
<td>intI2</td>
<td>AMP, NAL, CIP, LEV, GAT, NOR, GM, TET, CHL, SXT</td>
</tr>
<tr>
<td>S125</td>
<td>C</td>
<td>qnrS1</td>
<td>S83L</td>
<td>OXA-1</td>
<td></td>
<td>intI2</td>
<td>AMP, NAL, CIP, LEV, GAT, NOR, GM, TET, CHL, SXT</td>
</tr>
</tbody>
</table>
which prefer hydrolysing ceftazidime than ceftazidime, are the main type of ESBLs in Enterobacteriaceae isolates, including species of Shigella in China (Xiong et al., 2010). However, only 58.3% of the PMQR-positive isolates were positive for the blaCTX-M gene in this study. In addition, 66.7% of the aac(6’)-Ib-cr-positive isolates harboured blaCTX-M or blaCTX-M genes, and 50% of the aac(6’)-Ib-cr-positive isolates harboured class 1 or class 2 integrons, indicating that the presence of the aac(6’)-Ib-cr gene showed a correlation with the prevalence of blaCTX-M and blaCTX-M genes and integrons. Although only three (25%) of the 12 PMQR-positive isolates carried 16S rRNA methylase genes, all the isolates exhibited high-level amikacin resistance (MICs >256 µg ml⁻¹), indicating that 16S rRNA methylase genes have an important effect on the emergence of amikacin-resistant strains amongst clinical S. flexneri isolates.

The plasmids (~23 kb) of 10 (83.3%) of the 12 PMQR-positive S. flexneri isolates were successfully transferred to the recipients, suggesting that the dissemination of PMQR determinants is mostly due to the transmission of plasmids by horizontal exchange. Genotypic analysis of transconjugants showed that β-lactamase genes and 16S rRNA methylase genes were co-transferred with PMQR determinants to the recipients. Two intI1-positive isolates (S21, S43), which carried dfrA17-aadA5 gene cassettes, were also co-transferred. However, no class 2 integrons were transferable by conjugation, suggesting that class 2 integrons might be not located on plasmids. The co-existence of these resistance determinants on transferable plasmids may lead to the emergence and spread of MDR pathogens rapidly in various species in many countries. Furthermore, we also observed genetic relationships between the PMQR-positive S. flexneri isolates. In conclusion, continuous surveillance of the prevalence and correlation of resistance determinants amongst clinical isolates will be required to provide effective treatment and prevent the emergence and spread of MDR isolates.

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