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

Shigellosis remains 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 amongst 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-lactamase 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., 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 qnrS2-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 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 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 blaCTX-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 blaCTX-M-1 and class 2 integrons, suggesting that these determinants exhibited a significant correlation amongst the S. flexneri isolates. The blaCTX-M genes (especially blaCTX-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, gatifloxacin; 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></td>
<td></td>
<td></td>
<td>gyrA</td>
<td>parC</td>
<td></td>
<td></td>
<td></td>
</tr>
<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</td>
<td>S80I</td>
<td>OXA-1</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></td>
<td></td>
<td>RmtB</td>
<td>AMP, NAL, 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, intI2</td>
<td>AMP, CTX, FEP, NAL, CIP, GM, TET, CHL, SXT</td>
</tr>
<tr>
<td>S55</td>
<td>A</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>S69</td>
<td>H</td>
<td>qnrB4</td>
<td>D87N</td>
<td>S80I</td>
<td></td>
<td></td>
<td>AMP, CIP, LEV, NOR, GM, TET, CHL, SXT</td>
</tr>
<tr>
<td>S100</td>
<td>F</td>
<td>aac(6')-Ib-cr</td>
<td>S83L</td>
<td>CTX-M-14</td>
<td>OXA-1</td>
<td>ArmA</td>
<td>AMP, CTX, CAZ, NAL, CIP, LEV, GAT, NOR, GM, TET, CHL, SXT</td>
</tr>
<tr>
<td>S104</td>
<td>B</td>
<td>qnrS2</td>
<td>S83L</td>
<td>CTX-M-14</td>
<td>OXA-1</td>
<td>intI2</td>
<td>AMP, CTX, CAZ, NAL, CIP, LEV, GAT, NOR, GM, TET, CHL, SXT</td>
</tr>
<tr>
<td>S120</td>
<td>B</td>
<td>qnrS2</td>
<td>S83L</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>S121</td>
<td>B</td>
<td>qnrS2</td>
<td>S83L</td>
<td>CTX-M-14</td>
<td>OXA-1</td>
<td>DHA-1</td>
<td>intI1, intI2</td>
</tr>
<tr>
<td>S123</td>
<td>D</td>
<td>aac(6')-Ib-cr, qnrB6</td>
<td>S83L</td>
<td>CTX-M-14</td>
<td>OXA-1</td>
<td>RmtB</td>
<td>intI1, intI2</td>
</tr>
<tr>
<td>S125</td>
<td>C</td>
<td>qnrS1</td>
<td>S83L</td>
<td>OXA-1</td>
<td></td>
<td></td>
<td>intI2</td>
</tr>
</tbody>
</table>
which prefer hydrolysing cefotaxime 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
blaOXA-1 and 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 blaOXA-1 and blaCTX-M genes and integrons.
Although only three (25%) of the 12
PMQR-positive isolates harboured 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.

Acknowledgements
This study was supported by the Natural Science Foundation of China (no. 30972631)
and by the Provincial Natural Science Foundation Key Program of Higher Education
of China (no. KJ2010A344).

Yanyan Liu,1,2,4,1 Lifen Hu,1,2,3,4,1 Yachao Pan,1,2,4 jun Cheng,1,2,4 Yulin Zhu,1,2,4 Ying Ye1,2,4
and Jiabin Li1,2,4
1Department of Infectious Diseases, First Affiliated Hospital of Anhui Medical
University, Hefei, Anhui, PR China
2Institute of Bacterial Resistance, Anhui Medical University, Hefei, Anhui, PR
China
3Department of Center Laboratory, First Affiliated Hospital of Anhui Medical
University, Hefei, Anhui, PR China
4Anhui Center for Surveillance of Bacterial Resistance, Hefei, Anhui, PR
China
Correspondence
Jiabin Li
(lijiabin948@vip.sohu.com)
†These authors contributed equally to this work/paper.

Cattoir, V. & Nordmann, P. (2009). Plasmid-
mediated quinolone resistance in Gram-negative
bacterial species: an update. Curr Med Chem 16,
1028–1046.

CLSI (2010). Performance standards for
antimicrobial susceptibility testing. Twentieth
informal informational supplement. Document M100–
S20. Wayne, PA.

Dallenne, C., Da Costa, A., Decré, D., Favier, C.,
& Arlet, G. (2010). Development of a set of
multiplex PCR assays for the detection of genes
encoding important β-lactamases in
Enterobacteriaceae. J Antimicrob Chemother 65,
490–495.

RNA methylation: emerging resistance
mechanism against aminoglycosides. Clin Infect
Dis 45, 88–94.

Dutta, S., Kawamura, Y., Ezaki, T., Nair, G. B.,
GyrA subunit of DNA gyrase and the ParC
subunit of topoisomerase IV in quinolone-
resistant Shigella dysenteriae serotype 1 clinical
isolates from Kolkata, India. Antimicrob Agents
Chemother 49, 1660–1661.

Hu, L. F., Chang, X., Ye, Y., Wang, Z. X., Shao,
Stenotrophomonas maltophilia resistance to
trimethoprim/sulfamethoxazole mediated by
acquisition of sul and dfrA genes in a plasmid-
mediated class 1 integron. Int J Antimicrob Agents
37, 230–234.

for detection and isolation of large and small

Kotloff, K. L., Winickoff, J. P., Ivanoff, B.,
Clemens, J. D., Swerdlow, D. L., Sansonetti,
Global burden of Shigella infections: implications
for vaccine development and implementation of
control strategies. Bull World Health Organ 77,
651–666.

Luo, Y. P., Yang, J. Y., Zhang, Y. J., Ye, L. Y.,
Wang, L. L. & Guo, L. (2011). Prevalence of β-
lactamases and 16S rRNA methylase genes
amongst clinical Klebsiella pneumoniae isolates
carrying plasmid-mediated quinolone resistance

resistance – an emerging problem in the
treatment of shigellosis. Clin Microbial Infect
13, 1141–1143.

Wang, M., Tran, J. H., Jacoby, G. A., Zhang, Y.,
Wang, F. & Hooper, D. C. (2003). Plasmid-
mediated quinolone resistance in clinical isolates of
Escherichia coli from Shanghai, China. Antimicrob Agents Chemother 47,
2242–2248.

Xiong, Z., Li, J., Li, T., Shen, J., Hu, F. & Wang, M.
(2010). Prevalence of plasmid-mediated
quinolone-resistance determinants in Shigella
flexneri isolates from Anhui Province, China.
J Antibiot (Tokyo) 63, 187–189.

Yamane, K., Wachino, J., Suzuki, S., Kimura, K.,
Shibata, N., Kato, H., Shibayama, K., Konda, T.,
fluoroquinolone efflux pump, QepA, found in
an Escherichia coli clinical isolate. Antimicrob
Agents Chemother 51, 3354–3360.