Plasmid-Mediated qepA Gene among *Escherichia coli* Clinical Isolates, Japan

Kunikazu Yamane*, Jun-ichi Wachino, Satowa Suzuki, and Yoshichika Arakawa

Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases, Tokyo, Japan.

Key words: Fluoroquinolone resistance, *Escherichia coli, qnr, qepA*, plasmid-mediated efflux pump

*Corresponding author. Mailing address: Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashi-Murayama, Tokyo 208-0011, Japan. Phone: +81-42-561-0771. FAZ: +81-42-561-7173. E-mail: kazuwa@nih.go.jp
Abstract

751 Escherichia coli clinical isolates collected from 140 Japanese hospitals between 2002 and 2006 were screened for the qepA and qnr genes. Two E. coli (0.3%) harbored qepA, but no qnr was identified. The results suggested a low prevalence of E. coli harboring qepA or qnr in Japan. (48 words)
The most common chromosomal mechanism of resistance to fluoroquinolones (FQs) in pathogenic bacteria is amino acid substitutions in the quinolone-resistance determining region (QRDR) of DNA gyrase (GyrA) and/or topoisomerase IV (ParC), which are the main target molecules of FQs (7, 8). Efflux pumps and alteration in the outer membrane proteins also contribute to chromosomal FQ resistance (6).

Plasmid-mediated resistance mechanisms against FQs such as Qnr and AAC(6’)-Ib-cr have also been described (13). We recently identified qepA, a new plasmid-mediated gene responsible for reduced FQ susceptibility from *Escherichia coli* C316, which was isolated in 2002 from the urine of an inpatient in Japan (21) and *qepA* was also reported from *E. coli* 1450 which was isolated from Belgian hospital (13). *qepA* encodes an efflux pump belonging to the major facilitator subfamily (MFS). MICs of norfloxacin, enrofloxacin and ciprofloxacin were 32 to 64 fold higher in the experimental strains expressing QepA compared with the host strain (21).
The MICs of ampicillin, erythromycin, kanamycin, tetracycline, and chemical substances such as carbonyl cyanide \textit{m}-chlorophenylhydrazone (CCCP), acriflavine, rodamine 6G, crystal violet and sodium dodecyl sulfate were not affected, however, indicating that FQs are the specific substrates of QepA. Moreover, an accumulation assay of norfloxacin with or without CCCP, and efflux pump inhibitor, showed that QepA was an FQ-specific MSF type efflux pump (21).

\textit{qnrA} was the first plasmid-mediated gene that conferred resistance to quinolones such as nalidixic acid and increased MICs of FQs, originally reported in \textit{Klebsiella pneumoniae} clinical isolates from the United States (11, 17). Subsequently, two other groups of \textit{qnr} genes, \textit{qnrB} (9) and \textit{qnrS} (5) as well as their variants, have been reported. Qnrs belong to the pentapeptide repeat family and mimic DNA fragments bound to the DNA gyrase (17). The \textit{qnr} genes have been identified from various bacterial species belonging to the family \textit{Enterobacteriaceae} in many countries to
In Japan, *qnrS* was first identified in *Shigella flexneri* (5), and *qnrA* was also identified recently (15, 16). Clinically, *E. coli* is the most frequent cause of urinary tract infections, and FQs are some of the preferred antimicrobial agents for treatment (19). In this study, we investigated the prevalence of *qepA*, as well as *qnrA*, *qnrB* and *qnrS*, among *E. coli* clinical isolates collected from Japanese medical facilities. A total of 751 non-duplicate *E. coli* isolates isolated from patients admitted to 140 medical facilities in Japan between 2002 and 2006 were submitted to our reference laboratory for characterization of the genetic determinants responsible for antimicrobial resistance as well as their genetic relatedness. All isolates were suspended in Luria-Bertani (LB) broth supplemented with 25% glycerol and stored in a -80°C deep freezer until analysis. The isolates were initially screened by growth on LB agar plates containing 0.025 μg/ml of norfloxacin. PCR analyses for *qepA* and the three *qnr* genes were performed for all isolates that grew on the
norfloxacin-containing plates. DNA templates for the PCR were prepared by the standard boiling method. The primer sets used for detection of qnrA, qnrB and qnrS have been described by Cattoir et al (2) and Robicsek et al (14). The pairs of primers designed by Cattoir et al (2) were able to amplify internal fragments with qnrA1 to qnrA6, qnrB1 to qnrB8 and qnrS1 to qnrS2, respectively. A 199-bp fragment of qepA was amplified by PCR with primers QEPA-F: 5’-GCA GGT CCA GCA GCG GGT AG-3’ and QEPA-R: 5’-CTT CCT GCC CGA GTA TCG TG-3’. The pair of primer used for detection of rmtB have been described by Doi et al (4). rmtB is a 16S ribosomal RNA methylase gene that confers resistance to aminoglycosides and was located in close proximity to qepA on a transferable plasmid in E. coli C316 (21). Positive control strains for qnrA, qnrB and qnrS were E. coli J53 (pMG252) (11), E. coli J53(pMG298) (9) and E. coli DH10B(pBC-H2.6) (5), respectively, and that for qepA was E. coli KAM32(pSTVqepA) (21). PCR conditions for qepA were as follows:
initial denaturation at 96°C for 1 min followed by 30 cycles of amplification at 96°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. The final extension step was at 72°C for 5 min. The multiplex PCR condition for the qnr genes has been described previously (2, 14).

Of the 751 E. coli tested, 325 isolates grew on LB agar plates supplemented with 0.025 μg/ml of norfloxacin. Two isolates (0.3%) were positive for qepA and rmtB (E. coli MRY04-1030 and E. coli MRY05-3283). The two isolates were isolated from geographically distant hospitals. However, no qnr gene was detected among the E. coli tested in this study.

Transconjugation analysis was performed with E. coli DH10B as the recipient by the filter mating method (3). Transconjugants were selected on LB agar plates supplemented with streptomycin (50 μg/ml) and amikacin (50 μg/ml), because the plasmid carried rmtB that confers
resistance to amikacin. Plasmids were digested with EcoRI (New England Biolabs, Beverly, MA), and were electrophoresed through 1.0% agarose gel.

FQ resistance was successfully transferred from the two qepA-positive E. coli isolates to E. coli DH10B at a frequency of $10^{-5}$ to $10^{-6}$ cells per recipient cell by conjugation. EcoRI restriction patterns for qepA carrying plasmids are shown in Figure. The results of restriction patterns of pHPA from E. coli C316 and p05283 from E. coli MRY05-283 were very similar. However, those of p05283 and p041060 from E. coli MRY04-1060 were completely different from the other two. Neither of the qepA-positive plasmid conferred resistance to ampicillin.

PFGE was performed with the CHEF-Mapper system (BioRad Laboratories, Hercules, CA). Genomic DNA preparations from E. coli C316, E. coli MRY 04-1060 and E. coli MRY05-283 were digested with XbaI (New England Biolabs) (Figure). PFGE fingerprinting pattern of 3 qepA-positive strains were apparently different from each other.
Antimicrobial susceptibility testing was performed in the *qepA*-positive isolates and their transconjugants by the agar dilution method according to the guidelines recommended by the Clinical and Laboratory Standards Institute (1) (Table). Transconjugants of each *qepA*-positive isolate showed MICs of norfloxacin that were 4 to 5 fold higher than that for the recipient strain. The two *qepA*-positive isolates were also highly resistant to all aminoglycosides tested including amikacin, tobramycin and gentamicin, but susceptible to the third generation cephalosporins and imipenem.

In our previous study, *qepA* and *rmtB* were found to be encoded on the same transferable plasmid, and the analysis of the genetic environment of *qepA* in *E. coli* showed that *qepA* and *rmtB* were likely mediated by a composite transposon flanked by two copies of IS26 (21). Interestingly, an *E. coli* strain positive for both *qepA* and *rmtB* has been isolated also in Belgium (12). The genetic organization of the region containing *qepA* and *rmtB* was very similar to that of *E. coli* C316, suggesting the
qepA-harboring isolates demonstrating pan-resistance to aminoglycosides by production of RmtB may well have already spread worldwide.

Although qnr genes have been identified in *E. coli* and other members of the family *Enterobacteriaceae* isolated from other East Asian countries such as China, Korea and Taiwan (10, 18, 20), the results of our study indicate that *qnr*-harboring *E. coli* is still very rare in Japanese medical facilities.
Acknowledgements

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1 References


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FIG. A: EcoRI restriction profiles of *qepA*-carrying plasmids from the transconjugants. M, l-HindIII marker; lane 1, pHPA from *E. coli* C316, lane 2, p041060 form *E. coli* MRY04-1060, lane 3, p05283 from *E. coli* MRY05-283.

B: PFGE fingerprinting patterns of XbaI-digested total DNA preparation from 3 *E. coli* isolates. M, lambda ladder PFGE marker as a molecular size marker. Lanes 1 to 3, *E. coli* C316, *E. coli* MRY04-1030 and *E. coli* MRY05-3283.
TABLE. Antimicrobial susceptibility of the *qepA* donor, transconjugant and recipient strains

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th><em>E. coli</em> MRY04-1060</th>
<th><em>E. coli</em> MRY05-283</th>
<th><em>E. coli</em> DH10B (p041060)<em>a</em></th>
<th><em>E. coli</em> DH10B (p05283)<em>b</em></th>
<th><em>E. coli</em> DH10B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norfloxacin</td>
<td>&gt; 128</td>
<td>&gt; 128</td>
<td>0.25</td>
<td>0.25</td>
<td>≤ 0.008</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>64</td>
<td>128</td>
<td>0.008</td>
<td>0.015</td>
<td>≤ 0.008</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt; 128</td>
<td>&gt; 128</td>
<td>0.015</td>
<td>0.015</td>
<td>≤ 0.008</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Cefotaxim</td>
<td>0.13</td>
<td>0.13</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.13</td>
<td>0.13</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>&gt; 128</td>
<td>&gt; 128</td>
<td>&gt; 128</td>
<td>&gt; 128</td>
<td>0.5</td>
</tr>
<tr>
<td>Amikacin</td>
<td>&gt; 128</td>
<td>&gt; 128</td>
<td>&gt; 128</td>
<td>&gt; 128</td>
<td>2</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&gt; 128</td>
<td>&gt; 128</td>
<td>&gt; 128</td>
<td>&gt; 128</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*a*, Transconjugant of *E. coli* MRY04-1060.

*b*, Transconjugant of *E. coli* MRY05-283.