Short Communication

Plasmid-Mediated Quinolone Resistance Genes, aac(6’)-Ib-cr, qnrS, qnrB, and qnrA, in Urinary Isolates of Escherichia coli and Klebsiella pneumoniae at a Teaching Hospital, Thailand

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SUMMARY: A total of 121 Escherichia coli (47 extended-spectrum β-lactamase [ESBL] and 74 non-ESBL producers) and 75 Klebsiella pneumoniae isolates (49 ESBL and 26 non-ESBL producers) were collected from urine samples between October 2010 and April 2011 at a university hospital and assessed for the presence of plasmid-mediated quinolone resistance (PMQR) genes. Twenty-seven E. coli (22.3%) and 49 K. pneumoniae (65.3%) isolates harbored PMQR genes, which mostly consisted of aac(6’)-Ib-cr and qnrS, followed by qnrB and qnrA. Among the 76 PMQR-positive isolates, 15 (19.7%) and 2 (2.6%) carried 2 and 3 different PMQR genes, respectively. However, qnrC, qnrD, and qepA were not found in any isolate. The PMQR genes were more prevalent in ESBL producers than in non-ESBL producers (42.6% versus 9.5% in E. coli and 81.6% versus 34.6% in K. pneumoniae). Approximately 35%–60% of the PMQR-positive isolates were susceptible or intermediate susceptible to fluoroquinolones. The enterobacterial repetitive intergenic consensus-PCR method revealed that most PMQR-positive isolates belonged to different strains, indicating the spread of these resistance determinants. PMQR gene transfer by conjugation was successful in 10%–25% of the test donors. This study showed a high prevalence of PMQR genes among both organisms. Clinical use of fluoroquinolones for the treatment of infections caused by fluoroquinolone-susceptible strains harboring PMQR genes may lead to decreased therapeutic efficacy.

The primary mechanism of fluoroquinolone resistance in members of the family Enterobacteriaceae is mutations in the quinolone resistance-determining regions (QRDRs) of the topoisomerase genes encoded on bacterial chromosomes (1). However, plasmid-mediated quinolone resistance (PMQR) was first discovered in a Klebsiella pneumoniae isolate from the United States in 1994 (2). This protein was called the quinolone-resistance protein (later named QnrA1). It involves quinolone resistance by protecting DNA gyrase and topoisomerase IV from quinolones (3). Till date, five Qnr proteins have been identified; QnrA, QnrB, QnrS, QnrC, and QnrD (4). The second PMQR mechanism involves a variant of aminoglycoside acetyltransferase (AAC(6’)-Ib-cr), which can reduce ciprofloxacin activity by adding an acetyl group to this agent (5). The last mechanism of PMQR is the quinolone efflux pump (QepA), a proton-dependent transporter, which causes hydrophilic quinolone resistance, particularly to norfloxacin, ciprofloxacin, and enrofloxacin (6). These forms of PMQR have been reported in clinical isolates of Enterobacteriaceae worldwide (4,7). Because PMQR confers low-level resistance to fluoroquinolones, PMQR-positive isolates remain susceptible to these agents. However, in-vitro studies have shown that the selection of isolates with greater fluoroquinolone resistance can occur in isolates carrying PMQR genes after antimicrobial exposure (2,8). Therefore, the detection of PMQR in Enterobacteriaceae is required. The fluoroquinolone disk diffusion test, which is a simple routine laboratory method, could not detect PMQR-positive isolates because of the low-level resistance. Till date, molecular techniques such as PCR assays are required as confirmatory tests for PMQR detection in epidemiological studies.

In Thailand, QnrA was first reported in Escherichia coli, K. pneumoniae, Enterobacter cloacae, and Enterobacter sakazakii producing VEB-1 extended-spectrum β-lactamase (ESBL) in 1999 (9). Subsequently, QnrS1 was discovered in Salmonella enterica serovar Corvallis isolates from humans, chickens, pigs, cattle, and seafood imported from Thailand and in S. enterica serovar Stanley from Finnish tourists returning from Thailand (10–12), suggesting an international spread of these resistance determinants. In our hospital, susceptibility rates to fluoroquinolones using the disk diffusion tests in 2010 were 13%–22% and 16%–73% in ESBL- and non-ESBL-producing E. coli, respectively, and 24%–57% and 30%–93% in ESBL- and non-ESBL-producing K. pneumoniae, respectively. These ESBL-
PMQR-positive isolates by phenotypic and genotypicisms by PCR techniques and then characterized the detected PMQR genes in urinary isolates of both organ-

gnrae (49 ESBL and 26 non-ESBL producers) were obtained from positive urine cultures (showing bacte-

Among the 75 isolates, 49 (65.3

Table 1. MICs of nalidixic acid, ciprofloxacin, and ofloxacin for the PMQR-positive E. coli and K. pneumoniae isolates

<table>
<thead>
<tr>
<th>Organism (No. of isolates)</th>
<th>PMQR gene (No. of isolates)</th>
<th>Nalidixic acid (mg/L)</th>
<th>Ciprofloxacin (mg/L)</th>
<th>Ofloxacin (mg/L)</th>
<th>No. of ERIC patterns (type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (27) ESBL (20)</td>
<td>qnrS (12)</td>
<td>2–512</td>
<td>0.125–&gt;8</td>
<td>0.25–&gt;8</td>
<td>7 (A, B, F, G, H, I, J)</td>
</tr>
<tr>
<td></td>
<td>aac(6’)-Ib-cr (7)</td>
<td>4–512</td>
<td>0.064–&gt;8</td>
<td>0.125–&gt;8</td>
<td>6 (C, D, E, K, L, M)</td>
</tr>
<tr>
<td></td>
<td>qnrA, qnrS, aac(6’)-Ib-cr (1)</td>
<td>&gt;512</td>
<td>&gt;512</td>
<td>&gt;512</td>
<td>1 (N)</td>
</tr>
<tr>
<td>non-ESBL (7)</td>
<td>aac(6’)-Ib-cr (3)</td>
<td>&gt;512</td>
<td>&gt;512</td>
<td>&gt;512</td>
<td>4 (H, O, P, Q)</td>
</tr>
<tr>
<td>K. pneumoniae (49)</td>
<td>qnrS (12)</td>
<td>2–512</td>
<td>0.016–&gt;8</td>
<td>0.016–&gt;8</td>
<td>5 (B, E, F, G, H)</td>
</tr>
<tr>
<td>ESBL (40)</td>
<td>qnrB, aac(6’)-Ib-cr (9)</td>
<td>32–512</td>
<td>2–&gt;8</td>
<td>&gt;8</td>
<td>11 (D, H, O, R, T, U, V, W, X, Y, Z)</td>
</tr>
<tr>
<td></td>
<td>qnrS, aac(6’)-Ib-cr (2)</td>
<td>8–512</td>
<td>0.5–&gt;8</td>
<td>&gt;8</td>
<td>2 (K, L)</td>
</tr>
<tr>
<td></td>
<td>qnrS, aac(6’)-Ib-cr (1)</td>
<td>8–512</td>
<td>0.5–&gt;8</td>
<td>&gt;8</td>
<td>5 (A, C, M, N, O)</td>
</tr>
<tr>
<td></td>
<td>qnrB, aac(6’)-Ib-cr (1)</td>
<td>&gt;512</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td>2 (A, AG)</td>
</tr>
<tr>
<td></td>
<td>qnrB, aac(6’)-Ib-cr (1)</td>
<td>&gt;512</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td>1 (I, J)</td>
</tr>
<tr>
<td>non-ESBL (9)</td>
<td>qnrS, aac(6’)-Ib-cr (1)</td>
<td>8–512</td>
<td>0.5–&gt;8</td>
<td>&gt;8</td>
<td>5 (S, AA, AB, AC, AD)</td>
</tr>
<tr>
<td></td>
<td>qnrS, aac(6’)-Ib-cr (1)</td>
<td>8–512</td>
<td>0.5–&gt;8</td>
<td>&gt;8</td>
<td>1 (A)</td>
</tr>
<tr>
<td></td>
<td>qnrB, aac(6’)-Ib-cr (1)</td>
<td>&gt;512</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td>1 (AF)</td>
</tr>
</tbody>
</table>
In addition, the nucleotide sequences of the undigested 482-bp DNA fragments from 2 E. coli isolates were identical to that of aac(3′)-Ib-cr. The PMQR genes reported in our hospital were similar to those reported from many countries, particularly the People's Republic of China (7,18,19). They were found more frequently in the K. pneumoniae isolates than in the E. coli isolates (65.3% versus 22.3%). In addition, qnrS1-like was the most prevalent in the E. coli isolates, whereas aac(3′)-Ib-cr was a major PMQR gene found in the K. pneumoniae isolates. However, qnrS was the most common qnr found in both the organisms. QnrS1 was previously reported in non-Typhi Salmonella typhimurium and S. enterica qnrS1 genes (18,20,21). This study reported the presence of qnrA in Enterobacteriaceae and qnrS1 in Salmonella, as described previously (9–12). However, qnrC, qnrD, and qepA were not detected in any isolate.

PMQR gene transfer was performed by a broth culture mating method using E. coli UB1637 (streptomycin resistant) as a recipient (22). Donor and recipient strains were separately grown in Luria-Bertani (LB) broth (Hardy Diagnostics, Santa Maria, Calif., USA) at 37°C for 3–4 h with shaking. Following this, the donor culture was mixed with the recipient culture (1:25) and incubated for 3–4 h at 37°C with shaking. Subsequently, transconjugants were selected on MacConkey agar plates containing 1,600 mg/L of streptomycin (M & H Manufacturing, Samutprakarn, Thailand) with either 16 mg/L of nalidixic acid or 0.05–0.1 mg/L of ciprofloxacin or ofloxacin. The PMQR genes from 7 of 27 (25.9%) E. coli donors and 5 of 43 (11.6%) K. pneumoniae donors were transferred to the recipients as confirmed using PCR methods (Table 2). Only qnrS could be transferred from both organisms, with the exception that one E. coli donor transferred aac(3′)-Ib-cr in addition to qnrS. This study also showed cotransfer of ESBL genes with qnrS, similar to that reported previ-
ously (18,20,23). Resistance to other antimicrobials, including gentamicin, netilmicin, trimethoprim/sulfamethoxazole, and tetracycline, was also cotransferred (Table 2). In a study by Cai et al. (24), transfer of qnr to E. coli C600Lac-SMR was successful in 40% of isolates (10 of 25 donor strains), and qnrS (4 isolates), qnrB (4 isolates), and qnrA (2 isolates) were successfully transferred. The low transfer rate in this study may have been due to the use of different recipient strains or antimicrobials and their concentrations for selecting transconjugants. In addition, conjugation using the filter mating method was more effective than that using the broth mating method (25). The ability to transfer the PMQR genes indicated the potential spread of these resistance determinants among both the organisms.

Minimum inhibitory concentrations (MICs) of nalidixic acid (1–512 mg/L), ciprofloxacin (0.008–8 mg/L), and ofloxacin (0.008–8 mg/L) (Sigma-Aldrich, St. Louis, Mo., USA) for all the PMQR-positive isolates were determined using an agar dilution method, and their susceptibilities to other antimicrobial agents (Oxoid, Basingstoke, Hampshire, England) were determined using a disk diffusion test (26). E. coli ATCC 25922 was used as an antimicrobial-susceptible control. MICs of nalidixic acid, ciprofloxacin, and ofloxacin for the PMQR-positive E. coli isolates ranged from 2 to >512, 0.064 to >8, and 0.125 to >8 mg/L, respectively, with MIC₅₀ of >512, 4, and 8 mg/L, respectively, and MIC₉₀ of >512, >8, and >8 mg/L, respectively, whereas those for the PMQR-positive K. pneumoniae isolates were 2 to >512, 0.016 to >8, and 0.016 to >8 mg/L, respectively, with MIC₅₀ of 16, 2, and 2 mg/L, respectively, and MIC₉₀ of >512, >8, and >8 mg/L, respectively (Table 1). Among the PMQR- and ESBL-positive isolates, 45% (9 of 20) of the E. coli isolates and 32.5% (13 of 40) of the K. pneumoniae isolates had MICs of nalidixic acid, ciprofloxacin, and ofloxacin in susceptible or intermediately susceptible ranges according to the CLSI guidelines (26). All the PMQR-positive E. coli and K. pneumoniae isolates were susceptible to carbapenems; 88.9% and 95.9%, respectively, were susceptible to amikacin; and 33.3% and 36.7%, respectively, were susceptible to trimethoprim/sulfamethoxazole. Although all the transconjugants had quinolone and fluoroquinolone MICs higher than those of E. coli UB1637 (≥16-fold for nalidixic acid, ≥32- to 64-fold for ciprofloxacin, and ≥64- to 128-fold for ofloxacin), their MICs remained in susceptible ranges. These results revealed the low level of fluoroquinolone resistance due to PMQR, as reported previously (2,13,15,27). The treatment of infections caused by fluoroquinolone-susceptible PMQR-positive isolates may select high-level quinolone-resistant strains and lead to clinical failure. Unfortunately, a history of diagnosis, antimicrobial therapy, and outcome of treatment were not available for any patient included in this study. In case of the PMQR-positive fluoroquinolone-resistant strains, other resistance mechanisms such as mutations in the gyrase and topoisomerase genes may be involved in combination with PMQR (19,21). Unfortunately, the QDRD mutations of these resistant strains were not detected because of the limitation of the present study.

The PMQR-positive isolates were typed using an enterobacterial repetitive intergenic consensus (ERIC)-PCR method with the ERIC2 primer and the conditions described by Versalovic et al. (28). The ERIC-PCR fingerprints were compared by visual inspection. Variations in the band intensity or shape were not considered. The presence or absence of more than 2 distinct bands between 2 isolates is considered to indicate different strains (17). Nineteen ERIC-PCR patterns were observed among the 27 E. coli isolates, whereas 33 different patterns were obtained among the 49 K. pneumoniae isolates (Fig. 1 and Table 2). These findings indicated the dissemination of these resistance determinants as well as the clonal spread of these resistant strains within our hospital.

Although chromosomal QRDR mutations in topoisomerases play an important role in conferring a high level of quinolone resistance, the occurrence of PMQR in our hospital may contribute to an increase in quinolone resistance in clinical isolates of Enterobacteriaceae. The detection of PMQR may be useful for clinicians to ensure appropriate antimicrobial therapy.

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Conflict of interest None to declare.
REFERENCES


