Prevalence of plasmid-mediated quinolone resistance genes among ciprofloxacin-nonsusceptible *Escherichia coli* and *Klebsiella pneumoniae* isolated from blood cultures in Korea

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OBJECTIVES: To analyze the prevalence of plasmid-mediated quinolone resistance (PMQR) determinants in ciprofloxacin-nonsusceptible *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients at a tertiary care hospital in Korea.

METHODS: A total of 102 nonduplicate isolates of ciprofloxacin-intermediate or ciprofloxacin-resistant *E. coli* (n=80) and *K. pneumoniae* isolates from blood cultures were obtained. The qnr (qnrA, qnrB, qnrS), aac(6')-Ib-cr, qepA and oqxAB genes were detected using polymerase chain reaction (PCR) and confirmed using direct sequencing. To determine whether the PMQR-positive plasmid was horizontally transferable, conjugation experiments were performed.

RESULTS: Of the 102 isolates, 81 (79.4%) had one or more PMQR genes; these consisted of 59 (73.8%) *E. coli* and 22 (100%) *K. pneumoniae* isolates. The qnr genes were present in 15 isolates (14.7%); qnrB4 was detected in 10.8% and qnrS1 was detected in 3.9%. The aac(6')-Ib-cr, qepA and oqxAB genes were detected in 77.5%, 3.9% and 10.8%, respectively. In conjugation experiments, PMQR genes were successfully transferred from seven (8.6%) isolates. The range of minimum inhibitory concentrations of ciprofloxacin for these seven transconjugants increased to 0.5 mg/L to 1 mg/L, which was 16- to 33-fold that of the recipient *E. coli* J53 bacteria.

CONCLUSIONS: PMQR genes were highly prevalent among ciprofloxacin-nonsusceptible *E. coli* and *K. pneumoniae* from blood cultures in the authors’ hospital. Therefore, it is necessary to monitor for the spread of PMQR genes of clinical isolates and to ensure careful antibiotic use in a hospital setting.

Key Words: aac(6')-Ib-cr; oqxAB; Plasmid-mediated quinolone resistance genes; qepA; qnr

The quinolone class of antibiotics was introduced into clinical use in the 1960s (1) and has since been important for the treatment of bacterial infections. In the late 1980s, more systemically active drugs (eg, fluoroquinolone) became clinically available (2). Over the decades since the introduction of fluoroquinolones, resistance to these agents in *Enterobacteriaceae* has become common and widespread.

The main mechanisms of quinolone resistance arise from chromosomal mutations in genes encoding DNA gyrase and topoisomerase IV (3). Upregulation of efflux pumps and/or decreased expression of outer membrane porins are also classically described mechanisms resulting from chromosomal mutations (4,5). Recently, however, plasmid-mediated quinolone resistance (PMQR) genes have been detected in *Enterobacteriaceae* (6). Since the first PMQR determinant, termed Qnr (now known as QnrA1), was reported in a *Klebsiella pneumoniae* isolate in 1998 (6), two mechanisms of PMQR have been reported including the quinolone modification with a piperazinyl substituent by the acetyltransferase AAC(6')-Ib-cr and active efflux by QepA and OqxAB, which are pumps related to major facilitator superfamily transporters (7-10). The PMQR genes confer low-level quinolone resistance and supplement the level of resistance caused by other resistance mechanisms.
There are very few reports investigating these four different PMQR determinants (Qnr, AAC(6')-Ib-cr, QepA and OqxAB), especially OqxAB, from blood cultures in Korea. Therefore, in the present study, we determined the prevalence of PMQR determinants in ciprofloxacin-nonsusceptible Escherichia coli and Klebsiella pneumoniae isolated from patient blood cultures in Korea.

**METHODS**

**Bacterial isolates**

A total of 102 nonduplicate clinical isolates of ciprofloxacin-intermediate or ciprofloxacin-resistant E. coli (n=82) and K. pneumoniae (n=22) were obtained from blood cultures collected between January 2005 and December 2010 at the Kyung Hee Medical Center (Seoul, Republic of Korea). Bacterial identification and antimicrobial susceptibilities were determined according to routine laboratory protocols using conventional biochemical tests and the MicroScan WalkAway 96 (Dade Behring, USA), following the Clinical and Laboratory Standards Institute guidelines: ciprofloxacin susceptible, minimum inhibitory concentration (MIC) ≤1 μg/mL; intermediate, MIC 2 μg/mL; and resistant, MIC ≥4 μg/mL (11). Each isolate was obtained from an individual patient.

**Polymerase chain reaction amplification and sequencing for detection of PMQR genes**

Amplification of PMQR genes (qnrA, qnrB, qnrS, aac(6')-Ib, qepA, qnrA and qnrB) was performed using primers as described previously (12-15). Plasmid DNA was extracted from each isolate using a plasmid purification kit (SoLGenC Co, Daejeon, Korea) according to the manufacturer's instructions. All qnr (qnrA, qnrB and qnrS) genes were detected using multiplex polymerase chain reaction (PCR), and aac(6')-Ib, qepA, qnrA and qnrB were detected using PCR. Positive and negative controls were included for quality control. For the qnr PCR, 2 μL plasmid DNA was added to 50 μL reaction mixture containing 5 μL PCR buffer (5 mM MgCl2) (JMR Holdings, United Kingdom), 2.5 mM dNTPs (GeneACT Inc, Japan), 20 pM/μL of each primer and 1.5 U Taq polymerase. PCR conditions using the GeneAmp PCR system 9600 (Perkin-Elmer Centus Corp, USA) were: 5 min at 95°C; 35 cycles of amplification consisting of 45 s at 94°C, 60 s at 53°C and 60 s at 72°C; and 5 min at 72°C for the final extension. For aac(6')-Ib PCR, 1 μL plasmid DNA was added to 20 μL reaction mixture containing 2.0 μL PCR buffer, 2.5 mM dNTPs, 10 pM/μL primer and 0.4 U Taq polymerase. PCR conditions were: 12 min at 95°C; 35 cycles of amplification consisting of 45 s at 94°C, 60 s at 53°C and 60 s at 72°C; and 5 min at 72°C for the final extension. For qepA PCR, 3 μL plasmid DNA was added to 16 μL reaction mixture containing 10 pM/μL primer and 2× multiplex PCR premix (SoLGenC, Korea). PCR conditions were: 12 min at 95°C; 35 cycles of amplification consisting of 60 s at 96°C, 60 s at 60°C and 60 s at 72°C; and 5 min at 72°C for the final extension. For qnrA and qnrB PCR, 3 μL plasmid DNA was added to 16 μL reaction mixture containing 10 pM/μL primer and 2× multiplex PCR premix. PCR conditions were: 12 min at 95°C; 30 cycles of amplification consisting of 45 s at 94°C, 45 s at 64°C and 60 s at 72°C; and 5 min at 72°C for the final extension. The PCR products were analyzed using electrophoresis in a 2% agarose gel containing 0.5 μg/mL ethidium bromide at 130 V for 30 min. Positive and negative controls were included for quality control. Direct sequencing of the PCR products was used to confirm qnr, aac(6')-Ib and qepA positivity for PMQR genes. To identify aac(6')-Ib-cr, aac(6')-Ib-positive PCR products were confirmed by direct sequencing using a 3130XL DNA genetic analyzer (Applied Biosystems, USA). Isolates positive for both qnrA and qnrB were regarded as qepA-positive because the OqxAB protein is encoded by qepA and qepB genes located within the same operon. Nucleotide sequences were analyzed using the BLAST online database provided by the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov/BLAST).

**Conjugation experiments to determine PMQR transferability**

To determine whether quinolone resistance was transferable from the bacterial strains with plasmids carrying PMQR determinants, conjugation experiments were performed with azide-resistant E. coli J53 as the recipient. Each clinical strain was inoculated along with the recipient strain into tryptic soy broth and incubated at 37°C for 3 h. Transconjugants were selected on MacConkey agar containing sodium azide (100 mg/L) and ciprofloxacin (0.06 mg/L). To determine the presence of PMQR determinants, colonies were picked from the selection agar and analyzed by PCR.

**Antimicrobial susceptibility test**

MICs of various antibiotics (amikacin, gentamicin, tobramycin, nalidixic acid, ciprofloxacin, levofloxacin and olaquindox) were determined for the PMQR gene-positive donors and the recipient transconjugants using the broth microdilution method according to Clinical Laboratory Standards Institute guidelines (11) and using E. coli ATCC 25922 as a control.

**Statistical analysis**

Statistical analysis of species-specific distributions of PMQR genes was performed using Fisher's exact test; P<0.05 was considered to be statistically significant. MedCalc version 10.4.5 (MedCalc Software, Belgium) was used for calculations.

**RESULTS**

**Prevalence of PMQR genes**

Among the 102 total ciprofloxacin-intermediate or ciprofloxacin-resistant isolates, 81 (79.4%) were positive for at least one PMQR gene. PMQR genes were detected in 59 of 80 (73.8%) E. coli and all 22 (100%) K. pneumoniae isolates (Table 1).

| Gene | Prevalence | | |
|------|------------|---|---|
| qnrA | 36/81 (44.4%) | | |
| qnrB | 57/81 (70.4%) | | |
| qnrS | 1/22 (4.5%) | | |
| aac(6')-Ib-cr | 56/102 (54.9%) | | |

Of the PMQR genes, qnrA were present in 5 (14.17%) isolates and qnrB were detected in 11 (50.0%) K. pneumoniae isolates and qnrS was detected in two (2.5%) E. coli and two (9.1%) K. pneumoniae isolates. The sequences of qnrB and qnrS were identical to those of qnrB4 and qnrS1, respectively. Eighty-two of the 102 (80.4%) isolates were positive for aac(6')-Ib-cr and 79 of 102 (77.5%) isolates were positive for aac(6')-Ib-cr. The aac(6')-Ib-cr gene was detected in 59 of 80 (73.8%) E. coli and 20 of 22 (90.9%) K. pneumoniae isolates. The aac(6')-Ib-cr gene was present in four of 102 isolates (3.9%), all of which were E. coli strains. Eleven of the 102 (10.8%) isolates were positive for both qepA and qepB. The qepA gene was not found in any E. coli isolate; all 11 qepA-positive isolates were K. pneumoniae strains (Table 2).

Among the 102 isolates, 13 (12.7%) had two PMQR genes. Two E. coli isolates contained both qnrS1 and aac(6')-Ib-cr genes, and four were positive for both aac(6')-Ib-cr and qepA (Table 3). Of the K. pneumoniae isolates, one contained both qnrS1 and aac(6')-Ib-cr genes, three contained both qnrB4 and aac(6')-Ib-cr genes, and two contained both qnrB4 and qepA genes. Seven (6.9%) isolates, all of which were K. pneumoniae strains, had three PMQR genes; one of these possessed qnrS1, aac(6')-Ib-cr and qepA genes, and six contained qnrB4, aac(6')-Ib-cr and qepA genes (Table 4).

**Conjugation experiment**

Seven transconjugants were successfully obtained from the 81 PMQR-positive isolates used as donors in conjugation experiments. The qnr gene was successfully transferred in three of the 15 qnr-positive isolates (two were qnrS1 and one was qnrB4). The aac(6')-Ib-cr gene was transferred in six of 79 isolates and the qepA gene was transferred in one of 11 isolates; transconjugation produced no qepA-positive isolates.

Transconjugants were obtained from three of 59 (5.1%) PMQR-positive E. coli isolates and four of 22 (18.2%) PMQR-positive K. pneumoniae isolates. Of the three transconjugants with E. coli donors, transfer of the aac(6')-Ib-cr gene occurred in two and the transfer of qnrS1 occurred in one. Of the four transconjugants with K. pneumoniae donors, transfer of the aac(6')-Ib-cr gene occurred in one, and cotransfer of qnrB4 and...
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### TABLE 1
Annual distribution of plasmid-mediated quinolone resistance (PMQR) genes of *Escherichia coli* and *Klebsiella pneumoniae* isolates from 2005 to 2010

| Year of isolate | PMQR-positive isolates/total isolates, n/n (%) |
|-----------------|---------------------------------------------|
|                 | *E. coli* | *K. pneumoniae* | isolates with any PMQR genes, n (%) |
| 2005            | 1/10 (10.0) | 0 (0) | 1 (10.0) |
| 2006            | 1/5 (20.0)  | 0 (0) | 1 (20.0) |
| 2007            | 3/7 (42.9)  | 0 (0) | 3 (42.9) |
| 2008            | 14/17 (82.4)| 3/3 (100.0) | 17 (85.0) |
| 2009            | 18/20 (95.0)| 8/8 (100.0) | 28 (96.4) |
| 2010            | 21/21 (100.0)%| 11/11 (100.0)%| 32 (100.0)% |
| Total           | 59/80 (73.8)%| 22/22 (100.0)%| 61 (79.4)% |

### TABLE 2
Prevalence of plasmid-mediated quinolone resistance genes in *Escherichia coli* and *Klebsiella pneumoniae* isolates

| Species          | Isolates, n (%) |
|------------------|-----------------|
|                  | *qnrB4* | *qnrS1* | *aac(6')-Ib-cr* | *qepA* | *oqxAB* |
| *E. coli* (n=80) | 0 (0)   | 2 (2.5) | 59 (73.8)      | 4 (5.0) | 0 (0)   |
| *K. pneumoniae* (n=22) | 11 (50.0) | 2 (9.1) | 20 (90.9)     | 0 (0)   | 11 (50.0) |
| Total (n=102)    | 11 (10.8) | 4 (3.9) | 79 (77.5)     | 4 (3.9) | 11 (10.8) |

aac(6′)-Ib-cr, *qnrA* and *aac(6′)-Ib-cr*, or aac(6′)-Ib-cr and *qepA* occurred from different donors. Transferability was highest for *qnrS1* (two of four [50.0%]), followed by *qnrB4* (one of 11 [9.1%]) and *qepAB* (one of 11 [9.1%]), and aac(6′)-Ib-cr (six of 79 [7.6%]) (Tables 3 and 4).

### Antimicrobial susceptibility test
Among the 81 PMQR-positive isolates, the MIC of ciprofloxacin ranged from 2 mg/L to >256 mg/L. The resistance rates of PMQR-positive isolates to nalidixic acid, levofloxacin, amikacin, gentamicin and tobramycin were 100% (81 of 81), 96.3% (78 of 81), 14.8% (12 of 81), 43.2% (35 of 81) and 40.7% (33 of 81), respectively.

The MIC of ciprofloxacin for the seven transconjugants ranged from 0.5 mg/L to 1 mg/L, or to 33-fold higher than that for the *E. coli* J53 recipient bacteria (MIC 0.03 mg/L). All three *qnr*-containing transconjugants conferred decreased susceptibility to ciprofloxacin (MIC range 0.5 mg/L to 1 mg/L), nalidixic acid (MIC range 4 mg/L to 8 mg/L) and levofloxacin (MIC range 0.5 mg/L to 1 mg/L); these MICs are 16- to 33-fold, two- to four-fold and eight- to 16-fold the MICs for the preconjugated recipient *E. coli* J53 bacteria (0.03 mg/L, 2 mg/L and 0.0625 mg/L, respectively). The MIC of ciprofloxacin for six *aac(6′)-Ib-cr*-containing transconjugants ranged from 0.5 mg/L to 1 mg/L, or 16- to 33-fold the MIC for the preconjugated recipient. All *aac(6′)-Ib-cr*-containing transconjugants exhibited decreased susceptibility to nalidixic acid and levofloxacin. The two transconjugants with *qnr* and *aac(6′)-Ib-cr* exhibited increased MICs for ciprofloxacin (range 0.5 mg/L to 1 mg/L), which were 16- to 33-fold higher than the MIC for the preconjugated recipient. For one transconjugant with both *aac(6′)-Ib-cr* and *qepAB*, the MIC to ciprofloxacin was 0.5 mg/L, or 16-fold the MIC of the preconjugated recipient (Tables 3 and 4).

### DISCUSSION
We evaluated the incidence of *qnr*, *aac(6′)-Ib-cr*, *qepA* and *qepAB* genes in ciprofloxacin-nonsusceptible *E. coli* and *K. pneumoniae* strains isolated from patient blood cultures in Korea.

The *qnr* genes encode proteins that protect DNA gyrase and topoisomerase IV from inhibition by quinolones (16,17), and have recently been identified worldwide. The prevalence of the *qnr* genes in bacterial isolates may range from <1% to >50% (18-21), depending on the selection criteria and study period for bacterial isolates. Among ciprofloxacin-resistant *E. coli* and *K. pneumoniae* isolates, the incidences of *qnr* in China are 7.5% and 11.9%, respectively, *qnrA*, *qnrB* and *qnrS* were detected either alone or in combination in 3.8%, 4.7% and 3.8% of these isolates, respectively (18). In Korea, Shin et al (20) reported that 5.6% of *E. coli* and 55.9% of *K. pneumoniae* ciprofloxacin-resistant isolates contained only *qnrB* (*qnrB2*, *qnrB4* and/or *qnrB6*). Jeong et al (19) reported that the prevalence of *qnrA* in Korea was 0.8% in *E. coli* isolates (ciprofloxacin susceptible and resistant) between 2001 and 2003. Kim et al (21) determined that 0.5% of *E. coli* and 5.9% of *K. pneumoniae* (ciprofloxacin susceptible and resistant) isolates in Korea contained *qnr* (*qnrB* or *qnrS*). Of the *qnr* variants, we did not detect *qnrA*; *qnrB4* was the most common, followed by *qnrS1*. Epidemiological investigations, including the present study, have shown that *qnrB* (especially *qnrB4*) was common, while *qnrA* and *qnrS* are present in Korea at relatively low prevalences (19-21). In the present study, the prevalence of *qnrB* in *K. pneumoniae* (50%) was significantly higher than that in *E. coli* (0%) (Fisher's exact test, P<0.001), as noted previously (18,20).

The *aac(6′)-Ib-cr* gene, a variant of the gene encoding AAC(6′)-Ib, was first described in 2006 (7). The AAC(6′)-Ib-cr enzyme reduces only ciprofloxacin and norfloxacin activity by acetylation (7). Quinolones without piperacillin nitrogen were not affected by aac(6′)-Ib-cr (23). However, transconjugants containing only aac(6′)-Ib-cr also exhibited reduced susceptibilities to levofloxacin in the present study, suggesting it contributes to antimicrobial resistance through additional mechanisms. The prevalence of aac(6′)-Ib-cr was higher in our study (77.9%) than in previous studies (7,15,24-26). Among clinical *E. coli* isolates collected in China, 51% had aac(6′)-Ib-cr (7). In the United States, aac(6′)-Ib-cr was detected in 32% of *E. coli* and 16% of *K. pneumoniae* isolates (15). In Korea, aac(6′)-Ib-cr was detected in 3.4% of Enterobacteriaceae (24) and in 34.1% of extended-spectrum β-lactamase (ESBL)-producing *E. coli* and *K. pneumoniae* (26). In some reports, the presence of aac(6′)-Ib-cr was prevalent among *qnr*-positive isolates compared with *qnr*-negative isolates, suggesting a genetic association of quinolone resistance with aminoglycoside resistance (25,26). We also found that the prevalence of aac(6′)-Ib-cr in *qnr*-positive isolates (13 of 15 [86.7%]) was slightly higher than in *qnr*-negative isolates (66 of 87 [75.9%]).

The *qepA* gene encodes a novel efflux pump that resembles a 14-transmembrane-segment putative efflux pump belonging to the major facilitator superfamily (8). In 2007, *qepA* was first reported in clinical *E. coli* isolates from Japan (8) and Belgium (27). According to recent studies, *qepA* has a low prevalence (<1% in Korea [24,28]). In the present study, the prevalence of *qepA* among the 80 ciprofloxacin-nonsusceptible *E. coli* isolates (5%) was higher than that in previous studies (24,28). Another plasmid-mediated efflux pump gene belonging to the resistance-nodulation-cell division family, *qepAB*, confers reduced susceptibility to multiple agents including olaqunidox (a growth promoter in pigs), quinolones and fluoroquinolones (29,30). *OqxAB* is...
| Isolate | PMQR determinant | AMK | GEN | TOB | NAL | CIP | LEX | OLQ |
|---------|-----------------|-----|-----|-----|-----|-----|-----|-----|
| Ec 7    | aac(6’)-Ib-cr   | 8   | 4   | 4   | >256| 64  | 32  | 32  |
| Ec 13   | aac(6’)-Ib-cr   | 16  | 2   | 4   | >256| 64  | 32  | 32  |
| Ec 18   | aac(6’)-Ib-cr   | 8   | 128 | 4   | >256| 64  | 32  | 32  |
| Ec 19   | aac(6’)-Ib-cr   | 16  | 256 | 32  | >256| 64  | 32  | 32  |
| Ec 20   | qnrS1, aac(6’)-Ib-cr | 8   | 2   | 2   | 256 | 4   | 16  | 32  |
| Tc Ec 20| qnrS1          | 1   | 0.5 | 0.5 | 8   | 1   | 1   | 32  |
| Ec 23   | aac(6’)-Ib-cr   | 8   | 4   | 4   | >256| 64  | 32  | 32  |
| Ec 24   | aac(6’)-Ib-cr   | 32  | 8   | 8   | >256| 64  | 16  | 32  |
| Ec 25   | aac(6’)-Ib-cr   | 8   | 2   | 2   | >256| 128 | 32  | 32  |
| Ec 26   | aac(6’)-Ib-cr   | 16  | 4   | 4   | >256| 128 | 64  | 16  |
| Ec 30   | aac(6’)-Ib-cr   | 8   | 2   | 2   | >256| 128 | 64  | 64  |
| Ec 31   | aac(6’)-Ib-cr   | 8   | 4   | 4   | >256| 128 | 64  | 32  |
| Ec 32   | aac(6’)-Ib-cr   | 16  | 2   | 32  | >256| 256 | 16  | 16  |
| Ec 33   | aac(6’)-Ib-cr   | 16  | 128 | 16  | >256| 128 | 32  | 32  |
| Ec 34   | aac(6’)-Ib-cr   | 16  | 128 | 61  | >256| >256| 32  | 32  |
| Ec 35   | aac(6’)-Ib-cr, qepA | 16  | 64  | 32  | >256| >256| 16  | 32  |
| Ec 36   | aac(6’)-Ib-cr   | 8   | 64  | 16  | >256| 128 | 32  | 32  |
| Ec 37   | aac(6’)-Ib-cr   | 8   | 4   | 4   | >256| >256| 128 | 16  |
| Ec 38   | aac(6’)-Ib-cr   | 8   | 256 | 16  | >256| 128 | 32  | 32  |
| Ec 39   | aac(6’)-Ib-cr, qepA | 16  | 8   | 8   | >256| 256 | 32  | 32  |
| Ec 40   | aac(6’)-Ib-cr   | 8   | 4   | 4   | >256| 16  | 16  | 64  |
| Ec 41   | aac(6’)-Ib-cr   | 2   | 4   | 4   | >256| 64  | 32  | 16  |
| Ec 42   | aac(6’)-Ib-cr, qepA | 1   | 2   | 2   | >256| 16  | 4   | 32  |
| Ec 43   | aac(6’)-Ib-cr   | 4   | 2   | 2   | >256| 16  | 16  | 64  |
| Ec 44   | aac(6’)-Ib-cr   | 4   | 64  | 16  | >256| >256| >256| 16  |
| Ec 45   | aac(6’)-Ib-cr   | 16  | 2   | 32  | >256| >256| 64  | 32  |
| Tc Ec 45| aac(6’)-Ib-cr   | 1   | 0.5 | 1   | 128 | 64  | 32  |
| Ec 46   | aac(6’)-Ib-cr   | 16  | 128 | 16  | >256| >256| 128 | 32  |
| Tc Ec 46| aac(6’)-Ib-cr   | 1   | 0.5 | 1   | 64  | 1   | 0.5 | 32  |
| Ec 47   | aac(6’)-Ib-cr   | 8   | 2   | 4   | >256| >256| 64  | 32  |
| Ec 48   | aac(6’)-Ib-cr   | 16  | 4   | 4   | >256| >256| 64  | 64  |
| Ec 49   | aac(6’)-Ib-cr, qepA | 8   | >256| 16  | >256| 128 | 32  | 16  |
| Ec 50   | aac(6’)-Ib-cr   | 16  | 2   | 4   | >256| 128 | 16  | 32  |
| Ec 52   | aac(6’)-Ib-cr   | 8   | 2   | 2   | >256| 128 | 16  | 32  |
| Ec 53   | aac(6’)-Ib-cr   | 8   | 128 | 8   | >256| 256 | 32  | 32  |
| Ec 54   | aac(6’)-Ib-cr   | 4   | 64  | 4   | >256| 128 | 32  | 16  |
| Ec 55   | aac(6’)-Ib-cr   | 8   | 4   | 4   | >256| 128 | 32  | 32  |
| Ec 56   | aac(6’)-Ib-cr   | 8   | 4   | 32  | >256| >256| 32  | 32  |
| Ec 57   | aac(6’)-Ib-cr   | 4   | 128 | 16  | >256| 32  | 16  | 32  |
| Ec 58   | aac(6’)-Ib-cr   | 16  | 4   | 64  | >256| >256| 32  | 16  |
| Ec 59   | aac(6’)-Ib-cr   | 8   | 2   | 4   | >256| 128 | 64  | 32  |
| Ec 60   | aac(6’)-Ib-cr   | 8   | 2   | 4   | >256| 64  | 32  | 32  |
| Ec 61   | aac(6’)-Ib-cr   | >256| 2   | 4   | >256| 64  | 16  | 256 |
| Ec 62   | aac(6’)-Ib-cr   | 8   | 32  | 4   | >256| 128 | 32  | 32  |
| Ec 63   | aac(6’)-Ib-cr   | 16  | 32  | 16  | >256| 128 | 32  | 16  |
| Ec 64   | aac(6’)-Ib-cr   | 8   | 4   | 4   | >256| >256| 32  | 32  |
| Ec 65   | aac(6’)-Ib-cr   | 8   | 2   | 4   | >256| 128 | 32  | 32  |
| Ec 66   | aac(6’)-Ib-cr   | 8   | 4   | 4   | >256| 128 | 32  | 32  |
| Ec 67   | aac(6’)-Ib-cr   | 32  | 32  | 64  | >256| >256| 32  | 32  |
| Ec 68   | aac(6’)-Ib-cr   | 16  | >256| 64  | >256| >256| 32  | 32  |
| Ec 69   | aac(6’)-Ib-cr   | 8   | 2   | 4   | >256| 2   | 2   | 32  |
| Ec 70   | qnrS1, aac(6’)-Ib-cr | 8   | 128 | 16  | >256| 4   | 4   | 32  |
| Ec 71   | aac(6’)-Ib-cr   | 16  | 2   | 4   | >256| 128 | 64  | 32  |
| Ec 72   | aac(6’)-Ib-cr   | 16  | 2   | 4   | >256| 64  | 32  | 32  |
| Ec 73   | aac(6’)-Ib-cr   | 16  | 4   | 4   | >256| >256| 32  | 32  |
| Ec 74   | aac(6’)-Ib-cr   | 4   | 2   | 4   | >256| 256 | 128 | 16  |
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TABLE 3 – CONTINUED
Plasmid-mediated quinolone resistance (PMQR) genes and minimum inhibitory concentrations of antimicrobial agents for donors and their transconjugants in *Escherichia coli* isolates

| Isolate | PMQR determinant | AMK | GEN | TOB | NAL | CIP | LEX | OLQ |
|---------|------------------|-----|-----|-----|-----|-----|-----|-----|
| Ec 75   | aac(6')-Ib-cr    | 16  | 4   | 4   | >256| >256| 64  | 32  |
| Ec 76   | aac(6')-Ib-cr    | 32  | 128 | 16  | >256| 256 | 32  | 32  |
| Ec 77   | aac(6')-Ib-cr    | 4   | 4   | 4   | >256| 128 | 64  | 64  |
| Ec 78   | aac(6')-Ib-cr    | 8   | 2   | 4   | >256| 128 | 32  | 32  |
| Ec 79   | aac(6')-Ib-cr    | 8   | 128 | 16  | >256| 256 | 32  | 32  |
| Ec 80   | aac(6')-Ib-cr    | 32  | 256 | 64  | >256| >256| 64  | 64  |

Recipient

| Ec J53  | None            | 1   | 0.5 | 1   | 2   | 0.03| 0.06| 16  |

AMK Amikacin; CIP Ciprofloxacin; Ec E coli; GEN Gentamicin; LEX Levofloxacin; OLQ Olaquindox; NAL Nalidixic acid; TOB Tobramycin

TABLE 4
Plasmid-mediated quinolone resistance (PMQR) genes and minimum inhibitory concentrations of antimicrobial agents for donors and their transconjugants in *Klebsiella pneumoniae* isolates

| Isolate | PMQR determinant | AMK | GEN | TOB | NAL | CIP | LEX | OLQ |
|---------|------------------|-----|-----|-----|-----|-----|-----|-----|
| Kp 1    | qnrB4, aac(6')-Ib-cr | 4   | >256| >256| >256| 16  | 256 | >256|
| Tc Kp 1 | qnrB4, aac(6')-Ib-cr | 2   | 0.5 | 1   | 4   | 0.5 | 0.5 | 32  |
| Kp 2    | aac(6')-Ib-cr      | 4   | 2   | 2   | >256| >256| 16  | 16  |
| Kp 3    | aac(6')-Ib-cr      | 1   | 1   | 2   | >256| >256| 128 | >256|
| Kp 4    | qnrB4, aac(6')-Ib-cr | >256| >256| >256| >256| 128 | 16  | >256|
| Kp 5    | qnrS1, aac(6')-Ib-cr, oqxAB | 2   | 64  | 8   | >256| >256| 128 | >256|
| Kp 6    | qnrB4, aac(6')-Ib-cr, oqxAB | >256| >256| >256| >256| >256| >256| >256|
| Kp 7    | qnrB4, aac(6')-Ib-cr | 2   | 1   | 1   | >256| 6   | 16  | 256 |
| Tc Kp 7 | qnrB4, aac(6')-Ib-cr | 1   | 0.5 | 0.5 | 4   | 1   | 0.5 | 16  |
| Kp 8    | qnrB4, oqxAB       | >256| 256 | 32  | >256| >256| 256 | >256|
| Kp 9    | qnrB4, aac(6')-Ib-cr, oqxAB | >256| >256| >256| >256| >256| >256| >256|
| Kp 10   | qnrB4, aac(6')-Ib-cr, oqxAB | >256| >256| >256| >256| >256| >256| >256|
| Kp 11   | qnrB4, aac(6')-Ib-cr | >256| >256| >256| >256| >256| 32  | 256 |
| Kp 12   | aac(6')-Ib-cr      | 2   | 1   | 1   | >256| 6   | 8   | 63  |
| Kp 13   | qnrB4, aac(6')-Ib-cr, oqxAB | >256| >256| >256| >256| >256| >256| >256|
| Kp 14   | qnrB4, oqxAB       | >256| >256| >256| >256| >256| >256| >256|
| Kp 15   | aac(6')-Ib-cr      | 2   | 0.5 | 1   | >256| 16  | 32  | >256|
| Kp 16   | aac(6')-Ib-cr, oqxAB | 2   | 16  | 4   | >256| 64  | 128 | 128 |
| Tc Kp 16| aac(6')-Ib-cr      | 1   | 0.5 | 1   | 4   | 0.5 | 0.5 | 32  |
| Kp 17   | qnrB4, aac(6')-Ib-cr, oqxAB | >256| >256| >256| >256| >256| >256| >256|
| Kp 18   | aac(6')-Ib-cr      | 16  | 256 | 16  | >256| 64  | 64  | 256 |
| Kp 19   | aac(6')-Ib-cr, oqxAB | >256| 1   | >256| 16  | 128 | 128 | >256|
| Kp 20   | qnrB4, aac(6')-Ib-cr, oqxAB | >256| >256| >256| >256| >256| >256| >256|
| Tc Kp 20| aac(6')-Ib-cr, oqxAB | 16  | 0.5 | 1   | >256| 0.5 | 128 | 256 |
| Kp 21   | aac(6')-Ib-cr      | 8   | 32  | 4   | >256| 128 | 64  | >256|
| Kp 22   | aac(6')-Ib-cr      | 16  | 2   | 16  | >256| 32  | 32  | 256 |

Recipient

| Ec J53  | None            | 1   | 0.5 | 1   | 2   | 0.03| 0.06| 16  |

AMK Amikacin; CIP Ciprofloxacin; Ec Escherichia coli; GEN Gentamicin; Kp K pneumoniae; LEX Levofloxacin; NAL Nalidixic acid; OLQ Olaquindox; TOB Tobramycin

The PMQR genes, which include *aac(6')-Ib-cr*, *qnrB4*, *qnrS1*, *oqxAB*, and *aac(6')-Ib-cr*, are encoded by the *oqA* and *oqB* genes, which are located in the same operon. The *oqAB* genes are chromosomally located in *K. pneumoniae*. Thus, the plasmid containing *oqAB* appears to be the result of the capture of a chromosomal cassette from *Klebsiella* species (30). Also, Rodriguez-Martinez et al. (31) found simultaneous *oqA* and *oqB* signals in both chromosomal and large plasmid locations. The prevalence of the *oqAB* gene was 74% to 100% in other studies; thus, the detected prevalence of 50% among *K. pneumoniae* isolates in the present study was a relatively low value (12,32). However, we obtained only plasmid DNA using a plasmid purification kit; other studies obtained the chromosomal and/or plasmid DNA for detection of *oqAB* gene. Plasmid-mediated OqxAB was first detected in a human clinical isolate of *E. coli* from Korea (12). However, none of the *E. coli* isolates in the present study possessed *oqAB*. In previous studies, *oqAB*-positive *K pneumoniae* isolates yielded no transconjugants. However, one transconjugant with a *K pneumoniae* donor obtained the *oqAB* gene, which conferred decreased susceptibility to ciprofloxacin and olaquindox. There is still a lack of epidemiological information about *oqAB* gene in humans, and this requires further study.

Park et al. (33) reported that the prevalence of *qnr* determinants or *aac(6')-Ib-cr* was 97.4% in isolates with ciprofloxacin MICs of 1 mg/L, but 6.7% in isolates with ciprofloxacin MICs of 0.25 mg/L among ciprofloxacin-susceptible isolates of *K pneumoniae* in Korea. In this study, the prevalence of *qnr* determinants or *aac(6')-Ib-cr* was 100% in ciprofloxacin-nonsusceptible isolates of *K pneumoniae*, PMQR genes were remarkably high in isolates with ciprofloxacin MICs >1 mg/L (33).
Nam et al (34) studied mutations in the DNA gyrase and topoisomerase IV gene in the same isolates as included in the present study, and the mutation of the gyrA and parC genes were 98.0% and 91.1%, respectively, in these ciprofloxacin-nonsusceptible E. coli and K. pneumoniae. Of these, K. pneumoniae exhibited no mutations in the DNA gyrase and topoisomerase IV genes, but both had PMQR genes.

Conjugation experiments demonstrated that PMQR was transferable. The MICs of ciprofloxacin for seven transconjugants were 98.0% and 91.1%, respectively, in these K. pneumoniae exhibiting the mutation of the parC gyrA genes were 98.0% and 91.1%, respectively, in these ciprofloxacin-nonsusceptible E. coli and K. pneumoniae. Of these, K. pneumoniae exhibited no mutations in the DNA gyrase and topoisomerase IV genes, but both had PMQR genes.

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In the present study, we investigated a variety of PMQR genes in E. coli and K. pneumoniae and provided additional information about the actively investigated qepA and qpxAB genes. Analysis of the genes over several years made it possible to predict the presence of PMQR genes, and offers important information for antimicrobial selection and infection control.

It is important to note that the present study had several limitations. It was conducted at a single hospital and did not analyze the clonal relationships among PMQR-positive isolates. Also, it is necessary to confirm the colocalization of the qnr gene and other PMQR genes by PCR or Southern blot hybridization with both DNA probes of a single plasmid. Further nationwide epidemiological surveys and additional molecular studies for the possibility of horizontal transmission are required to support our results.

CONCLUSION

We identified PMQR genes in 79.4% (81 of 102) of ciprofloxacin-nonsusceptible E. coli and K. pneumoniae isolated from a tertiary-care hospital in Korea. The prevalent PMQR gene was aac(6’)-Ib-cr, followed by qnrB4 and qnrAB, and qnrS1 and qepA. PMQR genes were highly prevalent among ciprofloxacin-nonsusceptible E. coli and K. pneumoniae isolated from blood cultures in our hospital. Therefore, it is necessary to monitor for spread of PMQR genes of clinical isolates and to ensure careful antibiotic use in a hospital setting.

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