ORIGINAL ARTICLE

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* (n=22) 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

La prévalence des gènes de résistance à la quinolone à médiation plasmidique en cas d'*Escherichia coli* et de *Klebsiella pneumoniae* non susceptibles à la ciprofloxacine isolés dans des cultures sanguines en Corée

OBJECTIFS : Analyser la prévalence des déterminants de la résistance à la quinolone à médiation plasmidique (RQMP) en cas d'*Escherichia coli* et de *Klebsiella pneumoniae* non susceptibles à la ciprofloxacine, isolés chez des patients d'un hôpital de soins tertiaires de la Corée.

MÉTHODOLOGIE : Au total, les chercheurs ont obtenu 102 isolats non dupliqués d'*E coli* (n=80) et de *K pneumoniae* (n=22) moyennement résistants ou résistants à la ciprofloxacine dans les hémocultures. Ils ont décelé les gènes *qnr* (*qnrA*, *qnrB*, *qnrS*), *aac(6′)-Ib-cr*, *qepA* et *oqxAB* au moyen de la réaction en chaîne de la polymérase (PCR) et les ont confirmés par séquençage direct. Pour déterminer si les plasmides ayant une RQMP pouvaient opérer un transfert horizontal, les chercheurs ont effectué des expériences de conjugaison.

RÉSULTATS : Sur les 102 isolats, 81 (79,4 %) avaient au moins un gène de RQMP. De ce nombre, 59 (73,8 %) étaient des isolats d'*E coli* et 22 (100 %), de *K pneumoniae*. Les gènes *qnr* étaient présents dans 15 isolats (14,7 %), soit 10,8 % de gène *qnrB4* et 3,9 % de gène *qnrS1*. Les gènes *aac(6′)-Ib-cr*, *qepA* et *oqxAB* ont été décelés dans 77,5 %, 3,9 % et 10,8 % des isolats, respectivement. Dans les expériences de conjugaison, sept isolats (8,6 %) ont entraîné un transfert des gènes de RQMP. La plage de concentrations inhibitrices minimales de la ciprofloxacine de ces sept produits de transconjugaison est passée de 0,5 mg/L à 1 mg/L, soit 16 fois à 33 fois plus que celles des bactéries d'*E coli* J53 des receveurs.

CONCLUSIONS : Les gènes de RQMP étaient hautement prévalents dans les hémocultures d'*E coli* et de *K pneumoniae* non susceptibles à la ciprofloxacine à l'hôpital des auteurs. Par conséquent, il faut surveiller la propagation des gènes de RQMP dans les isolats cliniques et vérifier attentivement l'utilisation des antibiotiques en milieu hospitalier.

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.

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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=80) 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*, *oqxA* and *oqxB*) was performed using primers as described previously (12-15). Plasmid DNA was extracted from each isolate using a plasmid purification kit (SolGent 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*, *oqxA* and *oqxB* 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 (15 mM $MgCl_2$) (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 Gene AmpPCR system 9600 (Perkin-Elmer Centus Corp, USA) were: 5 min at 95°C; 35 cycles of amplification consisting of 60 s at 95°C, 60 s at 54°C and 60 s at 72°C; and 10 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 (SolGent, 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 *oqxA* and *oqxB* PCRs, 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; 32 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 *oqxA* and *oqxB* were regarded as *oqxAB*-positive because the OqxAB protein is encoded by *oqxA* and *oqxB* genes located within the same operon. Nucleotide sequences were analyzed using the BLAST online service 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).

Of the PMQR genes, *qnr* genes were present in 15 (14.7%) isolates. The *qnrA* gene was not detected in any isolate; however, *qnrB* was 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*, 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 *qepA* 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 *oqxA* and *oqxB*. The *oqxAB* gene was not found in any *E coli* isolate; all 11 *oqxAB*-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 *oqxAB* 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 *oqxAB* genes, and six contained *qnrB4*, *aac(6′)-Ib-cr* and *oqxAB* 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 *oqxAB* 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, the transfer of *aac(6′)-Ib-cr* 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

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 (%)		Isolates with any PMQR genes, n (%)
	E coli	*K pneumoniae*	
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	19/20 (95.0)	8/8 (100.0)	27 (96.4)
2010	21/21 (100.0)	11/11 (100.0)	32 (100.0)
Total	59/80 (73.8)	22/22 (100.0)	81 (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, *qnrS1* and *aac(6')-Ib-cr*, or *aac(6')-Ib-cr* and *oqxAB* occurred from different donors. Transferability was highest for *qnrS1* (two of four [50.0%]), followed by *qnrB4* (one of 11 [9.1%]) and *oqxAB* (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 16- 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 fourfold 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 *oqxAB*, 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 *oqxAB* 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*) (22) is common, while *qnrA* and *qnrS* are present in Korea at relatively low prevalences (19-21). In our 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 piperazinyl 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, *oqxAB*, confers reduced susceptibility to multiple agents including olaquindox (a growth promoter in pigs), quinolones and fluoroquinolones (29,30). OqxAB is

TABLE 3
Plasmid-mediated quinolone resistance (PMQR) genes and minimum inhibitory concentrations of antimicrobial agents for donors and their transconjugants in *Escherichia coli* isolates

		Minimum inhibitory concentration, mg/L						
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	32	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*	4	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	64	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	64	16	32
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	32
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	0.5	2	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	16
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	128	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	128	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

		Minimum inhibitory concentration, mg/L						
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; Tc Transconjugant; 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

		Minimum inhibitory concentration, mg/L						
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	*qnrS1*, *aac(6')-Ib-cr*	2	1	1	>256	8	16	256
Tc Kp 7	*qnrS1*, *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	1	2	>256	>256	256	>256
Kp 10	*qnrB4*, *aac(6')-Ib-cr*, *oqxAB*	>256	>256	>256	>256	128	256	>256
Kp 11	*qnrB4*, *aac(6')-Ib-cr*	>256	>256	>256	>256	8	32	256
Kp 12	*aac(6')-Ib-cr*	2	1	2	>256	8	8	63
Kp 13	*qnrB4*, *aac(6')-Ib-cr*, *oqxAB*	>256	>256	>256	>256	256	128	>256
Kp 14	*qnrB4*, *oqxAB*	>256	>256	>256	>256	>256	128	>256
Kp 15	*aac(6')-Ib-cr*	2	0.5	2	>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	128	>256
Kp 18	*aac(6')-Ib-cr*	16	256	16	>256	64	64	256
Kp 19	*aac(6')-Ib-cr*, *oqxAB*	>256	1	1	>256	16	128	128
Kp 20	*qnrB4*, *aac(6')-Ib-cr*, *oqxAB*	>256	>256	>256	>256	256	128	>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; Tc Transconjugant; TOB Tobramycin*

encoded by the *oqxA* and *oqxB* genes, which are located in the same operon. The *oqxAB* genes are chromosomally located in *K pneumoniae*. Thus, the plasmid containing *oqxAB* appears to be the result of the capture of a chromosomal cassette from *Klebsiella* species (30). Also, Rodriguez-Martinez et al (31) found simultaneous *oqxA* and *oqxB* signals in both chromosomal and large plasmid locations. The prevalence of the *oqxAB* 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 *oqxAB* 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 *oqxAB*. In previous studies, *oqxAB*-positive *K pneumoniae* isolates yielded no transconjugants. However, one transconjugant with a *K pneumoniae* donor obtained the *oqxAB* gene, which conferred decreased susceptibility to ciprofloxacin and olaquindox. There is still a lack of epidemiological information about *oqxAB* 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 ciproxacin 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, two *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 16- to 33-fold higher than the MIC for the unconjugated recipient *E coli* J53 strain, and the MICs of ciprofloxacin for three transconjugants carrying multiple PMQR genes (*qnr* and *aac[6']-Ib-cr*, or *aac[6']-Ib-cr* and *oqxAB*) were 16- to 33-fold higher than the MIC for the unconjugated recipient. The MICs of ciprofloxacin for transconjugants carrying *aac(6')-Ib-cr* in combination with *qnr* or *oqxAB* were not significantly higher than those for transconjugants carrying *aac(6')-Ib-cr* only, suggesting the presence of additional mechanisms contributing to fluoroquinolone resistance. These PMQR determinants confer low-level fluoroquinolone resistance and may facilitate higher-level resistance under selective pressure from antimicrobial agents at therapeutic levels (35,36). PMQR has been closely associated with ESBL, AmpC-type β-lactamase and aminoglycoside resistance mechanisms (5). In our study, the prevalence of ESBL-producing isolates in PMQR-positive isolates (28 of 81 [34.6%]) was higher than in PMQR-negative isolates (three of 21 [14.3%]), but the difference was not statistically significant (Fisher's exact test, P=0.109). Cotransfer of PMQR genes may contribute to the spread of multidrug resistance. Clinicians should be careful in prescribing quinolone and fluoroquinolone to prevent the spread of multidrug resistance.

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 *oqxAB* 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 *oqxAB*, 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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