Short Communication

Prevalence of Plasmid-Mediated Quinolone Resistance Genes and Ciprofloxacin Resistance in Pediatric Bloodstream Isolates of *Enterobacteriaceae* over a 9-Year Period

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SUMMARY: A total of 279 isolates of *Escherichia coli* ($n = 128$) and *Klebsiella pneumoniae* ($n = 151$) were obtained from blood samples from children at the Seoul National University Children's Hospital, Seoul, Korea from 1999 to 2007. Five plasmid-mediated quinolone resistance (PMQR) genes, *qnrA*, *qnrB*, *qnrS*, *qepA*, and *aac(6')-Ib-cr*, and the minimal inhibitory concentration (MIC) values for ciprofloxacin were tested for all the strains. Mutations in both *gyrA* and *parC* were analyzed in 57 representative strains. Twenty-seven strains (9.7%) had at least 1 of the 5 PMQR genes: *qnrB* in 20 isolates, *qnrS* in 1, *aac(6')-Ib-cr* in 5, and both *qnrB* and *aac(6')-Ib-cr* in 1 isolate. The overall PMQR prevalence rates tended to increase over time ($P = 0.001$). The non-susceptibility rate to ciprofloxacin was 11.0% (31/279). PMQR-harboring isolates tended to have increased ciprofloxacin MIC values among both quinolone resistance-determining region (QRDR) mutation-present ($P = 0.016$) and QRDR mutation-absent isolates ($P < 0.001$). The increasing prevalence of PMQR genes was associated with increase in quinolone use over time ($P < 0.001$) and increasing frequency of non-susceptibility to ciprofloxacin ($P < 0.001$).

Quinolone resistance in *Enterobacteriaceae* has increased in line with the increased number of quinolone prescriptions being administered for the treatment of infections by multidrug-resistant *Enterobacteriaceae* isolates. Mutations in the quinolone resistance-determining region (QRDR) and the presence of plasmid-mediated quinolone resistance (PMQR) genes contribute to quinolone resistance in complex and diverse ways (1–3). However, only limited studies have addressed the quinolone resistance of *Enterobacteriaceae* in pediatric isolates so far; long-term studies involving bloodstream isolates are even fewer in number (4–6).

In this study, we identified PMQR genes from *Escherichia coli* and *Klebsiella pneumoniae* isolates obtained from pediatric patients with bloodstream infections. We analyzed the isolates for changes in the prevalence of the PMQR genes and also regarding whether the presence of the PMQR genes is related to the amount of quinolones prescribed and to quinolone-resistance profiles.

A total of 279 consecutive *E. coli* ($n = 128$) and *K. pneumoniae* ($n = 151$) blood isolates were obtained from hospitalized children at the Seoul National Univer-

sity Children's Hospital, Seoul, Korea, from January 1999 to December 2007. Positive controls for the detection of the PMQR genes, *qnrA*, *qnrB*, *qnrS*, *qepA*, and *aac(6')-Ib-cr*, were used from previously published data (7–9). Distilled water was used as a negative control. Positive results for the PMQR genes, *qnrA*, *qnrB*, *qnrS*, and *qepA* were confirmed by direct sequencing of PCR products. All PCR products positive for *aac(6')-Ib* were further digested with *BtsCI* (New England Biolabs, Ipswich, Mass., USA) to identify *aac(6')-Ib-cr*, which lacks the *BtsCI* restriction site present in the wild-type gene (10). Mutations in both *gyrA* and *parC* found in the QRDRs were analyzed by PCR, and 57 strains, including all PMQR-positive strains and 30 PMQR-negative strains, were sequenced.

Antimicrobial susceptibility was determined by the disc diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI) (11). Minimal inhibitory concentration (MIC) values were measured for all strains using ciprofloxacin E Test Strips (AB Biodisk, Solna, Sweden). In accordance with CLSI guidelines, MIC $\leq 1 \mu\text{g/mL}$ was used to define susceptibility and MIC $\geq 2 \mu\text{g/mL}$ was used for non-susceptibility to ciprofloxacin (11). MIC₅₀ and MIC₉₀ indicated ciprofloxacin MIC values at which 50% and 90% of the isolates, respectively, were inhibited. ATCC *E. coli* 25922 was used as the control. The amounts of quinolones used from 1999 to 2007 at the study center was presented as days of antibiotic use per 1,000 patient

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Fig. 1. Correlation between the prevalence of PMQR genes and the duration of treatment with quinolones (Spearman's coefficient = 0.909, $P < 0.001$) and the correlation between the prevalence of PMQR genes and ciprofloxacin non-susceptibility (Spearman's coefficient = 0.849, $P < 0.001$) among 279 isolates of *E. coli* and *K. pneumoniae* from 1999 to 2007. Antibiotic usage has been presented in terms of days of antibiotic use per 1,000 patient days each year.

admission days each year, using data obtained from a computerized database of pharmacy records (12).

PMQR genes were detected in 27 (9.7%) of 279 isolates: *qnrB* in 20 isolates, *qnrS* in 1, *aac(6')-Ib-cr* in 5; and both *qnrB* and *aac(6')-Ib-cr* in 1 isolate. The PMQR prevalence rate for *K. pneumoniae* (13.9%) was significantly higher than for *E. coli* (4.7%; $P = 0.014$). No isolates were positive for the *qnrA* and *qepA* genes. The prevalence rate of *qnrB* was higher in *K. pneumoniae* (12.5%) than in *E. coli* (1.6%) ($P < 0.001$). *qnrB* subtypes were determined by sequencing, which showed the *qnrB2* subtype in 4 isolates, *qnrB4* in 15, and *qnrB10* and *qnrB13* in 1 isolate each.

As shown in Fig. 1, PMQR prevalence rates tended to increase over the 9-year period ($P = 0.001$): 7.5% in 2001, 10.7% in 2006, and 20.7% in 2007. Closer analysis of the changes in the prevalence of the commonly detected genes *qnrB* and *aac(6')-Ib-cr* showed that *qnrB*-harboring isolates were prevalent throughout the study period, whereas *aac(6')-Ib-cr*-carrying isolates were detected during the later period, that is, after 2004. All 4 isolates carrying the *qnrB2* subtype appeared during the earlier study period, while isolates with the *qnrB4* subtype appeared throughout the study period.

During the earlier study period, the PMQR genes seemed more prevalent in 2001 than in other years. In 2001, the *qnrB* gene was detected in 1 *E. coli* isolate and in 4 *K. pneumoniae* isolates. However, the *qnrB* subtypes were *qnrB2*, *qnrB4*, *qnrB10*, and *qnrB13*, thereby ruling out any epidemiological relationship. In addition, no epidemiological relationship was found on considering the time and place of isolation.

The overall non-susceptibility rate to ciprofloxacin was 11.0% (31/279). Twenty-four (18.8%) *E. coli* and 7 (4.6%) *K. pneumoniae* isolates demonstrated non-susceptibility (Table 1). Ciprofloxacin non-susceptibility

was significantly higher in the PMQR-positive isolates (25.9%, 7/27) than those without PMQR (9.5%, 24/252) ($P = 0.019$). The MIC values for ciprofloxacin, according to the presence of the PMQR genes, are also shown in Table 1. Overall, the MIC₅₀ of ciprofloxacin among the PMQR-positive isolates (MIC₅₀, 0.25 µg/mL) was approximately 8.3-fold higher than that of the PMQR-negative isolates (MIC₅₀, 0.03 µg/mL; $P < 0.001$). The median ciprofloxacin MIC of 30 PMQR-negative isolates (0.023 µg/mL) was similar to that of all the 252 PMQR-negative strains (0.032 µg/mL, $P = 0.849$). Amino acid mutations in the QRDRs were found in 15 isolates (8 of the 27 PMQR-positive and 7 of the 30 PMQR-negative isolates). Six isolates had *gyrA* mutations alone (codons 83 and/or 87) and the remaining 9 isolates had mutations in both *gyrA* (codons 83 and/or 87) and *parC* (codon 58 or 80).

The MIC values of ciprofloxacin for the PMQR-positive strains tended to be higher than those without PMQR genes among both the QRDR mutation-absent ($P < 0.001$) and QRDR mutation-present isolates ($P = 0.016$). Although the ciprofloxacin MIC values of PMQR-positive isolates tended to increase, non-susceptibility to ciprofloxacin was seen only in 7 strains which demonstrated the presence of mutations in the QRDRs among the 27 PMQR-positive isolates, whereas none of the PMQR-positive strains which did not have mutations in the QRDRs, were resistant.

A trend was observed toward the increased use of quinolone over time at the Seoul National University Children's Hospital. In this study, majority of cases of quinolone use were seen in the case of ciprofloxacin use for treating hematopoietic malignancies or immunodeficiency in children. The number of days of quinolone use in 1999 was 2.5 days per 1,000 patient admission days, which increased gradually later, although it was

Table 1. Ciprofloxacin non-susceptibility and MIC values of ciprofloxacin, according to the presence of plasmid-mediated quinolone resistance (PMQR) genes

Strain (<i>n</i>)	Ciprofloxacin non-susceptibility No. of strains (%)	<i>P</i> ¹⁾	Ciprofloxacin MIC $\mu\text{g/mL}$		<i>P</i> ²⁾
			MIC ₅₀	MIC ₉₀	
Overall (279)	31 (11.0)				
PMQR-negative (252)	24 (9.5)	0.019	0.03	2	<0.001
PMQR-positive (27)	7 (25.9)		0.25	> 32	
<i>E. coli</i> (128)	24 (18.8)				
PMQR-negative (122)	21 (17.2)	0.079	0.03	> 32	0.015
PMQR-positive (6)	3 (50.0)		0.25	> 32	
<i>K. pneumoniae</i> (151)	7 (4.6)				
PMQR-negative (130)	3 (2.3)	0.008	0.03	0.25	<0.001
PMQR-positive (21)	4 (19.0)		0.25	4	

MIC₅₀ and MIC₉₀ refer to the ciprofloxacin MIC values at which 50% and 90% of the isolates, respectively, were inhibited.

¹⁾: Ciprofloxacin non-susceptibility rates were compared between PMQR-negative and PMQR-positive stains using the Fisher's exact test.

²⁾: The MIC values were compared between PMQR-negative and PMQR-positive stains using the Mann Whitney *U* test.

maintained at less than 10 days per 1,000 patient admission days until 2005. In 2007, the consumption of quinolone increased to 15 days per 1,000 patient admission days. In concordance with the yearly positivity rate of PMQR genes, the amino acid mutation rate of the QRDRs *gyrA* and *parC* increased over time ($P < 0.001$). The increase in prevalence of PMQR genes correlated with the increased use of quinolone (Spearman's coefficient = 0.909, $P < 0.001$) and increasing ciprofloxacin non-susceptibility (Spearman's coefficient = 0.849, $P < 0.001$) (Fig. 1).

Both the prevalence of PMQR genes and the production of extended-spectrum β -lactamase (ESBL) were tested in 222 isolates. Among the 18 PMQR-positive isolates, 66.7% (12/18) produced ESBLs, whereas among the 204 PMQR-negative isolates, 29.4% (60/204) produced ESBLs. PMQR-positive strains were more likely to produce ESBLs, suggesting an association between PMQR prevalence and ESBL production ($P = 0.003$). Non-susceptibility rates to gentamicin, amikacin, and cefotaxime were significantly higher among the PMQR-positive strains than in those without PMQR genes. A plasmid carrying a PMQR gene is transferable to other organisms, which facilitates the selection of high-level resistance (13). Previous studies showed an association between PMQR gene-bearing plasmids and plasmids with ESBL, suggesting the association with resistance to other agents (4).

PMQR genes were first described in a clinical isolate of *K. pneumoniae* in 1998 (14). Since then, 5 groups of *qnr* determinants, *qnrA*, *qnrB*, *qnrC*, *qnrD* variant, and *qnrS*, have been identified (2). Three additional PMQR genes have also been described; *aac(6')-Ib-cr*, a variant of *aac(6')-Ib*, which encodes a variant aminoglycoside acetyltransferase that modifies ciprofloxacin (9); *qepA*, which encodes an efflux pump (8,15); and plasmid-mediated *oqxAB* (16), expanding the research field.

To date, no studies have been performed on prevalence trends of PMQR genes among pediatric isolates. This study has merit because a relatively large number of consecutive pediatric isolates were obtained over a long period to assess trends in PMQR prevalence and

ciprofloxacin non-susceptibility. We have demonstrated that the prevalence of PMQR genes increased over time, which was related to the increment in quinolone use and ciprofloxacin non-susceptibility. Hence, an increase in quinolone use may have been a crucial factor that led to an increased prevalence rate of PMQR genes and subsequently an increase in quinolone resistance. Currently, ciprofloxacin, levofloxacin, ofloxacin, and moxifloxacin are the 4 most commonly prescribed fluoroquinolones in Korea; however, none of these have been approved for use in children under 18 years. Tosufloxacin and norfloxacin have not yet been approved in Korea.

Although the role of PMQR in quinolone resistance remains unclear, it can help microorganisms to develop resistance mutations when exposed to therapeutic concentrations of quinolones (17,18). Notably, PMQR-positive isolates had significantly higher ciprofloxacin MIC values (8.3-fold higher when comparing the MIC₅₀ values) and ciprofloxacin non-susceptibility (2.7-fold higher) than the PMQR-negative strains, regardless of the presence of QRDR mutations (Table 1). These data suggest that the presence of PMQR genes could facilitate the accumulation of multiple mutations that lead to quinolone resistance. It will be intriguing to expand research into a new area by determining capsular serotypes and multilocus sequence typing in association with quinolone resistance.

In conclusion, this study demonstrated that selective pressure from the increased use of quinolone, together with the horizontal transmission of PMQR-positive strains prevalent in adults in the community, has contributed to the emergence of PMQR genes and increasing frequencies of ciprofloxacin non-susceptibility among *E. coli* and *K. pneumoniae* strains in the pediatric population.

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Conflict of interest None to declare.

REFERENCES

1. Hooper, D.C. (1992): Mechanisms of fluoroquinolone resistance. *Drug Resist. Updat.*, 2, 38–55.
2. Jacoby, G.A., Cattoir, V., Hooper, D.C., et al. (2008): Qnr gene nomenclature. *Antimicrob. Agents Chemother.*, 52, 2297–2299.
3. Strahilevitz, J., Jacoby, G.A., Hooper, D.C., et al. (2009): Plasmid-mediated quinolone resistance: a multifaceted threat. *Clin. Microbiol. Rev.*, 22, 664–689.
4. Wang, A., Yang, Y., Lu, Q., et al. (2008): Occurrence of qnr-positive clinical isolates in *Klebsiella pneumoniae* producing ESBL or AmpC-type β -lactamase from five pediatric hospitals in China. *FEMS Microbiol. Lett.*, 283, 112–116.
5. Han, C., Yang, Y., Wang, M., et al. (2010): The prevalence of plasmid-mediated quinolone resistance determinants among clinical isolates of ESBL or AmpC-producing *Escherichia coli* from Chinese pediatric patients. *Microbiol. Immunol.*, 54, 123–128.
6. García-Fulgueiras, V., Bado, I., Mota, M.I., et al. (2011): Extended-spectrum β -lactamases and plasmid-mediated quinolone resistance in enterobacterial clinical isolates in the paediatric hospital of Uruguay. *J. Antimicrob. Chemother.*, 66, 1725–1729.
7. Kim, H.B., Park, C.H., Kim, C.J., et al. (2009): Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. *Antimicrob. Agents Chemother.*, 53, 639–645.
8. Périchon, B., Courvalin, P. and Galimand, M. (2007): Transferable resistance to aminoglycosides by methylation of G1405 in 16S rRNA and to hydrophilic fluoroquinolones by QepA-mediated efflux in *Escherichia coli*. *Antimicrob. Agents Chemother.*, 51, 2464–2469.
9. Robicsek, A., Strahilevitz, J., Sahm, D.F., et al. (2006): Qnr prevalence in ceftazidime-resistant *Enterobacteriaceae* isolates from the United States. *Antimicrob. Agents Chemother.*, 50, 2872–2874.
10. Park, C.H., Robicsek, A., Jacoby, G.A., et al. (2006): Prevalence in the United States of *aac(6′)-Ib-cr* encoding a ciprofloxacin-modifying enzyme. *Antimicrob. Agents Chemother.*, 50, 3953–3955.
11. Clinical and Laboratory Standards Institute (CLSI) (2007): Performance Standards for Antimicrobial Susceptibility Testing-15th Informational Supplement: Approved Standard M100-S15. CLSI, Wayne, Pa., USA.
12. Patterson, J.E., Hardin, T.C., Kelly, C.A., et al. (2000): Association of antibiotic utilization measures and control of multiple-drug resistance in *Klebsiella pneumoniae*. *Infect. Control Hosp. Epidemiol.*, 21, 455–458.
13. Robicsek, A., Strahilevitz, J., Jacoby, G.A., et al. (2006): Fluoroquinolone modifying enzyme: a novel adaptation of a common aminoglycoside acetyltransferase. *Nature Med.*, 12, 83–88.
14. Martínez-Martínez, L., Pascual, A. and Jacoby, G.A. (1988): Quinolone resistance from a transferable plasmid. *Lancet*, 351, 797–799.
15. Perichon, B., Tankovic, J. and Courvalin, P. (1997): Characterization of a mutation in the *parE* gene that confers fluoroquinolone resistance in *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.*, 41, 1166–1167.
16. Kim, H.B., Wang, M., Park, C.H., et al. (2009): *oqxAB* encoding a multidrug efflux pump in human clinical isolates of *Enterobacteriaceae*. *Antimicrob. Agents Chemother.*, 53, 3582–3584.
17. Rodríguez-Martínez, J.M., Velasco, C., Pascual, A., et al. (2006): Correlation of quinolone resistance levels and differences in basal and quinolone-induced expression from three *qnrA*-containing plasmids. *Clin. Microbiol. Infect.*, 12, 440–445.
18. Rodríguez-Martínez, J.M., Velasco, C., García, I., et al. (2007): Mutant prevention concentrations of fluoroquinolones for *Enterobacteriaceae* expressing the plasmid-carried quinolone resistance determinant *qnrA1*. *Antimicrob. Agents Chemother.*, 51, 2236–2239.