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# Prevalence of Plasmid-mediated Quinolone Resistance and Its Association with Extended-spectrum Beta-lactamase and AmpC Beta-lactamase in *Enterobacteriaceae*

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**Background:** We investigated the prevalence of plasmid-mediated quinolone resistance and its association with extended-spectrum beta-lactamase (ESBL) and AmpC beta-lactamase in *Enterobacteriaceae*.
**Methods:** A total of 347 non-duplicated isolates of *Enterobacteriaceae* were collected between August and October 2006 from 2 hospitals. *Qnr* determinant screening was conducted using PCR amplification, and all positive results were confirmed by direct sequencing. *Qnr*-positive strains were determined on the basis of the presence of ESBL and AmpC beta-lactamase genes.
**Results:** The *qnr* gene was detected in 47 of 347 clinical *Enterobacteriaceae* isolates. Among the 47 *qnr*-positive strains, *Klebsiella pneumoniae* (N=29) was the most common, followed by *Escherichia coli* (N=6), *Enterobacter cloacae* (N=6), *Citrobacter freundii* (N=5), and *Enterobacter aerogenes* (N=1). These isolates were identified as *qnrA1* (N=6), 8 *qnrB* subtypes (N=40), and *qnrS1* (N=1). At least 1 ESBL was detected in 38 of the 47 *qnr*-positive strains. *Qnr*-positive strains also showed high positive rates of ESBL or AmpC beta-lactamase, such as TEM, SHV, CTX-M, and DHA. DHA-1 was detected in 23 of 47 *qnr*-positive strains, and this was co-produced with 1 *qnrA1* and 22 *qnrB4*. Strains harboring MIR-1T and CMY were also detected among the *qnr*-positive strains. Antimicrobial-resistance rates of *qnr*-positive strains to ciprofloxacin, levofloxacin, norfloxacin, nalidixic acid, and moxifloxacin were 51.1%, 46.8%, 46.8%, 74.5%, and 53.2%, respectively.
**Conclusions:** The *qnr* genes were highly prevalent in *Enterobacteriaceae*, primarily the *qnrB* subtypes. They were closely associated with EBSL and AmpC beta-lactamase.

**Key Words:** *Qnr*, Beta-lactamase, Quinolone, *Enterobacteriaceae*

## INTRODUCTION

The first plasmid-mediated quinolone resistance determinant, *qnrA*, was reported in 1998 in a *Klebsiella pneumoniae* strain from the United States [1]. Since then, 2 additional *qnr* determinants, *qnrB* and *qnrS*, have been identified in *Enterobacteriaceae* species [2, 3]. These determinants increase the minimum inhibitory concentrations (MICs) of quinolones by 8- to 32-fold, preventing the inhibition of DNA gyrase [4]. These 3 major groups of *qnr* determinants have since been detected in various *Enterobacteriaceae* species worldwide [5-9], and many *qnr* variants are listed in GenBank. Among them, *qnrB* sequences are particularly numerous; hence, *qnrB* sequences with the same allele number and dissimilar sequences submitted by different investigators or with the same sequences and different allele numbers can be found [10]. This situation results in confusion for many investigators when numbering their *qnr* sequences, particularly *qnrB* variants; thus, Jacoby et al. [10] adjusted

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all previously submitted sequences and proposed a new, well-organized numbering system for *qnr* alleles.

Association of *qnr* genes with extended-spectrum beta-lactamases (ESBLs) or AmpC beta-lactamases is also noteworthy; some strains containing a *qnrA* gene express class A or class C beta-lactamase, such as SHV, CTX, VEB-1, or DHA-1 [11, 12]. Some reports show an association of *qnrB* with an ESBL and AmpC beta-lactamase [3, 13].

Although there are some reports examining the prevalence of *qnr* determinants in Korea, these were restricted to specific bacterial species such as *Escherichia coli*, *K. pneumoniae*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Serratia marcescens* regardless of the type of specimen [8, 13-16]. Additionally, there are few data regarding the differences in *qnr* prevalence according to the type of clinical specimen and bacterial species and its association with various ESBLs and AmpC beta-lactamases. The aims of this study were to investigate the prevalence of 3 groups of plasmid-mediated quinolone resistance determinants (*qnrA*, *qnrB*, and *qnrS*) and to examine the association of these determinants with ESBLs and AmpC beta-lactamases in *Enterobacteriaceae*.

## MATERIALS AND METHODS

### 1. Strains

A total of 347 non-duplicated clinical strains of *Enterobacteriaceae* were collected from various clinical specimens from 2 hospitals, namely, the 902-bed tertiary-care Busan Paik Hospital (N=267) and the 200-bed secondary-care Dongrae Paik Hospital (N=80) in 2006. Sampling locations included urine (N=150), sputum or bronchial washings (N=59), abscess or wound pus (N=52), drainage discharge (N=30), peritoneal fluid (N=14), cervical discharge (N=10), gastric juice (N=6), blood culture (N=6), tissue (N=4), stool (N=4), bile (N=3), vaginal discharge (N=3), pleural fluid (N=2), catheter tip (N=2), intrauterine device (N=1), and cerebrospinal fluid (N=1). The strains were identified using routine laboratory protocols, including conventional biochemical tests and the VITEK system (bioMèrieux Vitek Inc., Hazelwood, MO, USA). These strains were identified as *E. coli* (N=159), *K. pneumoniae* (N=100), *E. cloacae* (N=30), *S. marcescens* (N=13), *Proteus mirabilis* (N=10), *Morganella morganii* (N=6), *C. freundii* (N=7), *Enterobacter aerogenes* (N=4), *Klebsiella oxytoca* (N=3), *Citrobacter braakii* (N=2), *Proteus vulgaris* (N=2), *Proteus penneri* (N=2), *Citrobacter amalonaticus* (N=1), *Enterobacter asburiae* (N=1), *Enterobacter hormaechei* (N=1), *Enterobacter intermedius* (N=1), *Escherichia fergusonii* (N=1), *Klebsiella rhinoscleromatis* (N=1), *Klebsiella* species (N=1), *Providencia rettgeri* (N=1), and *Providencia stuartii* (N=1). All isolates were stored in skim milk at -70˚C until further molecular and microbiological evaluation.

### 2. PCR and sequencing for *qnr* genes

Genomic DNA of the bacterial strains was amplified by PCR for the detection of 3 groups of *qnr* determinants (*qnrA, qnrB,* and *qnrS*) as described by Gay et al. [5]. All procedures for PCR and sequencing were the same as those described in our previous report [8]. Briefly, colonies were suspended in 50 μL of water in a microcentrifuge tube and boiled to prepare DNA templates. The primers used to amplify *qnrA* to yield a 516-bp product were 5´-ATTTCTCACGCCAGGATTTG-3´ and 5´-GATCGGCAAAGGTTAGGTCA-3´. Those used for *qnrB* to yield a 469-bp product were 5´-GATCGTGAAAGCCAGAAAGG-3´ and 5´-ACGATGCCTGGTAGTTGTCC-3´. Those used for *qnrS* to yield a 417-bp product were 5´-ACGACATTCGTCAACTGCAA-3´ and 5´-TAAATTGGCACCCTGTAGGC-3´. The 3 primer pairs were added to a template and PCR premix (Bioneer, Daejeon, Korea). PCR conditions were 94˚C for 45 sec, 53˚C for 45 sec, and 72˚C for 60 sec, with the cycle repeated 32 times. Reaction mixtures without a DNA template served as negative controls. All positive amplicons were sequenced to confirm the subtypes of the 3 *qnr* genes. Amplified DNA was isolated using the Qiagen gel extraction kit (Qiagen Inc., Hilden, Germany), and sequencing was performed using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 3130xl genetic analyzer (Applied Biosystems). Both amplicon strands were sequenced using the same primers as for PCR amplification. We numbered the *qnr* allele subtypes according to the *qnr* gene nomenclature of Jacoby et al. [10].

### 3. PCR and sequencing for ambler class A beta-lactamase and plasmid-mediated AmpC beta-lactamase among *qnr*-positive strains.

Plasmid DNA was extracted using a Qiagen Plasmid Purification kit (Qiagen Inc.) following the manufacturer's recommendations to detect ESBL and AmpC beta-lactamase in *qnr*-positive strains. In previous experiments, this plasmid DNA was used as a template for PCR with a series of primers designed to detect class A beta-lactamase genes and their extended-spectrum derivatives (Table 1): $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{PER-1}}$, $bla_{\text{VEB}}$, $bla_{\text{IBC/GES}}$, $bla_{\text{TEM}}$, and $bla_{\text{SHV}}$ [17, 18]. PCR products were subjected to direct sequencing, with both strands being sequenced to reduce error. We also investigated the presence of plasmid-mediated AmpC beta-lactamase among *qnr*-positive strains

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**Table 1.** Primers used for the detection and sequencing of class A beta-lactamases and plasmid-mediated AmpC beta-lactamases

| PCR target | Primer name | Primer sequence | Used for | Reference |
|---|---|---|---|---|
| *bla*$_{CTX-M}$ (CTX-M-1 cluster) | CTX-M-1F | 5´-CCG TCA CGC TGT TGT TAG G-3´ | | [17] |
| | CTX-M-1R | 5´-GAC GAT TTT AGC CGC CGA C-3´ | | |
| | BM-1F | 5´-ACT ATG GCA CCA CCA ACG AT-3´ | | [17] |
| | FM-1R | 5´-TTC GGT TCG CTT TCA CTT TT-3´ | | |
| *bla*$_{CTX-M}$ (CTX-M-2 cluster) | CTX-M-2F | 5´-CGG TGC TTA AAC AGA GCG AG-3´ | | [17] |
| | CTX-M-2R | 5´-CCA TGA ATA AGC AGC TGA TTG CCC-3´ | | |
| *bla*$_{CTX-M}$ (CTX-M-8 cluster) | CTX-M-8F | 5´-ACG CTC AAC ACC GCG ATC-3´ | | [17] |
| | CTX-M-8R | 5´-CGT GGG TTC TCG GGG ATA A-3´ | | |
| *bla*$_{CTX-M}$ (CTX-M-9 cluster) | CTX-M-9F | 5´-GAT TGA CCG TAT TGG GAG TTT-3´ | | [17] |
| | CTX-M-9R | 5´-CGG CTG GGT AAA ATA GGT CA-3´ | | |
| *bla*$_{PER-1}$ | PER-1F | 5´-GTT AAT TTG GGC TTA GGG CAG-3´ | | [17] |
| | PER-1R | 5´-CAG CGC AAT CCC CAC TGT-3´ | | |
| *bla*$_{VEB}$ | VEB-F | 5´-ACC AGA TAG GAG TAC AGA CAT ATG A-3´ | | [17] |
| | VEB-R | 5´ -TTC ATC ACC GCG ATA AAG CAC-3´ | | |
| *bla*$_{GES}$ and *bla*$_{IBC}$ | GES/IBC-F | 5´-GTT AGA CGG GCG TAC AAA GAT AAT-3´ | | [17] |
| | GES/IBC-R | 5´-TGT CCG TGC TCA GGA TGA GT-3´ | | |
| *bla*$_{TEM}$ | TEM-F | 5´-ATG AGT ATT CAA CAT TTC CGT-3´ | | [18] |
| | TEM-R | 5´-TTA CCA ATG CTT AAT CAG TGA-3´ | | |
| *bla*$_{SHV}$ | SHV-F | 5´-CCG GGT TAT TCT TAT TTG TCG CT-3´ | | [18] |
| | SHV-R | 5´-TAG CGT TGC CAG TGC TCG-3´ | | |
| *bla*$_{MOX-1}$, *bla*$_{MOX-2}$, *bla*$_{CMY-1}$, *bla*$_{CMY-8}$ to *bla*$_{CMY-11}$ | MOXMF | 5´-GCT GCT CAA GGA GCA CAG GAT-3´ | | [19] |
| | MOXMR | 5´-CAC ATT GAC ATA GGT GTG GTG C-3´ | | |
| *bla*$_{LAT-1}$ to *bla*$_{LAT-4}$, *bla*$_{CMY-2}$ to *bla*$_{CMY-7}$, *bla*$_{BIL-1}$ | CITMF | 5´-TGG CCA GAA CTG ACA GGC AAA-3´ | | [19] |
| | CITMR | 5´-TTT CTC CTG AAC GTG GCT GGC-3´ | | |
| *bla*$_{DHA-1}$, *bla*$_{DHA-2}$ | DHAMF | 5´-AAC TTT CAC AGG TGT GCT GGG T-3´ | | [19] |
| | DHAMR | 5´-CCG TAC GCA TAC TGG CTT TGC-3´ | | |
| *bla*$_{ACC}$ | ACCMF | 5´-AAC AGC CTC AGC AGC CGG TTA-3´ | | [19] |
| | ACCMR | 5´-TTC GCC GCA ATC ATC CCT AGC-3´ | | |
| *bla*$_{MIR-1T}$, *bla*$_{ACT-1}$ | EBCMR | 5´-TCG GTA AAG CCG ATG TTG CGG-3´ | | [19] |
| | EBCMR | 5´-CTT CCA CTG CGG CTG CCA GTT-3´ | | |
| *bla*$_{FOX-1}$ to *bla*$_{FOX-5b}$ | FOXMF | 5´-AAC ATG GGG TAT CAG GGA GAT G-3´ | | [19] |
| | FOXMR | 5´-CAA AGC GCG TAA CCG GAT TGG-3´ | | |
| *bla*$_{DHA-1}$ | DHA-1-LF | 5´-GGG GAG ATA ACG TCT GAC CA-3´ | Sequencing | [27] |
| | DHA-1-LR | 5´-TAG CCA GAT CCA GCA ATG TG-3´ | | |
| | DHA-1-MF | 5´-AAC TTT CAC AGG TGT GCT GGG T-3´ | | |
| | DHA-1-MR | 5´-CCG TAC GCA TAC TGG CTT TGC-3´ | | |
| | DHA-1-RF | 5´-GCT GGG GTT ATC TCA CAC CT-3´ | | |
| | DHA-1-RR | 5´-AAA TTA CGG CCC CGG CGT GT-3´ | | |
| *bla*$_{ACT-1}$ | PCACT-1F | 5´-CTT GAA CTG CTA TTA CGG-3´ | Sequencing | [28] |
| | PCACT-1R | 5´-GCA ATG TTT ACT ACA GCG-3´ | | |
| *bla*$_{MIR-1}$ | PCACT-1F | 5´-CTT GAA CTG CTA TTA CGG-3´ | Sequencing | [28] |
| | MIR-1R | 5´-GCA ATG TTT TAC TGC AGC G-3´ | | |
| *bla*$_{CMY-2}$ | CMY-2XF | 5´-AAC ACA CTG ATT GCG TCT GA-3´ | Sequencing | [19, 29] |
| | CMY-2XR | 5´-CTG GGC CTC ATC GTC AGT TA-3´ | | |

using the primers developed by Perez-Perez and Hanson for 6 groups: ACC, FOX, MOX/CMY, DHA, CIT, and EBC [19]. These groups could be used to detect the following target genes: $bla_{DHA}$, $bla_{MOX}$, $bla_{CMY}$, $bla_{LAT}$, $bla_{BIL}$, $bla_{ACC}$, $bla_{MIR}$, $bla_{ACT}$, and $bla_{FOX}$. We used 2 multiplex PCR methods described by Lee et al. [20] that were modified from the method of Perez-Perez and Hanson [19] to amplify the plasmid-mediated AmpC beta-lactamase genes. We used additional primers for sequencing the plasmid-mediated AmpC beta-lactamase genes and identified them by sequencing the PCR-amplified products. Primers used for the detection and sequencing of ESBL and the plasmid-mediated AmpC beta-lactamase are shown in Table 1.

### 4. Antimicrobial susceptibility tests

Susceptibility testing against quinolones was determined using the broth microdilution method recommended by the CLSI [21]. Isolates were tested against the following 5 agents in cation-adjusted Mueller-Hinton broth (BD, Sparks, MD, USA): ciprofloxacin (Sigma, Steinheim, Germany), levofloxacin (Sigma), norfloxacin (Sigma), nalidixic acid (Sigma), and moxifloxacin (Bayer AG, Wuppertal, Germany). MICs were determined after incubation at 35°C for 24 hr in ambient air. *E. coli* ATCC 25922 was used as the control strain. The interpretive criteria were those published in the relevant CLSI document [22]. For other antimicrobial agents, susceptibility testing was in accordance with routine laboratory protocols performed using the VITEK system or the disk diffusion assay.

## RESULTS

### 1. Prevalence of *qnr* genes and their subtypes

The *qnr* gene was detected in 47 of the 347 *Enterobacteriaceae* isolates by using PCR and sequencing. The positive rates were higher among organisms from the tertiary-care hospital (15.7%, N=42) than in the secondary-care facility (6.3%, N=5). The specimens showing *qnr*-positive results included sputum (N=15), urine (N=13), wound pus (N=10), drainage discharge (N=4), peritoneal fluid (N=2), cervical discharge (N=1), pleural fluid (N=1), and stool (N=1). *Qnr* genes were detected at a higher rate in respiratory specimens (15/59, 25.4%) than in urine (13/150, 8.7%). The most commonly observed *qnr*-positive pathogen was *K. pneumoniae* (N=29), followed by *E. coli* (N=6), *E. cloacae* (N=6), *C. freundii* (N=5), and *E. aerogenes* (N=1).

Ten *qnr* subtypes, including 1 *qnrA*, 8 *qnrB*, and 1 *qnrS*, were detected. There was a considerable difference in subtypes according to the specific species (Table 2). Thus, *qnrA* was primarily detected in *E. cloacae* and *E. coli*; on the other hand, *qnrB* was highly prevalent in *K. pneumoniae*. The *qnrA* gene was detected in 6 of the 47 *qnr*-positive isolates, and the sequence was identical for all strains. The *qnrB* gene was detected in 40 of the 47 *qnr*-positive isolates. These isolates showed considerable multiplicity of subtypes and sequences. The high rates of the *qnrB4* subtype among *qnrB* subtypes (29/40) should be noted. In addition to these *qnrB4* subtypes, 1 isolate containing *qnrB1*, 3 having *qnrB2*, 1 having *qnrB6*, 1 having *qnrB7*, 2 having *qnrB8*, 2 having *qnrB9*, and 1 having *qnrB12* were detected. The *qnrS1* gene was found in only 1 strain and exhibited 100% identity with the reference *qnrS1* gene sequence.

**Table 2.** Prevalence of *qnr* subtypes and variants according to species

| *qnr* subtype | *C. freundii* | *E. aerogenes* | *E. cloacae* | *E. coli* | *K. pneumoniae* | Total |
|---|---|---|---|---|---|---|
| *qnrA1* | | | 4 | 2 | | 6 |
| *qnrB1* | | | | | 1 | 1 |
| *qnrB2* | | | 1 | 1 | 1 | 3 |
| *qnrB4* | | | 1 | 3 | 25 | 29 |
| *qnrB6* | | | | | 1 | 1 |
| *qnrB7* | 1 | | | | | 1 |
| *qnrB8* | 1 | 1 | | | | 2 |
| *qnrB9* | 2 | | | | | 2 |
| *qnrB12* | 1 | | | | | 1 |
| *qnrS1* | | | | | 1 | 1 |
| Total | 5 | 1 | 6 | 6 | 29 | 47 |

Abbreviations: *qnr,* quinolone resistance gene; *C. freundii, Citrobacter freundii; E. aerogenes, Enterobacter aerogenes; E. cloacae, Enterobacter cloacae; E. coli, Escherichia coli; K. pneumoniae, Klebsiella pneumoniae.*

### 2. Association of *qnr*-positive strains with ambler class A beta-lactamase and plasmid-mediated AmpC beta-lactamase

At least 1 ESBL or AmpC beta-lactamase was detected in 38 of the 47 *qnr*-positive isolates (Table 3). The beta-lactamase production rates of *qnr*-positive *K. pneumoniae*, *E. coli*, *E. cloacae*, and *C. freundii* were 82.8% (24/29), 66.7% (4/6), 100% (6/6), and 80.0% (4/5), respectively. No ESBLs were detected in *E. aerogenes* harboring *qnrB8*. The *qnr*-positive strains showed high positive rates of beta-lactamases such as TEM, SHV, CTX-M, and DHA. CTX-M-3, CTX-M-9, and SHV-12 were highly prevalent among ambler class A beta-lactamases. In plasmid-mediated AmpC beta-lactamase, DHA-1 was detected in 23 of 47 *qnr*-positive strains and was co-produced with 1 *qnrA1* and 22 *qnrB4*. Strains harboring MIR-1T and CMY primers were also detected among *qnr*-positive isolates. There were no *qnr*-positive clinical isolates harboring MOX, ACC, FOX, PER-1, VEB, or GES.


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**Table 3.** *Qnr* subtype and association with class A beta-lactamase and plasmid-mediated AmpC beta-lactamase

| *Qnr* variant | Species | N | CTX-M | SHV | TEM | DHA-1 | MIR-1T | CMY |
|---|---|---|---|---|---|---|---|---|
| *qnrA1* | *E. coli* | 1 | | | | | | |
| | *E. coli* | 1 | CTX-M-3+9 | | | | | |
| | *E. cloacae* | 2 | CTX-M-9 | | | | | |
| | *E. cloacae* | 1 | CTX-M-9 | SHV-12 | | | | |
| | *E. cloacae* | 1 | | SHV-12 | | DHA-1 | | |
| *qnrB1* | *K. pneumoniae* | 1 | | | | | | |
| *qnrB2* | *E. coli* | 1 | | | | | | |
| | *K. pneumoniae* | 1 | | SHV-12 | | | | |
| | *E. cloacae* | 1 | | SHV-12 | | | ACT-1 | |
| *qnrB4* | *K. pneumoniae* | 2 | | | | | | |
| | *E. coli* | 1 | CTX-M-15 | | | DHA-1 | | |
| | *K. pneumoniae* | 3 | CTX-M-3 | SHV-12 | | DHA-1 | | |
| | *K. pneumoniae* | 1 | CTX-M-3 | SHV-12 | | | | |
| | *K. pneumoniae* (3)/*E. cloacae* (1) | 4 | CTX-M-3 | | | DHA-1 | | |
| | *K. pneumoniae* | 1 | CTX-M-9 | | | | | |
| | *K. pneumoniae* (2)/*E. coli* (1) | 3 | | | | DHA-1 | | |
| | *K. pneumoniae* | 2 | | SHV-12 | | | | |
| | *K. pneumoniae* | 9 | | SHV-12 | | DHA-1 | | |
| | *K. pneumoniae* | 2 | | SHV-2a | | DHA-1 | | |
| | *E. coli* | 1 | | | TEM-31 | | | |
| *qnrB6* | *K. pneumoniae* | 1 | | | | | | |
| *qnrB7* | *C. freundii* | 1 | | | | | | CMY-34-like (P201Q) |
| *qnrB8* | *E. aerogenes* | 1 | | | | | | |
| | *C. freundii* | 1 | CTX-M-3 | | | | | |
| *qnrB9* | *C. freundii* | 2 | | | | | | CMY-2 |
| *qnrB12* | *C. freundii* | 1 | | | | | | |
| *qnrS1* | *K. pneumoniae* | 1 | | | | | | |

Abbreviations: *qnr*, quinolone resistance gene; *E. coli, Escherichia coli; E. cloacae, Enterobacter cloacae; K. pneumoniae, Klebsiella pneumoniae; C. freundii, Citrobacter freundii; E. aerogenes, Enterobacter aerogenes.*

### 3. Antimicrobial Susceptibility to Quinolone Agents

Of the 47 *qnr*-positive strains examined, the resistance rates to ampicillin, ampicillin-sulbactam, aztreonam, cefazolin, tobramycin, amikacin, ceftriaxone, cefepime, and trimethoprim-sulfamethoxazole were 100%, 84.8%, 69.6%, 87.0%, 69.6%, 43.5%, 45.7%, 34.8%, and 47.8%, respectively. The antimicrobial-resistance rates of *qnr*-positive strains to ciprofloxacin, levofloxacin, norfloxacin, nalidixic acid, and moxifloxacin were 51.1%, 46.8%, 46.8%, 74.5%, and 53.2%, respectively (Table 4). The plasmid-mediated quinolone resistance rate was very high in both quinolone-resistant (24/96) and quinolone-susceptible strains (22/248). No imipenem- or meropenem-resistant strains of *Enterobacteriaceae* were observed.

## DISCUSSION

In this study, 47 of 347 (12.4%) *Enterobacteriaceae* showed *qnr* positive results. There was a considerable difference in *qnr* prevalence between the tertiary-care (15.7%) and secondary-care hospitals (6.3%) evaluated in this study. Generally, antimicrobial resistance rates are higher in tertiary-care hospitals [23, 24], and these differences are thought to be applicable to *qnr*-positive rates. Although several articles regarding *qnr* genes have been published, they focus on a specific species and do not report specimen types. In the present study, we isolated *qnr*-positive strains from various clinical specimens. We also observed a characteristically high *qnr*-positive rate of respiratory specimens (25.4%) compared with other specimens, including urine (8.7%), wound pus (19.2%), discharge (13.3%), and peritoneal fluid (14.3%). This

**Table 4.** MICs of 47 *qnr*-positive isolates to quinolones

| Species and *qnr* gene | N | MIC range ($MIC_{50}$/$MIC_{90}$) (μg/mL) | | | | |
|---|---|---|---|---|---|---|
| | | CIP | LEV | NOR | NAL | MOX |
| *K. pneumoniae* (N = 29) | | | | | | |
| *qnrB1* | 1 | < 0.03 | 0.06 | 0.12 | 2 | 0.06 |
| *qnrB2* | 1 | 2 | 2 | 8 | 256 | 2 |
| *qnrB4* | 25 | 0.12- > 256 (128/256) | 0.12-256 (32/128) | 1- > 256 (256/ > 256) | 16- > 256 ( > 256/ > 256) | 0.06- > 256 (8/256) |
| *qnrB6* | 1 | > 256 | 64 | > 256 | > 256 | 256 |
| *qnrS1* | 1 | 256 | 128 | > 256 | > 256 | > 256 |
| *E. coli* (N = 6) | | | | | | |
| *qnrA1* | 2 | 16-128 | 8-64 | 32- > 256 | > 256- > 256 | 16-128 |
| *qnrB2* | 1 | 0.25 | 0.5 | 1 | > 256 | 2 |
| *qnrB4* | 3 | 0.12- > 256 | 0.03-64 | 0.25- > 256 | 2- > 256 | 0.06-256 |
| *E. cloacae* (N = 6) | | | | | | |
| *qnrA1* | 4 | 0.05-1 | 0.5-1 | 0.5-2 | 16-32 | 2-4 |
| *qnrB2* | 1 | 4 | 2 | 4 | > 256 | 4 |
| *qnrB4* | 1 | 256 | 64 | > 256 | > 256 | 128 |
| *C. freundii* (N = 5) | | | | | | |
| *qnrB7* | 1 | < 0.03 | 0.12 | 0.12 | 8 | 1 |
| *qnrB8* | 1 | < 0.03 | 0.06 | 0.5 | 4 | 0.25 |
| *qnrB9* | 2 | < 0.03- < 0.03 | 0.12-0.12 | 0.25-1 | 8-32 | 1-1 |
| *qnrB12* | 1 | 0.25 | 0.25 | 1 | 8 | 1 |
| *E. aerogenes* (N = 1) | | | | | | |
| *qnrB8* | 1 | 0.25 | 0.06 | 1 | 4 | 0.25 |

Abbreviations: *qnr*, quinolone resistance gene; MIC, minimum inhibitory concentration; *K. pneumoniae, Klebsiella pneumoniae; E. coli, Escherichia coli; E. cloacae, Enterobacter cloacae; C. freundii, Citrobacter freundii; E. aerogenes, Enterobacter aerogenes*.

may be because *K. pneumoniae* is highly prevalent in respiratory specimens. Because *qnr*-positive strains can spread in hospital environments and cause fatal nosocomial infection, the prevalence of *qnr* genes should be periodically investigated.

The numbers of *qnrB* subtypes were diverse compared with those found in our previous study [8]. Additionally, 6 *qnrA* strains were different from those in our previous study. We confirmed that *qnrA1* (6/47) and *qnrB4* (29/47) strains were common, with these 2 subtypes accounting for 74.5% (35/47) of the total *qnr*-positive strains.

The prevalence of the *qnr* gene was caused by *qnrB4*-positive *K. pneumoniae* in our hospital; a significant number of the *qnr*-positive strains were of this unique subtype. Although the rate of *qnr* in *C. freundii* (42.9%) was relatively high, the numbers of *C. freundii* were small. Few reports have investigated *qnr* prevalence in *C. freundii*; hence, further evaluation is necessary [16, 25].

The presence of *qnr* was significantly related to the co-production of ESBL or AmpC beta-lactamase. Surprisingly, at least 1 ESBL was detected in 38 of 47 *qnr*-positive isolates examined in this study. The 14, 21, and 3 *qnr*-positive strains showed co-production of ESBL or AmpC beta-lactamase in 1, 2, and 3 genes, respectively. This is primarily attributable to the *qnrA1* and *qnrB4* subtypes.

There are some reports of an association between *qnr* and ESBL. Wang et al. [11] reported that several *qnrA*-positive strains produced SHV-7 and CTX-M-9. In our study, CTX-M-9 was common (4 of 6) among *qnrA1*-positive strains, but 1 strain produced SHV-12 instead of SHV-7. Another report revealed positive results for both *qnrA* and VEB-1 in *E. coli* [6]. The investigators subsequently detected the *qnrA* gene in 11 of 23 $bla_{VEB-1}$-positive *Enterobacter sakazakii* strains from Bangkok [26]. They also stated that the *qnrA* gene was not identified in positive enterobacterial isolates from any country except in previous 2 reports. No VEB-1-positive strain was identified among the 47 *qnr*-positive *Enterobacteriaceae* examined in our study.

In Korea, Pai et al. [13] described a clear association between *qnrB4* and DHA-1 strains. In our study, many *qnrB4*-positive strains contained DHA-1 and, except for *qnrB4* strains, only 1 strain produced DHA-1 in *qnrA1* among all *qnr*

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subtypes. Thus, we presumed that an association exists between *qnrB4* and DHA-1. However, this association does not appear to be limited to DHA-1. Although Pai et al. [13] stated that *qnrB*-positive strains were not closely related to SHV-12, we obtained different results. The co-production rates of SHV were very high in the *qnrB4* strain. SHV was produced in 17 strains among the 29-*qnrB4* positive strains. Additionally, 12 SHV were co-produced among 22 strains that were positive for both DHA-1 and *qnrB4*, and 20 of 23 DHA-1-positive strains harbored either SHV or CTX-M ESBLs. SHV-12 and DHA-1 were the most commonly observed strains in this study. In a previous report, CTX-M was found to be rare among *qnr*-positive strains, and these strains were primarily *qnrA*-positive ones [13]. However, 14 of 47 *qnr*-positive strains were CTX-M positive in the present study. These strains and 10 CTX-M strains, including 3 CTX-M-3, 1 CTX-M-9, and 1 CTX-M-15, harbored *qnrB4*. This was a characteristic finding in our series. Investigating the genetic environments, including CR1, to understand the mechanisms of association between *qnr* and ESBL is important. Additional evaluation is required to obtain a precise understanding of these mechanisms.

In the present study, we verified that the *qnr* genes were prevalent among quinolone-susceptible strains as well as among quinolone-resistant strains. This suggests that the *qnr* gene is widely dispersed among *Enterobacteriaceae*. This is because of the high prevalence of *qnrB4* strains, which are closely related to various ESBL and plasmid-mediated AmpC beta-lactamases.

### Authors' Disclosures of Potential Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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