Quinolones are broad-spectrum antimicrobial agents for the treatment of a variety of clinical and veterinary infections (1). Widespread use of these agents has heavily contributed to the rise of bacterial quinolone resistance. Bacterial resistance to quinolones is usually due to mutational changes in the chromosomally encoded type II topoisomerases, and the expression of efflux pumps as well as the loss of porins are expected to contribute to the development of quinolone resistance (2). However, low-level quinolone resistance was recently found to be transferable by a plasmid. After detection of the first valid plasmid-mediated quinolone-resistance determinant (*qnrA*) (3), two other plasmid-mediated resistant genes (*qnrB* and *qnrS*) were discovered (4,5). Plasmid-carrying genes could contribute to the development of higher-level fluoroquinolone resistance and might pose a threat, allowing the rapid spread of resistance among organisms (6). Thus, this and related findings might be important for treatment of patients in intensive care units (ICUs), where infection control is notoriously difficult.

In this study, we investigated isolates for the presence of *qnrA*, *qnrB*, and *qnrS* determinants among ciprofloxacin-resistant and susceptible strains isolated from ICU patients at two points in time, which is a 6-year interval. A total of 460 non-duplicate strains isolated from clinical samples (*n* = 347, from tracheal aspirates, abscesses, catheters, urine, sputum, blood, peritoneal fluid, and cerebrospinal fluid) and from non-clinical samples (*n* = 113, from rectal swabs) isolated in the years 2000 and 2006 were investigated. The following strains were examined: 117 *Escherichia coli*, 108 *Klebsiella pneumoniae*, 81 *Acinetobacter* spp., 72 *Pseudomonas* spp., 30 *Enterobacter* spp., 22 nonfermenting Gram-negative bacteria, 9 *Proteus mirabilis*, 8 *Serratia* spp., 6 *Klebsiella oxytoca*, 5 *Proteus vulgaris*, and 2 *Citrobacter* spp. isolates. The isolates belonged to 11 different genera of Gram-negative bacteria, and 40% of them were ciprofloxacin-resistant. Since strains carrying *qnr* may be detected as quinolone-susceptible, the samples contained both ciprofloxacin-susceptible and resistant isolates. Antimicrobial susceptibility tests were carried out by disc diffusion on Mueller-Hinton agar plates according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (7). The MICs of ciprofloxacin, cefotaxime, and ceftazidime were determined using the E-test (AB Biodisk, Solona, Sweden) method according to the manufacturer’s recommendations. *E. coli* ATCC 25922 was used as the control for susceptibility testing. Colonies of clinical strains were transferred into a sterile distilled water solution in an Eppendorf tube and the samples were boiled in order to prepare the DNA templates for polymerase chain reaction (PCR). The *qnrA*, *qnrB*, and *qnrS* genes were screened with recently described primer sets (5,8,9). *Enterobacter cloacae* 14300, *K. pneumoniae* Kp15*, and *E. cloacae* S2* were used as positive controls for *qnrA*, *B*, and *S*, respectively, and these controls were included with each group of tested strains. The amplification reaction consisted of 35 cycles of 60 s of denaturation at 94°C, 60 s of annealing at 55°C, and 60 s of extension at 72°C, with a final extension cycle of 7 min at 72°C. Products of amplification were detected by electrophoresis on 1% agarose gel with ethidium bromide staining, and the products were then photographed under UV light. Both strands of the amplification products obtained were sequenced with an Applied Biosystems sequencer (ABI PRISM 310 Genetic Analyzer; Applied Biosystems, Foster City, Calif., USA). The nucleotide sequences were analyzed with software available at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov). Detection and identification of extended-spectrum β-lactamase (ESBL) was performed for the *qnr*-positive strains and their transconjugants using specific primers of sequences encoding the TEM, SHV, CTX-M, and VEB-type ESBLs (9,11). Conjugation experiments using an azide-resistant *E. coli* J53
(AzR) as the recipient were performed in liquid media culture as previously described (12). Transconjugants were selected on trypticase soy agar plates containing sodium azide (100 μg/ml) for counter-selection and amoxicillin (100 μg/ml), trimethoprim-sulfamethoxazole (300 μg/ml), and cefoxitin (2 μg/ml). Plasmid analyses of clinical isolates, transconjugants, and reference strains were carried out according to methods described by Kado and Liu (13). Then, agarose gel electrophoresis analysis was conducted. E. coli NCTC50192 harboring four plasmids of 154, 66, 38, and 7 kb was used as the size marker for the plasmids. The total DNA from each qnrS-positive isolate was analyzed by a random amplified polymorphic DNA assay using the primers ERIC-2 and M13 as previously described (14).

The qnr genes were detected in three (one qnrB1 and two qnrS1) (0.65% of all strains, 1.09% of Enterobacteriaceae) out of 460 strains. Ciprofloxacin-susceptible E. cloacae (Ec9) and E. cloacaee1 (Ec11) were isolated from urine cultures in 2006, and these were qnrS1-positive. Ciprofloxacin-susceptible E. cloacae397 (Ec397) came from a culture of tracheal aspirate in 2006, and this specimen was qnrB1-positive. Each strain was isolated from different patients, these strains were all ESBL-producers, according to the results of a double disc-synergy test. Ec9 and Ec11 have similar antibiotic patterns, i.e., they are resistant to ampicillin, amoxicillin-clavulanic acid, ceftazolin, cefoxitin, cefotaxime, rifampicin, and trimethoprim-sulfamethoxazole. Ec397 was also resistant to gentamicin and chloramphenicol (Table 1). Genomic analysis by random amplified polymorphic DNA (RAPD) showed that two qnrS-positive E. cloacae strains were clonally related (data not shown). The pattern of susceptibility to the β-lactams of transconjugants corresponded to the expression of a clavulanic acid-inhibited ESBL. Ec9 and Ec11 harbored two non-conjugative plasmids with lengths of approximately 80 and 60 kb, respectively. Ec397 harbored an approximately 60-kb conjugative plasmid. PCR assays were used to detect β-lactamases, and identified a CTX-M-3 β-lactamase gene in Ec9 and Ec11, and a CTX-M-15 β-lactamase gene in Ec397. Transfer of the qnrB and bla<sub>CTX-M-15</sub> gene in E. cloacae397 to the azide-resistant E. coli J53 occurred following conjugation experiments and selection on agar containing 100 mg/L azid and 100 mg/L amoxicillin. TCEc397 was resistant to ampicillin, ceftazolin, gentamicin, rifampicin, chloramphenicol, and trimethoprim-sulfamethoxazole (Table 1). Transconjugants of Ec9 and Ec11 produced CTX-M-3 β-lactamase, but the qnrS gene was not identified.

Although qnr determinants have been screened in many countries around the world, they have not been investigated in detail in Turkey. Recently, Oktem et al. (15) screened for qnr genes among 78 quinolone-resistant blood culture isolates belonging among the Enterobacteriaceae, and these isolates from different locations in Turkey produced ESBL. The researchers found 5 qnrA-positive strains (6.4%), although neither qnrB nor qnrS were detected. Their results are similar to those of our previous study showing a rate of 4% qnrA among 49 ESBL-positive strains (10). In the present study, no qnrA was detected, and qnrB and qnrS were detected only infrequently. Another study by Assaroglu et al. reported finding qnrS1 in Salmonella enterica serovar Virchow isolated from Turkish food of avian origin (16). Recent studies that have detected qnr-positive and quinolone-susceptible clinical isolates have raised concerns about a hidden spread of qnr-mediated resistance (17). Three qnr-positive/ciprofloxacin-susceptible strains identified in our study provide support for such concerns. In Turkey, strain counts may be higher and affected locations more numerous, which may help estimate the prevalence of qnr.

This study provides the first report of the detection of qnrB and qnrS among clinical strains isolated from samples obtained in Turkey. The prevalence of qnrA, qnrB, and qnrS appears to be low in Turkey, but these strains may nonetheless initiate a rapid increase in bacterial resistance to valuable antimicrobial agents. Screening and detection of these strains may ultimately contribute to a revision of guidelines for antibiotic use, especially in ICUs.

Acknowledgments

We thank Laurent Poirel for kindly providing the strains K. pneumoniae Kp15, (qnrB positive) and E. cloacae S2 (qnrS positive).

References


Table 1. Drug susceptibility of three qnr-positive E. cloacae and their transconjugants

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<th>Strain</th>
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1) Data for E. coli J53 azid resistant (EcJ53AzR) are included for comparison.