Detection of *qnr* in Clinical Isolates of *Escherichia coli* from Korea

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***qnr* was detected in 2 of 260 *Escherichia coli* clinical isolates collected from a Korean hospital during the period 2001 to 2003. The two strains were not clonally related. *qnr* was located in In4 family class 1 integrons of original structure, downstream of *orf513* and upstream from another resistance gene (*dfrA3b*) and a gene of unknown function (*orf105*). Transfer of the *qnr* determinant by conjugation could be detected from only one strain.**

Quinolone resistance is usually caused by various chromosomal mutations that alter the target enzymes, such as DNA gyrase and topoisomerase IV, or activate efflux systems (2). Plasmid-mediated quinolone resistance has only recently been discovered (5). The plasmid gene responsible for quinolone resistance, termed *qnr*, is carried on class 1 integrons of the In4 family, downstream of the conserved region containing the *orf513* recombinase gene (4, 10, 12), and encodes a 218-amino-acid protein that belongs to the pentapeptide-repeat family of proteins (10). The presence of *qnr* increases the resistance to nalidixic acid and fluoroquinolones by four- to eightfold (5).

The presence of the *qnr* gene in clinical isolates from Korea has not yet been reported. Therefore, in this study we screened for the presence of the *qnr* gene in clinical isolates of *Escherichia coli* from patients in Korea and analyzed the transferability and the genetic context of the *qnr* gene.

The presence of the *qnr* gene was screened by PCR using specific primers in plasmid DNAs extracted from 260 consecutive nonduplicate *E. coli* isolates (194 ciprofloxacin resistant and 66 ciprofloxacin susceptible), which were obtained from patients admitted to the division of infectious diseases, Asan Medical Center, Seoul, Korea, during the period 2001 to 2003. Plasmid DNAs were extracted with a QIAGEN plasmid purification kit (QIAGEN, Valencia, CA), according to the manufacturer's recommendations. Plasmid DNA was amplified by PCR with primers 5'-GATAAAGTTTTTCAGCAAGAGG and 5'-ATCCAGATCGGCAAAGGTTA as described previously (3). DNA sequencing was performed directly on both strands of PCR products using a BigDye terminator sequencing kit and a 3700 DNA analyzer (Applied Biosystems, Foster City, CA).

The *qnr* gene was detected in two *E. coli* isolates from different patients: isolate 56 (from cultures of an open wound) and isolate 97 (from bile). Arbitrarily primed PCR (randomly amplified polymorphic DNA [RAPD]) fingerprinting was performed on DNA from both strains carrying *qnr* to assess the

clonal relationship. RAPD analysis was performed according to procedures described previously (8) by using primers 254, 255, 256, and 257. A different DNA banding pattern was obtained from each isolate, indicating that they were not clonally related (Fig. 1). DNA sequencing showed that the nucleotide sequence of *qnr* in isolate 97 was identical to that of the originally reported *qnr* sequence (5), but a single-nucleotide change (A → G at position 537), which did not change the amino acid sequence, was detected in *qnr* in isolate 56, as also detected in an earlier study (12).

Antimicrobial susceptibility of the two *E. coli* isolates carrying *qnr* was determined by agar dilution in accordance with the guidelines of the NCCLS (7). MICs are reported in Table 1. The level of quinolone resistance was lower for isolate 56 than for isolate 97. Both isolates were investigated for the presence of mutations in the quinolone resistance-determining regions of *gyrA*, *gyrB*, *parC*, and *parE* genes as described previously (1). In isolate 97, a point mutation was identified in *gyrA*, leading to the replacement of Ser83 by Tyr, while no mutations were detected in the quinolone resistance-determining regions of *gyrA*, *gyrB*, *parC*, and *parE* of isolate 56.

To determine whether the *qnr*-carrying plasmid is transferable, conjugation experiments were performed using azide-

TABLE 1. Resistance profiles of *E. coli* isolates and transconjugant

Strain or transconjugant	MIC (μg/ml) ^{a,b}									
	CIP	AMP	CTX	GEN	SMZ	TMP	CHL	TET	NAL	SPAR
Clinical strains										
56	1	>512	>512	8	>512	>512	4	64	64	2
97	8	>512	128	1	>512	>512	8	8	>512	16
Recipient										
J53Az	0.008	16	0.06	0.125	8	0.5	4	1	4	0.016
Transconjugant^b										
C56	0.25	>512	4	2	>512	>512	4	32	32	1

^a CIP, ciprofloxacin; AMP, ampicillin; CTX, cefotaxime; GEN, gentamicin; SMZ, sulfamethoxazole; TMP, trimethoprim; CHL, chloramphenicol; TET, tetracycline; NAL, nalidixic acid; SPAR, sparflaxacin.

^b MICs of other transconjugants were identical or different by no more than 1 dilution.

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FIG. 1. Arbitrarily primed PCR (RAPD) fingerprinting patterns of strains 56 and 97. M, molecular marker (1 kb).

resistant *E. coli* J53 as the recipient. With isolate 56, *qnr*-positive transconjugants were easily selected on Trypticase soy agar plates containing sodium azide (100 μ g/ml) for counter-selection and ampicillin (100 μ g/ml) for selection of plasmid-encoded resistance. PCR tests confirmed that transconjugants carried *qnr*. The conjugation frequency (number of transconjugants/number of donor cells) was about 4×10^{-4} . As shown in Table 1, the transconjugants obtained from isolate 56 were resistant to ampicillin, sulfamethoxazole, tetracycline, and trimethoprim and showed decreased susceptibility to quinolones compared with *E. coli* J53. However, no transconjugants were obtained using isolate 97 as a donor, in repeated experiments with ampicillin (100 μ g/ml), trimethoprim (40 μ g/ml), or sulfamethoxazole (300 μ g/ml) as a selecting agent.

The genetic context of *qnr* in *E. coli* isolates 56 and 97 was determined by sequencing the flanking regions on plasmid DNA. Results showed that *qnr* was carried on class 1 integrons of the In4 family that were different from each other and from

previously described *qnr*-containing integrons at the level of the gene cassette array and/or at the level of the unique region located downstream the conserved region containing *orf513* (Fig. 2). In particular, unlike previously described *qnr*-containing integrons, in those from Korean isolates the *qnr* gene was followed by another resistance gene (*dfrA3b*) and by a putative gene of unknown function (named *orf105*) whose putative protein product exhibits 70% homology with the N-terminal sequence of thymidylate synthase of *Salmonella enterica* serovar Paratyphi A strain ATCC9150 (6).

In conclusion, although a previous study showed that *qnr* was present in 8% (6 of 78) ciprofloxacin-resistant clinical isolates of *E. coli* from Shanghai, China (12), *qnr* has rarely been found in clinical strains from other areas of the world (3, 4, 9, 11). In the present study, the *qnr* gene was found in less than 1% of *E. coli* isolates from a collection of clinical isolates from Seoul, Korea. Although the *qnr* gene is currently uncommon, its identification indicates the emergence of plasmid-mediated resis-

FIG. 2. Comparison of structures of the *qnr*-containing integrons from isolates 56 and 97 with those of In36 and In37 and that of the *qnr*-containing integron from plasmid pQR1 (4, 11).

tance to quinolones. This mechanism may contribute to the increase in fluoroquinolone resistance in Korea.

Nucleotide sequence accession numbers. The nucleotide sequences of the *qnr*-positive integrons in strain 56 and 97 have been submitted to GenBank and have been assigned accession numbers AY878718 and AY878717, respectively.

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