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Emergence of the Plasmid-Mediated Quinolone Resistance Gene *qnrS1* in *Escherichia coli* Isolates in Greece[▽]

Three major groups of the plasmid-mediated quinolone resistance (Qnr) determinants have been identified so far in *Enterobacteriaceae*: the QnrA group, which includes 6 variants, the QnrB group, which includes 19 variants, and the QnrS group, which includes 3 variants (5). Although Qnr proteins produce only low-level resistance, they provide a favorable background for higher resistance to occur at quinolone concentrations that would be lethal in their absence, through secondary changes in DNA gyrase and topoisomerase IV, porin, or efflux systems (6). The purpose of our study was to investigate the presence and dissemination of the *qnr* genes among ciprofloxacin-resistant *Escherichia coli* isolates from different hospitals in Greece, a region with a relatively high frequency of quinolone resistance (1), where *qnr* genes had not been reported previously.

A total of 113 nonrepetitive ciprofloxacin-resistant *E. coli* clinical isolates were taken at random from the laboratory collections of four unrelated hospitals in northern and central Greece between 2006 and 2007 and analyzed for *qnr* genes. Ciprofloxacin MICs were estimated by the Etest (AB Biodisk, Solna, Sweden) and the agar dilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (3) by using the breakpoints 1 and 4 μg/ml for susceptibility and resistance, respectively. *E. coli* ATCC 25922 was used as a control in all susceptibility assays; positive controls for the genes *qnrA*, *qnrB*, and *qnrS* were kindly provided by J. Sanchez-Cespedes. Susceptibilities to all antimicrobials tested were defined according to the CLSI interpretative criteria (3).

PCR was performed with primers amplifying all known *qnr* gene variants. The primer pair for the gene *qnrA* was 5′-AGA GGATTTCTCACGCCAGG-3′ and 5′-CCAGGCACAGATC TTGAC-3′ (yielding a 580-bp product), that for *qnrB* was 5′-GGGTATGGATATTATTGATAAAG-3′ and 5′-CTAATCC GGCAGCACTATTA-3′ (yielding a 264-bp product), and the primer pair for *qnrS* was 5′-GCAAGTTCATTGAACAGGG T-3′ and 5′-TCTAAACCGTCGAGTTCGGC-3′ (yielding a 428-bp product). The gene *gyrA* was amplified with primers 5′-TTAATGATTGCCGCCGTCGG-3′ and 5′-TACACCGG TCAACATTGAGG-3′ (yielding a 648-bp product) and *parC* was amplified with primers 5′-AAACCTGTTCAGCGCCGC ATT-3′ and 5′-GTGGTGCCGTTAAGCAAA-3′ (yielding a 395-bp product) to evaluate possible coexisting chromosomal mutations. The corresponding specific PCR products were sequenced by LARK Technologies, Essex, United Kingdom.

For the *qnr*-positive isolates, synergy experiments were also performed using ciprofloxacin and the efflux pump inhibitor CCCP (carbonyl cyanide *m*-chlorophenylhydrazone) (8) to check the contribution of efflux pump overexpression to ciprofloxacin resistance. Pulsed-field gel electrophoresis (PFGE) analysis of XbaI-digested genomic DNA was performed, and the banding patterns of the strains were compared visually according to the criteria proposed by Tenover et al. (9). Filter mating experiments were performed with *qnr*-positive isolates by using *E. coli* 26R793 (*lac* negative and rifampin resistant) as the recipient. Transconjugants were selected on MacConkey agar plates containing 100 mg of rifampin/liter and 6 mg of nalidixic acid/liter, tested for *qnr* genes by PCR, and analyzed for plasmids by alkaline lysis.

Eleven of the 113 *E. coli* isolates (10%) derived from three independent hospitals in Thessaly (Larissa, central Greece) and Macedonia (Thessaloniki, northern Greece) and exhibiting nine unrelated PFGE strain patterns were *qnr* positive. The ciprofloxacin MICs for these isolates were 16 to 128 μg/ml; the characteristics of the isolates are presented in Table 1. One Qnr-positive isolate (isolate 3) was an extended-spectrum β-lactamase producer carrying the gene $bla_{\text{CTX-M-15}}$. No synergy between CCCP and ciprofloxacin in any isolate was observed. Sequencing of the PCR products showed that all 11

TABLE 1. Characteristics of the 11 *qnrS1*-positive study isolates

Isolate	PFGE type	Hospital[a]	Ward	Specimen	Date of isolation (mo/day/yr)	Ciprofloxacin MIC[b] (μg/ml)	Mutation(s) detected in:		Antibiotic(s) to which isolate exhibited phenotypic resistance[b,c]
							gyrA	*parC*	
1	I	A	Outpatient	Urine	3/7/07	32	S83L, D87N	S80R	AMP
2	II	B	Medical	Urine	2/8/06	32	S83L, D87N	S80I, E84V	AMP
3	III	A	Outpatient	Urine	2/24/06	64	S83L, D87N	S80I	AMP, AMC, CTX, CAZ, ATM, FEP, TCC
4	I	A	Outpatient	Urine	2/24/06	16	S83L, D87N	S80I	AMP
5	IV	C	Medical	Urine	4/5/07	64	S83L, D87N	S80I	AMP, AMC, CTX, CAZ, ATM, FEP, TZP, TCC
6	V	C	Medical	Blood	4/6/07	64	S83L, D87N	S80I	AMP, AMC
7	VI	B	Medical	Urine	1/15/07	128	S83L, D87N	S80I	AMP
8	VII	A	Surgical	Trauma	2/18/06	128	S83L, D87N	S80I	AMP
9	VI	B	Medical	Ascitic fluid	3/9/06	128	S83L, D87N	S80I	AMP, AMC, TCC
10	VIII	A	Medical	Urine	3/24/06	128	S83L, D87N	S80I	AMP, AMC, TZP, TCC
11	IX	A	Medical	Pus	5/31/06	32	S83L, D87N	S80I	AMP

[a] Hospital A, AHEPA University Hospital, Thessaloniki; hospital B, Hippokration Hospital, Thessaloniki; and hospital C, University Hospital of Larissa, Larissa, Greece.
[b] The susceptibility status was defined according to CLSI interpretative criteria (3).
[c] Antibiotics: AMP, ampicillin; AMC, amoxicillin-clavulanate; ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CTX, cefotaxime; FEP, cefepime; TCC, ticarcillin-clavulanate; and TZP, piperacillin-tazobactam.

isolates carried the allele *qnrS1*, that none carried *qnrA* or *qnrB*, and that all had the mutations in the genes *gyrA* and *parC* that commonly confer ciprofloxacin resistance on *E. coli* isolates (2, 4) (Table 1). Mating experiments revealed that *qnrS1* gene-carrying plasmids of various molecular sizes in 5 of the 11 isolates were transferable to the susceptible host. Ciprofloxacin MICs for the transconjugants were 0.25 to 0.5 μg/ml, while the MIC for the susceptible *E. coli* recipient was 0.032 μg/ml.

The presence of the *qnr* genes in clinical isolates from Greece had not been reported previously. In this study, a considerably high proportion, 10%, of quinolone-resistant *E. coli* isolates were found to carry the gene *qnrS1*. The predominance in Greece of the *qnr* variant *qnrS1*, which was up to now detected mainly among salmonellae (4) and more rarely in *E. coli* (7) in Europe, indicates its possibly wide distribution. The carriage of *qnrS1* in unrelated isolates of *E. coli* indicates either the natural existence of this gene in microbial populations or its wide horizontal spread through plasmids or integrons.

In conclusion, *qnr* genes seem to be common in ciprofloxacin-resistant clinical *E. coli* isolates and may contribute to the alarming rates of quinolone resistance in Greece.

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