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LETTER TO THE EDITOR

Plasmid-Mediated Quinolone Resistance in Different Diarrheagenic *Escherichia coli* Pathotypes Responsible for Complicated, Noncomplicated, and Traveler's Diarrhea Cases

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Diarrheagenic *Escherichia coli* (DEC) organisms are important agents of endemic and epidemic diarrhea worldwide, as well as significant contributors of traveler's diarrhea (TD) in industrialized countries (1, 2). Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), further divided into typical EPEC (tEPEC) and atypical EPEC (aEPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC) are considered the most important DEC pathotypes (2). STEC are food-borne pathogens responsible for important outbreaks of hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS) in industrialized countries (2). EAEC, ETEC, EPEC, and EIEC are generally considered major causes of TD in adults from developed countries and the leading causes of infant diarrhea in developing ones (2).

The first-choice agents for treating DEC infections are quinolones (3), although their use concretely in complicated STEC infections remains controversial (4). However, plasmid-mediated quinolone resistance (*qnr*) genes encoding small pentapeptide repeat proteins that protect type II DNA topoisomerases from quinolones, including five *qnr* families (*qnrA1-7*, *qnrB1-74*, *qnrC*, *qnrD1-2*, and *qnrS1-9*), have been described. *qnr* genes by themselves are able to confer only a low level of quinolone resistance, but they have been proposed to promote the emergence of chromosomal mutations leading to levels of resistance of clinical significance (5). Although their occurrence has been widely documented in extraintestinal *E. coli* isolates (6), studies concerning *qnr* occurrence in DEC isolates are scarce.

A routine screening for susceptibility to 13 different antimicrobials was carried out with 54 STEC, 16 aEPEC, 9 EAEC, 6 ETEC, and 2 EIEC strains (87 strains in total) isolated from complicated (HC and HUS) and noncomplicated endemic diarrhea and TD cases in the Spanish National Reference Laboratory (SNRL) during 2012 and 2013. The susceptibility testing was performed by the disk diffusion method as previously described (7), and results were interpreted according to CLSI guidelines. For strains showing a decrease in the diameter of the inhibition halo of ciprofloxacin (≤27 mm), the MICs of ciprofloxacin and nalidixic acid were determined by Etests. Additionally, to evaluate the possible association between *qnr* genes and the production of extended-spectrum β-lactamases (ESBLs), the ESBL phenotype was detected by the double-synergy test. Resistance genes were identified by PCR and DNA sequencing, plasmid analysis was carried out by S1 nuclease–pulsed-field gel electrophoresis (S1-PFGE) and PCR-based replicon typing, and conjugation assays were performed to link resistance genes to plasmids, as previously described (7).

Overall, four DEC strains out of 87 (4.6%) exhibited a decreased ciprofloxacin susceptibility (MIC, 0.38 to 1.5 μg/ml), with three of them being still susceptible to nalidixic acid (MIC, 6 to 16 μg/ml) (Table 1). Among them, *qnrB19* was identified in an EAEC strain isolated from an adult with diarrhea traveling from Mexico and also in an STEC O157:H7 strain isolated from a 7-year-old boy suffering from HUS after diarrhea (Table 1). Likewise, *qnrS1* was detected in an aEPEC strain isolated from a 1-year-old boy with noncomplicated diarrhea and also in an EIEC strain isolated from an adult with diarrhea traveling from Southeast Asia (Table 1). This EIEC strain showed a resistance phenotype indicating ESBL production and harbored $bla_{\text{CTX-M-15}}$ (Table 1). Conjugation experiments were positive for the EAEC, aEPEC, and EIEC strains. Plasmid analysis showed that *qnrB19* was transferred on a ColE_{Tp} plasmid of ~3 kb in the EAEC strain (Table 1). In the aEPEC strain, *qnrS1* was transferred on a nontypeable plasmid of ~48 kb, and cotransfer of the bla_{TEM1} gene was observed (Table 1). In the ESBL-producing EIEC strain, *qnrS1* was transferred with $bla_{\text{CTX-M-15}}$ and bla_{TEM1} on an IncK plasmid of ~97 kb (Table 1). Finally, in the STEC O157:H7 strain, *qnrB19* was harbored on a nonconjugative ColE_{Tp} plasmid of ~3.5 kb (Table 1).

To our knowledge, this is the first report of the occurrence of *qnr* genes in STEC, aEPEC, and EIEC clinical strains. Our study also confirms the occurrence of *qnr* genes in EAEC strains reported by Riveros et al. (8) and Kim et al. (9), which might have contributed to the increasing trend of fluoroquinolone resistance recently observed in this *E. coli* pathotype worldwide (8, 10). As for the plasmids, although *qnrB19* has previously been found in ColE-like plasmids (8, 11), *qnrS1* has rarely been identified in IncK plasmids. The presence of $bla_{\text{CTX-M-15}}$ in IncK plasmids from *E. coli* has been recently reported (12), despite these plasmids being involved mainly in the spreading of $bla_{\text{CTX-M-14}}$ (13), but to our knowledge, no IncK plasmid simultaneously harboring *qnrS1* and $bla_{\text{CTX-M-15}}$ has been reported yet. Although the clinical implications of our findings are still unknown, it may be speculated that *qnr* genes might play a significant role in therapeutic failures in DEC infections. In addition, epidemiologic surveillance and correct use of antimicrobial

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TABLE 1 Features of the four *qnr*-positive diarrheagenic *Escherichia coli* strains[a]

Strain	Pathotype	Origin	Serotype	*qnr* gene	Resistance phenotype or genotype	MIC (μg/ml) of NAL, CIP	Plasmid size (kb), incompatibility group
2384/12	EAEC	TD	O65/O71:H1[b]	*qnrB19*	AMP CHL TET AMC	12, 0.38	3, $ColE_{Tp}$
4425/12	STEC	CD	O157:H7	*qnrB19*	AMP SSS STR TET SXT	16, 0.38	3.5, $ColE_{Tp}$
4472/12	aEPEC	NCD	O49:H−	*qnrS1*	AMP SSS NAL TET bla_{TEM1}	>256, 1.5	48, NT
2113/13	EIEC	TD	O96:H19	*qnrS1*	AMP SSS STR CEF CTX SXT AMC bla_{TEM1} $bla_{CTX-M-15}$	6, 0.38	97, IncK

[a] NAL, nalidixic acid; CIP, ciprofloxacin; EAEC, enteroaggregative *E. coli*; STEC, Shiga toxin-producing *E. coli*; aEPEC, atypical enteropathogenic *E. coli*; EIEC, enteroinvasive *E. coli*; TD, traveler's diarrhea; CD, complicated endemic diarrhea; NCD, noncomplicated endemic diarrhea; H−, nonmotile; AMP, ampicillin; CHL, chloramphenicol; TET, tetracycline; AMC, amoxicillin-clavulanic acid; SSS, sulfonamides; STR, streptomycin; SXT, trimethoprim-sulfamethoxazole; CEF, cephalothin; CTX, cefotaxime; NT, nontypeable.
[b] This strain cross-reacted with the respective O antisera.

agents are needed to limit the spread of plasmid-mediated quinolone resistances.

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