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

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Title

Plasmid-mediated quinolone resistance in different diarrheagenic *Escherichia coli* pathotypes responsible for complicated, non-complicated, and travelers' diarrhea cases

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Diarrheagenic *Escherichia coli* (DEC) are important agents of endemic and epidemic diarrhea worldwide, as well as significant contributors of travelers' diarrhea in industrialized countries (1, 2). The most important DEC pathotypes are Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), further divided into typical (tEPEC) and atypical (aEPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC) (2). STEC are foodborne 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 travelers' diarrhea 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, together with rifaximin and azithromycin (3), although the use of quinolones concretely in STEC complicated infections remains controversial, as they have been postulated to increase the risk of development of HUS (4). However, plasmid-mediated quinolone resistance genes (*qnr*) encoding small pentapeptide-repeat proteins that protect type II DNA topoisomerases from quinolones have been described, including five *qnr* families [*qnrA1–7*, *qnrB1–74*, *qnrC*, *qnrD1-2* and *qnrS1–9* (http://www.lahey.org/qnrstudies)]. *qnr* genes by themselves are able to confer only a low-level quinolone resistance, but they have been proposed to promote the emergence of chromosomal mutations leading to resistance levels of clinical significance (5). Although the occurrence of *qnr* genes has been widely documented in extraintestinal *E. coli* (6), studies concerning *qnr* occurrence in DEC are scarce and, as far as we know, it has not been reported yet in clinical DEC strains other than EAEC (7, 8).

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 non-complicated endemic diarrhea and travelers' diarrhea cases in the Spanish National Reference Laboratory (SNRL) during 2012 and 2013. The susceptibility testing was performed by the disk diffusion method and results were interpreted according to CLSI guidelines. The panel included ampicillin, cefalotin, cefotaxime, amoxicillin/clavulanic acid, tetracycline, streptomycin, kanamycin, gentamicin, nalidixic acid, ciprofloxacin, chloramphenicol, trimethoprim/sulfamethoxazole, and a sulphonamide compound. 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 ESBLs, the ESBL phenotype was detected by the double synergy test. PCR and DNA sequencing were used to confirm the presence of *qnr* genes and identify the *qnrA*,

qnrB, *qnrC*, *qnrD*, and *qnrS* alleles, as well as β-lactamase (*bla*) alleles, as previously described (9). Conjugation experiments were used to determine the transfer of resistance using a rifampicin-resistant *E. coli* as recipient and all *qnr*-harbouring strains as donors and rifampicin (50 μg/ml) and ampicillin/streptomycin (100 μg/ml) to select transconjugants (9). The presence of plasmids and plasmid sizes were assessed by S1-PFGE and plasmid extraction with the QIAprep Spin Miniprep Kit (Qiagen) from every parental and transconjugant strain, and their incompatibility groups were established by PCR-based replicon typing (10). The location of *qnr* and *bla* genes was determined by Southern blot hybridization using PCR-generated digoxigenin-labelled probes (9).

Overall, four DEC strains out of 87 (4.6%) exhibited a decreased ciprofloxacin susceptibility (MIC 0.38-1.5 μg/ml), with three of them being still susceptible to nalidixic acid (MIC 6-16 μg/ml) (Table 1). As these values have been previously proposed to identify *qnr*-positive strains (5, 9), the presence of *qnr* genes was confirmed on the four strains. Concretely, *qnrB19* was identified in an EAEC strain isolated from an adult with diarrhea travelling from Mexico and also in a 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 non-complicated diarrhea and also in an EIEC strain isolated from an adult with diarrhea travelling from South-East Asia (Table 1). This latter EIEC strain showed a resistance phenotype indicating ESBL production and harbored the ESBL gene $bla_{\text{CTX-M-15}}$ (Table 1). Conjugation experiments were positive for the EAEC, aEPEC, and EIEC strains, and therefore three transconjugants were obtained. 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 non-typeable plasmid of ≈48 kb, and co-transfer of 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 harboured on a non-conjugative $ColE_{Tp}$ 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.* (7) and Kim *et al.* (8), which might have contributed to the increasing trend of fluoroquinolone resistance recently observed in this *E. coli* pathotype worldwide (7, 11). As for the plasmids, although *qnrB19* has previously been found in ColE-like plasmids (7, 12), *qnrS1* has rarely been found in incK plasmids, mainly involved in the spreading of $bla_{CTX\text{-}M\text{-}14}$ (13). Many surveys have shown *qnr*-positive Enterobacteriaceae simultaneously expressing plasmid-encoded β-lactamases, because genes encoding ESBLs and AmpC β-lactamases are often found on the same plasmid than *qnr* genes (5, 9). Nevertheless, although the presence of $bla_{CTX\text{-}M\text{-}15}$ in incK plasmids from *E. coli* has been recently reported (14) and *qnrS1* has been recently found linked to the AmpC β-lactamase $bla_{CMY\text{-}2}$ in multiresistance incK plasmids from *E. coli* (15), to our knowledge no IncK plasmid simultaneously harboring *qnrS1* and $bla_{CTX\text{-}M\text{-}15}$ 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 and so this is very important to take into consideration when working with diarrhea cases and their treatment. In addition, epidemiologic surveillance and correct use of antimicrobial agents are needed to limit the spread of plasmid-mediated quinolone resistances.

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157 TABLE 1 Features of the four *qnr*-positive diarrheagenic *Escherichia coli* strains

Strain	Pathotype	Origin	Serotype	*qnr* gene	Resistance pheno/genotypes	MIC (μg/ml) NAL/CIP	Plasmid size (kb)/incompatibility group
2384/12	EAEC	TD	O65/O71:H1[a]	*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

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, travelers' diarrhea; CD, complicated endemic diarrhea; NCD, non-complicated endemic diarrhea; H−, non-motile; AMP, ampicillin; CHL, chloramphenicol; TET, tetracycline; AMC, amoxicillin/clavulanic acid; SSS, sulphonamides; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; CEF, cefalotin; CTX, cefotaxime; NT, non-typeable.

[a] The strain cross-reacted with the respective O antisera.

158

159