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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) 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 (\textit{qnr}) encoding small pentapeptide-repeat proteins that protect type II DNA topoisomerases from quinolones have been described, including five \textit{qnr} families [\textit{qnrA1–7}, \textit{qnrB1–74}, \textit{qnrC}, \textit{qnrD1-2} and \textit{qnrS1–9} (http://www.lahey.org/qnrstudies)]. \textit{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 \textit{qnr} genes has been widely documented in extraintestinal \textit{E. coli} (6), studies concerning \textit{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 \textit{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 \textit{qnr} genes and identify the \textit{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 blaCTX-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 ColETp 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 blaTEM1 gene was observed (Table 1). In the ESBL-producing EIEC strain, qnrS1 was transferred with blaCTX-M-15 and blaTEM1 on an IncK plasmid of ≈97 kb.
Finally, in the STEC O157:H7 strain, qnrB19 was harboured on a non-conjugative ColE₁₉ 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 blaCTX-M-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 blaCTX-M-15 in incK plasmids from E. coli has been recently reported (14) and qnrS1 has been recently found linked to the AmpC β-lactamase blaCMY-2 in multiresistance incK plasmids from E. coli (15), to our knowledge no IncK plasmid simultaneously harboring qnrS1 and blaCTX-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 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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References


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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.

<sup>a</sup>The strain cross-reacted with the respective O antisera.