Letter to the Editor to: ANTIMICROBIAL AGENTS AND CHEMOTHERAPY

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 (TD) in industrialized countries (1, 2). Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), further divided into typical (tEPEC) and atypical (aEPEC), enteroxotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC) are considered the most important DEC pathotypes (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 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 STEC complicated infections remains controversial (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 \(qnrS1–9\)). \(qnr\) 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 their occurrence has been widely documented in extraintestinal \textit{E. coli} (6), studies concerning \(qnr\) occurrence in DEC 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 non-complicated 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 (\(\leq 27\) 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. Resistance genes were identified by PCR and DNA sequencing, plasmid analysis was carried out by 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 0.38-1.5 \(\mu\)g/ml), with three of them being still susceptible to nalidixic acid (MIC 6-16 \(\mu\)g/ml) (Table 1). Among them, \(qnrB19\) 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 $bla_{CTX-M-15}$ (Table 1). Conjugation experiments were positive 
for the EAEC, aEPEC, and EIEC strains. Plasmid analysis showed that $qnrB19$ was 
transferred on a 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 $bla_{TEM1}$ gene 
was observed (Table 1). In the ESBL-producing EIEC strain, $qnrS1$ was transferred with 
$bla_{CTX-M-15}$ and $bla_{TEM1}$ on an IncK plasmid of ≈97 kb (Table 1). Finally, in the STEC 
O157:H7 strain, $qnrB19$ was harbored on a non-conjugative ColETp 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 
$bla_{CTX-M-15}$ in incK plasmids from $E. coli$ has been recently reported (12), despite being 
mainly involved in the spreading of $bla_{CTX-M-14}$ (13), but to our knowledge no IncK plasmid 
simultaneously harboring $qnrS1$ and $bla_{CTX-M-15}$ 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 agents are needed to limit the spread of plasmid-
mediated quinolone resistances.
Acknowledgements

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References


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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pathotype</th>
<th>Origin</th>
<th>Serotype</th>
<th>qnr gene</th>
<th>Resistance phenotypes</th>
<th>MIC (μg/ml)</th>
<th>Plasmid size (kb)/incompatibility group</th>
</tr>
</thead>
<tbody>
<tr>
<td>2384/12</td>
<td>EAEC</td>
<td>TD</td>
<td>O65/O71:H1†</td>
<td>qnrB19</td>
<td>AMP, CHL, TET, AMC</td>
<td>12/0.38</td>
<td>3/ColE&lt;sub&gt;Tp&lt;/sub&gt;</td>
</tr>
<tr>
<td>4425/12</td>
<td>STEC</td>
<td>CD</td>
<td>O157:H7</td>
<td>qnrB19</td>
<td>AMP, SSS, STR, TET, SXT</td>
<td>16/0.38</td>
<td>3.5/ColE&lt;sub&gt;Tp&lt;/sub&gt;</td>
</tr>
<tr>
<td>4472/12</td>
<td>aEPEC</td>
<td>NCD</td>
<td>O49:H-</td>
<td>qnrS1</td>
<td>AMP, SSS, NAL, TET</td>
<td>&gt;256/1.5</td>
<td>48/NT</td>
</tr>
<tr>
<td>2113/13</td>
<td>EIEC</td>
<td>TD</td>
<td>O96:H19</td>
<td>qnrS1</td>
<td>AMP, SSS, STR, CEF, CTX, SXT, AMC</td>
<td>6/0.38</td>
<td>97/IncK</td>
</tr>
</tbody>
</table>

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.

† The strain cross-reacted with the respective O antisera.