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# First report of plasmid-mediated fluoroquinolone efflux pump QepA in *Escherichia coli* clinical isolate ST68, in South America

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ABSTRACT

This is the first report of the presence of *qepA1* efflux pump gene in *Escherichia coli* clinical isolate from Argentina, which was associated to other plasmid-mediated quinolone resistance determinants, such as *aac (6′)-Ib-cr* and *qnrB10* and also quinolone resistance determining regions mutations.



Quinolones and fluoroquinolones are antibacterial agents extensively used in the treatment of bacterial infections in humans, as well as in food-producing animals. However, their intensive use has prompted the emergence of resistance worldwide (Dalhoff, 2012) Traditionally, quinolone-resistant mechanisms included mutations in the *gyrA* and *parC* quinolone resistance determining regions (QRDR) and decreased accumulation of the drug due to impermeability of the outer membrane and/or overexpression of chromosomally encoded efflux pump systems. More recently, several plasmid-mediated quinolone resistance (PMQR) genes have been described, coding for Qnr proteins, an aminoglycoside acetyltransferase variant (AAC(6′)-Ib-cr) and efflux pumps (QepA and OqxAB) (Ruiz et al., 2012b). Qnr are proteins that interfere with quinolone binding to DNA gyrase and topoisomerasa IV. Different *qnr* genes have been described as *qnr*A, *qnr*B, *qnr*C, *qnr*D, *qnr*S, and *qnr*VC. The enzyme codified by *aac(6′)-Ib-cr*, an *aac(6′)-Ib* variant, modifies fluoroquinolones by N-acetylation of piperazinyl amine, reducing the susceptibility to ciprofloxacin and norfloxacin (Ruiz et al., 2012b). Plasmid-mediated QepA was initially identified in *Escherichia coli* clinical isolates from Belgium and Japan, in 2007. This efflux pump belongs to the major facilitator superfamily–type group and confers decreased susceptibility to hydrophilic fluoroquinolone. The *qepA1* gene encodes a 511 amino-acid protein (53 kDa) (Perichon et al., 2007; Yamane et al., 2007). Later, in 2008, a new *qepA* gene was reported in France coding for QepA2 that differs from QepA1 in 2 amino acids (Cattoir et al., 2008).

The aim of this study was to investigate the presence of PMQR determinants and QRDR mutations in a high-level fluoroquinolone-resistant *E. coli* isolated in October 2010 from a urine sample of an 88-year-old female inpatient at the Hospital Británico, Buenos Aires, Argentina.

Antibiotic susceptibility was determined by the agar dilution method according to the CLSI recommendations. *E. coli* B2 strain showed a multiresistance phenotype, including resistance to ampicillin, amoxicillin–clavulanic acid, cephalothin, nalidixic acid, fluoroquinolones, amikacin, tobramycin, and tetracycline. The isolate remained susceptible to third-generation cephalosporins, carbapenems, and gentamicin. MICs values of quinolones and aminoglycosides are summarized in Table 1.

Molecular detection of PMQR genes and QRDR mutations was conducted by allele-specific PCR amplification using total heat extracted genomic DNA as template and direct sequencing of PCR products in both strands (Cruz et al., 2013). The *qepA1* gene was detected in *E. coli* B2, as well as *aac(6′)-Ib-cr* and *qnrB10* genes. No *qnrA*, *qnrS*, *qnrC*, *qnrD*, *qnrVC*, *oqxA*, and *oqxB* were amplified. In addition, 2 amino acid changes were identified in both GyrA (S83L and D87N) and ParC (S80I and E84G) proteins.

Plasmid profiles of *E. coli* B2 were achieved by pulsed-field gel electrophoresis of S1 nuclease (Fermentas; Thermo Fisher Scientific Inc. Waltham, MA, USA) total digested DNA, displaying 3 different plasmids whose molecular sizes were approximately 97 kb, 80 kb and 40 kb.

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**Table 1**
Genetic characterization of resistance markers and antibiotic profile.

| *E. coli* strain | Approx. plasmid size (kb) | Typing method Inc/rep | PMQR genes | MIC (μg/mL) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | NAL | CIP | LVX | GAT | AMK | TOB | GEN |
| B2 | 97<br>80<br>40 | *FIB, F*<br>*N, I1* | *qnrB10*,<br>*aac(6′)-Ib-cr*,<br>*qepA* | >512 | >64 | >64 | >64 | 4 | 32 | 1 |
| B2-A7[a] | 40 | *N* | *qnrB10*,<br>*aac(6′)-Ib-cr* | 2 | 0.06 | 0.06 | 0.06 | 4 | 16 | 0.5 |
| B2-A10[a] | 97 | *FIB, F* | *qepA* | 1 | 0.015 | <0.031 | <0.031 | 1 | 0.25 | 0.5 |
| TOP10[b] | | | —— | 1 | <0.0019 | <0.031 | <0.031 | 0.5 | 0.25 | <0.125 |
| B2-J53[c] | 80<br>40 | *N, I1* | *qnrB10*<br>*aac(6′)-Ib-cr* | 8 | 1 | 0.25 | <0.25 | NT | 8 | <0.5 |
| J53[b] | | | ——— | 4 | <0.06 | 0.015 | <0.25 | NT | <0.5 | <0.5 |

Note: NAL = nalidixic acid; CIP = ciprofloxacin; LVX = levofloxacin; GAT = gatifloxacin; AMK = amikacin; TOB = tobramicin; GEN = gentamicin; NT = not tested.
[a] Transformant strains.
[b] Recipient strains.
[c] Transconjugant strain.

Bacterial conjugation was performed by the solid mating-out assay using azide-resistant *E. coli* J53 as recipient strain and BHI agar plates containing sodium azide (200 μg/mL) and nalidixic acid (2.5 μg/mL). The 2 smaller plasmids (80 kb and 40 kb) could be transferred to B2–J53 transconjugant, harboring *qnrB10* and *aac(6′)-Ib-cr* determinants but not *qepA1*.

*E. coli* TOP10 was used as recipient strain for electrotransformation, and different transformants were selected on trypticase soy agar plates containing appropriate antibiotic systems. *E. coli* B2-A7 transformant contained a 40-kb plasmid carrying both *qnrB10* and *aac(6′)-Ib-cr*. A 97-kb plasmid coding for *qepA* was observed in B2-A10 transformant, displaying smaller size than the *qepA*-plasmid reported by Ruiz et al. (2012a) and Rocha-Gracia et al. (2010).

According to previously reports, this transformant displayed an increase of at least 8-fold in the MIC value for ciprofloxacin with respect to the recipient strain (Table 1) (Kim et al., 2009; Tian et al., 2011).

The *qepA1* determinant has been rarely described in human isolates worldwide, achieving only a prevalence rate of 0.3% in a collection of *E. coli* isolated from 2002 to 2006 in Japan (Yamane et al., 2008), and 1.7% in a multicenter study conducted on extended spectrum β-lactamase (ESBL) producing enterobacteria in Mexico (Silva-Sanchez et al., 2011). Moreover, recent surveillance reports from Argentina underlined the absence of *qepA* as a PMQR mechanism despite the different bacterial selection criteria used in those studies (Andres et al., 2013; Cruz et al., 2013).

PCR-based plasmid replicon typing was performed (Table 1) (Carattoli et al., 2005). The *qepA* containing plasmid corresponded to the IncFIB incompatibility group. IncF group plasmids, harboring this gene, have been reported in isolates recovered from animals and humans (Cattoir et al., 2008; Deng et al., 2011; Perichon et al., 2007; Rocha-Gracia et al., 2010; Ruiz et al., 2012a). These incompatibility group plasmids possess great versatility, besides they play an important role in the dissemination of antibiotic resistance and virulence coding genes (Deng et al., 2011).

The *qepA* genetic context was performed by PCR mapping and sequencing. An IS26 tnpA, followed by a truncated class 1 integrase gene (682 bp) and a truncated *dfr2* gene (189 bp), was located upstream *qepA*, identical to the arrangement observed in *qepA* plasmids from Japan (pHPA), Belgium (pIP1206), South China (pHN3A11), and Korea (Fig. 1) (Kim et al., 2009; Perichon et al., 2007; Yamane et al., 2008). Downstream *qepA* region seems to be different from those observed in the plasmids mentioned above, as no PCR amplicons could be obtained using primers designed on those sequences.

*E. coli* B2 was classified into phylogenetic group D (Clermont et al., 2000), and it was assigned to ST68 according multilocus sequence typing scheme (http://mlst.ucc.ie/mlst/dbs/Ecoli), whereas other

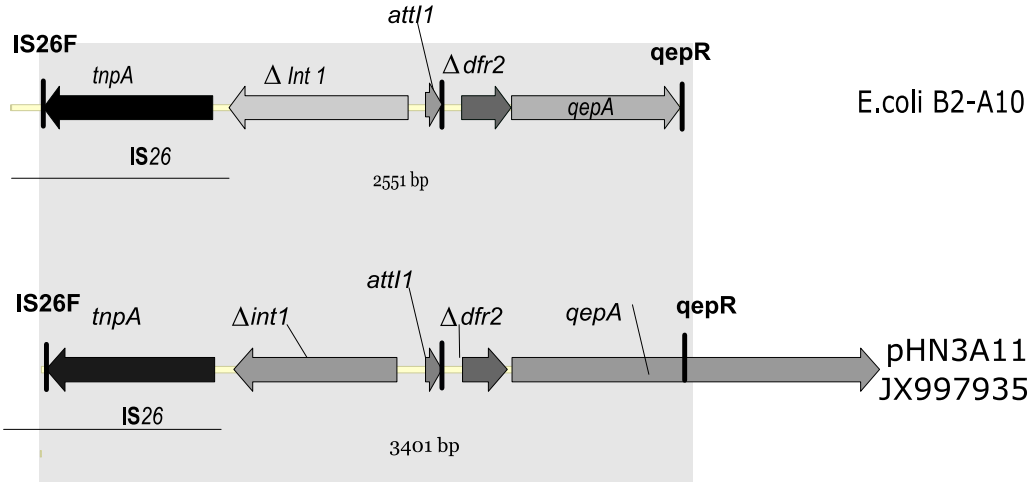


**Fig. 1.** Genetic context of *qepA* gene in *E. coli* B2-A10. Arrows represent coding sequences and indicate the direction of transcription. The Δ*dfr2*: truncated *dfr2* gene, *attI1*: attachment site recognized by IntI1, Δ*Int1*: truncated class 1 integrase gene, tnpA: IS*26* transposases. Shaded area (corresponding to 2551 bp) showed 100% identity with the same region of pHN3A11 plasmid. IS26F (5′-CTTACCAGGCGCATTTCGCC) and qepR (5′-AACTGCTTGAGCCCGTAGATC) were primers used for PCR amplification and sequencing.

*qepA*-positive *E. coli* isolates from United States and México corresponded to ST405 and ST205, respectively (Rocha-Gracia et al., 2010; Tian et al., 2011).

The *qepA* determinant has been mainly associated to $bla_{TEM-1}$ and $bla_{CTX-M-15}$ β-lactamase coding genes (Baudry et al., 2009; Rocha-Gracia et al., 2010; Silva-Sanchez et al., 2011). Furthermore, diverse studies highlight a putative association between *qepA* and *rmtB*, a 16S rRNA methylase gene that confers resistance to aminoglycosides, which was located in close proximity to *qepA* on the same transferable plasmid (Perichon et al., 2007; Tian et al., 2011; Yamane et al., 2008). However, in this study, $bla_{TEM-1}$ and $bla_{OXA-1}$ were detected in *qepA*-positive *E. coli* B2 strain but neither ESBL coding genes nor *rmtB* were found. In good agreement with previously reports, the presence of $bla_{TEM-1}$ was confirmed in B2-A10 transformant.

In South America, *qepA*-harboring isolates have been only reported in commensal *E. coli* recovered from healthy children in the Bolivian Chaco (Bartoloni et al., 2013), corresponding to CTX-M-15 and/or CTX-M-14 producing isolates. Co-existence of 3 different PMQR genes in the same strain has been previously detected among *E. coli* isolates recovered from pigs in China, harboring *qepA*, *aac(6′)-Ib-cr*, and *qnrS1* or *qnrS2* (Ma et al., 2009). To the best of our knowledge, this is the first report of *qepA1* efflux pump gene in a clinically significant isolate from Argentina and even South America. Also, this is the first time where 3 different PMQR determinants (*qepA1*, *aac(6′)-Ib-cr*, and *qnrB10*) were identified in single *E. coli* strain recovered from human samples.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.diagmicrobio.2014.01.007.

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