Detection of the plasmid-borne quinolone resistance determinant qepA1 in a CTX-M-15-producing Escherichia coli strain from Mexico

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Sir,

Three plasmid-mediated quinolone resistance (PMQR) mechanisms have been recently described: (i) qnr genes (implicated in target protection against quinolone inhibition); (ii) the aac(6’)-Ib-cr gene, which encodes an acetyltransferase that modifies both aminoglycosides and fluoroquinolones; and (iii) the qepA gene, which encodes an efflux pump.1 The qepA gene confers decreased susceptibility to hydrophilic fluoroquinolones,2,3 and has been previously detected in a few Escherichia coli isolates from humans in Europe, Asia and Canada,4–2 but, as far as we know, never before in Latin America.

E. coli strain C1550 showed a multiresistance phenotype and was recovered from a faecal sample of a 50-year-old ambulatory patient in Puebla (Mexico), who had abdominal inflammation and was diagnosed with a presumed gastrointestinal infection. Susceptibility to 11 antibiotics was tested by disc diffusion and agar dilution methods, and phenotypical detection of extended-spectrum β-lactamase (ESBL) activity was also carried out.6 The presence of blaCTX-M, blaTEM, blaOXA, blaSHV, qepA, qnrA, qnrB, qnrS, aac(6’)-Ib-cr, aac(3’)-II, aadA5, dfr2d and rmtB genes was determined by specific PCRs (see Table S1, available as Supplementary data at JAC Online) and sequencing. Specific point mutations were searched for by PCR and sequencing in the promoter region of the chromosomal ampC gene as well as in gyrA and parC genes. The presence of integrases of class 1 and class 2 integrons as well as of their gene cassettes was determined by PCR and sequencing. E. coli C1550 strain was typed by multilocus sequence typing (MLST) (http://mlst.ucc.ie/mlst/dbs/Ecoli) and its phylogenetic group was determined by multiplex PCR. The presence of shiga toxin genes as well as of the serotype 0157 was checked by specific PCRs.

E. coli strain C1550 showed a multiresistance phenotype and exhibited a positive test for ESBL production. MICs of nalidixic acid, ciprofloxacin and levofloxacin were >1024, >256 and 64 mg/L, respectively (Table 1). The qepA gene was detected in E. coli C1550, as well as the genes encoding CTX-M-15 and TEM-1b β-lactamasases. Nevertheless, this strain did not harbour the aac(6’)-Ib-cr, aac(3’)-II, blaOXA, blaOXA, qnrA, qnrB, qnrS and rmtB genes. The study of the genetic environment of the blaCTX-M-15 gene showed the ISEcp1 element upstream and the orf477 sequence downstream of the β-lactamase gene. Mutations in the +22, +26, +27 and +32 positions of the attenuator–promoter region of the ampC chromosomal gene were identified in E. coli C1550. In addition, amino acid changes were detected in both GyrA (S83L + D87N) and ParC (S80I) proteins. This strain harboured a class 1 integron containing the dfr2d gene cassette in its variable region, which confers resistance to trimethoprim, as well as the aac(6’)-Ib-cr genes in its 3′ conserved region. Likewise, a class 2 integron with the gene cassettes dfrA1, sat2 and aadA1, which confer resistance to trimethoprim, streptothricin and streptomycin, respectively, was also identified in this strain (Table 1).

E. coli strain C1550 was classified into the phylogenetic group D, and results of MLST showed an allele combination corresponding to the sequence type ST205. PCRs for shiga toxin genes and for serotype 0157 were negative in this strain.

Transconjugants of C1550 were obtained when E. coli strain J53 (rifampicin resistant) was used as recipient. They showed a resistance phenotype that included ampicillin, cefotaxime, aztreonam, cephalotaxime, aztreonam and trimethoprim/ sulfamethoxazole, and exhibited a positive ESBL test. Transconjugants showed 36- and 4-fold increases in the MICs of ciprofloxacin and levofloxacin, respectively, with respect to the recipient strain. They contained the following resistance genes: qepA1, blaCTX-M-15, blaTEM-1b, dfrA1, sat2, aadA1 and dfr2d (Table 1), but did not show amino acid changes in GyrA or ParC proteins. The results of the PFGE with DNA digested with S1 endonuclease showed the presence of one plasmid of ~145 kb in the donor C1550 and in the transconjugant strains. Plasmids were classified according to their incompatibility group using the PCR-based replicon typing method described by Carattoli,7 and the conjugative plasmid IncF with its FIA and FIB replication origins was detected, which suggests that E. coli C1550 is a reservoir of plasmids that contain multiresistance genes. The blaCTX-M-15 gene, often associated with the blaTEM-1, blaOXA-1 and aac(6’)-Ib-cr resistance genes, has been mainly located on plasmids belonging to the IncF group,7 however, in this work we did not detect the presence of
OXA-1 and aac(6')-Ib-cr genes in the *E. coli* C1550 isolate in spite of the presence of the conjugative plasmid IncF.

The ciprofloxacin MIC for *E. coli* strain C1550 was >256 mg/L, which suggests an additive effect on resistance to fluoroquinolones that might be attributed to the production of QepA and to the chromosomal mutations in the quinolone resistance-determining regions of the *gyrA* and *parC* genes. On the other hand, the increase in the MICs of fluoroquinolones observed for the transconjugants could be due to the acquisition of the qepA gene. Similarly to the qepA2-containing *E. coli* from France, our *E. coli* strain C1550 harboured *bla*CTX-M-15*, *bla*TEM-1, and *dfr2d* genes. Unlike the qepA-containing *E. coli* identified in Japan and Belgium, our strain did not carry the *rmtB* gene, and it remained susceptible to the aminoglycosides, as observed in France. Further studies on the genetic environment of the qepA gene of *E. coli* strain C1550 should be carried out.

The first clinical *E. coli* strain containing the qepA gene in Mexico and Latin America is described in this work, and the association of this emerging resistance determinant with a multidrug-resistant CTX-M-15 producer is worrisome, because it may spread in both the community and hospital settings, where co-selection with various antimicrobial agents may contribute to its dissemination. More studies should be carried out in the future to obtain knowledge on the prevalence of this determinant in *E. coli* isolates of different environments.

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**Transparency declarations**

None to declare.

**Supplementary data**

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

**References**


Detection of Escherichia coli harbouring extended-spectrum β-lactamases of the CTX-M classes in faecal samples of common buzzards (Buteo buteo)

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Sir,

In recent years, infections due to Escherichia coli harbouring extended-spectrum β-lactamases (ESBLs) of the CTX-M classes have dramatically increased among human populations, particularly in the community setting. There exist just a few previous reports in which ESBLs were detected in faecal E. coli isolates of wild animals, but, to our knowledge, never in faecal E. coli isolates of buzzards (Buteo buteo).

Thirty-three faecal samples from buzzards of Portugal were recovered from September 2007 to February 2008 and were studied for the presence of ESBL-producing E. coli isolates. All the faecal samples were collected individually from each buzzard and obtained in collaboration with CRATAS (Centre of Collecting, Welcome and Handling of Wild Animals). This centre is located in the University of Trás-os-Montes and Alto Douro and receives injured animals. None of the buzzards had been previously fed by humans or had received antibiotics. Most of the animals inhabited the Pênseda Geres National Park or other rural conservation areas of Portugal. Faecal samples were screened for the presence of ESBLs using Levina agar (Oxoid Limited, UK) supplemented with 2 mg/L cefotaxime (Levine–CTX) (Sigma–Aldrich, USA). Two colonies with typical E. coli morphology were selected and identified by classical biochemical methods (Gram, catalase, oxidase, indole, Methyl Red–Voges–Proskauer, citrate and urease) and by the API 20E system (BioMérieux, La Balme Les Grottes, France) from each positive faecal sample. Susceptibility to 16 antibiotics (ampicillin, amoxicillin/clavulanic acid, cefoxitin, cefotaxime, ceftazidime, aztreonam, imipenem, gentamicin, amikacin, tobramycin, streptomycin, nalidixic acid, ciprofloxacin, sulfamethoxazole/thrimethoprim, tetracycline and chloramphenicol) (Oxoid Limited, UK) was determined by the CLSI disc diffusion method for all recovered E. coli isolates. E. coli ATCC 25922 was used as a quality-control strain. Isolates resistant to third-generation cephalosporins (i.e. cefotaxime or ceftazidime) were selected for further studies (one per faecal sample, or two if they presented different phenotypes of antibiotic resistance). The double disc diffusion test (ceftazidime, ceftazidime and aztreonam in the presence or absence of amoxicillin/clavulanic acid) was performed to detect ESBL production.

The presence of genes encoding TEM-, SHV-, OXA- and CTX-M-type β-lactamases was studied by specific PCRs, and positive amplicons were sequenced to determine the specific type of β-lactamase gene. The genetic environment of blaCTX-M genes was studied by PCR and sequencing in all blaCTX-M-containing isolates using previously reported primers. The following antibiotic resistance genes were also sought by PCR: tet(A) and tet(B) (in tetracycline-resistant isolates); adaA (in streptomycin-resistant isolates); ace(3)-II and ace(3)-IY (in gentamicin-resistant isolates); and sul1, sul2 and sul3 (in sulfamethoxazole/thrimethoprim-resistant isolates). The presence of the intI1 and intI2 genes, encoding class 1 and 2 integrases, respectively, and the detection of phylogenetic groups of E. coli isolates were studied by PCR.

E. coli colonies were isolated from 5 of the 33 (15.2%) faecal samples in the Levine–CTX screen. Two E. coli isolates from each positive sample were recovered, showing different phenotypic and genomic profiles. All 10 of these isolates exhibited a resistant phenotype to cefotaxime and/or ceftazidime, and gave a positive ESBL production test. The β-lactamase genes detected in the ESBL-positive E. coli isolates were the following: blaCTX-M-32 + blatem-1 (seven isolates); and blaxCTX-M-1 + blatem-1 (three isolates) (Table 1). The high prevalence of blaCTX-M-32 in commensal E. coli isolates of buzzards in this study is remarkable (15.2% of total buzzards), as this gene is not frequently found in animal isolates, having been reported in only a few studies previously. Additionally, some of the birds were colonized with both CTX-M-1- and CTX-M-32-producing E. coli. The orfQ77 sequence was detected downstream of the blaxCTX-M-1 and blaxCTX-M-32 genes, and ISEcp1 was found upstream of the blaxCTX-M-1 gene in all our isolates; similar molecular arrangements incorporating CTX-M genes have been previously studied for E. coli isolates from wild animals. The presence of the CTX-M-1 gene in all our isolates; similar molecular arrangements incorporating CTX-M genes have been previously studied for E. coli isolates from wild animals. The presence of the CTX-M-1 and CTX-M-32 genes will be further investigated to confirm their role in ESBL production.