First report of the ceftazidimase CTX-M-19 in South America

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Abstract

We report the first detection of blaCTX-M-19 in South America, harboured in an Escherichia coli isolate obtained from a urine sample; such an isolate belonged to phylogenetic group A, ST603, and showed a ceftazidimase profile. blaCTX-M-19 was encoded in an approximately 100 kb IncI1/IncF conjugative plasmid, featuring pndAC and hok/sok addiction systems; the β-lactamase gene was flanked upstream by three tandem-like transposons (IS26, IS10 and IScep1), inserted one inside the other, and downstream by IS903.

Keywords: Ceftazidimase, CTX-M-19, ESBL, IS26, IS903

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Worldwide dissemination of CTX-M-derived extended spectrum β-lactamases (ESBLs) is a well-known concern [1]. Although this process probably began simultaneously at the beginning of the 1990s in Europe and South America [2], differences in antibiotic pressure forces resulted in different evolutionary routes. Thus, while CTX-M-9, CTX-M-14 and CTX-M-15 were frequently detected in Europe [1], CTX-M-2 was predominant in many countries of South America [3–5].

Nevertheless, this situation has been gradually changing, and the arrival of CTX-M-2 in Europe [1] was accompanied by the progressive detection of CTX-M-9, CTX-M-14 and CTX-M-15 in our continent [3–7]. However, so far, the ceftazidimase CTX-M-19 has only been reported in Europe [8].

In December 2010, Escherichia coli strain EC1737 was isolated from a urine sample from a 10-year-old girl admitted to the paediatric hospital Centro Hospitalario Pereira Rossell (CHPR) of Montevideo, Uruguay.

Identification and antibiotic susceptibility profile were determined using the VITEK® 2 Compact system (bioMérieux, Marcy l’Etoile, France). Minimal inhibitory concentration values for ciprofloxacin, ceftaxime, ceftazidime, gentamicin, and amikacin were determined by E-test; results were interpreted according to EUCAST guidelines (http://www.eucast.org).

Strain EC1737 displayed a ceftazidimase-like profile, being resistant to gentamicin, nalidixic acid, ciprofloxacin, nitrofurantoin and trimethoprim–sulfamethoxazole; nevertheless, EC1737 remained susceptible to amikacin, imipenem and meropenem (Table 1).

The genes blaCTX-M, blaSHV, blaTEM and blaPER-2 were sought by polymerase chain reaction (PCR) and sequencing [5–7], confirming the presence of blaCTX-M-19 and blaTEM, respectively.

In order to identify other mechanisms responsible for the observed resistance profile, we used PCR and sequencing to study the presence of (a) class-1 and 2 integrons [5, 9], (b) sul1, 2 and 3 genes, (c) plasmid-mediated quinolone-resistance genes (qnrABCDS, aac(6′)Ib-cr and qepA), and (d) mutations in the quinolone-resistance determining region (QRDR) [10].

In this sense, strain EC1737 harboured sul1 and sul2 genes and a class-1 integron with a 1500 bp variable region featuring a dfr17-adaA5 array. These genes usually determine resistance to trimethoprim–sulfamethoxazole, streptomycin and spectinomycin.

No plasmid-mediated quinolone resistance genes were detected. Nevertheless, the analysis of the QRDR showed two modifications in gyrA (Ser83Leu and Asp87Asn) and one in parC (Glu84Lys), compared to wild-type alleles in E. coli K-12 (GenBank accession NP_416734 and NP_417491, respectively). These mutations have previously been highlighted as responsible for resistance to ciprofloxacin [11, 12].

The probable association of blaCTX-M-19 to insertion sequences such as IScep1, IS26, IS903, ISCR1 was sought by PCR and sequencing [6]. In this regard, blaCTX-M-19 was flanked by IS26 and IS903 (upstream and downstream, respectively). IS26 and blaCTX-M-19 were separated by an 819 bp segment; interestingly, this segment was formed by 544 bp corresponding to a truncated IS10 insertion sequence and another 275 bp belonging to a fragment of IScep1, a genetic element commonly found upstream from blaCTX-M-14 alleles [13] (Fig. 1).
Conjugation assays were carried out using *E. coli* J53-2 (rifampin resistant, non-motile and ornithine negative) as recipient; transconjugants were selected on MacConkey agar supplemented with rifampin (150 mg/L) and ceftriaxone (1 mg/L), or gentamicin (4 mg/L) [6].

Two different sets of transconjugants were obtained (Fig. 2): (a) ceftriaxone-selected transconjugants (TcEC1737-CRO), displaying only a similar *β*-lactam resistance pattern as the donor strain, and positive PCR results for *bla*\textsubscript{CTX-M} (Table 1); and (b) gentamicin-selected transconjugants (TcEC1737-CN), showing resistance to *β*-lactams, aminoglycosides, and trimethoprim–sulfamethoxazole, but remaining susceptible to nitrofurantoin and quinolones. PCR results were positive for *bla*\textsubscript{CTX-M}, *bla*\textsubscript{TEM}, *intI1*, qacE\textsubscript{delta}-1, *sul1* and *sul2*, and confirmed the transfer of a class-I integron with a 1500 bp variable region.

The plasmid incompatibility group was determined by PCR according to Carattoli et al. [14].

Incl1, IncF, IncFIA and IncFIB, were detected in EC1737 and TcEC1737-CN but only Incl1 and IncF were detected in TcEC1737-CRO.

Plasmid size was estimated, for the donor strain and transconjugants, by treatment with S1 nuclease (Fermentas, Life Sciences, Vilnius, Lithuania) followed by pulsed-field gel electrophoresis (PFGE) as described previously [15].

Both strain EC1737 and TcEC1737-CN harboured two plasmids of 100 kb and 110 kb, approximately, whereas TcEC1737-CRO only harboured a 100 kb plasmid (Fig. 2).

The presence of plasmid maintenance mechanisms (i.e. addiction systems) in the donor strain and transconjugants TcEC1737-CRO and TcEC1737-CN was sought by PCR, as reported elsewhere [16]. Results were confirmed by amplicon sequencing.

EC1737 and TcEC1737-CN showed the presence of *pndAC*, *vagCD*, *cddAB*, *hok/sok* and *pemK1*, whereas TcEC1737-CRO only showed the presence of *pndAC* and *hok/sok* systems.

### TABLE 1. Antibiotic susceptibility profile of *Escherichia coli* EC1737 and transconjugants TcEC1737CRO and TcEC1737CN

| Antibiotic(s)                  | Minimum inhibitory concentration (mg/L) |
|--------------------------------|-----------------------------------------|
|                                | EC1737 | TcEC1737CRO | TcEC1737CN | E. coli J53-2 |
| Amoxicillin                    | (≥32)  | (≥32)       | (≥32)      | (≥32)         |
| Tazobactam piperacillin        | (≥4)   | (≥4)        | (≥4)       | (≥4)          |
| Cephalothin                    | (≥64)  | (≥64)       | (≥64)      | (≥64)         |
| Cefazolin                      | 4      | 8           | 6          | 0.38          |
| Cefotaxime                     | 2      | 1           | 1          | 0.12          |
| Cefepime                       | (≥1)   | (≥1)        | (≥1)       | (≥1)          |
| Meropenem                      | 0.02   | 0.02        | 0.02       | 0.02          |
| Imipenem                       | 0.25   | 0.25        | 0.25       | 0.25          |
| Amikacin                       | 1      | 0.20        | 0.20       | 0.20          |
| Gentamicin                     | 32     | 0.06        | 6          | 0.06          |
| Nalidixic acid                 | (≥32)  | (≥32)       | (≥32)      | (≥32)         |
| Ciprofloxacin                  | 4      | 0.03        | 0.03       | 0.03          |
| Trimethoprim–sulfamethoxazole  | (≥320) | (≥320)      | (≥320)     | (≥320)        |

Values in parentheses were determined by the Vitek-2 system.
Genetic characterization of strain EC1737 was done by: (a) determination of the phylogenetic group, according to Clermont et al. [17]; (b) screening for virulence determinants, according Johnson et al. [18]; and (c) multiple locus sequence typing (MLST), following the guidelines described in http://mlst.ucc.ie/mlst/dbs/Ecoli.

In this sense, EC1737 belongs to phylogenetic group A; screening for pathogenicity genes only yielded positive results for iutA, whereas MLST assay showed that this strain belongs to sequence type 603 (ST603; allelic profile, 6, 4, 4, 16, 43, 8, 6).

The occurrence of human isolates harbouring blαCTX-M-19 has been reported only once, namely from a faecal isolate of Klebsiella pneumoniae from a hospitalized girl in France, co-colonized by E. coli and K. pneumoniae harbouring CTX-M-14 (a likely precursor of CTX-M-19) [8].

Although there is no description of the plasmid bearing the blαCTX-M-19 allele, such a gene was found to be flanked by two full insertion sequences, namely ISEcplB and IS903D [13].

Interestingly, Ho et al. [19] and Kim et al. [20] have described alternative surroundings for blαCTX-M-14 involving the interruption of ISEcpl I by the insertion in different sites of IS10 or IS26.

Contrary to previous reports regarding CTX-M-9-derived genes, blαCTX-M-19 in pEC1737 was preceded by three tandem-like transposons, which appear to have inserted one inside the other; this reflects the plasticity of insertion sequences to mobilize antibiotic resistance genes. Regardless of the different events of insertion and deletion of the various insertion sequences, the expression of blαCTX-M-19 seems to be driven by the promoter sequence present in ISEcpl I, previously described by Poirel et al. [13].

Although E. coli EC1737 is not an ExPEC strain, this type of microorganism could act as a reservoir or carrier of antibiotic resistance genes, as suggested by the presence in this strain of two transferable plasmids. Additionally, the presence of at least two insertion sequences flanking blαCTX-M-19 could account for self-transfer events between different plasmids, or even from plasmids to the bacterial chromosome.

The sequence of blαCTX-M-19 and its surrounding region was deposited in the EMBL database (European Bioinformatics Institute) under accession number HG000669.

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