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

V. García-Fulgueiras[1], I. Bado[1], N. F. Cordeiro[1], G. Algorta[1,2] and R. Vignoli[1,]

1) Depto. de Bacteriología y Virología, Instituto de Higiene, Facultad de Medicina, Universidad de la República and 2) Laboratorio Central del Hospital Pereira Rossell-Ministerio de Salud Pública, Montevideo, Uruguay

Abstract

We report the first detection of $bla_{CTX\text{-}M\text{-}19}$ 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. $bla_{CTX\text{-}M\text{-}19}$ 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 (IS*26*, IS*10* and IS*Ecp1*), inserted one inside the other, and downstream by IS*903*.

Keywords: Ceftazidimase, CTX-M-19, ESBL, IS26, IS903
Original Submission: 6 September 2013; **Revised Submission:** 24 October 2013; **Accepted:** 4 November 2013
Article published online: 22 December 2013
New Microbe New Infect 2013; **1:** 44–47

Corresponding author: R. Vignoli, Depto. de Bacteriología y Virología, Instituto de Higiene, Facultad de Medicina, Universidad de la República, Alfredo Navarro 3051, CP:11600 Montevideo, Uruguay
E-mail: rvignoli@higiene.edu.uy

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, cefotaxime, 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 $bla_{CTX\text{-}M}$, bla_{SHV}, bla_{TEM} and $bla_{PER\text{-}2}$ were sought by polymerase chain reaction (PCR) and sequencing [5–7], confirming the presence of $bla_{CTX\text{-}M\text{-}19}$ and $bla_{TEM\text{-}1}$, 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-aadA5* 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 $bla_{CTX\text{-}M\text{-}19}$ to insertion sequences such as IS*Ecp1*, IS*26*, IS*903*, IS*CR1* was sought by PCR and sequencing [6]. In this regard, $bla_{CTX\text{-}M\text{-}19}$ was flanked by IS*26* and IS*903* (upstream and downstream, respectively). IS*26* and $bla_{CTX\text{-}M\text{-}19}$ were separated by an 819 bp segment; interestingly, this segment was formed by 544 bp corresponding to a truncated IS*10* insertion sequence and another 275 bp belonging to a fragment of IS*Ecp1*, a genetic element commonly found upstream from $bla_{CTX\text{-}M\text{-}14}$ alleles [13] (Fig. 1).

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

	Minimum inhibitory concentration (mg/L)			
Antibiotic(s)	**EC1737**	**TcEC1737CRO**	**TcEC1737CN**	***E. coli* J53-2**
Ampicillin	(≥32)	(≥32)	(≥32)	(4)
Tazobactam piperacillin	(≤4)	(≤4)	(≤4)	(≤4)
Cephalothin	(≥64)	(≥64)	(≥64)	(8)
Ceftazidime	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)	(4)	(4)	(4)
Ciprofloxacin	4	0.03	0.03	0.03
Trimethoprim–sulfamethoxazole	(≥320)	(≤20)	(≥320)	(≤20)

Values in parentheses were determined by the Vitek-2 system.

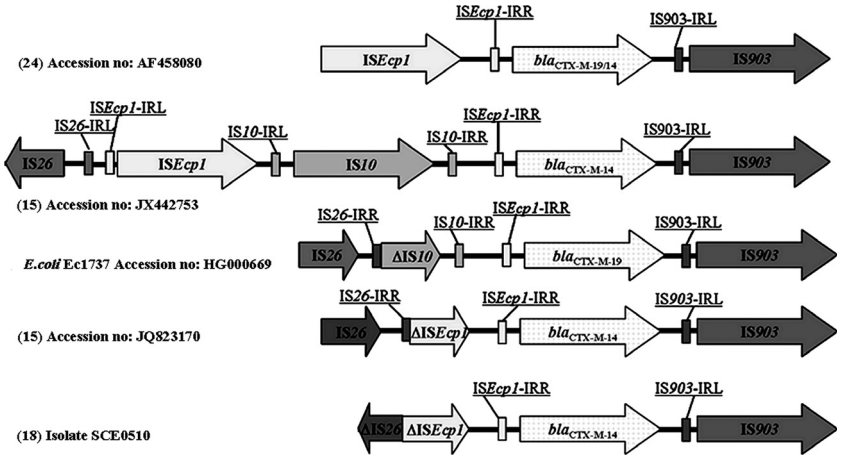

FIG. 1. Comparison of various genetic surroundings of similar bla_{CTX-M} genes, and the one described in pEC1737. IRL, left inverted repeat; IRR, right inverted repeat. Numbers in brackets indicate bibliographical references. Images are not drawn in scale.

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_{CTX-M} (Table 1); and (b) gentamicin-selected transconjugants (TcEC1737CN), showing resistance to β-lactams, aminoglycosides, and trimethoprim–sulfamethoxazole, but remaining susceptible to nitrofurantoin and quinolones. PCR results were positive for bla_{CTX-M}, bla_{TEM}, *intI1*, *qacEdelta-1*, *sul1* and *sul2*, and confirmed the transfer of a class-1 integron with a 1500 bp variable region.

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

IncI1, IncF, IncFIA and IncFIB, were detected in EC1737 and TcEC1737CN but only IncI1 and IncF were detected in TcEC1737CRO.

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 TcEC1737CN harboured two plasmids of 100 kb and 110 kb, approximately, whereas TcEC1737CRO only harboured a 100 kb plasmid (Fig. 2).

The presence of plasmid maintenance mechanisms (i.e. addiction systems) in the donor strain and transconjugants TcEC1737CRO and TcEC1737CN was sought by PCR, as reported elsewhere [16]. Results were confirmed by amplicon sequencing.

EC1737 and TcEC1737CN showed the presence of *pndAC*, *vagCD*, *ccdAB*, *hok/sok* and *pemKI*, whereas TcEC1737CRO only showed the presence of *pndAC* and *hok/sok* systems.

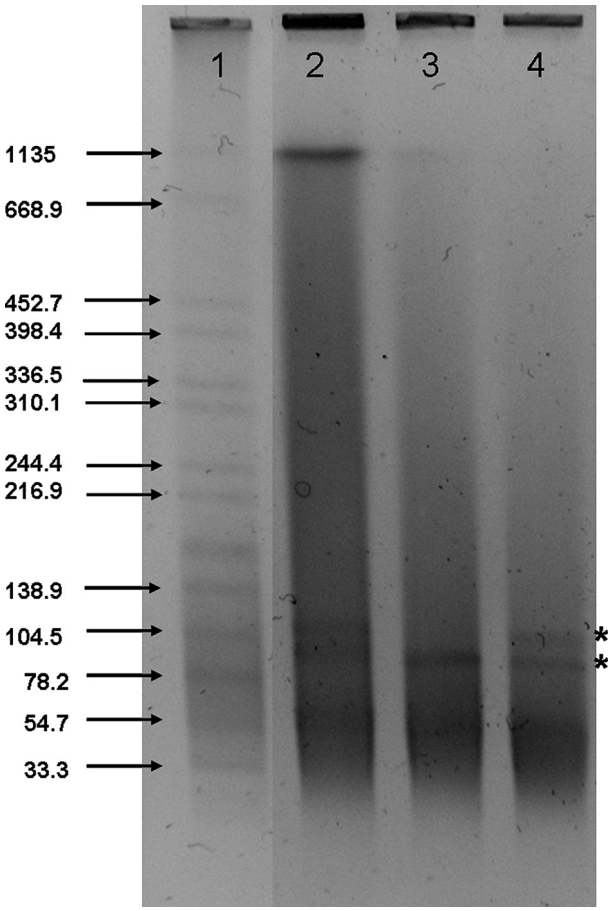

FIG. 2. Plasmid size estimation by S1 nuclease treatment and PFGE. Line 1: *Salmonella* Braenderup H9812; Line 2: EC1737; Line 3: TcEC1737CRO; Line 4: TcEC1737CN. Arrows indicate fragment sizes (in kpb) of *Salmonella* Braenderup DNA digested with *Xba*I. Plasmids are marked by asterisks.

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 $bla_{\text{CTX-M-19}}$ 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 $bla_{\text{CTX-M-19}}$ allele, such a gene was found to be flanked by two full insertion sequences, namely IS*Ecp1B* and IS*903D* [13].

Interestingly, Ho *et al.* [19] and Kim *et al.* [20] have described alternative surroundings for $bla_{\text{CTX-M-14}}$, involving the interruption of IS*Ecp1* by the insertion in different sites of IS*10* or IS*26*.

Contrary to previous reports regarding CTX-M-9-derived genes, $bla_{\text{CTX-M-19}}$ 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 $bla_{\text{CTX-M-19}}$ seems to be driven by the promoter sequence present in IS*Ecp1*, 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 $bla_{\text{CTX-M-19}}$ could account for self-transfer events between different plasmids, or even from plasmids to the bacterial chromosome.

The sequence of $bla_{\text{CTX-M-19}}$ and its surrounding region was deposited in the EMBL database (European Bioinformatics Institute) under accession number HG000669.

Funding

This work was partially supported by grants from CSIC (Comisión Sectorial de Investigación Científica, Uruguay) to R. V.

References

1. Cantón R, González-Alba JM, Galán JC. CTX-M enzymes: origin and diffusion. *Front Microbiol* 2012; 3: 110.
2. Radice M, Power P, Di Conza J, Gutkind G. Early dissemination of CTX-M-derived enzymes in South America. *Antimicrob Agents Chemother* 2002; 46: 602–604.
3. Bartoloni A, Pallecchi L, Riccobono E *et al.* Relentless increase of resistance to fluoroquinolones and expanded-spectrum cephalosporins in *Escherichia coli*: 20 years of surveillance in resource-limited settings from Latin America. *Clin Microbiol Infect* 2013; 19: 356–361.
4. Sennati S, Santella G, Di Conza J *et al.* Changing epidemiology of extended-spectrum beta-lactamases in Argentina: emergence of CTX-M-15. *Antimicrob Agents Chemother* 2012; 56: 6003–6005.
5. Bado I, Cordeiro NF, Robino L *et al.* Detection of class 1 and 2 integrons, extended-spectrum β-lactamases and *qnr* alleles in entero-

bacterial isolates from the digestive tract of intensive care unit inpatients. *Int J Antimicrob Agents* 2010; 36: 453–458.
6. García-Fulgueiras V, Bado I, Mota MI *et al.* Extended-spectrum β-lactamases and plasmid-mediated quinolone resistance in enterobacterial clinical isolates in the paediatric hospital of Uruguay. *J Antimicrob Chemother* 2011; 66: 1725–1729.
7. Bado I, García-Fulgueiras V, Cordeiro NF *et al.* First human isolate of *Salmonella enterica* serotype enteritidis harboring $bla_{CTX-M-14}$ in South America. *Antimicrob Agents Chemother* 2012; 56: 2132–2134.
8. Poirel L, Naas T, Le Thomas I, Karim A, Bingen E, Nordmann P. CTX-M-type extended-spectrum beta-lactamase that hydrolyzes ceftazidime through a single amino acid substitution in the omega loop. *Antimicrob Agents Chemother* 2001; 45: 3355–3361.
9. Kõljalg S, Truusalu K, Vainumäe I, Stsepetova J, Sepp E, Mikelsaar M. Persistence of *Escherichia coli* clones and phenotypic and genotypic antibiotic resistance in recurrent urinary tract infections in childhood. *J Clin Microbiol* 2009; 47: 99–105.
10. Kim HB, Park CH, Kim CJ, Kim EC, Jacoby GA, Hooper DC. Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. *Antimicrob Agents Chemother* 2009; 53: 639–645.
11. Fu Y, Zhang W, Wang H *et al.* Specific patterns of *gyrA* mutations determine the resistance difference to ciprofloxacin and levofloxacin in *Klebsiella pneumoniae* and *Escherichia coli*. *BMC Infect Dis* 2013; 13: 8.
12. Lin CC, Chen TH, Wang YC *et al.* Analysis of ciprofloxacin-resistant *Salmonella* strains from swine, chicken, and their carcasses in Taiwan and detection of parC resistance mutations by a mismatch amplification mutation assay PCR. *J Food Prot* 2009; 72: 14–20.
13. Poirel L, Decousser JW, Nordmann P. Insertion sequence ISEcp1B is involved in expression and mobilization of a bla(CTX-M) beta-lactamase gene. *Antimicrob Agents Chemother* 2003; 47: 2938–2945.
14. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; 63: 219–228.
15. Barton BM, Harding GP, Zuccarelli AJ. A general method for detecting and sizing large plasmids. *Anal Biochem* 1995; 226: 235–240.
16. Mnif B, Vimont S, Boyd A *et al.* Molecular characterization of addiction systems of plasmids encoding extended-spectrum beta-lactamases in *Escherichia coli*. *J Antimicrob Chemother* 2010; 65: 1599–1603.
17. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; 66: 4555–4558.
18. Johnson JR, Kuskowski MA, Owens K, Gajewski A, Winokur PL. Phylogenetic origin and virulence genotype in relation to resistance to fluoroquinolones and/or extended-spectrum cephalosporins and cephamycins among *Escherichia coli* isolates from animals and humans. *J Infect Dis* 2003; 188: 759–768.
19. Ho PL, Chan J, Lo WU *et al.* Dissemination of plasmid-mediated fosfomycin resistance fosA3 among multidrug-resistant *Escherichia coli* from livestock and other animals. *J Appl Microbiol* 2013; 114: 695–702.
20. Kim J, Bae IK, Jeong SH, Chang CL, Lee CH, Lee K. Characterization of IncF plasmids carrying the blaCTX-M-14 gene in clinical isolates of *Escherichia coli* from Korea. *J Antimicrob Chemother* 2011; 66: 1263–1268.