Emergence of CTX-M-27-producing Escherichia coli of ST131 and clade C1-M27 in an impacted ecosystem with international maritime traffic in South America

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Sir,

Surveillance studies of ESBL-producing Escherichia coli have identified a globally disseminated high-risk clone named ST131, with strains belonging to three clades (A, B and C) and three different sub-clades (C1, C1-M27 and C2). While C2 is associated with CTX-M-15, clade C1-M27 has been associated with CTX-M-27. Nowadays, the MDR and CTX-M-27-producing ST131-C1 cluster has been considered a novel epidemic clone. In South America, neither bla<sub>CTX-M-27</sub> nor <i>E. coli</i> ST131 C1-M27 have been reported so far. During a local surveillance study conducted to monitor the presence of WHO critical priority pathogens in impacted marine ecosystems, brown mussels (<i>Perna perna</i>) and oysters (<i>Crassostrea</i> spp.) were collected from 14 near-shore sites located at different distances from the port of Santos (the largest port of Latin America). Mussel (<i>n</i> = 10) and oyster (<i>n</i> = 10) samples, collected from each site, were placed into sterile plastic bags. The samples were kept refrigerated and processed within 3 hours after collection. Following standard methods for the examination, 25 g of bivalves were distributed in sterile plastic bags containing 225 mL of Brain Heart Infusion broth and incubated at 37°C for 24 hours. Subsequently, the samples were streaked onto MacConkey agar plates supplemented with ceftriaxone (2 mg/L), meropenem (2 mg/L) or colistin (2 mg/L), following incubation at 37°C for 24 hours.

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Two ceftriaxone-resistant *E. coli* isolates were recovered from mussel (*E. coli* 6M) and oyster (*E. coli* MO) samples collected from two different sites (23.987125S, 46.308609 W and 23.976040S, 46.372580 W) close to the port. Antimicrobial susceptibility testing, performed by disc diffusion and/or Etest methods, revealed that both strains were resistant to amoxicillin/clavulanic acid, aztreonam, trimethoprim/sulfamethoxazole, ceftiofur (32 mg/L), ceftazidime (32 mg/L), cefotaxime (32 mg/L) and tetracycline. Additionally, *E. coli* MO was resistant to nalidixic acid (32 mg/L) and ciprofloxacin (4 mg/L). PCR screening and Sanger sequencing revealed that these isolates were positive for the *bla*<sub>CTX-M-27</sub> ESBL gene.

*E. coli* strains were subjected to WGS using the Illumina NextSeq (2 x 150 bp) platform (Illumina, USA). De novo assemblies were performed using Spades v. 3.11. WGS data were analysed using bioinformatics tools available from the Center for Genomic Epidemiology (www.cge.dtu.dk).

*E. coli* 6M (accession number: NCWA00000000.1) belonged to serotype O86:H18 and sequence type ST38/CC38, whereas *E. coli* MO (accession number: NCVZ00000000.1) belonged to serotype O25b:H4 and ST131/CC131. These STs have been globally disseminated among humans, animals and aquatic environments, being commonly associated with CTX-M variants. Both strains belonged to the high-virulence phylogenetic group B2. In this regard, virulome analysis of *E. coli* 6M revealed the presence of *iss* (increased serum survival), *astA* (EAST-1 toxin), *eatA* (enterotoxigenic autotransporter A), *capU* (hexosyltransferase homologue), *nfaE* (diffuse adherence fibrillar adhesin) and *eilA* (*Salmonella* HilA homologue) genes, whereas *iha* (adherence protein), *sat* (secreted autotransporter toxin), *gad* (glutamate decarboxylase), *senB* (enterotoxin) and *iss* genes were found in *E. coli* MO. Moreover, *E. coli* MO carried *fimH30* (associated with ST131) and the C1 subclade-specific prophage-like region (M27PP1). On the other hand, both strains displayed an identical resistome for aminoglycosides (*strA*, *strB* and *aadA5*), β-lactams (*bla*<sub>CTX-M-27</sub>), sulphonamides (*sul1* and *sul2*), trimethoprim (*dfrA17*) and tetracycline (*tetA*), as previously observed in *E. coli* of ST131 and C1-M27 clade, whereas mutations in the quinolone

![Figure 1](image.png)
resistance-determining regions of gyrA (Ser83Leu, Asp87Asn), parC (Ser801le, Glu84Val) and parE (Ile529Leu) genes were only identified in the E. coli MO strain. FIB and FII, and Col156, FIA, FIB and FII replicon types were identified in E. coli 6M and MO strains, respectively.

Mobilization of plasmids ~130 kb in size (named pMO and p6M), bearing bla_{CTX-M-27} genes, was achieved by bacterial transformation using E. coli TOP10. FIB and FII replicons were identified in p6M (FAB formula F2: A−:B10), whereas FIA, FIB and FII replicon types were confirmed in pMO (FAB formula F1: A2: B20). The complete sequence of the pMO plasmid (GenBank accession no. MG886288) was obtained using de novo assembly, followed by gap closure by PCR and Sanger sequencing.

The pMO plasmid was 131 016 bp in length, containing 52.1% GC and 171 coding regions (CDS), of which 129 CDS encoded proteins with known functions (i.e. proteins related to plasmid replication, partition, maintenance, conjugation, toxin–antitoxin systems and antimicrobial resistance). Besides bla_{CTX-M-27}, the pMO plasmid harboured aadA5, sul1, dfrA17, tet(A) and mphA resistance genes, similarly to F1:A2:B20 plasmids harboured by the C1-M27 clade (Figure 1a). In fact, pMO showed a high nucleotide identity (>95%) to other F1:A2:B20 plasmids harboured by CTX-M-27-producing E. coli strains of ST131 and clade C1-M27, identified in European, Asian and North American countries (Figure 1a), which could support intercontinental dissemination of this sort of plasmid.

Although analysis of the genetic environment of bla_{CTX-M-27} genes, carried by both E. coli strains, revealed the presence of IS26 and IS903 mobile elements, E. coli MO presented a truncated IS903 upstream of the bla_{CTX-M-27} gene, whereas E. coli 6M presented a truncated IS{Ecp1} downstream of the bla_{CTX-M-27} gene, and tonB and ORF genes (Figure 1b).

In summary, to our knowledge, we report the first identification of CTX-M-27-producing E. coli strains, of ST131 and clade C1-M27, in Brazil. In this regard, since CTX-M-27-positive E. coli strains were recovered from areas impacted by intensive maritime traffic and transoceanic shipping activities, a possible introduction of international clones via commercial shipping routes could be speculated. Another option could be polluted effluents with previously unnoticed presence of CTX-M-27-positive strains. In fact, in Brazil, aquatic environments receiving large quantities of urban wastewater, animal waste and hospital effluents have been recognized as potential sources for the dissemination of CTX-M- and carbapenemase-producing Enterobacteriales. Therefore, continued monitoring of ESBL-producing E. coli in South American countries remains necessary to elucidate the local epidemiology and dynamics of the transmission of high-risk clades with pandemic potential.

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Transparency declarations
None to declare.

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