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Letter to the Editor

International high-risk clone of multidrug-resistant CTX-M-8-producing *Escherichia coli* C-ST410 infecting an elephant (*Loxodonta africana*) in a zoo

Sir,

Escherichia coli ST410 lineage is classified as an international high-risk clone owing to its capacity to cause recurrent infections and its high transmissibility. Extended-spectrum β-lactamase (ESBL)-producing *E. coli* ST410 is an extraintestinal pathogen that has been reported worldwide, being associated with resistance to extended-spectrum cephalosporins, fluoroquinolones and carbapenems [1]. Multidrug-resistant (MDR) bacteria have generated a great threat to public health and have been reported in different spheres (e.g. human, animal, food and the environment). To the best of our knowledge, this study reports for the first time the occurrence of MDR CTX-M-8-producing *E. coli* in a captive elephant (*Loxodonta africana*), raising an alert about the emergence of ESBL producers in zoo animals.

In May 2018, a 34-year-old elephant, donated by a circus in the 1990s, showed apathy, decreased appetite and secretion in the trunk and eyes. In December 2018, the elephant started showing serious signs of respiratory distress leading to the suspicion of pneumonia. In January 2019, the signs did not improve after the administration of antimicrobials and the animal died. A microbiological investigation was begun and samples from different organs were collected. A strain of *E. coli* (A240) was obtained from a uterine sample and exhibited an MDR profile to ampicillin (MIC ≥ 32 mg/L), ampicillin/sulbactam (MIC ≥ 32 mg/L), cefuroxime (MIC ≥ 64 mg/L), ceftazidime (MIC ≥ 32 mg/L), cefotaxime (MIC ≥ 64 mg/L), ceftriaxone (MIC ≥ 64 mg/L), cefepime (MIC > 32 mg/L), gentamicin (MIC ≥ 16 mg/L), tetracycline (MIC ≥ 64 mg/L), sulfamethoxazole/trimethoprim (MIC ≥ 4/76 mg/L), ciprofloxacin (MIC > 32 mg/L) and levofloxacin (MIC > 32 mg/L), as determined by VITEK® 2 (bioMérieux, USA) and Etest methods (BioMérieux, Marcy l'Étoile, France) [Clinical Laboratory Standards Institute (CLSI); M100, 28th ed.]. The A240 strain was also resistant to enrofloxacin by the disk diffusion method (CLSI; VET08, 4th ed.).

Total DNA was sequencing using Illumina MiSeq Platform (Illumina Inc., EUA) paired-end reads (250 bp) and *de novo* assembly was performed using CLC Genomics Workbench v.11.0.1 (Qiagen, Aarhus, Denmark). The contigs were curated using the Geneious v.11.1.5 (Biomatters Ltd., Auckland, New Zealand). The resistome, virulome, mobilome, multilocus sequencing typing (MLST), serotype, *fimH*-type, phylogenetic group, metal resistance genes (MRGs), antibacterial biocides resistance genes (ABRGs) and phylogenetic analysis were performed using tools available from the Center for Genomic Epidemiology (http://genomicepidemiology.org/), BacMet v.2.0 (http://bacmet.biomedicine.gu.se/) and EnteroBase v.1.1.2 (https://enterobase.warwick.ac.uk/species/index/ecoli). The minimum threshold of antimicrobial resistance genes (ARGs), MRGs and ABRGs was set to 90% identity and 80% coverage.

MDR *E. coli* A240 (GenBank accession no. VFBH01000000) belonged to ST410 (CC23), serotype ONT:H9, phylogroup C and *fimH*24. *Escherichia coli* ST410 has a pandemic distribution among foods, animals, humans and the environment, being closely associated with carbapenemases [i.e. New Delhi metallo-β-lactamase (NDM) and *Klebsiella pneumoniae* carbapenemase (KPC)] and ESBLs (i.e. CTX-M-15 and CMY) [1]. Curiously, the minimum spanning tree (MST) based on cgMLST revealed that MDR *E. coli* A240 (C-ST410-*fimH*24) was arranged (55-loci variant) together with humans KPC-producing *E. coli* lineages (BioSample accession numbers SAMN05511179 and SAMN05511171) isolated in the state of Rio de Janeiro, southeastern Brazil in 2011 (Fig. 1) [2]. The virulome analysis revealed the presence of increased serum survival (*iss*), long polar fimbriae (*ipfA*), microcin M part of colicin H (*mcmA*) and high-pathogenicity island (HPI), being classified as an extraintestinal pathogenic *E. coli* (ExPEC) lineage [1].

The resistome of MDR *E. coli* A240 showed the presence of clinically significant resistance genes to β-lactams ($bla_{\text{CTX-M-8}}$, $bla_{\text{TEM-1B}}$), aminoglycosides [*aph(3′)-Ia, aph(3′′)-Ib, aph(6)-Id, aac(3)-IId*, *aadA2*], tetracyclines [*tet*(B)], sulfonamides (*sul2*), trimethoprim (*dfrA12*), phenicols (*floR*) and macrolides [*mdf*(A), *Inu*(G)]. The mutation points in GyrA (Ser83Leu and Asp87Asn), ParC (Ser80Ile) and ParE (Ser458Ala) were associated with resistance to quinolone and fluoroquinolones. Additionally, MDR *E. coli* A240 co-carried MRGs to silver (*silABCEP*), copper (*pcoADR*, *copA*), cadmium/zinc/cobalt (*cusA*), arsenic (*arsBC*), chromium (*chrA*) and ABRGs (*sugE, mdfA*, *emrE*), which may co-select resistant-bacteria to antimicrobials.

MDR *E. coli* A240 harboured IncFIA-ST8, IncHI1-ST2 and IncI1-ST113 and *in silico* analysis showed the IncI1/ST113 plasmid carrying the IS*26* upstream of the $bla_{\text{CTX-M-8}}$ gene. IncI1-ST133 carrying $bla_{\text{CTX-M-8}}$ has been previously reported in *E. coli* lineages from humans and animals [3]. Fluoroquinolone-resistant *E. coli* ST410 has already been reported harbouring the colistin-resistant *mcr-1* and $bla_{\text{CTX-M-like}}$ (i.e. $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-8}}$) in humans from Brazil and an Asian black bear located in a zoo from the Czech Republic [4,5].

In conclusion, to the best of our knowledge, this is the first report of an MDR CTX-M-8-producing *E. coli* C-ST410-*fimH*24 isolated from a zoo animal. These findings reinforce that zoo animals have been acting as a reservoir of clinically important bacteria and ARGs. Further epidemiological studies are required to determine the routes of transmission of MDR pathogens to wild animals in captivity. Therefore, the occurrence of an international high-risk clone of MDR ESBL-producing strain in zoo animals may

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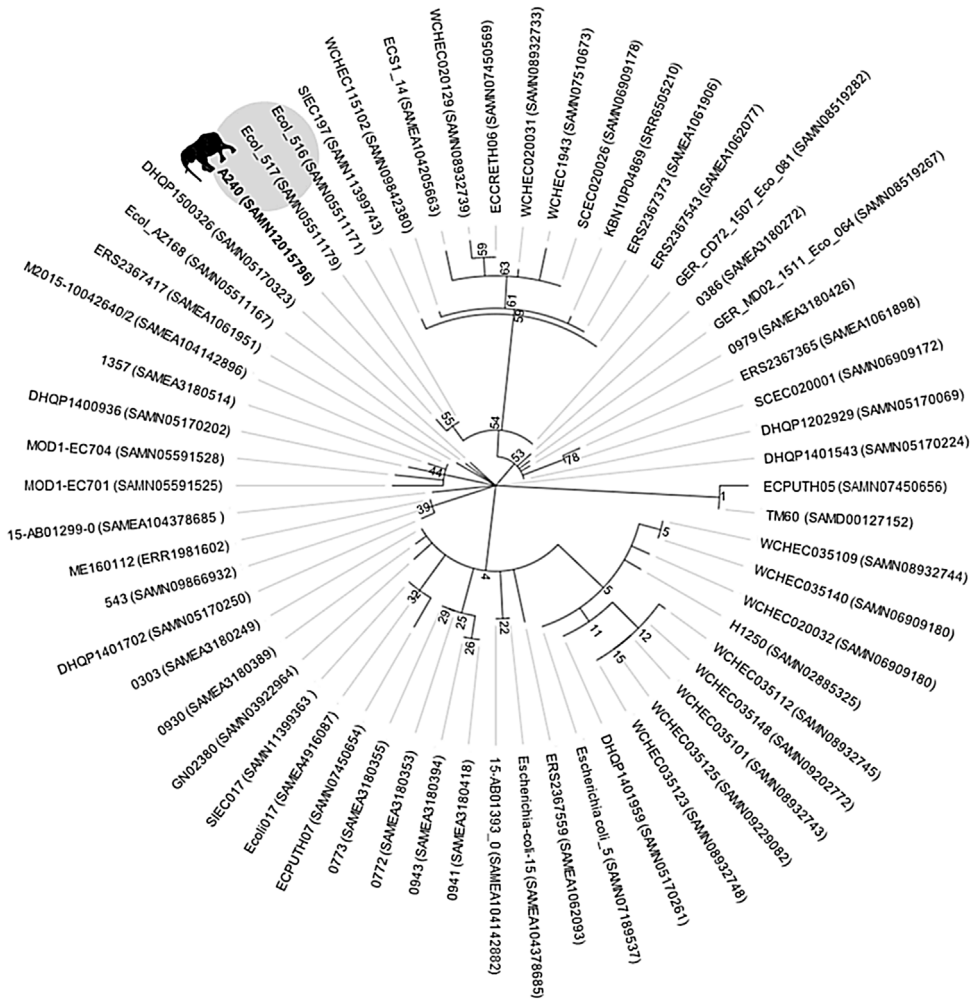

Fig. 1. MST of 60 *E. coli* lineages (C-ST410-*fimH*24) distributed worldwide based on cgMLST (2513 loci) constructed by the MSTree V2 algorithm available in EnteroBase (http://enterobase.warwick.ac.uk/species/index/ecoli). The BioSample accession numbers are shown in parentheses. Elephant illustration shows the A240 strain. The grey circle represents the clustered lineages (A240, Ecol_156 and Ecol_157).

represent a potential threat to public health as well as a challenge for veterinary medicine.

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Conflict of interests

None to declare.

Ethical approval

This study was approved by the technical and scientific committee of São Paulo Zoo Foundation (Document nº 89/2017/FPZSP/DTC).

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