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Genome Note

Draft genome sequence of a multidrug-resistant CTX-M-65-producing *Escherichia coli* ST156 colonizing a giant anteater (*Myrmecophaga tridactyla*) in a Zoo

João Pedro Rueda Furlan[a], Quézia Moura[b], Irys Hany Lima Gonzalez[c], Patrícia Locosque Ramos[c], Nilton Lincopan[b], Eliana Guedes Stehling[a,*]

[a] *Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil*
[b] *Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil*
[c] *Fundação Parque Zoológico de São Paulo, São Paulo, Brazil*

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ABSTRACT

Objectives: This study aimed to report the draft genome sequence of a multidrug-resistant (MDR) *Escherichia coli* colonizing a giant anteater (*Myrmecophaga tridactyla*) in a Brazilian Zoo.
Methods: The genome was sequenced using the Illumina MiSeq Platform and *de novo* genome assembly was performed using SPAdes v. 3.9. The draft genome sequence was annotated using NCBI Prokaryotic Genome Annotation Pipeline. Antibiotic resistance genes, virulence genes, sequence type, serotype and plasmid incompatibility groups were identified using tools from the Center for Genomic Epidemiology.
Results: The genome presented 4970 coding sequences and a GC content of 50.2%. Several antimicrobial resistance genes associated with resistance to β-lactams (bla_{TEM-1A} and $bla_{CTX-M-65}$), aminoglycosides [*aph(6)-ld*,*aph(3″)-lb*, *aph(4)-la*, *aac(3)-IVa*, *aadA1* and *aadA2*], tetracyclines (*tetB*), sulphonamides (*sul2* and *sul3*), trimethoprim (*dfrA8* and *dfrA12*) and phenicols (*floR* and *cmlA1*) were identified. Moreover, mutations in quinolone resistance-determining regions (QRDR) were found. This *E. coli* isolate also presented virulence genes and belonged to serotype ONT:H25 and ST156 (CC156).
Conclusion: This is the first report of a draft genome sequence of a CTX-M-65-producing *E. coli* ST156 obtained from a zoo animal, which can be used by genomic surveillance platforms, in order to track transmission dynamics of extended-spectrum β-lactamase (ESBL)-producing *E. coli* at the human–animal interface.

Emergence and dissemination of multidrug-resistant (MDR) bacteria in different ecological spheres (human, animal, food, and environment) have been considered a global threat to public health. Although in the last years special attention has been paid to the role of animals as reservoirs and disseminators of antimicrobial resistance, only few studies have evaluated the occurrence of MDR bacteria in zoo animals. Since zoos are environments with a high density of animals from different species in close proximity with each other and with humans (animal keepers, veterinarians, visitors), zoo animals can be colonized with MDR bacteria and spread them to different hosts and to the environment. Therefore, the aim of this study was to perform the genomic characterization of a MDR *Escherichia coli* strain recovered from a giant anteater kept in captivity [1].

In 2017, during a laboratory diagnostic routine in a Brazilian zoo, an *E. coli* isolate (A102) was obtained from a fecal sample of a giant anteater (*Myrmecophaga tridactyla*) presenting weight loss. The isolate was submitted to antimicrobial susceptibility test by disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI; M100, 27th ed.), displaying a MDR profile to ampicillin, cefazolin, cefoxitin, cefuroxime, cefaclor, cefotaxime, ceftriaxone, cefixime, aztreonam, gentamicin, tobramycin, streptomycin, tetracycline, doxycycline, minocycline, trimethoprim/sulfamethoxazole, chloramphenicol, ciprofloxacin, levofloxacin, norfloxacin, lomefloxacin, ofloxacin and nalidixic acid.

* Corresponding author. Present address: Av. do Café S/N. Monte Alegre, 14040-903, Ribeirão Preto, São Paulo, Brazil.
E-mail address: elianags@usp.br (E. Guedes Stehling).

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The genome was sequenced on an Illumina MiSeq Platform (Illumina Inc., San Diego, CA), using 250 bp paired-end reads, and *de novo* genome assembly was performed using SPAdes v. 3.9. The draft genome sequence was annotated using NCBI Prokaryotic Genome Annotation Pipeline v.3.2. Acquired antimicrobial resistance genes and chromosomal point mutations, plasmid replicons, sequence type, serotype, *fim*H-type, and virulence genes were analyzed using ResFinder 3.0, PlasmidFinder 2.0, MLST 2.0, SerotypeFinder 2.0, FimTyper 1.0, and VirulenceFinder 2.0, respectively, which are available at the Center for Genomic Epidemiology (http://genomicepidemiology.org/). Prediction of *E. coli* phylogenetic group was carried out using the online tool Clermont Typing (http://clermontyping.iame-research.center/).

A total of 2455.844 (2×250 bp) paired-end reads were generated with 122× coverage. In total, 4970 protein coding sequences, 204 pseudogenes, 82 tRNAs, 36 rRNAs and 9 ncRNAs were identified, with GC content of 50.2%. Resistome analysis showed a diversity of acquired antimicrobial resistance genes for β-lactams ($bla_{\text{TEM-1A}}$ and $bla_{\text{CTX-M-65}}$), aminoglycosides [*aph(6)-Id*, *aph(3″)-Ib*, *aph(4)-Ia*, *aac(3)-IVa*, *aadA1* and *aadA2*], tetracyclines (*tetB*), sulphonamides (*sul2* and *sul3*), trimethoprim (*dfrA8* and *dfrA12*) and phenicols (*floR* and *cmlA1*).

Interestingly, the $bla_{\text{CTX-M-65}}$ gene has become widely reported in *Enterobacteriaceae* from humans, animals, and food products in Asian countries and, more recently, it has been described in *Salmonella enterica* serovar Infantis isolates from humans, food animals, and retail chickens, in the United States, in *S. enterica* serovar Infantis isolates from humans, in Ecuador, and in *E. coli* isolates from humans in Bolivia and Peru [2–4]. However, until now there is no report of $bla_{\text{CTX-M-65}}$ in Brazil.

Additionally, mutations in quinolone resistance-determining regions (QRDR) of GyrA (Ser83Leu; Asp87Tyr) and ParC (Ser80Ile) were also detected. The IncFIA (new allele 23) and IncHI1 (allele 5) plasmid incompatibility groups were detected; however, it was not possible to determine the location of the $bla_{\text{CTX-M-65}}$ gene in these plasmids due to limitations of short sequencing read technology.

Multilocus sequence typing (MLST) analysis revealed that *E. coli* A102 belonged to sequence type (ST) 156 (CC156), which has been identified in humans, companion and wild animals, livestock, and environment, from different continents, according to data available at EnteroBase (http://enterobase.warwick.ac.uk). In Brazil, ST156 has been previously reported in an *E. coli* strain carrying *mcr-1* gene, isolated from a patient with bloodstream infection, in São Paulo State [5]. *E. coli* A102 was assigned to serotype ONT:H25-*fim*H1127 and commensal phylogroup B1, harboring *gad* (glutamate descarboxylase), *iss* (increased serum survival) and *lpfA* (long polar fimbriae) virulence genes.

In summary, we report the first draft genome sequence of a MDR CTX-M-65-producing *E. coli* ST156 recovered from a giant anteater (*M. tridactyla*), highlighting the role of this animal as reservoir of clinically important bacteria. Considering the $bla_{\text{CTX-M-65}}$ gene has only been detected in isolates from humans in countries of Latin America, other than Brazil, and *E. coli* ST156 has been previously reported in Brazil only once, in a strain obtained from human infection, further studies are necessary to better understand the transmission dynamics of MDR bacteria in the zoonotic context.

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number RCHK00000000. The version described in this paper is version RCHK01000000.

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Competing interests

None declared.

Ethical approval

Not required.

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