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Genome Note

Multidrug-resistant CTX-M-15-positive *Klebsiella pneumoniae* ST307 causing urinary tract infection in a dog in Brazil

Luciana Sartori[a], Fábio P. Sellera[b], Quézia Moura[c], Brenda Cardoso[c], Louise Cerdeira[a], Nilton Lincopan[a,c,*]

[a] Department of Clinical Analysis, School of Pharmacy, University of São Paulo, São Paulo, Brazil
[b] Department of Internal Medicine, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil
[c] Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

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ABSTRACT

Objectives: *Klebsiella pneumoniae* sequence type 307 (ST307) has emerged as a new high-risk clone worldwide. The aim of this study was to report the draft genome sequence of a multidrug-resistant CTX-M-15-positive *K. pneumoniae* strain causing urinary tract infection in a companion animal in Brazil.
Methods: Whole-genome sequencing was performed on an Illumina NextSeq platform and the genome was de novo assembled using SPAdes. Data analysis was performed using online tools from the Center for Genomic Epidemiology.
Results: The genome size of *K. pneumoniae* strain PRETA was calculated at 5 450 575 bp, containing a total of 5355 protein-coding sequences. The strain was assigned to the high-risk ST307 and carried the clinically relevant $bla_{CTX\text{-}M\text{-}15}$ gene, besides other genes conferring resistance to aminoglycosides, tetracyclines, quinolones, trimethoprim and fosfomycin.
Conclusion: These data offer novel information for comparative genomic analyses in order to track the transmission dynamics and epidemiology of the newly emerging high-risk clone ST307.

Klebsiella pneumoniae is an opportunistic pathogen responsible for human and animal infections worldwide, being frequently associated with resistance to critically important antimicrobial agents [1,2]. Recently, the World Health Organization (WHO) classified extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* as a high-priority pathogen (https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf). These bacteria have been commonly associated with human nosocomial infections and, more recently, have gained special attention as clinically important pathogens for small-animal medicine [1,2]. *K. pneumoniae* sequence type 307 (ST307) has emerged as a new high-risk clone worldwide. In fact, some epidemiological and molecular surveillance studies have suggested that ST307 is a candidate to replace CC258, currently the most prevalent clonal complex associated with multidrug resistance [3–5]. Here we present the first draft genome sequence of a multidrug-resistant (MDR) CTX-M-15-producing *K. pneumoniae* strain belonging to ST307, isolated from an infected companion animal in the city of São Paulo, Southeastern Brazil.

In 2018, a 6-year-old mixed-breed female dog presenting with urinary tract infection (UTI) was admitted to a private veterinary hospital in Brazil. A urine sample was collected by cystocentesis and was submitted to urine culture. A *K. pneumoniae* isolate was recovered from the urine sample, which was identified using a VITEK®2 system (bioMérieux, USA) and was further confirmed by whole-genome sequencing. *K. pneumoniae* strain PRETA displayed a MDR profile to amoxicillin/clavulanic acid, ceftiofur [minimum inhibitory concentration (MIC) >32 μg/mL], cefotaxime, ceftazidime, cefepime, gentamicin, ciprofloxacin, enrofloxacin, levofloxacin, norfloxacin, ofloxacin and tetracycline but remained susceptible to trimethoprim/sulfamethoxazole, ertapenem, imipenem, meropenem and fosfomycin as determined by disk diffusion (for fosfomycin, a 200 μg disk containing 50 μg of glucose-6-phosphate was used), Etest and/or agar dilution methods and according to breakpoints approved by the Clinical and Laboratory Standards Institute (CLSI supplement M100, 27th ed).

* Corresponding author at: Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.
E-mail address: lincopan@usp.br (N. Lincopan).

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Total DNA was extracted from *K. pneumoniae* PRETA using a PureLink™ Quick Gel Extraction Kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. DNA quality and quantity were evaluated by agarose gel electrophoresis and using a Qubit® 2.0 fluorometer (Life Technologies). A genomic library was constructed using a Nextera XT DNA Library Preparation Kit (Illumina Inc., Cambridge, UK), and genomic DNA was sequenced on an Illumina NextSeq platform (Illumina Inc.) using 150-bp paired-end reads. A total of 2 741 710 reads were generated with 100× coverage, which were de novo assembled using SPAdes into 378 contigs. Quality filters were applied and a Phred20 quality score was used. The draft genome was automatically annotated by the NCBI Prokaryotic Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The genome size was calculated at 5 450 575 bp with a GC content of 56.7%, comprising 5355 protein-coding sequences. In addition, 5619 total genes, 32 tRNAs, 3 rRNAs, 6 ncRNAs and 222 pseudogenes were identified.

Multilocus sequence typing (MLST) was performed using MLST 2.0 (https://cge.cbs.dtu.dk/services/MLST/) and *K. pneumoniae* strain PRETA was assigned to ST307, which has been recently recognised as an emerging high-risk clone, being responsible for several nosocomial outbreaks worldwide [3,4]. Moreover, ST307 has been closely associated with ESBL (mostly CTX-M-15) and/or carbapenemase production (i.e. KPC-, OXA- and NDM-type enzymes) [3–5]. In animals, the occurrence of *K. pneumoniae* ST307 has been restricted to dogs and cats suffering from UTI in Japan [2].

Virulence genes were detected using the Institut Pasteur database (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html) and revealed the presence of type 3 fimbriae genes (*mrkA*, *mrkB*, *mrkC*, *mrkA*, *mrkD*, *mrkF*, *mrkH*, *mrkI* and *mrkJ*), which have been associated with biofilm growth.

Genes conferring resistance to β-lactams ($bla_{CTX\text{-}M\text{-}15}$, $bla_{SHV\text{-}28\text{-}like}$ and $bla_{OXA\text{-}1}$), aminoglycosides [*aph(3")-Ib*, *aph(6)-Id* and *aac (3)-IIa*], tetracyclines (*tetA*), quinolones [*aac(6')-Ib*-cr, *qnrB1*, *oqxA* and *oqxB*], trimethoprim (*dfrA*) and fosfomycin (*fosA*) were identified by ResFinder 3.1 (https://cge.cbs.dtu.dk/services/ResFinder/), with a 90% threshold for gene identification.

Interestingly, the *qacE* and *sugE* quaternary ammonium compound resistance genes were also detected. Furthermore, the presence of an IncF incompatibility group plasmid was identified using PlasmidFinder 2.0 (https://cge.cbs.dtu.dk//services/PlasmidFinder/). In this regard, in silico analysis confirmed that the $bla_{CTX\text{-}M\text{-}15}$ gene was located on this plasmid.

To our knowledge, this is the first draft genome sequence of *K. pneumoniae* ST307 harbouring $bla_{CTX\text{-}M\text{-}15}$ and other medically important resistance genes, recovered from an infected companion animal in South America. Considering the increasing rates of CTX-M-producing *K. pneumoniae* infecting dogs and cats as well as the emergence and rapid dissemination of this new high-risk clone, surveillance strategies and genomic studies are urgently required. Moreover, since human–pet bonds could become a critical issue for the transmission of high-risk MDR pathogens, collaborative efforts between human and veterinary medicine are essential to determine transmission routes of these bacteria. Finally, this draft genome might provide additional information for comparative genomic analyses of molecular mechanisms of high-risk MDR *K. pneumoniae* at the human–animal interface.

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under accession no. **QITI00000000.1**. The version described in this paper is version **QITI00000000.1**.

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Competing interests

None declared.

Ethical approval

Not required.

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