



## Genome Note

# Genomic analysis of multidrug-resistant CTX-M-15-positive *Klebsiella pneumoniae* belonging to the highly successful ST15 clone isolated from a dog with chronic otitis

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## ABSTRACT

**Background:** Extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Klebsiella pneumoniae* have been increasingly reported worldwide as a frequent cause of human and animal infections. *K. pneumoniae* belonging to the K24 capsular serotype and sequence type (ST) ST15 has been considered a global successful clone responsible for the spread of the *bla*<sub>CTX-M-15</sub> gene.

**Objective:** To report the draft genome sequence of a multidrug-resistant CTX-M-15-positive *K. pneumoniae* K24-ST15 strain (L3KP1), which was isolated from a dog with chronic otitis.

**Methods:** Genomic DNA was extracted and sequenced using Illumina NextSeq platform. De novo assembly was performed by SPAdes and in silico prediction accomplished by curated bioinformatics tools.

**Results:** The genome size was calculated at 5 642 348 bp, with a GC content of 57.11%, and comprising 5601 total genes, 52 tRNAs, 8 rRNAs, 9 ncRNAs and 105 pseudogenes. The *K. pneumoniae* L3KP1 strain belonged to ST15 and carried the yersiniabactin biosynthetic gene cluster [*ybt* 10 (YbST28)] in the integrative conjugative element ICEKp4, and the KL24 locus encoding capsular serotype K24. Besides the *bla*<sub>CTX-M-15</sub> ESBL gene, other clinically important resistance genes to  $\beta$ -lactams, aminoglycosides, fosfomicin, macrolides, phenicol, quinolones, sulfonamides, tetracyclines and trimethoprim were detected. Additionally, heavy metals and disinfectant resistance genes were also identified.

**Conclusion:** This draft genome might be useful for comparative genomic analyses of the international clone of *K. pneumoniae* K24-ST15-CTX-M-15. In addition, information presented in this study also shed light on the urgent need to monitor ESBL-producing *K. pneumoniae* in veterinary hospitals.

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## 1. Introduction

Although extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Klebsiella pneumoniae* clones, including CTX-M producers, have been primarily related to human infections worldwide [1–3], there

are increasing reports of their occurrence in companion animals [1–3]. Otitis externa is an inflammatory condition of the external ear canal that is frequently complicated by secondary bacterial infections, requiring antibiotic treatment [4]. Methicillin-resistant *Staphylococcus* spp. and multidrug-resistant (MDR) *Pseudomonas aeruginosa* have been the most prevalent antibiotic-resistant pathogens isolated from dogs with otitis externa [4], however, the identification of ESBL- and carbapenemase-producing Enterobacterales has been also documented [5]. Here, we present the draft genome sequence of a CTX-M-15-producing *K. pneumoniae*

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strain belonging to the international K24 capsular serotype and sequence type (ST) ST15 isolated from a dog with chronic otitis, in southeast Brazil.

## 2. Methods

In March 2019, a 5-year-old male Rottweiler dog was admitted to a veterinary teaching hospital in Santos city, southeast Brazil. The dog presented with head shaking, pruritus, pain on palpation of the right ear pinna, purulent discharge and erythema of the ear. Veterinary medical records revealed unsuccessful treatment with enrofloxacin and recurrent history of otitis for 6 months. A sample was collected from the affected ear and was submitted for microbiological culture. A *K. pneumoniae* isolate (L3KP1) was recovered from the ear sample, identified using BD Phoenix (BD Diagnostics, Sparks, MD, USA) and further confirmed using whole-genome sequencing. The *K. pneumoniae* strain L3KP1 displayed a resistant profile to amoxicillin/clavulanic acid, aztreonam, ceftiofime, ceftiofur, ceftriaxone [mean inhibitory concentration (MIC > 32 µg/mL)], nalidixic acid, ciprofloxacin (MIC 64 µg/mL), enrofloxacin (MIC 32 µg/mL), levofloxacin (MIC 8 µg/mL), sulfamethoxazole/trimethoprim and tetracycline. However, it remained susceptible to cefoxitin, amikacin, gentamicin, fosfomicin, ertapenem, imipenem and meropenem; as determined by disc diffusion, Epsilonometer (E)-test and/or agar dilution methods [CLSI 2020, supplement M100 and CLSI 2018, standard VET01].

Genomic DNA of the L3KP1 strain was extracted using PureLink™ Quick Gel Extraction Kit (Life Technologies, Carlsbad, CA, USA) and Qubit 2.0 fluorometer (Life Technologies) was used to measure DNA concentration. Library preparation was performed using the Nextera DNA Flex Kit (Illumina, San Diego, CA, USA) and sequenced by using NextSeq550 platform (Illumina) paired-end reads (2 × 75-bp) (Illumina). De novo assembly was performed using SPAdes version 3.9 (<https://cge.cbs.dtu.dk/services/SPAdes/>). Assembly was curated using Geneious v.R9 (Biomatters Ltd., Auckland, New Zealand) and submitted for annotation using NCBI Prokaryotic Genome Annotation Pipeline version v4.10 ([http://www.ncbi.nlm.nih.gov/genome/annotation\\_prok/](http://www.ncbi.nlm.nih.gov/genome/annotation_prok/)).

## 3. Results and Discussion

The genome size was calculated at 5 642 348 bp, with a GC content of 57.11%, and comprising 5601 total genes, 8 rRNAs, 52 tRNAs, 9 ncRNAs and 105 pseudogenes. Multilocus sequence typing analysis, predicted using MLST 2.0 (<https://cge.cbs.dtu.dk/services/MLST/>), revealed that the *K. pneumoniae* strain L3KP1 was assigned to ST15. The ST15 is a well-documented international clone (most frequently reported in Asian and European countries), associated with ESBL (CTX-M-15) and/or carbapenemase production [1–3].

Resistome analysis identified several genes encoding resistance to β-lactams (*bla*<sub>CTX-M-15</sub>, *bla*<sub>SHV-28</sub>-like and *bla*<sub>OXA-1</sub>), aminoglycosides [*aadA2* and *aph(3')-Ia*], fosfomicin (*fosA*), macrolides (*mphA*), phenicol (*catB3*), quinolones [*aac(6')Ib-cr*, *oqxA* and *oqxB*], sulfonamides (*sul1*), tetracyclines (*tetA*) and trimethoprim (*dfrA12*), which were predicted by ResFinder 3.2 (<https://cge.cbs.dtu.dk/services/ResFinder/>), with a 90% threshold for gene identification. Moreover, in silico analysis revealed chromosomal point mutations in GyrA (Ser83Phe and Asp87Ala) and ParC (Ser80Ile), previously identified in the *K. pneumoniae* strain FDAARGOS\_775 (GenBank accession number CP040993.1), which could explain the fluoroquinolone resistance profile.

In this study, it was not possible to determine the complete sequence of plasmids, as an Illumina short read sequencing was used. However, in silico analysis and PlasmidFinder 2.1 (<https://cge.cbs.dtu.dk/services/PlasmidFinder/>) allowed us to identify the

partial sequence of a single plasmid carrying the *bla*<sub>CTX-M-15</sub> gene, belonging to the IncFIB and IncFII replicon types, which shares 99.9% sequence identity with other IncFIB–IncFII plasmids (GenBank accession numbers: <https://www.ncbi.nlm.nih.gov/nucleotide/CP036302>, <https://www.ncbi.nlm.nih.gov/nucleotide/LR025089> and <https://www.ncbi.nlm.nih.gov/nucleotide/CP029591>) identified in *K. pneumoniae* strains isolated from human infections.

K24 capsular serotype, virulence genes [*mrkABCFHIJ*] (type 3 fimbriae), *kfuABC* (ferric uptake system), *fyuA* (yersiniabactin receptor), and *irp1–2* and *ybtAEPQSTUX* (yersiniabactin synthesis), disinfectant resistance genes (*cpxA*, *kdeA*, *kexD*, *kpnE*, *oqxB* and *phoB*), and heavy metal resistance genes, [*arsRDABC* (arsenic), *pcoABCDERS* (copper) and *silABCEFGPRS* (silver)], were also detected using the Pasteur database (<http://bigsdbs.pasteur.fr/klebsiella/klebsiella.html>) and BacMet (<http://bacmet.biomedicine.gu.se/>). Phylogenetic analysis of the ybt locus revealed ybt 10 lineage (ybt locus sequence type YbST 28) and integrative element ICEKp4 (<https://github.com/katholt/Kleborate>), with K-locus KL24 and O-locus O1v1, being further identified.

In summary, we report the draft genome sequence of a CTX-M-15-producing *K. pneumoniae* ST15–KL24 (ICEKp4/ybt 10) isolated from an infected dog in Brazil. The rapid adaptation of this successful epidemic clone carrying *bla*<sub>CTX-M-15</sub> in veterinary settings represents an epidemiological alert deserving close monitoring. However, considering that cross-transmissions of high-priority bacteria could occur owing to the close contact between humans and their pets, continuous surveillance of such pathogens in small animal practice should be strongly encouraged. Although the short-read sequencing technology used in this study limits genome assembly, including complete replicons and repetitive regions, the data provided might be useful for comparative genomic analyses of K24–ST15–CTX-M15 *K. pneumoniae* and other international clones of *K. pneumoniae*, which have been emerging from both human and veterinary medicine.

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAAVWZ000000000. The version described in this paper is version JAAVWZ000000000.1.

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## Ethical approval

Not required.

## Competing interests

None declared.

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