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Genomic features of a polymyxin-resistant *Klebsiella pneumoniae* ST491 isolate co-harbouring $bla_{CTX\text{-}M\text{-}8}$ and *qnrE1* genes from a hospitalised cat in São Paulo, Brazil

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ABSTRACT

Objectives: Klebsiella pneumoniae has emerged as one of the major pathogens of humans and companion animals. Moreover, polymyxin resistance in *K. pneumoniae* is increasingly reported worldwide, mainly among extended-spectrum β-lactamase (ESBL)- and/or carbapenemase-producing isolates. The aim of this study was to report the draft genome sequence of a polymyxin-resistant, ESBL-producing *K. pneumoniae* isolate (14CSI) from a hospitalised domestic cat in Brazil.
Methods: Whole-genome sequencing of strain 14CSI was performed on an Illumina NextSeq platform and the genome was de novo assembled using Velvet v.1.2.10. Data analysis was performed using bioinformatics tools available from the Center for Genomic Epidemiology and the Institut Pasteur database.
Results: The genome size of strain 14CSI was calculated at 5 260 459 bp, with a GC content of 57.3% and comprising 5294 total genes, 28 tRNAs, 7 rRNAs, 8 ncRNAs and 237 pseudogenes. *Klebsiella pneumoniae* strain 14CSI belongs to sequence type 491 (ST491), presents a mutation (A14S) in the *mgrB* gene and co-harbours $bla_{CTX\text{-}M\text{-}8}$ and *qnrE1* genes. Genes conferring resistance to heavy metals were further identified.
Conclusion: This draft genome could be used as a reference sequence for comparative analysis of polymyxin-resistant and/or CTX-M-8-producing *K. pneumoniae* strains circulating at the human–animal interface.

Klebsiella pneumoniae is a leading cause of hospital- and community-acquired infections in humans, while their occurrence in companion animals has also raised serious concerns [1–3]. Worryingly, polymyxin resistance in *K. pneumoniae* has begun to be increasingly reported worldwide, mainly among extended-spectrum β-lactamase (ESBL)- and/or carbapenemase-producing isolates [4]. Although the presence of plasmid-mediated resistance associated with *mcr*-type genes has been frequently reported in polymyxin-resistant *K. pneumoniae* isolates, chromosomal mutations predominate. Among the chromosomal genes, *mgrB* has been frequently associated with several mutations and disruption by insertion sequence (IS) elements (mostly IS*5*-like and IS*903*) [4]. Here we present the draft genome sequence of a polymyxin-resistant *K. pneumoniae* strain belonging to sequence type 491 (ST491), presenting a mutation (A14S) in the *mgrB* gene and co-harbouring $bla_{CTX\text{-}M\text{-}8}$ and *qnrE1* genes, isolated from a hospitalised domestic cat in Brazil.

In 2017, a 5-year-old mixed breed male cat presenting with dyspnoea was admitted to a veterinary hospital in São Paulo city. The cat was diagnosed with feline viral rhinotracheitis, an upper respiratory or pulmonary infection of cats caused by Felid alphaherpesvirus 1 (FeHV-1). Enrofloxacin treatment was administered empirically to prevent secondary bacterial infection. A rectal swab was collected at the time of admission for ESBL

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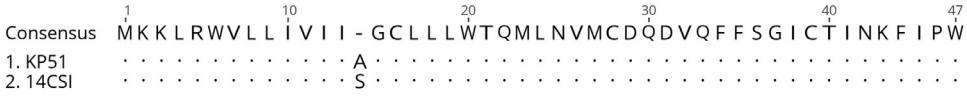

Fig. 1. DNA sequence alignment for wild-type MgrB protein from colistin-susceptible *Klebsiella pneumoniae* strain KP51 (GenBank accession no. MF431845) and from the polymyxin-resistant *K. pneumoniae* strain 14CSI (ST491) isolated from a companion animal in Brazil (this study). The A14S amino acid substitution was identified in *K. pneumoniae* 14CSI.

surveillance purposes. The sample was streaked on a MacConkey agar plate supplemented with ceftriaxone (2 μg/mL). In this respect, a ceftriaxone-resistant *K. pneumoniae* isolate (14CSI) was recovered, being identified by BD Phoenix (BD Diagnostics, Sparks, MD, USA) and further confirmed by whole-genome sequencing (WGS) analysis.

K. pneumoniae strain 14CSI displayed a resistant profile to amoxicillin/clavulanic acid, ceftiofur, ceftriaxone [minimum inhibitory concentration (MIC) > 32 μg/mL], cefoxitin and cefepime, whereas it remained susceptible to amikacin, gentamicin, nalidixic acid, enrofloxacin, norfloxacin, levofloxacin (MIC = 0.25 μg/mL), ciprofloxacin (MIC ≤ 0.125 μg/mL), ofloxacin, ertapenem, imipenem, meropenem, trimethoprim/sulfamethoxazole, tetracycline and fosfomycin (MIC = 16 μg/mL) as determined by disk diffusion, Etest and/or agar dilution methods and adopting breakpoints approved by the Clinical and Laboratory Standards Institute (CLSI M100, 27th ed). Moreover, strain 14CSI was resistant to polymyxin B (MIC = 8 μg/mL) as determined by broth microdilution according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_Tables.pdf).

Genomic DNA was extracted using a PureLink™ Quick Gel Extraction Kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The DNA concentration was evaluated 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 75-bp paired-end sequencing was carried out using an Illumina NextSeq platform. Multilocus sequence typing (MLST) as well as analysis of resistance genes and plasmid incompatibility groups were performed using bioinformatics tools (MLST 2.0, ResFinder 3.2 and PlasmidFinder 2.1, respectively) available from the Center for Genomic Epidemiology (http://genomicepidemiology.org/), whereas the virulome and presence of heavy metal genes were evaluated using the Institut Pasteur database (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html).

A total of 28 633 616 reads were generated with 200× coverage, which were de novo assembled in 323 contigs using Velvet v.1.2.10. The draft genome was automatically annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The genome size was calculated as 5 260 459 bp, with a GC content of 57.3% and comprising 5294 total genes, 28 tRNAs, 7 rRNAs, 8 ncRNAs and 237 pseudogenes. According to MLST analysis, *K. pneumoniae* strain 14CSI belongs to ST491, which was previously identified in a *K. pneumoniae* strain from a hospitalised human patient in England, being associated with KPC production [5]. Interestingly, ST491 has been also isolated from healthy individuals in Laos, being associated with colistin resistance [4].

The antimicrobial resistance genes $bla_{\text{CTX-M-8}}$, $bla_{\text{SHV-224}}$ (manually curated, GenBank accession no. AYD75397.1), $bla_{\text{TEM-1A}}$, *qnrE1* and *fosA* were identified with 100% identity. Furthermore, in silico analysis revealed a mutation (Ala14Ser) in the *mgrB* gene (Fig. 1) previously identified in a colistin-resistant *K. pneumoniae* ST491 strain from Laos [4]. Heavy metal resistance genes to copper (*pcoA–pcoE*) and silver (*silC*, *silE* and *silS*) as well as the *mrkABCDF* operon encoding type 3 fimbriae, which play an important role in biofilm formation, were also identified.

Plasmid incompatibility groups IncFIB, IncL/M and IncR were detected, and in silico analysis revealed that the *qnrE1* gene was located on the IncL/M plasmid. As a limitation of this study, since short-read sequencing technology was used, it was not possible to confirm the exact location of the $bla_{\text{CTX-M-8}}$ gene.

In summary, we report the identification and genomic features of a polymyxin-resistant *K. pneumoniae* strain co-harbouring $bla_{\text{CTX-M-8}}$ and *qnrE1* genes, colonising a companion animal. This draft genome can be used as a reference sequence for comparative analysis of polymyxin-resistant and/or CTX-M-8-producing *K. pneumoniae* strains. Lastly, WGS data provided in this study can expand our understanding about mechanisms of dissemination of resistance genes in *K. pneumoniae* lineages circulating at the human–animal interface.

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. PIUN00000000. The version described in this paper is version PIUN00000000.1.

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Ethical approval: Not required.

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