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Genome Note

Draft genome sequence analysis of a high carbapenem-resistant *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* strain isolated from an HIV-positive patient with pneumonia

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ABSTRACT

Objectives: The rapid spread of *Klebsiella* spp. is recognised as a major threat to public health owing to a rise in the number both of healthcare- and community-acquired infections. Here we report the draft genome sequence of a high carbapenem-resistant *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* strain (Cln185) isolated from a human immunodeficiency virus (HIV)-positive patient with pneumonia.
Methods: Classical microbiological methods were applied to isolate and identify the strain. Genomic DNA was sequenced using an Illumina HiSeq platform and the reads were de novo assembled into contigs using CLC Genomics Workbench. The assembled contigs was annotated and whole-genome sequencing (WGS) was performed.
Results: WGS analysis revealed that the genome comprised a circular chromosome of 5 406 774 bp with a GC content of 57.73%. Three important antimicrobial resistance genes (bla_{IMP-38}, $bla_{OKP-B-6}$ and bla_{DHA-1}) were detected. In addition, genes conferring resistance to aminoglycosides, β-lactams, fluoroquinolones and tetracycline were also identified.
Conclusion: The draft genome sequence reported here will lay the foundation for future research on antimicrobial resistance and pathogenic mechanisms in *K. quasipneumoniae* subsp. *quasipneumoniae* and also will promote comparative analysis with genomic features among different sources of clinically important multidrug-resistant strains.

Klebsiella spp. are prominent infectious agents widely distributed in the environment and associated with hospital- and community-acquired infections [1]. This is a matter of concern since *Klebsiella* spp. harbour many clinically relevant resistance genes and may lead to bacterial metastatic spread. In recent years, a novel species of the genus, namely *Klebsiella quasipneumoniae* subsp. *quasipneumoniae*, was further classified [2]. Research has confirmed that *K. quasipneumoniae* subsp. *quasipneumoniae* is also a human pathogen and poses a considerable threat, resulting in mortality to high-risk patients undergoing life-threatening procedures. *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* is frequently found in persons with hospital-acquired infection or colonisation [3]. In the last decade, carbapenem-resistant *Klebsiella* spp. have become a growing threat to public health. However,

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there has been much less research on antimicrobial resistance based on genome analysis of *K. quasipneumoniae* subsp. *quasipneumoniae*. In this study, whole-genome sequencing (WGS) was performed on *K. quasipneumoniae* subsp. *quasipneumoniae* strain Cln185, a high carbapenem-resistant strain isolated from a human immunodeficiency virus (HIV)-positive patient with pneumonia.

A 31-year-old HIV-infected man with cough and intermittent fever for >3 weeks was admitted to the First Affiliated Hospital of Kunming Medical University (Kunming, China). Bacteriology tests of sputum and blood samples confirmed that the pathogen was *Klebsiella* spp. Following treatment with trimethoprim/sulfamethoxazole, the patient experienced significant improvement within a few days. One colony was selected and was named Cln185. The antibiogram pattern of the strain was identified using a BD Phoenix?? 100 full-automatic microbial identification and drug susceptibility system (BD, Franklin Lakes, NJ, USA). Strain Cln185 displayed an extremely drug-resistant profile, except to polymyxin B, piperacillin/sulbactam and trimethoprim/sulfamethoxazole (Supplementary Table S1). In particular, strain Cln185 exhibited an antibiogram pattern with resistance to imipenem [minimum inhibitory concentration (MIC) =6 μg/mL], meropenem (MIC = 4 μg/mL) and ertapenem (MIC = 1 μg/mL).

To determine the whole-genome sequence of strain Cln185, bacterial genomic DNA was extracted using a HiPure Bacterial DNA Kit (Magen, Guangzhou, China) according to the manufacturer?"s instructions. DNA quality and concentration were determined using NanoDrop?? (Thermo Fisher Scientific, Wilmington, DE, USA). WGS was performed on an Illumina HiSeq PE150 platform (Illumina Inc., San Diego, CA, USA). A-tailed fragmented DNA, ligated to paired-end adaptors and PCR amplified with a 350-bp insert, was used for library construction at Beijing Novogene Bioinformatics Technology Co., Ltd. (Beijing, China). Illumina PCR adapter reads and low-quality reads from the paired-end reads were filtered by a quality control step using our own compiling pipeline. All good quality paired-end reads were assembled using SOAPdenovo (http://soap.genomics.org.cn/soapdenovo.html) into a number of scaffolds. Subsequently, the filter reads were handled by the next step of gap closing. Genome annotation was performed by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Finally, the genome sequence was acquired after correction using Pilon software. Prediction of genome function elements included the prediction of coding genes, non-coding RNA and clustered regularly interspaced short palindromic repeats (CRISPRs). The PHI (Pathogen Host Interactions), VFDB (Virulence Factors of Pathogenic Bacteria) and ARDB (Antibiotic Resistance Genes Database) were used to perform the above analyses. Carbohydrate-active enzymes were predicted by the Carbohydrate-Active Enzymes (CAZy) database.

A total of 66 contigs were assembled with an N_{50} contig size of 239 676 bp. The total size of the complete genome is 5 406 774 bp with a GC content of 57.73%. Using PGAP analysis, a total of 5147 protein-coding sequences, 89 tRNAs and 25 rRNAs were identified. MLST 1.8 (multi-locus sequence typing) (https://cge.cbs.dtu.dk/services/MLST/) analysis showed that strain Cln185 belongs to ST1308. Comparative genome analysis data was assessed by Microbial Nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE = BlastSearch&BLAST_SPEC = MicrobialGenomes). Strain Cln185 presents high identity with *K. quasipneumoniae* ATCC 700603 and *K. quasipneumoniae* strain D120-1. The evolutionary relationship of strain Cln185 and homologous genomes from different *K. quasipneumoniae* strains is summarised in Supplementary Fig. S1.

Consistent with the results of antimicrobial susceptibility testing, strain Cln185 encodes a carbapenemase gene (bla_{IMP-38}). IMP-38 is most similar to IMP-4, with one point mutation. In China, a variety of carbapenemases have been reported in *Klebsiella* spp. The bla_{IMP-4} gene now appears to be the most prevalent carbapenemase-encoding gene [4]. In addition, strain Cln185 encodes extensive antimicrobial resistance determinants conferring resistance to tetracycline (TetG), β-lactams (DHA-1, OKP-B-6), fluoroquinolones (GyrA, PatA) and aminoglycosides (KdpE). Antimicrobial susceptibility testing demonstrated that the genotype and phenotype matched. Furthermore, several multidrug resistance efflux pump families were also identified. On the other hand, Cln185 does not contain plasmids and the drug resistance genes are encoded on the chromosome.

Collectively, precise identification of antimicrobial resistance genes is critical for epidemiological purposes and infection control [5]. The objectives of the present work were to demonstrate that important antimicrobial resistance genes coexisted in a high carbapenem-resistant *K. quasipneumoniae* subsp. *quasipneumoniae* strain isolated from an HIV-positive patient with pneumonia, which would culminate in a difficult-to-treat infection. This study contributes to the description of the importance of monitoring infected patients, which represent a serious potential risk for the spread of clinically significant antibiotic resistance genes in community and hospital environments.

This Whole Genome project has been deposited at DDBJ/ENA/GenBank under the accession no. RSCI00000000. The version described in this paper is the first version (RSCI00000000).

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Competing interests

None declared.

Ethical approval

Ethical approval was obtained from the Medical Ethics Committee for Research of the First Affiliated Hospital of Kunming Medical University (Kunming, China).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.09.004.

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