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Genome Note

Genome sequence of a multidrug-resistant *Klebsiella pneumoniae* ST78 with high colistin resistance isolated from a patient in India

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ABSTRACT

Objectives: Multidrug-resistant (MDR) *Klebsiella pneumoniae* isolates with colistin resistance are a major concern in healthcare settings. This study aimed to evaluate the genome-wide distribution of antimicrobial resistance genes in *K. pneumoniae* CRKP I with high colistin resistance isolated from a patient in India.
Methods: The whole genome of *K. pneumoniae* CRKP I was sequenced on an Illumina MiSeq platform. De novo genome assembly was performed using SPAdes v.3.0.0, and the genome sequence was analysed using bioinformatics tools available from the Center for Genomic Epidemiology.
Results: The genome of *K. pneumoniae* CRKP I is 5.1 Mb in size and contains different classes of antimicrobial resistance genes. The isolate is highly resistant to colistin owing to a point mutation in *mgrB* gene, encoding a negative regulator of the PhoP/PhoQ two-component system. Multilocus sequence typing (MLST) showed that *K. pneumoniae* CRKP I belongs to ST78.
Conclusion: These data provide useful information for comparative genomic analysis regarding the dissemination of antimicrobial resistance genes in *K. pneumoniae*. To our knowledge, this is the first report of a MDR *K. pneumoniae* with high colistin resistance belonging to ST78 causing infection in a human.

The rapid emergence and global spread of antimicrobial resistance hamper the effective treatment of infections caused by antimicrobial-resistant bacteria. Hypervirulent strains of colistin-resistant *Klebsiella pneumoniae* have recently emerged and are reported from geographically diverse regions, causing community- and hospital-acquired infections both in healthy individuals and immunocompromised patients [1,2]. Since it is grouped as a 'priority pathogen' by the World Health Organization (WHO) with a critical urgency of need for new antibiotics, antimicrobial resistance in this bacteria brings a dangerous threat to the human race [3].

Klebsiella pneumoniae CRKP I was isolated from a blood sample of a female patient with chronic kidney disease admitted to the nephrology intensive care unit of a tertiary hospital in Kerala, India, in 2018. Following overnight culture at 37 °C in Luria–Bertani agar (HiMedia, Mumbai, India), the isolate was identified as *K. pneumoniae* using standard biochemical tests and was confirmed by 16S rRNA gene sequencing. Antimicrobial susceptibility testing was performed by the disk diffusion method, and colistin resistance was confirmed by broth microdilution. The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2016), and the minimum inhibitory concentration (MIC) breakpoint of colistin was determined according to the guidelines jointly recommended by CLSI-EUCAST (2016) [4,5]. The isolate was resistant to all classes of antibiotics tested and showed intermediate susceptibility to tigecycline, a last-resort antibiotic. The isolate showed high colistin resistance (MIC > 64 μg/mL) by broth microdilution. The high MIC of colistin and multidrug resistance of the isolate prompted us to sequence its genome to understand the antimicrobial resistance determinants.

Total genomic DNA was isolated using a Wizard® Genomic DNA Purification Kit (Promega) according to the manufacturer's protocol. Paired-end sequencing was performed on an Illumina MiSeq platform (Illumina Inc., San Diego, CA) generating 5,864,738

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reads at 140× coverage. Reads were trimmed and assembled de novo using SPAdes v.3.0.0. The sequences were annotated using the Rapid Annotation using Subsystem Technology (RAST) server. The isolate showed a G + C content of 56.7%. The annotation process identified 5869 coding sequences and 117 RNAs.

The sequence type (ST) of the isolate was analysed using the MLST 1.7 (multilocus sequence typing) server (https://cge.cbs.dtu.dk//services/MLST/). MLST analysis showed that the strain belongs to ST78. To our knowledge, this is the first report of a multidrug-resistant (MDR) *K. pneumoniae* with high colistin resistance belonging to ST78 causing infection in a human. Three conjugative plasmids [IncFIB(K), IncFIB(Mar) and IncHI1B] were identified by PlasmidFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/).

Antimicrobial resistance genes of the isolate were analysed using multiple computational tools including ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/) and The Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/). The resistome of CRKP I consists of genes responsible for resistance to aminoglycosides [*aac(6')-Ib*-cr, *armA*, *aadA2*, *aph(3')-V*, *aac(3)-IId* and *baeR*], β-lactams (bla_{NDM-1}, bla_{OXA-1}, bla_{SHV-11}, bla_{SHV-13}, bla_{TEM-1A} and $bla_{CTX-M-15}$), sulphonamides (*sul1*), tetracycline (*tetD*), trimethoprim (*dfrA1* and *drfA2*), fosfomycin (*fosA*), phenicols (*catB3* and *catA1*), macrolides [*msr(E)*, *mph(D)* and *mph(E)*] and fluoroquinolones [*oqxA*, *oqxB*, *aac(6')-Ib*-cr and *qnrB1*]. CARD analysis revealed that the isolate possesses target alterations in the proteins ParC (S80I), PBP3 (S357N and D350N), EF-Tu (R234F), UhpT (E350Q) and GyrA (D87G), conferring resistance to different classes of antibiotics. It was identified that colistin resistance was not plasmid-mediated as the isolate did not carry *mcr-1* or *mcr-2* genes. However, amplification and sequencing of the *mgrB* gene revealed a point mutation (G76A) leading to premature termination of the transmembrane protein MgrB. This truncated protein facilitates activation of lipopolysaccharide modification and thus high colistin resistance in CRKP I. Similar observations have been reported previously [6].

Meticulous analysis revealed that the genome of *K. pneumoniae* CRKP I lacks a functionally active CRISPR-Cas defence system, which may help the isolate to evolve as a MDR pathogen by acquiring a vast variety of antimicrobial resistance genes. The genome also possesses mobile genetic elements that confer high drug resistance and virulence. Stringent genome-wide analysis using multiple computational tools also proved the occurrence of resistance genes to all of the major antibiotics analysed.

In this context, the genome sequence of CRKP I will provide a suitable platform to validate the effect of multiple drug resistance genes and the pattern of co-resistance. It also helps us to understand and mine suitable drug targets to investigate the possibilities of developing new combination therapies that may ultimately lead to successful treatment of infections. The data can be also used to explore the genome-wide distribution of single nucleotide polymorphisms (SNPs) and its phenotypic outcomes and this will provide an insight into polymorphisms associated with altered antimicrobial susceptibility.

GenBank accession nos

The nucleotide sequences of the 16S rRNA gene and the mutated *mgrB* gene have been deposited in the GenBank nucleotide sequence database under the accession nos. **MK640671** and **MK639597**, respectively. The draft genome sequence has been deposited in GenBank under the accession no. **CP037927**.

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Competing interests

None declared.

Ethical approval

Not required.

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