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Genome Note

Hypermucoviscous polymyxin-resistant *Klebsiella pneumoniae* from Kolkata, India: Genomic and phenotypic analysis

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ABSTRACT

Objectives: Here we report the draft genome sequence of a colistin-resistant hypermucoviscous *Klebsiella pneumoniae* isolated from a hospitalised patient with acute kidney injury in Kolkata, India.
Methods: Whole genomic DNA was sequenced using an Illumina HiSeq platform. The generated reads were de novo assembled using SPAdes v.3.7.1. Genome annotation was performed using the NCBI Prokaryote Genome Annotation Pipeline (PGAP) v.4.6. The sequence type (ST), capsular type, antimicrobial resistance and virulence-related genes were predicted from the genome sequence.
Results: Klebsiella pneumoniae KP26 belonged to ST147. The assembly comprised 63 contigs (>1000 bp) with a total read length of 5 560 935 bp and a total of 5399 coding sequences. The isolate was resistant to most β-lactams, aminoglycosides, quinolones, fosfomycin, trimethoprim, sulphonamides and polymyxins. No *mcr* genes were detected in the genome.
Conclusion: Isolate KP26 is a hypermucoviscous, multidrug-resistant *K. pneumoniae* strain that may represent an emerging high-risk clone associated with severe infections in India.

1. Introduction

Hypervirulent (HV) strains of *Klebsiella pneumoniae* are often associated with a hypermucoviscous (HM) phenotype, which is determined by the 'string test' for isolates grown on agar plates. These strains are associated with serious infections and typically express capsular serotypes K1 or K2. Here we report a HM *K. pneumoniae* isolate predicted to express the capsular K54 serotype.

HM *K. pneumoniae* strain KP26 was recovered from the urine of a 58-year-old renal transplant patient admitted to hospital with sepsis and acute kidney injury. Initial urine cultures yielded colistin-resistant HM *Klebsiella*. The patient was treated with meropenem. Further blood and urine cultures were negative during the patient's hospital stay. The patient gradually developed multiorgan failure and subsequently died despite treatment with meropenem and teicoplanin (discontinued on Day 7). In addition, minocycline, fosfomycin and, eventually, tigecycline and echinocandins were administered for empirical management of sepsis. The patient did not receive any treatment with polymyxin B or colistin. This isolate was the only clinically significant culture result for the patient during his hospital stay.

2. Methods

The isolate was identified as *K. pneumoniae* using the VITEK 2 system. And the sensitivities performed using the AST-N280 panel. Colistin and polymyxin B MICs were determined by broth microtitre dilution. Sequencing was performed on an Illumina HiSeq platform (Illumina Inc., San Diego, CA) and the closest reference genome was identified using Kraken (https://ccb.jhu.edu/software/kraken/). The reads were mapped to the reference genome using Burrows–Wheeler aligner 'mem' (BWA-mem) algorithm v.2. De novo assembly of the reads was performed using SPAdes v.3.7.1, and the reads were again mapped back to the resultant contigs using BWA-mem. Annotation was performed using the NCBI Prokaryote Genome Annotation Pipeline (PGAP) v.4.6.

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Antimicrobial resistance genes were predicted using ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/); the capsular serotype was predicted using Kaptive (http://kaptive.holtlab.net); virulence genes were predicted using VFanalyzer for *K. pneumoniae* (http://www.mgc.ac.cn/VFs/main.htm); and the sequence type (ST) was determined using MLST 2.0 (multilocus sequence typing) (https://cge.cbs.dtu.dk/services/MLST/).

3. Results

The genome assembly comprised 63 contigs (>1000 bp) with a total read length of 5 560 935 bp, a GC content of 56.97%, an N_{50} value of 247 870 bp, and a total of 5399 coding sequences. The isolate was identified as ST147. Capsular and lipopolysaccharide serotypes were predicted to be KL54 and O2v1, respectively.

Strain KP26 was extensively drug-resistant and harboured genes encoding resistance to most β-lactam and β-lactamase inhibitor combinations (bla_{TEM-1B}, $bla_{CTX-M-15}$ and bla_{SHV-67}), aminoglycosides [*rmtF*, *aac(6')-Ib3*, *aph(3'')-Ib* and *aph(6)-Id*], quinolones [*qnrB1*, *aac(6')-Ib*-cr, *oqxA* and *oqxB*], fosfomycin (*fosA*), trimethoprim (*dfrA12*) and sulphonamides (*sul2*). The strain was susceptible to imipenem and meropenem but not to ertapenem (MIC = 4 μg/mL). The isolate showed polymyxin B and colistin MICs of 8 μg/mL. No *mcr* genes were detected but the isolate carried a known colistin-resistant D150 G substitution in PhoQ [1].

Despite displaying the HM phenotype (viscous string >5 mm), the genome sequence lacked many genes thought to be important for the HV phenotype, notably *rmpA*/*rmpA2* [2] and *iucABCD* genes for aerobactin synthesis [3]. However, the isolate was shown to be highly virulent in a *Galleria mellonella* model of *K. pneumoniae* infection [50% lethal dose (LD_{50}) = 10^2 CFU/larvae] (Fig. 1). The isolate was predicted to contain an alternative siderophore locus: yersiniabactin (*fyuA*/*irp12*/*ybtAEPQSTUX*).

4. Discussion

Klebsiella pneumoniae KP26 further highlights whether HM and HV phenotypes are synonymous [2]. This isolate lacked many genes described as important for pathogenicity in HM/HV isolates, most notably the *rmpA*/*rmpA2* genes. This corroborates what other studies have found in HM isolates from India. Shankar et al. examined a collection of 27 string-positive isolates and found that 0% and 11% were positive by PCR for the *rmpA* and *rmpA2* genes, respectively [4].

Isolate KP26 tested non-susceptible to ertapenem but did not harbour traditional carbapenem-hydrolysing enzymes. Analysis of the genome sequence identified a lesion (an IS*1380*-family transposase) in the outer membrane porin (OMP) gene *ompK*35. These OMP defects are known to impart ertapenem resistance due to impaired drug uptake, particularly when combined with extended-spectrum β-lactamases such as CTX-M-15 [5].

To the best of our knowledge, this is the first fully sequenced genome of a multidrug-resistant, HM, ST147 *K. pneumoniae* isolate.

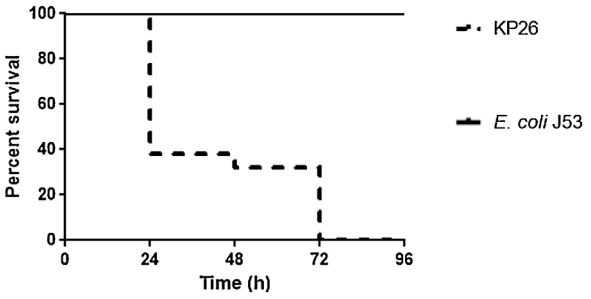

Fig. 1. Percent survival of *Galleria mellonella* inoculated with 10^2 CFU of *Klebsiella pneumoniae* KP26 or *Escherichia coli* J53. Each experiment was performed in triplicate with 10 larvae.

This may represent an emerging high-risk clone associated with severe infections in India that warrants enhanced surveillance.

5. Nucleotide sequence accession no

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. REFT00000000. The version described in this paper is version REFT01000000.

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Competing interests

None declared.

Ethical approval

Not required.

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