

Draft genome sequence of carbapenem-resistant *Klebsiella pneumoniae* ST6260 isolated from the catheter tip of a female patient in Nepal

Suchitra Thapa,¹ Basudha Shrestha,² Dev Raj Joshi,³ Reshma Tuladhar,³ Maria Getino,⁴ Manisha Shrestha,³ Yojana Pokhrel,³ Elita Jauneikaite⁴

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT *Klebsiella pneumoniae* is an opportunistic human pathogen, particularly associated with nosocomial infections and multidrug resistance. Here, we present a draft genome sequence of a carbapenem-resistant *K. pneumoniae* ST6260 isolated from the catheter tip of a female patient in a referral case received at Kathmandu Model Hospital, Kathmandu, Nepal.

KEYWORDS antibiotic resistance, *Klebsiella*

Klebsiella pneumoniae was isolated from the catheter tip of a 27-year-old female. A 5 cm distal tip was received in a sterile container at the hospital (N27°42'9.1656", E85°19'12.5832"), cultured on MacConkey agar (HiMedia, India) plate using the rolled plate method (1), and then incubated at 37°C for 24 hours. A single colony from the plate was grown in Nutrient broth (HiMedia, India) at 37°C for 4 hours and turbidity matched with 0.5 McFarland for antibiotic susceptibility testing. Antibiotic susceptibility was detected by the disc diffusion method, and carbapenemase production was confirmed by the modified carbapenem inactivation method, as described by CLSI guidelines (2). The isolate was preserved in Tryptic Soy Broth (HiMedia, India) (with 20% glycerol) at –80°C. Later, it was revived onto a Nutrient agar (HiMedia, India) plate for DNA extraction. A single colony from the plate was transferred into Luria-Bertani (LB) (HiMedia, India) broth for overnight incubation at 37°C. The DNA was extracted from LB broth using the CTAB method (3), and quality was determined through 260/280 nm absorbance measures using a NanoDrop spectrophotometer 1000 (Thermo Scientific, USA). Then, the genomic DNA was sent to MicrobesNG for whole-genome sequencing.

Genomic DNA libraries were prepared using the Nextera XT Library Prep Kit (Illumina, USA) following the manufacturer's protocol with the following modifications: input DNA was increased twofold, and PCR elongation time was increased to 45 seconds. DNA quantification and library preparation were carried out on a Hamilton Microlab STAR automated liquid handling system (Hamilton Bonaduz AG, Switzerland). Libraries were sequenced on an Illumina NovaSeq 6000 (Illumina, USA) using a 250 bp paired end protocol (~890,000 reads per pair, average coverage 70×). Raw reads were checked using FastQC version 0.11.9 (4). Trimmomatic version 0.39 (5) was used to trim reads with the following parameters: LEADING:20 TRAILING:20 SLIDING WINDOW:5:30 MINLEN:50. Species identification was done with Kraken2 version 2.1.43 (6) and Bracken version 2.9 (7). *De novo* assembly was performed using SPAdes version 3.15.2 (8) with careful mode activated and selecting k-mers up to 127. Bioawk version 1.0 (9) was then used to filter out contigs with size <200 bp and SPAdes coverage <2. Assembly statistics were assessed using QUAST version 5.0.2 (10) and CheckM2 version 1.2.3 (11). PGAP version 6.9 (12) was used for annotation. Kleborate version 2.5 (13) was used to report MLST (13)

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Address correspondence to Suchitra Thapa, suchitra.thapa@ac.tu.edu.np.

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TABLE 1 Summary of AMR determinants identified in the *K. pneumoniae* ST6260 genome from Nepal^a

	Size (bp)	No. of contigs	AMR determinants
Chromosome	5,318,348	91	<i>blaSHV-1</i> , <i>dfrA12</i> , <i>aadA2</i> , <i>fosA</i> , <i>oqxA</i> , <i>oqxB</i> ; GyrA-83Y, GyrA-87G, ParC-80I
Plasmid 1 (IncFIB, IncFII, IncR; mobilizable)	148,151	18	<i>blaTEM-1A</i> , <i>blaOXA-9</i> , <i>ant(3'')-Ia</i> , <i>aac(6'')-Ib</i> , <i>sul1</i> , <i>blaNDM-5</i> , <i>erm(B)</i> , <i>mph(A)</i> , <i>rmtB</i>
Plasmid 2 (IncFIB; non-mobilizable)	43,101	2	– ^b
Plasmid 3 (no rep; mobilizable)	23,345	2	–
Plasmid 4 (no rep; non-mobilizable)	19,072	1	–

^aIn total, seven plasmids were reported by MOB-suite analysis; only plasmids larger than 10 kbp have been summarized in the table.

^b– indicates not found.

(BIGSdb database), virulence loci, AMR genes, and K and O locus via Kaptive version 2.0.4 (14). Plasmid reconstruction was performed using MOB-suite version 3.1.8 (15). Default parameters were used except where otherwise stated.

Draft genome assembly stats were as follows: 5,568,701 bp, 117 contigs, 57% GC content, and *N*₅₀ of 187,282 bp. The draft genome contains a total of 5,585 genes, of which 5,321 were coding DNA sequences, 79 tRNA, 27 rRNA (6 complete and 21 partial), and 149 pseudogenes. *K. pneumoniae* genotype was ST6260. The predicted capsule type was K17 (locus K17) and O type O1 (locus O1/O2v1). Kleborate predicted a virulence score of 1 with yersiniabactin *ybt9* and truncated ICEKp3, and a resistance score of 2, indicating that carbapenemase without colistin resistance was detected, regardless of ESBL genes or OmpK mutations. AMR determinants and plasmids identified are summarized in Table 1.

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S.T. conceptualized and validated the study, drafted the manuscript, and reviewed and edited the manuscript; M.G., M.S., and Y.P. contributed to lab investigation, review, and editing; S.T. and E.J. acquired funding and curated the data; D.R.J., B.S., R.T., and E.J. supervised the study and reviewed and edited the manuscript.

AUTHOR AFFILIATIONS

¹Department of Microbiology, Tribhuvan University, Kirtipur, Central Development Region, Nepal

²Department of Microbiology, Kathmandu Model Hospital, Kathmandu, Nepal

³Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal

⁴NIHR Health Protection Research Unit in Healthcare Associated Infections and Antimicrobial Resistance, Department of Infectious Disease, Imperial College London, London, United Kingdom

AUTHOR ORCIDS

Suchitra Thapa  <http://orcid.org/0000-0001-5995-1909>

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Suchitra Thapa, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing | Basudha Shrestha, Supervision, Validation, Writing – review and editing | Reshma Tuladhar, Supervision, Validation, Writing – review and editing | Maria Getino, Data curation, Formal analysis, Resources, Software, Validation, Writing – review and editing | Manisha Shrestha, Investigation, Methodology, Writing – review and editing | Yojana Pokhrel, Investigation, Methodology, Writing – review and editing | Elita Jauneikaite, Funding acquisition, Resources, Software, Supervision, Validation, Writing – review and editing.

DATA AVAILABILITY

The raw reads for *K. pneumoniae* isolate are submitted to European Nucleotide Archive under sample accession number SAMEA115926202, <https://www.ebi.ac.uk/ena/browser/view/SAMEA115926202>, while draft assembly has been deposited in GenBank under the BioProject accession number PRJEB78979, <https://www.ncbi.nlm.nih.gov/bioproject/1155534>.

ETHICS APPROVAL

Ethical approval was obtained from the Nepal Health Research Council (Approval ID: 378/2021).

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