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# Draft Genome Sequences of the Clinical Isolates Kp 23 and KPM 20

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**ABSTRACT** *Klebsiella pneumoniae* strains are capable of becoming resistant through multiple mechanisms. Here, we announce draft sequences for Kp 23, a clinical isolate with no plasmid-encoded $\beta$-lactamases, and KPM 20, a clinical isolate with no plasmid-encoded $\beta$-lactamases and no detectable OmpK35, OmpK36, or PhoE in the outer membrane.

*K*lebsiella pneumoniae confers multidrug resistance through the loss of the outer-membrane porins OmpK35, OmpK36, and PhoE (1–3). However, many of these isolates retain intact structural genes that encode full-length porin proteins (4, 5). While an important factor in multidrug resistance, the exact contribution of porin loss when combined with other resistance mechanisms is unknown. This genome announcement describes the whole-genome sequences of Kp 23, a reference strain in our laboratory collected in 1973, and KPM 20, a cephalosporin-susceptible strain lacking OmpK35, OmpK36, and PhoE in the outer membrane.

Cultures were grown in Mueller-Hinton broth (MHB) at 37°C with shaking. DNA was extracted with the Qiagen MagAttract kit following manufacturer instructions. DNA was quantified using Qubit with the high-sensitivity (HS) DNA assay. Libraries were prepared using the Illumina Nextera Flex DNA kit with 250 ng DNA, optimized for 300-bp paired-end reads (6). Sequencing was performed on the Illumina MiSeq platform. Low-quality reads were filtered and Nextera adapter sequences removed using TrimmomaticPE version 0.36 (7).

The genomes were assembled as previously described by Harrison and Hanson (8, 9). Default parameters were used for all software unless otherwise specified. Initial contig libraries were created using SPAdes version 3.14.1 with the parameters "-k 21, 33, 55, 77, 88, 127," "-careful," and "-cov-cutoff 5" (10) and SGA version 0.10.15 with default parameters and "-pe-mode" set to 1 (11). These assemblies were merged using SPAdes and ordered with Ragout version 2.0 (12) using reference genomes (Kp 23, GenBank accession numbers CP021165.1, CP020071.1, CP018671.1, and CP025639.1; KPM 20, CP061700.1, CP056216.1, and CP024542.1). The contigs were joined using AlignGraph version 1.0 with the "distanceLow" and "distanceHigh" parameters set to 300 and 1,602, respectively (13). Using this assembly as a scaffold and the initial trimmed sequence files, a final reference-guided genome assembly was generated using SPAdes. Contigs less than 500 bases were filtered. The genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline version 5.0 and further assessed using PlasmidFinder version 4.1 (14) and ResFinder version 2.1 (15).

The assembly statistics for Kp 23 and KPM 20 are listed in Table 1. Upon annotation, the Kp 23 genome was found to contain 5,518 genes encoding 5,293 protein-coding sequences, 120 pseudogenes, 79 tRNAs, 12 rRNAs, and 14 noncoding RNAs (ncRNAs). The plasmid sequences were consistent with IncFIB(K), IncFII(K), and IncFII(pKP91). The resistance genes were *bla*$_{SHV-40}$, *fosA*, *oqxA*, and *oqxB*. Annotation of the KPM 20

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**TABLE 1** Assembly statistics for Kp 23 and KPM 20

| Isolate | No. of reads | Genome size (bp) | No. of contigs | G+C content (%) | $N_{75}$ (bp) | $N_{50}$ (bp) | $L_{75}$ | $L_{50}$ | Accession no. |
|---------|-------------|------------------|----------------|-----------------|---------------|---------------|----------|----------|----------------|
| Kp 23 | 4,459,218 | 5,596,095 | 58 | 57.2 | 168,132 | 361,162 | 11 | 5 | JAEVHE000000000 |
| KPM 20 | 4,451,906 | 5,562,045 | 119 | 57.08 | 64,450 | 134,417 | 25 | 11 | JAEVHD000000000 |

assembly showed 5,540 genes, with 5,257 protein-coding sequences, 183 pseudogenes, 78 tRNAs, 11 rRNAs, and 11 ncRNAs. The plasmid sequences Col(MG828), Col (pHAD28), and IncFIB(K)(pCAV1099-114) were identified. The resistance genes $bla_{SHV-56}$, $fosA$, $oqxA$, and $oqxB$ were detected.

*K. pneumoniae* resistance can emerge through the loss of outer membrane porins. Here, we provide two genome sequences with similar acquired resistance genes. Kp 23 serves as a useful reference for genome assemblies and as a comparator strain for further research. KPM 20 provides the genome sequence of a porin-deficient strain to examine the role of porins in antimicrobial resistance.

**Data availability.** The whole-genome shotgun assemblies have been deposited at DDBJ/ENA/GenBank under the BioSample accession numbers JAEVHE000000000 and JAEVHD000000000 for Kp 23 and KPM 20, respectively. The versions described in this paper are JAEVHE010000000 and JAEVHD010000000, respectively. The raw data can be accessed under the SRA accession numbers SRX9980334 and SRX9980335 for Kp 23 and KPM 20, respectively.

## REFERENCES

1. Doménech-Sánchez A, Martínez-Martínez L, Hernández-Allés S, del Carmen Conejo M, Pascual Á, Tomás JM, Albertí S, Benedí VJ. 2003. Role of *Klebsiella pneumoniae* OmpK35 porin in antimicrobial resistance. Antimicrob Agents Chemother 47:3332–3335. https://doi.org/10.1128/aac.47 .10.3332-3335.2003.

2. Kaczmarek FM, Dib-Hajj F, Shang W, Gootz TD. 2006. High-level carbapenem resistance in a *Klebsiella pneumoniae* clinical isolate is due to the combination of $bla_{ACT-1}$ β-lactamase production, porin OmpK35/36 insertional inactivation, and down-regulation of the phosphate transport porin PhoE. Antimicrob Agents Chemother 50:3396–3406. https://doi .org/10.1128/AAC.00285-06.

3. Albertí S, Rodríquez-Quiñones F, Schirmer T, Rummel G, Tomás JM, Rosenbusch JP, Benedí VJ. 1995. A porin from *Klebsiella pneumoniae*: sequence homology, three-dimensional model, and complement binding. Infect Immun 63:903–910. https://doi.org/10.1128/IAI.63.3.903-910.1995.

4. Wong JLC, Romano M, Kerry LE, Kwong H-S, Low W-W, Brett SJ, Clements A, Beis K, Frankel G. 2019. OmpK36-mediated carbapenem resistance attenuates ST258 Klebsiella pneumoniae in vivo. Nat Commun 10:3957. https://doi.org/10.1038/s41467-019-11756-y.

5. Doumith M, Ellington MJ, Livermore DM, Woodford N. 2009. Molecular mechanisms disrupting porin expression in ertapenem-resistant *Klebsiella* and *Enterobacter* spp. clinical isolates from the UK. J Antimicrob Chemother 63:659–667. https://doi.org/10.1093/jac/dkp029.

6. Illumina. 2018. Nextera XT DNA library prep reference guide. Document no. 15031942 v03. https://support.illumina.com/downloads/nextera_xt_ sample_preparation_guide_15031942.html.

7. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10 .1093/bioinformatics/btu170.

8. Harrison LB, Selmecki A, Hanson ND. 2019. Draft genome assemblies of clinical isolates of *Klebsiella pneumoniae* V901162 and *Enterobacter hormaechei*

Entb306. Microbiol Resour Announc 8:e00091-19. https://doi.org/10.1128/ MRA.00091-19.

9. Harrison LB, Hanson ND. 2017. Draft genome sequence of the mucoid *Pseudomonas aeruginosa* clinical isolate PA34. Genome Announc 5: e01307-17. https://doi.org/10.1128/genomeA.01307-17.

10. Prjibelski A, Antipov D, Meleshko D, Lapidus A, Korobeynikov A. 2020. Using SPAdes de novo assembler. Curr Protoc Bioinformatics 70:e102. https://doi.org/10.1002/cpbi.102.

11. Simpson JT, Durbin R. 2012. Efficient de novo assembly of large genomes using compressed data structures. Genome Res 22:549–556. https://doi .org/10.1101/gr.126953.111.

12. Kolmogorov M, Raney B, Paten B, Pham S. 2014. Ragout—a reference-assisted assembly tool for bacterial genomes. Bioinformatics 30:i302–i309. https://doi.org/10.1093/bioinformatics/btu280.

13. Bao E, Jiang T, Girke T. 2014. AlignGraph: algorithm for secondary de novo genome assembly guided by closely related references. Bioinformatics 30:i319–i328. https://doi.org/10.1093/bioinformatics/btu291.

14. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/ AAC.02412-14.

15. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykäsenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. 2020. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother 75:3491–3500. https://doi.org/10.1093/jac/dkaa345.