



Complete Genome Sequence of *Klebsiella quasipneumoniae* subsp. *similipneumoniae* Strain IF3SW-P1, Isolated from the International Space Station

 Natasha S. Sushenko,^{a,b}  Nitin K. Singh,^c  Daniel L. Vellone,^d  Scott W. Tighe,^d  Brian P. Hedlund,^{b,e}
 Kasthuri Venkateswaran,^c  Duane P. Moser^a

^aDepartment of Hydrologic Sciences, Desert Research Institute, Las Vegas, Nevada, USA

^bSchool of Life Sciences, University of Nevada Las Vegas, Las Vegas, Nevada, USA

^cJet Propulsion Laboratory, California Institute of Technology, Pasadena, California, USA

^dVermont Integrative Genomics Resource, University of Vermont, Burlington, Vermont, USA

^eNevada Institute of Personalized Medicine, University of Nevada Las Vegas, Las Vegas, Nevada, USA

ABSTRACT The 5.2-Mb circular genome of *Klebsiella quasipneumoniae* subsp. *similipneumoniae* strain IF3SW-P1, isolated from the International Space Station, was sequenced using Oxford Nanopore Technologies. The genome lacks a megaplasmid typical of hypervirulent and multidrug-resistant *Klebsiella* strains but does contain a chromosomally encoded OqxAB efflux pump associated with carbapenem resistance.

In 2014, two phylogroups of the opportunistic pathogen *Klebsiella pneumoniae* were described as the novel species *Klebsiella quasipneumoniae* (1). Since its definition as a species, *K. quasipneumoniae* has emerged as an understudied human pathogen with hypervirulent, multidrug-resistant (MDR), carbapenem-resistant, and hypermucoviscous strains isolated from both hospital-borne and community-acquired infections (2–5). Considering its prevalence on the International Space Station (ISS) (6), the newly recognized pathogenicity of *K. quasipneumoniae* increases concerns about the consequences of this species being exposed to the stresses of spaceflight, which are known to trigger bacterial virulence and antimicrobial resistance (7–10).

Strain IF3SW-P1 was isolated from the surface of the foot panel of the Advanced Resistive Exercise Device (ARED) on the ISS on 4 March 2015 (11) using a standard spread plate method on Reasoner's 2A (R2A) agar and archived in glycerol cryostocks (6). For this study, strain IF3SW-P1 was subcultured from cryostock and grown to late exponential phase in Trypticase soy broth (TSB) at 37°C; genomic DNA was then extracted using the DOE Joint Genome Institute bacterial genomic DNA isolation protocol (12).

Oxford Nanopore Technologies sequencing was performed using a GridION MK1 sequencer on a R10.4 flow cell (FLO-MIN112) with a library synthesized from Q20+ EA (early access) ligation reagents (SQK-LSK112-XL). The raw reads were base called using MinKNOW v29.10.8, with a mean quality score of 16.3 and a mode of 18.03. The genome was assembled, circularized, and polished using Flye v2.9 with the parameters *-nano-hq* and *-read-error 0.03* for the Q20+ data (13). The Flye-generated assembly contains two contigs, one 5.2-Mb circular chromosome and one 3-kb linear fragment confirmed via BLASTN v2.12.0 to be 99.8% identical to *Escherichia coli* strain Q4552 plasmid pECQ4552_IHU08 (GenBank accession number CP077071.1) (14). Notably, the genome does not encode any virulence- or drug resistance-associated plasmids, such as *bla*KPC and *Inc*(FII), which are known to occur in *Klebsiella* species (15).

The genome was identified as *K. quasipneumoniae* subsp. *similipneumoniae* by calculating the average nucleotide identity (ANI) using the EzBioCloud calculator compared to the two subspecies' type strains, *K. quasipneumoniae* subsp. *quasipneumoniae* 01A030^T (ANI, 96.63%) and *K. quasipneumoniae* subsp. *similipneumoniae* 07A044^T (ANI, 99.03%) (16). Strain IF3SW-P1

Editor Steven R. Gill, University of Rochester School of Medicine and Dentistry

Copyright © 2022 Sushenko et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Duane P. Moser, duane.moser@dri.edu.

The authors declare no conflict of interest.

Received 19 May 2022

Accepted 9 June 2022

Published 23 June 2022

TABLE 1 Assembly and annotation information

Characteristic	Data
Strain name	IF3SW-P1
ISS sampling date	4 March 2015
Location	ARED foot panel
Nearest species	<i>K. quasipneumoniae</i> subsp. <i>similipneumoniae</i> 07A044 ^T
ANI (%)	99.03
No. of raw reads	364,351,976
Genome size (bp)	5,238,176
<i>N</i> ₅₀ (bp)	5,238,176
No. of contigs	2 (1 chromosomal, 1 linear fragment)
Median coverage (×)	62
G+C content (%)	58.06
No. of coding sequences	4,998
GenBank accession no.	CP092121
SRA accession no.	SRR17974437
GeneLab accession no.	GLDS-470

is also related to but distinct from eight previously published draft genomes of *K. quasipneumoniae* strains isolated from the ISS, with >99% ANI for all (6).

The assembly was annotated using RASTtk v1.3.0 (17) as part of the Pathosystems Resource Integration Center (PATRIC) v3.6.12 (18). Predicted virulence genes on the chromosome include *iutA*, which encodes a ferric aerobactin receptor, although the gene encoding the associated siderophore aerobactin (*iucA*) is not present (19). The IF3SW-P1 genome also contains genes for the multidrug resistance efflux pump OqxAB, associated with carbapenem resistance in *K. pneumoniae* (20, 21). OqxAB is reported to be associated with resistance to benzalkonium chloride, a quaternary ammonium compound used as a disinfectant on the ISS (11). Default parameters were used for all software unless otherwise specified. Additional assembly and annotation information is listed in Table 1.

Data availability. The genomic assembly and raw reads have been deposited at GenBank (accession number CP092121) and the Sequence Read Archive (SRR17974437). These data are also available at NASA GeneLab (GLDS-470).

ACKNOWLEDGMENTS

We thank astronaut Terry Virts for collecting samples aboard the ISS, Aleksandra Checinska-Sielaff for isolating the strain, and the implementation team at NASA Ames Research Center (Fathi Karouia) for coordinating the sampling effort.

Part of this research was performed at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with NASA. This work was supported through 2012 Space Biology (NNH12ZTT001N) grant number 19-12829-26 under task order NNN13D111T awarded to K.V.; by NASA EPSCoR Rapid Response Research Cooperative Agreement (NNH18ZHA005C) award number 80NSSC19M0169 to D.P.M.; and by project MANGO, a 2018 Space Biology (NNH16ZTT001N) grant under task order 80NM0018F0589. Additional support was provided through the Nevada Space Grant Consortium Graduate Research Opportunity Fellowship award (number 13584) to N.S.S. and the UNLV MSI Open Article Fund.

REFERENCES

1. Brisse S, Passet V, Grimont PA. 2014. Description of *Klebsiella quasipneumoniae* sp. nov., isolated from human infections, with two subspecies, *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* subsp. nov. and *Klebsiella quasipneumoniae* subsp. *similipneumoniae* subsp. nov., and demonstration that *Klebsiella singaporensis* is a junior heterotypic synonym of *Klebsiella varicicola*. *Int J Syst Evol Microbiol* 64:3146–3152. <https://doi.org/10.1099/ijms.0.062737-0>.
2. Arena F, De Angelis LH, Pieralli F, Di Pilato V, Giani T, Torricelli F, D'Andrea MM, Rossolini GM. 2015. Draft genome sequence of the first hypermucoviscous *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* isolate from a bloodstream infection. *Genome Announc* 3:e00952-15. <https://doi.org/10.1128/genomeA.00952-15>.
3. Breurec S, Melot B, Hoen B, Passet V, Schepers K, Bastian S, Brisse S. 2016. Liver abscess caused by infection with community-acquired *Klebsiella quasipneumoniae* subsp. *quasipneumoniae*. *Emerg Infect Dis* 22:529–531. <https://doi.org/10.3201/eid2203.151466>.
4. Gan HM, Rajasekaram G, Eng WW, Kaniappan P, Dhanoa A. 2017. Whole-genome sequences of two carbapenem-resistant *Klebsiella quasipneumoniae* strains isolated from a tertiary hospital in Johor, Malaysia. *Genome Announc* 5:e00768-17. <https://doi.org/10.1128/genomeA.00768-17>.
5. Arabaghian H, Salloum T, Alousi S, Panossian B, Araj GF, Tokajian S. 2019. Molecular characterization of carbapenem resistant *Klebsiella pneumoniae* and *Klebsiella quasipneumoniae* isolated from Lebanon. *Sci Rep* 9:531. <https://doi.org/10.1038/s41598-018-36554-2>.

6. Solomon SA, Bharadwaj AR, Singh NK, Wood JM, Debieu M, O'Hara NB, Mason CE, Venkateswaran K. 2020. Draft genome sequences of *Klebsiella* species isolated from the International Space Station. *Microbiol Resour Announc* 9:e00923-20. <https://doi.org/10.1128/MRA.00923-20>.
7. Taylor PW. 2015. Impact of space flight on bacterial virulence and antibiotic susceptibility. *Infect Drug Resist* 8:249–262. <https://doi.org/10.2147/IDR.S67275>.
8. Klaus DM, Howard HN. 2006. Antibiotic efficacy and microbial virulence during space flight. *Trends Biotechnol* 24:131–136. <https://doi.org/10.1016/j.tibtech.2006.01.008>.
9. Crucian B, Sams C. 2009. Immune system dysregulation during space-flight: clinical risk for exploration-class missions. *J Leukoc Biol* 86:1017–1018. <https://doi.org/10.1189/jlb.0709500>.
10. Crucian B, Babiak-Vazquez A, Johnston S, Pierson DL, Ott CM, Sams C. 2016. Incidence of clinical symptoms during long-duration orbital space-flight. *Int J Gen Med* 9:383–391. <https://doi.org/10.2147/IJGM.S114188>.
11. Checinska Sielaff A, Urbaniak C, Malli Mohan GB, Stepanov VG, Tran Q, Wood JM, Minich J, McDonald D, Mayer T, Knight R, Karouia F, Fox GE, Venkateswaran K. 2019. Characterization of the total and viable bacterial and fungal communities associated with the International Space Station surfaces. *Microbiome* 7:50. <https://doi.org/10.1186/s40168-019-0666-x>.
12. Department of Energy Joint Genome Institute. 2012. Bacterial genomic DNA isolation using CTAB. <https://jgi.doe.gov/wp-content/uploads/2014/02/JGI-Bacterial-DNA-isolation-CTAB-Protocol-2012.pdf>.
13. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long error-prone reads using repeat graphs. *Nat Biotechnol* 37:540–546. <https://doi.org/10.1038/s41587-019-0072-8>.
14. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. *BMC Bioinformatics* 10:421. <https://doi.org/10.1186/1471-2105-10-421>.
15. Nicolás MF, Ramos PIP, Marques de Carvalho F, Camargo DRA, de Fátima Morais Alves C, Loss de Morais G, Almeida LGP, Souza RC, Ciapina LP, Vicente ACP, Coimbra RS, Ribeiro de Vasconcelos AT. 2018. Comparative genomic analysis of a clinical isolate of *Klebsiella quasipneumoniae* subsp. *similipneumoniae*, a KPC-2 and OKP-B-6 beta-lactamases producer harboring two drug-resistance plasmids from southeast Brazil. *Front Microbiol* 9:220. <https://doi.org/10.3389/fmicb.2018.00220>.
16. Yoon S-H, Ha S-M, Lim J, Kwon S, Chun J. 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van Leeuwenhoek* 110:1281–1286. <https://doi.org/10.1007/s10482-017-0844-4>.
17. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, 3rd, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci Rep* 5:8365. <https://doi.org/10.1038/srep08365>.
18. Davis JJ, Wattam AR, Aziz RK, Brettin T, Butler R, Butler RM, Chlenski P, Conrad N, Dickerman A, Dietrich EM, Gabbard JL, Gerdes S, Guard A, Kenyon RW, Machi D, Mao C, Murphy-Olson D, Nguyen M, Nordberg EK, Olsen GJ, Olson RD, Overbeek JC, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomas C, VanOeffelen M, Vonstein V, Warren AS, Xia F, Xie D, Yoo H, Stevens R. 2020. The PATRIC Bioinformatics Resource Center: expanding data and analysis capabilities. *Nucleic Acids Res* 48:D606–D612. <https://doi.org/10.1093/nar/gkz943>.
19. Russo TA, Olson R, MacDonald U, Metzger D, Maltese LM, Drake EJ, Gulick AM. 2014. Aerobactin mediates virulence and accounts for increased siderophore production under iron-limiting conditions by hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*. *Infect Immun* 82:2356–2367. <https://doi.org/10.1128/IAI.01667-13>.
20. Klontz EH, Tomich AD, Günther S, Lemkul JA, Deredge D, Silverstein Z, Shaw JF, McElheny C, Doi Y, Wintrodde PL, MacKerrell AD, Jr, Sluis-Cremer N, Sundberg EJ. 2017. Structure and dynamics of FosA-mediated fosfomycin resistance in *Klebsiella pneumoniae* and *Escherichia coli*. *Antimicrob Agents Chemother* 61:e01572-17. <https://doi.org/10.1128/AAC.01572-17>.
21. Rodriguez-Martinez JM, Diaz de Alba P, Briales A, Machuca J, Lossa M, Fernandez-Cuenca F, Rodriguez Bano J, Martinez-Martinez L, Pascual A. 2013. Contribution of OqxAB efflux pumps to quinolone resistance in extended-spectrum- β -lactamase-producing *Klebsiella pneumoniae*. *J Antimicrob Chemother* 68:68–73. <https://doi.org/10.1093/jac/dks377>.