



High-Quality Draft Genome Sequence of *Pseudomonas aeruginosa* 268 Isolated from a Patient with a Left Ventricular Assist Device

Logan J. Voegtly,^{a,b} Gregory K. Rice,^{a,b} Regina Z. Cer,^{a,b} Kenneth G. Frey,^a Biswajit Biswas,^a Theron Hamilton,^{a*} Saima Aslam,^c Kimberly A. Bishop-Lilly^a

^aGenomics & Bioinformatics Department, Biological Defense Research Directorate, Naval Medical Research Center, Fort Detrick, Maryland, USA

^bLeidos, Reston, Virginia, USA

^cDivision of Infectious Diseases and Global Public Health, School of Medicine, University of California, San Diego, California, USA

ABSTRACT *Pseudomonas aeruginosa* is known to cause persistent bloodstream infections associated with left ventricular assist devices (LVAD). Here, we present the high-quality draft genome assembly for a clinical isolate, *P. aeruginosa* 268. The genome sequence is available in GenBank under accession number [CP032761](#).

Pseudomonas aeruginosa is an ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) pathogen that exhibits intrinsic, acquired, and adaptive mechanisms of antibiotic resistance (1) and can cause persistent bloodstream infections associated with left ventricular assist devices (LVAD) (2). In one recent study, 22.6% of patients with LVAD implantation presented with infectious complications, among which almost a third were caused by *P. aeruginosa* (3). We present here the high-quality draft genome sequence of a *P. aeruginosa* isolate from a patient with a LVAD.

The isolate was cultured in tryptic soy broth overnight at 37°C. Genomic DNA was extracted with the Wizard genomic DNA kit (Promega, Madison, WI), and shotgun sequencing libraries were produced with the Nextera XT DNA library preparation kit (Illumina, Inc., San Diego, CA). Nextera mate-pair libraries were constructed from 1 μg of genomic DNA following the gel-free protocol. The mate-pair libraries were quantitated using NEBNext qPCR (NEB, Ipswich, MA). In the case of both shotgun and mate-pair sequencing, equimolar quantities of library were multiplexed and sequenced on the Illumina MiSeq platform using 2 × 300 v3 chemistry.

Illumina shotgun paired reads were processed with Sickle (4) using Phred at 30 or higher and a length of at least 50 bp and down-sampled to 100× coverage using bbnorm (5). Mate-pair sequencing reads were processed with NxTrim (6) (Table 1). Both mate-paired and shotgun paired-end libraries were *de novo* assembled with SPAdes 3.11.1 (7), using the “—careful” argument. Only true mate pairs were included, using the “—mp” argument. The assembly resulted in 27 contigs (N_{50} , 675,323 bp). This initial assembly was then manually closed to one contig with Bandage (8), Mauve (9), CLC Workbench 11.0 (Qiagen), and EDGE Bioinformatics (10).

Gene annotation was performed with a RAST server (11) with default settings; antibiotic resistance genes were identified with the Resistance Gene Identifier (RGI) from the Comprehensive Antibiotic Resistance Database (CARD) (12); virulence factors were identified with ShortBRED (13) with a customized database from the Virulence Factor Database (VFDB) (14). Sequence typing was determined with the *Pseudomonas aeruginosa* PubMLST database (15). Insertion sequences were identified with ISFinder (16). CLC Genomics Workbench was used to perform variant analysis.

P. aeruginosa 268 has a circular genome size of 7,030,474 bp and a G+C content of

Citation Voegtly LJ, Rice GK, Cer RZ, Frey KG, Biswas B, Hamilton T, Aslam S, Bishop-Lilly KA. 2019. High-quality draft genome sequence of *Pseudomonas aeruginosa* 268 isolated from a patient with a left ventricular assist device. *Microbiol Resour Announc* 8:e01403-18. <https://doi.org/10.1128/MRA.01403-18>.

Editor Irene L. G. Newton, Indiana University, Bloomington

This is a work of the U.S. Government and is not subject to copyright protection in the United States. Foreign copyrights may apply.

Address correspondence to Kimberly A. Bishop-Lilly, Kimberly.a.bishop-lilly.civ@mail.mil.

* Present address: Theron Hamilton, Navy Drug Screening Laboratory, Jacksonville, Florida, USA.

Received 15 October 2018

Accepted 24 November 2018

Published 3 January 2019

TABLE 1 Sequencing statistics

Library type	Raw data		Postquality control		SRA accession no.
	No. of reads	No. of base pairs	No. of reads	No. of base pairs	
Mate pair	13,056,854	3,098,317,963	429,477	96,162,258	SRR8183306
Shotgun	16,520,130	4,573,422,553	6,501,158	1,325,318,599	SRR8183307

65.9%. Annotation of *P. aeruginosa* 268 predicted a total of 6,578 coding sequences, 12 rRNA sequences, and 64 tRNA sequences. A total of 374 virulence factors and 63 antibiotic resistance genes were identified, including genes conferring resistance to beta-lactam, aminoglycosides, fluoroquinolones, macrolides, and tetracyclines. In addition, 21 insertion sequences were identified, notably 7 IS222 and portions of TnAs3. A 9-bp deletion was identified within acetyl coenzyme A dehydrogenase and occurs in 37% (106/287) of the reads; the biological significance of this potential deletion is not known. *P. aeruginosa* 268 strain contains two copies of the *acs* gene and therefore belongs to the two sequence types 235 (ST235), an international high-risk, multidrug-resistant clone, and 2613 (ST2613), which has the same profile as ST235 except with a different *acs* allele.

Data availability. The nucleotide sequence for *P. aeruginosa* 268 has been deposited at the NCBI under accession numbers CP032761 (GenBank) and SRR8183306 and SRR8183307 (SRA).

ACKNOWLEDGMENTS

This work was funded by work unit number A1417.

K.G.F., B.B., and K.A.B.-L. are employees of the U.S. Government, and T.H. is a military service member. This work was prepared as a part of official duties. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the U.S. Government.

REFERENCES

- Azam MW, Khan AU. 2018. Updates on the pathogenicity status of *Pseudomonas aeruginosa*. *Drug Discov Today* <https://doi.org/10.1016/j.drudis.2018.07.003>.
- Trachtenberg BH, Cordero-Reyes A, Elias B, Loebe M. 2015. A review of infections in patients with left ventricular assist devices: prevention, diagnosis and management. *Methodist Debakey Cardiovasc J* 11:28–32.
- Siméon S, Flécher E, Revest M, Niculescu M, Roussel J-C, Michel M, Leprince P, Tattevin P. 2017. Left ventricular assist device-related infections: a multicentric study. *Clin Microbiol Infect* 23:748–751. <https://doi.org/10.1016/j.cmi.2017.03.008>.
- Joshi NA, Fass JN. 2011. Sickle: a sliding-window, adaptive, quality-based trimming tool for FastQ files (version 1.33). <https://github.com/najoshi/sickle>.
- BBTools. <https://sourceforge.net/projects/bbmap>.
- O'Connell J, Schulz-Trieglaff O, Carlson E, Hims MM, Gormley NA, Cox AJ. 2015. NxTrim: optimized trimming of Illumina mate pair reads. *Bioinformatics* 31:2035–2037. <https://doi.org/10.1093/bioinformatics/btv057>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: interactive visualization of *de novo* genome assemblies. *Bioinformatics* 31:3350–3352. <https://doi.org/10.1093/bioinformatics/btv383>.
- Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 5:e11147. <https://doi.org/10.1371/journal.pone.0011147>.
- Li PE, Lo C-C, Anderson JJ, Davenport KW, Bishop-Lilly KA, Xu Y, Ahmed S, Feng S, Mokashi VP, Chain PSG. 2017. Enabling the democratization of the genomics revolution with a fully integrated Web-based bioinformatics platform. *Nucleic Acids Res* 45:67–80. <https://doi.org/10.1093/nar/gkw1027>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
- Jia B, Raphenya AR, Alcock B, Wagelchner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN, Doshi S, Courtot M, Lo R, Williams LE, Frye JG, Elsayegh T, Sardar D, Westman EL, Pawlowski AC, Johnson TA, Brinkman FSL, Wright GD, McArthur AG. 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic Acids Res* 45:D566–D573. <https://doi.org/10.1093/nar/gkw1004>.
- Kaminski J, Gibson MK, Franzosa EA, Segata N, Dantas G, Huttenhower C. 2015. High-specificity targeted functional profiling in microbial communities with ShortBRED. *PLoS Comput Biol* 11:e1004557. <https://doi.org/10.1371/journal.pcbi.1004557>.
- Chen L, Zheng D, Liu B, Yang J, Jin Q. 2016. VFDB 2016: hierarchical and refined dataset for big data analysis—10 years on. *Nucleic Acids Res* 44:D694–D697. <https://doi.org/10.1093/nar/gkv1239>.
- Jolley KA, Maiden MCJ. 2010. BIGSdb: scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics* 11:595. <https://doi.org/10.1186/1471-2105-11-595>.
- Siguié P, Perochon J, Lestrade L, Mahillon J, Chandler M. 2006. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res* 34:D32–D36. <https://doi.org/10.1093/nar/gkj014>.