





## Draft Genome Sequence of a bla<sub>NDM-1</sub>- and bla<sub>PME-1</sub>-Harboring Pseudomonas aeruginosa Clinical Isolate from Pakistan

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ABSTRACT We performed Illumina whole-genome sequencing on a carbapenemresistant Pseudomonas aeruginosa strain isolated from a cystic fibrosis patient with chronic airway colonization. The draft genome comprises 6,770,411 bp, including the carbapenemase  $\mathit{bla}_{\mathsf{NDM-1}}$  and the extended-spectrum beta-lactamase bla<sub>PMF-1</sub>. This isolate harbors 3 prophages, 14 antibiotic resistance genes, and 257 virulence genes.

seudomonas aeruginosa is a Gram-negative opportunistic pathogen frequently involved in nosocomial infections (1, 2). We isolated P. aeruginosa strain PA-81 from tracheal secretions of a cystic fibrosis patient admitted to a tertiary care hospital in Pakistan. The sample was plated on blood agar (Oxoid, UK) followed by substreaking of morphologically distinct colonies on Pseudomonas cetrimide agar (Oxoid). The isolate was transferred to St. Louis, MO, for further characterization. Distinct colonies of the isolate were used for identification by Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) with the library v2.3.3 (bio-Mérieux, Durham, NC) using default settings. Because the strain was found to be resistant to all available classes of antibiotics, including cefepime, meropenem, piperacillin-tazobactam, ceftolozane-tazobactam, ceftazidime-avibactam, ciprofloxacin, aztreonam, and trimethoprim-sulfamethoxazole, this strain was selected for further analysis from among more than 200 P. aeruginosa isolates collected in 2016.

Genomic DNA was extracted from overnight growth on blood agar (Hardy Diagnostics, Santa Maria, CA) using the QIAamp BiOstic bacteremia DNA kit (Qiagen, Germantown, MD). Illumina sequencing libraries were prepared using the Nextera DNA flex library prep kit (Illumina, San Diego, CA) with 0.5 ng genomic DNA (3). Wholegenome sequencing was performed on an Illumina NextSeg 500 instrument to obtain 2 imes 150-bp reads. Raw reads were processed and analyzed on the High Performance Computing Center cluster at Washington University in St. Louis School of Medicine, St. Louis, MO. Adapter sequences were trimmed using Trimmomatic v0.36 and decontaminated using DeconSeq v0.4.3 (4, 5). SPAdes v3.11.0 was used for de novo assembly of paired-end reads totaling 6,588,272 bp to produce an assembly with a mean genome coverage of  $22 \times$  (6). The quality of the resulting assembly was evaluated using QUAST v4.5 (7). The assembly produced 331 contigs (largest contig, 580,364 bp) with an  $N_{50}$  value of 265,381 bp. PATRIC v3.5.20 was used for annotation, which determined a total genome length of 6,770,411 bp with 6,607 coding

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sequences (CDSs). The strain had a 66% GC content, 65 tRNA genes, 3 rRNA genes, and the 16S, 23S, and 5S loci (8).

The genome of strain PA-81 contained 614 unique genes that were absent from the genome of the PAO1 reference strain (NCBI Reference Sequence number NC\_002516). The strain belongs to sequence type 357 (ST357) (https://cge.cbs.dtu.dk/services/MLST/) and serotype O11 (https://cge.cbs.dtu.dk/services/PAst/). Antibiotic resistance gene (ARG) annotation using the ResFinder and Comprehensive Antibiotic Resistance Database (CARD) servers (9, 10) revealed a number of ARGs against multiple classes of antibiotics, including beta-lactams (bla<sub>OXA-10</sub>, bla<sub>OXA-50</sub>, bla<sub>PAO</sub>, bla<sub>Amp-C</sub>, bla<sub>NDM-1</sub>, and bla<sub>PME-1</sub>), aminoglycosides [aph(3')-Ilb, aph(3')-Vla, ant(2")-la, and aadA1], fosfomycin (fosA), trimethoprim (dfrB2), amphenicols (catB7), and sulfonamides (sul1). The presence of bla<sub>NDM-1</sub> provides a likely mechanism of carbapenem resistance (11, 12). Further analysis using PlasmidFinder v1.3 did not reveal any known plasmid replicons (13). Three prophage regions of 21 kb, 12.6 kb, and 9.6 kb with identity scores of 90%, 85%, and 10%, respectively, were identified using PHAge Search Tool-Enhanced Release (PHASTER) (14). Default settings were used for all the employed tools and software.

To the best of our knowledge, this is the first draft genome sequence of a  $bla_{\text{NDM-1}}$ - and  $bla_{\text{PME-1}}$ -harboring *P. aeruginosa* strain from Pakistan. A more detailed analysis of this genome will be reported in a future publication.

**Data availability.** This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number QXJN00000000 (version QXJN01000000) and SRA accession number SRR8510690.

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## **REFERENCES**

- Gellatly SL, Hancock REW. 2013. Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. Pathog Dis 67:159–173. https://doi.org/10.1111/2049-632X.12033.
- Balasubramanian D, Schneper L, Kumari H, Mathee K. 2013. A dynamic and intricate regulatory network determines *Pseudomonas aeruginosa* virulence. Nucleic Acids Res 41:1–20. https://doi.org/10.1093/nar/gks1039.
- Baym M, Kryazhimskiy S, Lieberman TD, Chung H, Desai MM, Kishony R. 2015. Inexpensive multiplexed library preparation for megabase-sized genomes. PLoS One 10:e0128036. https://doi.org/10.1371/journal.pone .0128036.
- 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.
- Schmieder R, Edwards R. 2011. Fast identification and removal of sequence contamination from genomic and metagenomic datasets. PLoS One 6:e17288. https://doi.org/10.1371/journal.pone.0017288.
- 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.

- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.
- Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, Gillespie JJ, Gough R, Hix D, Kenyon R, Machi D, Mao C, Nordberg EK, Olson R, Overbeek R, Pusch GD, Shukla M, Schulman J, Stevens RL, Sullivan DE, Vonstein V, Warren A, Will R, Wilson MJC, Yoo HS, Zhang C, Zhang Y, Sobral BW. 2014. PATRIC, the bacterial bioinformatics database and analysis resource. Nucleic Acids Res 42:D581–D591. https://doi.org/10 .1093/nar/qkt1099.
- Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10.1093/jac/dks261.
- McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, Bhullar K, Canova MJ, De Pascale G, Ejim L, Kalan L, King AM, Koteva K, Morar M, Mulvey MR, O'Brien JS, Pawlowski AC, Piddock LJV, Spanogiannopoulos P, Sutherland AD, Tang I, Taylor PL, Thaker M, Wang W, Yan M, Yu T, Wright GD. 2013. The Comprehensive Antibiotic Resistance Database. Antimicrob Agents Chemother 57:3348–3357. https://doi.org/10.1128/AAC 00419-13
- 11. Teo JWP, La M-V, Jureen R, Lin RTP. 2015. Emergence of a New Delhi

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- metallo- $\beta$ -lactamase-1-producing *Pseudomonas aeruginosa* in Singapore. Emerg Microbes Infect 4:1. https://doi.org/10.1038/emi.2015.72. 12. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR.
- Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR. 2009. Characterization of a new metallo-β-lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother 53:5046–5054. https://doi.org/10.1128/AAC .00774-09.
- Carattoli A, Zankari E, García-Fernández A, Larsen MV, Lund O, Villa L, Aarestrup FM, 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.
- Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016.
  PHASTER: a better, faster version of the PHAST phage search tool.
  Nucleic Acids Res 44:W16-W21. https://doi.org/10.1093/nar/gkw387.

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