Draft Genome Sequence of a \textit{bla}_{NDM-1}\textendash{} and \textit{bla}_{PME-1}\textendash{}Harboring \textit{Pseudomonas aeruginosa} Clinical Isolate from Pakistan

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\textbf{ABSTRACT} We performed Illumina whole-genome sequencing on a carbapenem-resistant \textit{Pseudomonas aeruginosa} strain isolated from a cystic fibrosis patient with chronic airway colonization. The draft genome comprises \(6,770,411\) bp, including the carbapenemase \textit{bla}_{NDM-1} and the extended-spectrum beta-lactamate \textit{bla}_{PME-1}. This isolate harbors 3 prophages, 14 antibiotic resistance genes, and 257 virulence genes.

\textit{Pseudomonas aeruginosa} is a Gram-negative opportunistic pathogen frequently involved in nosocomial infections (1, 2). We isolated \textit{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 sub-streaking of morphologically distinct colonies on \textit{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 (bioMérieux, Durham, NC) using default settings. Because the strain was found to be resistant to all available classes of antibiotics, including cepafepime, meropenem, piperacillin-tazobactam, cefotaxime-tazobactam, ceftazidime-avibactam, ciprofloxacin, aztreonam, and trimethoprim-sulfamethoxazole, this strain was selected for further analysis from among more than 200 \textit{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). Whole-genome sequencing was performed on an Illumina NextSeq 500 instrument to obtain \(2 \times 150\)-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 Trimomatic v0.36 and decontaminated using DeconSeq v0.4.3 (4, 5). SPAdes v3.11.0 was used for \textit{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
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 (blaOXA-10, blaOXA-58, blaADC, blaAMPC, blaTEM, and blaPME), aminoglycosides [aph(3’)-Ia, aph(3’)-IIa, aph(2’)-Ia, and aadA1], fosfomycin (fosA), trimethoprim (dfrB2), amphenicols (catB7, catA, and catB), and sulfonamides (sulI). The presence of blaNDM-1 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.

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.

ACKNOWLEDGMENTS
This work was supported in part by an International Research Support Initiative Program (IRISIP) award of the Higher Education Commission (HEC) of Pakistan to S.I. and a United States Agency for International Development award (3220-29047) to S.A., C.-A.D.B., and G.D. R.F.P. received support from an NIGMS training grant through award T32 GM007067 (primary investigator, James Skeath) and the Monsanto Excellence Fund graduate fellowship.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

We acknowledge the pathology lab staff of PIMS, Islamabad, Pakistan, for providing the clinical isolate and associated metadata. We also thank The Edison Family Center for Genome Sciences & Systems Biology staff members Eric Martin, Brian Koebbe, and Jessica Hoisington-López for their technical support and sequencing expertise.

REFERENCES
11. Teo JWP, La M-V, Jureen R, Lin RTP. 2015. Emergence of a New Delhi

