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

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**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 quantified 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 (N₅₀, 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 32.7%.

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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).

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TABLE 1 Sequencing statistics

| Library type | Raw data | Postquality control |
|--------------|----------|---------------------|
| No. of reads | No. of base pairs | SRA accession no. |
| 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 |