



## Whole-Genome Sequence of Multidrug-Resistant *Pseudomonas aeruginosa* Strain BAMCPA07-48, Isolated from a Combat Injury Wound

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We report here the complete genome sequence of *Pseudomonas aeruginosa* strain BAMCPA07-48, isolated from a combat injury wound. The closed genome sequence of this isolate is a valuable resource for pathogenome characterization of *P. aeruginosa* associated with wounds, which will aid in the development of a higher-resolution phylogenomic framework for molecular-guided pathogen-surveillance.

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he rise of multidrug resistant (MDR) organisms in war wounds is of major concern to the health care community. Efforts are increasing to identify and characterize genotypes of war wound MDR pathogens for improved biosurveillance, woundcare response, and outcome (1-4). The Gram-negative opportunistic pathogen, Pseudomonas aeruginosa, is ubiquitously found in the environment (5, 6), exhibits intrinsic antibiotic resistance profile (7, 8), and is considered one of the primary etiological agents of wound and nosocomial infections (9-11). P. aeruginosa strain BAMCPA07-48 was kindly provided by San Antonio Military Medical Center (SAMMC) and was isolated from a wound caused by combat injury. Antimicrobial susceptibility testing (AST) of BAMCPA07-48 revealed resistance to antibiotic agents including fluoroquinolone, tetracycline, vancomycin, zeocin, trimethoprim, nitrofurantoin, aminoglycoside, and a wide range of  $\beta$ -lactams; susceptibility was only observed with tobramycin treatment ( $\geq 4 \, \mu g/mL$ ).

The BAMCPA07-48 genomic DNA was isolated from overnight culture using a Wizard Genomic DNA purification kit (Promega). Genomic DNA of BAMCPA07-48 was subjected to wholegenome sequencing using a hybrid sequencing method with PacBio and Illumina HiSeq platforms (12, 13). With  $200 \times$  coverage, the draft genome of BAMCPA07-48 was assembled and closed using BGI hybrid assembler (14). Genome annotation and visualization was conducted using GeneMarkS+ (15), resulting in the identification of 6,739 genes and 64 tRNA and 12 rRNA molecules. The genome sequence of BAMCPA07-48 revealed a G+C content of 66.03% and a genome size of 7.0 Mb.

Availability of high-quality closed-genome sequence enables rapid determination of virulence-state and phylogenomic profiling according to well-established genotypic classification methods using *in silico* computational approaches (16–20). The use of high-throughput next generation sequencing for whole-genome sequence analysis of BAMCPA07-48 will aid in high-resolution genotypic characterization and virulence-profiling while investigating the pathogenome evolution of this strain in relation to sequenced *P. aeruginosa* extant genotypes (21–24). Extending on and confirming our initial *in vitro* AST study, sequence-based analysis of the BAMCPA07-48 strain using an antibiotic resistance gene identifier (RGI) (25) revealed carriage of resistance genes against a wide range of antimicrobial classes including aminoglycoside, fosfomycin, triclosan, rifampin, trimethoprim, tetracycline, aminocoumarin, macrolide,  $\beta$ -lactams, chloramphenicol, polymyxin, and fluoroquinolone. Multilocus sequence typing (MLST) (16, 17) placed BAMCPA07-48 as sequence type 313 (26) and motility typing (*fliC*) classified it as a type A and *flaG*-positive strain (27–29). Further studies remain necessary to better understand genotypic variations and different virulence profiles present in *P. aeruginosa* isolates associated with combat injury wounds and when compared to extant genotypes.

In contrast to draft genomes, a complete genome sequence provides a higher-resolution finished product in which the order and accuracy of each single base pair of the genome is verified and there are no gaps in the genome (24, 30). The complete genome sequence of BAMCPA07-48 enables accurate downstream functional genomics analysis, precise identification of its genetic organization, and future comparative genomic studies with relation to the growing collection of sequenced *P. aeruginosa* strains.

**Nucleotide sequence accession number.** The complete genome sequence for *P. aeruginosa* strain BAMCPA07-48 has been deposited in GenBank under the accession number CP015377.

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## REFERENCES

- Levin AS, Barone AA, Penço J, Santos MV, Marinho IS, Arruda EA, Manrique EI, Costa SF. 1999. Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Clin Infect Dis 28:1008–1011. http:// dx.doi.org/10.1086/514732.
- Calhoun JH, Murray CK, Manring MM. 2008. Multidrug-resistant organisms in military wounds from Iraq and Afghanistan. Clin Orthop Relat Res 466:1356–1362. http://dx.doi.org/10.1007/s11999-008-0212-9.
- Yun HC, Murray CK, Roop SA, Hospenthal DR, Gourdine E, Dooley DP. 2006. Bacteria recovered from patients admitted to a deployed U.S. military hospital in Baghdad, Iraq. Mil Med 171:821–825. http:// dx.doi.org/10.7205/MILMED.171.9.821.
- Murray CK, Roop SA, Hospenthal DR, Dooley DP, Wenner K, Hammock J, Taufen N, Gourdine E. 2006. Bacteriology of war wounds at the time of injury. Mil Med 171:826-829. http://dx.doi.org/10.7205/ MILMED.171.9.826.
- Morales G, Wiehlmann L, Gudowius P, van Delden C, Tümmler B, Martínez JL, Rojo F. 2004. Structure of *Pseudomonas aeruginosa* populations analyzed by single nucleotide polymorphism and pulsed-field gel electrophoresis genotyping. J Bacteriol 186:4228–4237. http://dx.doi.org/ 10.1128/JB.186.13.4228-4237.2004.
- Trautmann M, Lepper PM, Haller M. 2005. Ecology of *Pseudomonas* aeruginosa in the intensive care unit and the evolving role of water outlets as a reservoir of the organism. Am J Infect Control 33:S41–S49. http:// dx.doi.org/10.1016/j.ajic.2005.03.006.
- Murray JL, Kwon T, Marcotte EM, Whiteley M. 2015. Intrinsic antimicrobial resistance determinants in the superbug *Pseudomonas aeruginosa*. MBio 6:e01603-01615. http://dx.doi.org/10.1128/mBio.01603-15.
- Okamoto K, Gotoh N, Nishino T. 2001. Pseudomonas aeruginosa reveals high intrinsic resistance to penem antibiotics: penem resistance mechanisms and their interplay. Antimicrob Agents Chemother 45:1964–1971. http://dx.doi.org/10.1128/AAC.45.7.1964-1971.2001.
- Lyczak JB, Cannon CL, Pier GB. 2000. Establishment of *Pseudomonas* aeruginosa infection: lessons from a versatile opportunist. Microbes Infect 2:1051–1060. http://dx.doi.org/10.1016/S1286-4579(00)01259-4.
- Schaber JA, Triffo WJ, Suh SJ, Oliver JW, Hastert MC, Griswold JA, Auer M, Hamood AN, Rumbaugh KP. 2007. *Pseudomonas aeruginosa* forms biofilms in acute infection independent of cell-to-cell signaling. Infect Immun 75:3715–3721. http://dx.doi.org/10.1128/IAI.00586-07.
- Rice LB. 2008. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. J Infect Dis 197:1079–1081. http:// dx.doi.org/10.1086/533452.
- 12. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6:1621–1624. http://dx.doi.org/10.1038/ismej.2012.8.
- Lin HH, Liao YC. 2015. Evaluation and validation of assembling corrected PacBio long reads for microbial genome completion via hybrid approaches. PLoS One 10:e0144305. http://dx.doi.org/10.1371/ journal.pone.0144305.
- Henson J, Tischler G, Ning Z. 2012. Next-generation sequencing and large genome assemblies. Pharmacogenomics 13:901–915. http:// dx.doi.org/10.2217/pgs.12.72.
- Besemer J, Lomsadze A, Borodovsky M. 2001. GeneMarkS: a selftraining method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res 29:2607–2618. http://dx.doi.org/10.1093/nar/29.12.2607.
- 16. Jolley KA, Maiden MC. 2010. BIGSdb: scalable analysis of bacterial ge-

nome variation at the population level. BMC Bioinformatics 11:595. http://dx.doi.org/10.1186/1471-2105-11-595.

- Curran B, Jonas D, Grundmann H, Pitt T, Dowson CG. 2004. Development of a multilocus sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa*. J Clin Microbiol 42:5644–5649. http:// dx.doi.org/10.1128/JCM.42.12.5644-5649.2004.
- Thrane SW, Taylor VL, Lund O, Lam JS, Jelsbak L. 20 April 2016. Application of WGS data for O-specific antigen analysis and in silico serotyping of *Pseudomonas aeruginosa* isolates. J Clin Microbiol. [Epub ahead of print.] http://dx.doi.org/10.1128/JCM.00349-16.
- Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. Nucleic Acids Res 39:W347–W352. http://dx.doi.org/ 10.1093/nar/gkr485.
- Boratyn GM, Camacho C, Cooper PS, Coulouris G, Fong A, Ma N, Madden TL, Matten WT, McGinnis SD, Merezhuk Y, Raytselis Y, Sayers EW, Tao T, Ye J, Zaretskaya I. 2013. BLAST: a more efficient report with usability improvements. Nucleic Acids Res 41:W29–W33. http://dx.doi.org/10.1093/nar/gkt282.
- Ozer EA, Allen JP, Hauser AR. 2014. Characterization of the core and accessory genomes of *Pseudomonas aeruginosa* using bioinformatic tools spine and AGEnt. BMC Genomics 15:737. http://dx.doi.org/10.1186/1471 -2164-15-737.
- 22. Jeukens J, Boyle B, Kukavica-Ibrulj I, Ouellet MM, Aaron SD, Charette SJ, Fothergill JL, Tucker NP, Winstanley C, Levesque RC. 2014. Comparative genomics of isolates of a *Pseudomonas aeruginosa* epidemic strain associated with chronic lung infections of cystic fibrosis patients. PLoS One 9:e87611. http://dx.doi.org/10.1371/journal.pone.0087611.
- Kwan T, Liu J, Dubow M, Gros P, Pelletier J. 2006. Comparative genomic analysis of 18 *Pseudomonas aeruginosa* bacteriophages. J Bacteriol 188:1184–1187. http://dx.doi.org/10.1128/JB.188.3.1184-1187.2006.
- Köser CU, Ellington MJ, Cartwright EJP, Gillespie SH, Brown NM, Farrington M, Holden MTG, Dougan G, Bentley SD, Parkhill J, Peacock SJ. 2012. Routine use of microbial whole genome sequencing in diagnostic and public health microbiology. PLoS Pathog 8:e1002824. http://dx.doi.org/10.1371/journal.ppat.1002824.
- 25. 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 LJ, 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. http:// dx.doi.org/10.1128/AAC.00419-13.
- 26. Libisch B, Watine J, Balogh B, Gacs M, Muzslay M, Szabó G, Füzi M. 2008. Molecular typing indicates an important role for two international clonal complexes in dissemination of VIM-producing *Pseudomonas aeruginosa* clinical isolates in Hungary. Res Microbiol 159:162–168. http:// dx.doi.org/10.1016/j.resmic.2007.12.008.
- Feldman M, Bryan R, Rajan S, Scheffler L, Brunnert S, Tang H, Prince A. 1998. Role of flagella in pathogenesis of *Pseudomonas aeruginosa* pulmonary infection. Infect Immun 66:43–51.
- Arora SK, Ritchings BW, Almira EC, Lory S, Ramphal R. 1998. The *Pseudomonas aeruginosa* flagellar cap protein, *FliD*, is responsible for mucin adhesion. Infect Immun 66:1000–1007.
- Brimer CD, Montie TC. 1998. Cloning and comparison of *fliC* genes and identification of glycosylation in the flagellin of *Pseudomonas aeruginosa* a-type strains. J Bacteriol 180:3209–3217.
- Fraser CM, Eisen JA, Nelson KE, Paulsen IT, Salzberg SL. 2002. The value of complete microbial genome sequencing (you get what you pay for). J Bacteriol 184:6403-6405. http://dx.doi.org/10.1128/ JB.184.23.6403-6405.2002.