



Complete Genome Sequence of *Pseudomonas aeruginosa* CMC-115, a Clinical Strain from an Acute Ventilator-Associated Pneumonia Patient

Adenike Adenikinju,^a Roderick V. Jensen,^b Thomas M. Kerkerling,^{a,c} Dorothy C. Garner,^{a,c}  Jayasimha Rao^{a,c}

^aDivision of Infectious Disease, Virginia Tech Carilion School of Medicine, Roanoke, Virginia, USA

^bDepartment of Biological Sciences, Virginia Tech, Blacksburg, Virginia, USA

^cInternal Medicine, Division of Infectious Disease, Carilion Medical Center, Roanoke, Virginia, USA

ABSTRACT We report the complete genome of clinical strain *Pseudomonas aeruginosa* CMC-115, which was isolated from an acute ventilator-associated pneumonia patient. Illumina sequencing reads were assembled using Geneious to yield a 6,375,262-bp circular chromosome that exhibited an unusual ferrichrome receptor in the pyoverdine synthesis locus and the absence of type 3 secretion system genes.

Pseudomonas aeruginosa is an increasingly common opportunistic pathogen causing infection in immunocompromised and hospitalized patients, leading to health care-associated pneumonia. Patients on ventilators are particularly vulnerable; *P. aeruginosa* invades the lower respiratory tract and leads to ventilator-associated pneumonia (VAP), increasing the risk of death by as much as 30% in intensive care units (1–3). The complete genome of a *Pseudomonas* strain from an acute VAP case provides useful information for identifying novel virulence factors and antimicrobial resistance (AMR) genes.

A prospective study was conducted in 2010 to 2012 with approval from the Carilion Clinic Institutional Review Board. In that study, CMC-115 was obtained from the Quest Diagnostics Microbiology Laboratory at Carilion Clinic. The strain was isolated from a tracheal aspirate sample from an acute VAP patient, and its identity was confirmed by biochemical tests. Antimicrobial susceptibility testing was performed using a Vitek 2 automated system (bioMérieux) according to Clinical and Laboratory Standards Institute guidelines (4). The isolate was grown on a blood agar plate and transported to the Carilion Basic Science Laboratory (CBSL); glycerol stocks were prepared and stored in a –80°C freezer. The patient was treated at Carilion Roanoke Memorial Hospital and survived.

A single colony of CMC-115 was grown for 18 h in 25 ml lysogeny broth at 37°C, at 200 rpm. The cell pellet was used for genomic DNA isolation with a Genomic-tip 20/G (Qiagen) (5). The genome was sequenced on the Illumina NextSeq platform at the Virginia Tech Genomics Resource Center, with a library constructed using the Illumina TruSeq DNA preparation kit. Sequencing generated 68,209,004 paired-end reads with a length of 150 bp, which were assembled with the Geneious v11.0.4 (Biomatters, Ltd.) *de novo* algorithm to produce 40 contigs with a length of >1,000 bp and an N_{50} value of 423,776 bp. The Geneious map to reference algorithm, with fine tuning set to iterate up to 10 times, was then used to iteratively map reads to the ends of the 40 contigs until the contigs overlapped and closed into a circular genome. A final use of the Geneious map to reference algorithm aligned >95% of the reads to the complete circular genome, with a uniform average coverage of 1,500×. All Geneious assemblies and alignments were performed with the default low-sensitivity/fastest settings, which allow at most 10% base mismatches.

Citation Adenikinju A, Jensen RV, Kerkerling TM, Garner DC, Rao J. 2020. Complete genome sequence of *Pseudomonas aeruginosa* CMC-115, a clinical strain from an acute ventilator-associated pneumonia patient. *Microbiol Resour Announc* 9:e00595-20. <https://doi.org/10.1128/MRA.00595-20>.

Editor Catherine Putonti, Loyola University Chicago

Copyright © 2020 Adenikinju et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Roderick V. Jensen, rjensen@vt.edu, or Jayasimha Rao, jrao@carilionclinic.org.

Received 20 May 2020

Accepted 22 June 2020

Published 23 July 2020

TABLE 1 Transposase family sequences in *Pseudomonas aeruginosa* CMC-115

Transposase family	Gene location	Locus tag	IS ^a	Origin ^b	Accession no.
I53 (ISPa57)	296203..297536	GO602_01345	IRL: TGTCTAGTCTGAAATAGGTTTACACTGCTCAGTGTAGCGGATAGCCCTCAAACGCCCGAT; IRR: TGTCCCGCCAGATGGAGTTTTTCGTACAGTACAAAGACAGAGTACCTACAAGC	<i>P. aeruginosa</i> DK1, <i>P. aeruginosa</i> PA7	LN870292
I53 (ISPa57)	410967..412186	GO602_01860	IRL: TGTCCCGTCTGAAATAAGGTTTACACTCCATCCGATTTCCGGAGAGTATGGAGCAA; IRR: TGTCTAGTCTGAAATAGGTTTACACTGCTCAGTGTAGCGGATAGCCCTCAAAC	<i>P. aeruginosa</i> DK1, <i>P. aeruginosa</i> PA7	LN870292
I53 (ISPa57)	2029342..2030561	GO602_09640	IRL: TGTCTAGTCTGAAATAGGTTTACACTGCTCAGTGTAGCGGATAGCCCTCAAACGCCCGAT; IRR: TGTCCCGTCTGAAATAAGGTTTACACTCCATCCGATTTCCGGAGAGCTAGA	<i>P. aeruginosa</i> DK1, <i>P. aeruginosa</i> PA7	LN870292
I53 (ISPa57)	2674283..2675502	GO602_12475	IRL: TGTCTAGTCTGAAATAGGTTTACACTGCTCAGTGTAGCGGATAGCCCTCAAACGCCCGAT; IRR: TGTCCCGTCTGAAATAAGGTTTACACTCCATCCGATTTCCGGAGAGCTAGA	<i>P. aeruginosa</i> DK1, <i>P. aeruginosa</i> PA7	LN870292
I53 (ISPa57)	4870826..4872045	GO602_22695	IRL: TGTCTAGTCTGAAATAGGTTTACACTGCTCAGTGTAGCGGATAGCCCTCAAACGCCCGAT; IRR: TGTCCCGTCTGAAATAAGGTTTACACTCCATCCGATTTCCGGAGAGCTAGA	<i>P. aeruginosa</i> DK1, <i>P. aeruginosa</i> PA7	LN870292
I53 (ISPa57)	4953930..4955149	GO602_23110	IRL: TGTCTAGTCTGAAATAGGTTTACACTGCTCAGTGTAGCGGATAGCCCTCAAACGCCCGAT; IRR: TGTCCCGTCTGAAATAAGGTTTACACTCCATCCGATTTCCGGAGAGCTAGA	<i>P. aeruginosa</i> DK1, <i>P. aeruginosa</i> PA7	LN870292
I521 (ISDev1)	858147..859679	GO602_04065	IRL: TGTCTAGTCTGAAATAGGTTTACACTGCTCAGTGTAGCGGATAGCCCTCAAACGCCCGAT; IRR: TGTCCCGTCTGAAATAAGGTTTACACTCCATCCGATTTCCGGAGAGCTAGA	<i>Devosia</i> sp. I507, <i>Aquamicrobium</i> sp. <i>P. aeruginosa</i>	CP026747
I53 (ISPa86)	11568120..1157562	GO602_05530	IRL: TGTCTAGTCTGAAATAGGTTTACACTGCTCAGTGTAGCGGATAGCCCTCAAACGCCCGAT; IRR: TGTCCCGTCTGAAATAAGGTTTACACTCCATCCGATTTCCGGAGAGCTAGA	<i>P. aeruginosa</i> PAO-muc	MF344569
ISNCY (ISPa6)	1327230..1327763	GO602_06370	IRL: TGTCTAGTCTGAAATAGGTTTACACTGCTCAGTGTAGCGGATAGCCCTCAAACGCCCGAT; IRR: TGTCCCGTCTGAAATAAGGTTTACACTCCATCCGATTTCCGGAGAGCTAGA	<i>P. aeruginosa</i> PAO-muc	U16784
I54 (ISPen1)	2789284..2790672	GO602_12805	IRL: TGTCTAGTCTGAAATAGGTTTACACTGCTCAGTGTAGCGGATAGCCCTCAAACGCCCGAT; IRR: TGTCCCGTCTGAAATAAGGTTTACACTCCATCCGATTTCCGGAGAGCTAGA	<i>Pseudomonas entomophila</i> L48	NC_008027
I53 (ISPa32)	3154955..3156092	GO602_14355	IRL: TGTCTAGTCTGAAATAGGTTTACACTGCTCAGTGTAGCGGATAGCCCTCAAACGCCCGAT; IRR: TGTCCCGTCTGAAATAAGGTTTACACTCCATCCGATTTCCGGAGAGCTAGA	<i>P. aeruginosa</i> PAO1	NC_002516
I53 (ISAeca4)	5918861..5919667	GO602_27515	IRL: TGTCTAGTCTGAAATAGGTTTACACTGCTCAGTGTAGCGGATAGCCCTCAAACGCCCGAT; IRR: TGTCCCGTCTGAAATAAGGTTTACACTCCATCCGATTTCCGGAGAGCTAGA	<i>Aeromonas caviae</i>	CP024198 ^c
I53 (ISPa32)	5919740..5920878	GO602_27520	IRL: TGTCTAGTCTGAAATAGGTTTACACTGCTCAGTGTAGCGGATAGCCCTCAAACGCCCGAT; IRR: TGTCCCGTCTGAAATAAGGTTTACACTCCATCCGATTTCCGGAGAGCTAGA	<i>P. aeruginosa</i> PAO1	NC_002516

^a IRL, left inverted repeat; IRR, right inverted repeat.

^b ISfinder tool (<http://www-isb.toul.fr>) with the standard parameters (10).

^c Accession number from an NCBI protein BLAST search.

The complete assembly of CMC-115 resulted in a circular genome of 6,375,262 bp with a G+C content of 66.4%. The NCBI Prokaryotic Genome Annotation Pipeline v4.2 (6, 7) identified 5,930 genes, including 5,850 protein-coding genes and 80 RNA genes (64 tRNAs, 12 rRNAs, and 4 noncoding RNAs), along with 68 pseudogenes and 2 CRISPR arrays.

Interestingly, the type 3 secretion system and its effector genes *exoS*, *exoT*, *exoU*, and *exoY* are absent in the genome of CMC-115 (8, 9). The genome of CMC-115 contains many insertion sequence (IS) elements (10), representing the IS3, IS4, IS21, IS63, and ISNCY families (11, 12) (Table 1). We found an ~101-kb locus for pyoverdine synthesis that contains an unusual *tonB*-dependent siderophore (ferrichrome) receptor (GO602_12470), the *fvpAIIb* (13–15) linked to IS3 (GO602_12475). Chromosomal AMR genes were identified using the RGI v5.1.0 and CARD v3.0.8 (16) Web servers with default parameters. Ten genes were found, including two β -lactamase genes (*bla*_{OXA-50} and/or *bla*_{OXA-395} and *bla*_{PDC-167} and/or *bla*_{PAO}), two phenicol resistance genes (*catB2* and *catB7*), an aminoglycoside resistance gene [*aph(3')IIIb*], a fosfomycin resistance gene (*fosA*), a vancomycin resistance gene (*vanWG*), a fusaric acid resistance gene (*fusc*) (17), a sulfonamide resistance gene (*sul1*), and a bleomycin resistance gene (*acetyltransf_10*) (18). In addition, 33 resistance-nodulation-cell division (RND) genes that are involved in antibiotic efflux mechanisms (19) and 5 genes involved in antibiotic target alterations were identified.

Data availability. The annotated complete genome assembly of strain *Pseudomonas aeruginosa* CMC-115 is available in GenBank under accession numbers [CP046602](#), [SRR11592953](#), [PRJNA593818](#), and [SAMN13451386](#).

ACKNOWLEDGMENTS

We acknowledge the faculty members of the Division of Infectious Disease, Carilion Clinic, and the Carilion Clinical Microbiology Laboratory for their assistance in collecting patient samples in the Carilion Clinic institutional review board-approved study. Experiments were carried out at the CBSL and Carilion Roanoke Community Hospital. We are thankful to Sarah Cox, Radford University Carilion, for her valuable support in manuscript reading and suggestions.

This study was supported by a Carilion Clinic grant to D.C.G. and J.R., a Graduate Student Research Supply grant to A.A. was funded by the Virginia Polytechnic Institute and State University (VT), and the Illumina sequencing was completed with the support of the Fralin Life Science Institute, VT, to R.V.J.

REFERENCES

- Williams BJ, Dehnbostel J, Blackwell TS. 2010. *Pseudomonas aeruginosa*: host defence in lung diseases. *Respirology* 15:1037–1056. <https://doi.org/10.1111/j.1440-1843.2010.01819.x>.
- Rello J, Ausino V, Ricart M, Castella J, Prats G. 1993. Impact of previous antimicrobial therapy on the etiology and outcome of ventilator-associated pneumonia. *Chest* 104:1230–1235. <https://doi.org/10.1378/chest.104.4.1230>.
- Klompas M. 2019. Ventilator-associated events: what they are and what they are not. *Respir Care* 64:953–961. <https://doi.org/10.4187/respcare.07059>.
- Clinical and Laboratory Standards Institute. 2017. Performance standards for antimicrobial susceptibility testing, 27th ed. Document M100. Clinical and Laboratory Standards Institute, Wayne, PA.
- Rao J, Susanti D, Childress JC, Mitkos MC, Brima JK, Baffoe-Bonnie AW, Pearce SN, Grgurich D, Fernandez-Cotarelo MJ, Kerkering TM, Mukhopadhyay B. 2018. *Tn2008*-driven carbapenem resistance in *Acinetobacter baumannii* isolates from a period of increased incidence of infections in a Southwest Virginia hospital (USA). *J Glob Antimicrob Resist* 12:79–87. <https://doi.org/10.1016/j.jgar.2017.08.017>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44: 6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Haft DH, DiCuccio M, Badretdin A, Brover V, Chetvernin V, O'Neill K, Li W, Chitsaz F, Derbyshire MK, Gonzales NR, Gwadz M, Lu F, Marchler GH, Song JS, Thanki N, Yamashita RA, Zheng C, Thibaud-Nissen F, Geer LY, Marchler-Bauer A, Pruitt KD. 2018. RefSeq: an update on prokaryotic genome annotation and curation. *Nucleic Acids Res* 46:D851–D860. <https://doi.org/10.1093/nar/gkx1068>.
- Elsen S, Huber P, Bouillot S, Couté Y, Fournier P, Dubois Y, Timsit JF, Maurin M, Attrée I. 2014. A type III secretion negative clinical strain of *Pseudomonas aeruginosa* employs a two-partner secreted exolysin to induce hemorrhagic pneumonia. *Cell Host Microbe* 15:164–176. <https://doi.org/10.1016/j.chom.2014.01.003>.
- Toska J, Sun Y, Carbonell DA, Foster ANS, Jacobs MR, Pearlman E, Rietsch A. 2014. Diversity of virulence phenotypes among type III secretion negative *Pseudomonas aeruginosa* clinical isolates. *PLoS One* 9:e86829. <https://doi.org/10.1371/journal.pone.0086829>.
- Siguier 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>.
- Mahillon J, Chandler M. 1998. Insertion sequences. *Microbiol Mol Biol Rev* 62:725–774. <https://doi.org/10.1128/MMBR.62.3.725-774.1998>.
- He S, Hickman AB, Varani AM, Siguier P, Chandler M, Dekker JP, Dyda F. 2015. Insertion sequence IS26 reorganizes plasmids in clinically isolated multidrug-resistant bacteria by replicative transposition. *mBio* 6:e00762-15. <https://doi.org/10.1128/mBio.00762-15>.
- Adenikinju A, Garner DC, Kerkering T, Jensen R, Rao J. 2019. 2606. A

- divergent ferrichrome receptor associated with an insertional element (IS3) identified on novel locus in clinical strain of *Pseudomonas aeruginosa*. *Open Forum Infect Dis* 6(Suppl 2):S906. <https://doi.org/10.1093/ofid/ofz360.2284>.
14. Bodilis J, Ghysels B, Osayande J, Matthijs S, Pirnay JP, Denayer S, De Vos D, Cornelis P. 2009. Distribution and evolution of ferripyoverdine receptors in *Pseudomonas aeruginosa*. *Environ Microbiol* 11:2123–2135. <https://doi.org/10.1111/j.1462-2920.2009.01932.x>.
 15. Kiriienko DR, Kang D, Kiriienko NV. 2018. Novel pyoverdine inhibitors mitigate *Pseudomonas aeruginosa* pathogenesis. *Front Microbiol* 9:3317. <https://doi.org/10.3389/fmicb.2018.03317>.
 16. Liu B, Pop M. 2009. ARDB—Antibiotic Resistance Genes Database. *Nucleic Acids Res* 37:D443–D447. <https://doi.org/10.1093/nar/gkn656>.
 17. Crutcher FK, Puckhaber LS, Stipanovic RD, Bell AA, Nichols RL, Lawrence KS, Liu J. 2017. Microbial resistance mechanisms to the antibiotic and phytotoxin fusaric acid. *J Chem Ecol* 43:996–1006. <https://doi.org/10.1007/s10886-017-0889-x>.
 18. Dortet L, Girlich D, Virlovet AL, Poirel L, Nordmann P, Iorga BI, Naas T. 2017. Characterization of BRP_{MBL}, the bleomycin resistance protein associated with the carbapenemase NDM. *Antimicrob Agents Chemother* 61:e02413-16. <https://doi.org/10.1128/AAC.02413-16>.
 19. Colclough AL, Alav I, Whittle EE, Pugh HL, Darby EM, Legood SW, McNeil HE, Blair JM. 2020. RND efflux pumps in Gram-negative bacteria: regulation, structure and role in antibiotic resistance. *Future Microbiol* 15: 143–157. <https://doi.org/10.2217/fmb-2019-0235>.