

Draft genome sequence of *Acinetobacter haemolyticus* strain MUWRP1017 isolated from the pus of a female inpatient at Bwera General Hospital in Uganda

Godfrey Wokorach,^{1,2} Bernard Erima,¹ Stephen Alafi,¹ Hope O. Kabatesi,¹ Julius T. Muhindo,³ Florence Najjuka,⁴ James Kiyengo,⁵ Hannah Kibuuka,¹ Ambrose K. Musinguzi,⁵ Fred Wabwire-Mangen,^{1,4} Denis K. Byarugaba^{1,4}

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT The bacterium *Acinetobacter haemolyticus*, with a genome size of 3.4 Mb, was isolated from a pus swab of a wound on the left lower limb above the ankle joint of a female patient. This strain carries the antimicrobial resistance genes cephalosporinase *blaADC-25*, oxallinase *blaOXA-264*, *floR*, and *sul2* and other resistance and virulence genes.

KEYWORDS *blaOXA-264*, *blaADC-25*, *Acinetobacter*, bacteria, Gram-negative bacteria, Uganda

Acinetobacter is a group of Gram-negative coccobacillus bacteria with increasing prevalence in hospitals worldwide, causing various infections (1). *Acinetobacter haemolyticus* is an emerging species with serious multidrug resistance agents like *A. baumannii*. It belongs to the haemolytic clade and has been implicated in nosocomial infections, with some carrying carbapenem-resistance determinants, making treatment difficult (2). This study describes the genome of strain MUWRP1017 isolated from a wound on the lower left limb of a female patient from the Democratic Republic of Congo. She received healthcare service at Bwera General Hospital in Kasese district, Uganda.

A swab obtained from pus was immediately inoculated on blood agar, and the resulting single colony was transferred to nutrient agar. The bacterial colony was presumptively identified as Gram-negative coccobacillus by Gram staining and was later grown on Luria broth to obtain cell mass for DNA extraction. All culturing procedures were done at 37°C for 24 hours under aerobic conditions. DNA was extracted using the DNeasy UltraClean microbial kit (Qiagen, Germantown, Maryland, USA) without modification. Extracted DNA was used to create libraries with the Kapa HyperPlus library preparation kit (Roche Diagnostics, Indianapolis, IN, USA). Paired-end sequencing (2 × 151 bp) of the libraries produced 11,677,660 total reads on Illumina NextSeq (Illumina, Inc., San Diego, CA). Raw reads were evaluated using FastQC v0.11.9 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and trimmed with Btrim v0.2.0 (3). Assembly with Newbler v2.7 generated a genome with 523 x coverage (4). The assembled genome was evaluated for completeness using CheckM v1.2.2 (5). The annotation of the draft genome was done using PGAP version 6.7 (6). The taxonomy assignment was done using the Genome Taxonomy Database (GTDB Release 214.1) considering an average nucleotide identity (ANI) index of ≥95% (7). Antimicrobial resistant genes were predicted with ResFinder v4.3.2 (8). The virulence factor database (VFDB) (<http://www.mgc.ac.cn/VFs/>) was used to predict the presence of virulence genes (9). All software utilized default settings, unless otherwise stated. Ethical approval was obtained from Makerere University School of Public Health Higher Degrees and Research Ethics Committee (HDREC 087) and Walter Reed Army Institute of Research IRB (WRAIR #1711).

Editor Vanja Klepac-Ceraj, Department of Biological Sciences, Wellesley College, Wellesley, Massachusetts, USA

Address correspondence to Denis K. Byarugaba, denis.byarugaba@mak.ac.ug.

The authors declare no conflict of interest.

Received 29 May 2024

Accepted 18 July 2024

Published 20 August 2024

Copyright © 2024 Wokorach 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/).

TABLE 1 Average nucleotide identity (ANI) index of type strains with strain MUWRP1017

Organism name	Type strain	Accession	Taxonomy ID	ANI (%)
<i>A. haemolyticus</i>	MTCC 9819	GCA_000430205.1	29430	98.13
<i>A. haemolyticus</i>	CIP 64.3	GCA_000369065.1	29430	98.25
<i>A. haemolyticus</i>	FDAARGOS_1392	GCA_019355995.1	29430	98.30
<i>A. haemolyticus</i>	NCTC10305	GCA_900444835.1	29430	98.30

CheckM v1.2.2 indicates the assembled genome was 99.99% complete. The assembly generated a genome of 3.4 Mb and 119 contigs with an N50 value of 70 kb. About 39.5% of the genome is made of GC bases. The genome was predicted to have 3,235 genes, of which 3,151 were protein-coding genes. The MUWRP1017 strain was identified as *Acinetobacter haemolyticus*, with the closest match to type strain (MTCC 9819), with an ANI value of 98.13% (Table 1). The MUWRP1017 strain carries *aac(6′)-Ig*, *aph(6)-Id*, and *aph(3′)-Ib* aminoglycoside resistant genes. The cephalosporinase *blaADC-25* and oxallinase *blaOXA-264* were the detected beta-lactam-resistant genes. Amphenicol (*floR*) and folate pathway antagonist (*sul2*) resistant genes were also detected. Adherence genes (*pilT*, *pilU*, *pilB*, *mshE*, *tapT*, and *htpB*) and Type VI secretion system tip protein (*vgrG/tssI*) were the virulence genes detected. The finding of *Acinetobacter*-derived cephalosporinase *blaADC-25*, the oxallinase *blaOXA-264*, and other resistance determinants is an alert for potential resistance threats in future.

ACKNOWLEDGMENTS

Whole-genome sequencing was performed by Walter Reed Army Research Institute (WRAIR)'s Multi-Drug-Resistant Organism Repository and Surveillance Network (MRSN) and are greatly acknowledged.

This research was funded by the United States Armed Forces Health Surveillance Division (AFHSD), Global Emerging Infections Surveillance (GEIS) Branch under PROMIS ID: USAMRD-K 17_KY_1.1.7.

AUTHOR AFFILIATIONS

¹Makerere University Walter Reed Project, Kampala, Uganda

²Department of Biology, Faculty of Science, Muni University, Arua, Uganda

³Bwera District Hospital, Kasese, Uganda

⁴Makerere University, Kampala, Uganda

⁵Uganda Peoples Defence Forces, Kampala, Uganda

AUTHOR ORCID*s*

Godfrey Wokorach  <http://orcid.org/0000-0001-8079-3474>

Denis K. Byarugaba  <http://orcid.org/0000-0002-4175-5659>

AUTHOR CONTRIBUTIONS

Godfrey Wokorach, Data curation, Formal analysis, Investigation, Validation, Writing – original draft | Bernard Erima, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Stephen Alafi, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review and editing | Hope O. Kabatesi, Data curation, Formal analysis, Investigation, Validation, Writing – review and editing | Julius T. Muhindo, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Writing – review and editing | Florence Najjuka, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Validation, Writing – review and editing | James Kiyengo, Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Validation, Writing – review and editing | Hannah Kibuuka, Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Validation, Writing – review and

editing | Ambrose K. Musinguzi, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Writing – review and editing | Fred Wabwire-Mangen, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Writing – review and editing | Denis K. Byarugaba, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Writing – review and editing

DATA AVAILABILITY

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [JBDPJG000000000](https://doi.org/10.1128/JBDPJG000000000). The version described in this paper is version [JBDPJG010000000](https://doi.org/10.1128/JBDPJG010000000). The GenBank assembly accession number is [GCA_039839735.1](https://doi.org/10.1128/GCA_039839735.1). The raw sequences were deposited in the Sequence Read Archive (SRA) under the accession number [SRR29081551](https://doi.org/10.1128/SRR29081551).

REFERENCES

1. Wong D, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B, Spellberg B. 2017. Clinical and pathophysiological overview of acinetobacter infections: a century of challenges. *Clin Microbiol Rev* 30:409–447. <https://doi.org/10.1128/CMR.00058-16>
2. Castro-Jaimes S, Bello-López E, Velázquez-Acosta C, Volkow-Fernández P, Lozano-Zarain P, Castillo-Ramírez S, Cevallos MA. 2020. Chromosome architecture and gene content of the emergent pathogen *Acinetobacter haemolyticus*. *Front Microbiol* 11:926. <https://doi.org/10.3389/fmicb.2020.00926>
3. Kong Y. 2011. Btrim: a fast, lightweight adapter and quality trimming program for next-generation sequencing technologies. *Genomics* 98:152–153. <https://doi.org/10.1016/j.ygeno.2011.05.009>
4. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen Y-J, Chen Z, et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437:376–380. <https://doi.org/10.1038/nature03959>
5. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>
6. 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>
7. Parks DH, Chuvochina M, Rinke C, Mussig AJ, Chaumeil PA, Hugenholtz P. 2022. GTDB: an ongoing census of bacterial and archaeal diversity through a phylogenetically consistent, rank normalized and complete genome-based taxonomy. *Nucleic Acids Res* 50:D785–D794. <https://doi.org/10.1093/nar/gkab776>
8. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, et al. 2020. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 75:3491–3500. <https://doi.org/10.1093/jac/dkaa345>
9. Liu B, Zheng D, Zhou S, Chen L, Yang J. 2022. VFDB 2022: a general classification scheme for bacterial virulence factors. *Nucleic Acids Res* 50:D912–D917. <https://doi.org/10.1093/nar/gkab1107>