





8 | Bacteriology | Announcement

# Draft genome sequence of carbapenems-resistant *Acinetobacter* baumannii Hakim RU\_CBWP strain isolated from a pond surface water in Bangladesh

Muhib Ullah Khan,<sup>1</sup> M. Romance,<sup>1</sup> Md. Arif-Uz-Zaman Polash,<sup>1</sup> Nusrat Zahan,<sup>1</sup> Md. Sumon Ali,<sup>1</sup> Jafor Raihan,<sup>1</sup> Subir Sarker,<sup>2</sup> Md. Hakimul Haque<sup>1,2</sup>

**AUTHOR AFFILIATIONS** See affiliation list on p. 3.

**ABSTRACT** We have revealed the genomic sequence of *Acinetobacter baumannii* strain Hakim RU\_CBWP isolated from pond surface water. Our assembled genome covers 3.787 Mb with 45.5629× coverage, showcasing an average GC content of 38.60%. This genome contains two CRISPR arrays, 17 prophages, 22 antibiotic resistance genes, and 20 virulence factor genes.

**KEYWORDS** whole genome, pond surface water, carbapenems resistant, *Acinetobacter baumannii*, Bangladesh

As an opportunistic and ESKAPE pathogen, *Acinetobacter baumannii* poses a significant nosocomial infection risk (1). Its propensity to develop resistance to last-resort antibiotics, including colistin, tigecycline, and carbapenems, raises grave public health concerns (2). Transferring resistant genes via mobile genetic elements complicates treatment strategies, particularly for critically ill and immunocompromised patients (3, 4). Multidrug-resistant *Acinetobacter baumannii* was noted in humans, animals, food, and the environment worldwide, underscoring the crucial need for continuous surveillance and monitoring through One Health approaches to understanding its molecular epidemiology and implementing effective control measures (3, 5, 6).

The Institute of Biological Science (IBSc) at the University of Rajshahi, Bangladesh, approved all research techniques and protocols under Memo No. 56/321/IAMEBBC/ IBSc. In September 2023, we collected pond surface water samples at the University of Rajshahi, Bangladesh, following standard procedures. The water samples were uniformly mixed, placed in sterile tubes, and transported to the laboratory (24.3733°N, 88.6049°E). We inoculated these samples on UTI agar (HiMedia, India) and incubated them aerobically at 37°C for 18-24 hours (7). Acinetobacter baumannii was isolated by streaking the cultures on MacConkey agar (HiMedia, India), followed by staining and biochemical tests (8). We performed antimicrobial susceptibility testing of the isolates using the disk diffusion method (9) following CLSI guidelines (10). The strain is resistant to amoxicillin, amoxicillin + clavulanic acid, cephradine, co-trimoxazole, azithromycin, and Gentamycin. We cultured the isolated strain in nutrient broth (HiMedia, India) overnight at 37°C and then extracted its genomic DNA using the Qiagen DNA Mini Kit (QIAGEN, Hilden, Germany). Genomic DNA underwent enzymatic fragmentation using the NEBNext dsDNA Fragmentase Kit (NEB, MA, USA), followed by size selection with SPRI beads (11). A sequencing library was prepared by the Nextera DNA Flex Library Preparation Kit (Illumina, San Diego, CA, USA), and the library was sequenced with 2 × 150 paired-end reads on the Illumina NextSeq2000 platform. Raw paired-end reads (n = 5,168,921) were trimmed with Trimmomatic.v0.39 (12), and genome assembly was conducted using Unicycler.v0.4.9 (13). Quality checks were performed using FastQC

**Editor** John J. Dennehy, Queens College Department of Biology, Queens, New York, USA

Address correspondence to Md. Hakimul Haque, hakim.ahvs@ru.ac.bd.

The authors declare no conflict of interest.

See the funding table on p. 3.

Received 28 April 2024 Accepted 10 May 2024 Published 12 June 2024

Copyright © 2024 Khan et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

July 2024 Volume 13 Issue 7

TABLE 1 Genomic attributes of the A. baumannii strain Hakim RU\_CBWP

Elements	Values	
Genome size	3,787,050 bp	
Genome coverage	45.5629×	
G + C content	38.60%	
Number of contigs	161	
Contig L50	11	
Contig N50	103,712 bp	
Total genes	3,672	
Coding sequences	3,621	
Coding genes	3,554	
RNA genes	51	
tRNA genes	41	
rRNAs genes	6	
ncRNAs genes	4	
Pseudo genes	67	
Genes assigned to SEED subsystems	3,679	
Number of subsystems	304	

v0.11.7 (14), and annotation was carried out using PGAP v3.0 (15). The assembled genome was analyzed for antibiotic resistance genes (ARGs) using CARD v.3.2.4 with RGI v6.0.2 (16) and ResFinder v.4.1 (17), mobile genetic elements (MGEs) using mobileOG-db (18), virulence factor genes using VFDB with VFanalyzer (19), pathogenicity index using PathogenFinder v.1.1 (20), sequence type using MLST v.2.0 (21), CRISPR arrays using CRISPRimmunity (22), prophages using PHASTER (23), and metabolic functional features using RAST v.2.0 (24). Unless otherwise specified, we used the default parameters for all tools.

The attributes of the draft genomes are noted in Table 1. Notably, 22 ARGs, 20 virulence genes, and 69 MGEs were identified. MLST classified the genome as sequence type unknown but nearest to 2168, 2133, 1938, 1379, 1422, 1452, 1447, 619, 2146, 2554, 1526, 1459, 25, 1450, 2145, 2831, and 1456, according to the PathogenFinder tool, which indicated a pathogenicity index of 0.861. The genome exhibited two CRISPR arrays with signature genes (*DEDDh*, *TnsC*, *WYL*, *cas3*, *TniQ*, *csa3*, and *cas3f*) and 17 prophages. RAST analysis uncovered 304 subsystems, comprising 3,679 genes with 28% coverage (Fig. 1).

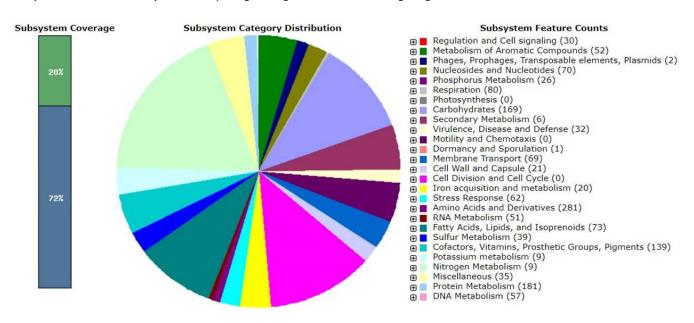


FIG 1 Metabolic functional features in the assembled genome of the A. baumannii Hakim RU\_CBWP strain in SEED viewer.

July 2024 Volume 13 Issue 7 10.1128/mra.00440-24 **2** 

# Downloaded from https://journals.asm.org/journal/mra on 28 October 2024 by 137.219.201.11.

## **ACKNOWLEDGMENTS**

The Rajshahi University funded this study under Bangladesh's University Grants Commission (UGC) (Financial Year 2023–2024).

M.U.K did the following: conceptualization, data curation, formal analysis, investigation, methodology, project administration, and writing–original draft; M.R. did the following: conceptualization, data curation, formal analysis, methodology, software, and writing–original draft; Md. A.-U.-Z.P. did the following: conceptualization, data curation, investigation, and methodology; N.Z. did the following: formal analysis, methodology, and software; Md. S.A. did the following: formal analysis, methodology, and software; S.S. did the following: formal analysis, writing—review and editing, and software; Md. H.H. did the following: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, resources, supervision, validation, writing–original draft, and writing–review and editing.

## **AUTHOR AFFILIATIONS**

<sup>1</sup>Department of Veterinary and Animal Sciences, University of Rajshahi, Rajshahi, Bangladesh

<sup>2</sup>Biomedical Sciences & Molecular Biology, College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Queensland, Australia

## **AUTHOR ORCIDs**

Md. Hakimul Haque http://orcid.org/0000-0002-3856-6478

## **FUNDING**

Funder	Grant(s) Author(s)
The Rajshahi University this study under Bangladesh's University Grant Commission (UGC) Financial year (2023-2024)	Md. Hakimul Haque

# **AUTHOR CONTRIBUTIONS**

Muhib Ullah Khan, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft | M. Romance, Conceptualization, Data curation, Formal analysis, Methodology, Software, Writing – original draft | Md. Arif-Uz-Zaman Polash, Conceptualization, Data curation, Investigation, Methodology | Nusrat Zahan, Formal analysis, Methodology, Software | Md. Sumon Ali, Formal analysis, Methodology, Software | Jafor Raihan, Formal analysis, Methodology, Software | Subir Sarker, Formal analysis, Software, Writing – review and editing | Md. Hakimul Haque, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review and editing

# **DATA AVAILABILITY**

The study on *Acinetobacter baumannii* Hakim RU\_CBWP, conducted using the WGS shotgun approach, was submitted to NCBI/GenBank, and it was assigned the accession number JBCDME000000000. The pertinent data, including the original readings, were stored with BioProject accession number PRJNA1101523, BioSample accession number SAMN40996234, and SRA accession number SRR28724258. The specific version mentioned in this document is labeled as JBCDME000000000.1.

July 2024 Volume 13 Issue 7 10.1128/mra.00440-24 **3** 

# Downloaded from https://journals.asm.org/journal/mra on 28 October 2024 by 137.219.201.11.

## **REFERENCES**

- Whiteway C, Breine A, Philippe C, Van der Henst C. 2022. Acinetobacter baumannii. Trends Microbiol 30:199–200. https://doi.org/10.1016/j.tim. 2021.11.008
- De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, Beatson SA, Paterson DL, Walker MJ. 2020. Antimicrobial resistance in ESKAPE pathogens. Clin Microbiol Rev 33:e00181-19. https://doi.org/10.1128/ CMR.00181-19
- Jeon JH, Jang KM, Lee JH, Kang LW, Lee SH. 2023. Transmission of antibiotic resistance genes through mobile genetic elements in *Acinetobacter baumannii* and gene-transfer prevention. Sci Total Environ 857:159497. https://doi.org/10.1016/j.scitotenv.2022.159497
- Castanheira M, Mendes RE, Gales AC. 2023. Global epidemiology and mechanisms of resistance of Acinetobacter baumannii-calcoaceticus complex. Clin Infect Dis 76:S166–S178. https://doi.org/10.1093/cid/ ciad109
- Thomsen J, Abdulrazzaq NM, AlRand H, Everett DB, Senok A, Menezes GA, Ayoub Moubareck C, UAE AMR Surveillance Consortium. 2023. Epidemiology and antimicrobial resistance trends of *Acinetobacter* species in the United Arab Emirates: a retrospective analysis of 12 years of national AMR surveillance data. Front Public Health 11:1245131. https: //doi.org/10.3389/fpubh.2023.1245131
- Cella E, Giovanetti M, Benedetti F, Scarpa F, Johnston C, Borsetti A, Ceccarelli G, Azarian T, Zella D, Ciccozzi M. 2023. Joining forces against antibiotic resistance: the one health solution. Pathogens 12:1074. https://doi.org/10.3390/pathogens12091074
- Romance M, Khan MU, Islam MS, Islam MF, Haque MH. 2024. Draft genome sequence of multidrug-resistant Klebsiella pneumoniae Hakim-RU strain isolated from a patient with urinary tract infections in Bangladesh. Microbiol Resour Announc 13:e0008924. https://doi.org/10. 1128/mra.00089-24
- Bergey DH, Buchanan RE, Gibbons NE. 1974. Bergey's manual of determinative bacteriology. 8th ed, p 966–1097. American Society for Microbiology, Washington, DC, USA.
- Bauer AW, Kirby WMM, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45:493– 496. https://doi.org/10.1093/ajcp/45.4\_ts.493
- M100-S32. 2022. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute, Wayne, PA, USA.
- Liu D, Li Q, Luo J, Huang Q, Zhang Y. 2023. An SPRI beads-based DNA purification strategy for flexibility and cost-effectiveness. BMC Genomics 24:125. https://doi.org/10.1186/s12864-023-09211-w
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/ 10.1093/bioinformatics/btu170
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595

- Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. Available from: http://www.bioinformatics.babraham.ac. uk/projects/fastqc/
- 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
- Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, Huynh W, Nguyen A-LV, Cheng AA, Liu S, et al. 2020. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 48:D517–D525. https://doi.org/ 10.1093/nar/gkz935
- Florensa AF, Kaas RS, Clausen PTLC, Aytan-Aktug D, Aarestrup FM. 2022.
  ResFinder an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. Microb Genom 8:000748. https://doi.org/10.1099/mgen.0.000748
- Brown CL, Mullet J, Hindi F, Stoll JE, Gupta S, Choi M, Keenum I, Vikesland P, Pruden A, Zhang L. 2022. mobileOG-db: a manually curated database of protein families mediating the life cycle of bacterial mobile genetic elements. Appl Environ Microbiol 88:e0099122. https://doi.org/10.1128/ aem.00991-22
- 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
- Cosentino S, Voldby Larsen M, Møller Aarestrup F, Lund O. 2013. PathogenFinder - distinguishing friend from foe using bacterial whole genome sequence data. PLoS ONE 8:e77302. https://doi.org/10.1371/journal.pone.0077302
- Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM, Lund O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 50:1355–1361. https://doi.org/10.1128/JCM.06094-11
- Zhou F, Yu X, Gan R, Ren K, Chen C, Ren C, Cui M, Liu Y, Gao Y, Wang S, Yin M, Huang T, Huang Z, Zhang F. 2023. CRISPRimmunity: an interactive web server for CRISPR-associated important molecular events and modulators used in genome editing tool identifying. Nucleic Acids Res 51:W93–W107. https://doi.org/10.1093/nar/gkad425
- Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016.
  PHASTER: a better, faster version of the PHAST phage search tool.
  Nucleic Acids Res 44:W16–W21. https://doi.org/10.1093/nar/gkw387
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, et al. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75. https:// doi.org/10.1186/1471-2164-9-75