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Draft genome sequence of biofilm-forming methicillin-resistant *Staphylococcus aureus* MTR_V1 strain isolated from a ready-to-eat food in Bangladesh

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ABSTRACT This announcement provides the genome sequence of the biofilm-forming methicillin-resistant *Staphylococcus aureus* MTR_V1 strain isolated from a ready-to-eat food sample in Bangladesh. Our assembled genome had a length of 2.8 Mb, 27 contigs, two CRISPR arrays, 38 predicted antibiotic resistance genes, and 66 predicted virulence factor genes.

KEYWORDS whole-genome sequencing, MRSA, biofilm formation, ready-to-eat food, CRISPR arrays, antibiotic resistance genes, virulence factor genes, Bangladesh

Excessive antibiotic use has caused antibiotic resistance, resulting in diverse multidrug-resistant (MDR) strains that pose a significant global health risk (1, 2). The development of resistance and biofilm in methicillin-resistant *Staphylococcus aureus* (MRSA) strains presents a severe threat to human health (3, 4).

Between June 2021 and March 2022, ready-to-eat food samples were collected from various vendors and restaurants in Mymensingh district, Bangladesh. The samples were processed using previous protocols (4) and incubated overnight in nutrient broth (HiMedia, India) at 37°C. The samples were then streaked on Mannitol Salt Agar (HiMedia, India) media, and the resulting colonies underwent staining and biochemical tests to isolate *Staphylococcus aureus* (5). *S. aureus* was identified through the matrix-assisted laser desorption ionization time-of-flight mass spectrometry assay (6). MRSA was confirmed by detecting the *mecA* gene, which was amplified by the primers *mec*A1 (5′-AAAATCGATGGTAAAGGTTGGC) and *mec*A2 (5′-AGTTCTGCAGTACCGGATTTGC) (7). Biofilm formation in MRSA was determined using Congo Red Agar (8) and Crystal Violet (9) assays. Finally, a biofilm-forming MRSA strain, *S. aureus* MTR_V1, was selected and incubated in nutrient broth (HiMedia, India) overnight at 37°C, and the harvested culture was used for DNA extraction with a Qiagen DNA mini kit (Qiagen, Hilden, Germany). The extracted DNA from the isolate was then subjected to enzymatic fragmentation using NEBNextdsDNA Fragmentase Kit (NEB, MA, USA) according to the manufacturer's instructions. The fragmented DNA was subjected to size selection using SPRI beads, which helped in isolating DNA fragments of the desired size range for sequencing (10). The sequencing library was created using the Nextera DNA Flex library prep kit (Illumina, San Diego, CA, USA) and sequenced on the Illumina NextSeq2000 platform with paired-end reads (2 × 150). The genome was assembled using the Unicycler.v0.4.9 (11), and the raw paired-end reads (n = 9,253,470) were trimmed using Trimmomatic.v0.39 (12). FastQC.v0.11.7 (13) was used to evaluate the quality of the trimmed reads. The genome was annotated using PGAP.v3.0 (14). SCCmecFinder.v1.2 (15) was employed for determining the predicted *SCCmec* element; PlasmidFinder.v2.1 (16) for plasmid typing; CARD.v3.2.4 (17), NDARO.v2023 (18), ResFinder.v4.1 (19), and

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PATRIC.v3.2.76 (20) for antibiotic resistance genes (ARGs); VFDB (21), VirulenceFinder.v2.0 (22), and Victors (23) for virulence factor genes (VFGs); MLST.v2.0 (24) for sequence type; DrugBank.v4.0 (25) and TTD (26) for drug target genes (DTGs); TCDB (27) for transporter genes (TGs); and RAST.v2.0 (28) for metabolic functional features. Default parameters were used for all software used in this study, unless otherwise specified.

The *S. aureus* MTR_V1 genome assembly contained 27 contigs with a G + C content of 32.65% and three contig L50s with an N50 value of 280,895 bp. It had a genome size of 2,766,668 bp and a coverage of 30.0×. Our genome also harbored 2,703 CDS, 64 RNA genes, and 89 pseudogenes. The *SCCmec* element of the *S. aureus* MTR_V1 strain was predicted as SCCmec_type_IVa(2B), and the MLST analysis assigned it as sequence type ST1930. Additionally, the assembled genome contained two CRISPR arrays, three plasmids, 38 predicted ARGs, 66 predicted VFGs, 35 DTGs, 97 TGs, and 273 subsystems (with 33% coverage and 1,231 genes).

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AUTHOR CONTRIBUTIONS

Fatimah Muhammad Ballah, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft | Md. Saiful Islam, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review and editing | Samina Ievy, Investigation | Farhana Binte Ferdous, Investigation | Md. Abdus Sobur, Writing – review and editing | AMM Taufiquer Rahman, Conceptualization, Writing – review and editing | Marzia Rahman, Supervision | M. Nazmul Hoque, Formal analysis | Jayedul Hassan, Writing – review and editing | Md. Tanvir Rahman, Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review and editing

DATA AVAILABILITY

The Whole Genome Sequencing (WGS) shotgun analysis of *S. aureus* MTR_V1 was formally submitted to GenBank and can be accessed using the accession number JAPKJV000000000. The associated data, comprising the raw reads, have been

deposited with the following accession numbers: BioProject—PRJNA902494, BioSample—SAMN31761769, and SRA—SRR25110733. In this article, the specific version indicated is JAPKJV000000000.1.

REFERENCES

1. Ahmed T, Islam MS, Nuruzzaman M, Sadekuzzaman M, Kabir SML, Rahman MT, Khan MSR. 2023. Draft genome sequence of the multidrug-resistant Citrobacter freundii 132-2 strain isolated from a domestic duck in Bangladesh. Microbiol Resour Announc 12:e0037823. https://doi.org/10.1128/mra.00378-23
2. Islam MS, Rahman AMMT, Hassan J, Rahman MT. 2023. Extended-spectrum beta-lactamase in *Escherichia coli* isolated from humans, animals, and environments in Bangladesh: a one health perspective systematic review and meta-analysis. One Health 16:100526. https://doi.org/10.1016/j.onehlt.2023.100526
3. Ballah FM, Islam MS, Rana ML, Ullah MA, Ferdous FB, Neloy FH, Ievy S, Sobur MA, Rahman AT, Khatun MM, Rahman M, Rahman MT. 2022. Virulence determinants and methicillin resistance in biofilm-forming *Staphylococcus aureus* from various food sources in Bangladesh. Antibiotics (Basel) 11:1666. https://doi.org/10.3390/antibiotics11111666
4. Ballah FM, Islam MS, Rana ML, Ferdous FB, Ahmed R, Pramanik PK, Karmoker J, Ievy S, Sobur MA, Siddique MP, Khatun MM, Rahman M, Rahman MT. 2022. Phenotypic and genotypic detection of biofilm-forming *Staphylococcus aureus* from different food sources in Bangladesh. Biology (Basel) 11:949. https://doi.org/10.3390/biology11070949
5. Lancette G. A, Tatini S. R. 1992. *Staphylococcus aureus*, p 533–550. In C. Vanderzant, Splittstoesser D F (ed), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, DC, USA.
6. Dubois D, Leyssene D, Chacornac JP, Kostrzewa M, Schmit PO, Talon R, Bonnet R, Delmas J. 2010. Identification of a variety of *Staphylococcus* species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 48:941–945. https://doi.org/10.1128/JCM.00413-09
7. Lee JH. 2003. Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl Environ Microbiol 69:6489–6494. https://doi.org/10.1128/AEM.69.11.6489-6494.2003
8. Arciola CR, Baldassarri L, Montanaro L. 2001. Presence of icaA and icaD genes and slime production in a collection of staphylococcal strains from catheter-associated infections. J Clin Microbiol 39:2151–2156. https://doi.org/10.1128/JCM.39.6.2151-2156.2001
9. Kouidhi B, Zmantar T, Hentati H, Bakhrouf A. 2010. Cell surface hydrophobicity, biofilm formation, adhesives properties and molecular detection of adhesins genes in *Staphylococcus aureus* associated to dental caries. Microb Pathog 49:14–22. https://doi.org/10.1016/j.micpath.2010.03.007
10. 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 24:452. https://doi.org/10.1186/s12864-023-09551-7
11. 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
12. 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
13. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. Online. Available from: http://www.bioinformatics.babraham.ac.uk/projects/fastqc
14. 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
15. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). 2009. Classification of Staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. Antimicrob Agents Chemother 53:4961–4967. https://doi.org/10.1128/AAC.00579-09
16. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. 2014. *In silico* detection and typing of plasmids using plasmidfinder and plasmid multilocus sequence typing . Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/AAC.02412-14
17. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, Huynh W, Nguyen A-L, Cheng AA, Liu S, Min SY, Miroshnichenko A, Tran H-K, Werfalli RE, Nasir JA, Oloni M, Speicher DJ, Florescu A, Singh B, Faltyn M, Hernandez-Koutoucheva A, Sharma AN, Bordeleau E, Pawlowski AC, Zubyk HL, Dooley D, Griffiths E, Maguire F, Winsor GL, Beiko RG, Brinkman FSL, Hsiao WWL, Domselaar GV, McArthur AG. 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
18. Feldgarden M, Brover V, Gonzalez-Escalona N, Frye JG, Haendiges J, Haft DH, Hoffmann M, Pettengill JB, Prasad AB, Tillman GE, Tyson GH, Klimke W. 2021. Amrfinderplus and the reference gene catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. Sci Rep 11:12728. https://doi.org/10.1038/s41598-021-91456-0
19. 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
20. Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, Gillespie JJ, Gough R, Hix D, Kenyon R, Machi D, Mao C, Nordberg EK, Olson R, Overbeek R, Pusch GD, Shukla M, Schulman J, Stevens RL, Sullivan DE, Vonstein V, Warren A, Will R, Wilson MJC, Yoo HS, Zhang C, Zhang Y, Sobral BW. 2014. PATRIC, the bacterial bioinformatics database and analysis resource. Nucleic Acids Res 42:D581–91. https://doi.org/10.1093/nar/gkt1099
21. 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
22. Kleinheinz KA, Joensen KG, Larsen MV. 2014. Applying the resfinder and virulencefinder web-services for easy identification of acquired antibiotic resistance and *E. coli* virulence genes in bacteriophage and prophage nucleotide sequences. Bacteriophage 4:e27943. https://doi.org/10.4161/bact.27943
23. Sayers S, Li L, Ong E, Deng S, Fu G, Lin Y, Yang B, Zhang S, Fa Z, Zhao B, Xiang Z, Li Y, Zhao X-M, Olszewski MA, Chen L, He Y. 2019. Victors: a web-based knowledge base of virulence factors in human and animal pathogens. Nucleic Acids Res 47:D693–D700. https://doi.org/10.1093/nar/gky999
24. 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
25. Law V, Knox C, Djoumbou Y, Jewison T, Guo AC, Liu Y, Maciejewski A, Arndt D, Wilson M, Neveu V, Tang A, Gabriel G, Ly C, Adamjee S, Dame ZT, Han B, Zhou Y, Wishart DS. 2014. DrugBank 4.0: shedding new light on drug metabolism. Nucleic Acids Res 42:D1091–7. https://doi.org/10.1093/nar/gkt1068
26. Zhu F, Han B, Kumar P, Liu X, Ma X, Wei X, Huang L, Guo Y, Han L, Zheng C, Chen Y. 2010. Update of TTD: therapeutic target database. Nucleic Acids Res 38:D787–91. https://doi.org/10.1093/nar/gkp1014
27. Saier MH, Reddy VS, Tsu BV, Ahmed MS, Li C, Moreno-Hagelsieb G. 2016. The transporter classification database (TCDB): recent advances. Nucleic Acids Res 44:D372–9. https://doi.org/10.1093/nar/gkv1103
28. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:1–15. https://doi.org/10.1186/1471-2164-9-75