




Genome Sequence of a Dengue Virus Serotype 2 Strain Identified during the 2019 Outbreak in Bangladesh

Roly Malaker,^a Mohammad S. I. Sajib,^a Apurba R. Malaker,^a Hafizur Rahman,^a Yogesh Hooda,^{a,b} Md Hasanuzzaman,^a Samir K. Saha,^{a,c,d}  Senjuti Saha^a

^aChild Health Research Foundation, Dhaka, Bangladesh

^bMRC Laboratory of Molecular Biology, Cambridge, United Kingdom

^cDhaka Shishu (Children) Hospital, Dhaka, Bangladesh

^dBangladesh Institute of Child Health, Dhaka, Bangladesh

ABSTRACT A nearly complete genome sequence of a dengue virus serotype 2 strain detected in the serum of a patient in 2019 during the largest outbreak of dengue fever in Bangladesh is reported.

Dengue is a mosquito-borne viral disease caused by dengue virus, a single-stranded positive-sense RNA virus of the family *Flaviviridae* (1). Here, we report the genome sequence of a dengue virus detected in a clinically symptomatic patient during the 2019 dengue outbreak in Bangladesh (2).

All protocols were approved by the ethical review board of the Bangladesh Institute of Child Health. Samples were collected for clinical care and diagnostic testing at the discretion of the attending physicians, and written consent was obtained from the caregiver for additional laboratory analysis.

For this study, a subset of dengue virus-positive serum specimens, tested using the NS1 antigen kit (product no. 09DEN10D; SD Biosensor, South Korea), were retested using reverse transcription-quantitative PCR (RT-qPCR) to estimate the dengue viral load (3). One specimen collected on 27 July 2019 in Dhaka, Bangladesh, had the highest viral load, with a cycle threshold value of 24, and was selected for next-generation sequencing. For library preparation, viral nucleic acid was extracted from a 200- μ l serum sample using the Quick-DNA/RNA microprep extraction kit (product no. D7005; Zymo, CA, USA) according to the manufacturer's protocol. The NEBNext Ultra II RNA library preparation kit (product no. E7770; New England Biolabs, MA, USA) was used to prepare the sequencing library. External RNA Controls Consortium Collection spike-in control mix (product no. 4456740; Thermo Fisher Scientific, MA, USA) was used as a marker of potential library preparation errors. The final library was sequenced on an Illumina iSeq100 sequencer using 150-nucleotide paired-end sequencing.

The library generated 7,688,000 reads, and 2,467,976 reads passed the default filter of BaseSpace (Illumina). Raw fastq files were uploaded to the IDseq portal to identify the pathogenic organisms, as described elsewhere (4, 5). In brief, the human reads were filtered out from the fastq file by alignment with a reference database using the Spliced Transcripts Alignment to a Reference (STAR) algorithm. Next, a series of quality control steps were performed to exclude low-quality reads, duplicates, and low-complexity reads with the help of the Paired-Read Iterative Contig Extension (PRICE) package, the CD-HIT-DUP tool (v4.6.8), and the Lempel-Ziv-Welch (LZW) algorithm. Another round of host filtering was executed by applying the Bowtie2 package. The remaining reads were queried against the most recent version of the NCBI nucleotide and nonredundant protein databases using Genomic Short-read Nucleotide Alignment Program (GSNAP) (v2018-10-26) and RAPSearch 2 (v2.24), respectively. Finally, the

Citation Malaker R, Sajib MSI, Malaker AR, Rahman H, Hooda Y, Hasanuzzaman M, Saha SK, Saha S. 2021. Genome sequence of a dengue virus serotype 2 strain identified during the 2019 outbreak in Bangladesh. *Microbiol Resour Annot* 10:e01246-20. <https://doi.org/10.1128/MRA.01246-20>.

Editor Kenneth M. Stedman, Portland State University

Copyright © 2021 Malaker 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 Senjuti Saha, senjutisaha@gmail.com.

Received 29 October 2020

Accepted 25 November 2020

Published 7 January 2021



FIG 1 Phylogenetic trees of dengue virus serotype 2 (DENV2) strains rendered using Nextstrain. (A) Phylogenetic tree of CHRF_DenV002 (GenBank accession no. [MN328061](https://www.ncbi.nlm.nih.gov/nucl/MN328061)) contextualized with 1,202 dengue virus serotype 2 genomes available on Nextstrain. The circle bordered in red represents the position of CHRF_DenV002 in the tree. (B) Phylogenetic tree of CHRF_DenV002 contextualized with the closest genomes sequenced from Southeast and East Asian countries (access date, 18 October 2020).

matched reads and their relative abundance were calculated and mapped at the genus level and visualized.

The final nearly complete genome of the dengue virus consisted of 10,699 bases, with a GC content of 45.8% and an average coverage depth of 237×. It had 92.5% nucleotide and 97.5% amino acid sequence identity to the reference dengue virus serotype 2 genome (GenBank accession no. [NC_001474.2](https://www.ncbi.nlm.nih.gov/nucl/NC_001474.2)). The genome was submitted to the NCBI GenBank database (accession no. [MN328061](https://www.ncbi.nlm.nih.gov/nucl/MN328061)) and subsequently contextualized in the dengue virus serotype 2 build of Nextstrain (<https://nextstrain.org/dengue/>

denv2) among 1,202 available dengue virus serotype 2 genomes sampled between 1944 and 2019 (6) (Fig. 1A). The sequenced genome is closely related to dengue viruses seen in other Southeast and East Asian countries, specifically, Singapore, Malaysia, Indonesia, South Korea, and China (Fig. 1B).

Data availability. The dengue viral genome from Bangladesh has been deposited in GenBank (accession no. [MN328061](https://doi.org/10.1016/S0022-3476(97)70054-4)). The raw reads have been deposited in the NCBI Sequence Read Archive (SRA accession no. [SRR12901070](https://doi.org/10.1016/S1473-3099(19)30616-4)). The BioProject and BioSample accession numbers are [PRJNA672124](https://doi.org/10.1016/S1473-3099(19)30616-4) and [SAMN16557024](https://doi.org/10.1016/S1473-3099(19)30616-4), respectively.

ACKNOWLEDGMENTS

We are grateful to the Chan Zuckerberg Biohub and the Chan Zuckerberg Initiative for their intellectual support and guidance. We specifically thank Madeline Y. Mayday and Lillian M. Khan for their support with library preparation and Joseph L. DeRisi, Cristina M. Tato, and Rebacca Eggar for overall supervision and guidance with genome analysis.

This project was funded by the Bill and Melinda Gates Foundation (grant OPP1198769).

REFERENCES

1. Kautner I, Robinson MJ, Kuhnle U. 1997. Dengue virus infection: epidemiology, pathogenesis, clinical presentation, diagnosis, and prevention. *J Pediatr* 131:516–524. [https://doi.org/10.1016/S0022-3476\(97\)70054-4](https://doi.org/10.1016/S0022-3476(97)70054-4).
2. Hsan K, Hossain MM, Sarwar MS, Wilder-Smith A, Gozal D. 2019. Unprecedented rise in dengue outbreaks in Bangladesh. *Lancet Infect Dis* 19:1287. [https://doi.org/10.1016/S1473-3099\(19\)30616-4](https://doi.org/10.1016/S1473-3099(19)30616-4).
3. Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, Medina F, Colon C, Margolis H, Munoz-Jordan JL. 2013. Analytical and clinical performance of the CDC real time RT-PCR assay for detection and typing of dengue virus. *PLoS Negl Trop Dis* 7:e2311. <https://doi.org/10.1371/journal.pntd.0002311>.
4. Kalantar KL, Carvalho T, de Bourcy CFA, Dimitrov B, Dingle G, Egger R, Han J, Holmes OB, Juan Y-F, King R, Kislyuk A, Lin MF, Mariano M, Morse T, Reynoso LV, Cruz DR, Sheu J, Tang J, Wang J, Zhang MA, Zhong E, Ahlyong V, Lay S, Chea S, Bohl JA, Manning JE, Tato CM, DeRisi JL. 2020. IDseq: an open source cloud-based pipeline and analysis service for metagenomic pathogen detection and monitoring. *Gigascience* 9:giaa111. <https://doi.org/10.1093/gigascience/giaa111>.
5. Saha S, Ramesh A, Kalantar K, Malaker R, Hasanuzzaman M, Khan LM, Mayday MY, Sajib MSI, Li LM, Langelier C, Rahman H, Crawford ED, Tato CM, Islam M, Juan YF, de Bourcy C, Dimitrov B, Wang J, Tang J, Sheu J, Egger R, De Carvalho TR, Wilson MR, Saha SK, DeRisi JL. 2019. Unbiased metagenomic sequencing for pediatric meningitis in Bangladesh reveals neuroinvasive chikungunya virus outbreak and other unrealized pathogens. *mBio* 10:e02877-19. <https://doi.org/10.1128/mBio.02877-19>.
6. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, Sagulenko P, Bedford T, Neher RA. 2018. Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics* 34:4121–4123. <https://doi.org/10.1093/bioinformatics/bty407>.