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Complete genome sequence of dengue virus serotype 2 obtained from Chattogram, Bangladesh

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ABSTRACT The whole genome sequence of a dengue virus serotype 2 strain (GenBank accession number PQ657766) obtained from a dengue-infected hospitalized patient in Chittagong, Bangladesh, is classified as the cosmopolitan genotype V, with genetic alterations observed in several structural proteins. The genomic data were generated using Oxford Nanopore sequencing technology.

KEYWORDS Dengue, Bangladesh, Chattogram, WGS, DENV-2

D engue virus (DENV), an Orthoflavivirus in the Flaviviridae family, is a mosquito-borne virus that causes dengue fever and has an ~10.7 kb positive-sense RNA genome. In rare cases, infection progresses to dengue hemorrhagic fever or fatal dengue shock syndrome (1–3). Bangladesh's 2023 dengue outbreak was the deadliest, with 1,705 deaths (4), while over 14 million dengue cases and more than 10,000 deaths have been recorded globally in 2024 (5). Enhanced genomic surveillance is crucial to track DENV lineages (6) and aid preventive measures (7).

Blood serum samples were collected from dengue patients in government tertiary hospitals in Chittagong during the 2023 outbreak. Viral RNA was extracted using Chemagic Viral NA/gDNA H96 Kit (CMG-1049) on the Chemagic 360 system (PerkinElmer, USA) following the manufacturer's instructions. A real-time RT-PCR assay using the TagPath 1-Step Multiplex Master Mix (No ROX) Kit (Thermo Fisher Scientific, USA) was performed on the Bio-Rad CFX96 Touch real-time PCR system (Bio-Rad, USA) to determine the DENV serotypes. DENV serotyping primers and probes were sourced from a published article (8). Among the identified DENV serotypes, a DENV-2 positive (severe, hospitalized) case with a Ct value < 25 underwent whole-genome sequencing. cDNA was prepared from the isolated viral RNA using the PrimeScript cDNA Synthesis Kit (Takara Bio Inc., Japan) as per the manufacturer's protocol, followed by a multiplex PCR that was performed with GoTaq G2 Hot Start Polymerase (Promega Corporation, USA) and specific primer pools (9). Oxford Nanopore sequencing technology was utilized for the whole genome sequencing of the DENV-2 serotype. Sequencing libraries were prepared according to the manufacturer's V14 Ligation Sequencing protocol (Native Barcoding Kit V14 96, SQK-NBD114.96). The barcoded library (50 fmol) was loaded onto a MinION R10.4.1 Flowcell (FLO-MIN114) and sequenced on the MinION MK 1C platform. Base calling was performed using bcl2fastg, yielding 19.1M bases, with an average read length of 1.1 Kb, assessed by FastQC. Adapters/primers were removed using Trimmomatic software. Minimap2 version 2.28 (https://github.com/lh3/minimap2) was used for genome mapping against the reference genome NC_001474. The number of reads that were mapped to the reference genome was 10,723 bp, with a coverage depth of 82.5×.

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TABLE 1	List of genes	in the	analyzed	DENV-2	sequence,	including	amino	acid	alterations	in s	structural	
proteins (a	accession num	ber of t	the referen	nce geno	ome: NC_00)1474)						

Protein name	Gene name	Amino acid changes	List of alterations in the			
			protein sequence			
Structural protein	Polyprotein (POLY)	12	K9R (122A > G), S101T (397T			
			> A 399T > C), M104I (408G			
			> A), L108M (418C > A),			
			D143N (523G > A), V234A			
			(797T > C 798C > A), I241V			
			(817A > G), M249I (843G >			
			A), H262Y (880C > T), A266I			
			(892G > A 893C > T), T276A			
			(922A > G), M286I (954G >			
			A)			
	Anchored capsid protein	4	K9R (122A > G), S101T (397T			
	(ancC)		> A 399T > C), M104I (408G			
			> A), L108M (418C > A)			
	Capsid protein (C)	1	K9R (122A > G)			
	Membrane glycoprotein	7	D29N (523G > A), V120A			
	precursor (prM)		(797T > C 798C > A), I127V			
			(817A > G), M135I (843G >			
			A), H148Y (880C > T), A152I			
			(892G > A 893C > T), T162A			
			(922A > G)			
	Protein (pr)	1	D29N (523G > A)			
	Membrane glycoprotein (M)	6	V29A (797T > C 798C > A),			
			I36V (817A > G), M44I (843G			
			> A), H57Y (880C > T), A61I			
			(892G > A 893C > T), T71A			
			(922A > G)			
	Envelope protein (E)	1	M6I (954G > A)			

The completeness of the sequenced genome was determined by comparing it with the reference genome (NC_001474). The coverage breadth of our sequence was around 99%.



FIG 1 The randomly selected reference sequences were aligned with the study sequence using the Augur pipeline (https://github.com/nextstrain/augur). The tree was built using IQ-TREE 2 (https://github.com/iqtree/iqtree2) with 1,000 bootstraps (maximum likelihood). The analyzed sequence clusters within the DENV-2 serotype were classified into genotype V (cosmopolitan genotype) after comparison with representative global sequences.

The Kraken2 viral database was utilized to obtain a taxonomic identification. Analysis with BLASTn showed that the viral genome exhibited 99.57% similarity with the genomic sequence of the DENV-2 strain from Thailand (GQ868591). Phylogenetic analysis of whole genome sequences placed the Chittagong isolate PQ657766 within DENV-2 genotype-V (Fig. 1), linking it to a Southeast Asian/American lineage found in Southeast Asia and Central/South America. Notable genetic alterations were found in the DENV-2 serostructural proteins, including polyprotein, anchored capsid protein, membrane glycoprotein precursor, and membrane glycoprotein (Table 1).

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DATA AVAILABILITY

The assembled genome was deposited in GenBank under accession number PQ657766, and the reads were submitted under SRA ID SRR32137766.

ETHICS APPROVAL

The Ethical Review Committee of the Chittagong Medical College, Chattogram, Bangladesh approved the study (memo no: 59.27.0000.013.19.PG.2023.009/296).

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