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Epidemiology and evolution of dengue outbreaks in Bangladesh (2020−2023)

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ABSTRACT

Objective: The aim of the study is to provide a comprehensive overview of the epidemiology and evolution of dengue virus (DV) in Bangladesh from 2020 to 2023.

Methods: From January 1, 2020, to December 31, 2023, we analyzed the daily press releases on the dengue situation made public by the government. The published sequences from the period were then subjected to phylogenetic analysis, amino acid substitutions, evolutionary rate, and selection pressure analysis.

Results: In Bangladesh, dengue fever usually peaks during the monsoon season. However, the increase in cases began considerably earlier, at the end of April 2023 and the disease had claimed 1 705 lives, marking its highest annual death toll ever. Dhaka is the city most severely affected. The phylogenetic analysis revealed that the Bangladeshi DV-1 strains clustered far from any known genotype, indicating the circulation of a novel genotype, whereas the DV-3 strains clustered within the existing circulating genotype I. The DV-1 strains had a total of 107 amino acid alterations and two deletions, while the DV-3 strains had 17 alterations. The Bangladeshi DV-1 and DV-3 strains evolve rapidly. Additionally, selection pressure analysis revealed one positive selection site in DV-1 and three in DV-3, respectively, which require further research.

Conclusions: The circulation of novel genotypes, a higher mutation rate, and positive selection of DV might result in the recent dengue outbreaks in the country. The study will help in the development of strategies to control outbreaks in Bangladesh and other nations with similar conditions in the future.

KEYWORDS: Dengue; Outbreaks; Epidemiology; Evolution; Bangladesh

1. Introduction

The dengue virus (DV) causes dengue fever in humans and is transmitted through the bites of infected mosquitoes, primarily *Aedes aegypti*[1,2]. The number of dengue virus infection cases worldwide has surged over the past 20 years. In 2000, there were 505 430 reported cases; by 2010, that number had risen to over 2 400 138, and by 2015, it reached 3 312 040 cases. Additionally, the number of deaths increased from 960 to over 4 032 between 2000 and 2015[2].

Significance

Bangladesh experienced the deadliest outbreak of dengue fever in 2023, breaking the country's 24-year history of dengue. This study provides a comprehensive epidemiological overview of dengue epidemics in the country from 2020 to 2023. It also presents evidence of recent year-round dengue outbreaks in Bangladesh by reporting on novel genotype circulation, higher mutations, and positive selection in the dengue virus genome.

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Of all dengue cases worldwide, 75% occur in Southeast Asia and the Western Pacific region[3]. With an estimated 100-400 million infections occurring each year[2], dengue fever causes outbreaks globally and epidemics in tropical and subtropical regions, posing an imminent public health hazard, especially in underdeveloped nations like Bangladesh.

DV is a positive-sense, single-stranded RNA virus with an envelope, belonging the genus flavivirus within the Flaviviridae family[4]. Three structural proteins—the capsid (C), membrane (M), and envelope (E)—as well as seven non-structural (NS) proteins—NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5—are encoded by a single open reading frame in its genome[4]. The E protein makes up the outermost layer of the virus. This glycoprotein is associated with several crucial biological characteristics of dengue viruses, such as the induction of immunological responses and neutralizing antibodies[5]. Human dengue virus is classified into four serotypes (DV-1, DV-2, DV-3, and DV-4) based on a 30% variation in their total amino acid sequences[6,7]. The fifth dengue serotype, DV-5, was discovered in Malaysia in 2007[8]. Throughout their lifetime, a patient may contract more than one dengue virus serotype. Secondary infections with a heterologous serotype are more serious than the original infections[9].

In Bangladesh, dengue was first recorded as “Dacca fever” in 1964[10]. However, the first outbreak began in 2000[11], caused by DV-3[12], and continued with the circulating serotype. Bangladesh has reported cases of four DV serotypes, with DV-1 and DV-2 predominating until 2016[13]. Since 2019, DV-3 has dominated the serotype landscape; in 2023, DV-2 took over that position[14]. Bangladesh experienced the worst dengue outbreak on record in 2023. As of December 31, 2023, a total of 321 179 cases and 1 705 deaths had been reported[15], however, cases began rapidly increasing in late June. Compared to previous years, the reported case fatality rate is higher, at 0.53%[14]. The rise of dengue cases in Bangladesh can be attributed to the unusually high temperatures, high humidity, and sporadic rainfall, which have led to an increase in mosquito populations.

The study aimed to provide a comprehensive epidemiological overview of the dengue outbreaks in Bangladesh from 2020 to 2023. It also focused on the evolutionary analysis of the E protein. A more precise understanding of the epidemiology and evolution of the virus may help control future outbreaks.

2. Methods

2.1. Setting and subjects for epidemiological study

The data were obtained from the Directorate General of Health Services (DGHS) database (https://old.dghs.gov.bd/index.php/bd/home/5200-daily-dengue-status-report), which had released the confirmed dengue cases and associated deaths through a press release from the government. The information covers the period from January 1, 2020 to December 31, 2023 for the inhabitants of Bangladesh. The curves were plotted using Microsoft Excel 2016.

2.2. Phylogenetic analysis

Two phylogenetic trees (DV-1 and DV-3) for the *E* gene were generated in order to analyze the molecular characterization during the most recent outbreaks of dengue in Bangladesh. Due to the unavailability of viral sequences from 2020 to 2023 in the country on the National Center for Biotechnology Information (NCBI) website, no phylogenies for DV-2 and DV-4 were constructed. The phylogenetic study of DV-1 employed 110 sequences in total. In addition to the 57 sequences from 2020 to 2023, we also added nine sequences reported earlier from Bangladesh. A total of 113 sequences were used for the DV-3 phylogenetic analysis. This included 79 sequences from the study period and nine previously reported sequences from Bangladesh. In both instances, sequences with low gene coverage were excluded, and contemporaneous *E* gene sequences from other countries were obtained from the NCBI website in GenBank format and then converted into FASTA format to analyze the molecular evolution of Bangladeshi strains.

Phylogenetic analysis was conducted using MEGA X (version 10.0.5)[16]. The Maximum-likelihood method was used to construct the phylogenetic tree. Genetic distances were estimated using the Tamura-Nei model with gamma-distributed rates of variation among sites and a proportion of invariable sites (TN93+G+I), based on the Bayesian Information Criterion (BIC) score, and bootstrapping with 1000 replicates provided statistical support for the tree.

2.3. Amino acid substitutions

Amino acid alterations were analyzed by comparing the entire E

protein sequences with the reference one, the reference sequence for DV-1 was NC_001477 and NC_001475 for DV-3. A total of 57 sequences of DV-1 and 79 sequences of DV-3 from 2020 to 2023 were compared to the reference sequence.

2.4. Evolutionary rate

The Bayesian Markov Chain Monte Carlo (MCMC) approach in the BEAST2 package (v2.6.7)[17] was used to calculate the rate of nucleotide substitutions per site per year of the E proteins of DV-1 and DV-3 in Bangladesh from 2020 to 2023. In this case, sequences of 57 and 79 nucleotides from the E protein of DV-1 and DV-3, respectively, from Bangladesh from 2020 to 2023 were used along with the corresponding reference sequences. According to the BIC score, the TN93+G+I model was found to be the best-fit model for the datasets. For all the datasets, an uncorrelated relaxed molecular clock with a Bayesian skyline coalescent prior was employed. The chain lengths were run for 10 million generations, and trees were sampled every 1 000 generations to achieve a sufficient level of sampling, as assessed using the Tracer (v1.7.1).

2.5. Selection pressure

The impact of the selection pressure acting on each codon in the viral *E* gene was assessed using the online prediction web server Datamonkey (https://www.datamonkey.org/). The selection pressure for DV-1 and DV-3 was assessed using the same dataset employed to estimate the evolutionary rate. Three different approaches, including single-likelihood ancestor counting (SLAC)[18], mixed effects model of evolution (MEME)[19], and fast unconstrained Bayesian approximation (FUBAR)[20], were carried out with default parameters to estimate the ratio of non-synonymous to synonymous substitution rate (dN/dS) per codon site. Negative selection was considered to have occurred when the dN/dS ratio was≤1[21]. Positive and negative selection at each site were analyzed using at least two methods, based on statistical significance (P<0.1 or posterior probability>0.9).

3. Results

3.1. Seasonal trend of dengue outbreaks

The data collection faced limitations in 2020 due to COVID-19. However, in 2020, dengue cases have been reported every month throughout the year. The number of dengue cases increased significantly in the subsequent years. In 2021 and 2022, there were 28 429 and 61 089 cases of dengue fever respectively. The deadliest months in 2021 were August and September, while in 2022 it was October and November. In 2023, there were five times as many dengue cases as the previous year, shattering all previous records. The worst month ever for the country was September 2023 when the disease had spread to nearly 80 000 individuals (Figure 1).

The number of dengue-related deaths has been increasing every year, as has the total number of cases. Despite the lack of dengue-related data in 2020 as of COVID-19, three deaths were reported among 1 405 dengue cases (Figure 2A). On the contrary, there were 105 and 281 fatalities in 2021 and 2022, respectively (Figure 2B and 2C). In 2023, a total of 1705 deaths were recorded, making it the deadliest year on record. August, September and October had the highest number of deaths with 342, 396, and 359, respectively (Figure 2D).

3.2. Demographic characteristics of dengue in 2023

The geographic area depicted in Figure 3 represents the number of dengue fever cases reported in the country in 2023. With 169 321 cases (52.7%), the dengue epidemic has been most severe in the Dhaka division, followed by Chattogram with 44 435 cases (13.8%). These may be attributed to the city's dense population. Dengue fever has ravaged the inhabitants of Dhaka city alone, with 110 008 confirmed cases, accounting for 34.3% of all dengue incidences in the entire country (Figure 4A). Dengue fever is less prevalent in Sylhet, with 1 435 confirmed cases. The number dengue cases have varied between 5 540 and 38 049 in other divisions, including Mymensingh, Khulna, Rajshahi, Rangpur, and Barishal.

Furthermore, in 2023, nearly two-thirds of the affected people were male (Figure 4B). Every age group has been affected by the DV. However, dengue fever predominantly affected people aged 21-30 (27.0%), while most fatalities (18.8%) were reported in people aged 31-40 (Table 1).

3.3. Amino acid substitutions

To analyze the variability in E protein, the amino acid sequences of the Bangladeshi DV-1 and DV-3 isolates from 2020 to 2023 were compared with those of the reference strains of each type,

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NC_001477 and NC_001475, respectively. Any of the 495 DV-1 and 493 DV-3 sites in the alignment were considered as variable sites. In DV-1, a total of 107 substitution sites were observed in all the viruses circulating from 2020 to 2023. Among them, 21 substitutions were in domain I, 34 in domain II, 29 in domain III, and the remaining 23 were in the transmembrane domain. Two deletions of amino acids (T156 and E157) were also found in domain I (Figure 5A). These significant number of changes, particularly the deletion of two amino acids from the DV-1 E protein, further support the widespread dissemination of the novel genotype in Bangladesh. However, there were only 17 substitutions among the 493 variable sites in DV-3. The lowest number of substitutions (two) were in domain I, whereas seven and five substitutions were noticed in domain II and III, respectively (Figure 5B).

3.4. Phylogenetic analysis

Based on the nucleotide sequences of E protein, we investigated the evolutionary relationships between the DV sequences in Bangladesh from 2020 to 2023, in comparison to other dengue viruses that had previously circulated in both the country and other countries. The DV-1 strains studied were highly phylogenetically distinct from any known genotypes circulating globally (Figure 6). Even so, they clustered distantly from the sylvatic genotype (VI), and two isolates from India in 2019 clustered with these strains. Notably, the study identified two sub-clusters within these strains, likely attributable to their numerous distinctive mutations, which means the emergence and spread of a novel genotype throughout the country. In contrast, the DV-1 strains isolated in Bangladesh before 2020 were clustered with DV-1 genotype V, while all of the isolates for DV-3 from this study belonged to genotype I (Figure 7). Moreover, the study strains showed a high degree of genetic similarity to strains identified during earlier outbreaks in Bangladesh in the recent past decade (2010s), indicating a circulation of existing genotype.

3.5. Evolutionary rate

The *E* gene sequences of DV-1 and DV-3 in Bangladesh from 2020 to 2023 were analyzed to calculate the evolutionary rate. The rate was found to be higher for DV-1, which was 2.01×10^{-3} substitutions per site per year. Additionally, the rate for DV-3 was determined to be 1.05×10^{-3} substitutions per site per year (Table 2).

3.6. Selection pressure

Negative selection (dN/dS<1) was found to occur for the majority of codons in the *E* gene of DV-1 and DV-3. One site (475) of DV-1 and three sites (367, 368, 447) of DV-3 were detected to be under positive or diversifying selection pressure by only one method, MEME (Table 2).

Table 1. Distribution of dengue cases and deaths by age in 2023 [*n* (%)].

Age (years)	Dengue cases	Deaths
0-10	32 228 (10.0)	124 (7.3)
11-20	60 724 (19.0)	144 (8.5)
21-30	86 627 (27.0)	273 (16.0)
31-40	58 944 (18.4)	320 (18.8)
41-50	38 449 (12.0)	272 (16.0)
51-60	25 924 (8.1)	256 (15.0)
61-70	13 477 (4.2)	205 (12.0)
>70	4 806 (1.5)	111 (6.5)
Total	321 179	1 705

Note: The percentage (%) may be greater than 100% due to rounding.

Table 2. Evolutionary analysis and selection pressure of *E* gene of dengue virus (DV-1 and DV-3) in Bangladesh.

Types	Rate	Total sites	dN/dS		(AA position)		
			SLAC	MEME	SLAC	MEME	FUBAR
DV-1	2.01×10^{-3}(0.48-4.12)	495	**0.074**	**0.037**	0	1 (475)	0
DV-3	1.05×10^{-3}(0.81-1.30)	493	**0.078**	**0.075**	0	3 (367, 368, 447)	0

Rate: Evolutionary rate (subs./site/year) (95% highest probability density); dN/dS: Ratio of non-synonymous and synonymous sites; SLAC: Single-likelihood ancestor counting; MEME: Mixed effects model of evolution; FUBAR: Fast, unconstrained Bayesian approximation; AA position: Amino acid position.

P<0.1 are considered statistically significant and indicated by bold.

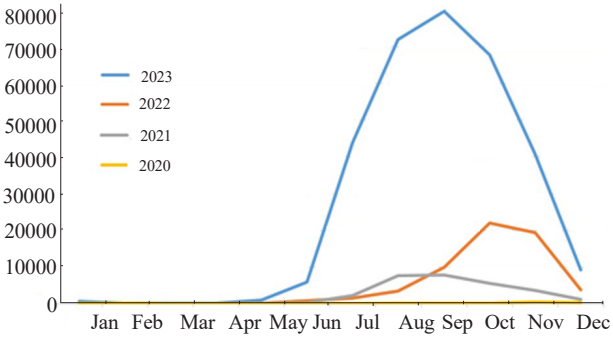

Figure 1. Monthly distribution of dengue cases from January 2020 to December 2023.

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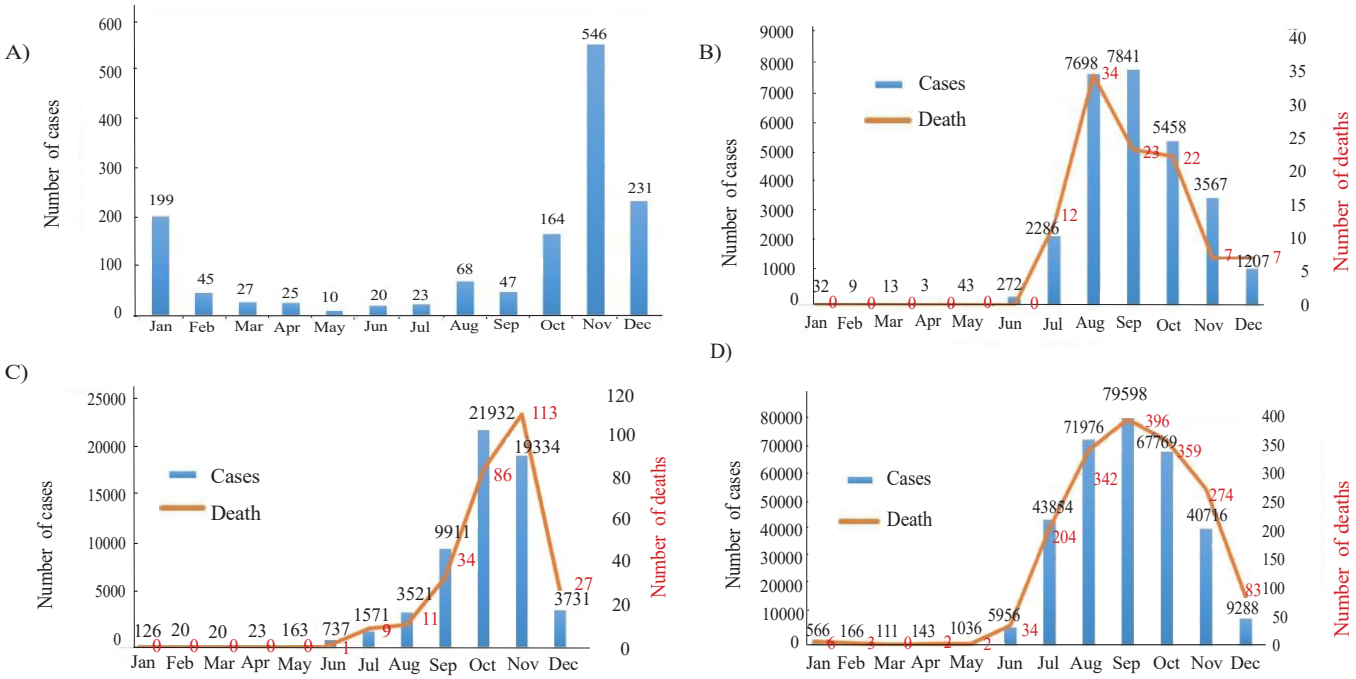

Figure 2. A) Monthly cases of dengue in 2020; Due to COVID-19, no monthly fatality data was available, with a total of 3 deaths reported. B) Monthly cases and mortality of dengue in 2021; C) Monthly cases and mortality of dengue in 2022; D) Monthly cases and mortality of dengue in 2023.

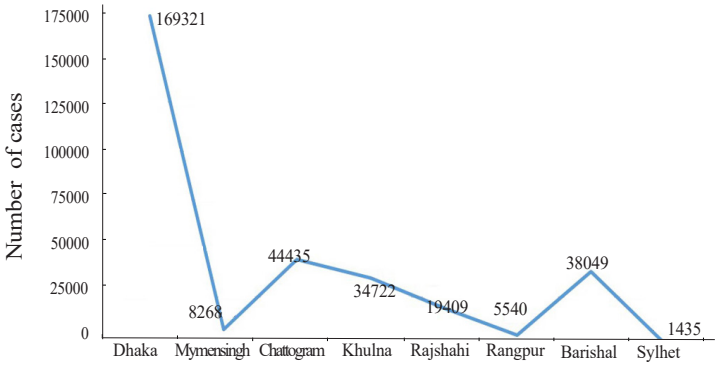

Figure 3. Distribution of dengue cases by division in 2023.

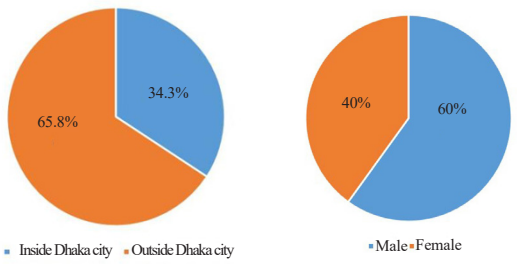

Figure 4. A) Dengue incidences within and outside of Dhaka city in 2023. B) Gender distribution of dengue cases in 2023.

A)

Isolate name	Domain Ⅰ																								
	6	29	37	46	50	52	138	141	156	157	158	160	163	169	171	173	174	176	177	180	182	184	293
Reference sequence	I	S	D	L	V	N	S	V	T	E	H	T	T	P	S	I	Q	T	D	A	T	D	I
Bangladeshi DV-1 strains	V	G	N	Q	A	Q	T	I			Q	V	E	S	V	A	I	P	E	T	G	E	E

Isolate name	Domain Ⅱ																										
	53	55	63	66	68	81	83	88	90	93	94	96	114	120	122	123	124	125	129	197	203	205	207	210	213	225	227
Reference sequence	P	V	A	S	T	T	V	T	F	R	R	F	I	K	V	T	K	L	I	V	K	S	L	K	L	S	S
Bangladeshi DV-1 strains	L	T	G	T	V	I	P	Q	Y	K	H	Y	V	Q	L	E	L	I	V	I	N	A	M	R	F	I	E

Isolate name	228	229	234	235	242	275	277
Reference sequence	Q	E	Q	D	T	T	T
Bangladeshi DV-1 strains	T	P	K	E	N	G	S

Isolate name	Domain Ⅲ																								
	300	304	305	307	309	313	320	322	323	325	327	329	339	340	342	343	345	346	347	357	360	363	377	380	382	384	389
Reference sequence	V	G	S	K	E	A	V	V	Q	K	E	T	S	Q	E	K	V	T	Q	I	D	K	Y	V	A	E	L
Bangladeshi DV-1 strains	A	N	A	V	K	S	I	I	K	E	K	E	T	E	G	Q	K	A	H	V	K	E	N	I	I	D	I

Isolate name	390	392
Reference sequence	S	F
Bangladeshi DV-1 strains	N	Y

Isolate name	Transmembrane domain																								
	425	429	430	432	435	436	442	445	446	454	461	466	471	472	475	477	478	481	482	484	485	491	492
Reference sequence	I	F	T	V	L	I	T	G	V	T	I	L	R	S	L	M	T	A	V	M	V	V	M
Bangladeshi DV-1 strains	V	L	N	L	M	V	S	T	A	I	V	I	K	N	M	F	S	V	I	I	I	A	V

B)

Isolate name	Domain Ⅰ		Domain Ⅱ							Domain Ⅲ					Transmembrane domain		
	132	169	68	81	124	219	223	231	270	301	303	362	377	383	404	452	480
Reference sequence	Y	T	I	V	P	A	T	R	N	T	T	P	V	N	E	V	A
Bangladeshi DV-3 strains	H	V	V	I	L	T	I	K	T	S	A	L	I	K	A	I	V

Non-polar Polar Polar (Basic) Polar (Acidic) Deletion

Figure 5. Amino acid substitutions of A) DV-1 and B) DV-3 E protein. The numbers represent the amino acid position.

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Figure 6. The maximum-likelihood (ML) phylogenetic tree of the nucleotide sequences of the *E* gene of DV-1 from various geographic locations, including Bangladesh, was constructed using MEGA-X. Study isolates were represented by the black circle (●). Bootstrap values for 1000 replicates were shown on major nodes of the tree, and values<70% were omitted. Genotypes of DV-1 were shown on the right, 'X' indicated novel genotype. The horizontal bar at the lower left indicated nucleotide substitutions per site.

Figure 7. The maximum-likelihood (ML) phylogenetic tree of the nucleotide sequences of the *E* gene of DV-3 from various geographic locations, including Bangladesh, was constructed using MEGA-X. Study isolates were represented by the black circle (●). Bootstrap values for 1 000 replicates were shown on major nodes of the tree, and values<70% were omitted. Genotypes of DV-3 were shown on the right. The horizontal bar at the lower left indicated nucleotide substitutions per site.

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4. Discussion

The incidence rate of dengue has risen in recent decades globally, with Southeast Asia, the western Pacific, and America being the most impacted regions[22]. In 2023, Bangladesh experienced the most severe dengue outbreak on record. Despite dengue being an endemic disease in Bangladesh, the most recent outbreak is exceptional due to its seasonality and early abrupt spike compared to previous years[23]. We have discussed the epidemiology and evolution of dengue from 2020 to 2023, providing insights into recent dengue outbreaks in Bangladesh.

In Bangladesh, the dengue season typically begins in early July and lasts until December, but in 2023, the outbreak began in late June. This might be due to climate change, which contributes to the spread of such mosquito-borne diseases. Aedes aegypti, the main dengue fever carrier in the area, finds new breeding grounds every time it rains[24]. A 1 mm increase in rainfall throughout the summer and monsoon seasons can lead to an 8% rise in dengue cases. In a recent study, dengue infections in Bangladesh were positively and significantly correlated with temperature and rainfall throughout the year[25]. Based on previous studies, the vast majority (over 90%) of dengue fever cases occur in August and September[26].

From 2020 to 2023, dengue surpassed COVID-19 as the leading cause of mortality in Bangladesh. As of December 31, 2023, there were 412 102 cases of dengue fever reported in this decade, with 2 094 of those cases resulting in fatalities due to its severity. The total number of deaths in 2023 is 1 705, accounting for more than 81%. The severity of the disease is demonstrated by this statistic. Moreover, with 34.3% of all cases, Dhaka city is the epicenter of the outbreak. The increasing population density is probably one of the main causes of this problem. Recently, incidences in Dhaka started to stabilize, but they have been rising in other parts of the country. Besides Dhaka, Chattogram and Barishal have experienced the most severe dengue outbreaks.

In the recent dengue outbreak in Bangladesh, gender distribution revealed a definite male predominance (60.0%), with nearly twice as many cases occurring as in males compared to females. The ratio of male to female dengue cases was comparable to earlier outbreaks noted in Bangladesh[27]. India likewise came up with a similar outcome[28, 29]. In contrast, findings in South America showed that the proportion of female patients was either equal to or higher than that of male cases[30].

The majority of dengue cases in Bangladesh have historically affected young adults. More than 80% of infections during the first pandemic in 2000 were in adults (>18 years old), with cases most frequently occurring in people between the ages of 18 and 33[11]. Likewise, in 2023, the most confirmed cases (27.0%) belonged to the 21-30 age group. India[28], Sri Lanka[31], Brazil[32], and Ethiopia[33] have reported comparable results.

The phylogenetic analysis conducted in this study for DV-1 has shown some fascinating phenomena. According to the phylogeny based on the entire DV-1 *E* gene sequences, a distinct genotype (X) may have been circulating throughout the country since the beginning of this decade. This genotype was even grouped distantly with the sylvatic genotype (VI). It completely displaced the genotype V strains that were previously prevalent in Bangladesh[34], and some identical strains were dispersedly found in India in 2019. In contrast, all strains of DV-3 in Bangladesh from 2020 to 2023 were clustered together under the same genotype (I). According to previous reports, genotype I has displaced genotype II in Bangladesh as of 2017, which was also supported by our study[34].

For amino acid sequence comparisons, the reference strains for each serotype (DV-1 and DV-3) were compared with the entire E protein sequences in Bangladesh from 2020 to 2023. The analysis revealed that both the DV-1 and DV-3 strains had distinctive amino acid changes. In addition, two more amino acid deletions at positions 156 and 157 were found in DV-1. The host immune response, may be significantly affected by these alterations and deletions since the E protein is the primary antigenic determinant[35]. The structural stability, host range, tropism, and virulence of DV-1 in the country may also be impacted[36]. A severe type of dengue fever could evolve as a result of these modifications. This could also affect the development of strain-specific drugs and vaccines against dengue. To fully comprehend the importance of substitutions in amino acids, further research is necessary.

In this study, a higher evolutionary rate was observed. For DV-1 and DV-3, the rate was estimated to be 2.01×10^{-3} and 1.05×10^{-3} substitutions per site per year, respectively. According to some previous studies, this was higher in both cases[22, 35, 36]. This indicates that, compared to some other RNA viruses like HIV or influenza, which typically have evolutionary rates considerably in excess of 1×10^{-3} substitutions per site per year[37], dengue viruses in Bangladesh evolves slightly faster.

The selection pressure on the virus can demonstrate whether or not

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the mutations can be retained. dN/dS>1 indicates positive selection pressure, while dN/dS≤1 denotes neutral selection. The majority of codons were found to be under negative selection, according to the selection pressure analysis, which was related to the constraints of arbovirus evolution[38]. However, three sites in DV-3 and one positive selection site in DV-1 were screened. Further research is required to fully understand the effects of these positively selected sites.

The significant drawback of this study was the analysis of only the DV-1 and DV-3 sequences of the *E* gene due to the lack of data for DV-2 and DV-4. Additionally, there were no sequences of DV-1 and DV-3 for 2023. However, this study includes important findings that require corroboration through further analyses.

5. Conclusions

The study demonstrates that Bangladesh is at risk for catastrophic dengue outbreaks due to the co-circulation of new genetic variants with existing genotypes of distinct types of dengue viruses. Therefore, it is crucial to continuously monitor the virus and its variants in order to comprehend the epidemiological and evolutionary dynamics and get ready for any future outbreaks.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

Hasan M conceived, designed, and supervised the study, as well as revised the draft. Islam S performed the experiment and analyzed the data, did literature search, and wrote the first draft. Both authors approved the final draft.

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