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Molecular epidemiology of circulating dengue serotypes in Dhaka, Bangladesh: 2023 outbreak

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ABSTRACT

Objectives: Dengue fever is a significant global health concern, especially in tropical regions, including Bangladesh, which reported 316,773 cases and 1652 deaths in 2023. This study intends to explore the circulating serotypes of the dengue virus (DENV) and their clinical associations across Bangladesh.
Methods: The study enrolled 1317 febrile patients presenting with at least two symptoms (nausea or vomiting, rash, aches and pains, positive tourniquet test, and leukopenia) at Dhaka Medical College and Hospital (DMCH) between July and August 2023. They provided demographic data, clinical features, and blood samples. DENV was tested using a rapid detection test and real-time reverse transcription-polymerase chain reaction.
Results: Of 1317 patients, 300 (23%) tested positive by rapid detection test and 775 (59%) by real-time reverse transcription-polymerase chain reaction. The median age of participants was 20 years, with 61% of confirmed cases being male. The DENV-2 serotype was found in 88%, DENV-3 in 12%, and only one was found positive for DENV-4. Overall, 80 percent of dengue-positive cases showed warning signs (abdominal tenderness, persistent vomiting, rash, and bleeding). The severity of cases was significantly higher with DENV-2.
Conclusions: This study generates insights into the distribution of dengue serotypes and their association with disease severity. The DENV-2 was found as the dominant serotype in the 2023 outbreak.

Introduction

Dengue viral infection is a vector-borne disease spread by the *Aedes aegypti* mosquito. The disease burden is more prevalent in tropical regions, especially Asia, followed by Africa and America [1–3]. It has increased the global threat, affecting 400 million dengue cases annually in subtropical and tropical areas, and making approximately 50% of the worldwide population susceptible to dengue infection [4–6]. Bangladesh has experienced several outbreaks since 2000, the first one reported in 1964. Population growth, unplanned urbanization, climate change, and inadequate vector control measures have been linked to a favorable environment for *Aedes* mosquito breeding and viral transmission [7]. A large outbreak was first recorded in 2000, with 5551 hospitalized cases and 93 deaths [8,9]. One of the severe outbreaks was in 2019 when 101,354 confirmed cases and 164 deaths were reported [10]. Two major outbreaks occurred in Dhaka city in 2021 (28,429 cases and 85 deaths) [11] and in 2022 (61,732 cases and 281 deaths) [12,13]. Between 2000 and 2022, Bangladesh recorded 788 dengue-related deaths, of which 492 (over 62%) occurred between 2019 and 2022 [14]. In 2023, the Directorate General of Health Services (DGHS) published a report that revealed alarming data of 316,773 dengue-positive cases and 1652 deaths, yielding a case fatality rate (CFR) of 0.5%. This was the highest record of cases in the recent dengue outbreak history in Bangladesh [15]. This unprecedented surge emphasizes the need for a wide range of scientific insights to manage the dengue outbreak and its impacts on society effectively.

Dengue manifests from a spectrum of a mild fever to a severe illness. Approximately 25% of infected individuals present symptoms that include sudden high fever, body pain, myalgia, arthralgia, rash, headache, retro-orbital pain, nausea, and vomiting. The significant findings associated with dengue fever are thrombocytopenia and increased hematocrit level by 20% which may proceed to shock [15]. The febrile illness starts to subside between 3-7 days; however, some may progress to severe disease associated with, intravascular volume depletion due to increased blood vessel permeability and plasma extravasation. It can be fatal if left unmanaged and untreated in time [1,16,17].

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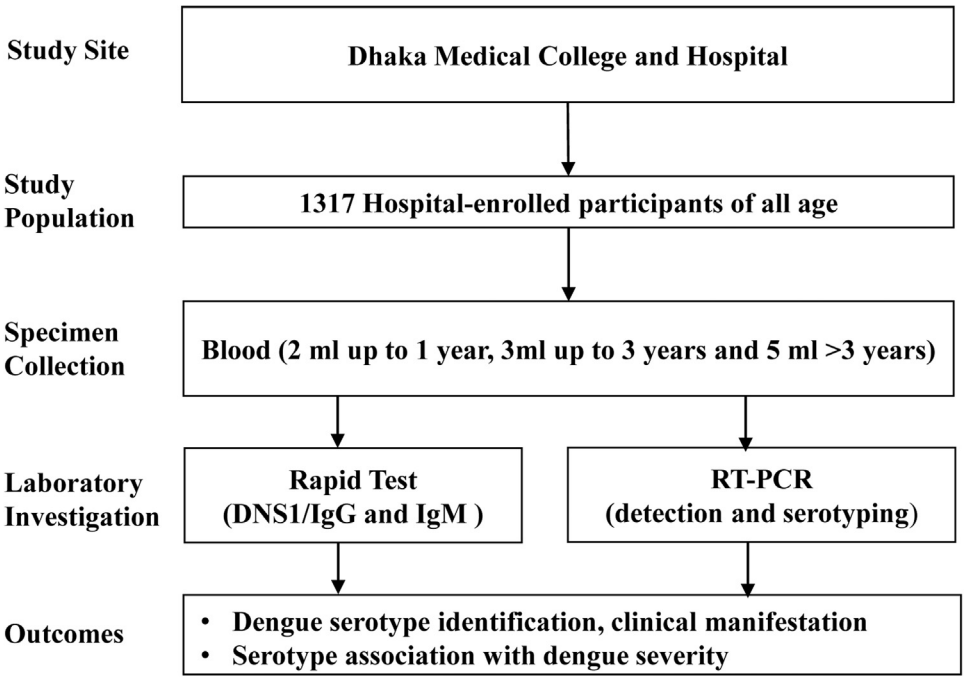

Figure 1. Study design of the dengue outbreak in 2023.
DNS1, dengue non-structural protein-1; Ig, immunoglobulin; RT-PCR, reverse transcription-polymerase chain reaction.

Moreover, circulating dengue serotypes (DENV 1-4) are associated with the severity of the dengue infection [18]. Four DENV serotypes with varying degrees of severity are responsible for dengue viral fever. Serotype dominance has fluctuated in recent years leading to changing impacts on the disease severity and transmission pattern of dengue viral infection. Secondary or repeated infections by different serotypes pose a risk of developing severe illness through antibody-dependent enhancement (ADE) [19]. In Bangladesh, the initial outbreak in 2000 was attributed to DENV-3 [20,21]. In subsequent years until 2016, all four serotypes were detected in the reported cases being DENV-1 and DENV-2 the most prominent serotypes [22]. In 2017 and 2018, the re-emergence of DENV-3 was identified although DENV-2 was the most prevalent serotype in 2018 [23]. But DENV-3 re-emerged as the dominant serotype in 2019 in the country again and continued till 2022 (Supplementary Figure 1) [24,25]. However, some studies claimed DENV-2 to be the country's most prevalent circulating serotype in 2022 [26].

Considering the complex dengue epidemiology and the challenges in patient management, this study was designed to explore the distribution of serological types of the dengue virus (DENV) and their relationship with disease severity levels.

Materials and methods

Study design

In the 2023 dengue outbreak, a cross-sectional study was carried out at Dhaka Medical College and Hospital (DMCH) (Figure 1). All febrile patients who visited the virology lab (outdoor settings) for testing as well as those admitted to the medicine ward (indoor settings) were selected for this study. The inclusion criteria encompassed individuals of all ages and sexes who met the World Health Organization (WHO) 2009 case definition. Specifically, patients presenting with fever and at least two symptoms, such as nausea or vomiting, rash, aches and pains, positive tourniquet test, leukopenia, or any warning signs, were included. Conversely, patients undergoing surgical procedures at the time of enrollment were excluded. All participants, irrespective of age and gender, provided informed written consent to donate blood and aid in filling up a questionnaire before enrollment. Consent from a parent or legal guardian was obtained for minors. Additionally, blood samples were not collected from individuals who declined to provide written consent. We enrolled 1317 suspected dengue cases between July 22 and August 31, 2023. In Bangladesh, dengue infection typically peaks between May and December. While the virus continued to circulate beyond August 2023, we ceased sample collection upon reaching our target number of participants and due to funding limitations. The assigned trained medical technologist collected blood samples from all participants: 2 ml from participants up to 1 year old, 3 ml from those aged 2-3 years, and 5 ml from participants older than 3 years. Blood collection volumes were determined based on children's age, weight, and health status, following WHO guidelines and icddr,b Institutional Review Board (IRB) recommendations. Additionally, we plan to conduct future research, focusing on immunological analysis and biomarker investigations.

Dengue fever definition

This study defined dengue fever, dengue with and without warning signs, dengue hemorrhagic fever (DHF), and severe dengue fever according to the WHO 2009 revised criteria. The patient had confirmed dengue fever with bleeding manifestations; therefore, it was considered DHF. Additionally, symptoms such as restlessness, mucosal bleeding, abdominal pain or tenderness, persistent vomiting, lethargy, and any sign of clinical fluid accumulation were considered warning signs. Moreover, severe bleeding, fluid accumulation with respiratory distress, dengue shock syndrome, or severe organ involvement were defined as severe dengue fever. Utilizing the WHO 2009 criteria and available clinical data, we categorized cases into severe and non-severe dengue fever groups.

Laboratory investigation

Rapid detection test

Blood samples were tested for DNS1 (dengue non-structural protein-1) antigen/IgG (immunoglobulin G) and IgM (immunoglobulin M) antibodies using rapid test kits (SD BIOLINE Dengue DUO rapid tests) at the Virology Laboratory, DMCH, and sent to the Virology Laboratory,

Table 1
Sequence of the primers-probes used for the DENV screening and serotyping.

Test type	Oligonucleotide	Sequence (5'-3')
DENV screening	Forward primer	GARAGACCAGAGATCCTGCTGTCT
	Reverse primer	ACCATTCCATTTTCTGGCGTT
	Probe	AGCATCATTCCAGGCAC
DENV-1	Forward primer	CAAAAGGAAGTCGYGCAATA
	Reverse primer	CTGAGTGAATTCTCTCTGCTRAAC
	Probe	CATGTGGYTGGGAGCRCGC
DENV-2	Forward primer	CAGGCTATGGCACYGTCACGAT
	Reverse primer	CCATYTGCAGCARCACCATCTC
	Probe	CTCYCCRAGAACGGGCCTCGACTTCAA
DENV-3	Forward primer	GGACTRGACACACGCACCCA
	Reverse primer	CATGTCTCTACCTTCTCGACTTGYCT
	Probe	ACCTGGATGTCGGCTGAAGGAGCTTG
DENV-4	Forward primer	TTGTCCTAATGATGCTRGTCG
	Reverse primer	TCCACCYGAGACTCCTTCCA
	Probe	TYCCTACYCCTACGCATCGCATTCCG

Abbreviation: DENV, dengue virus.

icddr,b for molecular testing. Patients with either DNS1 or IgM positive were regarded as positive, both DNS1 and IgM negative were considered negative, and patients with only IgM negative results but no DNS1 results were treated as invalid by rapid tests.

Molecular test

After receiving the samples at icddr,b, we processed them under BSL-2 laboratory conditions with BSL-3 practices within a certified Class II biological safety cabinet (BSC). We extracted and purified the dengue viral RNA using a Chemagic Viral NA/gDNA kit from blood samples following recommended protocols. Real-time reverse transcription-polymerase chain reaction (Dengue virus genesig detection kit, UK) was used to identify dengue viral nucleic acid. We used DENV-specific consensus primers and probes to detect DENV-RNA and their serotypes. The sequences of primers and probes of this study are listed in Table 1. The master mix was prepared using the following components and compositions: TaqPath 1-Step Multiplex Master Mix, 5.0 µl; forward and reverse primers (final concentration 0.5) and probes (final concentration 0.25), 0.5 µl; and nuclease-free water, 8.5 µl. The thermal program for both screening and serotyping was set as follows: 1st step, 25°C for 2 minutes; 2nd step, 53°C for 10 minutes; 3rd step, 95°C for 2 minutes; followed by 45 cycles at 95°C for 15 seconds and 60°C for 1 minute. A sample generating a threshold cycle (C_t) value ≤40 was considered positive.

Data analysis

We analyzed the data using SPSS version 21. Frequencies and percentages were used to express categorical variables. The $\chi 2$ test was used to compare categorical data whereas Fisher's exact test was used if expected cell sizes were <5. Analyses were conducted at a 95% CI (confidence interval) level along with P <0.05 significance level.

Results

Detection and serotype distribution

In this study, 1317 participants (1056 outpatients and 261 inpatients) were enrolled between July 22 and August 31, the peak season for dengue infection in Bangladesh in 2023. The median age of all participants was 20 years, and approximately 61% of the confirmed dengue cases were male. DENV-RNA was positive in 300 (23%) participants, found in rapid detection test, and 775 (59%) participants, found in real-time polymerase chain reaction. The remaining samples could not be serotyped due to their low viral load, indicated by a high C_t value of 36.63 ± 1.56. Among the serotyped 674 samples, 593 (88%) were DENV-2, 80 (12%) were DENV-3, and only one was positive for DENV-4. The distribution of dengue-positive cases across age groups was as follows: 29% (n = 221) in 1-10 years, 20% (n = 154) in 11-20 years, 30% (n = 231) in 21-30 years, 11% (n = 86) in 31-40 years, 5% (n = 39) in 41-50 years, 4% (n = 27) in 51-60 years, and 2% (n = 12) in those older than 60 years. The highest number of cases was observed in the 21-30 years age group, followed by 1-10 years and 11-20 years, with lower case numbers in older age groups.

Regarding serotype distribution across different age groups, it was observed that DENV-2 was responsible for over 80% of positive cases in all age groups. The highest incidence was found in the 31-40 years age group, with 94% of cases, followed by 91% in the 21-30 years and over 60 years age groups. The 1-10 years and 51-60 years age groups had 87% of cases, while the 11-20 years and 41-50 years age groups had 84% (Figure 2). In men, 90% (n = 387) tested positive for DENV-2, 10% (n = 44) for DENV-3, and only one sample was positive for DENV-4. Among women, 85% (n = 206) were positive for DENV-2, while 15% (n = 36) tested positive for DENV-3 (Figure 3). We compared the distribution of clinical symptoms between DENV-2 and DENV-3-associated severe and non-severe cases whose complete data were available (n = 443). Overall, 18% (80/443) progressed to severe dengue. The comparison revealed that cough (43%), rash (18%), and retro-

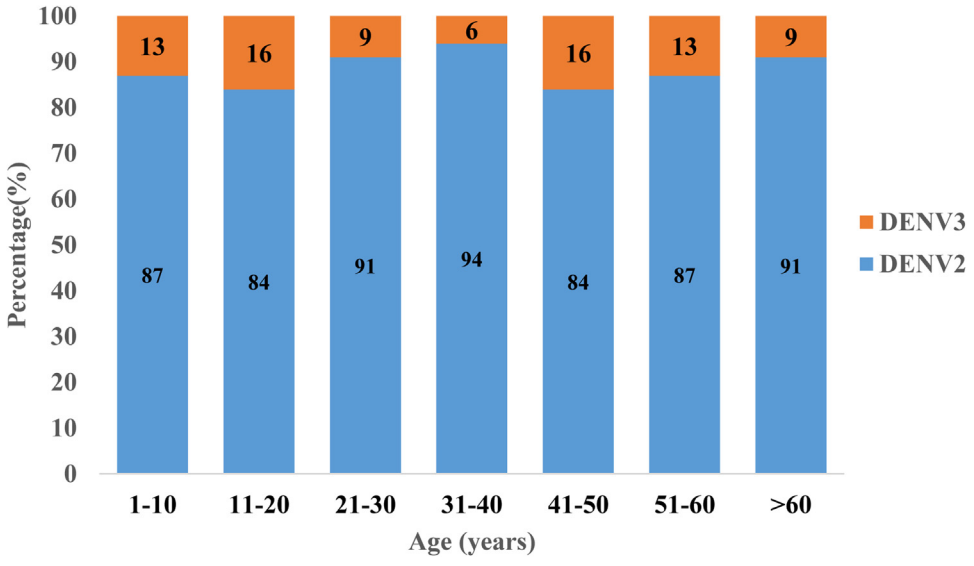

Figure 2. Distribution of DENV serotypes among age groups.
DENV, dengue virus.

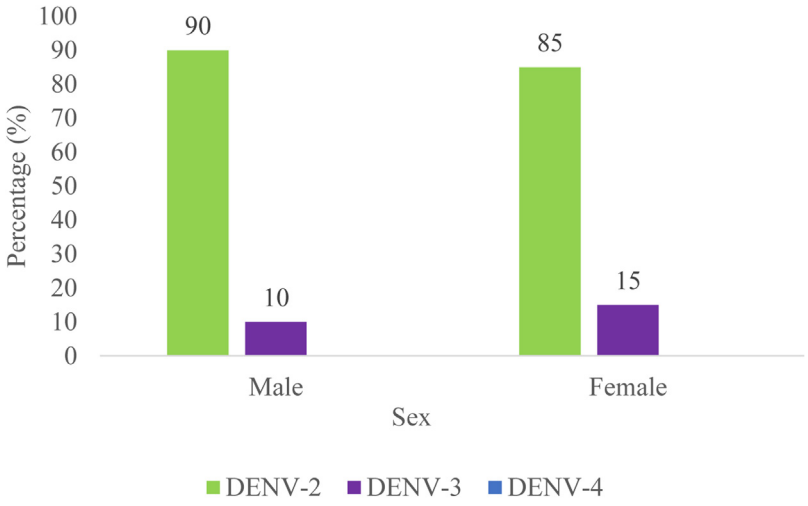

Figure 3. Distribution of DENV serotypes based on sex. DENV, dengue virus.

Table 2
Distribution of DENV serotypes in severe and non-severe cases according to warning signs.

Clinical Symptoms	Severe	Non-severe	*P*-value	DENV-2			DENV-3		
	n = 80	n = 363		Severe n = 70	Non-severe n = 280	*P*-value	Severe n = 6	Non-severe n = 40	*P*-value
Without warning sign									
Headache	34 (43)	180 (50)	NS	27 (39)	137 (49)	NS	4 (67)	20 (50)	NS
Retro orbital pain	13 (16)	14 (4)	**<0.001**	11 (16)	9 (3)	**<0.001**	2 (33)	5 (13)	NS
Body pain	38 (48)	173 (48)	NS	33 (47)	136 (49)	NS	4 (67)	21 (53)	NS
Running nose	9 (11)	54 (15)	NS	9 (13)	43 (15)	NS	00 (0)	5 (13)	NS
Cough	34 (43)	94 (26)	**0.003**	32 (46)	73 (26)	**<0.001**	2 (33)	10 (25)	NS
Lose motion	23 (29)	95 (26)	NS	20 (29)	75 (27)	NS	2 (33)	12 (30)	NS
Rash	14 (18)	12 (4)	**<0.001**	13 (19)	11 (4)	**<0.001**	0 (0)	1 (3)	NS
Taste for food	20 (25)	140 (39)	NS	18 (26)	107 (38)	0.04	1 (17)	15 (38)	NS
With warning sign									
Abdominal Tenderness	53 (66)	110 (30)	**<0.001**	49 (70)	93 (33)	**<0.001**	2 (33)	10 (25)	NS
Persistent vomiting	61 (76)	204 (56)	**0.004**	54 (77)	167 (60)	**<0.001**	4 (67)	17 (43)	NS
Mucosal bleeding	39 (49)	48 (13)	**<0.001**	36 (51)	39 (14)	**<0.001**	1 (17)	6 (15)	NS
Pregnancy	0 (1)	1 (1)	NS	0 (0)	1 (0.4)	NS	0 (0)	0 (0)	NS
Diabetes mellitus	7 (9)	19 (5)	NS	6 (7)	11 (4)	NS	1 (17)	6 (15)	NS

Results are presented as n (%). Abbreviation: DENV, dengue virus; NS, non-significant.

orbital pain (16%) appeared as non-warning signs and were significantly (P <0.001) higher in the severe cases compared to non-severe cases (Table 2). Moreover, persistent vomiting (76%), abdominal tenderness (66%), and bleeding (49%) were manifested as warning signs and were significantly higher (P <0.001) in severe cases compared to non-severe cases.

In total, 70 out of 350 (20%) were severe dengue cases among DENV-2 positive cases, and 6 out of 46 (13%) were severe dengue cases among DENV-3 positive cases. Additionally, among those without warning signs, cough (46%), altered taste for food (26%), rash (19%), and retro-orbital pain (16%) were more frequent in DENV-2-associated severe cases than in DENV-3. However, headache (67%), body aches (67%), and loose motion (33%) were more prevalent in DENV-3-associated severe cases. Furthermore, among those with warning signs, persistent vomiting (77%), abdominal tenderness (70%), and bleeding (51%) were significantly higher (P <0.001) in DENV-2-associated severe cases than in DENV-3. No clinical symptoms significantly differed between DENV-3-associated severe and non-severe cases.

DENV-2 vs DENV-3 serotypes

Next, we explored the severity of dengue fever with either DENV-2 or DENV-3 serotypes and the symptoms associated with severe dengue. Severe organ involvement (23%) and severe bleeding (31%) were higher in DENV-2 than in DENV-3 (17% severe organ involvement and 14% severe bleeding) positive cases, whereas dengue shock syndrome (83%) and clinical fluid accumulation with respiratory distress (66%) were higher in DENV-3 cases than in DENV-2 (77% dengue shock syndrome and 41% clinical fluid accumulation with respiratory distress). Furthermore, the symptoms associated with dengue shock syndrome, clinical fluid accumulation with respiratory distress, severe organ involvement, and severe bleeding did not change significantly between DENV-2 and DENV-3 (Table 3).

Discussion

This study examined the distribution of circulating dengue serotypes and their association with disease severity. Throughout the study period, DENV-2 serotypes were more prevalent among all patients than DENV-3 serotypes. However, DENV-2 cases exhibited a higher frequency of severe bleeding and organ involvement, while DENV-3 cases showed a greater prevalence of plasma leakage and breathlessness.

It is notable that although previous studies have shown a higher prevalence of dengue among older people [27], the present study found a more significant proportion of participants aged 1-30 years. This underscores the importance of strengthening public health awareness campaigns to ensure wider access to testing facilities, thereby effectively limiting disease transmission. Furthermore, the higher representation of male participants aligns with earlier research, indicating potential

Table 3
Comparison of severe and non-severe cases between DENV-2 and DENV-3.

Severe dengue symptoms (yes)	DENV-2 n = 350	DENV-3 n = 46	*P-value*
Dengue shock syndrome	54 (77)	5 (83)	NS
Low blood pressure	69 (20)	6 (13)	NS
Rapid breathing	43 (12)	10 (22)	NS
Cold moist skin hands	40 (11)	5 (11)	NS
Decreased urine output	39 (11)	5(11)	NS
Clinical fluid accumulation with respiratory distress	29(41)	4(66)	NS
Pleural effusion	20 (6)	2 (4)	NS
Edema	39 (11)	5 (11)	NS
Abdominal fluid	19 (5)	4 (9)	NS
Breathlessness	159 (44)	24 (52)	NS
Severe organ involvement	16 (23)	1 (17)	NS
Liver enlargement	6 (2)	1 (2)	NS
Central nervous system involvement (Lethargy)	10 (3)	0 (0)	NS
Renal failure	3 (1)	0 (0)	NS
Severe bleeding	22 (31)	1(14)	NS
Gum bleeding	8 (11)	0 (0)	NS
Conjunctival bleeding	31(42)	5 (71)	NS
Melena/Fresh blood per rectum	9 (12)	1 (14)	NS

Results presented as n (%). Abbreviation: DENV, dengue virus.

sex-specific susceptibilities to dengue attributed to diverse working environments and biological disparities [28,29].

Consistent with previous research, our findings confirm a shift in dengue serotype prevalence in Bangladesh since 2016 [30]. However, the resurgence of DENV-2 observed in this study underscores the dynamic nature of dengue circulation patterns in Dhaka. Secondary infections with heterologous serotypes are known to be responsible for the ADE effect and may increase the risk of severe dengue. As DENV-2 circulation re-emerged in 2023, we found that several clinical symptoms such as cough, retro-orbital pain, abdominal tenderness, persistent vomiting, rash, severe organ involvement, and bleeding were higher at a significant rate in DENV-2-associated severe cases in comparison with non-severe cases. This result is consistent with the ADE mechanism and suggests that DENV-2 re-emergence was responsible for the severity of the 2023 outbreak, as DENV-3 had been predominantly co-circulating in the previous years. Moreover, this result highlights the need for mass screening and continuous genomic surveillance to guide targeted intervention strategies to reduce disease severity [31].

This study has certain limitations, although it offers valuable insights into dengue epidemiology and diagnostic hurdles. First, only focusing on Dhaka districts restricts the applicability of our findings to the broader national context. Second, challenges in participant follow-up, the inability to evaluate secondary dengue infections, and the reliance on clinical symptoms to identify severe cases emphasize the necessity for extensive biomarker testing in future research. Third, we could not diagnose whether the patients had secondary infections caused by heterologous serotypes; thus, comprehensive genomic analyses are essential to uncover the underlying mechanisms of dengue severity. However, the prevalence of DENV-2 and DENV-3 is incomparable due to the limited number of DENV-3 positive cases.

DENV-2 re-emerged in Bangladesh after 2 decades, playing a significant role in the severity of the outbreak. From 2000 to 2022, DENV-3 was the predominant circulating serotype, leading to the development of immunity against it. However, in 2023, DENV-2 became the dominant serotype, coinciding with an increase in fatality rates, possibly due to ADE. In contrast, neighboring countries such as India and Nepal have experienced more frequent DENV-2 outbreaks, resulting in partial population immunity, which has mitigated infection severity and reduced mortality rates. Continuous surveillance with larger sample sizes across various geographical regions would offer a more accurate understanding of the association between clinical manifestations and dengue serotypes. Despite these limitations, the study adds to the knowledge of dengue fever and sets the stage for future research to mitigate its impact in Bangladesh.

In conclusion, our study addresses current gaps in dengue outbreak management by elucidating diagnostic challenges and the distribution of serotypes with clinical outcomes. These insights will assist policymakers in making informed decisions about mass screening to reduce the transmission and burden of the disease.

Declarations of competing interest

The authors have no competing interests to declare.

Funding

icddr,b

Ethical approval

The study protocol (Protocol Number – ERC-DMC/ECC/2023/221) was authorized by the institutional review board (IRB) of Dhaka Medical College and Hospital. The parents or legal guardians of children and adult patients' consents were obtained before filling out a questionnaire and collecting the blood samples for laboratory analysis.

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Author contributions

MR, SSB, NS, and KN contributed to the conception and study design. SRT, KN, and MNB conducted data analysis, interpreted the results, and were involved in drafting the manuscript. SA, AR, YK, and SBH performed laboratory activities and provided intellectual input. AKG and AHB assisted in the collection of clinical information. Additionally, all authors carefully reviewed the draft of the manuscript and approved it for publication.

Use of AI

During the preparation of this work, we used ChatGPT 4.0 to edit the language of this manuscript. After using this tool, we reviewed and edited the content as needed and took full responsibility for the content of the publication.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2025.100597.

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