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Research Article

Dengue dynamics: Unraveling serotype trends over a decade at Rawalpindi Medical University

Muhammad Khurram[1], Muhammad Umar[2], Kehkshan Jabeen[3*], M. Mujeeb Khan[4], Arshad Rabbani[1], Shahzad Manzoor[1], Saima Ambreen[1]

[1]Department of Medicine, Holy Family Hospital, Rawalpindi Medical University, Rawalpindi, Pakistan.
[2]Department of Gastroenterology, Rawalpindi Medical University, Rawalpindi, Pakistan.
[3*] Department of Pathology, Rawalpindi Medical University, Rawalpindi, Pakistan.
[4]Department of Infectious Diseases, Holy Family Hospital, Rawalpindi Medical University, Rawalpindi, Pakistan.

***Corresponding author's email:** kehkashan.jabeen@yahoo.com

Abstract

Dengue fever poses a persistent health challenge in Pakistan, marked by cyclic epidemics driven by serotype shifts. This study analyses decade-long trends (2013–2024) in Dengue Virus (DENV) serotype prevalence at Rawalpindi Medical University (RMU) to inform predictive models and improve prevention and control strategies. Our study looked at dengue cases confirmed by lab tests at RMU from 2013 to 2024. We identified the most common dengue serotypes infecting the population each year and tracked changes in serotype patterns over time. A chi-square test was used to analyse the data trends. From 2013 to 2024, the dengue serotype landscape at RMU was predominantly shaped by DENV-2, which began as the sole serotype in 2013 and maintained its dominance, peaking at 72% in 2017. Notable shifts occurred with DENV-3 taking precedence in 2014 and a co-dominance of DENV-1 and DENV-2 in 2019. By 2023, DENV-1 surged to become the dominant serotype at 71%, only for DENV-2 to reclaim supremacy in 2024 with a striking 90%. While data gaps for certain years limit a comprehensive analysis, the study underscores significant trends: the persistent prevalence of DENV-2, the emergence of DENV-1, and the episodic presence of DENV-3. DENV-2 has been predominant in a statistically significant way ($p = 0.018$) over these years. These findings align with regional dengue patterns, underscoring the interconnected nature of its transmission. The dominance of DENV-2 highlights critical implications for disease severity and targeted public health responses. The data reveals shifting patterns in dengue virus serotypes over time. To stay ahead of potential outbreaks, it's crucial to continuously monitor these changes and adjust our strategies accordingly.

Keywords: Dengue virus, Serotype prevalence, Rawalpindi Medical University, Epidemiology, Public health.

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Introduction

Dengue virus (DENV), a major global health concern, poses a significant threat to over 3.9 billion people residing in more than 100 endemic countries, primarily in resource-limited settings [1]. Worldwide, close to 50% of the population faces the risk of dengue virus infections [2, 3]. Over the last 50 years, the incidence of this disease has surged by 30 times [4]. Dengue is a serious viral illness that poses a significant threat to public health, particularly in tropical and subtropical areas across the globe [5, 6]. The elevated mortality and morbidity rates linked to dengue fever highlight its importance as a critical global health concern. The dengue virus (DENV), which is an enveloped RNA virus belonging to the Flavivirus genus in the Flaviviridae family, possesses a single-stranded positive-sense RNA genome that is approximately 10.8 kb long. This genome encodes a polyprotein that is processed into 10 viral proteins, which include three structural proteins (capsid, pre-membrane/membrane, and envelope) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The four serotypes of DENV (DENV-1, DENV-2, DENV-3, and DENV-4) exhibit 30–35% variation in their amino acid sequences and are further categorized into genotypes that show less than 10% diversity in the envelope protein. This genotypic classification is based on differences in the viral envelope gene [7 - 12].

The World Health Organization (WHO) initially classified dengue into classic dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) in 1997. To improve early detection of severe cases, the classification was updated in 2009 to include dengue without warning signs, dengue with warning signs (e.g., abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, hematocrit increase with platelet decrease), and severe dengue (severe plasma leakage, bleeding, or organ failure). Dengue virus infections range from asymptomatic or mild cases to severe forms like DHF and DSS, which can be life-threatening [13 - 16].

Dengue virus (DENV) has been endemic in Pakistan since 1995, driven by its temperate and subtropical climate, which supports vector-borne diseases [5, 17 - 20]. The first outbreak in 1994 involved DEN-1 and DEN-2, followed by a significant DEN-3 outbreak in Karachi in 2005 [21]. Annual outbreaks with co-circulating serotypes have persisted, including major epidemics in Lahore (2011) and Khyber Pakhtunkhwa (2013) [6, 22 - 23]. The escalating burden was marked by outbreaks in 2019 (47,120 cases), 2021 (52,000 cases), and 2022, when 75,450 cases were reported, with DENV-2 (62%) and DENV-1 (37%) predominating. In 2023, another outbreak recorded 20,072 cases, prompting alerts in major cities. These cyclic serotype shifts are influenced by factors such as population immunity, vector ecology, climate, and viral evolution. Secondary infections with different serotypes can lead to more severe outcomes due to immune-mediated mechanisms like antibody-dependent enhancement (ADE). The prevalence is further driven by breeding sites, including artificial water reservoirs, irrigation systems, and post-rainfall conditions [6, 16]. While primary DENV infection induces serotype-specific immunity, secondary infections heighten the risk of severe forms, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), due to ADE [12, 24 - 25].

Previous research hasn't fully explored dengue fever trends in Rawalpindi and Islamabad from 2013 to 2024. This study investigates dengue cases and serotype shifts in these areas to inform prevention and management strategies for future outbreaks.

Methodology

Study design and setting

This cross-sectional observational study at RMU analyzed laboratory-confirmed dengue cases from 2013 to 2024, identifying the predominant DENV serotype each year and examining annual shifts in serotype distribution. The findings were compared to regional and global serotype trends to contextualize the observed patterns.

Ethical approval

current cross-sectional study received approval from the Ethical Review Committee of Rawalpindi Medical University. No direct patient interactions or interventions were involved, ensuring that patient anonymity was maintained throughout the data analysis process. Therefore, informed consent was not required.

Data collection sources

Data were collected from the Department of Infectious Disease (DID) HFH dengue surveillance system, that records laboratory-confirmed cases of dengue from RMU-affiliated hospitals. The data included:

- Annual records of predominant DENV serotypes from 2013 to 2024.
- Circulation percentages for each serotype per year.
- Data on secondary circulating serotypes, when available.

Laboratory analysis

As part of standard practice, blood samples from patients testing positive for the NS1 antigen were sent to the National Institute of Health (NIH) in Islamabad and the Provincial Public Health Reference Laboratory in Lahore for serotype analysis.

Data analysis

Yearly Trend Analysis: The data were analyzed annually to identify trends in the prevalence of different dengue virus serotypes. A chi-square goodness-of-fit test was applied to determine whether the observed distribution of predominant serotypes across the years differed significantly from what would be expected by chance. A p-value of less than 0.05 was considered statistically significant.

Comparative Analysis: Stacked bar chart illustration used to represent the percentage distribution of predominant and secondary dengue virus serotypes from 2013 to 2024. Each bar segment corresponds to a specific serotype, highlighting the contribution of both primary and secondary serotypes each year.

Results

Our analysis of RMU data from 2013 to 2024 showed that dengue virus serotypes changed over time. DENV-2 was the most common serotype for most years, but there were some shifts. In 2013, DENV-2 accounted for all detected cases, and it remained prevalent between 2015 and 2017, peaking at 72% in 2017. A chi-square goodness-of-fit test was applied to determine whether the observed distribution of predominant serotypes across the years differed significantly from what would be expected by chance. DENV-2 has been predominant in a statistically significant way ($p = 0.018$) over these years. However, in 2014, DENV-3 took over, making up 85.9% of cases, while DENV-2 accounted for 14.1%.

The year 2019 marked an unusual pattern with DENV-1 and DENV-2 showing equal prevalence and co-dominance. Another major shift occurred in 2023 when DENV-

1 surged to become the dominant serotype, constituting 71% of cases. This change was short-lived, as DENV-2 regained its dominance in 2024, comprising 90% of the reported cases, with DENV-1 reduced to 10%.

Data availability was inconsistent, with gaps noted in 2018 and 2020, and in 2021, only 23% of cases were attributed to DENV-2, with no specified secondary serotype reported. These findings, detailed in Table 01, underscore the complex and evolving landscape of dengue serotype distribution over the study period.

This stacked bar chart illustrates the percentage distribution of predominant and secondary dengue virus serotypes from 2013 to 2024 (Figure 1). Each bar segment corresponds to a specific serotype, highlighting the contribution of both primary and secondary serotypes each year.

Table 01: Trends in dengue virus serotypes over the years 2013 to 2024 significant predominance of DENV-2.

Year	Predominant Serotype	Percentage of Predominant Serotype	Secondary Serotype (if available)	Secondary Serotype Percentage
2013	DENV-2	100%	None	NA
2014	DENV-3	85.90%	DENV-2	14.10%
2015	DENV-2	62%	DENV-3	38%
2016	DENV-2	45.16%	DENV-3	42.18%
2017	DENV-2	72%	None	NA
2018	Not Available	NA	NA	NA
2019	DENV-1 and DENV-2	Co-dominant	-	-
2020	Not Available	NA	NA	NA
2021	DENV-2	23%	Not specified	Not specified
2022	DENV-2	60%	DENV-1	40%
2023	DENV-1	71%	DENV-2	29%
2024	DENV-2	90%	DENV-1	10%

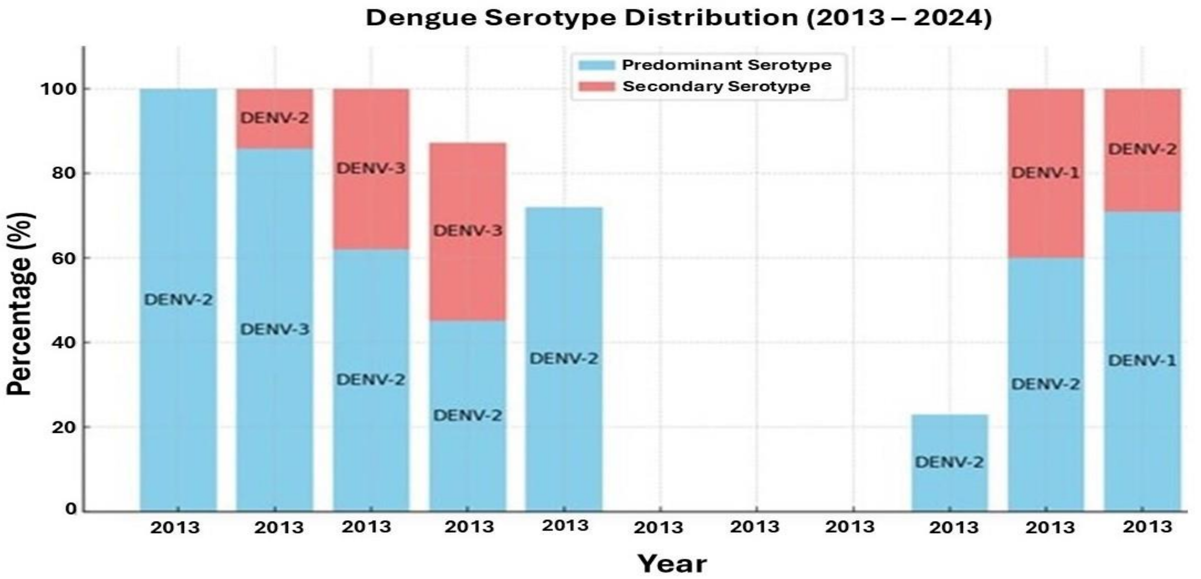

Figure 1: Stacked bar chart of dengue serotype distribution (2013-2024).

Graph analysis

- **2013**: Exclusive prevalence of DENV-2 at 100%, with no secondary serotypes detected.
- **2014**: Dominance of DENV-3 at 85.9%, accompanied by DENV-2 at 14.1%, indicating mixed serotype circulation.
- **2015-2016**: DENV-2 maintained higher prevalence, with a significant presence of DENV-3, suggesting active co-circulation.
- **2017**: Predominant presence of DENV-2 at 72%, without notable secondary serotypes.
- **2018 & 2020**: No data available, reflecting possible surveillance or reporting gaps.
- **2019**: Co-dominance of DENV-1 and DENV-2, marking an unusual year of equal serotype prevalence.
- **2021**: DENV-2 recorded the lowest dominance at 23%, with secondary data unspecified.
- **2022**: DENV-2 re-established as the predominant serotype (60%), with DENV-1 as the secondary (40%).
- **2023**: Shift to DENV-1 dominance at 71%, indicating a significant change in serotype trends.
- **2024**: DENV-2 surged back to 90% prevalence, with DENV-1 at 10%, suggesting a return to DENV-2 predominance.
- This chart offers a comprehensive visual representation of serotype fluctuations and their impact over the studied years.

Discussion

Dengue continues to be a significant public health issue globally, with Asia shoulder approximately 75% of the global burden [26, 27]. Pakistan, as a developing nation within this region, has experienced a marked increase in dengue cases since the virus's first detection in 1994 [28]. Notably, annual dengue epidemics became evident in Karachi by November 2005 [29], evolving into larger outbreaks and major epidemics in subsequent years, particularly in 2011, 2017, 2018, 2019, and most recently, 2022 [30 - 32]. The rising number of cases—from 47,120 in 2019 to 75,450 by 2022—demonstrates a significant upward trend, reflecting a 44% increase over just three years [32]. The surge in 2022 was largely driven by severe rainfall and flooding, creating optimal breeding conditions for *Aedes* mosquitoes and displacing large numbers of people [32]. In October 2023, the IOM Pakistan Situation Report highlighted extensive flooding and adverse environmental conditions that impacted over 1.3 million people and declared 84 districts as calamity-hit zones [33]. In response, the Pakistan Meteorological Department issued a high dengue alert in September 2023 for major cities [34], citing optimal temperature and humidity conditions for Aedes mosquito breeding and virus transmission [35 - 37]. As Pakistan reports its highest confirmed dengue cases in 2023 (20,072 cases), the urgency for targeted seroepidemiological studies and intervention strategies has become vital. This research aims to fill that gap by examining NS1-positive dengue cases to identify the serotypes that are circulating. Understanding the prevailing serotypes and their variations is crucial for healthcare professionals since such changes can significantly impact disease severity and the overall burden. By analyzing serotype trends over the past decade among patients at RMU Allied Hospitals, this study offers valuable insights that can enhance predictive models, inform preventative measures, and improve resource distribution to help reduce the risk of severe outbreaks in the future.

The examination of RMU data from 2013 to 2024 shows fluctuating trends in dengue virus serotypes, with DENV-2 consistently leading in most years. It was the sole serotype in 2013 (100%) and remained prevalent from 2015 to 2017, peaking at 72% in 2017. A significant change took place in 2014 when DENV-3 became the most common serotype (85.9%), pushing DENV-2 to a secondary position (14.1%). An unusual co-dominance of DENV-1 and DENV-2 was noted in 2019. In 2023, another major shift occurred with DENV-1 taking the lead (71%), before DENV-2 regained its dominance in 2024 (90%), while DENV-1 dropped to 10%. Data gaps in certain years, such as 2018 and 2020, and limited serotype information in 2021 (23% DENV-2), highlight the need for more consistent surveillance. Detailed findings are summarized in Table 01.

The serotype shifts observed in our study at RMU reflect patterns reported in neighboring South Asian countries, where DENV-2 and DENV-1 frequently alternate as dominant serotypes. Similar to the trends at RMU in 2023, DENV-1 has periodically emerged as the leading serotype in India, while DENV-2 has been predominantly linked to severe outbreaks. Such fluctuations in serotype prevalence are characteristic of dengue-endemic regions and result from complex interactions between viral dynamics, host immunity, and environmental factors. The periodicity of serotype alternation, as seen in Thailand with shifts every 7–9 years and epidemic surges every 3–5 years, further underscores the importance of continuous surveillance to anticipate and mitigate outbreaks. [37 - 40]. This supports the RMU observation of a temporary DENV-I surge in 2023 before reverting to DENV-II dominance in 2024.

Table 02: RMU serotype data and regional/international data comparison year wise.

Year	RMU Predominant Serotype	Regional Comparison (South Asia)	International Comparison
2013	DENV-2 (100%)	India: DENV-2 predominant	Brazil: High DENV-2 prevalence
2014	DENV-3 (85.9%), DENV-2 (14.1%)	Sri Lanka & Bangladesh: Increase in DENV-3, India: DENV-2	Thailand & Malaysia: Rise in DENV-3
2015	DENV-2 (62%), DENV-3 (38%)	India & Bangladesh: Predominant DENV-2	Brazil: High DENV-2 prevalence
2016	DENV-2 (45.16%), DENV-3 (42.18%)	Sri Lanka: DENV-2 dominant, India: DENV-2 prevalent	Mexico: Increase in DENV-2 cases
2017	DENV-2 (72%)	Bangladesh & India: Predominant DENV-2	Thailand & Philippines: DENV-2 prevalent
2018	Data Not Available	Sri Lanka: DENV-3 dominant, India: DENV-2 & DENV-3	Singapore & Malaysia: High DENV-3
2019	DENV-1 & DENV-2 (Co-dominant)	India & Nepal: Co-circulation of DENV-1 & DENV-2	Brazil: DENV-1 and DENV-2 co-circulation
2020	Data Not Available	COVID-19 impact, India & Sri Lanka: Predominant DENV-2	Limited data due to COVID-19
2021	DENV-2 (23%)	India: DENV-2 widespread, Sri Lanka: Similar trend	Brazil & Colombia: Resurgence in DENV-2
2022	DENV-2 (60%), DENV-1 (40%)	India: DENV-2 dominant, Sri Lanka: High DENV-2 circulation	Mexico & Brazil: Predominant DENV-2
2023	DENV-1 (71%), DENV-2 (29%)	India & Bangladesh: Shift to DENV-1, Sri Lanka: DENV-1 rise	Thailand & Singapore: Increase in DENV-1
2024	DENV-2 (90%), DENV-1 (10%)	Preliminary data: India & Bangladesh: Return of DENV-2	Brazil & Thailand: Resurgence of DENV-2

Sri Lanka also saw a shift in dengue serotypes, with DENV-1 becoming more common in 2023, similar to RMU. This suggests that regional factors like climate and mosquito movement might be driving these changes [41- 43]. Some South Asian countries, like Bangladesh and Nepal, have mostly seen DENV-3 and DENV-4, which is different from RMU's pattern of DENV-1 and DENV-2[44 - 46]. In Southeast Asia, countries like Thailand and Singapore have seen serotypes switch back and forth, with DENV-1 and DENV-2 taking turns being dominant [47 - 49]. This is similar to what we've seen at RMU, and it suggests that bigger regional factors like human movement, trade, and climate might be at play as shown in Table 02.

Latin American countries like Brazil and Mexico often see DENV-2 as a dominant serotype, with DENV-1 sometimes taking over [50 - 52]. This pattern is similar to what we've seen at RMU, suggesting a possible global cycle in dengue serotype prevalence. DENV-2's dominance at RMU is concerning due to its link to severe disease. The shift to DENV-1 in 2023 might have reduced severe cases, but DENV-2's return in 2024 could increase the risk of severe infections. Our study highlights the need for ongoing surveillance and adaptive public health strategies.

However, our study has some limitations. It's based on a single institution, and incomplete testing might affect serotype accuracy. Expanding the study to multiple centers and improving data collection could make the results more reliable.

Conclusion

Our study reveals shifting dengue serotype patterns, with DENV-2 dominant at RMU. We need enhanced surveillance and adaptive public health strategies due to the severity of DENV-2. Larger, multi-center studies are needed for a better understanding of dengue dynamics.

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