Section: Microbiology

Original Research Article

A PROSPECTIVE STUDY OF SEROTYPING OF DENGUE POSITIVE PATIENT IN A TERTIARY CARE HOSPITAL, AHMEDABAD

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Received *: 06/10/2026*
Received in revised form *: 08/11/2026*
Accepted *: 20/11/2026*

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DOI: 10.70034/ijmedph.2026.4.392

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2026; 16 (4); 2607-2611

ABSTRACT

Background: Dengue virus infection has a varied clinical presentation, and information on circulating serotypes can support local surveillance. This study examined dengue serotype distribution and selected epidemiological characteristics among patients attending a tertiary care hospital in Ahmedabad, Gujarat.

Materials and Methods: This prospective laboratory-based study was conducted at the virology laboratory, Department of Microbiology, GMERS Medical College and Hospital, Sola, Ahmedabad, from July 2022 to December 2023. Patients of all ages presenting within five days of illness were considered. Serum samples were screened for dengue non-structural protein 1 (NS1) antigen by enzyme-linked immunosorbent assay. NS1-positive samples underwent further real-time reverse transcription polymerase chain reaction testing, including RNase P detection and serotyping. The study reports that 165 NS1-positive samples were tested for RNase P and that 100 RNase P-positive samples were included in the analysis. Demographic characteristics, geographic distribution, serotypes and clinical categories were described.

Results: Out of the 100 participants, 64 were male and 36 were female. Forty-two were aged 19–34 years, 38 were aged 0–18 years and 20 were aged 35–64 years. DENV-1 was reported in 49 cases, DENV-4 in 36, DENV-2 in 13 and DENV-3 in two. The study's geographic table reported that 83 participants were from the northwest zone of Ahmedabad. Clinical categories were dengue fever in 76 patients, dengue hemorrhagic fever in 23 and dengue shock syndrome in one.

Conclusion: All four dengue serotypes were reported in the study population, with DENV-1 and DENV-4 predominating. The findings describe selected patients attending one hospital and should not be interpreted as population-wide prevalence estimates or evidence of a causal association between serotype and disease severity.

Keywords: dengue; dengue virus; serotypes; NS1 antigen; reverse transcription polymerase chain reaction; Ahmedabad; surveillance.

INTRODUCTION

Dengue is a mosquito-borne viral disease with a broad clinical spectrum. Infection may result in an uncomplicated febrile illness or progress to manifestations involving bleeding, plasma leakage and shock. Recognition of warning signs and appropriate clinical monitoring are important because disease severity can change during the course of illness.[1,2]

Four dengue virus serotypes, designated DENV-1 to DENV-4, are generally considered in epidemiological surveillance. Infection with one serotype produces serotype-specific immunity, whereas a subsequent infection with a different serotype may be associated with an increased risk of severe disease. The relationship between immune responses, viral characteristics and clinical outcome is complex; serotype alone does not determine the course of an individual infection.[1,3]

Dengue transmission depends on interactions among the virus, mosquito vectors and human populations.

Changes in circulating serotypes have implications for surveillance because populations may have different histories of exposure to each serotype. Describing local serotype patterns can therefore complement clinical and laboratory surveillance.[4,5]

Laboratory testing supports the assessment of suspected dengue, particularly when clinical symptoms overlap with those of other febrile illnesses. Detection of dengue non-structural protein 1 (NS1) antigen and molecular detection of viral RNA are approaches used in the early phase of infection. Assays that distinguish serotypes can provide additional information for epidemiological studies.[1,6,7]

The predominant dengue serotype may differ across geographic areas and study periods. Reports cited in the study include a study of changing serotype patterns in an endemic region and a pediatric study from Uttarakhand in which DENV-2 circulation was described.[5,8] Such findings highlight the importance of interpreting local results in relation to the population studied and the period of observation.

The present study aimed to identify dengue virus serotypes among dengue-positive patients attending a tertiary care hospital in Ahmedabad and to describe their distribution alongside demographic, geographic and clinical characteristics.

MATERIALS AND METHODS

Study design and setting: This prospective study was conducted at the virology laboratory, Department of Microbiology, GMERS Medical College and Hospital, Sola, Ahmedabad, from July 2022 to December 2023. The study population comprised patients attending the hospital with suspected dengue infection. The study reports a final sample of 100 dengue-positive patients who underwent the described laboratory testing and serotyping pathway.

Eligibility and participant information: Patients of all ages and both sexes were considered for inclusion. The study described inclusion of serum samples from dengue-positive patients presenting between the first and fifth days of illness. Patients presenting after the fifth day of symptoms were excluded. The inclusion criteria referred to NS1 antigen or reverse transcription polymerase chain reaction (RT-PCR) positivity, while the testing protocol stated that NS1-positive samples proceeded to molecular testing.

The study form recorded patient age, sex, address and area, date of illness, presenting complaints and selected laboratory information. It also included fields for past or travel history, comorbidities and consent for blood testing.

Ethical Consideration: This study was reviewed and approved by the Institutional Ethics Committee of GMERS Medical College and Civil Hospital, Sola, Ahmedabad (DCGI Registration No. ECR/404/Inst/GJ/2013/RR-20) under Reference No. GMERSMCS/IEC/99/2022, dated 02/08/2022. The research was conducted in accordance with the Declaration of Helsinki, the Indian Good Clinical Practice (GCP) guidelines, and the ICMR National Guidelines for Biomedical and Health Research Involving Human Participants (2017)

Sample collection and processing: Approximately 5 mL of blood was collected by venipuncture into a plain container using aseptic precautions. Samples were transported to the microbiology laboratory within two hours, accompanied by a request form containing patient and specimen details. Hemolyzed, lipemic or turbid samples were rejected, as were specimens in inappropriate containers or those with a mismatch between the request form and the collection tube.

After receipt in the laboratory, blood was centrifuged at 2,000 rpm for 10 minutes to separate serum. The study reported storage at 2–8°C for up to seven days and at −20°C for longer periods when immediate testing was not performed.

NS1 antigen detection and molecular testing: Samples were screened for dengue NS1 antigen using a sandwich enzyme-linked immunosorbent assay kit manufactured by Oscar Medicare Pvt. Ltd., New Delhi. According to the stated testing protocol, NS1-positive serum samples underwent further real-time RT-PCR testing. The study reports that 165 NS1-positive samples were tested for RNase P and that 100 were RNase P-positive.

RNA extraction was described as a spin-column procedure using a Qiagen viral RNA extraction kit. The extracted material was subjected to a real-time assay using an RNase P primer–probe mix and TaqPath one-step master mix. A cycle threshold below 40 was stated as the criterion for RNase P positivity. The study reports that RNase P-positive samples proceeded to serotyping.

For the serotyping assay, the study described a 20 µL reaction and 40 amplification cycles. Separate fluorescent channels were reported for DENV-1, DENV-2, DENV-3 and DENV-4, and a cycle threshold below 35 was interpreted as positive for the corresponding serotype. The study also labels the primer–probe mix in the serotyping section as an RNase P mix. The assay documentation must therefore be checked to confirm the reagents, controls and targets used.

Clinical categories and analysis: The study classified cases as dengue fever, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Its description of DHF included fever, clinical findings, thrombocytopenia and a rise in hematocrit; the description of DSS included signs of circulatory compromise alongside laboratory findings. These categories are retained as reported, without independent reassessment of individual patient records.

Results are presented as counts and percentages. Because the final sample contained 100 participants, each count corresponds numerically to its percentage of the study sample. The results presented here are

descriptive; no inferential statistical results are reported.

RESULTS

Participant characteristics: The final analysis included 100 patients: 64 males and 36 females. The largest age group was early adulthood, defined in the study as 19–34 years, with 42 participants. There were 38 participants aged 0–18 years and 20 aged 35–64 years. The study reported no participants in its late-adulthood category, labeled as older than 65 years; the stated age-category boundaries did not explicitly account for age 65.

Table 1: Demographic characteristics of the study sample

Characteristic	Number	Percentage
Sex		
Male	64	64
Female	36	36
Age group, years		
0–18	38	38
19–34	42	42
35–64	20	20
>65	0	0

Percentages are based on the reported sample of 100 patients.

Distribution of dengue serotypes: All four serotypes were reported in the final sample. DENV-1 was the most frequently identified serotype, accounting for 49 cases, followed by DENV-4 in 36 cases. DENV-2 was reported in 13 cases and DENV-3 in two. These proportions describe the selected study sample rather than all suspected dengue cases or the population of Ahmedabad.

Table 2: Reported dengue serotype distribution

Serotype	Number of cases	Percentage
DENV-1	49	49
DENV-2	13	13
DENV-3	2	2
DENV-4	36	36
Total	100	100

Percentages are based on the reported sample of 100 patients.

Geographic distribution: The study's geographic table reported 83 patients from the northwest zone of Ahmedabad, 12 from the southwest zone, four from the west zone and one from the south zone. The study noted the hospital's location when discussing the concentration of cases in the northwest zone. These counts describe the reported addresses of participating patients and do not establish incidence or relative risk across zones.

The geographic table indicated that DENV-1 and DENV-4 were common among patients from the northwest zone. Interpretation of zone-specific patterns is limited by small numbers in several zones and inconsistencies between the geographic table and the accompanying narrative. The geographic data should be checked before detailed zone-specific comparisons are made.

Clinical categories: Of the 100 patients, 76 were classified as having dengue fever, 23 as having DHF and one as having DSS. The study reported DENV-2 in the single DSS case. Because only one patient was classified as having DSS, this observation cannot establish a relationship between DENV-2 and shock in the study population.

Table 3: Reported clinical categories

Clinical category	Number of cases	Percentage
Dengue fever	76	76
Dengue hemorrhagic fever	23	23
Dengue shock syndrome	1	1
Total	100	100

Percentages are based on the reported sample of 100 patients.

DISCUSSION

This prospective hospital-based study described four dengue serotypes among 100 selected dengue-positive patients. DENV-1 accounted for nearly half of the reported cases, while DENV-4 accounted for more than one-third. DENV-2 and DENV-3 were reported less frequently. The identification of all four serotypes in the study sample supports the value of documenting local serotype patterns, although the findings cannot establish how those patterns changed over time.

The study discussed its findings alongside reports from other settings in which predominant serotypes differed. The cited Kolkata study examined changing circulating serotypes, while the Uttarakhand pediatric study described DENV-2 circulation.[5,8] Differences in study period, patient selection, age distribution and

laboratory methods should be considered before comparing their proportions directly with those observed in Ahmedabad.

Demographic and geographic observations: Male patients accounted for 64% of the study sample. The study noted male predominance in several other hospital-based studies. The present participants were selected from patients attending one hospital and undergoing the specified testing pathway; consequently, the observed sex distribution cannot by itself establish a difference in susceptibility or infection risk between males and females in the wider population.

Participants aged 19–34 years formed the largest age group, followed by those aged 0–18 years. This distribution describes the patients included in the study but does not represent an age-specific infection rate. The numbers of people in each age group in the hospital's source population, and the numbers tested for suspected dengue, were not provided in the results used for this article. Differences in healthcare attendance and testing could also affect the observed distribution.

Most participants were reported from the northwest zone. The hospital's location is important context for this finding, as the study noted. Without population denominators and a consistent account of the catchment area, the number of cases from each zone should not be interpreted as community incidence. Small numbers from zones other than the northwest further limit comparisons of their serotype distributions.

Serotype and clinical presentation: Most participants were classified as having dengue fever, while 23 were classified as having DHF and one as having DSS. These results describe a selected hospital sample and do not indicate the proportion of all dengue infections in the community that progress to each clinical category. The use of the study's reported clinical categories also makes it important to verify the criteria applied to each patient.

The study explored serotype distribution within age and clinical groups, but inconsistencies across the relevant tables prevent a dependable account of those subgroups in this article. The single reported DSS case with DENV-2 is an observation, not evidence that DENV-2 caused shock or carried a higher risk than other serotypes. Studies cited in the study have examined associations between serotype and severity, including a meta-analysis and observational studies from Brazil and Malaysia.[9–11] Their inclusion in the discussion does not resolve the inconsistencies in the present dataset.

Previous infection is an important consideration when interpreting dengue severity. The study discusses secondary infection with a different serotype as a possible contributor to severe disease, but the results extracted here do not establish individual participants' prior infection status or infection sequence. Neither secondary infection nor a mechanism of severity can therefore be inferred for an individual case from the reported serotype alone.[3,9]

Laboratory pathway and interpretation: The reported workflow first screened samples for NS1 antigen, after which NS1-positive samples underwent molecular testing. The results table lists 165 NS1-positive samples tested for RNase P, of which 100 were reported as RNase P-positive. The final sample was therefore selected through more than one laboratory step. Its serotype proportions should not be generalized to NS1-negative patients, patients who did not meet the subsequent testing criteria, or all people with suspected dengue.

The study describes RNase P detection as a step preceding dengue serotyping, but its wording about the role of RNase P and the primer–probe mix in the serotyping assay is ambiguous. The investigators should verify the assay targets, controls, kit documentation and criteria used to designate a sample dengue-positive and assign a serotype. The disposition of the 65 NS1-positive samples not reported as RNase P-positive should also be clarified.

The distinction between diagnostic and serotyping methods matters when comparing studies. The study bibliography includes investigations of serotype-specific NS1 detection and serotype-specific immunoglobulin M assays.[6,7]

Strengths and limitations: The study's strengths include its prospective design, an explicit early-illness sampling window, and descriptions of specimen collection and laboratory handling. Reporting serotypes alongside patient characteristics provides a starting point for describing the hospital's dengue case mix during the study period.

Dengue reviews emphasize the breadth of clinical and immunopathological factors relevant to disease presentation.[3,12]

CONCLUSION

Among 100 selected dengue-positive patients attending a tertiary care hospital in Ahmedabad, all four dengue serotypes were reported. DENV-1 was most frequent, followed by DENV-4, DENV-2 and DENV-3. Most patients were classified as having dengue fever, and most were reported from the northwest geographic zone.

The findings provide a hospital-based description of serotype distribution during the study period.

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