



# Circulation of DENV-3 Genotype 3 during 2017 to 2018 in Delhi: A Single-Center Hospital-Based Study

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## Abstract

**Introduction** Delhi is hyperendemic for dengue virus (DENV) where all the four DENV have previously been reported. A constant vigilance of circulating DENV serotypes is important in surveillance, since the introduction of a new variant to areas affected by preexisting serotypes constitutes a risk factor for dengue hemorrhagic fever and dengue shock syndrome.

**Objectives** This retrospective study was performed with an objective to determine the circulating serotype and genotype of DENV in acute phase blood samples of patients who have reported to a tertiary liver care hospital in New Delhi during the last 2 years (2017–2018).

**Methods** The data of clinician-initiated testing for dengue nonstructural protein 1 (NS1) antigen (Ag) was searched in the institutional hospital information system. The serum sample of dengue NS1 Ag-positive cases confirmed by enzyme-linked immunosorbent assay (ELISA; PANBIO, Gyeonggi-do, ROK) and a fever duration of less than 5 days were retrieved from the laboratory archive. The DENV serotyping on these sample was performed by reverse transcriptase polymerase chain reaction (RT-PCR). Sequencing and phylogenetic analysis was done for the capsid premembrane (CprM) region to determine the genotype.

**Results** A total of 440 acute-phase samples were received. Twenty one (4.77%) were positive for dengue NS1 Ag with a mean age of 35.1 years and male-to-female ratio of 1.1:1. Eight cases (38.09%) were positive by dengue RT-PCR and all belonged to DENV-3 serotypes. Phylogenetic tree analysis revealed DENV-3 clustered to genotype III with 100% homology with 2008 Indian subcontinent strain.

**Conclusion** This study revealed circulation of DENV-3, genotype III in Delhi from 2017 to 2018, similar to the 2008 viral type. Virological surveillance is an important exercise to be done for viral infections with public threat and outbreak potential.

## Keywords

- ▶ dengue
- ▶ DENV-3
- ▶ genotype 3
- ▶ surveillance

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## Introduction

Dengue is a vector borne acute arboviral infection widely prevalent in the tropics and subtropics. According to the World Health Organization (WHO) estimates, 3.6 billion people reside in dengue endemic areas and 70% of the actual burden are in Asia.<sup>1,2</sup>

Symptoms of dengue infection is wide ranging from subclinical disease to severe flu like symptoms, and some people develop severe dengue associated with number of complications such as severe bleeding, organ impairment, and/or plasma leakage. Over the past two decades, the burden of dengue cases has increased almost 15 folds.<sup>1</sup>

Dengue infection is caused by a virus belonging to the family flaviviridae, genus *Flavivirus*, and consists of a single stranded positive sense RNA approximately 11 kb as its genome encodes for three structural (capsid [C], premembrane [prM], and envelope [E]) and seven nonstructural proteins<sup>3</sup>. Dengue virus (DENV) can be classified into four antigenically distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) based on sequence diversity and each serotype is further grouped into various genotypes.<sup>4</sup>

A life-long immunity is attained after infection with a particular serotype and a cross-protective immunity against other serotypes for a short period of time.<sup>5</sup> Secondary infection by a different serotype is associated with severe dengue probably due to antibody-dependent enhancement (ADE) resulting in high viremia.<sup>6,7</sup>

Delhi is hyperendemic for DENV with cocirculation of all the four dengue serotypes.<sup>8</sup> Delhi has witnessed several dengue epidemics in the past two decades due to rapid boom in urbanization, monsoon-influenced subtropical climate, water storage practices, and use of water coolers. These factors provide the mosquito vector *Aedes aegypti* a favorable environment to thrive and transmit DENV among the human population which, at times, gives rise to major outbreaks depending on the circulating serotype and genotype of DENV.<sup>9,10</sup>

In the absence of an effective vaccine, virological surveillance and development of effective, locally adapted control programs are the two most important arms in the prevention of dengue infection. Hence, there is a need of continuous monitoring of the circulating DENV type in a given population to develop such locally adapted control programs. Furthermore, most studies focus on the circulating serotypes during epidemics and the studies on circulating strains during the interepidemic periods are neglected and there can be significant variations for which public health systems are unprepared.<sup>11</sup>

We performed the present study targeting the CprM gene junction of the virus to elucidate the molecular epidemiology of the circulating strain of DENV.

## Materials and Methods

### Patient Selection and Specimen Collection

This was a retrospective study performed from January 2017 to December 2018 in a tertiary care liver hospital in New Delhi, India. All consecutive patients with signs and symptoms

(according to WHO guidelines) suggestive of DENV infections visiting the outpatient department (OPD) or admitted in inpatient department (IPD) were tested for DENV infection.<sup>12</sup> Plasma was separated from blood samples and stored in the virology repository at  $-80^{\circ}\text{C}$  till further testing.

### Dengue Virus Diagnosis

Diagnosis of DENV was done with dengue NS1 antigen (Ag; PANBIO, Gyeonggi-do, ROK) if the sample was collected within the first 5 days of onset of clinical symptoms, and with dengue immunoglobulin (Ig)-M capture ELISA (PANBIO, Gyeonggi-do, ROK) if the sample was collected after 5 days of onset of clinical symptoms.

### RNA Extraction and cDNA Synthesis

All the DENV NS1 Ag-positive cases were further processed for RNA extraction using QIAamp Viral Mini kit (Qiagen, GmbH, Germany) according to the manufactures' instructions. The extracted viral RNA was reverse transcribed to cDNA using Quantitect reverse transcriptase kit (QIAGEN, GmbH Germany) according to manufacturer's instructions.

### Detection of Dengue Virus by Reverse Transcriptase Polymerase Chain Reaction

The cDNA was then amplified in the external round of reverse transcriptase polymerase chain reaction (RT-PCR) using DENV consensus primers (D1: 5' TCAATATGCT-GAAACGCGGAGAAAACC G 3'; D2: 5' TTGCACCAACAGT-CAATGTCT TCA GGT TC 3') in 25- $\mu\text{L}$  reaction volume. The DNA product obtained was of 511 bp.

Subsequent amplification of cDNA was performed with the DENV consensus forward primer (D1) and four dengue serotype-specific reverse primers (TS 1: 5' CGTCTCAGT-GATCCGGGGA 3'; TS 2: 5' CGCCACAAGGGCCATGAACAG 3'; TS 3: 5' TAACATCAT- CATGAGACAGAGC 3'; TS 4: 5' CTCTGTTGTCTTAAACAAGAGA 3') as described by Lanciotti et al,<sup>13</sup> but all the four serotype-specific primers were added in a single-reaction mixture. A 1:20 dilution of the external PCR product was used in the nested PCR reaction. DENV serotypes were identified by the size of the resulting DNA bands (DENV-1: 482 bp, DENV-2: 119 bp, DENV-3: 290 bp, and DENV-4: 392 bp). Amplicons were resolved on 2% agarose gel and visualized with ethidium bromide in ultraviolet (UV) light by using a gel documentation system (Wealtec, United States).

### DNA Sequencing

PCR amplicons were gel-purified using QIAquick gel extraction kit (QIAGEN, GmbH Germany) as per manufacturer's instructions. Purified amplicons were subjected to Sanger's sequencing using ABI 3730 DNA analyzer (ThermoFisher Scientific, Waltham, Massachusetts, United States).

### Phylogenetic Analysis

BLAST tool was used to confirm the identity of the obtained sequences (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>, NCBI). Forward and reverse sequence alignments were done manually and edited to resolve the nucleotide ambiguities and

consensus sequences were obtained using GeneDoc v.2.7 (<http://genedoc.software.informer.com/2.7/>) and BioEdit v.7.2 (<http://bioedit.software.informer.com/7.2/>). All the new sequences of DENV-3 genome were submitted to Genbank.

Maximum likelihood method using MEGA 6 v.6.06 software was used for constructing the phylogenetic tree for the DENV-3 sequences. Tamura–Nei model of nucleotide substitution was used to calculate genetic distances. A total of 1,000 bootstrap replicates were used to assess the robustness of the tree.

## Ethics

The study had the approval of the Institutional Ethical Committee and was conducted following the Declaration of Helsinki. Patient's consent was waived-off, as it was done on the deidentified and anonymized left over clinical samples.

## Results

A total of 440 acute-phase samples were received. Twenty one (4.77%) were positive for dengue NS1 Ag with a mean age of 35.1 years and male-to-female ratio of 1.1:1. Eight cases (38.09%) were positive by dengue RT-PCR and all belonged to DENV-3 serotypes. No case of concomitant infection with more than one serotype was observed. In cases where NS1 Ag was positive but RT-PCR was negative, duration of fever was 5 days, as by this time viremia declines and only NS1 Ag persists till IgM antibodies are formed. The window for viral RNA detection is limited to 4 to 5 days after the initial infection and NS1 Ag stays in the circulation for a period of 9 to 10 days.<sup>14,15</sup> Therefore, at day 5, postinfection RT-PCR might miss few samples and can be picked by Ag test.

### DNA Sequence Analysis

The CprM region of the DENV-3 genome was sequenced for eight positive samples. The samples were sequenced in both forward and reverse direction. All the obtained DENV-3 sequences were confirmed by BLAST. These sequences are submitted in Genbank database and accession number of the same are awaited.

### Phylogenetic Analysis

Phylogenetic analysis was done for DENV-3 serotype for the present study. The prototype strain used was H87 strain of genotype V of DENV-3 (GenBank Accession number M93130), corresponding to 131 to 370bp of the CprM region of full genome of the prototype strain. Eighty sequences (8 studied and 72 other sequences of different genotypes of DENV-3 (GenBank) were aligned to construct maximum likelihood tree. Phylogenetic analysis clustered all the studied sequences with the genotype III (Indian subcontinent; **Fig. 1**).

## Discussion

Delhi, the capital of India, is known to be hyperendemic for DENV infection due to the circulation of all the four serotypes

in the population.<sup>10</sup> In the past two decades, Delhi has witnessed several outbreaks and epidemics of DENV infection. Dense human population, rapid urbanization, increasing globalization, and the tropical climatic conditions have contributed toward the frequent outbreaks of DENV infection in Delhi. Hence, a continuous serosurveillance of the circulating DENV strain is a necessity to prevent further epidemics and help in the development of local control program.<sup>16</sup>

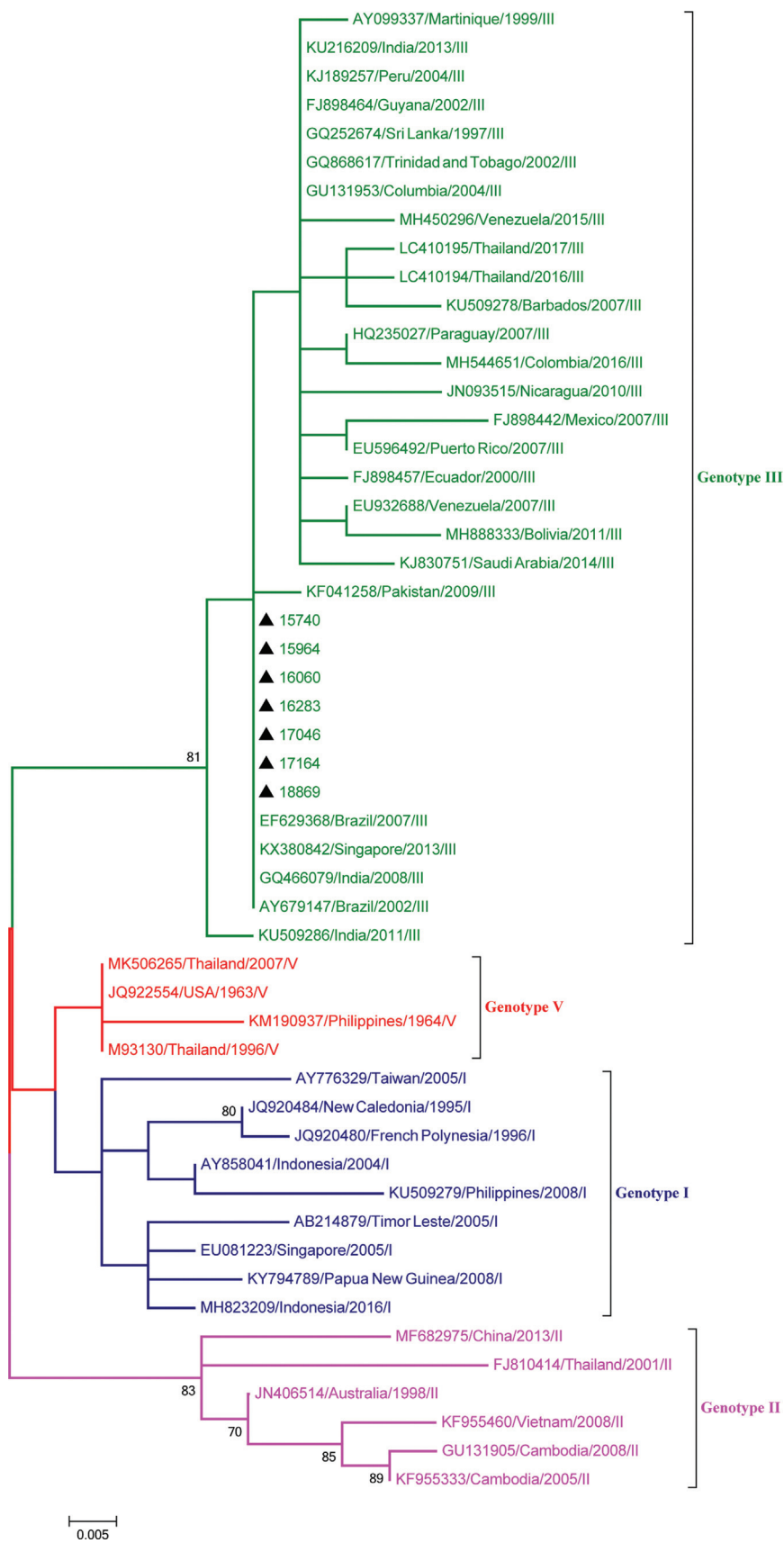
In the present study, molecular characterization of circulating DENV strains was performed targeting the CprM junction. Genotyping based on CprM junction is easier and economical because of the utilization of a single set of primer for both amplification and sequencing of any of the four DENV serotypes.<sup>9</sup>

The frequency of dengue observed in 2017 to 2018 in our tertiary care hospital was found to be 4.6%. Though Delhi is hyperendemic for dengue, a low positivity rate (4.6%) was found in our study. The aim of the study was to not only assess the prevalence of dengue in Delhi but also analyze the circulating serotype/genotype of DENV in the year 2017 to 2018 circulating in Delhi. So, the positivity of 4.6% is only reflective of the single-center footfall and not of entire Delhi. It is a single-center hospital-based study, therefore the positivity is lower. Moreover in the years 2017 and 2018, as there was no ongoing outbreak in the city, the overall positivity was lower. The numbers of dengue cases in 2017 and 2018 in Delhi were 9,271 and 7,136 as compared with 2015 when Delhi had experienced a dengue outbreak and the number of dengue cases reported were 15,867.<sup>17</sup>

A change in the circulation pattern of dengue serotypes has been reported from Delhi in terms of prevalent serotype. The DENV-2 serotype dominated the dengue fever during 2003 to 2006.<sup>13,18–21</sup> DENV-3 emerged as a dominant serotype in the year 2003 which continued in circulation till 2006.<sup>18</sup> In addition, 2006 also reported the emergence of DENV-1 in 30% of the cases.<sup>18</sup> Subsequently, the predominance of DENV-1 was reported till 2010. DENV-1 and DENV-2 serotypes cocirculated in almost equal proportions in 2011<sup>8</sup>. A switch in the prevailing serotype occurred again in 2012 due to codominant circulation of DENV-2 and DENV-3. DENV-2 dominated during 2012, 2013, and 2015.<sup>8,15</sup> Subsequently, DENV-1 dominated in 2014<sup>16</sup>. Further, during 2016, a change in the circulating serotype occurred leading to dominance of DENV-3 in this region (**Table 1**).

The most relevant finding of our study was the circulation of a single serotype, DENV-3 genotype III during 2017 to 2018 which was similar to the DENV-3 strain circulating in the year 2016.<sup>22</sup> Similar studies from Pakistan also reported the circulation of DENV-3 during 2015.<sup>23</sup> Studied strains of DENV-3 were clustered in genotype III (Indian subcontinent) by phylogenetic analysis. The analysis also concluded that the DENV-3 study sequences grouped with the strains from China (unpublished), Pakistan,<sup>24</sup> and India.<sup>24–26</sup> Furthermore DENV-3 serotype has been reported from different geographical regions including India.

The oldest DENV-3 strain, Philippines 56, was classified as genotype I along with other strains from the Philippines and



**Fig. 1** Maximum likelihood phylogenetic tree of DENV-3 strains. The study sequences are marked by the symbol▲. Bootstrap values are represented by the number on nodes generated by 1,000 replications. The strains used to construct the tree are represented by the strain name, followed by country/region and year of isolation/submission of strain. The study sequences clustered with genotype III (Indian subcontinent). DENV, dengue virus.

**Table 1** Circulation of various dengue serotypes in Delhi

Year	Dengue virus serotype	Epidemic/non-epidemic	References
1996	2	Epidemic	Dar et al <sup>28</sup>
1997	1	Non-epidemic	Vajpayee et al <sup>29</sup>
2003	1,2,3 (genotype III),4	Epidemic	Dar et al <sup>8</sup>
2004	1	Non-epidemic	Gupta et al <sup>10</sup>
2005	3 (genotype III)	Epidemic	Gupta et al <sup>10</sup>
2006	3 (genotype III)	Epidemic	Bharaj et al <sup>30</sup>
2007	3 (genotype III)	Non-epidemic	Kukreti et al <sup>18</sup>
2008	1	Non-epidemic	Chakravarti et al <sup>31</sup>
2010	1	Non-epidemic	Gupta et al <sup>11</sup>
2011–2014	1,2,3 (genotype III)	Non-epidemic	Afreen et al <sup>32</sup>
2015	2	Epidemic	Choudhary et al <sup>33</sup>
2016	3 (genotype III)	Non-epidemic	Parveen et al <sup>22</sup>
2017–2018	3 (genotype III)	Non-epidemic	Present study

Indonesia isolated over two decades, suggesting the persistence of this genotype in the area for a long time.

Genotype III of DENV-3 circulates throughout the world, whereas other genotypes are localized in particular geographic regions, indicating a higher potential of genotype III to spread and dominate in geographically diverse regions of the world. This genotype has also been implicated in major dengue epidemics in several parts of Asia, Africa and the Americas and has the potential to cause an international dengue pandemic.<sup>27</sup>

Furthermore our study also shows that in the non-epidemic period usually one strain circulates and cocirculation of multiple types with concurrent infection with more than one serotype is not seen.

## Limitations

The study had few limitations like the study was retrospective in nature, a small sample size, and it was representative of patients coming to a tertiary care hospital. The exact depiction of data from the community is not truly represented by hospital based studies. Therefore for virological surveillance, community-based prospective studies should be performed.

## Conclusion

The study provides crucial information highlighting the need for continuous monitoring of DENV genotypes in the endemic region. This study revealed circulation of DENV-3, genotype III in Delhi in the year 2017 to 2018 similar to 2008 viral type. Virological surveillance is an important exercise to be done for viral infections with public threat and outbreak potential.

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## Conflict of Interest

None declared.

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