# Co-circulation of all the Four Serotypes of Dengue Virus in Endemic Region of Saurashtra, Gujarat during 2019-2020 Season

BINITA JOSEPH ARING[1], DIPALI MAGANBHAI GAVALI[2], PUSHPA RAMJIBHAI KATESHIYA[3], HIRAL MODBHAI GADHVI[4], SUMMAIYA MULLAN[5], KAMAXI KIRITBHAI TRIVEDI[6], AKHLAKAHEMAD ALIBHAI NATHAMETHA[7], ANAND HITENBHAI NAKHAVA[8]

## ABSTRACT

**Introduction:** Dengue has rapidly emerged as a vector-borne viral disease in recent years and also endemic in all continents. The agent of dengue, i.e., dengue viruses, are categorised under the genus Flavivirus, with the four dengue virus serotypes: designated as Dengue Virus-1 (DENV-1), DENV-2, DENV-3 and DENV-4. These all four serotypes are in circulation either singly, or more than one at the same time.

**Aim:** To study the epidemiological update of dengue with circulating serotype and co-infection in Saurashtra region, Gujarat, India.

**Materials and Methods:** A cross-sectional study was conducted during January 2019 to December 2020 and total samples received were 12,563 which were clinically suspected dengue samples case. After receiving blood samples, serum was separated and proceeded for Dengue NS1Ag (non structural protein 1 antigen), and Dengue IgM Ab (Immunoglobulin M antibody). After serological confirmation, 151 samples from different geographical area were selected for Dengue specific Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR) for serotyping.

**Results:** Total 4069 (32.3%) had confirmed dengue positive by Enzyme Linked Immunosorbent Assay (ELISA). The ratio of male cases was higher than female, and in age group 21-35 year (47%). Seasonal trend showed a gradual increase in positivity from June with high peak in October. Circulation of all the four serotypes in area, higher monotypic infection by DENV-1 serotype (41.2%), followed by DENV-4, DENV-2 and DENV-3. Co-infection of different serotypes were also found.

**Conclusion:** The present study concluded that all four serotypes circulate with predominant being DENV-1 type and co-infection of different serotypes in the Saurashtra region. Dengue mainly affected adult male population, and seasonal peak during monsoon and post-monsoon period.

**Keywords:** Co-infection, Dengue haemorrhagic fever, Dengue shock syndrome, Enzyme linked immunosorbent assay, Immunity, Protein, Real-time polymerase chain reaction

## INTRODUCTION

Dengue is endemic in all continents in recent years and has, rapidly emerged and the most important mosquito-borne viral disease. The dengue viruses are arbovirus; categorised under genus Flavivirus. DENV genome is positive-sense, single stranded Ribonucleic Acid (RNA), codes for two types of viral proteins: (I) Structural proteins: Capsid (C) protein, Membrane (M) protein, Envelop (E) protein, (II) Nonstructural proteins like NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 [1-3]. Dengue virus has four serotypes as DENV-1 to DENV-4. Recently, 5th serotype was identified from Bangkok in 2013. All these serotypes are circulated either singly, or more than one; at the same time, so persons living in a dengue endemic area can have all four serotype infections during their lifetimes. DENV infection causes Dengue Fever (DF) with or without warning sign. A severe dengue includes Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) in which thrombocytopenia, haemorrhage and excessive plasma leakage occurs which leads to death [4,5].

Dengue virus are antigenically distinct and extensive cross-reactivity was seen in serological assays due to all flavivirus has common group epitopes on envelop protein. One DENV serotype infection provides lifelong immunity to that serotype but does not protect against infection from another DENV serotype [6]. In DENV infection, the antibody dependent enhancement phenomena production leads to increase in severity of secondary dengue infection [7]. The first epidemic dengue was recorded in 1780 at Chennai. The first epidemic of DF proved virologically in 1963-1964 at Calcutta and Eastern Coast area [8-10]. Generally, one dominating serotype causes outbreak, but circulation of all four serotypes are also found, that leads to mixed infection with more than one serotype and increased disease severity [11-14]. Co-infection of all four DENV serotypes with each other in single outbreak were reported in 2017, from Odisha and Hyderabad [15,16].

In recent year various method for diagnosis of DENV infection was developed like virus isolation, Molecular method (nucleic acid amplification tests; e.g., RT-PCR, Real-time RT-PCR), and serological method e.g., Antibody detection (by Haemagglutination Inhibition (HAI), Complement Fixation Test (CFT) and ELISA for IgM or IgG detection), Antigen detection (by ELISA and Immunochromatographic Test (ICT) or direct-Immunofluorescence (IF)) [17]. For DENV serotyping antigen detection immunofluorescence assay and plaque reduction neutralisation were available [18], but they need well-equipped laboratories so, not widely available. Molecular methods need high specificity, sensitivity and rapid, also be serotype specific so, World Health Organisation (WHO) recommended for the detection of DENV RNA [19]. Molecular methods are becoming more widely used, it provides results more quickly and more sensitive for serotyping, all four dengue serotypes detected by fluorogenic probe-based PCR assay [7].

Knowledge about geographic distribution of disease, their burden, prevalence and incidence are necessary, and also with this background, circulating DENV serotypes strains were detected. So, these information help in appropriate utilisation of existing and emerging prevention control strategies and introduction of dengue vaccine in near future. The present study was done with an aim to identify prevalent dengue serotype and co-circulation of all the four serotype of dengue virus in endemic region of Saurashtra, Gujarat, India.

## MATERIALS AND METHODS

The present study was a cross-sectional study conducted after obtaining permission from the Institutional Ethical Committee (MP Shah Govt. Medical College and Guru Gobind Singh Government Hospital, Jamnagar; IEC/Certi/138/08/2018). Cases were mainly referred from Outpatient Department (OPD) and indoor of GG hospital, Jamnagar, Gujarat. India.

**Sample size calculation:** A pilot study for clinically, suspected dengue cases conducted for period of one month gave estimated proportion, of 7.6% in Saurashtra region. Considering 7.6% of proportion sample size was calculated by formula, with vonfidence interval=95% and power of study=80%

$$n \geq \frac{NZ^2_{1-a/2}\,p(1-p)}{d^2(N-1)+Z^2_{1-a/z}\,p(1-p)}$$

n=12086 (where, P is estimated proportion=7.6%; Z=1.96 N is population size of Saurashtra=32,56,800 as per Census 2011 [20].

d is Marginal error rate taken as 10% of estimated proportion=0.76%

Alph (@) is type I error=5% power of study

**Inclusion criteria:** All dengue virus antigen- NS1 and antibody-IgM ELISA positive samples were included in this study.

**Exclusion criteria:** ELISA negative (antigen- NS1 and antibody-IgM) were excluded for serotyping.

### Study Procedure

Universal sampling method was adopted, where total 12,563 clinically suspected dengue samples were collected for period of two year (from January 2019 to December 2020) and analysed later after completion of collection of data. Samples were collected at central laboratory of GG Hospital and serotyping done at Microbiology Department of MP Shah Government Medical College, Jamnagar. After receiving blood samples, non-haemolysed, non-turbid and non-lipemic serum was selected and proceed for Dengue NS1 Ag by Microlisa test kit (if H/O of illness <5 days) and Dengue IgM Ab by NIV Pune test kit (if H/O of illness >5 days). After serological confirmation, 151 ELISA positive samples from different geographical area were selected for Dengue specific RT-PCR serotyping.

### Protocol for Dengue Specific RT-PCR is as Follows

**Viral RNA extraction:** RNA isolation was performed using QI Amp Viral RNA mini kit (Qiagen, Valencia, CA, USA).

Pipet 560 µL prepared Buffer AVL in to microcentrifuge tube, add 140 µL serum and mix by vertexing for 15 seconds, incubate at room temperature (15-25°C) for 10 minutes, centrifuge the tube to remove drops from the inside of the lid. This was followed by a brief centrifugation; RNA precipitation was done using 560 µL of ethanol (96%-100%) to the sample and mixing by pulse-vertexing for 15 seconds and the mixture was transferred to QIAamp ® Mini column for RNA isolation. The final RNA was eluted in 60 µL of the elution buffer.

**Master mix preparation for dengue differentiation**: Calculate the amount of each reagent to be added for Master mix. Use 12.5 µL buffer, 1.5 µL Primer-probe Mix and 1 µL Enzyme.

### Preparation of a 96 Well Plate

Take a 96 well plate, add 15 µL of the master mix in the wells, add 10 µL of the extracted RNA, negative control and the positive control. Mix properly, cover the well with adhesive film, slightly vortex the plate and centrifuge briefly afterward and put the strip in ABI-7500 RT-PCR.

### Detector Programming

For pathogen DEN-1 FAM dye at 520 nm, DEN-2 VIC dye at 550 nm, DEN-3 ROX dye at 610 nm and DEN-4 CY5 dye at 670 nm wavelength used.

### Temperature Cycle

1. Reverse Transcription at 15 minutes at 50°C;
2. Taq mix inhibitor inactivation at one minute at 94°C; and
3. PCR amplification (for 45 Cycles) at 94°C for 8 Seconds 60°C for one minute* (data collection).

### Interpretation

1. Negative controls: All primers and probes should not exhibit amplification curves that cross the threshold line.
2. Positive controls: Must show amplification with sigmoid curve for specific primer and probes sets: DEN-1, DEN-2, DEN-3 and DEN-4.
3. Internal/Extraction controls: Must show amplification to specific primer and probes.
4. RNase P (RP): All clinical specimen shows amplification curves in RP reaction and cross the threshold line, indicating the presence of the human RNase P gene. (If all primer and probes shows amplification but not RNase P result valid, but all marker and RP not amplified result as an inconclusive). If the criteria listed above are met, any patient sample displaying an exponential trace should be considered as positive for one of the pathogens targeted by the kit.

## STATISTICAL ANALYSIS

Percentage, Chi-square test (Yates correction applied where cell value below 5). Significance taken at p<0.05; data were entered and analysed in Microsoft excel 2016.

## RESULTS

Total 12,563 clinically suspected dengue samples tested, 4069 (32.3%) had confirmed Dengue positive by ELISA method. Of these, 151 dengue positive samples tested for RT-PCR serotyping and only 109 were dengue positive, negative result does not preclude infection because patient have low viral load, and that was beyond detectable limit of the assay.

In dengue serotyping study all the four serotypes in circulation. DENV-1 was 41.2%, followed by DENV-4 was 23%, DENV-2 was 22% and DENV-3 was 6.4%. Also, mixed infection of DENV-1 and DENV-2, DENV-1 and DENV-3, DENV-1 and DENV-4 and DENV-3 and DENV-4 were 8% [Table/Fig-1].
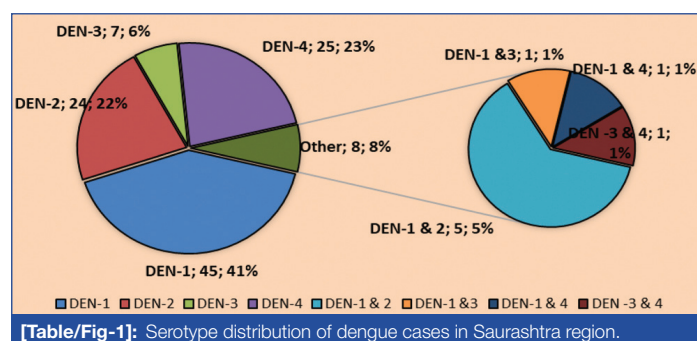


**[Table/Fig-1]:** Serotype distribution of dengue cases in Saurashtra region.

In male/female suspected dengue cases positivity percentage in male 65 (60%) was higher than female 44 (40%), This difference between male and female among specific DENV-1 to DENV-4 was statistically not significant (p>0.05) [Table/Fig-2]. Majority of samples were in age group 21-35 year (47%) followed by 6-20 year (27%). Most of co-infection were in age group 21-35 year and 36-50 year [Table/Fig-3,4].

Sporadic cases were observed throughout of year, but cases were gradually increasing with positivity from June and reach to peak in October (47%) than gradually declining of cases in November [Table/Fig-5].
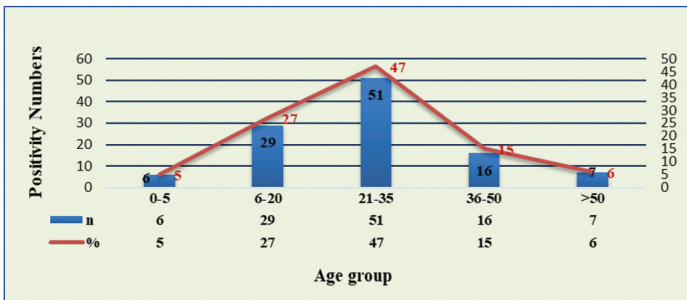
Majority showed dengue positive cases in three to four days duration from the onset of illness and co-infection with more than one serotype was after three days duration of illness noted [Table/Fig-6].

| Serotype | Male (%) | Female (%) |
|---|---|---|
| DENV-1 | 28 (25.7) | 17 (13.8) |
| DENV-2 | 12 (11) | 12 (11) |
| DENV-3 | 3 (2.7) | 4 (3.6) |
| DENV-4 | 16 (14.7) | 9 (8.2) |
| DENV-1 and DENV-2 | 3 (2.7) | 2 (1.8) |
| DENV-1 and DENV-3 | 1 (1) | 0 |
| DENV-1 and DENV-4 | 1 (1) | 0 |
| DENV-3 and DENV-4 | 1 (1) | 0 |
| Total | 65 (60) | 44 (40) |

**[Table/Fig-2]:** Gender wise distribution of dengue positive cases serotyping.
Chi-square ($c^2$)=1.97, d.f=3, p.0.05, statistically not significant. Chi-square calculated only for specific DENV-1 to DENV-4 infection and not for mixed infection



**[Table/Fig-3]:** Age wise positivity number (%) of dengue cases.
Chi-square value=1.82, p-value=0.77, statistically not significant
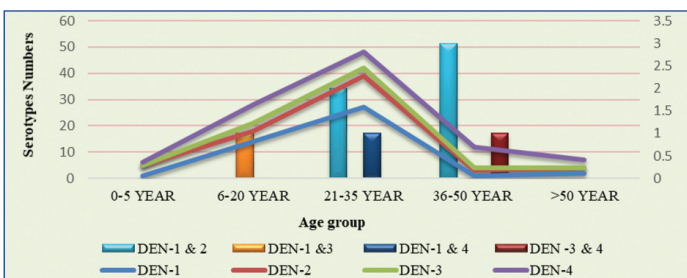


**[Table/Fig-4]:** Age wise serotyping of dengue cases.
Chi-square value =16.74, p=0.01, statistically significant. Calculation done between groups, 0-20 years, 21-35 years and >35 years: Chi-square calculated only for specific DENV-1 TO DENV-4 infection and not for mixed infection
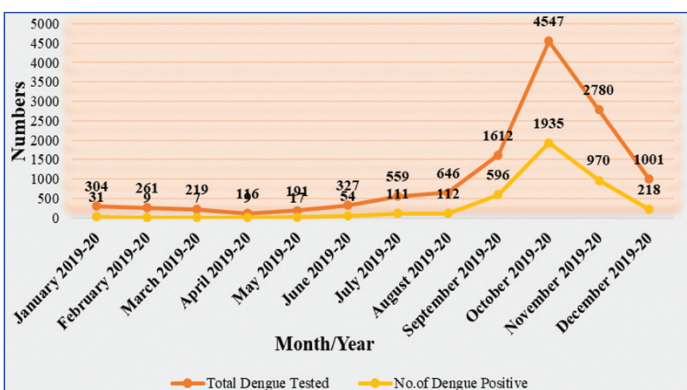


**[Table/Fig-5]:** Month wise distribution of dengue cases in Saurashtra region.
Chi-square value=493.43, p=0.0001, statistically significant. Chi-square calculated for quarters Jan-Mar 2019-2020, Apr-June 2019-2020, Jul-Sept 2019-2020, Oct-Dec 2019-2020.

## DISCUSSION

Genus flavivirus have highest mutation rate tends to have chances of co-infection much higher because all four dengue serotypes circulate in a population. Study shows increasing trend of co-circulation of different DENV serotypes in region that suggest Saurashtra becoming hyper endemic region; so, monitoring of circulatory strain is required. Since, DENV-2 and DENV-3 were causing severe diseases than the other [4] so, rapid and accurate detection of serotype can help in DENV infection detection. For this DENV RNA detection and with the real-time RT-PCR serotyping during the first five to six days after symptomatic onset. So, timely dengue virus infection detection, their

| Distribution of dengue serotypes as per duration (in days) from the onset of illness | | | | | | |
|---|---|---|---|---|---|---|
| Serotypes | 1 | 2 | 3 | 4 | 5 | >5 |
| DEN-1 | 0 | 9 | 10 | 21 | 5 | 0 |
| DEN-2 | 0 | 1 | 9 | 9 | 4 | 1 |
| DEN-3 | 2 | 0 | 2 | 2 | 1 | 0 |
| DEN-4 | 1 | 5 | 8 | 7 | 4 | 0 |
| DEN-1 and 2 | 0 | 0 | 2 | 3 | 0 | 0 |
| DEN-1 and 3 | 0 | 0 | 0 | 1 | 0 | 0 |
| DEN-1 and 4 | 0 | 1 | 0 | 0 | 0 | 0 |
| DEN -3 and 4 | 0 | 0 | 1 | 0 | 0 | 0 |

**[Table/Fig-6]:** Positivity in duration (in days) of onset of illness.

serotype status help to initiate timely community and vector control programs to mitigate the spread of DENV infection.

Present study, shows the highest monotypic infection was DENV-1 (41%), followed by DENV-4 (23%), DEN-2 (22%) and DENV-3 (6%) provides direct evidence of all four serotypes were circulated in Saurashtra region, similar finding were observed in Vicente CR et al., shows DENV-1 (77.3%), Rahman M et al., (72.7%) and Paul R et al., shows (57%), but not corelated with Racherla RG et al., and Sarkar A et al., study shows highest monotypic infection was DENV-2 (41%), and (60%), and Mehta TK and Shah PD, study shows DENV-3 (53%) [21-26].

In present study, DEN-4 (23%) were reported after DEN-1 monotypic infection, which was similar to Vicente CR et al., showed 16.1%, Racherla RG et al., showed 37% [21,24]; While Mehta TK and Shah PD, showed 3% [26], Paul R et al., and Sarkar A et al., study were no any GEN-4 serotype found [23,25]. All these finding shows that in different region different serotypes were circulated can shift from one to another, but in present study samples were obtained from hospitalised patients. So, result may not actually reflect the predominant serotype.

In this study, few cases DENV-3 (6%) infection were detected similar to Racherla RG et al., DENV-3 (12%) [24] and Vicente CR et al., DENV-3 (0.2%) [21] while in Sarkar A et al., [25], Paul R et al., and Mehta TK and Shah PD, shows higher infection of DEN-3 (29.7%), (40%) and (53%) [23,26]. Prevalence rate of all four serotypes were changed in different area. So, data on serotypes help in surveillance systems in that particular area to predict an impending dengue outbreak. DF were complex epidemiology so, prevalent strain substantially changed it may be due to urbanisation, population growth, lack of sanitisation, ineffective mosquito controls all these facilities fertile breeding ground to vector mosquitoes.

Mixed infection was also detected in present study of different serotype was 8 (7%) of this DENV-1 and DEN-2 (5%) was predominant than another mixed infection. Mehta TK and Shah PD, [26] showed co-infection DEN-2 and DEN-3 (7.4%) and DEN-3 and DEN-4, DEN-2 and DEN-4 was (0.7%), another study of Kerala [11] reported a DEN-1 and DEN-3 co-infection was predominant than the other combinations, Rahman M et al., also showed mixed infection of DEN-1 and DEN-2 was (15.1%) [22] and Sarkar A et al., shows DEN-2 and DEN-3 (30.6%) [25]. In India, dengue is hyperendemic and all DENV serotype circulate during the same period of time that tends to mixed infection was occurred with different serotypes in the patients [27].

Seropositivity in male (60%) was higher than female in present study which was similar to the studies from Rahman M et al., showed 73.6% [22], Mehta TK and Shah PD, (61.48%) [26] and Paul R et al., (64%) [23], in opposite Sarkar A et al., showed female (46.5%) were more affected than male [25] and Racherla RG et al., were no significant association found between male and females [24]. Among all the age group middle age group (47%) was most affected in showed study was comparable to most of studies like Rahman M et al., showed 73.6% [22], Mehta TK and Shah PD, showed 40.7% [26], Racherla RG et al., showed 52.7% [24] and Paul R et al., showed 42% [23]. This age groups highly comprises to working and student population and they were exposed to external environment and Ades mosquito vectors which are day biters.

Majority of dengue positivity was seen 2-5 days duration from the onset of illness in this study, which was similar to Mehta TK and

Shah PD [26]. Stage of diseases important like initial phase, first 5 days of illness high number of circulating viruses are present in serum to explore the prognostic markers of severe diseases and early detection of different dengue serotype.

In present study, dengue virus positivity increases from monsoon and at peak level in post-monsoon period were similar to various studies [22,26]. This can be explained by mosquito vector multiply during monsoon period and stagnant of water sources in post monsoon period leads to increases favoring of breeding mosquito vectors. So, appropriate and effective preventive measures should be taken during these time period.

### Limitation(s)

The sample was collected mostly from a single center, so serotype prevalent in present study may vary from the other region. The clinical requisition form of patients were received in our the center was not completely filled by the clinician so, clinical manifestation and severity of diseases could not be assessed.

## CONCLUSION(S)

All four dengue virus serotypes can co-circulate in region and the immunity to one serotype does not give protection to another heterotopous serotype. Seasonal peak which coincides with increased vector breeding's during monsoon and post-monsoon period. So, effective implementation of vector control measures and personal prophylaxis is effective to control diseases. This should be concerns to public health authority and should stimulate the arbovirus diagnostic method in public health laboratories. People should use mosquito repellents and nets to avoid being infected by mosquitoes spreading dengue, malaria, chikungunya and other diseases. Prevention is always better than cure, help themselves by keeping the surroundings clean and not letting garbage accumulate.

## REFERENCES

[1] Dwivedi VD, Tripathi IP, Tripathi RC, Bharadwaj S, Mishra SK. Genomics, proteomics and evolution of Dengue virus. Briefings in Functional Genomics. 2017;16(4):217-27, https://doi.org/10.1093/bfgp/elw040.

[2] Rodenhuis-Zybert IA, Wilschut J, Smit JM. Dengue virus life cycle: Viral and host factors modulating infectivity. Cellular and Molecular Life Sciences. 2010;67(16):2773-86. Doi:10.1007/s00018-010-0357-z. PMID 20372965. S2CID 4232236.

[3] Hanley KA, Weaver SC, eds. A ligand-binding pocket in the dengue virus envelope glycoprotein. Proceedings of the National Academy of Sciences of the United States of America. Frontiers in Dengue Virus Research. Caister Academic. 2010; ISBN 978-1-904455-50-9. Modis Y, Ogata S, Clements D, Harrison SC (June 2003).

[4] Gubler DJ, Clark GG. Dengue/dengue hemorrhagic fever: The emergence of a global health problem. Emerg Infect Dis. 1995;1(2):55-57. https://doi.org/10.3201/eid0102.950204.

[5] Shrivastava S, Tiraki D, Diwan A, Lalwani SK, Modak M, Mishra AC, et al. Co-circulation of all the four dengue virus serotypes and detection of a novel clade of DENV-4 (genotype I) virus in Pune, India during 2016 season. PLoS ONE. 2018;13(2):e0192672. https://doi.org/10.1371/journal.pone.0192672.

[6] Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev. 1998;11(3):480-96.

[7] Johnson BW, Russell BJ, Lanciotti RS. Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. J Clin Microbiol. 2005;43(10):4977-83.

[8] Sarkar JK, Chatterjee SN, Chakravarty SK. Haemorrhagic fever in Calcutta: Some epidemiological observations. Indian J Med Res. 1964;52:651-59.

[9] Gupta N, Srivastava S, Jain A, Chaturvedi UC. Dengue in India. Indian J Med Res. 2012;136(3):373-90. PMCID: PMC3510884.

[10] Carey DE, Myers RM, Reuben R, Rodrigues FM. Studies on dengue in Vellore, South India. Am J Trop Med Hyg. 1966;15:580-87.

[11] Reddy MN, Dungdung R, Valliyott L, Pilankatta R. Occurrence of concurrent infections with multiple serotypes of dengue viruses during 2013-2015 in northern Kerala, India. Peer J. 2017;5:e2970. https://doi.org/10.7717/peerj.2970 eCollection 2017. PMID: 28316881.

[12] Bharaj P, Chahar HS, Pandey A, Diddi K, Dar L, Guleria R, et al. Concurrent infections by all four dengue virus serotypes during an outbreak of dengue in 2006 in Delhi, India. Virol J. 2008;5:1. https://doi.org/10.1186/1743-422X-5-1. PMID: 18182120.

[13] Das B, Das M, Dwibedi B, Kar SK, Hazra RK. Molecular investigations of dengue virus during outbreaks in Orissa state, Eastern India from 2010 to 2011. Infect Genet Evol. 2013;16:401-10. https://doi. org/10.1016/j.meegid.2013.03.016 Epub 2013 Mar 22. PMID: 23523598.

[14] Tazeen A, Afreen N, Abdullah M, Deeba F, Haider SH, Kazim SN, et al. Occurrence of co-infection with dengue viruses during 2014 in New Delhi, India. Epidemiol Infect. 2017;145(1):67-77. Epub 2016 Sep 13. https://doi.org/10.1017/S0950268816001990. PMID: 27620341.

[15] Mishra B, Turuk J, Sahu SJ, Khajuria A, Kumar S, Dey A, et al. Co-circulation of all four dengue virus serotypes: First report from Odisha. Indian J Med Microbiol. 2017;35(2):293-95. https://doi. org/10.4103/ijmm.IJMM_15_536. PMID: 28681825.

[16] Vaddadi K, Gandikota C, Jain PK, Prasad VSV, Venkataramana M. Co-circulation and co-infections of all dengue virus serotypes in Hyderabad, India 2014. Epidemiol Infect. 2017;145(12):2563-74. Epub 2017 Jul 20. PMID: 28726595 https://doi.org/10.1017/S0950268817001479.

[17] Teles FR, Prazeres DM, Lima-Filho JL. Trends in dengue diagnosis. Rev Med Virol. 2005;15(5):287-302. https://doi.org/10.1002/rmv.461. PMID: 15672450.

[18] van Panhuis WG, Gibbons RV, Endy TP, Rothman AL, Srikiatkhachorn A, Nisalak A, et al. Inferring the serotype associated with dengue virus infections on the basis of pre- and postinfection neutralising antibody titers. J Infect Dis. 2010;202(7):1002-10. https://doi.org/10.1086/656141. PMID: 20738205; PubMed Central PMCID: PMCPMC2943243.

[19] WHO. World Health Organisation. Dengue guidelines for diagnosis, treatment, prevention and control: New edition. 2009.

[20] https://www.census2011.co.in/census/district/191-jamnagar.html.

[21] Vicente CR, Herbinger KH, Fröschl G, Romano CM, Areias Cabidelle ADS, Junior CC, et al. Serotype influences on dengue severity: A cross-sectional study on 485 confirmed dengue cases in Vitória, Brazil. BMC Infect Dis. 2016;16:320.

[22] Rahman M, Sharma A, Patgiri S, Hussain E, Borah AK, Saikia L. Serotyping of dengue viruses circulating during 2014-2015 in Assam, India. Indian J Med Microbiol. 2018;36:429-31. Doi: 10.4103/ijmm.IJMM_17_121.

[23] Paul R, Oberoi L, Singh K, Devi P. Molecular characterization and serological analysis of dengue viruses in amritsar district. Int J Contemporary Med Res. 2018;5(3):C10-14.

[24] Racherla RG, Pamireddy ML, Mohan A, Mudhigeti N, Mahalakshmi PA, Nallapireddy U, et al. Co-circulation of four dengue serotypes at South Eastern Andhra Pradesh, India: A prospective study. Indian J Med Microbiol. 2018;36:236-40. Available from: https://www.ijmm.org/text.asp?2018/36/2/236/238684.

[25] Sarkar A, Taraphdar D, Chatterjee S. Molecular typing of dengue virus circulating in Kolkata, India in 2010. Hindawi Publishing Corporation Journal of Tropical Medicine. 2012;2012:960329. 5 pages. Doi: 10.1155/2012/960329.

[26] Mehta TK, Shah PD. Identification of prevalent dengue serotypes by reverse transcriptase polymerase chain reaction and correlation with severity of dengue as per the recent World Health Organisation classification (2009). Indian J Med Microbiol. 2018;36:273-78.

[27] Islam A, Abdullah M, Tazeen A, Naqvi IH, Kazim SN, Ahmed A, et al. Circulation of dengue virus serotypes in hyperendemic region of New Delhi, India during 2011-2017. J Infect Public Health. 2020;13(12):1912-19.

PARTICULARS OF CONTRIBUTORS:
1.  Associate Professor, Department of Microbiology, Shri MP Shah Medical College, Jamnagar, Gujarat, India.
2.  Assistant Professor, Department of Microbiology, Shri MP Shah Medical College, Jamnagar, Gujarat, India.
3.  Tutor, Department of Microbiology, Shri M P Shah Medical College, Jamnagar, Gujarat, India.
4.  Assistant Professor, Department of Microbiology, Shri MP Shah Medical College, Jamnagar, Gujarat, India.
5.  Professor and Head, Department of Microbiology, SMC, Surat, Gujarat, India.
6.  Techanician, Department of Microbiology, Shri MP Shah Medical College, Jamnagar, Gujarat, India.
7.  Research Scientist, Department of Microbiology, Shri MP Shah Medical College, Jamnagar, Gujarat, India.
8.  Research Scientist, Department of Microbiology, Shri MP Shah Medical College, Jamnagar, Gujarat, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Dipali Maganbhai Gavali,
401, Rajyog Apartment, Maruti Nagar 3, Airport Road, Near Krishnam Apartment,
Race Course, Rajkot, Gujarat, India.
E-mail: dipaligavli8@gmail.com

AUTHOR DECLARATION:
• Financial or Other Competing Interests:    None
• Was Ethics Committee Approval obtained for this study?    Yes
• Was informed consent obtained from the subjects involved in the study?    Yes
• For any images presented appropriate consent has been obtained from the subjects.    Yes