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ABSTRACT

Dengue fever is the predominant mosquito borne disease across the globe. The disease is spread by *Aedes* mosquitoes. It is endemic in tropical and subtropical countries of the world. Common symptoms of dengue fever (DF) are fever, fatigue, rash, headache, retro-ocular pain, arthralgia, myalgia, nausea, vomiting and low platelet count. Most infections result in asymptomatic response or mild febrile illness (DF). A small percentage of cases result in potentially fatal dengue with warning signs and severe dengue which is characterized by plasma leakage. The etiologic agent of dengue fever, the dengue virus belongs to the family Flaviviridae and genus Flavivirus. It is a small enveloped RNA virus with a single stranded, positive-sense RNA genome of ~10.7 kb. The genome encodes three structural and seven non-structural proteins in a single open reading frame. The open reading frame is flanked by 5' and 3' untranslated regions.

Thousands of cases of dengue fever are reported from Delhi every year. After the monsoon season, mosquito growth increases due to water logging. In addition, Delhi has many ill planned areas and infrastructure and drainage facilities are poor contributing to rampant mosquito growth. As a result dengue fever cases increase in the city in the rainy season. In the present study, DENV detection and serotyping were carried out on acute blood samples collected during 2011-2014. Molecular characterization of the DENV strains was carried out by sequencing of the envelope gene followed by phylogenetic analysis. Evolutionary analysis of dengue strains was also done using Bayesian methods.

In the present study, blood samples were collected from suspected dengue patients from Dr. M. A. Ansari Health Centre, Jamia Millia Islamia, New Delhi. Detection of DENV infection was done by RT-PCR and/or ELISA. DENV infection was detected in 68.87% out of 604 samples tested by RT-PCR between 2011 & 2014. DENV-1 was detected in 25.48% samples, DENV-2 in 79.56% samples and DENV-3 in 11.29% samples. DENV-4 was not detected. Co-infection by more than one dengue

serotype was detected in 18.26% samples. Before the predominance of DENV-2 detected in the present study, DENV-1 and 3 were in dominant circulation in New Delhi. Thus a change in the dominant dengue serotype was detected in the present study.

Envelope (E) gene of 38 DENV-1 strains, 47 DENV-2 strains and 14 DENV-3 strains was sequenced in the study. Phylogenetic analysis of DENV-1 strains clustered the strains in the American African genotype. Nucleotide substitution rate of DENV-1 was estimated to be 6.28×10^{-4} substitution /site/year by Bayesian evolutionary analysis. Time to the most recent common ancestor (TMRCA) was also determined.

DENV-2 strains of the study clustered within Cosmopolitan genotype on phylogenetic analysis. A lineage replacement event was also detected in 2013 in which a dengue outbreak had occurred. Lineage replacements are often observed in case of DENV and have been suggested to result in increased transmission of the viral infection. A novel mutation Thr404Ile in the stem region of the E protein of DENV-2 was also detected in the study. The stem region of the E protein is essential for the formation of E protein trimers in response to low pH. This novel mutation involes replacement of Threonine a polar amino acid by Isoleucine which is non polar but retains the property of having a chiral carbon in the side chain. Role of this mutation in the virus life cycle is not clear at present and should be explored in the site directed mutagenesis studies. Nucleotide substitution rates of DENV-2 were found to be 7.7×10^{-4} substitution /site/year and TMRCAs were also estimated. DENV-3 strains of the study were found to cluster with Genotype III .Nucleotide substitution rates of DENV-3 were found to be 7.64×10^{-4} substitution /site/year by Bayesian evolutionary analysis and TMRCAs were also determined.

The study will monitor dengue strains circulating in Delhi and will shed light on origin and diversity of these strains. Molecular characterization will assist in development of vaccines and antivirals. Further, these epidemiological investigations will also contribute to efforts towards prevention and control of the outbreaks.