Molecular characterization of Umbre virus (Bunyaviridae)
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Abstract
Umbre (UMB) virus was first isolated from India in 1955 and classified as Orthobunyavirus (Turlock serogroup). Eight isolates of this virus, isolated from Culex mosquitoes were characterized on the basis of partial glycoprotein (G2) gene. Twenty-six percent differences at nucleotide level while 17% differences at amino acid level were noted within different isolates. Phylogenetic data shows that this virus represents a distinct group within the genus Orthobunyavirus.

Findings
The viruses of Bunyaviridae family are spherical particles, range 80 to 120 nm in diameter and share a common genetic organization of three predominantly negative stranded RNA segments (S, M and L). Based on antigenic, genetic and ecological relatedness, the Bunyaviruses are divided into five genera. The genus Orthobunyavirus includes approximately 60 viruses, which are known to cause disease in humans (Elliot, 1996). Virological surveillance of these viruses depends primarily on detecting the viruses in arthropod vector populations in nature. Although, serological test like immunoassays are available for antigen detection for a few viruses, cross-reaction in closely related viruses cannot be ignored (Artsob et al., 1984; Hildreth et al., 1982).

UMB viruses used in this study are listed in (Table 1) along with their geographical origin, host source and year of isolation. The available eight strains of this virus was procured from the virus registry of National Institute of Virology, Pune and propagated in VeroE-6 cells. Cytopathic effect (CPE) was observed during 4th - 6th post infection day. Infected cells were harvested, centrifuged and supernatant was used for molecular characterization of the virus.

RNAs were isolated using chloroform, isoamylalcohol and further purified using RNAaid kit (Biogene), according to the manufacturer's instructions. RNAs were dissolved in 50 μl nuclease free water. Different sets of primers were used to amplify partial N, L and M gene. Partial M gene of 570 bp could be amplified using primer pair M14C and M619R, as described by Bowen et al., (2001), represents the nucleotide sequences of the N-terminal half.
Table 1: Details of the virus strains used in the current study

| Strain no. | Year of isolation | Host association | Place of isolation | Accession No.  |
|------------|------------------|------------------|-------------------|---------------|
| G-1424     | 1955             | Culex bitaeniornychus | Umbre, Maharashtra | EU697948      |
| G-7441     | 1956             | Cx. vishnui       | Kammavanpet, Tamil Nadu | EU697945      |
| G-8335     | 1956             | Cx. vishnui       | Minnal, Tamil Nadu | EU697946      |
| G-9601     | 1956             | Cx. vishnui       | Sulari, Tamil Nadu | EU697947      |
| G-16283    | 1957             | Cx. vishnui       | Sathuperi, Tamil Nadu | EU678356      |
| G-16310    | 1957             | Cx. vishnui       | Sathuperi, Tamil Nadu | EU697942      |
| 631308     | 1963             | Cx. vishnui       | Vellore, Tamil Nadu | EU697944      |
| 809365     | 1980             | Cx. vishnui       | Muduvadi, Karnataka | EU697943      |

of the G2 glycoprotein. Superscript III single step RT-PCR with Platinum Taq DNA polymerase kit (Invitrogen) was used for amplification of partial M gene according to the manufacturer’s instructions.

Amplified products were detected in 2% agarose gel after staining with ethidium bromide in Tris/acetate/EDTA buffer (TAE). A desired size of 575 bp product was purified using QIAquick gel extraction kit (Qiagen), as per manufacturer’s instructions. The sequences of amplified products were determined by using ABI PRISM BigDye Terminator V3.1 cycle sequencing ready reaction kit (Applied Biosystems). Amplification primers were used to sequence the amplified products. Cycle sequencing PCR program was used for 96°C-1 min, 96°C-10 sec, 50°C-5 sec and extension of 2 min at 60°C for 30 cycles.

The partial M gene sequence was curedt with the help of KODON Software and aligned with known Gene Bank sequences of Bunyamwera serogroup, California serogroup, and Kaeng Khoi viruses using clastal W program. Phylogenetic analysis was performed using Mega 3.0 by using neighbor-joining algorithm with thousand bootstrap values.

Partial M gene sequences showed maximum homology with Bunyamwera serogroup virus. Nucleotide and amino acid similarity within eight isolates varied from 74–100% and 83–99% respectively. Isolate G1424 and 809365 come together with 6% and 1% difference of nucleotide and amino acid respectively, while other six isolates club together with 5% nucleotide and 4% amino acid differences (Figure 1). Nucleotide and amino acid homology in UMB viruses ranged from 49–75% and 25–84% respectively, while other six isolates club together with 6% and 1% difference of nucleotide and 83–99% respectively. Isolate G1424 and 809365 not only highly homologues on the genomic level but also in their cell infectivity pattern. These two strains took more time to show CPE in comparison of other six isolates. Complete genome sequencing may shed light, why these two isolates are separate from other six, which club together despite isolated from two different mosquito species.

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Turlock and Umbre virus are distinct from each other based on neutralization test (Calisher et al., 1984). Availability of more sequences of Turlock group may answer about placement of this group of viruses. Bunyaviruses being three segmented RNA viruses have the capacity to reassort their segments into new genetically distinct viruses, if the target cells are subject to dual infection. The possibility of drift, shift and UMB virus evolution towards an emerging disease pathogen cannot be predicted based on partial sequences. Complete genome sequencing of UMB virus can possibly suggest whether there is any reassortment between three genes of this virus as known for Ngeri, Batai and Jatobal virus (Briese et al., 2006; Yanase et al., 2006; Saeed et al., 2001).

**Competing interests**
The authors declare that they have no competing interests.

**Authors’ contributions**
PDY performed the PCR and sequencing. ACM helped in preparation of manuscript. DTM and PDY designed, coordinated the study and prepared the manuscript.
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Figure 1
Phylogenetic comparison of partial M RNA segments of the Umbre virus with other Orthobunyaviruses. Using Mega 3.0 software Neighbor-joining analysis performed with 1000 bootstrap replicates. Umbre virus forms a separate group within Orthobunyaviruses. Partial M segment source are: Umbre virus strain no G-16310 (EU697942), 809365 (EU697943), 631308 (EU697944), G-7441 (EU697945), G-8335 (EU697946), G-9601 (EU697947), G-1424 (EU697948) and G-16283 (EU678356).
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