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Elucidating Genomic Variation And Evolutionary Dynamics of Local Dengue Virus In Host And Vector

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ABSTRACT

OBJECTIVE: To evaluate the evolutionary dynamics and genomic variation of Dengue Virus (DENV), specifically the Capsid precursor membrane (CprM) gene, in both the mosquito vector Aedes aegypti and human hosts.

METHODOLOGY: The Vector (Aedes aegypti) was molecularly identified using the Cytochrome C oxidase (COI) gene, and mosquito collection sites were mapped using geographic information systems. The Vector samples and Host (Human) samples were tested for dengue virus presence using PCR amplification that targets the CprM gene and generates a 510 bp fragment. To understand the evolutionary dynamics, nucleotide sequences of the CprM gene were retrieved from the NCBI database for mosquito-derived (32 sequences) and human serum–derived (40 sequences) samples, aligned using MEGA11 to identify conserved regions. Maximum likelihood phylogenetic trees were constructed to reveal evolutionary links between Dengue virus strains.

RESULTS: Strong purifying selection acting on the CprM gene was found by selection pressure analysis with the Global MG94xREV model and DataMonkey, with a low dN/dS ratio. Regions of high genetic diversity were identified through Shannon entropy analysis, indicating adaptive evolution. The Integer Neighbor-Joining (NJ) Network provided a clear visualization of the genetic relationships, demonstrating that the viruses have both host-specific and shared lineages.

CONCLUSIONS: This study discovered conserved and diversified genomic regions by phylogenetic analysis and molecular identification, revealing adaptive evolution and purifying selection. These findings enhance our understanding of DENV adaptation in hosts and vectors, informing targeted preventive measures.

Key Words: Dengue virus; Dengue hemorrhagic fever; Aedes aegypti; Agarose gel electrophoresis; Sequence alignment; Phylogeny; Natural selection

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INTRODUCTION

Dengue is an arthropod-borne arboviral infection and increasing public health problem in subtropical and tropical regions of the world. An estimated 50 to 100 million dengue virus (DENV) infections are recorded each year; of these, 250,000 to 500,000 cases result in dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF), with approximately 20,000–25,000 deaths annually.[1] A total of 146 nations and territories reported the incidence of at least one arboviral disease, with 123 (49.2%) reporting multiple arboviral infections.[2]

An enveloped single-stranded RNA virus of the Flaviviridae family, and the Flavivirus genus, is the primary cause of dengue, a highly serious febrile disease. The DENV comprises four antigenically distinct serotypes: DENV-1, DENV-2, DENV-3, and DENV-4, which are antigenically similar and can cause dengue infection. The clinical presentation of DENV infection can vary from mild symptoms like the flu to dengue DHF and DSS.3 Possible symptoms include a high-grade fever of 40 °C, a strong headache, pain in the muscles and joints, swollen glands, vomiting, eye pain, and nausea.[3] Two mosquito species, Aedes aegypti and Aedes albopictus, are responsible for the transmission of the DENV among humans.[4] The primary arbovirus vector for numerous diseases (dengue, yellow fever, etc.) is the hematophagous insect Aedes aegypti.[5]

The very first dengue outbreak in Pakistan was documented in Karachi in 1994. Subsequently, the outbreak spread throughout

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the entire country.6 Three primary components are required for the dengue fever outbreak: the dengue virus, Aedes aegypti, which serves as its vector, and an abundance of vulnerable human hosts. Entomologists have developed several taxonomic keys based on the morphology of Aedes aegypti over the last decade.[7] However, molecular biology has developed several methods to boost identification accuracy. These technologies not only aid in investigations of genetic variation resulting from ongoing insecticide usage but also from ecological changes.[8] To monitor dengue patients, viral isolation and then the gold standard polymerase chain reaction (PCR) assay are performed from suspected blood samples.[9]

Phylogenetic analysis has elucidated the origins, epidemiology, and forces that shape DENV molecular evolution in nature.[10] Several mitochondrial genes are utilized for molecular identification, including the internal transcribed spacer (ITS2) gene, the mitochondrial NADH dehydrogenase subunit 4 (ND4) gene, as well as the major mitochondrial cytochrome c oxidase subunit I (COI) gene.[11,12]The evolutionary dynamics of DENV are influenced by a wide range of human and mosquito parameters, such as seasonal fluctuations, virus and mosquito conditions, population immunity, vectorial capacity and competence, and random stochastic occurrences. Because of its RNA-dependent RNA polymerase (RdRp), which is prone to error, DENV typically acquires one mutation in each complete genome replication cycle in its vertebrate host. This leads to genomic diversification within the infected host, which are known as variants or quasispecies. Recombination events are uncommon in inter- and intra-serotypic infections, despite the well-documented fact that numerous DENV serotypes can coinfect an individual .[13]

The World Health Organization estimates that every year, from 50-100 million cases of dengue are reported globally.6 Despite significant vector control efforts, the rapid spread of the disease and global spread remain unchecked, and currently, dengue vaccines exist with limitations or targeted treatments.[14] DENV is the most common cause of hospitalisation worldwide and is widespread in Pakistan.[15] In the last several decades, the dengue virus has spread quickly both inside and across countries, leading to a rise in the frequency of outbreaks and severe dengue infection, as well as the hyperendemicity of several dengue virus serotypes in several tropical regions.16 Since 2005, dengue has become a major public health concern in Pakistan, posing a threat to millions of lives.

Even though dengue virus genetic diversity has been extensively studied worldwide, little is known about the relative evolutionary dynamics of dengue virus strains that are concurrently circulating in Even though dengue virus genetic diversity has been extensively studied worldwide, little is known about the relative evolutionary dynamics of dengue virus strains that are concurrently circulating in Pakistani mosquito vectors and human hosts within the same community transmission setting. Pakistani mosquito vectors and human hosts within the same community transmission setting. This research aims to enhance our understanding of dengue transmission dynamics and provide insights into virus evolution in humans and mosquitoes as well as to determine the species of mosquitoes involved in dengue transmission along with their distribution in Lahore. There is a knowledge gap regarding host–vector virus adaptability and lineage continuity during transmission cycles because most of the regional research either concentrate on clinical isolates or vector monitoring separately. Therefore, to better understand viral evolution within a localised transmission ecosystem, this study aimed to compare the genomic variation along with selection pressures acting on the dengue virus's CprM gene in locally collected human and vector samples, followed by global sequence comparison.

METHODOLOGY

Firstly, An in-silico comparative genomic study utilising publicly accessible dengue virus CprM gene sequences obtained from the NCBI database; and secondly, Molecular detection of laboratory-based of DENV in locally collected mosquito and human serum samples. Database sequences were used to assess evolutionary links and selection pressures over a larger genetic context. In contrast, local samples were utilised to verify the existence of circulating viruses. From January 2023 to January 2024, a total of 20 serum samples with laboratory confirmation of dengue infection were obtained from the Institute of Public Health, Lahore. The patients had visited the hospitals for general problems like fever, headache, bleeding, and retro-orbital pain. With the consent of the patients, the collected samples were subjected to RNA isolation within 6 hours of collection. Convenience sampling was employed since there were not enough confirmed cases available during the collection period, and the study was deemed exploratory.

Mosquito pools were made up of mature female Aedes aegypti mosquitoes that were gathered from homes where dengue cases have been reported. For molecular analysis, only female specimens with morphological confirmation were used. Genomic DNA was isolated from mosquitoes using a Blood and Tissue DNA extraction kit (Qiagen, Germany) by following the manufacturer's instructions. Using Nanodrop (Thermo Fisher Scientific, USA), the concentration of DNA was determined. The extracted DNA was kept at -20°C until further use. The COI gene, which is 735 bp in size, was amplified according to the method described by Chan A et al. using Primer pair; Forward Primer (5'GGATTTGGAAATTGATTAGTTCCTT–3') and Reverse Primer (3'-AAAAATTTTAATTCCAGTTGGAACAGC– 5'). The

PCR recipe was used for a total reaction volume of 25 µL. The cycling conditions for PCR amplification, for 35 cycles are start with an initial denaturation phase that denatures the DNA strands for three minutes at 95°C. After that, there were 35 cycles of denaturation (for 30 seconds at 95°C), annealing (for 30 seconds at 65°C to enable primer binding to the DNA), and extension (for 1 minute at 72°C to produce new DNA strands). The last extension was done for ten minutes at 72°C to make sure all the PCR products were fully synthesized. 1.5% agarose gel electrophoresis was used to visualise the PCR results.

RNA extraction: The Favor PrepTM Viral Nucleic Acid Extraction Kit was used to extract RNA from adult Aedes mosquitoes and human samples in accordance with the manufacturer's instructions. For later use, RNA was kept in storage at -80 °C.17 All the RNA samples were subjected to NanoDrop (NanoDrop 2000, ThermoFisher, US) to check RNA concentration and purity. cDNA Synthesis: All the extracted RNA samples were subjected to cDNA synthesis using gene-specific primers. A master mix containing the required amounts of each component was prepared, resulting in a total of 600 µL of reaction mixture for all samples. The PCR tubes were then run on a thermocycler at 42 °C for 60 minutes and then at 72 °C for 10 minutes, for complementary DNA synthesis. The prepared samples were then stored at -20 degrees for further experimental purposes. Primer selection: The primers set used for DENV detection were forward (TCAATATGCTGAAACGCGAGAGAAACCG) and reverse primer (TTGCACCAGCAATCAATGTCTTCGGGTTC). PCR Amplification (for DENV identification): A PCR reaction mixture was prepared, which has a final volume of 25 µL. The cycling conditions for PCR amplification, for 35 cycles, were as follows: a three-minute initial denaturation at 95°C to denature the DNA, thirty seconds of denaturation at 95°C, and one minute of extension at 72°C to synthesize new DNA strands. The PCR products underwent a final extension phase, which involved 10 minutes at 72°C to guarantee full extension. Mutational and Data Analysis Methods: Dengue virus Capsid–pre-membrane (CprM) gene sequences from human and mosquito isolates that had previously been reported were obtained from the NCBI GenBank database in order to look into evolutionary trends. To determine conserved and variable regions, multiple sequence alignment was carried out using MEGA. The ML approach was used in MEGA11 to create phylogenetic trees that showed the evolutionary relationships between the various DENV strains. Utilizing models like Global MG94xREV and the Datamonkey service, the selection pressure on the CprM gene was evaluated by computing the dN/dS ratio. To identify episodic positive selection at specific sites, the Mixed Effects Model of Evolution (MEME) was utilized. Using BioEdit and additional entropy calculation tools, Shannon entropy was computed for amino acid sequences of the CprM region to evaluate sequence variability.

To evaluate the mutation patterns in the virus obtained from human hosts vs mosquito vectors, a comparative investigation was carried out. The evolutionary pressures operating on the virus in various habitats are identified by analyzing the variations and similarities in mutation rates and types. Using PopArt software, a neighbor-joining network was built to show the genetic links between the DENV sequences.

RESULTS

Mosquito Sample Collection and Species Identification

The research study was carried out in the district of Lahore. Dengue virus was detected in a subset of locally collected mosquito and human samples using PCR tests. The publicly available CprM gene sequences obtained from GenBank were then used for phylogenetic and evolutionary studies. Aedes aegypti mosquito samples were taken at random from the locations listed in Table 1. A total of ten Aedes aegypti pools were collected. After microscopy, a total of 74 Aedes aegypti mosquitoes were separated into ten pools, labelled, capped, and kept in 1.5 mL microcentrifuge tubes at −80°C until needed. Only female mosquitoes were then subjected to additional analysis (Figure 1).

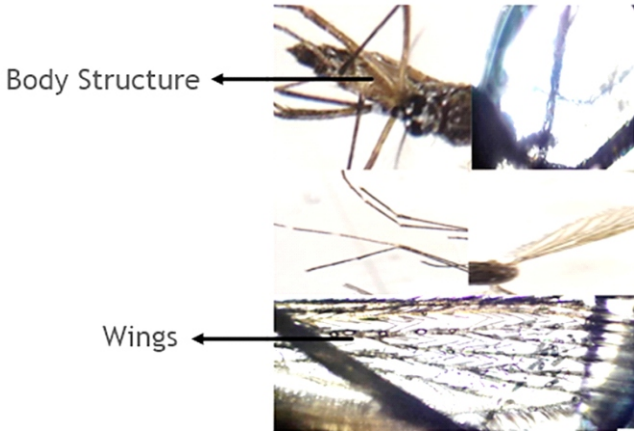
Multiple Sequence Alignment and Phylogenetic Analysis of Aedes aegypti COI gene

The sequences in FASTA format retrieved from NCBI were obtained based on a high identity percentage. The downloaded FASTA sequences were aligned using Clustal-W using the Molecular Evolutionary Genetics Analysis (MEGA-X version 11) program. A phylogenetic tree was generated with the assistance of the MEGA program. The evolutionary tree was generated by the Maximum Likelihood technique and bootstrapping with 1000 replications, utilizing the COI gene sequences from Aedes aegypti in the phylogenetic analysis. Following that, a BLAST search was conducted on the NCBI database using the COI gene sequences that were produced of Aedes aegypti's BLAST results showed 97.83–100% identity, which is almost similar to previously reported Aedes aegypti from GenBank from India (MK265724, MK265725, MK265726, MK265727, MK359842, MG770600, MK805532, and MK805535), Sri Lanka (MGH004693, MGH004696, MGH004699, MF993580, MG004703, MG004708, and MG384711), Brazil (MN018999, MN018985, MN019000, MN019002, and MN019006), Tokyo (LC482626 and LC482630), Sudan (MN893739 and MN893711), and West Africa (MK359830) which will be shown in phylogenetic tree (Figure 1 in Supplementary File 1).

Table 1: Mosquito Sample Collection and Identification Information

Pool ID	Species	Number of Mosquitoes	Collection Date	Collection Location	Identifier
1	Aedes aegypti	5	2023-10-28	Site A (Urban)	AEG-1
2	Aedes aegypti	8	2023-10-28	Site A (Urban)	AEG-2
3	Aedes aegypti	10	2023-10-15	Site B (Suburban)	AEG-3
4	Aedes aegypti	6	2023-10-26	Site C (Suburban)	ALB-1
5	Aedes aegypti	9	2023-10-26	Site C (Rural)	ALB-2
6	Aedes aegypti	7	2023-10-09	Site D (Forest)	ALB-3
7	Aedes aegypti	5	2023-10-15	Site B (Suburban)	AEG-4
8	Aedes aegypti	10	2023-10-28	Site A (Urban)	AEG-5
9	Aedes aegypti	8	2023-11-09	Site D (Forest)	ALB-4
10	Aedes aegypti	6	2023-10-26	Site C (Rural)	ALB-5

Figure 1: Morphological Identification of Mosquitos



Geographical distribution of Mosquito Sample Collection sites

Table 2 details the locations where the mosquito samples were collected. GPS coordinates, including latitude and longitude values, were precisely calculated and recorded GPS (Global Positioning System) for every sampling site. These sampling sites' geographic dispersion reflects the diverse array of habitats from which the mosquito samples have been collected.

Geographic Information Systems (GIS) Mapping of Collection Sites

GPS coordinates were obtained for each sampling location, which represented various habitats throughout Lahore (Table 2). GIS mapping was utilised to identify possible dengue hotspots (Figure 2).

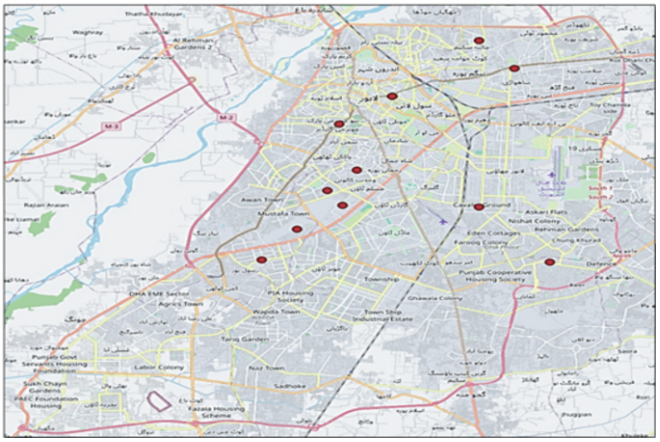
Molecular Identification of dengue virus in collected vector samples (Aedes aegypti) and Host Samples using PCR

The Vector (Aedes aegypti) samples and Host (Human) Samples were tested for dengue virus presence using PCR amplification that targets the CprM gene, which, when present, generates a 510 bp fragment. This indicates that the vector (Aedes aegypti) samples contained the dengue virus in human host serum samples from probable Aedes aegypti vector infections. Variable band presence indicated heterogeneous infection rates among mosquito pools. The specificity and precision of the PCR results are validated by the absence of the bands in the negative controls, indicating that the bands detected as a result of dengue virus DNA presence (Figure 2, 5 in Supplementary File 1).

Table 2: Sample Collection Locations

S.No	Sampling Location	Latitude (N)	Longitude (E)
1.	Bahria Town	31°20'28.1"	74°13'25.3”
2.	Johar Town	31°28'00.0"	74°22'00.0"
3.	Sabzazar	31°29'12.0"	74°16'48.0"
4.	Iqbal Town	31°30'15.2"	74°18'45.4"
5.	Mustafa Town	31°31'20.3"	74°18'15.1"
6.	Samanabad	31°31'00.0"	74°18'00.0"
7.	Data Gunj Baksh Town	31°34'00.0"	74°19'30.0"
8.	Shalimar Town	31°35'00.0"	74°23'00.0"
9.	Ravi Town	31°34'00.0"	74°19'30.0"
10.	Cantonment near Ravi River	31°36'00.0"	74°22'00.0"
11.	Lower part of Ravi Town	31°33'00.0"	74°18'00.0"
12.	Aziz Bhatti Town	31°28'00.0"	74°24'00.0"
13.	Nishtar Town	31°30'00.0”	74°22'00.0"

Figure 2: GIS Mapping of Dengue hotspots in Lahore
Molecular Identification of Aedes aegypti Mosquito



PCR amplification produced a 735 bp COI gene fragment. A 1.5% agarose gel was used to visualize the PCR results, as shown in Figure 3.

Lane-1-6, 11-16 = 735 bp specific band for Aedes aegypti (Vector) DNA (COI-gene)

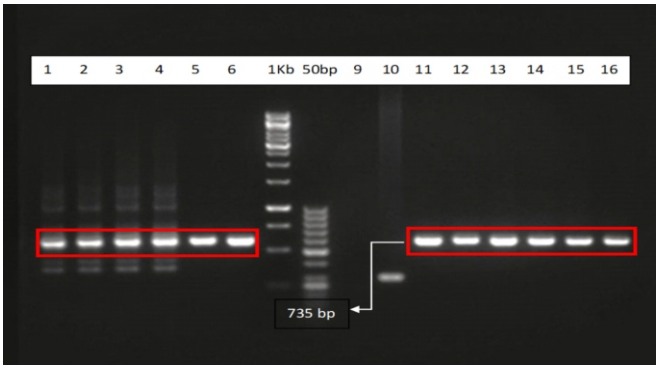
Lane 7= 1kb DNA Ladder

Lane 8= 50 bp DNA Ladder

Lane 9 = Negative control

Lane 10= Positive control

Figure 3: Molecular Identification of Aedes aegypti

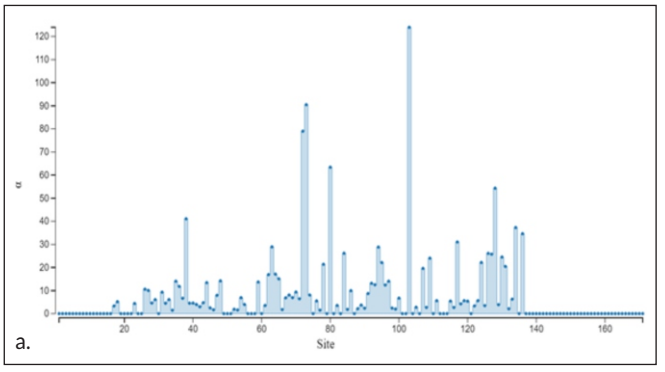


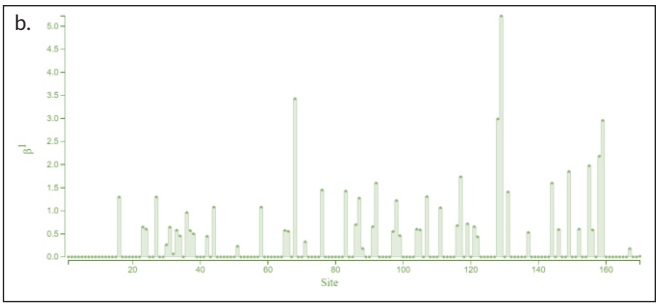
Selection Pressure Analysis of DENV (Aedes aegypti) CprM gene

Selection pressure analysis was conducted on the CprM gene of the DENV genome using the DataMonkey server, as shown in Figure 4a (Figure 4 in Supplementary File 1).

- The Global MG94xREV model was selected based on a higher log-likelihood (log L) value (-2428.79) and lower corrected Akaike Information Criterion AICC value (4983.38).Selection pressure analysis was conducted on the CprM gene of the DENV genome (Host) using the Data Monkey server, as in Figure 4b.

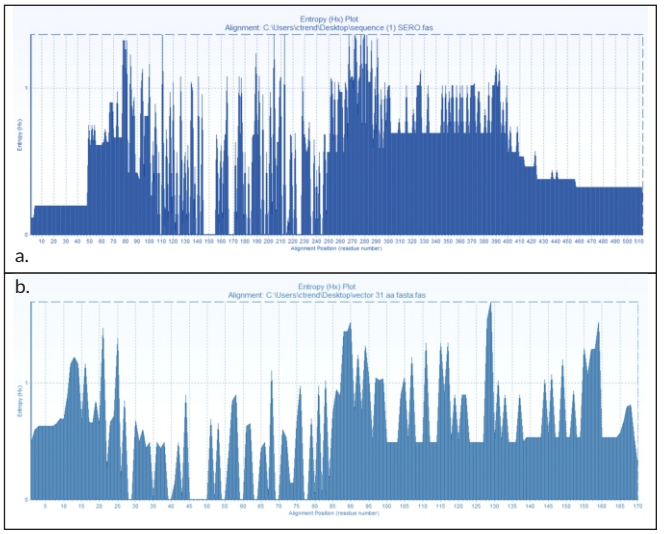
Figure 4: Selection pressure analysis conducted on the CprM gene DENV a. Host b. Aedes aegypti





The non-synonymous/synonymous rate ratio (dN/dS) for the sequences was calculated to be 0.125. This ratio, being significantly less than 1, suggests that the DENV sequences are under strong purifying selection. This means that synonymous mutations, which do not alter the amino acid sequence, are more prevalent than non-synonymous mutations, indicating selective pressure to maintain the protein-coding integrity of the viral genome. The findings underscore the evolutionary stability of the DENV genome, which is essential for its survival and pathogenicity. Shannon entropy analysis identified genetic variety hotspots in the CprM gene (Figure 5a). The Global MG94xREV model demonstrated a lower AICC value (3172.85) compared to the Nucleotide GTR model (3379.03), indicating a better balance between model fit and complexity. The dN/dS ratio (0.0942) is significantly less than 1, indicating that the CprM gene analyzed is under negative selection. This low ratio suggests strong selective pressure to preserve this gene region, as non-synonymous mutations (which alter protein function) are likely to be deleterious and are thus removed by natural selection. Shannon entropy analysis was conducted on the amino acid sequences of the CprM region of DENV, derived from human host samples. The entropy values (Hx) were calculated to quantify the degree of variability, with higher entropy values indicating greater variability at specific positions, as in Figure 5b.

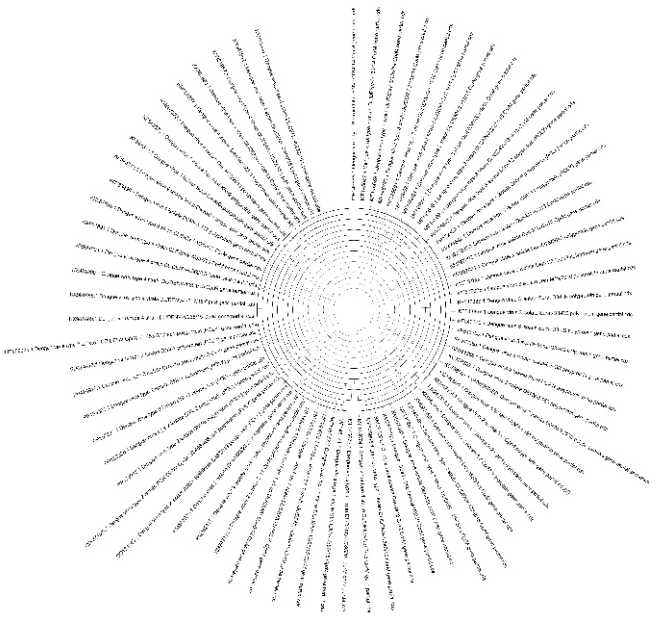
Figure 5: Shannon Entropy DENV CprM gene a. Host b. Aedes aegypti



Multiple Sequence Alignment and Phylogenetic Analysis of DENV (Aedes aegypti) and Host Samples of the CprM gene

The PCR primers used for dengue virus detection target the CprM gene region, which is the same genomic region analyzed in the retrieved GenBank sequences for evolutionary comparisons. No sequencing of local PCR products was performed. The similar sequences were downloaded from NCBI (Supplementary files 1 and 2). These sequences were aligned using the Clustal-W algorithm within the Molecular Evolutionary Genetics Analysis (MEGA-X version 11) software. MEGA was also used to generate a phylogenetic tree, where retrieved CprM gene sequences derived from human isolates and Aedes aegypti were analysed. An evolutionary tree was generated using the Maximum Likelihood method with bootstrapping performed with 1000 replications (Figure 3, 6 in Supplementary File 1).

Figure 6: The DENV (Host) and DENV (Vector) CprM phylogenetic tree bar representing a substitution rate of 0.0005 and created using the Maximum Likelihood approach with 1000 bootstrap replicates.



The Global MG94xREV model was preferred for analyzing the evolutionary dynamics of DENV in the human host based on its superior fit as indicated by the AICC and log likelihood values (Figure 7 in Supplementary File 1).

Neighbor Joining network of DENV (Host and vector)

Variations in branch lengths were observed across the network, reflecting the degree of genetic divergence between sequences. Shorter branches indicated closer genetic relationships, while longer branches suggested greater divergence. Certain sequences formed central nodes in the network, connecting multiple branches. These central nodes could represent ancestral sequences or sequences that have undergone less divergence compared to others in the network. These regions indicate hotspots of genetic diversity within the host population, reflecting the virus's adaptation to different environmental pressures or vector species.

Combined Analysis of Host and Vector CprM Gene Sequences

Phylogenetic analysis identified several subclades, indicating the DENV serotypes/strains that circulated in the population. Variations were detected for both hosts and vectors Figure 7. The Integer Neighbor-Joining Network analysis revealed genetic linkages and divergence trends, implying that geographical and environmental factors influence DENV evolution (Figure 7). These clusters suggest possible common ancestral origins or recent gene flow between these groups.

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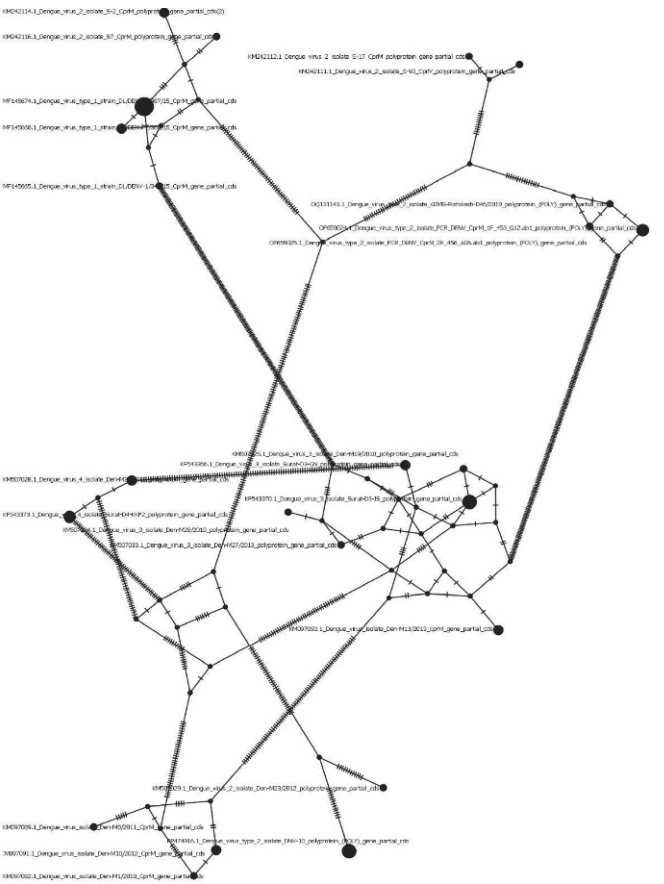
Figure 7- Neighbor Joining network of DENV (Host and vector)



DISCUSSION

In this study, we analyzed 20 serum samples for PCR detection of dengue virus, while evolutionary analysis was conducted using retrieved CprM gene sequences from human and vector isolates, to investigate the genomic variation and evolutionary dynamics of the dengue virus in both the host (human) and vector (mosquito). Additionally, we employed GIS mapping to visualize the geographical distribution of mosquito collection sites, providing a spatial context to the molecular findings. The identification of the DENV CprM gene in the serum samples confirmed the presence of the virus in the human hosts. This finding is consistent with previous studies that highlight the CprM gene as a critical target for molecular diagnostics of dengue virus due to its conserved nature across different strains.[18]

Islam et al. conducted a study in India in 2023 where patients enrolled presented with fever, frequently accompanied by rashes, headaches, nausea, vomiting, retro-orbital pain, and weakness. In 2018, 28 out of 89 serum samples tested positive DENV, representing 32% of the samples tested after the monsoon season, demonstrating the value of molecular methods for diagnosing dengue infection in instances that are clinically suspected.[19] The CprM gene was similarly analysed for the vector, and the COI gene was used for molecular identification, enabling accurate differentiation of Aedes Aegypti from other mosquito species. This shows that our analyses were focused on the primary vector responsible for DENV transmission. The GIS mapping of mosquito collection sites provided valuable insights into the spatial distribution of Aedes Aegypti populations in the study area. This spatial analysis, combined with molecular data, can help identify potential hotspots for DENV transmission and contribute to target the vector control strategies for DENV prevention.

The study conducted by Isa et al. aimed to detect DENV serotypes prevalent in Aedes mosquito populations which were gathered from certain locations in Nigeria. Using Rueda's coloured identification keys, the mosquitoes were gathered and morphologically classified down to the species level. In general, pools of 20 individuals from each Aedes species were used to test each discovered species. Sanger sequencing techniques were employed to characterize the DENV serotypes found in mosquitoes using RT-PCR and semi-nested PCR.[20]

Furthermore, one of the important and effective tools for detecting the onset of dengue outbreaks is the detection of DENV in infected mosquitoes. By combining PCR-based screening with the GIS, a useful tool for vector control may be possible, allowing the identification of high-risk locations. Méndez-Galván et al. demonstrated that it enhances the capacity of epidemiological surveillance systems to predict outbreaks and identify silent viruses. There is a strong likelihood of a dengue outbreak in the future because DENV is circulating in dengue vectors.[18] During the investigation, 37 different kinds of mosquitoes were collected from the Canadian provinces using the standard taxonomic keys for classification. After identification, their DNA was extracted using the Gene Elute-TM-Mammalian Genomic DNA Miniprep Kit, and the COI gene was amplified and sequenced.[21]

A study on the phytogeography and invasion history of Aedes Aegypti carried in West Africa in order to characterize molecules and analysing COI genes in the vector.[22]

Using light traps to monitor dengue illness, 950 specimens were gathered for investigation in Saudi Araia. Although this study demonstrated that Aedes Aegypti are present all year round, two peaks in abundance were noted from December to March due to ideal humidity and temperature.[23] Multiple sequence alignment of the CprM gene's nucleotide sequences from both human and mosquito samples revealed several mutations, with some conserved regions across all samples. In human hosts, certain mutations may be driven by the immune response by the host's defense mechanisms, leading to the selection of viral variants that can evade immune detection, suggesting host-specific adaptations that influence its transmission dynamics and virulence. Sequences of the CprM gene were partially examined with two DENV-4 samples and five DENV-3 samples were sequenced. Using NCBI BLAST, the consensus sequences were verified. Islam et al. also included sequences from 2017 (DENV-1, n = 2; DENV-3, n = 16) in their evolutionary study.[19]

The phylogenetic trees constructed using the maximum likelihood method in MEGA11 revealed distinct clades corresponding to different genotypes, with some strains showing close evolutionary relationships across both hosts. This indicates that the virus circulates between humans and mosquitoes, maintaining its genetic diversity. The selection pressure analysis, conducted using the Global MG94xREV model and the Datamonkey service, indicated that the CprM gene is generally under purifying selection in both hosts, as evidenced by the low dN/dS ratio. This suggests that most mutations in the CprM gene are deleterious and are selected against, preserving the gene's functional integrity. However, the application of the Mixed Effects Model of Evolution (MEME) identified episodic positive selection at specific sites in the CprM gene. These positively selected sites may be associated with adaptive changes that enhance the virus adapting in either the human host or mosquito vector. The Shannon entropy analysis of the amino acid sequences of the CprM region revealed regions with varying degrees of sequence variability. Higher entropy values were observed at certain positions, indicating areas of greater sequence diversity. This variation may reflect the different selective pressures acting on the virus in humans versus mosquitoes, with the virus possibly adopting

different evolutionary strategies to optimize its survival and replication in each host. In another study, selection pressure analysis revealed purifying or negative selection in the CprM region for specific codon locations under positive selection were found. Using BioEdit (v.7.2) software, entropy analysis was used to identify the locations in the CprM region of the DENV genome that are prone to mutation for DENV-1, DENV-3, and DENV-4. A threshold of 0.2 was established for the identification of a variable site.[19]

The construction of the Integer NJ (Neighbor-Joining) network for the CprM gene sequences from dengue virus (DENV) isolates obtained from both host and vector samples provided valuable insights into the evolutionary dynamics of the virus. The results demonstrated distinct clustering patterns, reflecting the potential influence of host and vector on viral genetic diversity. The distinct clusters observed in the NJ network suggest that DENV may undergo different evolutionary pressures depending on whether it is within a host or a vector. The clustering of specific sequences within host or vector groups indicates that certain genetic variations may be more favourable or adaptive in one environment over another. This host-vector differentiation could be a result of selective pressures such as immune response in the host or replication efficiency within the vector.

The topology of the NJ network, with its closely related sequences and few outliers, suggests that while most DENV isolates are relatively conserved, there are unique evolutionary events that contribute to the overall diversity of the virus. These outlier sequences could represent rare mutations or recombination events that might affect the virus's ability to infect hosts or vectors or to evade immune responses. The DENV-3 and DENV-4 networks demonstrated unique lineages and regional grouping, along with the appearance of haplotypes are also reported.[19,23]

There are several limitations to this study. First, results should be considered as exploratory rather than population-representative because there were not enough clinical samples gathered locally. Second, geographic sampling bias may have been introduced by the evolutionary analysis's partial reliance on publicly accessible sequences. Third, the entire viral genome was not examined. It is advised that future research employ whole-genome sequencing and longitudinal sampling to confirm these results.

It is recommended to employ continuous integrated surveillance that combines molecular characterisation of circulating dengue virus strains with vector monitoring. In endemic areas, routine genomic surveillance may enhance outbreak prediction and aid in the detection of new variations.

CONCLUSION

The comparative analysis of DENV sequences showed differences in mutation patterns, selection pressures, and genetic diversity between the hosts which underscores the virus ability to adapt to varying environments. This is critical for its persistence, spread and evolution of the DENV CprM gene. The Integer NJ network analysis of the CprM gene provides a deeper understanding of the evolutionary relationships between viral strains in hosts and vectors. The observed clustering patterns and genetic divergence emphasize the importance of considering both host and vector environments when studying DENV evolution. The detection of genetic variations would be helpful in reducing the risk Dengue virus strains may pose in the future, especially in areas where the virus is endemic.

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CONFLICT OF INTEREST

Author declared no conflict of interest

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AUTHORS CONTRIBUTIONS

FK: Conceptualization, study design, data collection, manuscript drafting, critical revision of the manuscript, final approval of the version to be published, and accountability for all aspects of the work.

MZ: Conceptualization, study design, data acquisition, data analysis, manuscript drafting, critical revision of the manuscript, final approval of the version to be published, and accountability for all aspects of the work.

SQ: Conceptualization, study design, data interpretation, manuscript drafting, critical revision of the manuscript, final approval of the version to be published, and accountability for all aspects of the work.

MM: Data collection, manuscript drafting, critical revision of the manuscript, final approval of the version to be published, and accountability for all aspects of the work..

DATA SHARING POLICY

The data that support the findings of this study are available from the corresponding author upon reasonable request.