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Molecular epidemiology and evolutionary trends of dengue virus serotype-2 strains in Sri Lanka

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Abstract

Background Dengue virus (DENV) infections in Sri Lanka have intensified over the past 3 decades. Surveillance and characterization of different DENV lineages of different serotypes causing outbreaks is crucial for initiation of timely dengue control measures and for implementing dengue vaccines. Therefore, we characterized the DENV-2 strains in Sri Lanka from 2016, until end of 2023 and their evolutionary dynamics to understand the geographical spread and mutations arising with the DENV-2 serotypes in Sri Lanka.

Methodology Sequencing was carried out on 80 DENV-2 samples collected from patients with acute dengue recruited in the years 2016 to 2018, and on 12 DENV-2 samples in patients recruited in years 2022 to 2023 using Oxford Nanopore Technology. Phylogenetic analysis was carried out using the IQ-TREE tool, and Galaxy to construct a phylogenetic tree from the aligned sequence data. The sequences were also analyzed for non-synonymous amino acid changes in the envelope and NS1 regions.

Results The Sri Lankan DENV-2 sequences circulating from 2016 to end of 2023 belonged to genotype II.F.1.1 lineage. They were closely related to strains circulating in the same period in South Asia and Southeast Asia. We identified 15 non-synonymous mutations within the envelope region and 22 non-synonymous mutations within the NS1 region, with 7 non-synonymous mutations within the E region (M6I, Q52H, E71A, V129I, N390S, I484V, T478S) and 10 non-synonymous mutations within the NS1 region (S80T, T117A, Q131H, K174R, F178S, N222S, L247F, I264T, T265A, K272R) seen in all sequenced samples. Some of these mutations were previously shown to be associated with increase in viral replication, NS1 secretion and immune evasion, while some have not been reported elsewhere.

Conclusions Given the increase in dengue transmission in many countries, it is important to further strengthen DENV surveillance for studying the evolutionary patterns of the DENV to initiate timely and appropriate control measures.

Keywords Dengue, Virus, Serotypes, Genotypes, Mutations, Transmission, Evolution, Pathogenicity, Virulence, Sequence

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Background

There has been an alarming rise of dengue infections in all regions of the world, with year 2024 reporting the highest number of dengue cases so far [1]. An estimated 3.83 billion people—more than 53% of the global population—live in areas suitable for dengue transmission, a figure that is expected to rise further in the future due to climate change [1, 2]. Yet, the number of dengue infections is forecasted to keep rising every year while spreading to new geographical regions due to multiple factors such as climate change, rapid urbanization, population displacements and possibly rapid evolution of the dengue virus (DENV) [3]. As a result, the World Health Organization has recently named it as an emerging pathogen of pandemic potential [4].

Dengue infections are caused by four related but genetically distinct dengue virus serotypes (DENV1 to 4), which share 60 to 70% homology with each other [5]. Each DENV serotype consists of multiple lineages with a nucleotide diversity of 6 to 8% [6]. Although DENVs are endemic in many countries in the tropical and subtropical regions, major shifts in the circulating DENV serotype leads to large outbreaks, overwhelming health care systems [7–10]. Moreover, due to increased transmission rates, many countries have reported co-circulation of multiple DENV serotypes and lineages resulting in outbreaks [11–13]. Sri Lanka has been experiencing dengue outbreaks for the last 3 decades, with the number of dengue cases increasing over the years [7]. While dengue is endemic in Sri Lanka, with one of the four DENV serotypes dominant during certain periods, sudden shift in the predominant circulating DENV serotypes has been associated with occurrence of large outbreaks [7]. For instance, the emergence of DENV-2 in mid-2016, was associated with the largest outbreak reported in Sri Lanka in year 2017, which was associated with increase in disease severity and mortality, resulting in 186,101 hospital admissions [14, 15]. This DENV-2 strain continued to cause outbreaks until the later part of 2019, when DENV-3 emerged causing a large outbreak [11]. However, the incidence of dengue in Sri Lanka drastically declined during 2020 to mid-2022, during the COVID-19 pandemic, as seen in many other countries, possibly due to school closures and lack of movement [16]. Following the relaxation of COVID-19 restrictions dengue emerged, with DENV-2 being the predominant serotype until the later part of 2022, where DENV-3 emerged, causing a large outbreak in year 2023 [11]. Furthermore, although all DENV serotypes are known to cause severe dengue, DENV-2 was shown to have a higher likelihood of causing severe dengue, compared to other DENV serotypes [17–19]. Therefore, it is important to study possible virological factors that led to higher morbidity and mortality and persistence of DENV-2 during the pandemic years and emergence of DENV-2 as the predominant serotype, while the COVID-19 restrictions were lifted.

Different DENV lineages have been shown to result in different magnitudes of infectivity, viral titers and pathogenicity in *in vitro* models [20], with certain lineages known to cause more severe infection and larger outbreaks [14, 21]. For instance, mice infected with the DENV-2 sub-genotype A of the cosmopolitan strain showed significantly higher weight loss and delay in clearance of the virus than mice infected with sub-genotype C of the cosmopolitan strain, showing that mutations and single nucleotide substitutions in DENV strains may cause differences in disease pathogenesis [22]. Different DENV strains cause varied pathogenicity in different mouse models, and viraemia, clinical features, disease severity, not only depend on the virus strain used, but also if the animal models are immunocompetent or not, whether the strain is mouse-adapted, neuro-adapted or closer to the clinical isolates [23, 24]. Certain changes in the DENV, such as single nucleotide substitution in an American DENV-2 genotype may explain the lack of outbreak in Tonga in contrast to large number of severe dengue cases elsewhere in the Pacific during the early 1970s [25]. These phenotypic differences between the DENV lineages may be attributed to sequence-dependent virus-host interactions. With the implementation of new vaccines and vector control strategies, immunity from dengue vaccination and the use of Wolbachia mosquitoes in vector control may impose new selection pressures on DENV [26], thus creating the possibility of new virus lineages. Therefore, surveillance and characterization of circulating DENV lineages of different serotypes would be important to understand how they may affect clinical disease severity and transmission dynamics. In this study, we characterized the DENV-2 strains in Sri Lanka from 2016, until end of 2023 and their evolutionary dynamics to understand the geographical spread and mutations arising with the DENV-2 serotype in Sri Lanka.

Methods

Recruitment of patients with acute dengue infection for serotyping PCR and DENV sequencing

Blood samples were collected by nursing officers and trained medical professionals using proper biosafety precautions, from adult patients who were clinically suspected to have an acute dengue infection, with a duration of illness of ≤4 days, following informed consent. 369 patients were recruited from July 2016 to October 2018, and 467 adult patients were recruited between November 2022 to December 2023, in the National Institute of Infectious Diseases (NIID), Angoda, Sri Lanka. Quantitative real time PCR for detection of the four DENVs were carried out in all samples to determine the infecting DENV serotype using modified CDC primers as

previously described [16, 27]. A multiplex PCR, which also identifies the DENV serotype was carried out in all samples collected from these patients, as previously described [16, 27]. Only samples in which DENV-2 was identified, were included in the subsequent analysis.

Ethical approval

Ethics approval (12/15 and 58/19) for the study was obtained from the Ethics Review Committee, University of Sri Jayewardenepura and the administrative clearance was obtained from the Ministry of Health, Sri Lanka. All individuals gave informed written consent.

Whole genomic sequencing of the DENV2 that circulated in Sri Lanka during 2017 to 2018 and 2023

As DENV-2 was the predominant circulating serotype since mid-2016 and was responsible for over 95% of infections in 2017 [14], 80/369 DENV2 positive samples from mid-2016 to end of 2018 and 12/467 DENV2 positive samples from November 2022 to December 2023 were selected for sequencing using an amplicon-based framework [28]. All samples with a cycle threshold value of <25 were selected for sequencing. Briefly, Viral RNA was extracted from clinical serum specimens using the QIAamp Viral RNA Mini Kit (Qiagen, USA, Cat: 52906) according to the manufacturer's protocol. The procedure involved lysis of samples in buffer AVL (containing carrier RNA), followed by the addition of ethanol and binding of RNA to the QIAamp mini spin column. After two sequential wash steps using buffers AW1 and AW2, purified viral RNA was eluted with Buffer AVE. The extracted RNA was either used immediately for cDNA synthesis or stored at -20 °C.

Whole genomic sequencing was carried out as previously described by us [11]. Briefly, the cDNA was synthesized from viral RNA using the SuperScript IV First-Strand Synthesis System (Life Technologies) – for the 80 samples collected from 2016 to 2018, or Lunascript (LunaScriptTM RT SuperMix Kit, New England Biolabs, E3010) – for the 12 samples collected between 2022 and 2023. Amplicons were then generated using Q5 Hot Start High-Fidelity DNA Polymerase (New England Biolabs) with DENV-2 specific primers. The PCR mixture was initially incubated for 30s at 98 ∘C for denaturation, followed by 35 cycles of 98 ∘C for 15 s and 65 ∘C for 5 min. The PCR products were then diluted 10-fold. Subsequently, the diluted amplicons were end-prepped and dA-tailed using NEBNext Ultra II End Repair/dA-Tailing Module. Native barcodes and sequencing adapters supplied in the EXP-NBD101/114 kit (Oxford Nanopore Technologies) were attached to the dA-tailed amplicons using NEB Blunt/TA Ligase 2x Master Mix and NEBNext Quick Ligation Kit, respectively. Finally, 15 ng of DNA library was loaded on to the R9.4.1 flow cell following the SQK-LSK109 ligation sequencing kit (Oxford Nanopore Technologies) protocol, the sequencing run was performed on the Minion Mk1b (Oxford Nanopore Technologies) for a total of 24h. Reads were basecalled using the MinKnow software.

Bioinformatics analysis

The details of all bioinformatics analysis including data acquisition, phylogenetic analysis, tree building, mutational analysis, molecular clock analysis and geospatial mapping can be found in the Supplementary methods.

Previous studies have identified the envelope (E) and non-structural protein 1 (NS1) genes as the most informative targets for DENV-2 genotyping. Accordingly, our mutational analysis was specifically focused on these two key genomic regions to investigate viral evolution [29].

Statistical analysis

Statistical analysis was carried out with R (version 4.4.1) using R packages stats, dplyr, ggplot2, ggpubr, tidyverse, patchwork, stringr. (version 3.12.8) using pandas, geopandas, matplotlib, Phylo, SeqI0,TreeTime. All the R and Python code used for analysis and figure generation can be accessed at: https://github.com/AICBU/DENV2_SriLanka.

Results

Full-length genomic sequence of the circulating DENV-2 in Sri Lanka

Quantitative real-time PCR was carried out in a total of 836 samples obtained from patients with a suspected dengue infection (collected between July 2016 to October 2018 and between November 2022 to December 2023). DENV-2 was identified in 284 samples (207 samples from 2018 to 2018 and 77 samples from 2022 to 2023) and 92/284 DENV-2 samples were subjected to sequencing. The mean age of the patients was 31 years (SD ± 14.2 years). Whole genome sequencing of DENV-2 positive samples collected during the outbreak between 2016 and 2018 (Supplementary Table 7) found the circulating DENV-2 strain to be genotype II, major lineage F and minor lineage 1 (genotype II.F.1.1) based on the latest classification of DENVs (Fig. 1) [6]. Phylogenetically, the 2017/18 sequences were closely related to strains circulating during the same period in China and other Asian countries such as Thailand, Singapore and Malaysia (Fig. 1b). DENV-2 positive samples were also collected between 2022 and 2023 (Supplementary Table 07). The whole genome sequencing on these samples revealed they were of the same genotype II F.1.1 strain as the 2017/2018 samples. The 2017/2018 and 2022/2023 sequences formed a new DENV-2 genotype II.F.1.1 clade, clustering with other previously reported 2017 Sri Lankan DENV-2 strains (MT180479, GenBank)

Fig. 1 Phylogeny of Dengue virus serotype 2 (DENV-2). **A** Maximum-likelihood phylogeny based on full-length DENV-2 genome sequences ($n = 1,098$) representing the global diversity of major DENV-2 genotypes and lineages. Major clades are color-coded according to the latest classification. This overview provides a global perspective on DENV-2 evolutionary relationships and illustrates where Sri Lankan sequences from this study fall within the broader phylogeny. **B** Magnified view of the 2II_F clade from panel A, showing finer-scale phylogenetic structure within this lineage. Global 2II_F sequences are shown in yellow, while Sri Lankan sequences from both outbreak periods (2016–2018 and 2022–2023) are highlighted in red. Time-scaled phylogenetic analysis was performed with TreeTime, and estimated times to the most recent common ancestor (TMRCA) are indicated on key nodes

[30], and genetically distant from the previous DENV-2 strains that circulated in Sri Lanka from 1996 to 2003, which belonged to DENV-2 genotype II_A (Sup Fig. 1). These DENV-2 strains from 1990s and early 2000s belong to the major lineage A, which was mainly reported from Africa in The 1990s, which has since evolved to II_A1 by 2007 and subsequently II_A2.2 by 2018 (Fig. 1B)(Fig. 2). The more recent F1.1 lineage is predominantly spread in East and Southeast Asia (Fig. 1B). Although the Sri Lankan sequences span 2016–2023, time-resolved phylogenetic analysis estimated the time to the most recent common ancestor (tMRCA) of the Sri Lankan II.F.1.1 sub-lineage to be approximately early 2013 (90% confidence interval: 2013.03–2014.02). This suggests that the lineage responsible for the 2017 outbreak may have been circulating in Sri Lanka several years before its detection. As these divergence times are inferred using a maximum-likelihood framework, they should be considered approximate (Fig. 1B).

Evolution of DENV-2 genotype II. F.1.1 strains in Sri Lanka (analysis of the molecular clock)

The 2II_F-focused dataset exhibited strong temporal structure, with root-to-tip regression yielding an R^2 of 0.83. The estimated substitution rate for the full clade was 6.90×10^{-4} substitutions per site per year ($\pm 2.11 \times 10^{-5}$), while visualization in Clockor2 indicated a similar rate of 7.68×10^{-4} substitutions per site per year ($R^2 = 0.842$).

The Sri Lankan 2II_F.1.1 sub-lineage exhibited a substitution rate of 8.53×10^{-4} substitutions per site per year in Clockor2, consistent with the overall temporal structure of the 2II_F clade. Visualization including both global and Sri Lankan sequences provided a clear depiction of evolutionary rates across the clade (Fig. 2).

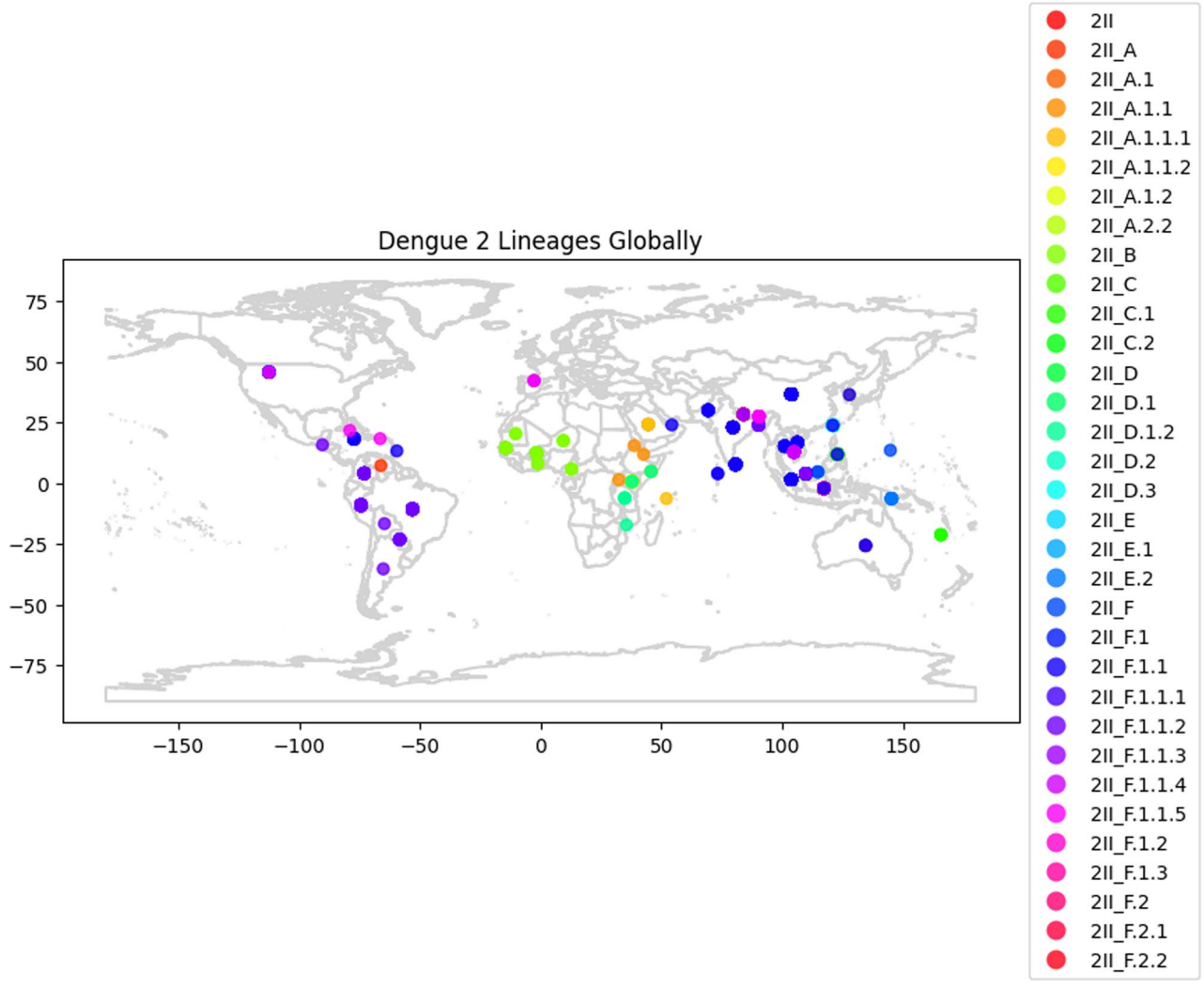

Fig. 2 Geographical distribution of Dengue 2 Genotype 2 Lineages. An illustration of the spatial distribution of the different DENV-2 genotype 2 lineages across the world. Centroids, colour coded according to the major and minor lineages (right-hand-side panel) appear in the geographical locations they have been reported from. Data was accessed from Nextstrain [31]

These rates align with previously reported DENV-2 substitution rates, supporting that the 2II_F-focused clade, including the 2II_F.1.1 sub-lineage, evolves at a rate comparable to the wider lineage [32, 33]. The observed temporal structure validates the use of molecular clock models to infer the recent evolutionary history and diversification of this clade (Fig. 3)(Supplementary Table 06) [34].

Characterization of mutations specific to E and NS1 in DENV-2 genotype II.F.1.1

Analysis of the mutations within DENV-2 genotype II_F.1.1 compared to the reference genome (GenBank: NC 001474.2) showed a total of 344 non-synonymous mutations across 221 amino acid positions within the envelope (E) region and 159 non-synonymous mutations across 132 amino acid positions within the NS1 region, occurring with varying frequencies. (Supplementary Table 01). The Sri Lankan DENV2 sequences showed a total of 243 non-synonymous mutations within E, across 184 amino acid positions and 236 non-synonymous NS1 mutations across 165 amino acid positions. The most frequent amino acid changes in the envelope were seen in in domain II (Fig. 4A, B) (Supplementary Tables 2 and 3) in both the global F1.1(n = 666) and Sri Lankan sequences(n = 204). The regions with the highest frequency NS1 mutations in both the F1.1 and Sri Lankan sequences were seen in the wing domain and spaghetti loop (Fig. 4C, D) (Supplementary Tables 4 and 5).

The Sri Lankan sequences only showed 2 unique amino acid changes (Q271K, T478A) in the E region compared to the main F1.1 clade. Conversely, the Sri Lankan sequences had more NS1 amino acid positions mutated compared to the main clade with 85 unique amino acid changes. The majority of these amino acid positions were in the beta-ladder (n = 43) and wing domain (n = 39), with

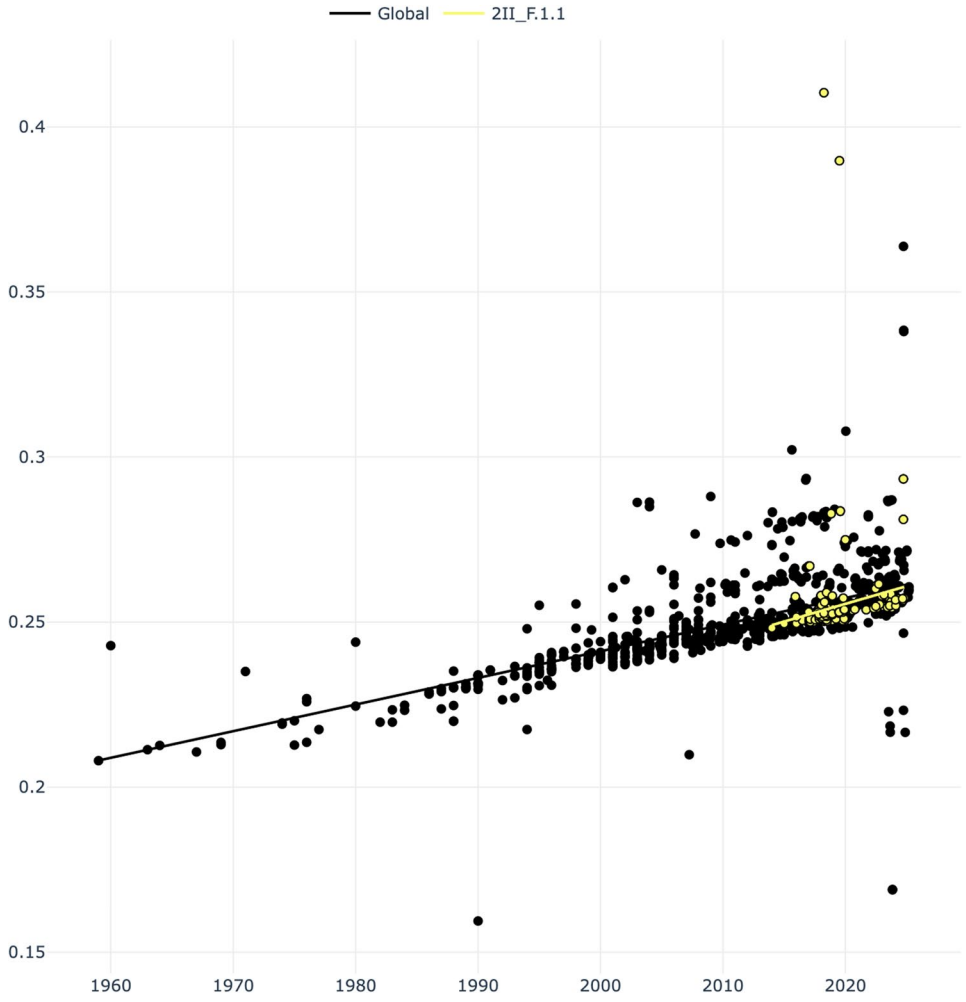

Fig. 3 Molecular clock analysis of the DENV-2 2II_F clade and Sri Lankan 2II_F.1.1 sub-lineage. Time-scaled phylogenetic analysis of the 2II_F clade revealed strong temporal structure ($R^2 = 0.83$), supporting reliable molecular clock inference. The global 2II_F dataset (black line) showed an estimated substitution rate of 6.90×10^{-4} substitutions per site per year ($\pm 2.11 \times 10^{-5}$). The Sri Lankan 2II_F.1.1 sub-lineage (green line) exhibited a rate of 8.53×10^{-4} substitutions per site per year

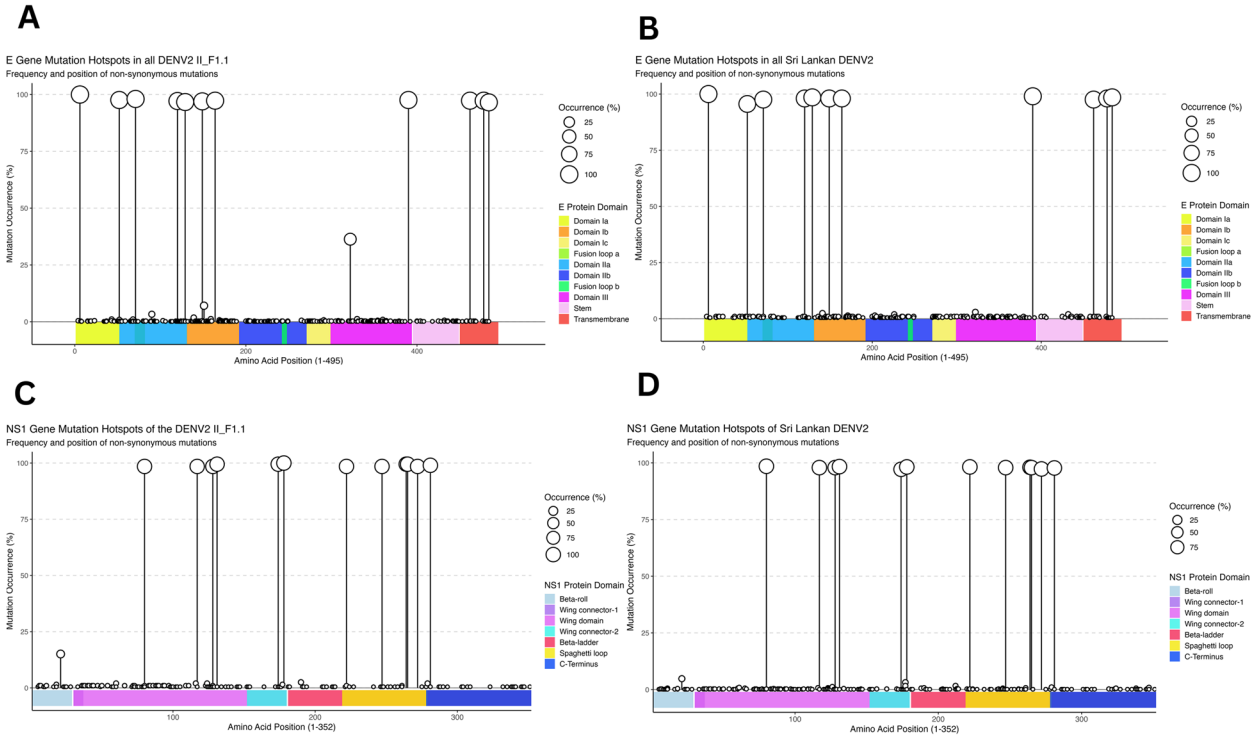

Fig. 4 Frquencies of non-synonymous mutations within the envelope and NS1 regions identified in the DENV-2 genotype II.F.1.1 strains from Sri Lanka. The plots display the frequency and positional distribution of non-synonymous mutations across the E/NS1 proteins. Mutation occurrence is represented by the y-axis value and the size of the points. The x-axis indicates the amino acid position. The colored bars at the base of the plot delineate the defined domains of the E/NS1 proteins. **A** Mutation hotspots in the E gene of the II_F1.1 DENV-2 global clade. **B** Mutation hotspots in the E gene of Sri Lankan DENV2. **C** Mutation hotspots in the NS1 gene of the II_F1.1 DENV-2 global clade. **D** Mutation hotspots in the NS1 gene of Sri Lankan DENV-2

only 3 in the hydrophobic [35] beta-roll. The frequencies of these unique mutations in the 204 Sri Lankan sequences were however very low (supplementary data file 08).

However, due to the relatively small sample size of a total of 204 Sri Lankan DENV-2 sequences used in this study, how these different sub-lineages arising out of the DENV-2 genotype II.F.I associated with clinical disease outcomes could not be analysed.

Comparison of Sri Lankan DENV-2 genotype II.F.I strains with the Indian genotype II.F.I strains

We compared the envelope and NS1 proteins of the Sri Lankan DENV-2 genotype II.F.1 strains (n = 199) with the most recent Indian F1.1 (n = 25) strains and all available global genotype II. F.1.1 clade (n = 366). While there were 16 mutations in the envelope protein common to all 3 groups, one mutation (T478A) was seen only in Sri Lankan strains and not in the rest of the F1.1 clade. When we compared the mutations within NS1, we found that 18 mutations were common to all 3 groups, with 6 mutations being seen only in Sri Lankan and Indian strains (H50Y, Q98P, S125P, S128F, G266A). There were 66 mutations unique to Sri Lanka compared to the rest of the F1.1 clade.

Discussion

Outbreaks due to DENVs have been reported in Sri Lanka since The 1960s [36], while dengue outbreaks resulting in dengue haemorrhagic fever (DHF) only occurred since 1989 [7]. All four DENV serotypes had been circulating in Sri Lanka with DENV-3 and DENV-2 serotypes co-circulating and causing outbreaks until year 2009, which was replaced by DENV-1 in 2009 [7, 37, 38]. DENV-2 remerged in mid-2016, completely replacing the dominant serotype DENV-1 in mid-2016 [7]. In this study we show that the introduction of a new DENV-2 genotype in mid-2016, were responsible for the largest dengue outbreak in Sri Lanka in 2017 [14, 39]. Interestingly, although the outbreaks with this DENV-2 genotype reduced in subsequent years, it was found to become endemic and continued to cause outbreaks in Sri Lanka [16]. Although these DENV-2, genotype II strains were initially classified as belonging to the cosmopolitan genotype [14], based on the earlier DENV genotype classification, using the new proposed classification, they were found to form a new lineage of genotype II.F.1.1 [6]. Therefore, although the cosmopolitan genotype of DENV-2 was reported from all regions in the world [21], DENV-2 genotype II.F.1.1 was only reported in South Asia and Southeast Asia. It was shownthat the DENV-2

cosmopolitan genotype reported from India, was significantly divergent from the DENV-2 genotypes from other regions in the world but had many similarities with the DENV-2 genotype II.F.1 strain reported from Sri Lanka [40]. Therefore, the new DENV classification provides more granularity regarding the evolution and geographical spread of the DENV genotypes as it enables characterization of sub-lineages of different genotypes.

The DENV-2 genotype II.F.1.1 strains in Sri Lanka showed a similar evolutionary rate as other DENV-2 strains [34]. We observed 2 unique non-synonymous mutations within the envelope region and 85 unique non-synonymous mutations within the NS1 region in the Sri Lankan strains. The NS1 domain of the Sri Lankan clade had more amino acid changes than the main F1.1 clade. The regions under the highest mutational pressure were the wing domain and spaghetti loop (Fig. 4C, D). Many of these mutations in the envelope and NS1 protein was also seen in the recent DENV-2 genotype II F.1.1 Indian strains [40], although there were a few unique mutations identified within the Sri Lankan strains. Therefore, it would be important to study further if these mutations could affect the binding of neutralizing antibodies and thereby possibly leading to re-infection with DENV-2 as described previously [41, 42]. Although these mutations have not been previously described as being unique to the cosmopolitan strain, some of these mutations have shown to be associated with pathogenicity. For instance, the T117A was shown to produce 12-fold more intracellular virus than the wild-type strains [43]. The K272R substitution in NS1, which was identified in the DENV-2 virus that led to a large outbreak in 2015 in Taiwan, was shown replicate faster, produce more secretory NS1 in cell culture supernatants and inhibited STAT1 phosphorylation thereby inhibiting interferon signaling pathways [44]. While some other mutations observed by us have been described in other DENV-2 genotypes [20], DENV-2 genotype III in Brazil [45] and in other genotypes [30], it would be important to further study the implications of these mutations in transmission of the virus and altering its pathogenicity. Unfortunately, we could not determine clinical significance of these mutations, of if any of these mutations increased pathogenicity and virulence in vitro or in mouse models as it was beyond the scope of this study.

The DENV-2 genotype II.F.1.1 strains in Sri Lanka had 2 unique mutations in the envelope region and 85 unique non-synonymous mutations within the NS1, which are not found in any of the circulating DENV-2_II.F1.1 viruses elsewhere. Therefore, it is possible that these genetic changes within Sri Lankan DENV-2 genotype II.F.1.1 strains could at least partially contribute to the change from this genotype transitioning from epidemic to endemic transmission. Although factors that lead to evolution and adaptation of the DENV are not entirely clear, it is likely that these mutations could have been driven by either human or Aedes hosts in Sri Lanka. As dengue case numbers in Sri Lanka report patients hospitalized with clinically suspected dengue infection, the actual infections dengue infections are very much underestimated. Importantly, asymptomatic infection is also known to cause transmission [46], and it could be the main selection factor for development of mutations to adapt to acquiring endemicity. As dengue pathogenesis is underpinned largely by virus-host interactions, local population genetic factors both of humans and Aedes may drive geographic dependent nuances in DENV diversity. The impact of such DENV diversity on dengue across the different ethnic groups living in the dengue endemic world should be investigated promptly.

The genetic diversity between different genotypes of DENV serotypes have also shown to affect vaccine efficacy. For instance, the CYD-TDV phase III vaccine trial showed different DENV2-specific vaccine efficacy in Asian and Latin American populations [47, 48]. These differences may, at least in part, have been influenced by the antigenic distance between the vaccine strain and circulating DENV reduced CYD-TDV vaccine efficacy across study sites [49, 50]. Therefore, given that the strains used to develop the currently licensed dengue vaccines composed of historical DENV strains, it is important to continuously carry our surveillance of different DENV strains in different parts of the world.

Conclusions

In summary, this study characterized DENV-2 strains circulating in Sri Lanka from 2016 to 2023, which caused the country's largest recorded dengue outbreak and were identified as genotype II.F.1.1. These isolates exhibited mutations linked to enhanced replication, NS1 secretion, and immune evasion, along with unique substitutions that may have promoted the shift from epidemic to endemic transmission. The observed genetic divergence, with potential implications for vaccine efficacy and transmission dynamics, underscores the need for continued genomic surveillance in highly endemic regions.

Abbreviations

CDC	Centers for Disease Control
DENV	Dengue virus
RNA	Ribonucleic acid
STAT 1	Signal transducer and activator of transcription 1

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-04584-2.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Supplementary Material 5.

Supplementary Material 6.

Supplementary Material 7.

Authors' contributions
Conceptualization: DA, GNM, TTPJ. Data curation: DA, HK, LG, TTPJ, AW. Experiments and investigations: DA, TTPJ, HK, LG, FB, AS. Data analysis: BS, MDS, DR. Project administration and supervision: GNM, CJ, EEO, AW. Funding acquisition: GNM, CJ, EEO. Writing the manuscript: DA, GNM, EEO. Reviewing the manuscript: EEO.

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Data availability
All data is available within the manuscript and the supporting files. The sequences and their NCBI and GISAID accession numbers are available in supplementary Table 07. The patient meta data is available in supplementary information. The NCBI accession numbers are: PV752083- PV752130, EPI_ISL_19901609, EPI_ISL_19906836, and EPI_ISL_19906837.

Declarations

Ethics approval and consent to participate
Ethics approval was obtained from the Ethics Review Committee of the University of Sri Jayewardenepura (12/15 and 58/19). All participants gave informed written consent. This study has adhered to the Declaration of Helsinki https://www.wma.net/policies-post/wma-declaration-of-helsinki/.

Consent for publication
Not applicable.

Competing interests
EEO has served in advisory capacities on dengue for Sanofi Pasteur, Takeda Pharmaceuticals, MSD, Johnson & Johnson and Novartis. GNM has served in advisory capacities on dengue for Johnson & Johnson, Novartis, Takeda and Abbott. The other authors have no competing interests to declare.

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