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Whole genome sequencing and phylogenetic analysis of dengue virus in Central Nepal from 2022 to 2023

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Abstract

Background In Nepal, dengue is an emerging disease of growing concern as outbreaks are increasing in both size and geographic reach and beginning to affect areas previously thought dengue-free. Dengue genomic surveillance has previously been limited within Nepal; however, with the increase in accessibility to sequencing technologies since the COVID-19 pandemic, it has recently become more feasible.

Methods This hospital-based retrospective study utilized banked samples from the 2022 and 2023 dengue seasons from Dhulikhel Hospital/Kathmandu University Hospital in Central Nepal. Next-generation sequencing was performed to obtain whole genome sequences of dengue virus which were analyzed phylogenetically using a maximum likelihood GTR + G model. Mutations were evaluated across viral particle region using the GISAID DengueServer.

Results We obtained 41 full-length sequences of DENV from 80 PCR-positive samples, including 24 sequences (58.5%) from 2022 and 17 sequences (41.5%) from 2023. We identified a shift in the majority serotype of our samples from DENV1 in 2022 to DENV3 in 2023, though 3 out of the 4 serotypes were identified in both years. Phylogenetic analysis revealed clusters within genotype III of DENV1 and genotype III of DENV3 closely related to strains from an outbreak of DENV in northern India in 2018–2019. DENV2 sequences fell into the cosmopolitan genotype IV-A1 and IV-B2 clades and were related to sequences from South and Southeast Asia and the USA, pointing to the global nature of dengue transmission. NS3 showed the highest frequency of mutation, whereas NS2B, NS4, NS5, and E were the most conserved. The most common mutations found were substitutions L17M and T20I in the 2 K peptide. A high number of mutations were observed in DENV3, followed by DENV2, with some mutations being unique to specific serotypes and others matching previously reported strains.

Conclusions We identified possible clade shifts in the DENV1 and 2 populations and a rising prevalence of DENV3. Our study showed a high level of serotype diversity of DENV circulating in Central Nepal. Furthermore, our results indicate that DENV populations in Nepal are related to a geographically diverse set of sequences but are most strongly influenced by Indian strains of DENV.

Keywords Dengue, Whole genome sequencing, Phylogenetics, Genomics, Neglected tropical diseases

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Background

Dengue fever is a vector-borne disease caused by dengue virus (DENV), which is a single-stranded RNA virus of the Flaviviridae family. Flaviviruses, such as DENV, West Nile virus, and Zika virus are primarily transmitted by mosquitoes of the *Aedes* genus, *Aedes aegypti* and *Aedes albopictus* [1]. Predominantly endemic in tropical and subtropical regions, the WHO estimated that about half of the world's population is at risk for dengue, and that 100–400 million infections occur each year [1].

The first recorded case of DENV in Nepal occurred in 2004 [2]. Since then, DENV has become endemic to the country with outbreaks occurring during the rainy season (August-November) on a regular 2- to 3-year cycle [3]. In 2022, the Epidemiology and Disease Control Division (EDCD; Kathmandu, Nepal) recorded 54,784 cases and 88 deaths across all 7 provinces. This trend continued in the 2023 season which recorded 51,243 cases [4, 5]. As per the EDCD, Nepal observed outbreak of dengue in 2022, but in 2023, a high case count but no outbreak was observed, hence our use of "season" in reference to the 2023 cases [4, 5]. Previous work has identified the co-circulation of all four serotypes of DENV in Nepal, which increases risk within this population of secondary infection and the subsequent complications [6].

The DENV genome is made up of one ~ 11 kilobase long open reading frame. It contains 10 protein encoding regions, including 3 structural regions (capsid [C], premembrane [preM], and envelope [E]) and 7 nonstructural (NS) regions (NS1, NS2A, NS2B,NS3, NS4A, NS4B, and NS5) which perform various functions as transcription factors, cofactors, and innate immune response inhibitors [7, 8]. DENV exists as four antigenically and immunologically distinct serotypes which share approximately 65% of their genome and can be further subdivided into several genotypes within each serotype [9]. DENV genotypes are defined as clusters of sequences which diverge up to 6% within a given region at the nucleotide level [10, 11]. Infection from one serotype confers lifetime immunity to the specific serotype and up to 6-month immunity to all serotypes of DENV [12]. However, secondary infection presents an increased risk of serious complications such as dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF) through antibody-dependent enhancement (ADE) [13].

Genomic surveillance provides unique insight into the proliferation of the virus throughout space and time. Tracking the prevalence and phylogeny of dengue virus genotypes sheds light on the origins and epidemic behavior of the virus in Nepal and can further provide insight into changes in virulence, vector competence, and transmission dynamics [14–16]. However, due to the high cost associated with sequencing, there have not been many phylogenetic studies focusing specifically on DENV in Nepal. Next-generation sequencing (NGS) has recently made the whole genome sequencing of DENV more feasible within Nepal [17]. In order to overcome this research gap, we utilized NGS to obtain whole genome sequences of DENV originating in Nepal. In this study, we sequenced the DENV found in banked samples collected during the outbreak of 2022 (August to October) and dengue season of 2023 (August to November) to investigate the circulation of DENV within Central and Eastern Nepal in 2022 and 2023 and to explore the origins and genetic variability of DENV within this region.

Methods

Study design

This retrospective study investigated the genomics of DENV in banked dengue samples. These anonymized samples were taken from patients who visited Dhulikhel Hospital/Kathmandu University Hospital (DHKUH) for diagnosis and treatment and had received a rapid antigen test at the DHKUH Department of Microbiology. Samples were included if complete personal demographic information (age, sex, geographic region) had been entered into the electronic medical record (EMR) and were NS1 + by rapid antigen test.

Sample collection and evaluation

Eighty-nine banked NS1 + whole blood samples of August to October 2022 and 62 from August to November 2023 were obtained. These samples were in storage at − 80 °C until retrieval for this study. Basic demographic information (age, sex, geographic region) was accessed through the DHKUH EMR. The serum from patients were retested for the NS1 antigen using the InBios NS1 *Detect*™ (DNS1-RD; InBios, Seattle, USA) rapid diagnostic test kit.

RNA extraction and quantitative PCR

RNA extractions were performed using the Zymo Quick-RNA Viral Kit (R1035; Zymo, Orange, USA), according to the manufacturer's instructions. A total of 200 µL of serum was used for RNA extraction. The genetic material was eluted in nuclease-free water and was stored at − 20 °C until sequencing or quantitative PCR (qPCR). For the verification of the presence of DENV RNA, qPCR was performed on the extracted samples before sequencing, using either the Dengue Virus Nucleic Acid Test Kit (M092T050B0C0; Mole Biosciences, Taizhou, People's Republic of China) or the *abTES*™ DEN 4 qPCR kit (300,185; Ait Biotechnology, Singapore), and was carried out according to the manufacturer's instructions. Samples that failed to exhibit a positive Ct value were not taken forward for sequencing. The cutoffs for

positivity were determined based on the manufacturer's instructions.

Whole genome sequencing

Viral libraries were synthesized using the Illumina COVIDSeq Test Kit (RUO Version) (20051274; Illumina, San Diego, USA). A version of the Illumina COVIDSeq protocol adapted for use in dengue virus samples described by Vogels et al. was closely followed [18]. Custom DENV-specific primers for pan-serotype amplification of dengue virus were designed and ordered from Integrated DNA Technologies (IDT) based on the Vogels et al. DengueSeq protocol. After amplification, libraries were evaluated for the presence of adapter dimers, concentration, and amplicon size of ~ 300 bp using the Agilent TapeStation 4150 (Agilent, Santa Clara, USA) and loaded for sequencing at a concentration of 120 pM. Pair-ended amplicon-based whole genome sequencing (2 × 150 bp) was performed on the iSeq100 (Illumina, San Diego, USA).

Sequence alignment and phylogenetic analysis

Consensus genomes were constructed using the Chan Zuckerberg ID viral consensus genome alignment pipeline v3.5.0 [19] using Nepalese genomes from 2022 as references for assembly (DENV1: OR821722.1; DENV2: OR821725.1; DENV3: OR821726.1). Utilizing regional sequences for our viral consensus genome assembly allowed us to achieve greater coverage breadth. Serotype and genotype of the viral genomes were determined using the Genome Detective Dengue Virus Typing tool [20]. Sequences with a viral genome coverage ≥ 70% were subjected to multiple sequence alignment in MEGA11 using MUSCLE (MUltiple Sequence Comparison by Log-Expectation) alignment. Maximum likelihood phylogenetic trees were constructed in MEGA11 using a general time-reversible model with gamma distributed rate variation (GTR + G) as identified by the IQ-TREE ModelFinder, with 1000 bootstrap replicates [21]. Phylogenetic trees were constructed by serotype using the self-produced sequences (accessible in the Global Initiative on Sharing All Influenza Data (GISAID) database; EPI_ISL_19081917—EPI_ISL_19081957) in combination with related full-length DENV sequences identified using BLAST search and accessed from GenBank [22]. GISAID accession numbers and alignment coverage of all samples included in phylogenetic analyses are available as Additional file 1: Table S1.

Mutation analysis

The mutations in all Nepalese genomes ($N = 134$ with 76 whole genomes), including genomes from this study ($N = 41$), were evaluated using DengueServer from GISAID (A*STAR Bioinformatics Institute (BII), Singapore) [23]. This platform uses well-characterized reference genomes (hDenV1/Nauru/NMRI-45AZ5PDK-0/1974 for DENV1, hDenV2/Thailand/CDC-16681/1964 for DENV2, and hDenV3/Sri Lanka/IMTSSA-1266/2000 for DENV3) with significant relevance (dominant genotype, phylogenetically distinct, and epidemiologically and clinically relevant: known to cause severe dengue, including DHF and DSS). In this study, newly observed "unique" mutations were evaluated across the viral particle region (envelope [E], capsid [C], pre-membrane [preM]) and replication complex (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).

Statistical analysis and data representation

All statistical analyses were performed using R software (v4.3.2, R Core Team 2023). Figure 1 was generated in R using leaflet v2.2.2.9000 [24].

Results

Study demographics

Sociodemographic characteristics and serotype distribution of the 80 qPCR-positive samples are presented in Table 1. In 2022, an equal number of samples were from men and women, whereas in 2023, more samples were from men (63%) than women. In both 2022 and 2023, most of the samples were from patients residing within Kavrepalanchok (60% and 75%, respectively) which is a peri-urban district in Central Nepal. This population represented 53 of the total 80 samples. The remaining patients predominantly resided in neighboring districts in Central Nepal including Bhaktapur [7], Kathmandu [6], Sindhu [4], Lalitpur [2], and others (Fig. 1). One sample collected in 2022 belonged to a patient from Banke district in western Nepal.

Serotype distribution

This study included a total of 80 qPCR-positive samples. These samples included 48 qPCR-positive samples from the dengue outbreak of 2022 and 32 samples from the dengue prevalent season of 2023 (Table 1). In 2022, most samples were identified as DENV1 (28, 58.3%) followed by DENV3 (11, 22.9%) and DENV2 (9, 18.7%), whereas in 2023 the majority of samples were identified as DENV3 (20, 62.5%) followed by DENV2 (8, 25%) and DENV1 (4, 12.5%). DENV4 was not detected in either year.

Phylogenetic analysis

Forty-one out of the 80 qPCR + samples exhibited successful sequencing, characterized by ≥ 70% genome coverage at 10 × coverage depth, and were subjected to phylogenetic analysis. Successful sequencing occurred in samples with an average Ct value of 17.84 ($SD = 6.68$).

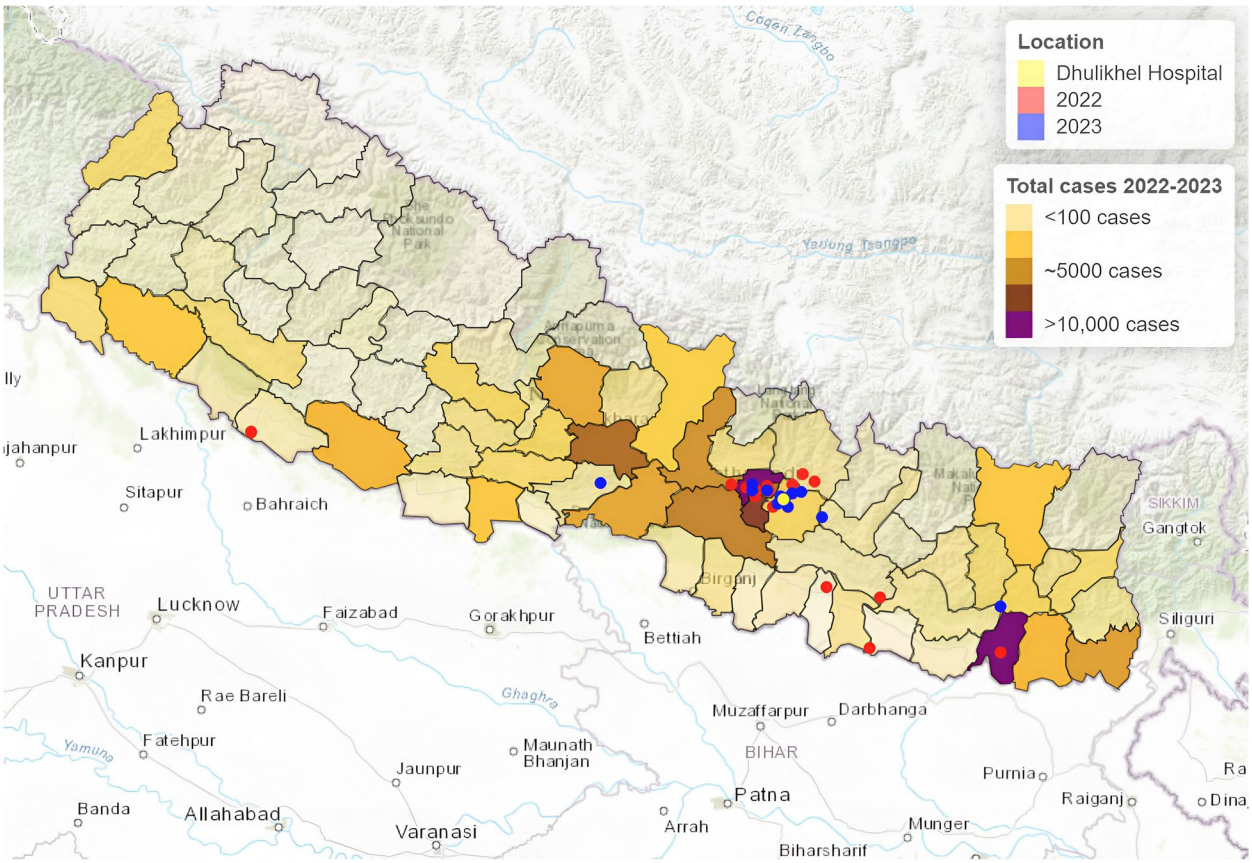

Fig. 1 Map of Nepal identifying the region of residence of study subjects ($N=80$) with districts shaded and colored based on total reported cases from 2022 to 2023. Circles indicate sites of unique subject regions of residency. Red circles represent sites associated with samples collected in 2022, and blue circles represent sites associated with samples collected in 2023. Yellow circle represents the location of the study site (Dhulikhel Hospital/ Kathmandu University Hospital). Location data was collected from the DHKUH EMR. Map generated in R using leaflet v2.2.2.9000 [24]

Table 1 Demographic and serotype distribution of qPCR-positive samples stratified by year ($N=80$). Statistics are shown as *n* (%) for categorical variables and mean (range) for continuous variables

Demography	2022 ($N=48$)	2023 ($N=32$)	Overall ($N=80$)
Sex			
Female	24 (50%)	12 (38%)	36 (45%)
Male	24 (50%)	20 (63%)	44 (55%)
Age	32 (7, 65)	40 (22, 71)	35 (7, 71)
Region			
Within Kavrepalanchok	19 (40%)	8 (25%)	27 (34%)
Outside of Kavrepalanchok	29 (60%)	24 (75%)	53 (66%)
Serotype			
DENV-1	28 (56%)	4 (13%)	32 (40%)
DENV-2	9 (19%)	8 (25%)	17 (21%)
DENV-3	11 (23%)	20 (63%)	31 (39%)

DENV1

A phylogenetic tree of DENV1 sequences analyzed in the manuscript is available as Fig. 2. Seven sequences from 2022 and two sequences from 2023 were phylogenetically analyzed. All 2022 and 2023 genomes of DENV1 from Nepal were identified as genotype III. The Nepalese clade was most closely related to a Chinese genome of DENV from a traveler returning from India in 2019 (MN923086.1) and other Indian DENV1 genomes from 2019. All DENV1 genomes of this study clustered into a discrete phylogenetic group with the remaining Nepalese DENV1 sequences isolated in 2022 and 2023.

DENV2

A phylogenetic tree of DENV2 sequences analyzed in the manuscript is available as Fig. 3. Seven sequences from 2022 and eight sequences from 2023 were phylogenetically analyzed. All DENV2 genomes from this study were identified as cosmopolitan genotype with subgenotypes IV-A1 and IV-B2 both represented.

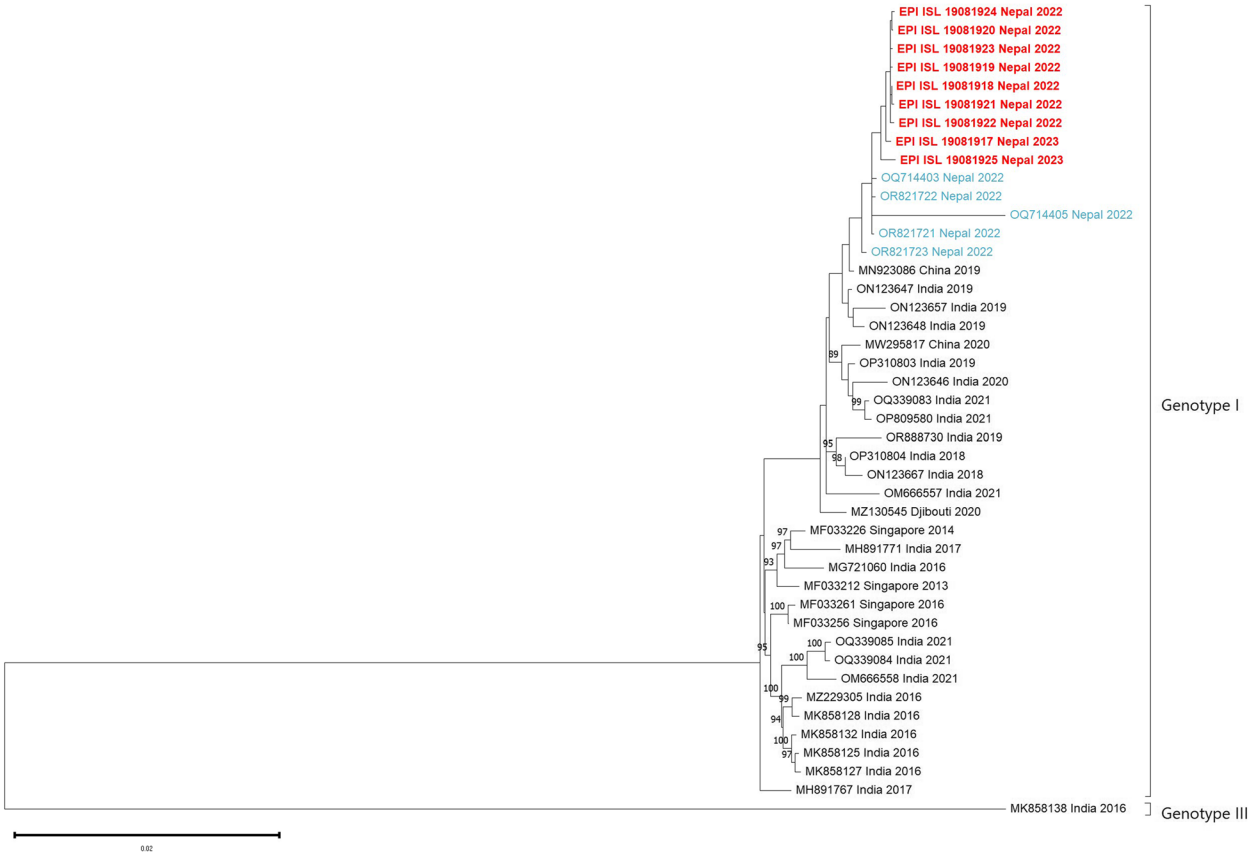

Fig. 2 Phylogeny of Nepalese DENV1 genomes and related sequences. A maximum likelihood phylogenetic tree was constructed with the general time reversible model with gamma distribution and 1000 bootstrap replicates in MEGA11. Nepalese DENV1 genomes obtained in this study and related whole genome sequences of DENV1 accessed through the GenBank database were included in the analysis. Genomes obtained in this study are shown in red, and other Nepalese genomes are colored in blue. Sequences in black are related strains accessed from GenBank. Genome names are displayed as "GISAID accession number_Country_Year" for self-produced sequences and "GenBank accession number_Country_Year" for all other sequences. Genotype is indicated to the right of the sequences. Numbers on the branches indicate bootstrap values greater than 70%. Distance scale is displayed at the bottom left of the tree

Nepalese DENV2 genomes from 2022 and 2023 grouped phylogenetically based on year. The Nepalese sequences from 2022 formed two clusters which were related to genomes from Singapore in 2017–2019 and India in 2021. Of the 2022 samples, two singletons were also observed which were associated with the lineage of Indian DENV from 2021 and a Singaporean genome from 2012. Additionally, genomes from 2023 formed one cluster and one singleton, which were related to DENV genomes from India in 2021 and the USA from 2022 and 2023. Further, one genome from 2023 was related to a 2023 genome from Bangladesh. Interestingly, most sequences from 2022 and 2023 did not group phylogenetically with previously sequenced Nepalese sequences from 2017, which formed distinct clades within both observed subgenotypes (IV-A1 and IV-B2).

DENV3

A phylogenetic tree of DENV3 sequences analyzed in the manuscript is available as Fig. 4. Nine sequences from 2022 and eight sequences from 2023 were analyzed. All DENV3 genomes, in this study, were identified as genotype III. Out of the 17 DENV3 genomes, 14 formed a cluster with the other Nepalese genomes of DENV3 from 2022. The major DENV3 cluster was most closely related to an Indian DENV genome from 2019. Further, another two-sequence cluster, comprising one sequence from 2022 and one from 2023, was also most closely related to the same 2019 Indian strain. Additionally, one singleton from 2023 was found to be related to Indian DENV3 from 2022.

Mutational analysis

The frequency of unique mutations was variable among the genes and their corresponding proteins (Table 2).

Fig. 3 Phylogeny of Nepalese DENV2 genomes and related sequences. A maximum likelihood phylogenetic tree was constructed with the general time reversible model with gamma distribution and 1000 bootstrap replicates in MEGA11. Nepalese DENV2 genomes obtained in this study and related whole genome sequences of DENV2 accessed through the GenBank database were included in the analysis. Genomes obtained in this study are shown in red, and other Nepalese genomes are colored in blue. Sequences in black are related strains accessed from GenBank. Sequence names are displayed as "GISAID accession number_Country_Year" for self-produced sequences and "GenBank accession number_Country_Year" for all other sequences. Genotype is indicated to the right of the sequences. Numbers on the branches indicate bootstrap values greater than 70%. Distance scale is displayed at the bottom left of the tree

Across serotypes, the most unique mutations were observed in DENV3, followed by DENV2. Across genes, the highest unique mutation frequency was observed in NS3 ($n=20$), followed by NS2A ($n=4$), while the lowest ($m=2$) was observed in NS2B, NS4, NS5, and E. The most frequently observed unique mutations, L17M and T20I, both occurred in the 2 K transmembrane domain of hydrophobic protein NS4A (2 K). These mutations were observed in four other genomes from Nepal and were restricted to the DENV3 serotype. Unique mutations D192B in DENV1 and L56J in DENV3 were identified in domain I and domain II of the E protein, respectively. The most common previously identified mutations in the E gene were A219T and E404A.

Mutations in NS3 were mostly substitutions with two leading to stop codons (D371stop and E93stop). One NS3 substitution mutation, T532V, which was observed in genomes belonging to DENV2 strain has also been identified in the Indian strain hDenV2/India/DL-IGIB-1130DD0465415D/2022 from October 2022. The NS2B mutation T52A seen in two of our DENV3 genomes (EPI_ISL_19081951 and EPI_ISL_19081956) was also observed in US strain hDenV3/USA/FL-BPHL-0187/2023 from November 2023. Previously identified mutations were observed mostly in NS5 ($n=623$), followed by E ($n=380$), NS1 ($n=360$), NS3 ($n=292$), NS2A ($n=221$), NS4B ($n=191$), preM ($n=176$), NS4A excluding 2 K ($n=135$), C ($n=130$), NS2B ($n=111$), and 2 K ($n=34$).

Discussion

This retrospective study utilized molecular methods to investigate the DENV of banked serum samples collected during 2022 and 2023 in Central Nepal. Phylogenetic analysis of the DENV whole genome sequences revealed three of the four DENV serotypes circulating in both 2022 and 2023 and a strong influence of northern Indian DENV populations on those within Central Nepal. In this set of samples, we observed a slightly higher proportion of men (55%) than women (45%) and an average age of 35 years. This finding is consistent with previous studies in Nepal, where dengue is a disease mostly observed in men and young adults (15–40 years old) [25]. The observed age and gender distribution could be attributed to the increased likelihood of these demographics of being involved in outdoor working activities [25, 26].

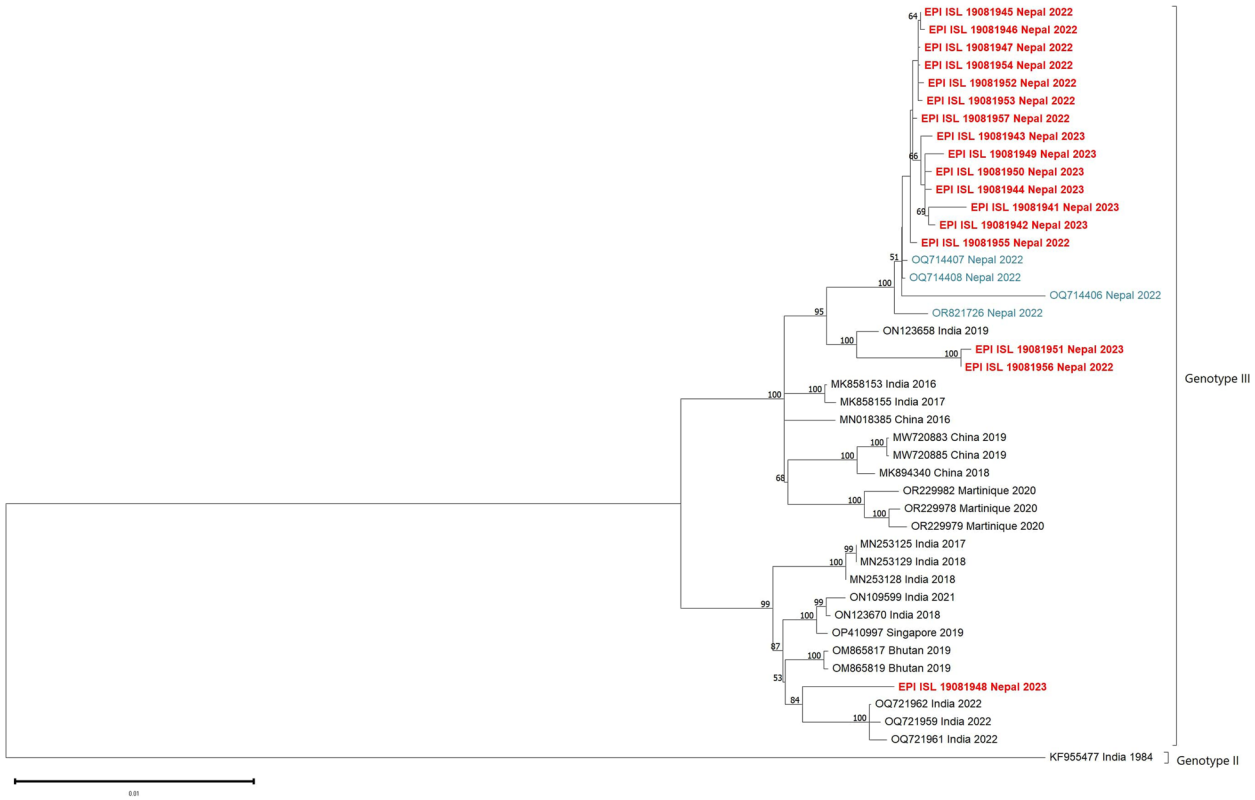

Fig. 4 Phylogeny of Nepalese DENV3 strains and related sequences. A maximum likelihood phylogenetic tree was constructed with the general time reversible model with gamma distribution and 1000 bootstrap replicates in MEGA11. Nepalese DENV3 strains obtained in this study and related whole genome sequences of DENV3 accessed through the GenBank database were included in the analysis. Genomes obtained in this study are shown in red, and other Nepalese genomes are colored in blue. Sequences in black are related strains accessed from GenBank. Sequence names are displayed as "GISAID accession number_Country_Year" for self-produced sequences and "GenBank accession number_Country_Year" for all other sequences. Genotype is indicated to the right of the sequences. Numbers on the branches indicate bootstrap values greater than 70%. Distance scale is displayed at the bottom left of the tree

In the 2022 study population, we identified DENV1 as the major serotype (28/48, 58%) followed by DENV3 (11/48, 23%), reflecting the general trend within the Kathmandu Valley [26]. This is consistent with the findings of our 2022 serological study which found DENV1 to be predominant in a similar population to our study [27]. In 2023, the most common serotype within our study population shifted to DENV3 (20/32, 63%). Previous serosurveillance studies have indicated that prior major outbreaks have been caused largely by DENV1 and 2, with the 2022 Kathmandu Valley outbreak being primarily attributable by DENV1 [26–32]. However, there has been a recent rise in the prevalence of DENV3, an observation corroborated in our study [26]. Interestingly, a survey of serotypes circulating during 2023 in the Dhading District, which lies in the north-eastern region of the Bagmati Province, identified the vast majority (97.5%) of PCR-positive dengue cases to be DENV2 [33]. This contrasts with the serotype distribution within our 2023 study population, which identified DENV2 as the second most common serotype (25%) after DENV3. The introduction of DENV3, which likely has low population immunity within Nepal, may impact the size or severity of the next outbreak. Because immunity against DENV is serotype specific, the dominant circulation of multiple serotypes within a small region leaves populations primed for serotype invasion if a secondary or tertiary serotype starts circulating in a nonimmune population, leading to increased likelihood of more severe outcomes [13, 34].

Although we are limited by the lack of historical genomic analysis of DENV within Nepal, these findings point to a likelihood of multiple or continuous introductions of DENV2 to the country. This study revealed that 2022 and 2023 Nepalese DENV2 genomes were more closely related to sequences from India, China, Singapore, Bangladesh, and the USA than previously sequenced DENV2 within Nepal [29]. Furthermore, the lineages and genotypes in the Nepalese DENV1 and 3 populations (genotype III of DENV1 and genotype III

Table 2 Mutations and their frequency among the DENV genomes, from this study, and Nepalese genomes from the GISAID database ($N=214$), separated based on their region of occurrence

Region	Gene_Mutation	Serotype	Occurrence across genomes from this study ($N=80$)	Occurrence across Nepal genomes ($N=134$)
Structural (viral particle region)	E_D192B	DENV1	1	1
	E_L56J	DENV3	1	1
Nonstructural (viral replication complex)	NS4A_2K_L17M	DENV3	16	20
	NS4A_2K_T20I	DENV3	16	20
	NS2A_F159J	DENV1	1	1
	NS2A_G130R	DENV2	1	1
	NS2A_L211J	DENV2	1	1
	NS2A_R55S	DENV2	1	1
	NS2B_I97T	DENV2	1	1
	NS2B_T52A	DENV3	2	2
	NS3_A373S	DENV3	1	1
	NS3_C375L	DENV3	1	1
	NS3_D371stop	DENV3	1	1
	NS3_E181D	DENV3	1	1
	NS3_E93stop	DENV3	1	1
	NS3_F362V	DENV3	1	1
	NS3_G369R	DENV3	1	1
	NS3_G380R	DENV3	1	1
	NS3_I366H	DENV3	1	1
	NS3_I372H	DENV3	1	1
	NS3_K367Q	DENV3	1	1
	NS3_K381E	DENV3	1	1
	NS3_N370K	DENV3	1	1
	NS3_N374K	DENV3	1	1
	NS3_N379E	DENV3	1	1
	NS3_R377A	DENV3	1	1
	NS3_S365Q	DENV3	1	1
	NS3_T532V	DENV2	1	1
	NS3_V363C	DENV3	1	1
	NS3_W361V	DENV3	1	1
	NS5_I162G	DENV3	1	1
	NS5_R248K	DENV3	1	1

of DENV3) closely mirror those circulating in Northern India in 2018 and 2019 as identified by Behera et al. [35] Indeed, we also observed phylogenetic grouping between recent Nepali sequences and Indian sequences from 2019. Given the short life span of the *Aedes* genus [36], it is probable that these lineages of DENV entered Nepal through human hosts rather than infected mosquito vectors, likely by tourists or other visitors. In 2022, the highest number of tourists to Nepal was from India and the USA, representing 34.1% and 12.5% of the total arrivals in Nepal [37]. Similarly, in 2023, the proportion of Chinese tourists rose to the third most common nationality of visitor at 6.0% after Indian and US citizens (31.5% and 9.9%, respectively) [38]. It should also be noted that the proportion of Indian visitors to Nepal is likely underrepresented by these numbers due to the open border between the two countries.

Our phylogenetic analysis also revealed intraserotype diversity between the Nepalese DENV1 and DENV2. In general, the consequence of intraserotype genetic diversity of DENV is not as well-established as that of interserotype differences. Historically, the extinct American genotype of DENV has been linked to lower virulence, milder disease presentation, and less viral replication in vectors than the Southeast Asian strain that replaced it in South America; however, differences between currently

circulating genotypes are less clear [39, 40]. Some studies have found a correlation between the presence of novel genotypes and a rise in DENV infection incidence and posited increased viral fitness as responsible for genotype replacement [15, 41]. Nevertheless, further studies are required to fully understand the impact of genetic diversity on dengue disease pathogenesis and epidemiology.

The role of mutations in any virus should not be dismissed, because viruses are rapidly evolving as a result of selection pressure which can lead to drug and immune escape pathways [11, 42]. For example, common mutations T478S and R120T present in the E protein of Nepalese genomes have been shown to respectively dramatically reduce vaccine efficacy and enhance receptor affinity [43, 44]. Although literature supporting the observed substitutions D192B and L56J in E protein were not found, these mutations are present in N terminal domain I and domain II, respectively. Domain II of the E protein contains a hydrophobic fusion peptide (residues 98 to 110) involved in attaching the virus to the target cell membrane [45–47]. The E protein residues critical for binding of mAb did not harbor mutations in any of the genomes of our study [48].

Mutations in the 2 K peptide region of NS4 were observed in several genomes in residues 17 and 20. Though the exact effects of substitutions in residues 17 and 20 are not known, the 2 K peptide serves as signal sequence for the translocation of the adjacent NS4B into the endoplasmic reticulum (ER) lumen [49–51]. We also observed several mutations in the NS2 protein. Mutations of the NS2 protein are not entirely modeled; however, they have started to garner attention due to emerging evidence of their essential roles in viral replication and immunomodulation [52–55]. The NS3 and NS5 regions are highly conserved regions of the dengue genome and perform enzymatic activities involved in the viral replication cycle [56–58]. NS5 in DENV2 has been detected frequently in the cellular nucleus and is ultimately linked to viral pathogenesis [58]. Although information on mutation frequency and supporting literature for mutations in NS3 and NS5 observed in this study was not found, it is possible that new mutations in conserved region could affect the viral pathogenesis by altering perturbations or protein–protein interactions [49]. The differential mutational frequency between NS proteins and structural proteins observed in this study could reflect negative pressure selecting against mutations to key proteins, as well as the difference in protein size given that the totaled size of the NS proteins (~2250 amino acids) is more than double that of the structural proteins (~840) [59].

We observed a higher mutation rate in our DENV3 samples than other serotypes. DENV3 has been a more prevalent serotype in Nepal as well as globally and has been associated with severe disease outcomes during primary infection [60]. While there are not any particular studies depicting higher mutation rates in DENV3, further investigation in relation to clinical outcomes is essential to understand the effects of this high mutation rate, if any.

Until now, the vast majority of dengue surveillance efforts within Nepal have been hospital-based studies investigating the disease over only 1 or 2 years within a single population [6, 24–29]. Similarly, this study was conducted using samples banked at a tertiary care hospital, and therefore did not obtain data on clinical parameters and risk factors. We acknowledge that such hospital-based studies introduce implicit bias. Further, the samples included in this study were largely from the Bagmati Province in Central Nepal, and thus may not be representative of the overall Nepalese DENV population. We also acknowledge that the mutation profiling could be better discussed with clinical outcomes, as prior studies have related mutations to severity including neurovirulence [11, 61]. Further, the effects of mutations could also be more effectively studied by functional analysis in silico (FoldX, I-TASSER, or DMPfold) to observe their role in folding patterns and protein–protein interaction [49, 62]. However, we believe that our extensive phylogenetic analysis and mutational analyses performed are valuable even without clinical metadata as we have chosen to focus primarily on our investigation of the genomic data presented in this study.

In Nepal, dengue has seen an exponential rise in cases in the past few years. In 2022, the majority of cases occurred in the Bagmati Province in Central Nepal with three districts within the Kathmandu Valley, Kathmandu, Lalitpur, and Bhaktapur districts, being the most affected [4]. This emergence of dengue in the hilly region, which was previously thought to be protected from dengue due to its temperate climate, poses a serious threat to public health in Nepal, particularly as climate change, unplanned urbanization, and poor waste management systems exacerbate outbreaks in urban areas [63]. As the affected region and population within Nepal expands, the need for disease and viral surveillance to help understand and control the problem becomes more pressing. As we demonstrated in this study, DENV in the hilly region of Central Nepal is closely related to viruses in India, Singapore, China, and the USA, likely due to international tourism and the open-border policy between Nepal and India, where dengue is also endemic [64]. Dengue transmission is a complex, multifaceted phenomenon which requires long-term monitoring in order to enact the most effective disease control measures. Furthermore, with multiple dengue vaccines

currently under development, ongoing genomic surveillance will also be critical in understanding the impact of genetic diversity on the efficacy of vaccines [65–67]. We were able to effectively profile the genetic diversity and displacement of DENV in a populous region of Central Nepal using whole genome sequencing during a period of high dengue incidence.

Conclusions

This retrospective study, which included banked samples from two consecutive years of high dengue burden in Nepal, leveraged amplicon-based whole genome sequencing to examine the genetic diversity of three serotypes of dengue virus circulating in Central Nepal. We identified a strong influence of Indian DENV populations on Nepalese DENV regardless of serotype, as well as several lineages of DENV2 related to those in neighboring countries in Asia and the USA. Furthermore, we identified possible clade shifts in the DENV1 and 2 populations and a high number of DENV3 cases, which is an emerging serotype in Central Nepal. Genomic surveillance and disease monitoring of dengue in Nepal are an ongoing task that requires continuous effort; however, our demonstration of successful whole genome sequencing of DENV using the iSeq100, a tool specifically designed for use in low resource settings, is encouraging.

Abbreviations

2K	2K transmembrane peptide
ADE	Antibody-dependent enhancement
BLAST	Basic Local Alignment Search Tool
C	Capsid
COVID	Coronavirus disease
DENV	Dengue virus (DENV1 = dengue virus serotype 1)
DHF	Dengue hemorrhagic fever
DHKUH	Dhulikhel Hospital/Kathmandu University Hospital
DSS	Dengue shock syndrome
E	Envelope
ECDC	Epidemiology and Disease Control Division
EMR	Electronic medical record
GISAID	Global Initiative on Sharing All Influenza Data
GTR + G	Generalized time reversible + gamma distributed rates
IDT	Integrated DNA Technologies
KUSMS IRC	Institutional Review Committee at Kathmandu University School of Medical Sciences
MEGA	Molecular Evolutionary Genetics Analysis
NGS	Next-generation sequencing
NHRC ERB	Ethical Review Board at Nepal Health Research Council
NS	Nonstructural region (e.g., NS1 = nonstructural region 1)
PCR	Polymerase chain reaction
preM	Pre-membrane
PRC	People's Republic of China
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
RUO	Research use only
SD	Standard deviation
WHO	World Health Organization
USA	United States of America

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44263-025-00135-z.

Additional file 1. Table S1. GISAID accession numbers with alignment coverage (%). Table containing the GISAID accession numbers of self-produced sequences included in phylogenetic analysis with percent alignment coverage of each sequence.

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Authors' contributions

MC: Conceptualization, design, data acquisition, analysis, interpretation, original draft, revision and review. NK: Conceptualization, design, data acquisition, analysis, interpretation, original draft, revision and review. AS: Data acquisition, interpretation, original draft, revision and review. SKM: Data acquisition, revision and review. DT: Interpretation, revision and review. RS: Conceptualization, design, interpretation, revision and review. All authors read and approved the final manuscript.

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Data availability

All sequences are accessible in the GISAID Dengue database (accession numbers available in Additional file 1: Table S1). Raw reads are available as NCBI BioProject PRJNA1155024 at https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1155024.

Ethics approval and consent to participate

Ethical approval for this study was obtained by the Ethical Review Board at Nepal Health Research Council (NHRC ERB, ref.: 107/2023) and Institutional Review Committee at Kathmandu University School of Medical Sciences (KUSMS IRC, ref.: 117/2024). This study did not directly contact the human subjects or patients and only investigated banked serum samples obtained during routine dengue diagnostic procedures with secondary metadata obtained from hospital's EMR. Therefore, informed consent from the patients was waived by the NHRC ERB and the KUSMS IRC. We declare that all research conducted in the study conformed to the principles of the Helsinki Declaration.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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