WITHDRAWN: Retrospective investigation of origin, epidemiology and genetic characteristics of the dengue fever outbreak in Yunnan, China from 2017 to 2018

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Abstract

Dengue has become a worldwide public health problem. In recent years, Dengue has broken out in multiple countries and regions. From 2017 to 2018, a dengue epidemic broke out in Yunnan of China and neighboring Southeast Asian countries (Laos and Myanmar). In this study, retrospective detection and genetic characterization of Dengue virus (DENV) strains were carried out between 2017 and 2018 in Yunnan, China. The dengue outbreak in Yunnan, China in 2017 was mainly caused by DENV1, while the dengue outbreak in 2018 was caused by DENV1 and DENV2. Among them, DENV2 plays a dominant role. Then, three complete sequence and ten envelope (E) gene sequences were obtained through PCR. YN/324 and YN/017 were isolated from Myanmar travelers, and YN/117 isolate from Laos travelers. In addition, YN/007 isolate has 13 nucleotides (10270 nt -10282 nt) deletions in the 3' UTRs, which leads to significant changes in the RNA secondary structure. Multiple sequence alignment E gene sequences revealed that these locally isolates share high homology (> 99.5%) with the isolates from Myanmar and Laos. The phylogenetic divergence analysis also revealed that most of the local isolates were closely related to the isolates from Myanmar and Laos. YN/033 strain belong to genotype V of DENV1, and it was detected in Yunnan after 2013. Other isolates of DENV1 in this study were clustered in a branch representing genotype I. These isolates of DENV2 in this study belonged to Asia I, Asia II and Cosmopolitan. Further analyzed the relationship between the evolutionary tree branching and amino acid substitution of genotype revealed that multiple amino acid substitutions are related to the genetic evolution of DENV1 or DENV2, respectively. Similarly, genotype 1 of DENV1 can be divided into 4 subgroups based on the amino acid substitutions. In addition, twelve amino acid mutations are unique to the isolate in this study and have never been reported. Interestingly, recombination analysis found that both DENV1 and DENV2 isolates in this study had widespread intra-serotype recombination. In summary, the results of the epidemiological investigation implies that the dengue outbreak in Yunnan of China was mainly caused by imported cases. This research provides new reference for further research on the prevalence and the molecular epidemiology of DENV in Yunnan, China, as well as the variation characteristics of these novel isolates.

1. Introduction

Dengue virus (DENV) is highly prevalent mosquito-borne virus, which enters the human body through the bite of Aedes aegypti and Aedes albopictus [1]. DENV lead to Dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2]. DENV belongs to *Flavivirus* in the family *Flaviviridae* is a enveloped, single-stranded, negative-sense and RNA virus that the genome(~ 11.0 kb) contains only one open reading frames (ORFs), which encoding a three structural proteins and seven non-structural proteins. In general, based on the antigenic difference of E protein, dengue virus is divided into four different serotypes (DENV 1-4), and its serotypes can be further divided into different genotypes. It is worth noting that there is difficult to have long-term complete and effective cross-protection between different serotypes of DENV [3, 4].

E protein (~495 amino acids) is the most fully exposed protein on the surface of mature infectious virus particles, and also contains immunologically important epitopes associated with virus neutralization [5]. It plays an important role in mediating the adsorption of viruses and host cells, membrane fusion and in the process of virus assembly and maturation. According to the determined crystal structure of E protein, three different domains (EDI, EDII and EDIII) are formed in the spatial structure. Among them, EDII is located at the amino terminus of the E protein and contains a fusion peptide structure that mediates the fusion of the virus and the target cell (fusion loop, 98~110 amino acids) [6]; EDIII mediates the adsorption of viruses to target cells and is considered to be the region containing the receptor binding region and the most important neutralizing epitope [7-9].

DENV has been spread to more than 100 countries and regions, especially in tropical and subtropical regions. About half of the world's population lives in dengue-affected areas, and there are more than 50 million infections every year [10]. The global DF epidemic is becoming more and more serious and has become an increasingly serious global public health problem [11]. In recent years, dengue epidemics have broken out in multiple regions of China, such as Guangdong, Zhejiang, Yunnan, Henan, Hunan, etc [12-15]. In particular, Yunnan province of China is endemic for dengue with numerous cases reported every year. In 2008, a dengue epidemic caused by DENV1 occurred in Ruili City, Yunnan Province, China, which is adjacent to Myanmar. In 2013 and 2015, large-scale dengue outbreaks occurred in Xishuangbanna, Yunnan Province, China, which is adjacent to Myanmar and Laos [15, 16]. From 2016 to 2019, Dengue outbreaks continue to occur in the border areas between China and Laos, Myanmar and Vietnam [17]. In this report, we investigated the prevalence of DENV in Yunnan Province, China from 2017 to 2018. It is important to understand the molecular characteristics and evolutionary trends of circulating DENV strains.

2. MATERIALS AND METHODS

2.1 Sample collection

172 serum samples (DENV NS1-positive and DENV IgG-negative) of human, which were used in this study, were obtained from the Yunnan institute of parasitic diseases and stored at −80˚C.

2.2 DNA extraction and polymerase chain reaction (PCR)

The viral RNA was extracted from human serum samples with the FastPure Cell/Tissue Total RNA Isolation Kit V2 (Vazyme Biotech Co. Nanjing) following the manufacturer's instructions. The primer pairs were designed based on the reference sequence from NCBI (Table S1, S2 and S3). HiScript® II One Step RT-PCR Kit (Dye Plus) was used for PCR amplification (Vazyme Biotech Co. Nanjing). 5min TA/Blunt-Zero Cloning Kit (Vazyme Biotech Co. Nanjing) were used to construct recombinant vectors, which were amplified in *Escherichia coli* (*E. coli,* DH5α) before sequencing.

2.3 Data sources and statistical analysis

The dengue reference sequences and numbers used in this study were downloaded from the NCBI database and counted. The reported cases in Viet Nam, Laos, Cambodia, Philippines, Singapore and Myanmar were obtained from the WHO Regional Office for the Western Pacific's Institutional Repository for Information Sharing (WPRO IRIS, http://apps.who.int/iris/). The reported cases in Yunnan of China were obtained from the National Health Commission of the People's Republic of China (http://www.nhc.gov.cn/). The data was analyzed by SPSS software.

2.4 Phylogenetic analysis

The E gene and the genome sequences of DENV obtained in this study have been deposited in GenBank under the accession numbers OM250374–OM250383 (E gene), and OM250394, OM256481 and OM257419 (Complete genome), respectively. Reference genome sequence for DENV was obtained from NCBI. Sequences were assembled using SeqMan software (DNASTAR Inc., Madison, Wisconsin, USA) and aligned using MegAlign (DNASTAR Inc., Madison, Wisconsin, USA) with the Clustal W alignment method for genomic similarity and amino acid similarity analyses. A phylogenetic tree was constructed using the maximum likelihood method with 1,000 bootstrap replicates and the aligned dataset using the MEGA7 program.

2.6 Recombination analysis

Recombination event analysis was carried out by analyzing the Egene sequnces of DENV1and DENV2. RDP4 (http://web.cbio.uct.ac.za/~darren/rdp.html) Software (RDP, Bootscan, MaxChi, GENECONV, Chimaera, SiScan and 3Seq) is used for preliminary screening of recombinant strains, SimPlot (http://sray.med.som.jhmi.edu/RaySoft) software, used to analyze breakpoints and the sequence of recombination and parental lineages.

3. Results

3.1 Epidemic trend of dengue in Yunnan of China and neighboring countries

According to statistics from the World Health Organization, from 2013 to 2019, the number of dengue cases in Yunnan of China and neighboring countries remained at a high level. However, after the outbreak of COVID-19 in 2019, the number of dengue cases in neighboring countries in 2020 has not decreased significantly, while the number of dengue cases in Yunnan province has decreased significantly (Figure 1a and 1b). Then, a comparative analysis of the distribution areas of local cases and imported cases found that the main outbreak areas overlapped and were mainly concentrated in Xishuangbanna and

Ruili in Yunnan Province, China (Figure 1c and 1d). In addition, the correlation analysis between the number of imported cases and the number of local cases showed that they showed a significant positive correlation (correlation coefficient = 0.936). In particular, since there is no publicly available detailed data, it is impossible to count the number of DENV1~4 isolates in dengue cases from 2013 to 2019. Therefore, this study counted the number of DENV1~4 isolates in Yunnan from 2013 to 2019 in Genbank data and found that the change trend of DENV1~4 strains is consistent with the trend of dengue epidemic (Figure 2a).

3.2 Screening for DENV prevalence in clinical samples

In this study, among the 172 serum samples, 38 (38/172, 22.1%) serum samples were positive for DENV. From which, 13 of 72 (13/72, 18.1% 9/DENV1, 4/DENV2) serum samples were positive in 2017, 25 of 100 (25/100, 25.0%, 12/DENV1, 13/DENV2) serum samples were positive in 2018. From which, the proportion of DENV2 strains detected in dengue cases rose from 30.8% to 52.0% from 2017 to 2018 (Figure 2). This result is consistent with the statistical data of DENV in GenBank. Then, after removing repeated sequences three complete sequence (MH107161–MH107163) and ten E gene sequences (MH107141–MH107150) were obtained. From which, YN/324 and YN/017 isolate from Myanmar travelers, YN/117 isolate from Laos travelers.

3.3 Sequence Analysis

The complete genome sequences and the E gene sequences of these strains in this study were obtained by amplifying overlapping fragments. From which, YN/117 isolate were obtained from a Laos traveler, YN/017 isolate and YN/324 isolate were obtained from Myanmar travelers. Other strains in this study were obtained from local residents. The genome and the ORF of YN/RL isolate include 10735 nucleotides and 10179 nucleotides (3393 AA), respectively. The 5' and 3' UTRs of YN/RL strain were 94 and 465 nt in length, respectively. YN/RL isolate lacks 1 nucleotides (79 nt) in the 5' UTRs, which leads to significant changes in the RNA secondary structure (Supplementary Figures 1a and 2). Interestingly, the genomes of the YN/MH strain and the YN/017 isolate have different lengths. The ORF and 5' UTRs were 10176 nt (encoding 3391 AA) and 96 nt in length, respectively. The difference is that compared with YN/MH strain and DENV2-SS (New Guinea C) isolate, YN/017 isolate lacks 13 nucleotides (10270 nt -10282 nt) in the 3' UTRs, which leads to significant changes in the RNA secondary structure (Supplementary Figures 1b and 3). In addition, the E gene of these sequences in this study all contain 1485 nucleotides and encode 495 amino acids.

3.4 Multiple sequence alignment and phylogenetic analysis

In order to perform genetic evolution analysis, the DENV classic strains of various countries or regions (focus on the three countries bordering Yunnan, Myanmar, Laos and Vietnam) were obtained from NCBI

as reference sequences. The phylogenetic divergence analysis based on the E gene sequences of DENV1 revealed that YN/033 strain belong to genotype V, which was the first detected in Yunnan after 2013. Other isolates in this study (YN/251, YN/324, YN/336, YN/075, YN/RL) of DENV1 were clustered in a branch representing genotype I. YN/251 and YN/336 were located in one cluster of the ML tree, with the closest relationship to the YN/324 isolate (Yunnan/Vietnam traveler), Vietnam isolate (2010, KY971707) and Thailand isolate (2001, KY586543). It is worth noting that these isolates were continuously detected in Yunnan and neighboring countries (Laos, Myanmar and Vietnam) from 2013 to 2019. YN/RL strain were in another cluster, with the closest relationship to the Laos isolate (2015, MG894873), and China/Zhejiang isolate (2016, KY886977). Similarly, YN/RL isolate was continuously detected in Yunnan, other province (Zhejiang, Guangdong and Hubei) and neighboring countries (Laos, Myanmar and Singapore) from 2013 to 2019. Special, YN/075 isolate is genetically distant from other isolate in this study and has closeted relationship with Guangdong isolate (2010, JN029812) and USA/Hawaii isolate (1944, EU848545, DENV1-SS) (Figure 3a).

Further analyzed the relationship between the evolutionary tree branching and amino acid substitution of DENV1 revealed that amino acid variations at 10 positions determine the evolutionary differences of gene subtypes. From which, V(I)293M result in Genotype I type; and V436I result in Genotype II type; and K120E, E157G and V345I result in Genotype III type; M(V)293I result in Genotype IV type; F339I, A369T, and I439V result in Genotype V type. In additional, genotype 1 of DENV1 can be divided into 4 subgroups based on the amino acid variation (I122V- Genotype- Ib, N52D and V312L- Genotype Ic, I380V- Genotype Id) (Figure 3b and 3c).

Similarly, phylogenetic analyses revealed that these strains in this study belonged to Asia I (YN/002 isolate), Asia II (YN/017 isolate, YN/MH isolate) and Cosmopolitan (YN/011 isolate, YN/117 isolate, YN/020 isolate, YN/197 isolate). YN/020 isolate and YN/197 isolate has close relationship with YN/117 isolate (Yunnan/Laos traveler), Laos isolate (2018, MN44614) and Malaysia isolate (2014, KX452017). Compare with YN/117 isolate, YN/020 isolate and YN/197 isolate. YN/011 isolate were in another cluster of Cosmopolitan type, with the closest relationship to the India isolate (1992, FJ538925). It is worth noting that these isolates of Cosmopolitan type in this study have been detected in Yunnan (include foreign tourists) and multiple countries (Australia, Singapore, Myanmar, Thailand, Laos, Indonesia and Malaysia) from 2014-2019. YN/MH isolate has close relationship with YN/017 isolate (Yunnan/Myanmar traveler) and Papua New Guinea strain (1944, KM204118, DENV2-SS). Specially, YN/002 strain is genetically distant from other isolates in this study and has close relationship with Laos isolate (2018, MN44619) (Figure 4a).

Combined with multiple sequence alignment and evolutionary analysis results revealed that amino acid variations at 8 amino acid variations are related to genetic evolution. D390N result in America type; and E71K H149N and I462V result in Cosmopolitan type; and V491A result in America/Asia type; and G128E result in Asia- I type; and E126K and M492V result in Asia II type (Figure 4b and 4c).

3.5 Homology and amino acid mutation analysis

The comparison of the complete ORF sequences revealed that the YN/RL isolate share 90.5–97.2% identity to the reference sequence of DENV1. Based on nucleotide similarity analysis of the E protein of DENV1, these isolates of DENV1 in the study shared 89.7%–100.0% similarity with the reference sequence of DENV1 (Table S4). Compared with the E protein of DENV1-SS isolate, these isolates of DENV1 in this study has 22 amino acid mutations, of which 8 amino acid mutations had never been reported (Figure 5a). In particular, YN/324 (Yunnan/Myanmar traveler) strain contain 4 amino acid mutations (H89Q, N92A, V91A, C92G) occurred in the first ED II domain of E protein. Compared to the closest China/Hubei strain (2011, KP772252), a total of 7 amino acid mutations occurred in the CDS of the YN/RL strain (Supplementary Figures 4a).

The complete ORF of YN/MH strain shared 91.9%– 93.9% nucleotide identity with the reference sequence of DENV2. Multiple sequence alignment revealed that the E protein strains of DENV2 in this study showed 89.2%–99.9% identity to the reference sequence (Table S5). Compared with the E protein of Papua New Guinea (1944, KM204118, DENV2-SS), these strains of DENV2 in this study has 17 amino acid mutations, of which 3 amino acid mutations had never been reported (Figure 5b). Compared to the closest DENV2-SS strain, a total of 4 amino acid mutations occurred in the CDS of the YN/MH strain and YN/017 strain (Yunnan/Myanmar traveler). The difference is that compared with DENV2-SS strain and the YN/MH strain, YN/017 strain (Yunnan/Myanmar Traveler) has two amino acids mutations in the NS3 protein (Supplementary Figures 4b).

3.6 Recombination analysis

Multiple studies have confirmed that DENV has widespread intra-serotype and inter-serotype recombination. Combined with RDP4 and SimPlot analysis showed that four strains in this study existed intra-serotype recombination. Among them, the genome sequence of YN/075 strain has a single breakpoint, which is composed of the parental strain (Indonesia/Surabaya strain, AB915384, 2013) and the minor strain (Thailand strain, KY586543,2001). Interestingly, the genome sequence of YN/002 strain, YN/011 strain and YN/017 strain all contained multiple breakpoints. In addition, no recombination between different serotypes has been detected (Figure 6).

4. Discussion

Yunnan basically has a subtropical and tropical monsoon climate with numerous flora and fauna. The southern part of Yunnan Province (such as the Xishuangbanna area) is located on the northern edge of the tropics with lush vegetation. The annual average temperature is maintained between 18.9℃ 22.6℃, which is very suitable for the growth and reproduction of mosquitoes. Yunnan has close economic exchanges with Southeast Asian countries and is also a popular holiday and tourist destination. This has

resulted in very high population mobility in the border areas of Yunnan, which facilitated the spread of mosquito-borne viruses such as dengue, Japanese encephalitis, chikungunya and Zika virus, etc. [18, 19].

Since Liu et al. [20] first reported the detection of dengue virus in Yunnan Province in 2006, large-scale dengue outbreak shave occurred in Yunnan every 2 years since 2013, involving different serum types of DENV. Among them, DENV1 is the main serotype that caused dengue epidemics in 2013-2015, 2016-2017, and 2019; DENV 2 was mainly in 2015, 2017-2019; DENV 3 was mainly concentrated in 2013 and 2015; DENV 4 is only scattered sporadically [21-23]. Specially, the outbreak area of dengue is mainly border port cities (such as Jinghong, Mengla, Ruili, Jiangcheng and Menglian, etc.) with Myanmar, Vietnam and Laos. In the past, it was generally believed that the dengue outbreak in Yunnan was caused by local cases spreading through mosquito bites or mosquitoes spreading across borders. In response to the COVID-19 pandemic, China implemented strict restrictions on cross border travel to prevent disease importation. Yunnan, a Chinese province that borders dengue-endemic countries in Southeast Asia, experienced unprecedented reduction in dengue, from 6840 recorded cases in 2019 to 260 in 2020. On the contrary, the number of dengue cases in Southeast Asian dengue endemic countries adjacent to Yunnan has not dropped significantly. Subsequently, a correlation analysis of the regional distribution and increase/decrease trends of local cases and imported cases revealed that there was a significant positive correlation. In order to further study the source of the dengue outbreak in Yunnan of China, the results of homology comparison and genetic evolution analysis revealed that there are have high homology and closest relationship between the local strains and the strains from Laos and Myanmar travelers. Based on the above results, it is speculated that the dengue outbreak in Yunnan was mainly caused by imported cases. Li et al. [24] used the 2013-2020 epidemiological and viral genome data in Yunnan and neighboring countries to establish a multivariate statistical model for analysis. It is concluded that Yunnan is a regional sink for DENV lineage movement, dengue incidence between 2013-2019 in Yunnan was closely linked with international importation of cases. This is consistent with the conclusions of this research.

Another noteworthy phenomenon is the positive rate of DENV2 has been significantly increased from 2017 to 2018 in Yunnan, China. Statistics show that before 2015, DENV1 was the main prevalent serotype in the world. However, since 2015, the scale and frequency of dengue epidemics caused by DENV2 in various countries and regions have been increasing. DENV2 has maintained a high infection rate in Yunnan after 2014. Especially in 2015 and 2018, the number of DENV2 dengue cases far exceeded other serotypes. This result suggests that DENV2 and DENV1 may alternately become the main epidemic serotypes in Yunnan Province, China. Genetic evolution analysis revealed that the DENV strains circulating in Yunnan were mainly genotype 1 of DENV1 and Cosmopolitan of DENV2. It is particularly important to note that some strains have existed stably in Yunnan and neighboring countries for a long time. Such as DENV1 isolates (YN/RL, YN/251, YN/324 and YN/326) and DENV2 isolated (YN/002, YN/011, YN/117, YN/197, YN020) were repeatedly detected in Yunnan and neighboring countries from 2013 to 2019. On the contrary, genotype 5 isolates (such as YN/033) disappeared after 2013 in Yunnan and reappeared in 2017. Further research is needed to trace the origin of these strains.

Mutation and recombination play a decisive role in the evolution of viruses, especially RNA viruses. The 5' and 3' UTRs of virus play an important role in efficient viral RNA translation and genome replication. Song et al. [25] confirmed that the 5'-UTRs of flaviviruses have the function of internal ribosome entry sites. Yan et al. [26] using a virus-induced reporting gene system reveal that the deletion of nucleotides 10663-10677 and 10709-10723 facilitates translation. In the present research, compared with the DENV1-SS isolate or DENV2-SS isolate, YN/RL isolate and YN/MH isolate has multiple base deletions or mutations at the 5'-UTRs and 3'-UTRs; YN/017 isolate has 13 nucleotides (10270 nt -10282 nt) deletions in the 3' UTRs. These nucleotide changes in lead to significant changes in the RNA secondary structure. Whether to change the level of replication and translation of the isolate remains to be confirmed by further research.

Present research indicated that prM protein can promote the maturation of the virus and increase its infectivity, and induce the production of protective antibodies. E protein contains immunologically important epitopes associated with virus neutralization and has become a potential target protein for vaccine and neutralizing antibody therapy. Deng et al. [27] identified a broad flavivirus cross-neutralizing monoclonal antibody that can recognize a new epitope in the E protein fusion loop (EDII, ^{98}DRXW^{101}). Thullier et al. [28] indicated that the murine monoclonal antibody 4E11 neutralizes dengue viruses of all serotypes by binding to the 296-400 segment of the major dengue virus envelope glycoprotein (DE). Chin et al. [29] found that the E protein domain III of dengue virus can competitively inhibit virus entry. In the present research, multiple amino acid mutation was found on the E protein of these isolates. In particular, twelve amino acid mutations are unique to the isolate in this study and have never been reported. A single mutation (S139N) in the PrM protein of YN/ML and YN/017 isolate. Thus, amino acid substitution in both prM/E proteins may greatly affect the the immunogenicity and receptor affinity. It is worth noting that the E genes of these isolates have extensive intra-serotype recombination, which will accelerate the mutation and evolution of the virus.

In this study, these isolates also have multiple amino acid mutation in non-structural protein, mainly in NS3 and NS5. Non-structural proteins also play an important role in virus replication and evasion of host immune response. Such as, Ye etc. [30] indicated a single silent mutation G66A in the NS2A gene of JEV abolished the production of NS1’ in vitro and reduced neurovirulence and neuroinvasiveness in mice. NS3 is a multifunctional protein with helicase, RNA-stimulated nucleoside triphosphatase (NTPase) and RNA 5'-triphosphatase (RTPase) activities. NS5 is responsible for replication of the viral genome, RNA capping and suppression of host interferon responses [31]. Thus, amino acid substitution in non-structural proteins may greatly affect the efficiency of viral replication.

From 2013 to 2020, there have been many large-scale dengue outbreaks in Yunnan. However, multiple studies have been divergent on the source and prevalence of dengue in Yunnan. This research conducted a systematic analysis of dengue epidemic trends, geographical distribution, and genetic evolution in Yunnan and surrounding countries. Multiple evidence implies the dengue outbreak in Yunnan was mainly caused by imported cases; moreover, DENV2 and DENV1 may alternately become the main epidemic

serotypes in Yunnan Province, China. We hope that this research can provide references for future research on the tracing, epidemic trend, and variation of dengue virus in Yunnan province, China.

Declarations

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CONFLICTS OF INTEREST

The authors have no conflicts of interest regarding the research, authorship, and/or publication of this article.

AVAILABILITY OF DATA AND MATERIALS

The data set supporting the conclusions of this article is available in GenBank.

Authors' contributions

WW, YML, and XFG performed the research, analyzed the data, and wrote the manuscript. NY, CW, and CHL helped to write the manuscript and participated in sample collection. FLN, MZ and WCS participated research testing. TRL, HNZ, HJL and NYJ designed the research. All authors read and approved the final manuscript.

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Figures

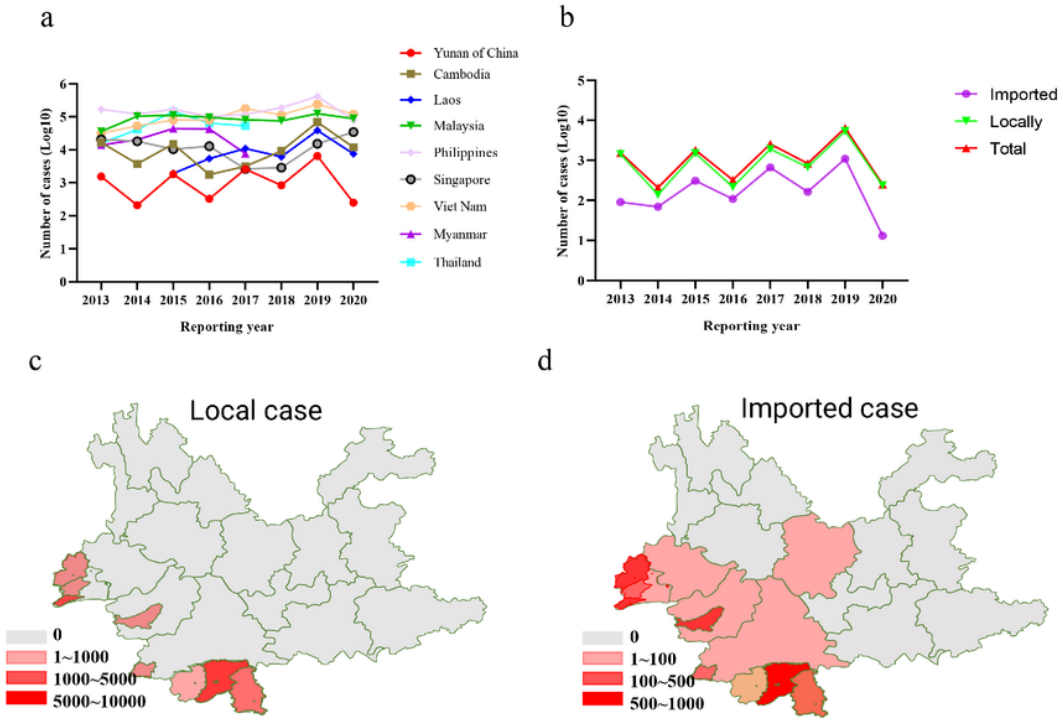

Figure 1

(a) The number of dengue cases in Yunnan and neighboring countries from 2013 to 2020 (b) The number of local cases and imported cases in Yunnan from 2013 to 2020. (c) Regional distribution map of local cases from 2013 to 2020. (d) Regional distribution map of Imported cases from 2013 to 2020.

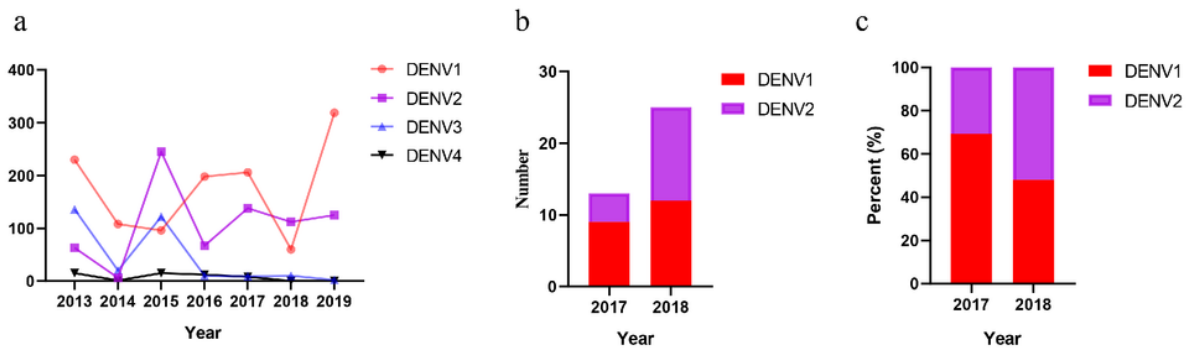

Figure 2

(a) The number of DENV1-4 strains in the GenBank from 2013 to 2019. (b) The number of differently DENV strains in this study. (c) The percentage of differently DENV strains in this study.

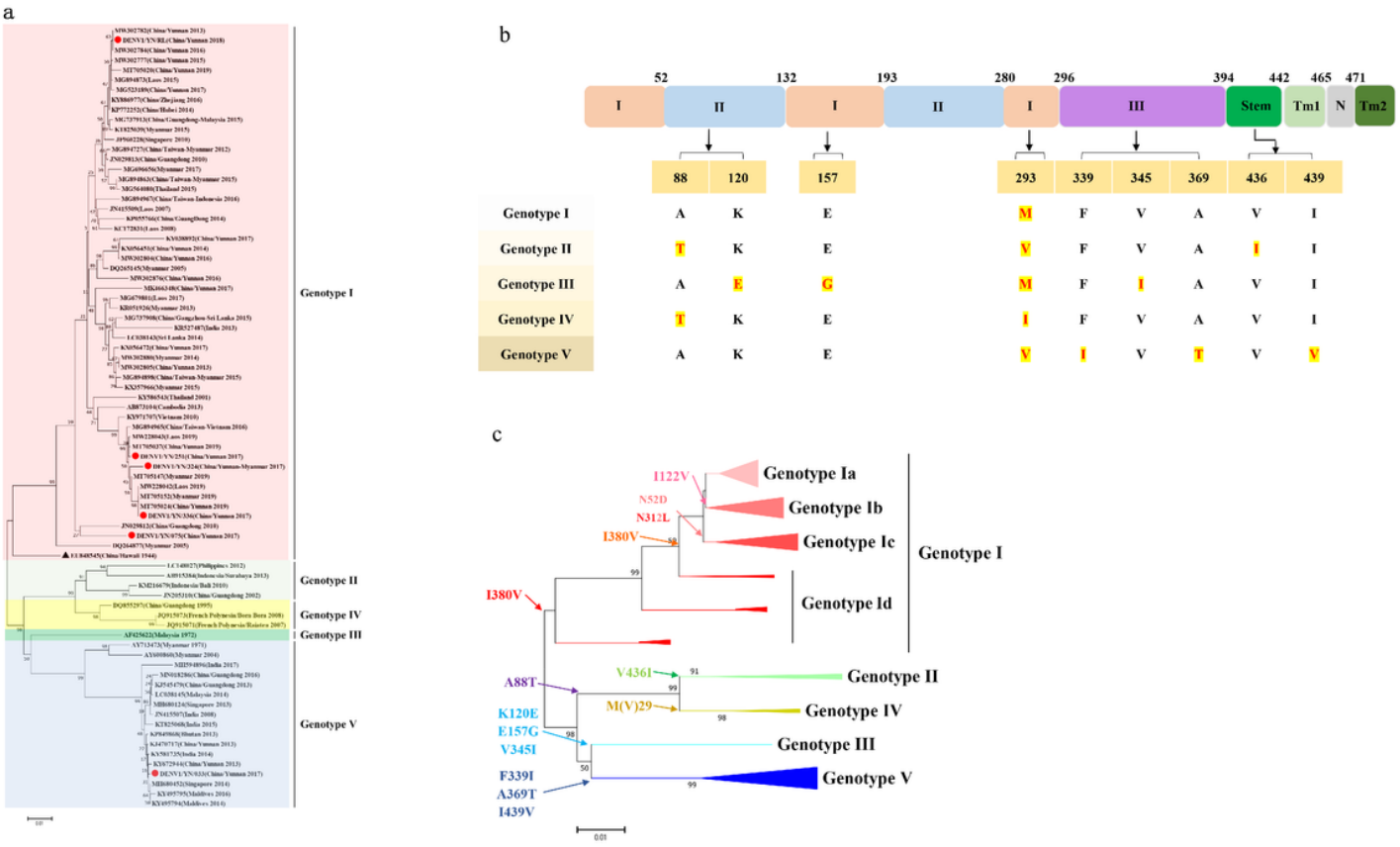

Figure 3

(a) Phylogenetic tree of DENV-1 epidemic strains in Yunnan, China. Maximum likelihood (ML) trees were built using 1,000 bootstrap replicates. Red circles indicate the strains detected in this study. (b) The characteristics of amino acid variation among different genotypes of DENV1. (c) Relationship between evolutionary tree branching and amino acid mutations in genotype I of DENV-1.

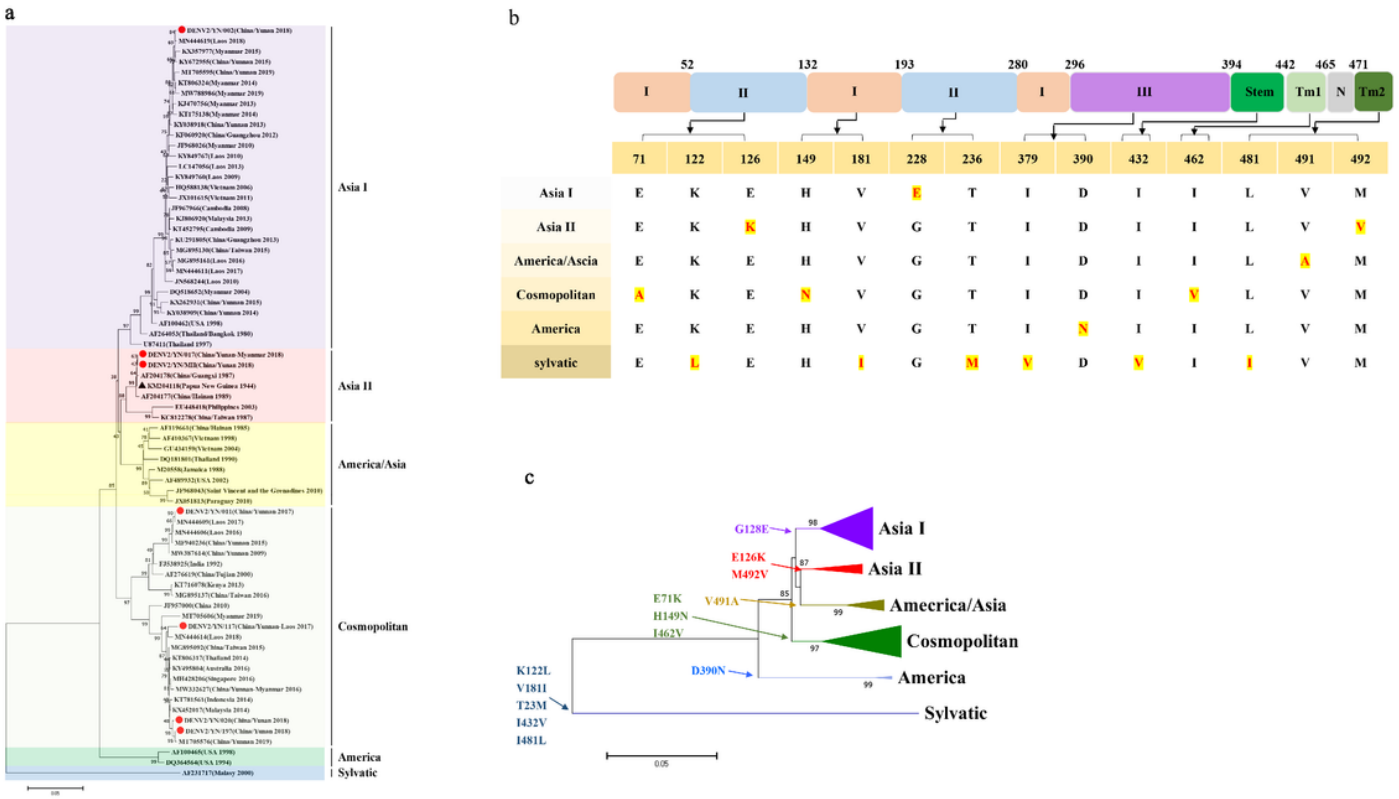

Figure 4

(a) Phylogenetic tree of DENV-2 epidemic strains in Yunnan, China. Maximum likelihood (ML) trees were built using 1,000 bootstrap replicates. Red circles indicate the strains detected in this study. (b) The characteristics of amino acid variation among different genotypes of DENV2 (c) Relationship between evolutionary tree branching and amino acid mutations in genotype I of DENV-2.

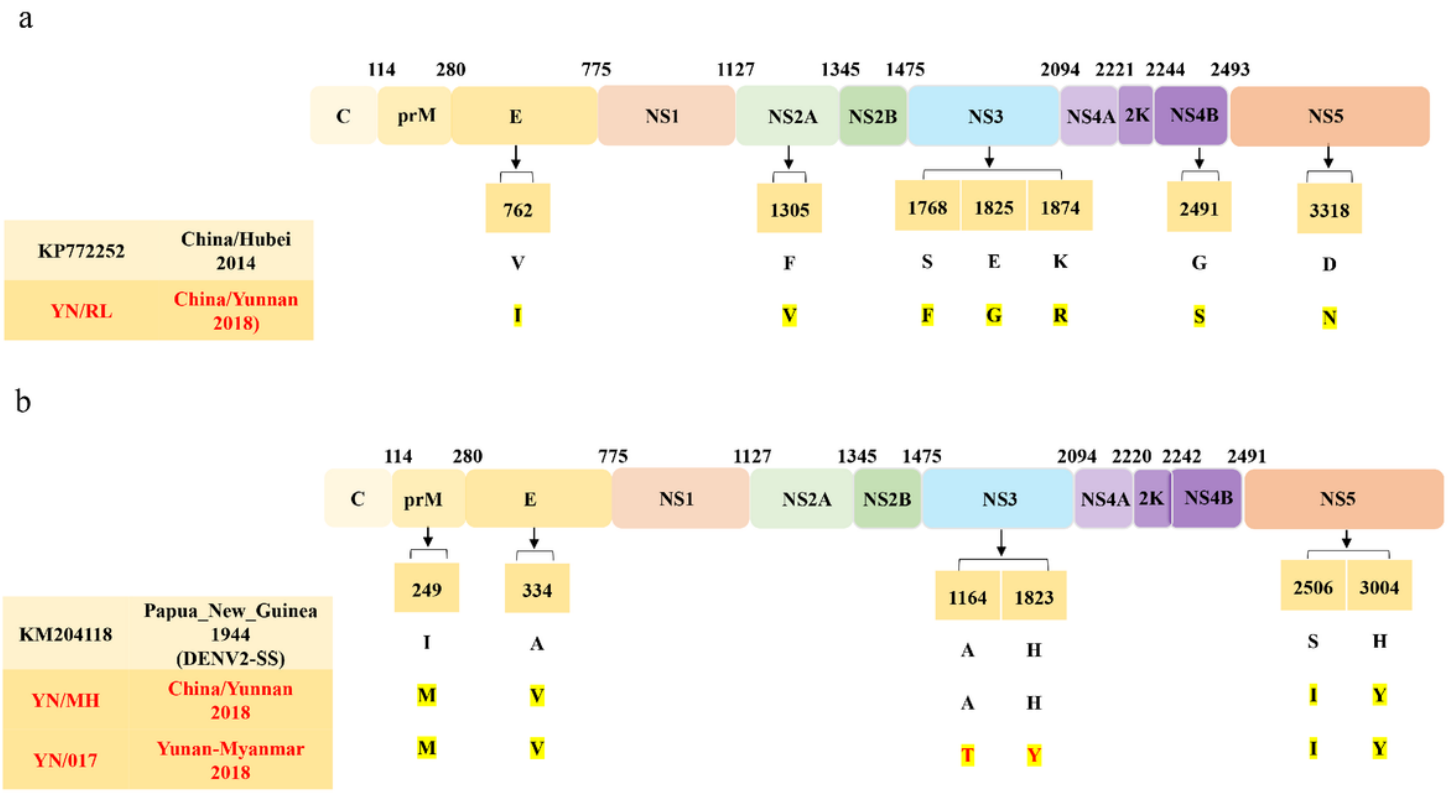

Figure 5

(a) Amino acid mutations in the E gene of DENV1. (b) Amino acid mutations in the E gene of DENV2.

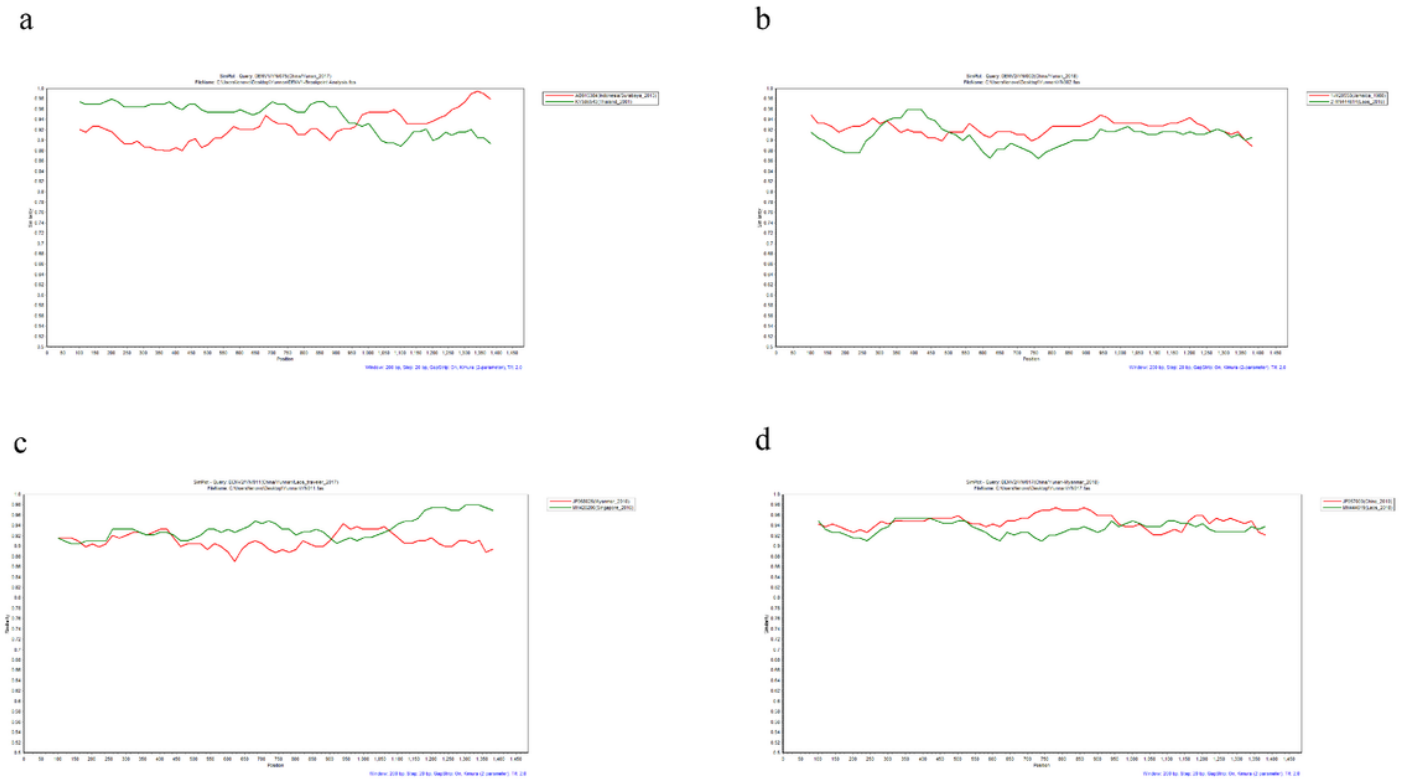

Figure 6

(a) Recombination analyses of YN/075 isolate, which is composed of the parental strain (Indonesia/Surabaya, AB915384) and the minor strain (Thailand, KY586543). (b) Recombination analyses of YN/002 isolate, which is composed of the parental strain (Jamaica, M20558) and the minor strain (Laos, MN444614). (c) Recombination analyses of YN/011 isolate, which is composed of the parental strain (Myanmar, JF968026) and the minor strain (Singapore, MH428206). (d) Recombination analyses of YN/017 isolate, which is composed of the parental strain (China, JF957000) and the minor strain (Laos, MN444619).

Supplementary Files

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