

Emergence And Circulation of Dengue Virus Serotype 2 In Guangzhou Over A Period of 20 Years

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Abstract

Objectives The dengue epidemic in Guangzhou has imposed a rising burden on society and health infrastructure. Here we present the genotype data for dengue virus serotype 2 (DENV-2) to improve the understanding of dengue epidemic.

Methods We sequenced the envelope gene of DENV-2 obtained from patient serum sample, and subsequently performed the maximum-likelihood phylogenetic analysis using PhyMLv3.1, the maximum clade credibility analysis using BEAST v.1.10.4 and selection pressure analysis using Datamonkey 2.0 .

Results The DENV-2 prevalent in Guangzhou region related to the strains of Southeast Asian countries. Our results suggest that the Malaysia/Indian subcontinent genotype is prevalent in Guangzhou and no genotype shift has occurred during the last 20 years. Episodic positive selection was detected at one site.

Conclusions Prevention and monitoring imported cases are important for local control. The shift between the lineages of the Malaysia/Indian subcontinent genotype, which originated at different time points, may be the underlying cause of rising DENV-2 cases in Guangzhou. The low rate of dengue haemorrhagic fever in Guangzhou may be explained by the dominance of the less virulent Malaysia/Indian subcontinent genotype.

1. Introduction

Dengue is caused by infection with dengue virus (DENV), which is a member of the genus *Flavivirus*, family *Flaviviridae*. Approximately 96 million dengue infections were estimated globally in 2010, of which 70% were in Asia [1]. The clinical manifestations of dengue can range from mild fever, known as dengue fever, to the lethal forms of dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Despite extensive efforts, no effective vaccines or drugs against DENV have been developed to date. Therefore, prevention strategies such as preventing further mosquito bites in patients, cleaning vector breeding sites, and using pesticides are the main methods to control dengue at present.

There are four serotypes of DENV, each of which can be divided into several genotypes based on phylogenetic analysis. Dengue virus serotype 2 (DENV-2) is considered to have greater potential to cause DHF/DSS and spread than the other three serotypes. Some studies have shown that secondary infection with DENV-2 after the first heterotypic infection is more likely to result in DHF/DSS than the secondary infection of the other three serotypes[2].

Since dengue fever was first recorded in 1978, dengue has been an epidemic in Guangzhou, China for nearly 40 years, imposing an increasing burden over time. Over the past decade, more than 40,000 cases of dengue have been reported in the region, dominated by DENV-1 [3,4]. However, DENV-2 is also detected in Guangzhou, which has been associated with outbreaks in some communities over the last two decades. With the spread and epidemic of DENV-2, the risk of a DHF/DSS outbreak is increasing. Furthermore, a specific genotype may have the propensity to cause DHF/DSS and be transmitted efficiently by vectors[5,6]. Therefore, it is necessary to closely monitor the genetic diversity and genotype variations of DENV-2 to best understand dengue epidemics in Guangzhou.

Toward this end, we analysed serum samples of patients suspected to have dengue that were sent to the Guangzhou Center for Disease Control and Prevention for diagnostic purposes by hospitals from 2001 to 2020. By phylogenetic analysis of the DENV-2 strains presenting during this time, this investigation will provide reference information to scientists and public health officials dealing with prevention and control of dengue.

2. Materials And Methods

2.1 Sample collection

Blood samples were obtained from patients with symptoms suggestive of dengue, such as sudden high fever with headache, arthralgia, and/or myalgia, according to the diagnosis for dengue fever promulgated by the National Health Commission of the People's Republic of China[7].

2.2 Virus isolation and sequencing

The serum samples were analysed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) on arrival, using a dengue virus RT-qPCR kit (Jiangsu BioPerfectus Technologies Co., Ltd., China).

From 2001 to 2018, the samples that tested positive for DENV were diluted 1:50 in RPMI-1640 medium (Life Technologies Corporation, Grand Island, NY, USA). *Aedes albopictus* clone C6/36 (ATCC CRL-1660) cell monolayers (Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were inoculated with the diluted samples and incubated at 28°C. Supernatants of cultures with cytopathic effects (CPE) observed within 7 days were harvested for further sequencing. Supernatants of cell cultures with no CPE were added to new C6/36 monolayers. Inoculated cells without CPE after three generations were considered to be negative for a DENV isolation.

In 2019 and 2020, to improve the quality or fidelity of the sequences, RNA extracted from the serum samples was used for RT-qPCR and direct sequencing of the envelope gene without virus isolation, as recommended by Leitmeyer et al. [8]. Therefore, more sequences were acquired in 2019 than in other years.

Viral RNA was extracted from the supernatants of cell cultures (2001–2018) or sera of patients (2019–2020) using the QIAamp Viral RNA Mini kit (Qiagen, Germany) according to the manufacturer's instructions. The envelope gene was amplified using the SuperScript III One-Step RT–PCR System with Platinum Taq DNA polymerase (Invitrogen, USA). The sense primer was 5'-CCAGGCTTTACCATAATGGC-3' and the anti-sense primer was 5'-CCAGCTGCAACAACGCAACCAC-3'. The reaction was initiated at 50 °C for 30 min, followed by denaturation at 94 °C for 2 min; 40 cycles of denaturation at 94 °C

for 30 s, primer annealing at 52 °C for 30 s, and primer extension at 72 °C for 2 min; and a final extension step at 72 °C for 7 min. The product was sequenced using Sanger sequencing.

2.3 Phylogenetic and molecular clock analysis

The maximum-likelihood phylogenetic tree of the obtained envelope sequences acquired in Guangzhou along with the reference sequences retrieved from GenBank[9] was constructed using PhyML v3.1. The substitution model was determined by SMART model selection of the Bayesian information criterion[10]. The fast likelihood-based method of eBayes was applied owing to the large number of sequences. Genotypes were grouped according to the criteria defined by Rico-Hesse[11].

A maximum clade credibility (MCC) tree was constructed for the same sequences using BEAST v.1.10.4. The detected date for each sequence was used as the calibration date. The tree was visualised using Bayesian Evolutionary Analysis Utility (BEAUti) with the following settings: substitution model,GTR; base frequencies, estimated; site heterogeneity model,gamma + invariantsites; number of gamma categories, 4; clock type, uncorrelated relaxed clock; tree model, random starting tree; length of chain, 100,000,000; echo state to screen every 10,000;and log parameters every 10,000. The results of both the maximum-likelihood and MCC phylogenetic trees were visualised and edited using FigTree.

2.4 Selection pressure analysis

Episodic adaptive selection was evaluated using the Mixed Effects Model of Evolution (MEME) algorithm implemented by Datamonkey 2.0[12]. A signature of positive selection for each site can be determined when the β^+ parameter, representing the rate of nonsynonymous substitutions (dN), is greater than α , representing the rate of synonymous substitutions (dS).

3 Results

Table 1 summarises the DENV-2 cases detected by RT-qPCR in the serum samples of patients suspected to have dengue from 2001 to 2020. There were 416 cases of DENV-2 infection, comprising 373 domestic cases (89.66%) and 43(10.34%) imported cases. Cases from Southeast Asian countries constituted 83.72% (n=36) of imported cases. Before 2010, only one DENV-2 infection was detected in 2005. Since 2010, DENV-2 infections have been detected every year.

Over the 20 years, 148 DENV-2 envelope gene sequences were obtained from the sera of patients in Guangzhou. All sequences were deposited in GenBank. The phylogenetic tree shown in Figure 1 was constructed based on these 148 sequences detected in Guangzhou and 56 sequences downloaded from GenBank. Most of the sequences (n= 142, 95.95%) identified from 2001 to 2020 in Guangzhou belonged to the Malaysia/Indian genotype, while only six sequences (4.05%) clustered in. No American genotype or West African genotype was detected during the study period.

The Malaysia/Indian subcontinent genotype can be further divided into several lineages: GZ1, GZ2, GZ3, GZ4, and GZ5. The sequences acquired in 2005, 2010, and 2013 all belonged to lineage GZ4. The sequences then shifted to lineage GZ5 in 2014. In 2015, there were nine sequences detected, three of which (33.33%) clustered in lineage GZ2 and the other six (66.67%) clustered in lineage GZ5. Since then, most of the sequences belonged to lineage GZ5, and some were scattered among the GZ1, GZ2,GZ3, and GZ4 lineages.

When several identical sequences were detected in the same year, one sequence was retained as a representative for building the MCC tree (Figure 2). This tree was based on 80 sequences obtained in Guangzhou along with 53 sequences retrieved from GenBank. All of the Malaysia/Indian subcontinent genotype strains shared an ancestor in 1955. However, the different lineages of the Malaysia/Indian subcontinent genotype manifested different introduction times. Lineage GZ5, which was the most prevalent, emerged in 1995 and comprised sequences from 2014 to 2019.

Positive selection of the envelope gene for lineage GZ5 was analysed using MEME. The sequences of Guangzhou were compared with those of the reference sequence DQ518635 isolated from Malaysia in 2003. Episodic positive selection was detected at one site, codon position 364, with β^+ = 2160.36, α =0.00, P =0.06. When performing molecular characterisation, two amino acid differences were observed in all sequences from the Guangzhou isolates in comparison with the DQ518635 sequence from Malaysia in 2003:E329D and I439V.

4 Discussion

Over the last century, DENV-2 caused epidemics in 1986, 1987, 1988, and 1993 in Guangzhou[13,14], and has subsided since. Although no infection of DENV-2 was detected prior to 2005, the number of DENV-2 cases continued to increase annually between 2010 and 2018. At the same time, we found that the percentage of domestic cases increased from 80.95% to 95.31% between 2015 and 2018, reaching a peak in both number and percentage in 2018. Although the number and percentage of domestic cases declined slightly in 2019, they were the second highest compared to previous years. These findings indicate that DENV-2 is spreading in Guangzhou. Moreover, there was a sharp decrease in DENV-2 cases and in the number of total DENV cases in 2020, which might be related to the quarantine imposed due to the corona virus disease 2019 outbreak[15]. That is, limiting imported cases with the quarantine helped to restrain the local epidemic, highlighting the relevance of monitoring imported cases for local control.

This epidemiological investigation revealed that 83.72% of imported cases, most of which were returning travellers, originated from Southeast Asian countries. The results of a BLAST search in GenBank and the phylogenetic tree also showed that the DENV-2 strains sequenced in Guangzhou were closely related to the strains in Southeast Asian countries, which was similar to the characteristic of dengue epidemics involving the other three serotypes in Guangzhou[3,16,17]. The World Health Organization statistics revealed that infections in Southeast Asian countries account for half of the global dengue burden. From 2015 to 2019, dengue cases in Southeast Asia increased by 46% (from 451,442 to 658,301)[18]. China is contiguous with Southeast Asian countries, with active economic exchange. With the opening of private travel abroad, the number of travellers to Southeast Asian countries has increased steadily[19–21].

This situation is not unique to China, as the spread of dengue viruses by travellers has become a worldwide problem[22]. Therefore, better preparation is needed with strict regulations to prevent the spread of infection when travelling in endemic areas. For example, a convenient and rapid method for screening viremia that can be applied at customs may help to curb the importation and spread of DENV.

The phylogenetic tree showed that the dominant genotype in Guangzhou was the Malaysia/Indian subcontinent genotype, which comprised 142 of the 148 DENV-2 sequences (95.95%). Moreover, four (66.67%) of the six strains constituting the Southeast Asia genotype were detected among imported cases, indicating that the Malaysia/Indian subcontinent genotype was responsible for the domestic epidemic, and no genotype shift was observed in the 20 years. The most recent common ancestor of all Malaysia/Indian subcontinent strains was estimated to have appeared in 1955. However, when dividing the Malaysia/Indian subcontinent genotype into its different lineages, a shift was observed between 2013 and 2014. Lineage GZ5 has prevailed since 2014, and the number of DENV-2 cases began to rise after that point. Although there is no clear relationship between lineage and virulence in DENV, outbreaks, limited circulation, and spreading related to shifts in lineages have been reported[23–26]. Substitutions in the envelope gene that may result in maturation and activation of macrophages, with consequent enhancement of the immune response characterised by increased production of cytokines, are considered to be the likely cause of the prevailing differences among lineages. Positive selection analysis of the GZ5 lineage by MEME showed signs of directional selection. However, further research is needed to confirm whether the lineage shift is responsible for this rise in cases. Meanwhile within the same year, the strains could also be distributed in different lineages; for example, the sequences from isolates obtained in 2018 were distributed among lineages GZ1, GZ3, GZ4 and GZ5. This co-epidemic of different lineages showed different origins of DENV, which complicates the epidemic situation in Guangzhou.

The MCC tree revealed that the strains of Guangzhou also originated from different time periods. Strains that clustered into lineage GZ5 shared the eldest ancestor in 1995, whereas strains belonging to lineage GZ1 emerged in 2016. The domestic and foreign strains of lineage GZ2 detected in 2014 and 2016 shared the same ancestor in 2012. The strain then evolved during 2014 and 2016 in Guangzhou and spread not only in China but also in Thailand and Vietnam. The evolution and dissemination of DENV-2 were most obvious in this branch.

Secondary infection with DENV-2 after infection with heterotypic DENV is believed to be associated with an increased risk of DHF/DSS[2]. However, with the epidemic of DENV-1 in Guangzhou persisting for more than 20 years and the rising number of DENV-2 cases[3,4], the incidence of DHF remained relatively low compared with the global incidence or the incidence of Southeast Asian countries[27–30]. Some studies have revealed that secondary infection of the American genotype of DENV-2 failed to cause DHF[31], and other extensive studies indicated that the Southeast Asian genotype was more efficient at infection and was also more likely to cause DHF[5,6,31,32]. Of the 148 sequences detected in Guangzhou, only six sequences (4.05%) belonged to the Southeast Asia genotype, four of which were identified from imported cases. This revealed that the epidemic of the Southeast Asia genotype was rare, which may explain the low incidence of DHF in Guangzhou. Our results also suggest that the Malaysia/Indian subcontinent genotype may be less virulent than the Southeast Asian genotype. However, further studies are needed to determine whether the incidence of DHF/DSS is low in other areas with an epidemic dominated by the Malaysia/Indian subcontinent genotype. Determining the critical differences between genotypes and host immune mechanisms that may enhance the pathogenesis of genotypes may provide new insight to better understand the mechanism of DHF/DSS.

Declarations

Ethics approval and consent to participate: The Ethics Committee of the Guangzhou Centre for Disease Control and Prevention approved the present study. Written informed consent was obtained from all the participants in the study or from the parent/guardian when the participant was a minor (younger than 18 years old). All procedures involved in this work complied with the ethical standards of the relevant national and institutional committees on human experimentation and with the Declaration of Helsinki (2008 amendment).

Consent for publication: Not applicable.

Availability of data and materials: The sequences analysed during the current study are available in the GenBank repository, <https://www.ncbi.nlm.nih.gov/genbank/>. The data that support the findings of this study are available from the corresponding author.

Competing interests: The authors declare no conflict of interest.

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Authors' contributions: LJ conducted most of the experiments and drafted the manuscript. YL designed the experiments and analysed data. QJ collected the epidemiological information. WS and YC cultured the virus and sequenced the genes. XW and ZY participated in the detection of dengue virus in the samples. All authors read and approved the final manuscript.

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References

1. Bhatt S, et al. (2013) The global distribution and burden of dengue. *Nature*; 496:504–507.doi:10.1038/nature12060.
2. Sangkawibha N, et al. (1984) Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. *Am.J. Epidemiol*; 120:653–669.doi: 10.1093/oxfordjournals.aje.a113932.

3. Jiang L, et al. (2013) Molecular Epidemiological and virological study of dengue virus infections in Guangzhou, China, during 2001–2010. *Virology*;10: 4. doi: 10.1186/1743-422X-10-4.
4. Bai Z, et al. (2018) Evolutionary and phylodynamic analyses of dengue virus serotype I in Guangdong Province, China, between 1985 and 2015. *Virus Res*;256:201–208. doi: 10.1016/j.virusres.2018.07.005.
5. Cologna R, Rico-Hesse R. (2003) American genotype structures decrease dengue virus output from human monocytes and dendritic cells. *J. Virol*;77:3929–3938. doi: 10.1128/JVI.77.7.3929-3938.2003.
6. Anderson JR, Rico-Hesse R. (2006) Aedes aegypti vectorial capacity is determined by the infecting genotype of dengue virus. *Am. J. Trop. Med. Hyg*;75:886–892.
7. China National Health Commission of the People's Republic Of. Diagnosis for Dengue Fever. (2018) Available online: <http://www.nhc.gov.cn/wjw/s9491/201803/d524df26df28453eada8371dc3565818.shtml>.
8. Leitmeyer KC, et al. (1999) Dengue Virus Structural Differences That Correlate with Pathogenesis. *J. Virol*; 73:4738–4747. doi: 10.1128/JVI.73.6.4738-4747.
9. Guindon S, et al. (2010) New Algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst. Biol*;59:307–321. doi: 10.1093/sysbio/syq010.
10. Lefort V, Longueville JE, Gascuel O. (2017) SMS: Smart model selection in PhyML. *Mol. Biol. Evol*; 34: 2422–2424. doi: 10.1093/molbev/msx149.
11. Rico-Hesse RH. (2003) Microevolution and virulence of dengue viruses. *Adv. Virus Res*;59:315–341. doi:10.1016/S0065-3527(03)59009-1.
12. Murrell B, et al. (2012) Detecting individual sites subject to episodic diversifying selection. *PLOS Genet*;8: e1002764. doi: 10.1371/journal.pgen.1002764.
13. Pan ZM, et al. (2000) Epidemiology of dengue in Guangzhou, during 1990s. *South China J. Prev. Med*; 26:33–35.
14. Luo L, et al. (2008) The Analysis of the epidemiologic features of dengue fever from 1978 to 2006 in Guangzhou, China. *Chin. J. Infect. Dis*;26:490-3, 08–015. doi:CNKI:SUN:ZHCR.0.
15. Jiang L et al. (2021) Decreased dengue cases attributable to the effect of COVID-19 in Guangzhou in 2020. *PLOS Negl. Trop. Dis*;15: e0009441. doi:10.1371/journal.pntd.0009441.
16. Liu Y, et al. (2017) Phylogenetic Analysis of Envelope Gene of Dengue Virus Serotype 2 in Guangzhou, 2001-2015. *Chin. J. Epidemiol*;38:90-95. doi:CNKI:SUN:ZHLX.0.
17. Liu Y, et al. (2018) Molecular characterization and phylogenetic analysis of the envelope gene of dengue virus type 3 in Guangzhou, China 2001–2016. *Chin. J. Zoonoses*;3:487–490. doi:10.3969/ji.ssn.1002-2694.2018.00.072.
18. WHO. Dengue and Severe Dengue. (2021) Available online: <https://www.who.int/southeastasia/health-topics/dengue-and-severe-dengue>.
19. Bao FH. (2018) Disparity of size and rank for the outbound tourism of mainland China. *Area Res Dev*;37:92–97. doi: 10.3969/j.issn.1003-2363.2018.01.018.
20. Bao FH, Chen Y. (2017) Research on the spatial-temporal distribution characteristics of China's outbound tourism in the past 10 years. *World Reg. Stud*;26: 127–139. doi: 10.3969/j.issn.1004-9479.2017.02.014.
21. Ding J, Li LF. (2004) A Study on tourist behavior of outbound travel for Guangzhou residents. *Geogr. Res*;2: 705–714.
22. Wilder-Smith A, et al. (2019) Dengue. *Lancet*;393: 350–363. doi: 10.1016/S0140-6736(18)32560-1.
23. Oliveira MF, et al. (2010) Two lineages of dengue virus type 2, Brazil. *Emerg. Infect. Dis*;16: 576–578. doi: 10.3201/eid1603.090996.
24. Faria NR, et al. (2013) Twenty years of DENV-2 activity in Brazil: molecular characterization and phylogeny of strains isolated from 1990 to 2010. *PLOS Negl. Trop. Dis*;7: e2095. doi: 10.1371/journal.pntd.0002095.
25. McElroy KL, et al. (2011) Endurance, refuge, and reemergence of dengue virus type 2, Puerto Rico, 1986–2007. *Emerg. Infect. Dis*;17:64–71. doi: 10.3201/eid1701.100961.
26. Mir D, et al. (2014) Spatiotemporal dynamics of DENV-2 Asian-American genotype lineages in the Americas. *PLOS One*; 9:e98519. doi: 10.1371/journal.pone.0098519.
27. Lu JY, et al. (2018) Epidemic trend of dengue fever in Guangzhou City from 2008 to 2017. *J. Trop. Med*;18:973–976.
28. Karyanti MR, et al. (2014) The Changing incidence of dengue haemorrhagic fever in Indonesia: A 45-year registry-based analysis. *B.M.C. Infect. Dis*;14:412. doi: 10.1186/1471-2334-14-412.
29. Guo C, et al. (2017) Global epidemiology of dengue outbreaks in 1990–2015: A systematic review and meta-analysis. *Front. Cell. Infect. Microbiol*;7: 317. doi: 10.3389/fcimb.2017.00317.
30. Cummings DA, et al. (2009) The Impact of the Demographic Transition on Dengue in Thailand: Insights from a Statistical Analysis and Mathematical Modeling. *PLOS Med*;6: e1000139. doi: 10.1371/journal.pmed.1000139.
31. Watts DM, et al. (1999) Failure of secondary infection with American Genotype Dengue 2 to cause dengue haemorrhagic fever. *Lancet*;354: 1431–1434. doi: 10.1016/S0140-6736(99)04015-5.
32. Rico-Hesse R, et al. (1997) Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. *Virology*;230:244–251. doi: 10.1006/viro.1997.8504.

Tables

Table 1 Annual numbers of domestic and imported DENV-2 cases detected by RT-qPCR in suspected patients' serum samples from 2001 to 2020.

| year | DENV2 | Domestic cases (percentage) | Imported cases (percentage) | Bangladesh | Angola | Cambodia | Fiji | India | Indonesia | Malaysia | Maldives | Myanmar |
|-------|-------|-----------------------------|-----------------------------|------------|--------|----------|------|-------|-----------|----------|----------|---------|
| 2001 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2002 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2003 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2004 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2005 | 1 | 0 | 1 (100%) | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 2006 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2007 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2008 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2009 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2010 | 2 | 2 (100%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2011 | 2 | 0 | 2 (100%) | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2012 | 2 | 0 | 2 (100%) | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| 2013 | 2 | 2 (100%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2014 | 10 | 9 (90%) | 1 (10%) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015 | 21 | 17 (80.95%) | 4 (19.05%) | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| 2016 | 37 | 30 (81.08%) | 7 (18.92%) | 1 | 3 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| 2017 | 48 | 41 (85.42%) | 7 (14.58%) | 1 | 1 | 2 | 0 | 0 | 0 | 1 | 0 | 2 |
| 2018 | 192 | 183 (95.31%) | 9 (4.69%) | 0 | 2 | 0 | 0 | 0 | 0 | 4 | 0 | 0 |
| 2019 | 96 | 86 (89.58%) | 10 (10.42%) | 0 | 3 | 0 | 0 | 0 | 2 | 4 | 0 | 0 |
| 2020 | 3 | 2 (66.67%) | 1 (33.33%) | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| total | 416 | 372(89.42%) | 44(10.58%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Figures



Figure 1

Maximum-likelihood midpoint rooted phylogenetic tree of DENV-2 envelope sequences derived from 148 sequences detected in Guangzhou and 56 reference sequences retrieved from GenBank. Sequences of Guangzhou are identified according to the accession number, isolated year, and lab number. Reference sequences from GenBank (marked with a triangle) are identified using the accession number, country, and year. Bootstrap support, with >75%, is shown next to the branches. Different colours present different genotypes.

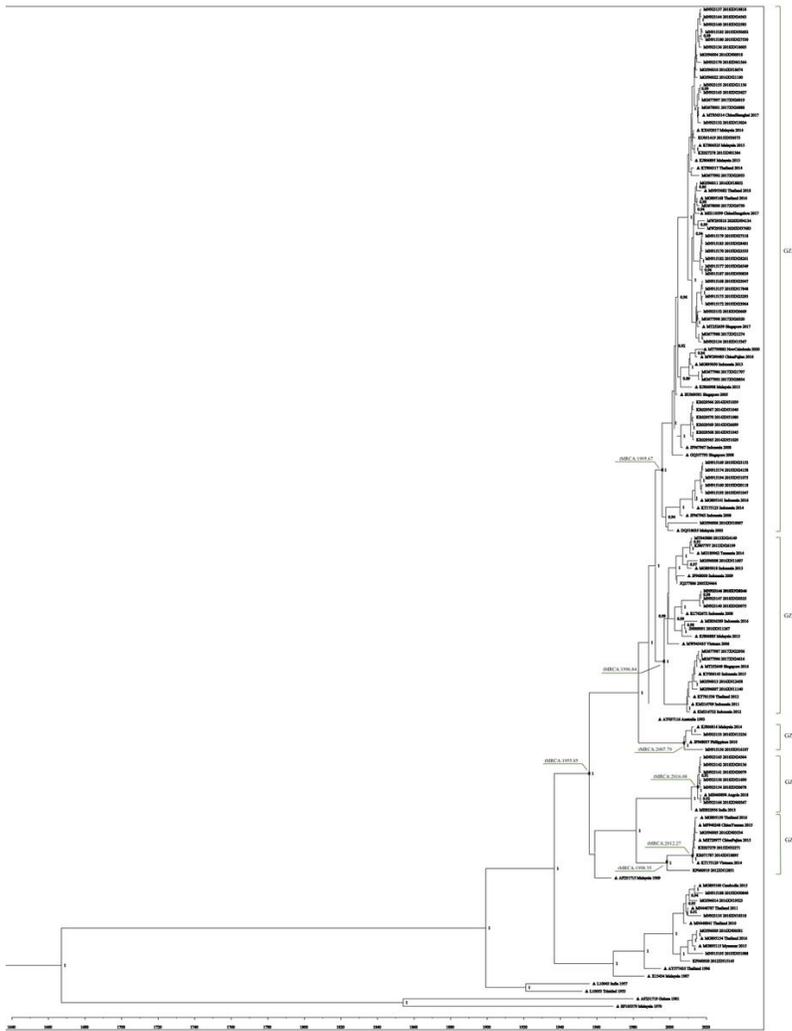


Figure 2
 Maximum clade credibility (MCC) tree of 80 envelope (E) gene nucleotide sequences collected in Guangzhou, along with 53 sequences retrieved from GenBank marked with a triangle. Posterior probabilities higher than 0.80 are shown at each node. tMRCA = time to the most recent common ancestor.