



HAL
open science

The Genetic Evolution of DENV2 in the French Territories of the Americas: A Retrospective Study from the 2000s to the 2024 Epidemic, Including a Comparison of Amino Acid Changes with Vaccine Strains

Alisé Lagrave, Antoine Enfissi, Sourakhata Tirera, Magalie Pierre Demar, Jean Jaonaso, Jean-François Carod, Tsiriniaina Ramavoson, Tiphanie Succo, Luisiane Carvalho, Sophie Devos, et al.

► **To cite this version:**

Alisé Lagrave, Antoine Enfissi, Sourakhata Tirera, Magalie Pierre Demar, Jean Jaonaso, et al.. The Genetic Evolution of DENV2 in the French Territories of the Americas: A Retrospective Study from the 2000s to the 2024 Epidemic, Including a Comparison of Amino Acid Changes with Vaccine Strains. *Vaccines*, 2025, 13 (3), pp.264. <10.3390/vaccines13030264>. <hal-05048358>

HAL Id: hal-05048358

<https://hal.science/hal-05048358v1>

Submitted on 28 Apr 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.





L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY 4.0 - Attribution - International License

Article

The Genetic Evolution of DENV2 in the French Territories of the Americas: A Retrospective Study from the 2000s to the 2024 Epidemic, Including a Comparison of Amino Acid Changes with Vaccine Strains

Alisé Lagrave¹, Antoine Enfissi¹, Sourakhata Tirera¹, Magalie Pierre Demar², Jean Jaonaso², Jean-François Carod³, Tsiriniaina Ramavoson³, Tiphane Succo⁴, Luisiane Carvalho⁴, Sophie Devos⁴, Frédérique Dorleans⁵, Lucie Leon⁵, Alain Berlioz-Arthaud⁶, Didier Musso⁶, Raphaëlle Klitting^{7,8}, Xavier de Lamballerie^{7,8}, Anne Lavergne¹ and Dominique Rousset^{1,*}

¹ Arbovirus National Reference Center, Virology Unit, Institut Pasteur de la Guyane, Cayenne, French Guiana; alagrave@pasteur-cayenne.fr (A.L.); aenfissi@pasteur-cayenne.fr (A.E.); stirera@pasteur-cayenne.fr (S.T.); anne.lavergne@pasteur.fr (A.L.)

² Laboratoire Centre Hospitalier de Cayenne, Cayenne, French Guiana; magalie.demar@ch-cayenne.fr (M.P.D.); jean.jaonaso@ch-cayenne.fr (J.J.)

³ Department of Biology, West French Guiana Hospital Center, Saint-Laurent-du-Maroni, French Guiana; jf.carod@ch-ouestguyane.fr (J.-F.C.); t.ramavoson@ch-ouestguyane.fr (T.R.)

⁴ Santé Publique France, Cellule Guyane, Cayenne, French Guiana; tiphane.succo@santepubliquefrance.fr (T.S.); luisiane.carvalho@santepubliquefrance.fr (L.C.); sophie.devos@santepubliquefrance.fr (S.D.)

⁵ Santé Publique France, Cellule Antilles, French Caribbean Islands; frederique.dorleans@santepubliquefrance.fr (F.D.); lucie.leon@santepubliquefrance.fr (L.L.)

⁶ Laboratoires Eurofins Guyane, French Guiana; alain.berlioz@biologie.eurofinseu.com (A.B.-A.); didier.musso@biologie.eurofinseu.com (D.M.)

⁷ National Reference Center for Arboviruses, Inserm-IRBA, Marseille, France; raphaelle.klitting@inserm.fr (R.K.); xavier.de-lamballerie@univ-amu.fr (X.d.L.)

⁸ Unité des Virus Émergents (UVE: Aix-Marseille Univ, Università di Corsica, IRD 190, Inserm 1207, IRBA), Marseille, France

* Correspondence: dominique.rousset@pasteur.fr



Academic Editors: Ralph A. Tripp, Salman L. Butt and Henry Kariithi

Received: 13 December 2024

Revised: 21 February 2025

Accepted: 24 February 2025

Published: 1 March 2025

Citation: Lagrave, A.; Enfissi, A.; Tirera, S.; Pierre Demar, M.; Jaonaso, J.; Carod, J.-F.; Ramavoson, T.; Succo, T.; Carvalho, L.; Devos, S.; et al. The Genetic Evolution of DENV2 in the French Territories of the Americas: A Retrospective Study from the 2000s to the 2024 Epidemic, Including a Comparison of Amino Acid Changes with Vaccine Strains. *Vaccines* **2025**, *13*, 264. <https://doi.org/10.3390/vaccines13030264>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Background: Dengue virus type 2 (DENV2) is endemic to hyperendemic in the French territories of the Americas (FTAs), including French Guiana, Guadeloupe, Martinique, Saint-Barthelemy, and Saint-Martin. In 2023–2024, French Guiana, Martinique, and Guadeloupe experienced unprecedented dengue epidemics partly associated with this serotype. In response, we conducted a retrospective study of the diversity of DENV2 strains circulating in the FTAs from 2000 to 2024. Methods: To this end, we selected DENV2 samples from the collection at the National Research Center for Arboviruses in French Guiana (NRCA-FG) and sequenced them using Oxford Nanopore Technologies (ONT)-based next-generation sequencing (NGS). Results: Phylogenetic analysis revealed that (i) the 77 DENV2 sequences from the FTAs belong to two distinct genotypes—Asian American and Cosmopolitan; (ii) from the 2000s up to the 2019 epidemic in French Guiana, all sequenced strains belonged to the Asian American genotype; (iii) and from 2019 to 2020, strains circulating in Martinique and Guadeloupe belonged to the Cosmopolitan genotype, specifically the Indian subcontinent sublineage, while (iv) strains from the 2023–2024 outbreak in Martinique, Guadeloupe, and French Guiana fall within a distinct sublineage of the same genotype—Other Cosmopolitan. Additionally, we analyzed amino acid (AA) changes in FTA sequences compared to the Dengvaxia[®] and Qdenga[®] vaccines. The analysis of amino acid changes in FTA sequences compared to the vaccines (Dengvaxia[®] and Qdenga[®]) identified 42 amino acid changes in the prM/E regions (15 in the prM region and 27 in the E region) relative to CYD-2 Dengvaxia[®] and 46 amino acid changes in the

prM/E regions relative to Qdenga[®], including 16 in the prM region and 30 in the E region. Some of these AA changes are shared across multiple genotypes and sublineages, with 8 substitutions in the prM region and 18 in the E region appearing in both analyses. This raises questions about the potential impact of these changes on vaccine efficacy. Conclusion: Overall, these findings provide a current overview of the genomic evolution of DENV2 in the FTA, which is crucial for developing more effective prevention and control strategies and for selecting future vaccines tailored to circulating strains.

Keywords: DENV2; French territories of the Americas; epidemic; genomic diversity; phylogenetic analysis; amino acid variations; vaccines; prevention strategies

1. Introduction

Dengue virus (DENV), a member of the *Flaviviridae* family, is the most common arthropod-borne acute febrile illness. The DENV genome is a 10,700-base single-stranded positive RNA encoding structural (capsid (C), pre-membrane (prM), and envelope (E)) and non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [1]. The outer surface of the viral structure is made up of structural proteins that facilitate viral entry into human cells, while non-structural proteins are associated with viral replication [2]. Four distinct serotypes (DENV1, DENV2, DENV3, DENV4) are defined by their genetic characteristics (i.e., 25% genetic differences between two serotypes) [3]. Within these serotypes, different genotypes co-exist, separated by a 6% genetic difference [4]. Amino acid sequence heterogeneity between serotypes is around 40%, and the divergence generated within serotypes is around less than 9% [2].

Dengue is endemic in tropical and subtropical regions. In November 2024, more than 14 million cases of dengue fever have been reported globally, including more than 10,000 deaths [5]. While a substantial increase in dengue cases has been reported worldwide over the past five years, this increase has been particularly pronounced in the region of the Americas, where the number of cases exceeded 12 million in November 2024, surpassing the annual peak of 4.6 million cases in 2023 [6]. In the FTAs, dengue circulation is endemo-epidemic to hyper-endemic (regular circulation with epidemic peaks and co-circulation of different serotypes). The various epidemics between 2005 and 2021 have been described in Bonifay et al., 2023 [7]. DENV2 caused epidemic outbreaks in French Guiana in 2006, co-circulated with DENV4 in 2012–2013, with DENV1 in 2020–2021, and with DENV3 in 2023–2024 [7,8]. In Martinique, DENV2 was the predominant serotype during the 2013–2014 epidemic and during the 2023–2024 outbreak [9,10]. In Guadeloupe, DENV2 circulated predominantly during the 2019–2021 outbreak and in the same way during the 2023–2024 outbreak [9,10]. The different genotypes of the DENV2 include one sylvatic genotype and five non-sylvatic genotypes (Asian I, Asian II, American, Asian-American, and Cosmopolitan) [11]. The sylvatic and American genotypes no longer circulate, the latter having been over-circulated in Central and South America in the 1950s and 1960s [12]. Asian I and II genotypes are specific to the Asian continent [13]. The Asian-American genotype has been spreading in Southeast Asia and in Central and South America since the 1960s, but has not been detected in Asia since the 2000s [12]. The last one, the Cosmopolitan genotype, is geographically widespread and contributes the most to the current global burden of DENV2 [13–15].

There are only a limited number ($n = 25$) of DENV2 sequences from French Guiana in public databases, and no study has examined the DENV2 strains circulating in the FTAs from 2000 to 2024. Herein, we conducted a retrospective analysis of the diversity of DENV2 strains in the region over this period. We also considered the potential impact of this

diversity by assessing amino acid (AA) changes in the sequences from French Guiana in comparison to the Dengvaxia[®] and Qdenga[®] vaccines.

2. Materials and Methods

2.1. Ethical Statement

This study was conducted as part of the public health surveillance program of the National Reference Center for Arboviruses in French Guiana (NRCA-FG), in collaboration with the French Public Health Agency (Santé Publique France, SPF). Accordingly, as an epidemiological record, consultation with the ethics committee was not required. Samples involved in this study were chosen among remaining human serum samples received as part of standard diagnostic and expertise activities of the NRCA-FG and kept by the NRCA-FG biobank according to the French legislation (article L.1211–2 and related of the French Public Health Code—FPHC). Human serum samples were anonymized, with no or minimal risk to patients within the terms of the European General Data Protection Regulation and the French National Commission on Informatics and Liberty (CNIL).

2.2. Clinical Samples and Study Design

The samples used in this study came from the NRCA-FG biobank, which comprises clinical specimens received from hospital and private laboratories in French Guiana and French Caribbean Islands as part of the arbovirus surveillance system. No information was available on the evolution of the cases (severity). Between 2001 and January 2024, 3616 samples tested DENV-2-positive, among which 624 had a cycle threshold (Ct) value below 26. Among these samples, a selection was made in each municipality and for each detection period, leading to 77 samples collected between 2001 and 2024 being selected for the analysis (Table 1), including 12 from Guadeloupe, 5 from Martinique, 3 from Saint-Barthelemy, 3 from Saint-Martin, 51 from French Guiana, and 3 from French Guiana imported from French Caribbean Islands.

Table 1. Number of generated sequences by territory/municipalities and years of sampling.

Territory	n	Municipality	Year
Guadeloupe	12		
	1	Baie-Mahault	2022
	1	Basse-Terre	2007
	1	Les Abymes	2012
	2	Pointe-à-Pitre	2022
	1	Sainte-Anne	2020
Martinique	6	Uninformed	2023
	5		
	4	Fort-de-France	2023
Saint-Barthelemy	1	Lamentin	2022
	3	Uninformed	2023
Saint-Martin	3	Uninformed	2023
French Guiana	51		
	9	Cayenne	2007/2013/2019/2023
	14	Kourou	2013/2019/2023/2024
	12	Remire-Montjoly	2013/2023/2024
	5	Matoury	2023/2024
	3	Tonate-Macouria	2019/2023
	2	Sinnamary	2023/2024
	2	St-Laurent-du-Maroni	2013/2023
	2	Montsinery-Tonnegrande	2023/2024
	1	Saint-Georges	2023
1	Roura	2024	

Table 1. *Cont.*

Territory	n	Municipality	Year
French Guiana	3		
imported from French	1	Guadeloupe	2023
Caribbean Islands	1	Martinique	2023
	1	Uninformed	2016
Total	77		

2.3. MinION Library Preparation and Multiplexed Nanopore Sequencing

Whole-genome sequencing was performed on an Oxford Nanopore MinION device using R9.4 flow cells (Oxford Nanopore Technologies, Oxford, UK) based on a protocol from Quick et al., 2017 [16]. RNAs were first extracted using the Macherey Nagel Virus 96 kit (Macherey Nagel, Düren, Germany; ref: 740452.4) according to the manufacturer's instructions. Then, 8 µL of RNA was reverse transcribed to complementary DNA using LunaScript RT SuperMix (NEB, Ipswich, MA, USA), and a serotype-specific multiplex PCR was performed with Q5 High-Fidelity DNA polymerase (NEB, Ipswich, MA, USA) in three separate pools, using a DENV2 primer scheme described in Appendix A Table A1. Primer schemes were designed using the modified CDC protocol [17–19] and empirically refined. Cycling conditions for the RT were 25 °C 2 min 1 cycle, followed by 55 °C 10 min 1 cycle and 95 °C 1 min 1 cycle. For the multiplex PCR, the cycling conditions were 30 s at 98 °C, followed by 35 cycles at 98 °C for 15 s, 63 °C (Pool 1 and 2) or 60 °C (Pool 3) for 5 min, and conservation at 4 °C. The resulting 400 bp PCR products were pooled, cleaned using AmpureXP magnetic beads (Beckman Coulter, High Wycombe, UK), and quantified using a Qubit dsDNA High Sensitivity assay on a Qubit 3.0 instrument (Thermo Fisher Scientific, Waltham, MA, USA). The samples were then barcoded using the Ultra II End Repair/dA-Tailing Module (New England Biolabs, Ipswich, MA, USA) and the native barcoding kits NBD104 and NBD196 (Oxford Nanopore Technologies), cleaned with magnetic beads, and pooled at equimolar ratios before ligation of the AMII adapters with blunt/TA ligase master mix (New England Biolabs, Ipswich, MA, USA). Sequencing libraries were loaded onto the R9.4 flow cell using the ligation sequencing kit SQK-LSK109 (Oxford Nanopore Technologies, Oxford, UK), and sequencing data were collected for 12 h. Sequence reads were base-called and demultiplexed using the Guppy algorithm v3.6 (Oxford Nanopore Technologies, Oxford, UK). The consensus genome sequences were produced using the Artic network's bioinformatics pipeline (<https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html>, accessed on the 17 July 2024), along the primer schemes used to produce amplicons, which read alignment to genomes of reference with minimap2, incorporating primer removal and the nanopolish algorithm (<https://github.com/jts/nanopolish>, accessed on the 17 July 2024) to improve the consensus sequence by signal level variant calling. Regions with insufficient coverage (minimum depth coverage set to 20) were masked with N characters.

2.4. Phylogenetic Analysis

The 77 whole-genome consensus sequences of DENV2 obtained in this study (Appendix A Table A2) have been aligned with CLC Main Workbench software (version 22; Qiagen, Hilden, Germany) to whole-genome DENV2 references sequences downloaded from GenBank (28 February 2024). A total of one hundred and twenty-two reference sequences were selected to ensure a balanced geographical and temporal distribution, encompassing all the genotypes described so far. For this, using GISAID, representatives of each genotype and sub-genotype were chosen to reflect the different isolation dates and localities. In a second step, the NRCA-FG generated sequences were subjected

to BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=GeoBlast&PAGE_TYPE=BlastSearch, accessed on the 20 July 2024), and the closest reference sequences for each were added to the dataset, resulting in a final dataset of 122 reference sequences (Appendix A Table A3). BEAST and BEAUTI (version 1.10.1) software were used to construct the maximum clade credibility (MCC) tree, using GTR + G + I (General Time Reversible with Gamma distribution and Invariant sites) substitution model, corresponding to the best-fit tested on CLC Main Workbench software (version 8.0), according to the corrected Akaike Information Criterion (AICc), with a strict clock model and Bayesian skyline prior [20–22]. The Markov Chain Monte Carlo (MCMC) analysis was run for 50 million generations with sampling every 1000 generations. The final MCMC sampling chains were checked using Tracer v1.7.1 with 10% burn-ins removed. Convergence was controlled by checking that an effective sample size (ESS) > 200 was obtained for all parameters using Tracer v1.7.1 [23]. The maximum clade credibility tree was generated by Tree Annotator 1.10.4, and the time-scaled phylogeny was visualized using FigTree v1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>, accessed on the 20 September 2024).

2.5. Amino Acid Changes Against Vaccinal Strains *Dengvaxia* and *Qdenga*

Amino acid changes were compared between the 77 FTA sequences generated in this study plus 17 reference sequences from Martinique and Guadeloupe (OR229958 to OR229960; OR229962 to OR229973; OR229975 to OR229976) and the *Dengvaxia* CYD-2 vaccine strain (Accession number: KX239895) and *Qdenga* vaccine strain (Accession number: U87411). A Python script was written to generate a tab-separated file with the position where amino acid changes occurred and the corresponding alternative amino acid, for each reference (*Dengvaxia* CYD-2 vaccine strain and *Qdenga* vaccine strain). The vaccine composition of *Dengvaxia* CYD-2 vaccine strain (KX239895) includes the prM (membrane glycoprotein precursor) and E (envelope) regions, while *Qdenga* vaccine strain (U87411) includes all genome regions (C, prM, E, NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). We focused only on the common regions (prM and E), as the majority of neutralizing antibodies target the viral E proteins [24,25].

3. Results

The global phylogenetic analysis was carried out on 199 sequences (Figure 1). FTA DENV2 sequences obtained from French Guiana (FG), Martinique (MTQ), Guadeloupe (GLP), Saint-Martin (SM), and Saint-Barthelemy (SB), having circulated between 2002 and 2024, belong to different genotypes—Asian American and Cosmopolitan (Figure 1).

3.1. DENV2 Strains Belonging to the Asian American Genotype

The Asian American genotype includes FTA strains from 2007 through the 2019 FG outbreak, as shown in Figure 2. Strains from French Guiana circulating in 2002 (EU920835) and 2006–2007 (EU920848 and R0113041) are intermingled with other American strains (Guyana, Suriname, Dominican Republic, Cuba, or Nicaragua). Strains from Martinique 2005 (EU9208037, EU9208043) and Guadeloupe 2006–2007 (EU9208049 and R0124144) appear in a different lineage, with the most recent common ancestor (MRCA) dating back to 2004 (95% HPD 2004–2005). Similarly, the Guadeloupe 2012 (W1221159) and French Guiana 2013 (PP719122–PP719125) strains appear in a different sub-lineage with a common ancestor dating from 2009 (95% HPD 2008–2011). The sequences from FG 2019 (PP719127 to PP719138) are also found in a distinct sublineage with a common ancestor dating from 2018 (95% HPD 2018–2019). These sequences are found in a monophyletic clade sharing nucleotide identity from 99.88 to 100% between each other and from 99.41 to 99.49% nucleotide identity with a sequence from Brazil PP234938 Brazil circulating in 2018.

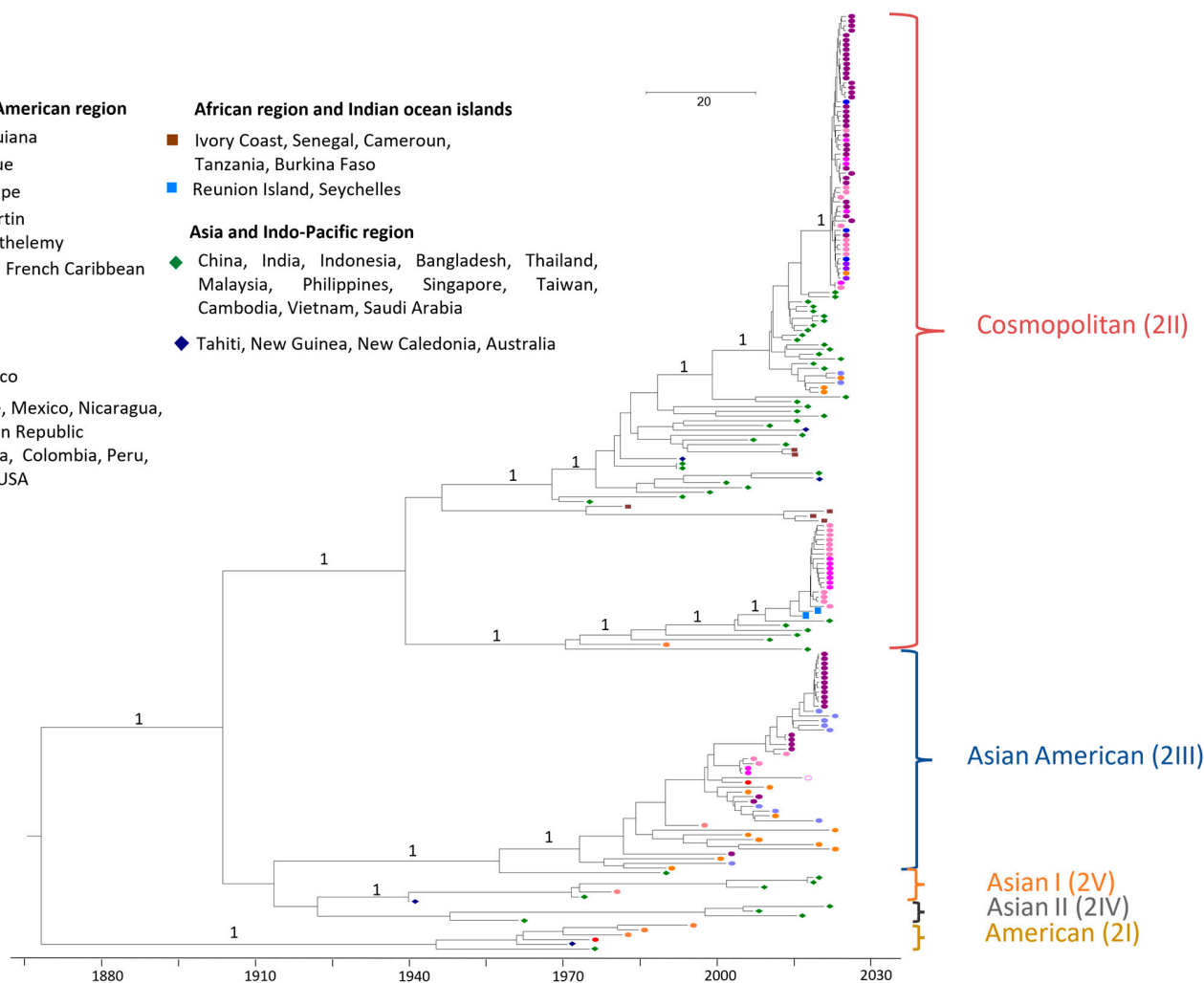


Figure 1. Bayesian phylogeny of DENV2 whole-genome sequences. The maximum clade credibility (MCC) tree, using GTR + G + I (General Time Reversible with Gamma distribution and Invariant sites) substitution model, with strict lognormal clock model and Bayesian skyline prior. The analysis included 199 sequences with 77 FTA sequences generated in this study and 122 reference sequences downloaded from GenBank. Sylvatic strains (OK605757 and EF105384) were used as the outgroup. The new classification proposed by Hill et al. has been added in brackets [26].

3.2. DENV2 Strains Belonging to the Cosmopolitan Genotype

Interestingly, at the same time (2019–2020), strains circulating in Martinique and Guadeloupe (2019–2020 epidemic) belong to another genotype (Cosmopolitan genotype, Indian sub-continent lineage), as shown in Figure 3. The sequences of this monophyletic clade are distributed according to their origin (MTQ or GLP) and share between 99.60 and 99.96% nucleotide identity with each other and 99.55 and 99.72% nucleotide identity with MN272404 Reunion Island 2018. The MRCA between MN272404 Reunion Island 2018 and this clade dates to 2015 (95% HPD 2014–2017), Figure 3. The Reunion Island strain was imported from the Seychelles [27]. Asian strains, notably Indian strains, are at the origin of this clade.

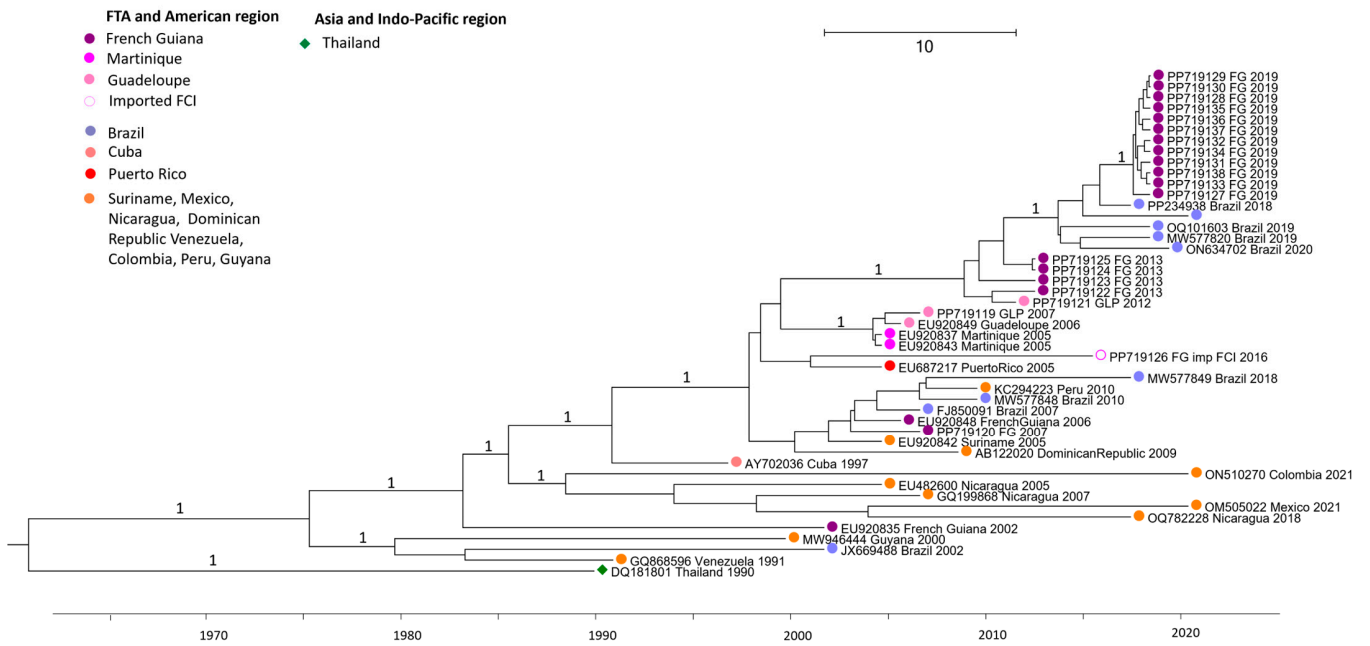


Figure 2. Bayesian phylogeny of DENV2 whole-genome sequences. Detailed on Asian American genotype. The maximum clade credibility (MCC) tree, using the GTR + G + I (General Time Reversible with Gamma distribution and Invariant sites) substitution model, was constructed with a strict clock model and Bayesian skyline prior.

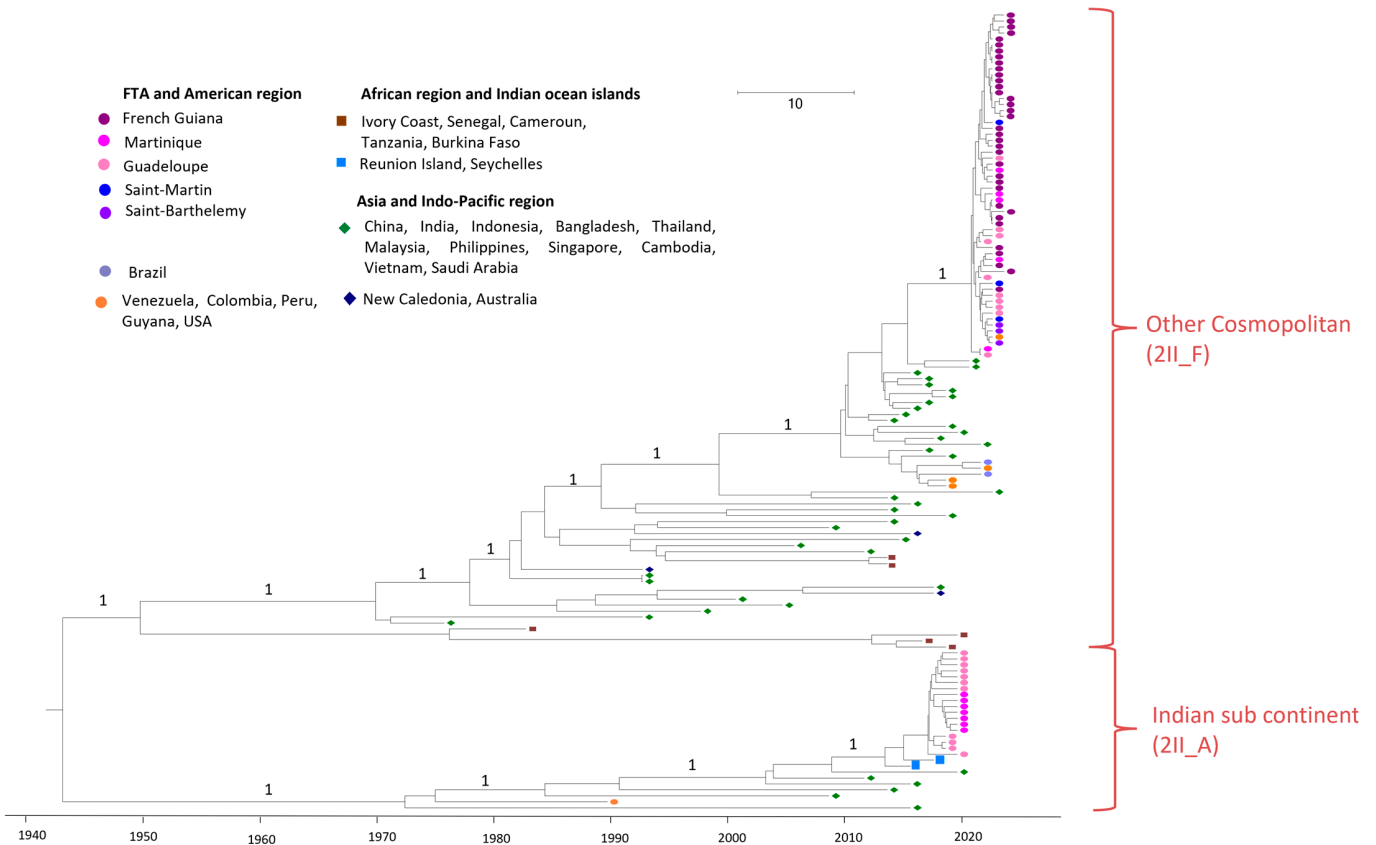


Figure 3. Bayesian phylogeny of DENV2 whole-genome sequences. Detailed on Cosmopolitan genotype. The maximum clade credibility (MCC) tree, using the GTR + G + I (General Time Reversible with Gamma distribution and Invariant sites) substitution model, was constructed with a strict clock model and Bayesian skyline prior. The new classification proposed by Hill et al. has been added in brackets [26].

Finally, the sequences of the 2023–2024 DENV2 epidemics in the FTAs, mainly in French Guiana, Guadeloupe, and Martinique, but also of a few sporadic cases in Saint-Martin and Saint-Barthelemy, belong to a well-supported monophyletic clade (posterior probability 100%) and are included in the Cosmopolitan genotype, the Other Cosmopolitan sublineage. These sequences share 99.78–100% nucleotide identity. In addition, sequences from this clade shared 99.11–99.30% nucleotide identity with Indian sequences from 2021 (OM639992 and OM639982). The MRCA between those sequences' dates back to 2016 (95% HPD 2014–2017), as shown in Figure 3.

3.3. Amino Acid Changes Against the Vaccines (*Dengvaxia* and *Qdenga*)

Comparison of amino acid variations between our dataset containing the 77 FTA sequences generated in this study added to the 17 sequences from Martinique and Guadeloupe 2019–2020 and the *Dengvaxia* CYD-2 vaccine strain (KX239895) showed 42 positions with AA substitutions, including 15 in the prM (membrane glycoprotein precursor) region and 27 in the E (envelope) region (Supplementary Data Table S1). The ratio between the number of substitutions and the AA size of each region, respectively, 166 AA for prM and 495 AA for E, shows a higher ratio for prM (9.04%) compared to E (5.45%). A closer look at the AA substitution specific to one or more genotypes and subgenotypes (Asian American, Cosmopolitan Indian sub-continent, and Cosmopolitan Other Cosmopolitan) compared with the *Dengvaxia* CYD-2 vaccine strain (KX239895) reveals 10 positions with AA variations in the prM region (ratio 6.02%) and 19 in the E region (ratio 3.83%), as shown in Figure 4 and Supplementary Data Table S1. Some are shared by all sequences of the Asian American genotype (n = 17), of the Cosmopolitan Indian subcontinent subgenotype (n = 14), or of the Other Cosmopolitan subgenotype (n = 17), as shown in Figure 4. Some are even shared between all genotypes (n = 5), as shown in Figure 4. It is therefore possible to define genotype-specific substitution profiles.

Analysis of amino acid changes between our dataset compared to the *Qdenga* vaccine strain (U87411) showed 46 AA positions with AA substitutions along the prM and E regions with, respectively, 16 and 30 AA changes, as shown in Supplementary Data Table S2. Looking more specifically at the AA substitution specific to one or more genotypes and subgenotypes (Asian American, Cosmopolitan Indian sub-continent, and Cosmopolitan Other Cosmopolitan) compared to the *Qdenga* vaccine strain, we observed 32 amino acid changes with 11 in the prM (ratio of 6.62%) and 21 in the E (ratio of 4.24%), as shown in Figure 5 and Supplementary Data Table S2. Some AA substitutions are even shared between all genotypes as for the Asian American genotype (n = 17) and for the Cosmopolitan genotype (n = 21), as shown in Figure 5. Within this Cosmopolitan genotype, the Cosmopolitan Indian subcontinent shows 20 shared AA changes, while the Other Cosmopolitan subgenotype shows 21, as shown in Figure 5. Some are shared by all genotypes (n = 8), as shown in Figure 5. It is therefore possible to define genotype-specific substitution profiles.

Moreover, some amino acid changes specific to one or more genotypes and subgenotypes are common to both analyses (*Dengvaxia* and *Qdenga* vaccine strains) in the prM (8 common amino acid changes) and E (18 common amino acid changes) regions, as shown in Table 2 and Figures 4 and 5 and Supplementary Figure S1.

AA positions/region	prM														E																
	15	24	29	39	52	82	120	125	148	152	23	52	71	91	129	141	149	164	170	203	251	308	322	340	380	390	447	462	491	494	
Dengvaxia_CYD2_KX239895	S	V	D	M	K	T	A	I	H	A	I	Q	E	V	V	V	H	V	I	N	F	V	I	M	I	N	A	I	V	G	
Asian American	PP719120 FG 2007	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V				A	Q	
	PP719119 GLP 2007	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V				A	Q	
	PP719121 GLP 2012	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V				A	Q	
	PP719122 FG 2013	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V				A	Q	
	PP719124 FG 2013	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V				A	Q	
	PP719125 FG 2013	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V				A	Q	
	PP719123 FG 2013	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V				A	Q	
	PP719126 FG 2016	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V				A	Q	
	PP719130 FG 2019	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V		V		A	Q	
	PP719127 FG 2019	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V		V		A	Q	
	PP719138 FG 2019	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V		V		A	Q	
	PP719136 FG 2019	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V		V		A	Q	
	PP719131 FG 2019	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V		V		A	Q	
	PP719137 FG 2019	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V		V		A	Q	
	PP719128 FG 2019	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V		V		A	Q	
	PP719129 FG 2019	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V		V		A	Q	
	PP719132 FG 2019	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V		V		A	Q	
PP719135 FG 2019	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V		V		A	Q		
PP719134 FG 2019	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V		V		A	Q		
PP719133 FG 2019	G	L	I	I	V	T				I	I	I	I	I	I	I	I	I	T	D	V	I	T	V		V		A	Q		
Cosmopolitan Indian sub-continent	PP719139 GLP 2020	L			N	A	T	Y	V		A	I	S							V	V			S	V						
	OR229968 MTQ 2020	L			N	A	T	Y	V		A	I	S							V	V			S	V						
	OR229969 MTQ 2020	L			N	A	T	Y	V		A	I	S							V	V			S	V						
	OR229970 MTQ 2020	L			N	A	T	Y	V		A	I	S							V	V			S	V						
	OR229959 GLP 2020	L			N	A	T	Y	V		A	I	S							V	V			S	V						
	OR229966 MTQ 2020	L			N	A	T	Y	V		A	I	S							V	V			S	V						
	OR229975 GLP 2019	L			N	A	T	Y	V		A	I	S							V	V			S	V						
	OR229967 MTQ 2020	L			N	A	T	Y	V		A	I	S							V	V			S	V						
	OR229962 GLP 2020	L			N	A	T	Y	V		A	I	S							V	V			S	V						
	OR229972 MTQ 2020	L			N	A	T	Y	V		A	I	S							V	V			S	V						
	OR229965 GLP 2020	L			N	A	T	Y	V		A	I	S							V	V		T	S	V						
	OR229973 GLP 2019	L			N	A	T	Y	V		A	I	S							V	V			S	V						
	OR229958 GLP 2020	L			N	A	T	Y	V		A	I	S							V	V			S	V						
	OR229964 GLP 2020	L			N	A	T	Y	V		A	I	S							V	V			S	V						
OR229963 GLP 2020	L			N	A	T	Y	V		A	I	S							V	V			S	V							
OR229971 MTQ 2020	L			N	A	T	Y	V		A	I	S							V	V			S	V							
OR229976 GLP 2019	L			N	A	T	Y	V		A	I	S							V	V			S	V							
OR229960 GLP 2020	L			N	A	T	Y	V		A	I	S							V	V		L	S	V							
Other Cosmopolitan	PP719143 GLP 2022	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719140 GLP 2022	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719141 MTQ 2022	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719142 GLP 2022	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP510618 MTQ 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP510617 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP510623 MTQ 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719171 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719155 SB 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719152 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719161 GLP 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP510625 MTQ 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719176 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP510621 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719163 GLP 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719145 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719148 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719169 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719172 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719144 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719173 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719162 GLP 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719150 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719170 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719175 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719168 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719174 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719166 GLP 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719167 GLP 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719149 MTQ 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719153 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP719164 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP510620 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						
	PP510619 FG 2023	L	N		N	A	T	Y	V	H	A	I	I	N						V	V			S	V						

AA positions/region	prM															E																		
	15	29	39	52	82	83	120	135	148	152	162	6	23	52	71	91	120	129	141	149	164	170	203	308	322	340	380	390	447	462	478	484	491	
Qdenga_D2_U87411.1	S	D	M	K	T	M	V	M	H	A	T	M	I	Q	E	V	R	V	I	H	I	I	N	V	I	M	I	N	A	I	T	I	V	
Asian American	G	I	I	I	T	I	I	I	I	A	A	I	I	I	I	I	I	I	I	I	I	I	T	D	I	I	T	V	I	I	S	V	A	A
Cosmopolitan Indian sub continent	N	A	T	A	I	Y	V	A	I	A	A	A	A	T	I	V	S	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
Other Cosmopolitan	N	N	N	N	A	I	V	A	I	V	H	A	T	I	N	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V

Figure 5. Mapping of observed AA substitution patterns specific to one or more genotypes and subgenotypes (Asian American, Cosmopolitan Indian Sub-continent, and Cosmopolitan Other Cosmopolitan) compared with the Qdenga vaccine strain (U87411). The A447V AA change is observed for a sublineage of the Asian American genotype.

Table 2. AA substitution found in both analyses (Dengvaxia and Qdenga vaccine strains) in the prM and E regions.

Region prM	n = 8	S15G, D29N, M39I, K52N, T82A, A120V or V120A, H148Y, A152V
Region E	n = 18	I23V, Q52H, E71A, V91I, V129I, I141V or V141I, H149S and N, I164V or V164I, I170T, N203D, V308I, I322V, M340T, I380V, N390S, A447V, I462V, V491A

4. Discussion

This study describes the evolution of DENV2 strains circulating in the FTAs over a 20-year period and the corresponding amino acid variations compared to vaccine strains (Dengvaxia[®] and Qdenga[®]). The phylogenetic analysis of 100 DENV2 sequences circulating in the FTAs, between 2002 and 2024, reveals the successive circulation of two distinct genotypes: Asian American and Cosmopolitan. The first one included all strains from 2000s to 2013, as well as strains circulating in 2019 in French Guiana (mainly affecting the municipality of Kourou). FG sequences are intermingled with other sequences from America, suggesting that significant exchanges occurred in the region during this period [28–30]. At the same time, in 2019–2020, strains from Martinique and Guadeloupe were shown to belong to the Cosmopolitan genotype under the Indian sub-continent lineage (or 2II_A according to Hill et al., 2024 [26]). The exact importation origin could not be determined, but these sequences presented high homology with sequences from the Indian Ocean (Reunion Island and the Seychelles) and India, which circulated between 2016 and 2018 [9]. This sub-lineage seems to have already circulated in the Americas (such as OR389333 Venezuela 1990), but its origin seems to be Asian (India) [31,32]. Finally, French Guiana, Guadeloupe, Martinique, Saint-Martin, and Saint-Barthelemy strains from the 2023–2024 epidemic were found to belong to the Cosmopolitan genotype, lineage Other Cosmopolitan (or 2II_F according to Hill et al., 2024 [26]). Other American strains over the same period belong to this lineage such as OM791800 and OM791801 Peru 2019, ON634745 and OP941843 Brazil 2022; and OR037352 Colombia 2022. These sequences are related to Bangladesh strains from 2017 and 2019 (LC436673 and MN328061) [9,33–35]. However, the 2023–2024 FTA outbreak clade is distinct from this one; therefore, new introductions from Asia (MRCA 2016) can be assumed to be the origin of this epidemic instead of a drift of strains already present on the American continent. The 2023–2024 FTA outbreak clade found the highest percentage of nucleotide identity with the Indian sequences of 2021 (OM639992 and OM639982) in GenBank. However, other routes of introduction cannot be excluded, as these data depend on what is available in databases, which are not exhaustive.

The brief interval between the 2019–2021 and 2023–2024 outbreaks of the same serotype (DENV2) in French Guiana and Guadeloupe was unexpected, as it contrasts with the typical historical pattern, where the predominant serotypes generally alternate between two consecutive outbreaks, with an inter-epidemic period of 3–4 years. This raises the question of what factors have driven the emergence and spread of this serotype. Different mechanisms could be at play. While population immunity evolves over time due to births and population turnover, a loss of immunity alone does not explain the rapid spread observed over such a short period (from 2019–2021 to 2023–2024). Other factors must also contribute to the spread and diversification of DENV, such as environmental and socioeconomic factors that facilitate vector adaptation and proliferation [29,36–44]. A transmission advantage due to vector-related factors could be involved, but no entomological data related to vector competence and more broadly to vectorial capacity are available. A transmission advantage due to higher viremia is difficult to evaluate because of possible sampling biases, but, based on our data, no significant difference in DENV2 Ct values was observed between the 2019–2020 and 2023–2024 epidemics in French Guiana. Finally, the genetic variability induced by

the genotype shift in French Guiana and subgenotype shift in Guadeloupe could have led to at least partial escape from population immunity. Thus, by comparing the amino acid changes in the envelope region of the 2023–2024 epidemic strains from French Guiana and Guadeloupe (Cosmopolitan Other Cosmopolitan) with those from the 2019 epidemic in French Guiana (Asian American genotype) and 2019–2020 in Guadeloupe (Cosmopolitan Indian Subcontinent genotype), variations are observed at positions V23I, H52Q, V91I, V141I, N149H, I170T, N203D, V308I, V322I, M340T, S363A, I380V, S390N, A447V, V462I, and V491A for French Guiana and V23I, H52Q, I141V, and N149S for Guadeloupe. Some of these amino acid changes are found in known epitopes of the DENV2 envelope, such as V308I, M340T, S363A, and S390N (IEDB Database). These AA changes could affect these specific epitopes and may impact the immune response against the virus. Therefore, these changes could contribute to this escape. The significant and increasing diversity observed has led the scientific community to reconsider the classification of DENV viruses in order to establish a more precise and accurate naming system [26]. In the future, it would be valuable to explore the relationship between genomics and clinical data, aiming to identify variants that may be associated with specific clinical data (e.g., severity).

The genetic diversity of DENV raises the question of its impact on antigenic characteristics, and therefore on infection- or vaccine-elicited antibodies, and this poses a major challenge for obtaining an effective vaccine [45]. Vaccine development against dengue has progressed during the last decade [46]. However, these efforts have been challenged by the need to induce effective protection against all four DENV serotypes and by the potential negative effect of partial immunity to DENV, which can worsen symptoms in subsequent heterotypic infection [46]. At present, the most advanced dengue vaccines are all tetravalent and based on live-attenuated recombinant viruses. CYD-TDV, Dengvaxia[®] developed by Sanofi Pasteur (Lyon, France), and TAK-003, DENVax, Qdenga[®] developed by Takeda (Cambridge, MA, USA), have been approved, although Dengvaxia[®] will no longer be marketed from August 2026. The original strain of Dengvaxia[®] is the Thailand DENV-2 PUO-218 isolated in 1980, while DENV-2 Thailand 16681 used in Qdenga[®] was isolated in 1984; both are of the Asian I genotype [47,48]. Consequently, these vaccine strains are genetically distant from the Cosmopolitan genotype, which is responsible for the most recent global dengue epidemics.

The amino acid variations between the FTA DENV2 strains and the two vaccines were analyzed. The results identified 42 positions with amino acid changes (15 in the prM region and 27 in the E region) compared to CYD-2 Dengvaxia[®], while 46 variable amino acid positions were found relative to Qdenga[®] in the same regions, including 16 in the prM region and 30 in the E region. Some amino acid changes were common across multiple genotypes and subgenotypes, appearing in both analyses (Dengvaxia[®] and Qdenga[®]), with 8 substitutions in the prM region and 18 in the E region. Among the positions showing conserved amino acid changes relative to the two vaccine strains, nine (Q52H, V91I, I141V, H149N, I164V, N203D, V308I, I322V and N390S) were also previously identified by Martinez et al. from a panel of DENV2 representative of global genetic diversity chosen to investigate the influence of genetic diversity on vaccine performance [48]. Martinez et al. identified several highly variable amino acid positions in the E protein of DENV2 (at positions 71, 129, 141, and 390) and demonstrated that these genetic variations have an impact on the extent of the neutralizing antibody activity following infection and vaccination [47]. Rabaa et al. and Velasco et al. identified significant amino acid changes and mismatches at mAb-targeted epitope sites (E71, E149, and E226) between circulating DENV-2 viruses and vaccine components, including two amino acid changes found in our study (at positions 71 and 149) [49,50]. To further investigate the impact of amino acid modifications on vaccine efficacy, it would be interesting to assess the level of neutralization efficiency of

post-vaccination immune sera against these different strains using the PRNT method. Thus, the diversity in the E protein could have several significant implications. (i) Escape from neutralizing antibodies: Alterations in these amino acid positions may modify the E protein's structure, making it harder for neutralizing antibodies to recognize and bind to the virus. This could impair the immune system's ability to effectively neutralize the virus. (ii) Immune pressure and evolution: The high variability at these positions suggests they are under immune pressure, likely due to prior host exposure to the virus. Variants with these mutations could have a selective advantage, as they are less susceptible to neutralizing antibodies generated by previous infections or vaccinations. (iii) Impact on vaccine development: The observed variability complicates the development of a universal vaccine. A vaccine targeting several genotypic variants may be needed to develop a universal vaccine [51–55].

5. Conclusions

In conclusion: the findings emphasize the significant regional and international exchanges that have led to multiple introductions of DENV2 into the FTAs, particularly from Asia, resulting in epidemic outbreaks. They underscore the importance of genomic research for understanding the evolutionary history of circulating strains and for tracing importation events. The analysis of amino acid changes between FTA strains and vaccine strains (Dengvaxia[®] and Qdenga[®]) identified numerous substitutions, some of which are shared across multiple genotypes and sub-lineages in both analyses. This raises questions about how these changes may affect vaccine-induced immunity and vaccine efficacy. Overall, this study highlights the critical need to sequence and analyze the genetic evolution of DENV in order to develop more effective prevention and control strategies, as well as to define vaccines best suited to circulating strains.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/vaccines13030264/s1>, Table S1: List of primers per pool used for DENV2 whole-genome amplification; Table S2: French Guiana, Guadeloupe and Martinique DENV2 sequences obtained by NGS (n = 77); Table S3: List of accession numbers, country, collection dates, genotype, and lineage for DENV2 used for the phylogenetic trees. Figure S1: AA substitution found in both analyses (Dengvaxia and Qdenga vaccine strains) annotated on DENV2 E protein (annotated from 9DOF).

Author Contributions: Conceptualization, A.E., A.L. (Anne Lavergne) and D.R.; Formal analysis, A.L. (Alisé Lagrave) and S.T.; Resources, M.P.D., J.J., J.-F.C., T.R., T.S., L.C., S.D., F.D., L.L., A.B.-A., D.M., R.K. and X.d.L.; Supervision, A.L. (Anne Lavergne) and D.R.; Writing—original draft, A.L. (Alisé Lagrave), A.L. (Anne Lavergne) and D.R.; Writing—review and editing, A.L. (Alisé Lagrave), A.E., S.T., M.P.D., J.J., J.-F.C., T.R., T.S., L.C., S.D., F.D., L.L., A.B.-A., D.M., R.K., X.d.L., A.L. (Anne Lavergne) and D.R. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financed by the NRCA-FG. The activity of the National Reference Centers is supported by the French national health agency (Santé publique France).

Institutional Review Board Statement: This study was part of a public health surveillance program of the National Reference Center for Arboviruses in French Guiana (NRCA-FG), which works in collaboration with the French Public Health Agency (Santé Publique France, SPF). Accordingly, as an epidemiological record, approval by an ethics committee was not required. The samples involved in this study were chosen among remaining human serum samples received as part of standard diagnostic and expertise activities of the NRCA-FG and stored in the NRCA-FG biobank according to French legislation (article L.1211-2 and related articles of the French Public Health Code—FPHC). Human serum samples were anonymized, with no or minimal risk to patient data privacy in accordance with the terms of the European General Data Protection Regulation and the French National Commission on Informatics and Liberty (CNIL).

Informed Consent Statement: Not applicable.

Data Availability Statement: All virus sequences are accessible on Genbank (accessions specified in the Supplementary Materials). The Python code and input files are available on Github at <https://github.com/aliselagrave/Python-script-to-compare-DENV-vaccine-strains-and-a-sequence-alignment-of-DENV2-.git> (accessed on 14 December 2024).

Acknowledgments: We thank the working group members of the arbovirus genomics diagnostic laboratories and their affiliations for their valuable contributions—Guadeloupe: Synergibio and INOVIE Biopôle Antilles; Martinique: Cerballiance and Eurofins; and French Guiana: Eurofins and French Guiana hospital laboratories. We thank also the technicians of the National Research Center for Arboviruses of French Guiana for their input. Our thanks also go to our intern Evina Grosdidier and our engineer Quentin Derian at NRCA-FG, who helped to write the Python scripts.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

AA	Amino acid
BEAST	Bayesian Evolutionary Analysis Sampling Trees
BEAUti	Bayesian Evolutionary Analysis Utility
cDNA	Complementary DNA
CDS	Coding sequence
CLC	CLC Main Workbench
CNIL	National Commission on Informatics and Liberty
DENV	Dengue virus
DENV2	Dengue virus serotype 2
ESS	Effective sample size
FCI	French Caribbean Islands
FG	French Guiana
FPHC	French Public Health Code
FTA	French Territory of the Americas
GTR + G + I	General Time Reversible with Gamma distribution and Invariant sites
95% HPD	95% high-probability densities
MRCA	Most recent common ancestor
NGS	Next-generation sequencing
NRCA-FG	National Research Center for Arboviruses French Guiana
NS	Nonstructural protein
MCC	Maximum clade credibility
ONT	Oxford Nanopore Technologies
PCR	Polymerase chain reaction
PRNT	Plaque Reduction Neutralization Test
SPF	Santé Publique France
WHO	World Health Organization

Appendix A

Table A1. List of primers per pool used for DENV2 whole-genome amplification.

Primer Name	Sequence	Size Amplicon	Pool
CDC-D2-F-1ter	AGTTGTTAGTCTACGTGGACCGAC	484	1
CDC-D2-R-1	TGYCTRCYRACGATCATGTGT		
CDC-D2-F-3	ACRCRYACAGGAGAACACAGAA	365	
CDC-D2-R-3	TTTTGCCATCGTYGTACRCA		

Table A1. *Cont.*

Primer Name	Sequence	Size Amplicon	Pool
CDC-D2-F-9	CACGYAGYACCTCACTGTCT	384	1
CDC-D2-R-9	GATCGTTTTCTGCTGCATGA		
CDC-D2-F-11	GAAACAGCAGAATGYCCCAA	474	
CDC-D2-R-11	AGGGTCCTCTAYTTCCACAGTC		
CDC-D2-F-13bis	TGGTCAACTCYTTGGTCACAG	409	
CDC-D2-R-13	CAAGGCYAACGCRTCAGTCA		
CDC-D2-F-17	GAACTGGAAGATGGAGCYTA	402	
CDC-D2-R-17	CCATAAAGRCCACRACYTTTCCT		
CDC-D2-F-19	ACAYTAATCCTGGCYCCYAC	345	
CDC-D2-R-19	TGATTGGTGCRITGCTYTGAGG		
CDC-D2-F-21bis	GTGGTCACRACWGACATTTTCAG	416	
CDC-D2-R-21	TGGTARGTCYCCTCTTCTCAT		
CDC-D2-F-23	TGGTGYTTTGATGGARTYAAGAACA	426	
CDC-D2-R-23	ACATTCCCAGRGTCATYTTCCC		
CDC-D2-F-25	ATAGCCATCCTYACAGTGGTGG	418	
CDC-D2-R-25	TTGCTTGRAGTCCTGGCCCTAT		
CDC-D2-F-29	GCAGRGGRRGGCTGGTCATA	396	
CDC-D2-R-29	GTGGARTTYCKTGAGAGTGG		
CDC-D2-F-31	GGCYTACCATGGCAGCTAYGAA	400	
CDC-D2-R-31	TGTCAACCARYTCCCAAAAYC		
CDC-D2-F-35bis	GAATGGCYATCAGYGGAGATGA	426	
CDC-D2-R-35	GCGTGTATGGACCARGTTGT		
CDC-D2-F-2bis	GGAAAAGAGATTGGAARGATGYTGA	425	
CDC-D2-R-2	ATGACATCCATGTTTCRGTTCGTG		
CDC-D2-F-4	GGAGGAAGYGGGTTGAYAT	395	
CDC-D2-R-4	CTCTCYCCTGARTGRGGTGT		
CDC-D2-F-6bis	GTCACGATGGARTGCTCYCC	412	
CDC-D2-R-6bis	CCATGTTGTGYTCTGCTATTTCC		
CDC-D2-F-8	GGAGTAGARCCGGGACAA	397	
CDC-D2-R-8	CCRCTRCCACATTTCACTTCTT		
CDC-D2-F-10	TTGTGGAATCCGYTCAGTAACA	398	
CDC-D2-R-10	GCYGCTGACATGAGTTTGAGT		
CDC-D2-F-14bis	AGACCAACTTTTGCAGCTGGA	452	
CDC-D2-R-14	AATGCTCACCATCCCDACTGC		
CDC-D2-F-16	TGYTAYGTGCTCACTGGA	430	
CDC-D2-R-16bis	CGTGTGACRTGCCACATTGT		
CDC-D2-F-20	TGATYATYATGGAYGAAGCCCA	420	
CDC-D2-R-20	TCAGCCTTGAAGTTRGCRC		
CDC-D2-F-22	CCAGTAYATATACATGGGGGAACC	407	
CDC-D2-R-22	GCCAGTGGRTCAGARTAGATCC		
CDC-D2-F-24	CATGCTCTYAGTGAAGTCC	399	
CDC-D2-R-24	TGCTCTCRGRTSCTGGGTT		
CDC-D2-F-26	ATGGACATYGGAGTCCCC	411	
CDC-D2-R-26	TGTTTRGCCATTGAYACTGCAA		

2

Table A1. Cont.

Primer Name	Sequence	Size Amplicon	Pool	
CDC-D2-F-28bis	AGAGGAGAAACGGACCAYCA	313	2	
CDC-D2-R-28	GACGACTCCCCTATRTCACAC			
CDC-D2-F-30	CTRCAAAGGAAATAYGGAGGAGC	413		
CDC-D2-R-30	GACGTCCCAAGGTTTTGTGTYAG			
CDC-D2-F-32	GAAARGTGAGAAGCAAYGCAGC	424		
CDC-D2-R-32bis	GATTCTTGTGTCCCATCCTGC			
CDC-D2-F-34	ACACCAAGAGGCACAGTAATG	369		
CDC-D2-R-34	GCACYGTGTCCARTCGTTC			
CDC-D2-F-36bis	CRTCACAYTGGGTCCAACAAG	403		
CDC-D2-R-36	ATGGCTTAATCCGACCTGACT			
CDC-D2-F-5	GAGGATGGGGAAAWGGRTG	363		
CDC-D2-R-5	CATGGYAAAYGGCAGGTCTA			
CDC-D2-F-7	GACATCTYAARTGYAGGCTGAG	420		
CDC-D2-R-7	TCCCARGCTGTRTCACCTARAA			
CDC-D2-F-12	GGCCAAARTCACACACYCT	401		
CDC-D2-R-12	TGTCAATCTGYCCATGTCCG			
CDC-D2-F-15	GGATACCAYTRGCRYTGACGA	371		
CDC-D2-R-15	TGGTCAGTGTITGTTCTTCYTCTT			
CDC-D2-F-18	AACYAACACYGGAACMATAGG	386		3
CDC-D2-R-18	GCTGGGGTTTGRITAYCTTATTGG			
CDC-D2-F-27	ATGATGAGGACYACATGGGCT	401		
CDC-D2-R-27	TCTCTCRACGAACCATCTCA			
CDC-D2-F-33	TAGAGTTTGAAGCCCTRGATT	430		
CDC-D2-R-33	TCCTTCTCCYTCCATCTGTCT			
CDC-D2-F-37	TAGGCARTGAGGRATACACAGA	503		
CDC-D2-R-37bis	TTTTCTGGCGTTCGTGCCTGG			

Table A2. French Guiana, Guadeloupe, and Martinique DENV2 sequences obtained by NGS (n = 77).

	Sequence ID	Accession Number	Size (PB)	Territory: Municipality	Collection Date
1	17023/DENV2/FG/14-07-2023	PP510620	10,677	French Guiana: Saint-Laurent-du-Maroni	2023-07-14
2	29053/DENV2/FG/27-08-2023	PP510619	10,688	French Guiana: Sinnamary	2023-08-27
3	07040/DENV2/FG/07-09-2023	PP510624	10,688	French Guiana: Cayenne	2023-09-07
4	04043/DENV2/FG/02-10-2023	PP510617	10,690	French Guiana: Remire-Montjoly	2023-10-02
5	25040/DENV2/MTQ/24-07-2023	PP510622	10,279	Martinique	2023-07-24
6	25047/DENV2/MTQ/24-07-2023	PP510625	10,686	Martinique	2023-07-24
7	25056/DENV2/MTQ/21-07-2023	PP510623	10,676	Martinique	2023-07-21
8	25112/DENV2/MTQ/26-07-2023	PP510618	10,690	Martinique	2023-07-26
9	22030/DENV2/GLP/21-09-2023	PP510621	10,688	French Guiana: imported from Guadeloupe	2023-09-21
10	24144/DENV2/GLP/2007-01-06	PP719119	10,279	Guadeloupe: Basse-Terre	2007-01-06
11	13041/DENV2/FG/2007-01-13	PP719120	10,279	French Guiana: Cayenne	2007-01-13
12	21159/DENV2/GLP/2012-09-29	PP719121	10,279	Guadeloupe: Les Abymes	2012-09-29
13	24196/DENV2/FG/2013-01-28	PP719122	10,279	French Guiana: Kourou	2013-01-28
14	26059/DENV2/FG/2013-03-06	PP719123	10,279	French Guiana: St Laurent du Maroni	2013-03-06
15	08056/DENV2/FG/2013-03-12	PP719124	10,279	French Guiana: Cayenne	2013-03-12
16	06154/DENV2/FG/2013-03-22	PP719125	10,279	French Guiana: Remire-Montjoly	2013-03-22

Table A2. Cont.

	Sequence ID	Accession Number	Size (PB)	Territory: Municipality	Collection Date
17	16285/DENV2/FG/2016-08-10	PP719126	10,279	French Guiana: Cayenne imported West French Indies	2016-08-10
18	18111/DENV2/FG/2019-04-11	PP719127	10,279	French Guiana: Kourou	2019-04-11
19	03092/DENV2/FG/2019-06-26	PP719128	10,279	French Guiana: Kourou	2019-06-26
20	31071/DENV2/FG/2019-07-26	PP719129	10,279	French Guiana: Tonate-Macouria	2019-07-26
21	19127/DENV2/FG/2019-08-13	PP719130	10,279	French Guiana: Kourou	2019-08-13
22	03143/DENV2/FG/2019-08-26	PP719131	10,279	French Guiana: Kourou	2019-08-26
23	12083/DENV2/FG/2019-09-07	PP719132	10,279	French Guiana: Kourou	2019-09-07
24	24099/DENV2/FG/2019-09-18	PP719133	10,279	French Guiana: Cayenne	2019-09-18
25	23042/DENV2/FG/2019-09-23	PP719134	10,279	French Guiana: Cayenne	2019-09-23
26	03014/DENV2/FG/2019-09-25	PP719135	10,279	French Guiana: Kourou	2019-09-25
27	14118/DENV2/FG/2019-11-04	PP719136	10,693	French Guiana: Kourou	2019-11-04
28	12084/DENV2/FG/2019-12-09	PP719137	10,693	French Guiana: Kourou	2019-12-09
29	27085/DENV2/FG/2019-12-22	PP719138	10,279	French Guiana: Kourou	2019-12-22
30	24068/DENV2/GLP/2020-02-24	PP719139	10,279	Guadeloupe: Sainte-Anne	2020-02-24
31	04007/DENV2/GLP/2022-09-24	PP719140	10,693	Guadeloupe: Baie-Mahault	2022-09-24
32	04008/DENV2/MTQ/2022-10-28	PP719141	10,279	Martinique: Lamentin	2022-10-28
33	04025/DENV2/GLP/2022-12-16	PP719142	10,693	Guadeloupe: Pointe-a-Pitre	2022-12-16
34	04026/DENV2/GLP/2022-12-21	PP719143	10,279	Guadeloupe: Pointe-a-Pitre	2022-12-21
35	16007/DENV2/FG/2023-10-14	PP719144	10,693	French Guiana: Cayenne	2023-10-14
36	24032/DENV2/FG/2023-10-22	PP719145	10,279	French Guiana: Remire-Montjoly	2023-10-22
37	26006/DENV2/FG/2023-10-24	PP719146	10,279	French Guiana: Matoury	2023-10-24
38	04011/DENV2/FG/2023-11-01	PP719147	10,693	French Guiana: Kourou	2023-11-01
39	07002/DENV2/FG/2023-11-02	PP719148	10,279	French Guiana: Remire-Montjoly imported from Martinique	2023-11-02
40	08028/DENV2/FG/2023-11-06	PP719149	10,693	French Guiana: Remire-Montjoly	2023-11-06
41	08057/DENV2/FG/2023-11-08	PP719150	10,693	French Guiana: Tonate-Macouria	2023-11-08
42	15006/DENV2/FG/2023-11-12	PP719151	10,279	French Guiana: Matoury	2023-11-12
43	21004/DENV2/FG/2023-11-18	PP719152	10,693	French Guiana: Remire-Montjoly	2023-11-18
44	22030/DENV2/FG/2023-11-21	PP719153	10,693	French Guiana: Cayenne	2023-11-21
45	25009/DENV2/FG/2023-11-23	PP719154	10,693	French Guiana: Montsinery-Tonnegrande	2023-11-23
46	27054/DENV2/SB/2023-10-02	PP719155	10,693	Saint-Barthelemy	2023-10-02
47	27058/DENV2/SB/2023-09-30	PP719156	10,693	Saint-Barthelemy	2023-09-30
48	27089/DENV2/SB/2023-10-05	PP719157	10,693	Saint-Barthelemy	2023-10-05
49	28056/DENV2/SM/2023-10-05	PP719158	10,693	St Martin	2023-10-05
50	28062/DENV2/SM/2023-10-06	PP719159	10,693	St Martin	2023-10-06
51	28071/DENV2/SM/2023-10-07	PP719160	10,279	St Martin	2023-10-07
52	30041/DENV2/GLP/2023-11-17	PP719161	10,693	Guadeloupe	2023-11-17
53	30063/DENV2/GLP/2023-11-18	PP719162	10,693	Guadeloupe	2023-11-18
54	01014/DENV2/GLP/2023-11-16	PP719163	10,693	Guadeloupe	2023-11-16
55	04074/DENV2/FG/2023-11-28	PP719164	10,279	French Guiana: Saint-Georges	2023-11-28
56	07018/DENV2/GLP/2023-11-09	PP719165	10,693	Guadeloupe	2023-11-09
57	08047/DENV2/GLP/2023-11-17	PP719166	10,693	Guadeloupe	2023-11-17
58	08137/DENV2/GLP/2023-11-08	PP719167	10,279	Guadeloupe	2023-11-08
59	09010/DENV2/FG/2023-12-06	PP719168	10,693	French Guiana: Cayenne	2023-12-06
60	12047/DENV2/FG/2023-12-11	PP719169	10,279	French Guiana: Remire-Montjoly	2023-12-11
61	19018/DENV2/FG/2023-12-18	PP719170	10,279	French Guiana: Remire-Montjoly	2023-12-18
62	20050/DENV2/FG/2023-12-17	PP719171	10,693	French Guiana: Cayenne	2023-12-17
63	20057/DENV2/FG/2023-12-16	PP719172	10,279	French Guiana: Tonate-Macouria	2023-12-16
64	27010/DENV2/FG/2023-12-26	PP719173	10,693	French Guiana: Matoury	2023-12-26
65	28051/DENV2/FG/2023-12-25	PP719174	10,279	French Guiana: Remire-Montjoly	2023-12-25
66	30025/DENV2/FG/2023-12-29	PP719175	10,693	French Guiana: Kourou	2023-12-29

Table A2. Cont.

	Sequence ID	Accession Number	Size (PB)	Territory: Municipality	Collection Date
67	01020/DENV2/FG/2023-12-30	PP719176	10,693	French Guiana: Matoury	2023-12-30
68	06028/DENV2/FG/2024-01-05	PP719177	10,279	French Guiana: Remire-Montjoly	2024-01-05
69	07009/DENV2/FG/2024-01-06	PP719178	10,279	French Guiana: Kourou	2024-01-06
70	08101/DENV2/FG/2024-01-05	PP719179	10,693	French Guiana: Montsinery-Tonnegrade	2024-01-05
71	09080/DENV2/FG/2024-01-05	PP719180	10,279	French Guiana: Roura	2024-01-05
72	12011/DENV2/FG/2024-01-08	PP719181	10,693	French Guiana: Matoury	2024-01-08
73	18044/DENV2/FG/2024-01-17	PP719182	10,693	French Guiana: Sinnamary	2024-01-17
74	19020/DENV2/FG/2024-01-19	PP719183	10,693	French Guiana: Kourou	2024-01-19
75	23066/DENV2/FG/2024-01-23	PP719184	10,279	French Guiana: Remire-Montjoly	2024-01-23
76	24011/DENV2/FG/2024-01-24	PP719185	10,279	French Guiana: Remire-Montjoly	2024-01-24
77	24059/DENV2/FG/2024-01-24	PP719186	10,279	French Guiana: Remire-Montjoly	2024-01-24

Table A3. List of accession numbers, country, collection dates, genotype, and lineage for DENV2 used for the phylogenetic trees.

	A. Number	Country	Collection Date	Genotype	Lineage
1	OR804009	Guadeloupe	2023		
2	OR821962	USA	2023		
3	OR125606	China	2023		
4	ON908244	China	2022		
5	OP941843	Brazil	2022		
6	ON634745	Brazil	2022		
7	OR037352	Colombia	2022		
8	OM639982	India	2021		
9	OM639992	India	2021		
10	OQ683881	Cambodia	2020		
11	OR029726	China	2019		
12	PP234928	Thailand	2019		
13	OM791800	Peru	2019		
14	OM791801	Peru	2019		
15	MN328061	Bangladesh	2019		
16	MW512491	Singapore	2019		
17	MW512481	Singapore	2018		
18	LC410191	Thailand	2017	Cosmopolitan	Other Cosmopolitan
19	LC436673	Bangladesh	2017		
20	MW721469	China	2017		
21	MH827541	China	2017		
22	LC410190	Thailand	2016		
23	MW512451	Singapore	2016		
24	KU517847	Philippines	2015		
25	KX452026	Malaysia	2014		
26	MH823208	Indonesia	2014		
27	OQ832622	Vietnam	2019		
28	MK578532	China	2016		
29	KU517846	Indonesia	2014		
30	MH985858	Australia	2016		
31	KX452015	Malaysia	2014		
32	KU509272	Thailand	2009		
33	MN018349	Indonesia	2015		
34	MG189962	Tanzania	2014		
35	MZ857210	Tanzania	2014		

Table A3. Cont.

	A. Number	Country	Collection Date	Genotype	Lineage
36	MW512376	Singapore	2012		
37	EU482640	Vietnam	2006		
38	AY037116	Australia	1993		
39	FJ196854	China	1993		
40	KC964094	China	1993		
41	MN566112	New Caledonia	2018		
42	MW512489	Singapore	2018		
43	JN851131	Singapore	2005		
44	KF744397	Philippines	2001		Other Cosmopolitan
45	AB189124	Indonesia	1998		
46	OK469352	Singapore	1993		
47	GQ398264	Indonesia	1976		
48	OM317565	Cameroun	2020		
49	MT981148	Senegal	2019		
50	MT982169	Ivory Coast	2017		
51	EU056810	Burkina Faso	1983		
52	OR229975	Guadeloupe	2020		
53	OR229962	Guadeloupe	2020		
54	OR229960	Guadeloupe	2020		
55	OR229959	Guadeloupe	2020		
56	OR229964	Guadeloupe	2020		
57	OR229963	Guadeloupe	2020	Cosmopolitan	
58	OR229958	Guadeloupe	2020		
59	OR229965	Guadeloupe	2020		
60	OR229968	Martinique	2020		
61	OR229966	Martinique	2020		
62	OR229969	Martinique	2020		
63	OR229970	Martinique	2020		
64	OR229967	Martinique	2020		
65	OR229972	Martinique	2020		Indian sub-continent
66	OR229971	Martinique	2020		
67	ON123651	India	2020		
68	OR229976	Guadeloupe	2019		
69	OR229973	Guadeloupe	2019		
70	MN272404	Reunion Island	2018		
71	MN272405	Seychelles	2016		
72	MN294937	Saudi Arabia	2016		
73	MG721054	India	2016		
74	KJ830750	Saudi Arabia	2014		
75	MH822944	India	2012		
76	JQ955623	India	2009		
77	OR389333	Venezuela	1990		
78	OM505022	Mexico	2021		
79	ON510270	Colombia	2021		
80	ON634730	Brazil	2021		
81	ON634702	Brazil	2020		
82	OQ101603	Brazil	2019		
83	MW577820	Brazil	2019	Asian American	
84	PP234938	Brazil	2018		
85	OQ782228	Nicaragua	2018		
86	MW577849	Brazil	2018		
87	MW577848	Brazil	2010		

Table A3. Cont.

	A. Number	Country	Collection Date	Genotype	Lineage
88	KC294223	Peru	2010		
89	FJ850091	Brazil	2007		
90	GQ199868	Nicaragua	2007		
91	EU920849	Guadeloupe	2006		
92	EU920848	French Guiana	2006		
93	EU920837	Martinique	2005		
94	EU920843	Martinique	2005		
95	EU687217	Puerto Rico	2005		
96	EU920842	Suriname	2005	Asian American	
97	EU482600	Nicaragua	2005		
98	EU920835	French Guiana	2002		
99	JX669488	Brazil	2002		
100	AB122020	Dominican Republic	2001		
101	MW946444	Guyana	2000		
102	AY702036	Cuba	1997		
103	GQ868596	Venezuela	1991		
104	DQ181801	Thailand	1990		
105	OQ652965	China	2018		
106	OM257419	China	2017		
107	HQ891024	Taiwan	2008		
108	KF704358	Cuba	1981	Asian I	
109	GQ398268	Indonesia	1975		
110	KM204118	New Guinea	1944		
111	OQ832627	Vietnam	2020		
112	KY672955	China	2015		
113	GU131897	Cambodia	2007	Asian II	
114	NC_001474	Thailand	1964		
115	EU056811	Peru	1995		
116	GQ868592	Colombia	1986		
117	GQ868589	Mexico	1983		
118	EU056812	Puerto Rico	1977	American	
119	GQ398257	Indonesia	1977		
120	HM582110	French Polynesia	1973		
121	OK605757	Senegal	1990		
122	EF105384	Senegal	1970	Sylvatic	

References

1. Murugesan, A.; Manoharan, M. Dengue Virus. In *Emerging and Reemerging Viral Pathogens*; Academic Press: Cambridge, MA, USA, 2020; pp. 281–359. [CrossRef]
2. Rahman, N.A.A.; Fuaad, A.A.-H.A.; Azami, N.A.M.; Amin, M.C.I.M.; Azmi, F. Next-Generation Dengue Vaccines: Leveraging Peptide-Based Immunogens and Advanced Nanoparticles as Delivery Platforms. *J. Pharm. Sci.* **2024**, *113*, 2044–2054. [CrossRef]
3. Tamura, T.; Zhang, J.; Madan, V.; Biswas, A.; Schwoerer, M.P.; Cafiero, T.R.; Heller, B.L.; Wang, W.; Ploss, A. Generation and Characterization of Genetically and Antigenically Diverse Infectious Clones of Dengue Virus Serotypes 1–4. *Emerg. Microbes Infect.* **2024**, *11*, 227–239. [CrossRef] [PubMed]
4. Shrivastava, S.; Tiraki, D.; Diwan, A.; Lalwani, S.K.; Modak, M.; Mishra, A.C.; Arankalle, V.A. Co-Circulation of All the Four Dengue Virus Serotypes and Detection of a Novel Clade of DENV-4 (Genotype I) Virus in Pune, India during 2016 Season. *PLoS ONE* **2018**, *13*, e0192672. [CrossRef]
5. Dengue Worldwide Overview. Available online: <https://www.ecdc.europa.eu/en/dengue-monthly> (accessed on 10 September 2024).
6. Situation Report No 44—Dengue Epidemiological Situation in the Region of the Americas—Epidemiological Week 36, 2024—PAHO/WHO | Pan American Health Organization. Available online: <https://www.paho.org/en/documents/epidemiological-alert-increase-dengue-cases-americas-region-7-october-2024> (accessed on 29 November 2024).

7. Bonifay, T.; Le Turnier, P.; Epelboin, Y.; Carvalho, L.; De Thoisy, B.; Djossou, F.; Duchemin, J.-B.; Dussart, P.; Enfissi, A.; Lavergne, A.; et al. Review on Main Arboviruses Circulating on French Guiana, An Ultra-Peripheral European Region in South America. *Viruses* **2023**, *15*, 1268. [CrossRef] [PubMed]
8. Lagrave, A.; Enfissi, A.; Tirera, S.; Demar, M.P.; Jaonaso, J.; Carod, J.-F.; Ramavoson, T.; Succo, T.; Carvalho, L.; Devos, S.; et al. Re-Emergence of DENV-3 in French Guiana: Retrospective Analysis of Cases That Circulated in the French Territories of the Americas from the 2000s to the 2023–2024 Outbreak. *Viruses* **2024**, *16*, 1298. [CrossRef]
9. Garcia–Van Smévorde, M.; Piorkowski, G.; Emboulé, L.; dos Santos, G.; Loraux, C.; Guyomard, S.; Joannes, M.-O.; Fagour, L.; Najioullah, F.; Cabié, A.; et al. Phylogenetic Investigations of Dengue 2019–2021 Outbreak in Guadeloupe and Martinique Caribbean Islands. *Pathogens* **2023**, *12*, 1182. [CrossRef] [PubMed]
10. SPF. Surveillance de la Dengue aux Antilles. Point au 14 Septembre. 2023. Available online: <https://www.santepubliquefrance.fr/regions/antilles/documents/bulletin-regional/2023/surveillance-de-la-dengue-aux-antilles.-point-au-14-septembre-2023> (accessed on 24 September 2024).
11. Twiddy, S.S.; Farrar, J.J.; Vinh Chau, N.; Wills, B.; Gould, E.A.; Gritsun, T.; Lloyd, G.; Holmes, E.C. Phylogenetic Relationships and Differential Selection Pressures among Genotypes of Dengue-2 Virus. *Virology* **2002**, *298*, 63–72. [CrossRef]
12. Weaver, S.C.; Vasilakis, N. Molecular Evolution of Dengue Viruses: Contributions of Phylogenetics to Understanding the History and Epidemiology of the Preeminent Arboviral Disease. *Infect. Genet. Evol.* **2009**, *9*, 523–540. [CrossRef]
13. Yenamandra, S.P.; Koo, C.; Chiang, S.; Lim, H.S.J.; Yeo, Z.Y.; Ng, L.C.; Hapuarachchi, H.C. Evolution, Heterogeneity and Global Dispersal of Cosmopolitan Genotype of Dengue Virus Type 2. *Sci. Rep.* **2021**, *11*, 13496. [CrossRef]
14. Gräf, T.; Ferreira, C.D.N.; de Lima, G.B.; de Lima, R.E.; Machado, L.C.; Campos, T.d.L.; Schemberger, M.O.; Faoro, H.; Paiva, M.H.S.; Bezerra, M.F.; et al. Multiple Introductions and Country-Wide Spread of DENV-2 Genotype II (Cosmopolitan) in Brazil. *Virus Evol.* **2023**, *9*, vead059. [CrossRef]
15. Hill, S.C.; Neto de Vasconcelos, J.; Granja, B.G.; Thézé, J.; Jandondo, D.; Neto, Z.; Mirandela, M.; Sebastião, C.D.S.; Cândido, A.L.M.; Clemente, C.; et al. Early Genomic Detection of Cosmopolitan Genotype of Dengue Virus Serotype 2, Angola, 2018. *Emerg. Infect. Dis.* **2019**, *25*, 784–787. [CrossRef]
16. Quick, J.; Grubaugh, N.D.; Pullan, S.T.; Claro, I.M.; Smith, A.D.; Gangavarapu, K.; Oliveira, G.; Robles-Sikisaka, R.; Rogers, T.F.; Beutler, N.A.; et al. Multiplex PCR Method for MinION and Illumina Sequencing of Zika and Other Virus Genomes Directly from Clinical Samples. *Nat. Protoc.* **2017**, *12*, 1261–1276. [CrossRef] [PubMed]
17. Jones, F.K.; Morrison, A.M.; Santiago, G.A.; Rysava, K.; Zimler, R.A.; Heberlein, L.A.; Kopp, E.; Saunders, K.E.; Baudin, S.; Rico, E.; et al. Introduction and Spread of Dengue Virus 3, Florida, USA, May 2022–April 2023. *Emerg. Infect. Dis. J.* **2024**, *30*, 376–379. [CrossRef] [PubMed]
18. Rowe, D.; McDermott, C.; Veliz, Y.; Kerr, A.; Whiteside, M.; Coss, M.; Huff, C.; Leal, A.; Kopp, E.; LaCrue, A.; et al. Dengue Outbreak Response during COVID-19 Pandemic, Key Largo, Florida, USA, 2020. *Emerg. Infect. Dis. J.* **2023**, *29*, 1643–1647. [CrossRef]
19. Naveca, F.G.; Santiago, G.A.; Maito, R.M.; Ribeiro Meneses, C.A.; do Nascimento, V.A.; de Souza, V.C.; do Nascimento, F.O.; Silva, D.; Mejía, M.; Gonçalves, L.; et al. Reemergence of Dengue Virus Serotype 3, Brazil, 2023. *Emerg. Infect. Dis.* **2023**, *29*, 1482–1484. [CrossRef] [PubMed]
20. Barido-Sottani, J.; Schwery, O.; Warnock, R.C.M.; Zhang, C.; Wright, A.M. Practical Guidelines for Bayesian Phylogenetic Inference Using Markov Chain Monte Carlo (MCMC). *Open Res. Eur.* **2023**, *3*, 204. [CrossRef]
21. Suchard, M.A.; Lemey, P.; Baele, G.; Ayres, D.L.; Drummond, A.J.; Rambaut, A. Bayesian Phylogenetic and Phylodynamic Data Integration Using BEAST 1.10. *Virus Evol.* **2018**, *4*, vey016. [CrossRef]
22. Baele, G.; Lemey, P.; Bedford, T.; Rambaut, A.; Suchard, M.A.; Alekseyenko, A.V. Improving the Accuracy of Demographic and Molecular Clock Model Comparison While Accommodating Phylogenetic Uncertainty. *Mol. Biol. Evol.* **2012**, *29*, 2157–2167. [CrossRef]
23. Rambaut, A.; Drummond, A.J.; Xie, D.; Baele, G.; Suchard, M.A. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Syst. Biol.* **2018**, *67*, 901–904. [CrossRef]
24. Rey, C.; Guéguen, L.; Sémon, M.; Boussau, B. Accurate Detection of Convergent Amino-Acid Evolution with PCOC. *Mol. Biol. Evol.* **2018**, *35*, 2296–2306. [CrossRef]
25. Tsai, W.-Y.; Lin, H.-E.; Wang, W.-K. Complexity of Human Antibody Response to Dengue Virus: Implication for Vaccine Development. *Front. Microbiol.* **2017**, *8*, 1372. [CrossRef]
26. Hill, V.; Cleemput, S.; Pereira, J.S.; Gifford, R.J.; Fonseca, V.; Tegally, H.; Brito, A.F.; Ribeiro, G.; de Souza, V.C.; Brcko, I.C.; et al. A New Lineage Nomenclature to Aid Genomic Surveillance of Dengue Virus. *PLoS Biol.* **2024**, *22*, e3002834. [CrossRef] [PubMed]
27. Pascalis, H.; Turpin, J.; Roche, M.; Krejbich, P.; Gadea, G.; Nten, C.A.; Desprès, P.; Mavingui, P. The Epidemic of Dengue Virus Type-2 Cosmopolitan Genotype on Reunion Island Relates to Its Active Circulation in the Southwestern Indian Ocean Neighboring Islands. *Heliyon* **2019**, *5*, e01455. [CrossRef]

28. Añez, G.; Morales-Betoulle, M.E.; Rios, M. Circulation of Different Lineages of Dengue Virus Type 2 in Central America, Their Evolutionary Time-Scale and Selection Pressure Analysis. *PLoS ONE* **2011**, *6*, e27459. [[CrossRef](#)] [[PubMed](#)]
29. Allicock, O.M.; Lemey, P.; Tatem, A.J.; Pybus, O.G.; Bennett, S.N.; Mueller, B.A.; Suchard, M.A.; Foster, J.E.; Rambaut, A.; Carrington, C.V.F. Phylogeography and Population Dynamics of Dengue Viruses in the Americas. *Mol. Biol. Evol.* **2012**, *29*, 1533–1543. [[CrossRef](#)]
30. Mir, D.; Romero, H.; de Carvalho, L.M.F.; Bello, G. Spatiotemporal Dynamics of DENV-2 Asian-American Genotype Lineages in the Americas. *PLoS ONE* **2014**, *9*, e98519. [[CrossRef](#)] [[PubMed](#)]
31. Walimbe, A.M.; Lotankar, M.; Cecilia, D.; Cherian, S.S. Global Phylogeography of Dengue Type 1 and 2 Viruses Reveals the Role of India. *Infect. Genet. Evol.* **2014**, *22*, 30–39. [[CrossRef](#)]
32. Uzcategui, N.Y.; Camacho, D.; Comach, G.; Cuello de Uzcategui, R.; Holmes, E.C.; Gould, E.A. Molecular Epidemiology of Dengue Type 2 Virus in Venezuela: Evidence for in Situ Virus Evolution and Recombination. *J. Gen. Virol.* **2001**, *82*, 2945–2953. [[CrossRef](#)]
33. Amorim, M.T.; Hernández, L.H.A.; Naveca, F.G.; Essashika Prazeres, I.T.; Wanzeller, A.L.M.; da Silva, E.V.P.; Casseb, L.M.N.; da Silva, F.S.; da Silva, S.P.; Nunes, B.T.D.; et al. Emergence of a New Strain of DENV-2 in South America: Introduction of the Cosmopolitan Genotype through the Brazilian-Peruvian Border. *Trop. Med. Infect. Dis.* **2023**, *8*, 325. [[CrossRef](#)]
34. Giovanetti, M.; Pereira, L.A.; Santiago, G.A.; Fonseca, V.; Mendoza, M.P.G.; de Oliveira, C.; de Moraes, L.; Xavier, J.; Tosta, S.; Fristch, H.; et al. Emergence of Dengue Virus Serotype 2 Cosmopolitan Genotype, Brazil. *Emerg. Infect. Dis.* **2022**, *28*, 1725–1727. [[CrossRef](#)]
35. Ciuoderis, K.; Usuga, J.; Moreno, I.; Perez-Restrepo, L.; Flórez, D.; Cardona, A.; Cloherty, G.; Berg, M.; Hernández-Ortiz, J.; Osorio, J. Characterization of Dengue Virus Serotype 2 Cosmopolitan Genotype Circulating in Colombia. *Am. J. Trop. Med. Hyg.* **2023**, *109*, 1298–1302. [[CrossRef](#)] [[PubMed](#)]
36. Santos, L.L.M.; de Aquino, E.C.; Fernandes, S.M.; Ternes, Y.M.F.; Feres, V.C.d.R. Dengue, Chikungunya, and Zika Virus Infections in Latin America and the Caribbean: A Systematic Review. *Rev. Panam. Salud Publica* **2023**, *47*, e34. [[CrossRef](#)]
37. Mavian, C.; Dulcey, M.; Munoz, O.; Salemi, M.; Vittor, A.Y.; Capua, I. Islands as Hotspots for Emerging Mosquito-Borne Viruses: A One-Health Perspective. *Viruses* **2019**, *11*, 11. [[CrossRef](#)]
38. Pollett, S.; Melendrez, M.C.; Maljkovic Berry, I.; Duchêne, S.; Salje, H.; Cummings, D.A.T.; Jarman, R.G. Understanding Dengue Virus Evolution to Support Epidemic Surveillance and Counter-Measure Development. *Infect. Genet. Evol.* **2018**, *62*, 279–295. [[CrossRef](#)]
39. Torres, J.R.; Orduna, T.A.; Piña-Pozas, M.; Vázquez-Vega, D.; Sarti, E. Epidemiological Characteristics of Dengue Disease in Latin America and in the Caribbean: A Systematic Review of the Literature. *J. Trop. Med.* **2017**, *2017*, e8045435. [[CrossRef](#)] [[PubMed](#)]
40. Wearing, H.J.; Robert, M.A.; Christofferson, R.C. Dengue and Chikungunya: Modelling the Expansion of Mosquito-Borne Viruses into Naïve Populations. *Parasitology* **2016**, *143*, 860–873. [[CrossRef](#)]
41. Rezza, G. Dengue and Chikungunya: Long-Distance Spread and Outbreaks in Naïve Areas. *Pathog. Glob. Health* **2014**, *108*, 349–355. [[CrossRef](#)] [[PubMed](#)]
42. Grange, L.; Simon-Loriere, E.; Sakuntabhai, A.; Gresh, L.; Paul, R.; Harris, E. Epidemiological Risk Factors Associated with High Global Frequency of Inapparent Dengue Virus Infections. *Front. Immunol.* **2014**, *5*, 280. [[CrossRef](#)]
43. Vega-Rúa, A.; Zouache, K.; Girod, R.; Failloux, A.-B.; Lourenço-de-Oliveira, R. High Level of Vector Competence of *Aedes Aegypti* and *Aedes Albopictus* from Ten American Countries as a Crucial Factor in the Spread of Chikungunya Virus. *J. Virol.* **2014**, *88*, 6294–6306. [[CrossRef](#)]
44. Flamand, C.; Quenel, P.; Ardillon, V.; Carvalho, L.; Bringay, S.; Teisseire, M. The Epidemiologic Surveillance of Dengue-Fever in French Guiana: When Achievements Trigger Higher Goals. In *User Centred Networked Health Care*; IOS Press: Amsterdam, The Netherlands, 2011; pp. 629–633.
45. Izmirly, A.M.; Alturki, S.O.; Alturki, S.O.; Connors, J.; Haddad, E.K. Challenges in Dengue Vaccines Development: Pre-Existing Infections and Cross-Reactivity. *Front. Immunol.* **2020**, *11*, 1055. [[CrossRef](#)]
46. Hou, J.; Ye, W.; Chen, J. Current Development and Challenges of Tetravalent Live-Attenuated Dengue Vaccines. *Front. Immunol.* **2022**, *13*, 840104. [[CrossRef](#)] [[PubMed](#)]
47. Martinez, D.R.; Yount, B.; Nivarthi, U.; Munt, J.E.; Delacruz, M.J.; Whitehead, S.S.; Durbin, A.P.; de Silva, A.M.; Baric, R.S. Antigenic Variation of the Dengue Virus 2 Genotypes Impacts the Neutralization Activity of Human Antibodies in Vaccinees. *Cell Rep.* **2020**, *33*, 108226. [[CrossRef](#)] [[PubMed](#)]
48. Phadungsombath, J.; Lin, M.Y.-C.; Srimark, N.; Yamanaka, A.; Nakayama, E.E.; Moolasart, V.; Suttha, P.; Shioda, T.; Uttayamakul, S. Emergence of Genotype Cosmopolitan of Dengue Virus Type 2 and Genotype III of Dengue Virus Type 3 in Thailand. *PLoS ONE* **2018**, *13*, e0207220. [[CrossRef](#)]
49. Rabaa, M.A.; Girerd-Chambaz, Y.; Duong Thi Hue, K.; Vu Tuan, T.; Wills, B.; Bonaparte, M.; van der Vliet, D.; Langevin, E.; Cortes, M.; Zambrano, B.; et al. Genetic Epidemiology of Dengue Viruses in Phase III Trials of the CYD Tetravalent Dengue Vaccine and Implications for Efficacy. *eLife* **2017**, *6*, e24196. [[CrossRef](#)]

50. Velasco, J.M.; Klungthong, C.; Chinnawirotpisan, P.; Diones, P.C.; Valderama, M.T.; Leonardia, S.; Manasatienkij, W.; Joonlasak, K.; Rodpradit, P.; Mateo, J.; et al. Genetic Diversity of Dengue Virus Circulating in the Philippines (2014-2019) and Comparison with Dengue Vaccine Strains. *PLoS Negl. Trop. Dis.* **2024**, *18*, e0012697. [[CrossRef](#)]
51. Natali, E.N.; Horst, A.; Meier, P.; Greiff, V.; Nuvolone, M.; Babrak, L.M.; Fink, K.; Miho, E. The Dengue-Specific Immune Response and Antibody Identification with Machine Learning. *npj Vaccines* **2024**, *9*, 16. [[CrossRef](#)]
52. Kok, B.H.; Lim, H.T.; Lim, C.P.; Lai, N.S.; Leow, C.Y.; Leow, C.H. Dengue Virus Infection—A Review of Pathogenesis, Vaccines, Diagnosis and Therapy. *Virus Res.* **2023**, *324*, 199018. [[CrossRef](#)]
53. Huang, A.T.; Salje, H.; Escoto, A.C.; Chowdhury, N.; Chávez, C.; Garcia-Carreras, B.; Rutvisuttinunt, W.; Maljkovic Berry, I.; Gromowski, G.D.; Wang, L.; et al. Beneath the Surface: Amino Acid Variation Underlying Two Decades of Dengue Virus Antigenic Dynamics in Bangkok, Thailand. *PLoS Pathog.* **2022**, *18*, e1010500. [[CrossRef](#)] [[PubMed](#)]
54. Bell, S.M.; Katzelnick, L.; Bedford, T. Dengue Genetic Divergence Generates Within-Serotype Antigenic Variation, but Serotypes Dominate Evolutionary Dynamics. *eLife* **2019**, *8*, e42496. [[CrossRef](#)] [[PubMed](#)]
55. Rouvinski, A.; Guardado-Calvo, P.; Barba-Spaeth, G.; Duquerroy, S.; Vaney, M.-C.; Kikuti, C.M.; Navarro Sanchez, M.E.; Dejnirattisai, W.; Wongwiwat, W.; Haouz, A.; et al. Recognition Determinants of Broadly Neutralizing Human Antibodies against Dengue Viruses. *Nature* **2015**, *520*, 109–113. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.