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Research paper

Detection of Dengue viruses among febrile patients in Lagos, Nigeria and phylogenetics of circulating Dengue serotypes in Africa

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ABSTRACT

Dengue fever, a mosquito borne viral disease, is caused by Dengue virus. This virus and its vector is endemic in most tropical countries including Nigeria. Dengue presents with febrile symptoms and is a major cause of morbidity and mortality in affected countries. The infection presently has no licensed drugs and vaccine is only available for previously exposed individuals. Despite the endemicity of Dengue in Nigeria, very few studies have identified circulating Dengue genotypes in the country. There is also sparse information on the occurrence, distribution and temporal patterns of circulating dengue virus serotypes as well as genotypes in Africa. This situation creates barriers to effective control of the infection in the continent.

This study identified Dengue serotypes and genotypes among febrile patients in two health centers in Lagos, Nigeria. Phylogenetic analysis of Dengue sequences previously collected from African countries and submitted to GenBank database from 1944 till date was also performed. One hundred and thirty febrile persons were recruited for the study between April and August 2018. Eleven (8.5%) persons were Dengue virus positive. Dengue virus serotypes 1 (genotype I) and 3 (genotype I) were identified as actively circulating in Lagos, Nigeria. DENV 1 genotype V, DENV 2 cosmopolitan genotype and DENV 3 genotype III has over the years been the predominant circulating Dengue strains in Africa. Relative genotypic stability of circulating Dengue serotypes in Africa occurred over the past five decades. This may be due to limited investigations on circulating Dengue serotypes among asymptomatic individuals in the region as most studies focused on disease outbreaks and imported cases.

There is the need to describe circulating Dengue genotypes in northern Africa, southern Africa as well as among asymptomatic individuals in other parts of Africa as this will provide further information on the diversity of Dengue genotypes circulating in the region.

1. Introduction

Dengue is the most important mosquito-borne viral disease in the world (Konongoi et al., 2016). The infection has rapidly spread in all WHO regions in recent years. It is estimated that 3.9 billion people, including 120 million travelers, in over 128 countries are at risk of Dengue virus infection. Also, Globally, about 500,000 persons require hospitalization each year due to Dengue infection, with an associated case fatality rate of 2.5% (WHO Fact Sheet, 2018). Dengue is an infection caused by Dengue virus (DENV), a positive sense, single stranded RNA of the family *Flaviviridae*. The family consists of about 70 mosquito-and tick-borne viruses which includes other medically important viral species like West Nile virus, Japanese encephalitis virus and Yellow fever virus (Klema et al., 2016).

The 11 kb DENV RNA genome encodes 10 proteins which includes three structural proteins-capsid (C), pre-membrane (prM) and envelope (E) -and seven nonstructural proteins, namely NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (Bujalowski et al., 2017). There are four distinct serotypes of DENV, which are DENV 1, 2, 3 and 4. The serotypes are antigenically distinct despite sharing 65% genomic similarity (Kolawole et al., 2017; Mustapha et al., 2017). DENV 1 and 2 serotypes has 8 distinct genotypes each, namely I, Ib, II, III, IV, V, Recombinant and Lab

Abbreviations: DENV, Dengue virus; DF, Dengue fever; DHF, Dengue hemorrhagic fever; DSS, Dengue shock syndrome

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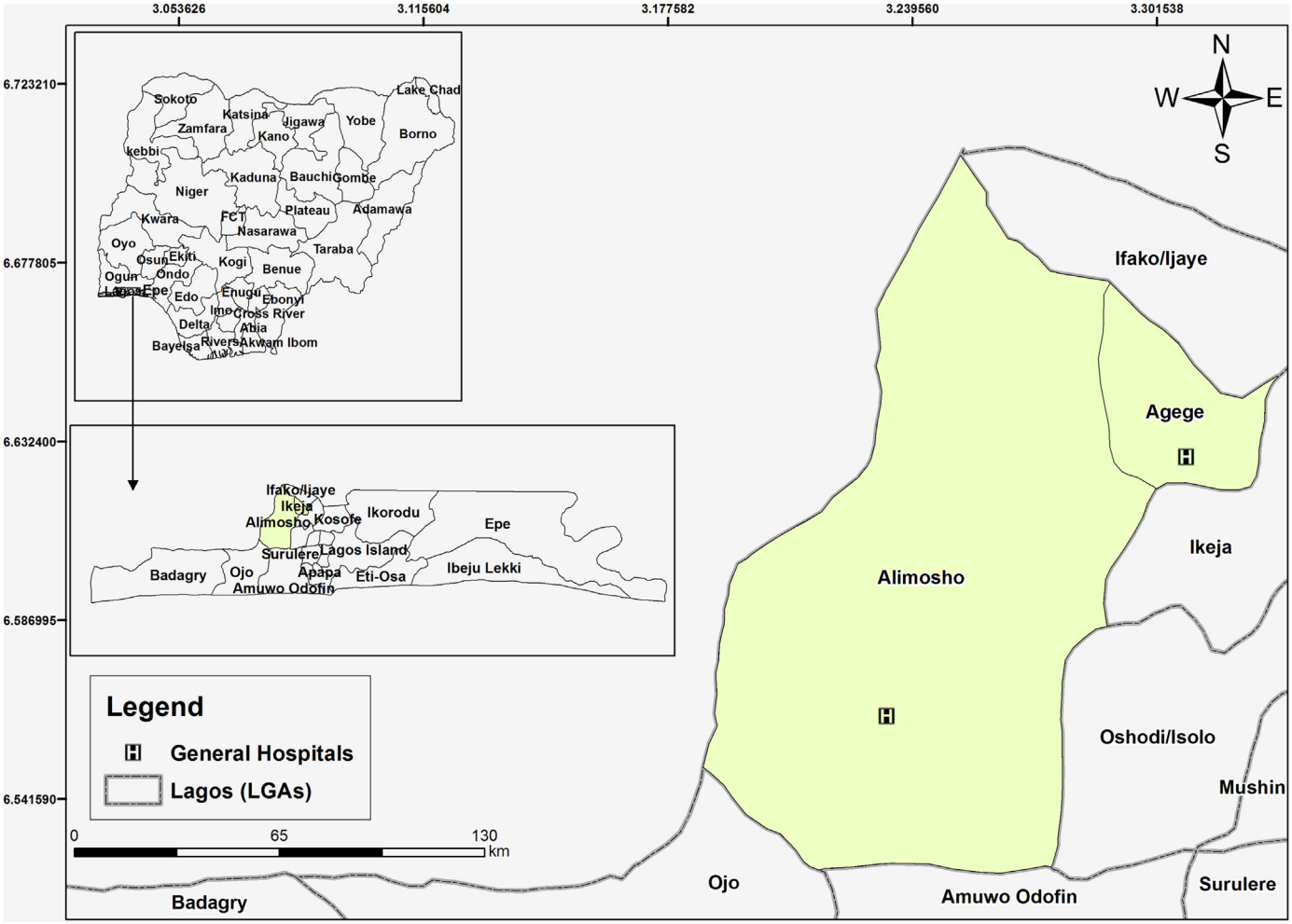


Fig. 1. Map of Lagos metropolis showing Alimosho and Agege LGA General Hospitals.

strains for DENV 1.

DENV 2 is subdivided into American, Asian-American, Asian I, Asian II, Cosmopolitan, Sylvatic, Recombinant and Unknown genotypes respectively. DENV 3 has 7 genotypes- I, II, III, IV, V, Recombinant and Unknown while DENV 4 has 6- I, IIA, IIB, III, Sylvatic and Recombinant genotypes(Yamashita et al., 2016). Transmission of DENV is by bite of Aedes species, specifically *Aedes aegyti* and *Aedes albopictus* mosquitoes. DENV is maintained in both urban and sylvatic cycles. The urban cycle involves humans, *Aedes aegyti* and *Aedes albopictus,* while the sylvatic cycle includes non-human primates and forest Aedes species (Fagbami and Onoja, 2018).

Symptomatic DENV infection have been grouped into Dengue fever (DF), Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS) based on disease severity (WHO Fact Sheet, 2018). DF is an acute febrile illness lasting 5–7 days characterized by headache, retroocular as well as muscle and joint pains, nausea, vomiting, and rash. The infection is endemic in most tropical and subtropical countries worldwide (Fagbami and Onoja, 2018). Based on 2009 WHO Dengue classification, severe dengue is defined as dengue with severe plasma leakage, severe bleeding, or organ failure. While Dengue fever do not involve these symptoms, it may be with or without warning signs. These warning signs include abdominal pain, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, restlessness and liver enlargement. Probable Dengue is defined as live in/travel to Dengue-endemic areas, fever and any two of the following criteria: nausea, vomiting, rash, aches and pains, leucopenia and any warning sign (Hadinegoro, 2012). Mild infection with a particular Dengue serotype leads to lasting immunity against the infecting serotype while repeated exposures to different serotypes increases the chances of developing DHF or DSS (Yamashita et al., 2016).

Several studies have reported high prevalence of Dengue infection in Nigeria, although most of these works used serological assays (Adedayo et al., 2013; Baba et al., 2009; Dawurung et al., 2010; Idris et al., 2013; Kolawole et al., 2017; Mustapha et al., 2017; Nasir et al., 2017; Oladipo et al., 2014; Onoja et al., 2016; Oyero and Ayukekbong, 2014). There is very limited information on the circulating Dengue serotypes and genotypes in Nigeria. From 1964 when Dengue virus was first detected in Nigeria till date, < 10 sequences have been successfully deposited into the GenBank database.

In sub Saharan Africa where the virus and its vector is also present, very few studies have described the occurrence of Dengue serotypes and genotypes(Yamashita et al., 2016). Most Dengue sequences collected from Africa and submitted in the GenBank were isolated during outbreaks as well as from foreign travelers and febrile patients (Caron et al., 2013; Goncalvez et al., 2002; Messer et al., 2003; Moi et al., 2010; Vasilakis et al., 2008a; Warrilow et al., 2012; Yousseu et al., 2018). Majority of these sequences were deposited arbitrarily from several sources. There is sparse information on the temporal and spatial distribution of circulating Dengue genotypes in the African region.

Studies have shown that emergence of new serotypes or shifts in circulating genotypes of Dengue viruses in a region increases the severities of infection during outbreaks (Shrivastava et al., 2018). Furthermore, since there are no licensed drugs for dengue infection and vaccines against the virus is restricted to persons with previous

Table 1
Oligonucleotide species-specific primers used for Dengue virus NS5 gene nested PCR amplification and serotyping.

Primer	Sequence (5′–3′)	Amplicon (bp)
nDENV1(−)	CGTTTTGCTCTTGTGTGCGC	472
nDENV2(−)	GAACCAGTTTGTTTDRTTTCATAGCTGCC	316
nDENV3(−)	TTCCTCGTCCTCAACAGCAGCTCTCGCACT	659
nDENV4(−)	GCAATCGCTGAAGCCTTCTCCC	222

Table 2
Patient characteristics based on sex, age groups and location showing the prevalence of Dengue virus infection.

	Negative	Positive (%)	Total	*P* value
Sex				
Male	53	1 (1.9)	54	
Female	66	10 (13.1)	76	.0256
Total	119	11 (8.5)	130	
Age groups				
≤5 years	56	5 (8.2)	61	
6–14 years	27	2 (6.9)	29	
15–24 years	9	0	9	
25–34 years	12	1 (7.7)	13	.47239
35–44 years	9	1 (10.0)	10	
45–60 years	6	2 (25.0)	8	
Total	119	11 (8.5)	130	
Location				
Alimosho HC	65	9 (12.2)	74	
Orile Agege HC	54	2 (3.6)	56	.1135
Total	119	11 (8.5)	130	

Statistical significance level was determined using Fisher's exact.

exposure (Galula et al., 2014; Rabaa et al., 2017; Villabona-Arenas et al., 2016), there is the need for continuous surveillance of circulating Dengue serotypes and genotypes in endemic regions. This information will prove very useful for vaccines and drugs development (Halstead, 2013; Usme-Ciro et al., 2014). It will also aid in response to Dengue outbreaks.

This study was designed to identify circulating DENV serotypes and genotypes among persons presenting with febrile illness in two health centers in Lagos, Nigeria. Phylogenetic analysis to determine spatial and temporal patterns of Dengue sequences collected previously from African countries and submitted to GenBank was also performed.

2. Materials and methods

2.1. Patient characteristics and study settings

This cross-sectional study was conducted in Alimosho and Orile-Agege local government areas of Lagos State, South western Nigeria. As shown in Fig. 1, Alimosho and Orile-Agege areas are located within the Lagos metropolitan area of Ikeja Division. Alimosho is the largest local government in Lagos state with over 1.2 million inhabitants as at 2006, most of which are Yorubas. On the other hand, Orile-Agege is a multi-ethnic community in which the Yorubas are predominant with the presence of sparse population of non-Yoruba speaking people. The area is home to over 650,000 people as at 2006.

The samples were collected from out and in patients presenting with fever (> 37.5 °C) at pediatrics, general medicine and gynecology units of Alimosho and Orile-Agege General Hospitals. These hospitals are the major public hospitals in these communities and open to everyone. Accessibility and availability of resident physicians were major requirements used for the selection of these hospitals. For phylogenetic analysis of previously deposited sequences, summary data for circulating Dengue serotypes and genotypes in Africa were retrieved from Dengue Geographic Viewer (Yamashita et al., 2016). This information was updated manually with available Dengue sequences in GenBank

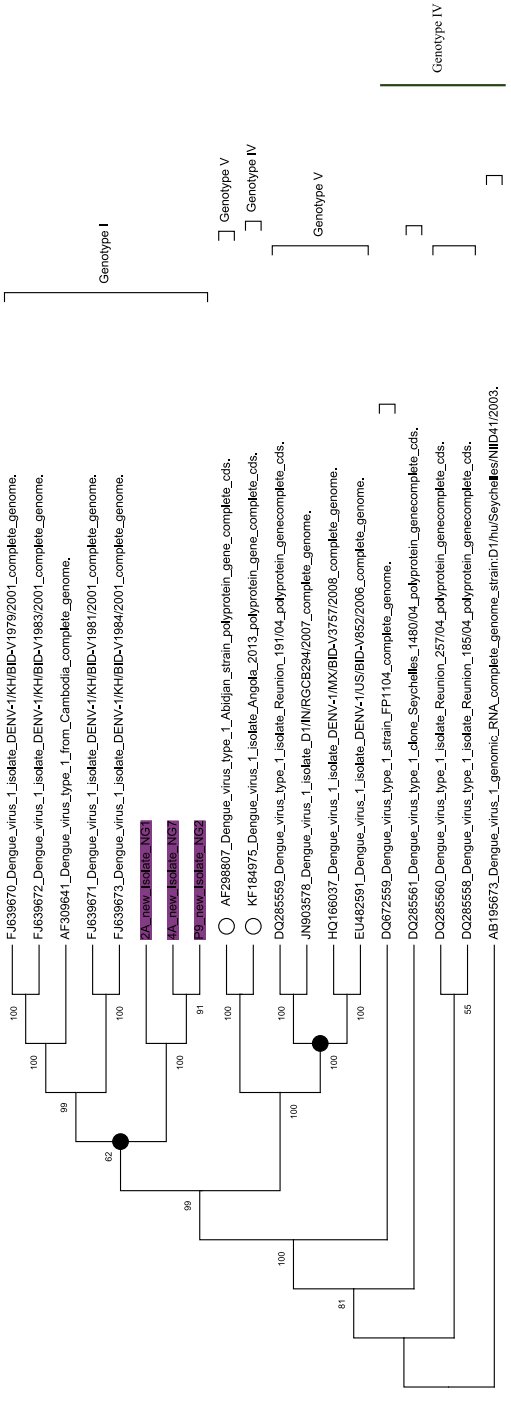


Fig. 2. Phylogenetic tree of the NS5 gene of DENV 1 genotypes isolated from Lagos, Nigeria.
The main genotypes are indicated using roman numerals at the node of the lineage. The countries of isolation of the strains are indicated next to the strain names. The serotypes circulating in Lagos, Nigeria are indicated by the sample ID, color and code-'new isolate NG'. Phylogenetic studies were conducted by using MEGA version 10. Genetic distances were calculated with the Tamura-Nei model at the nucleotide level. Phylogenetic tree was generated using the maximum likelihood method. The robustness of the tree was evaluated with 1000 bootstrap replicates. Due to the low numbers of alignable NS5 sequences collected from African countries, sequences from other regions were included in the phylogenetic analysis.

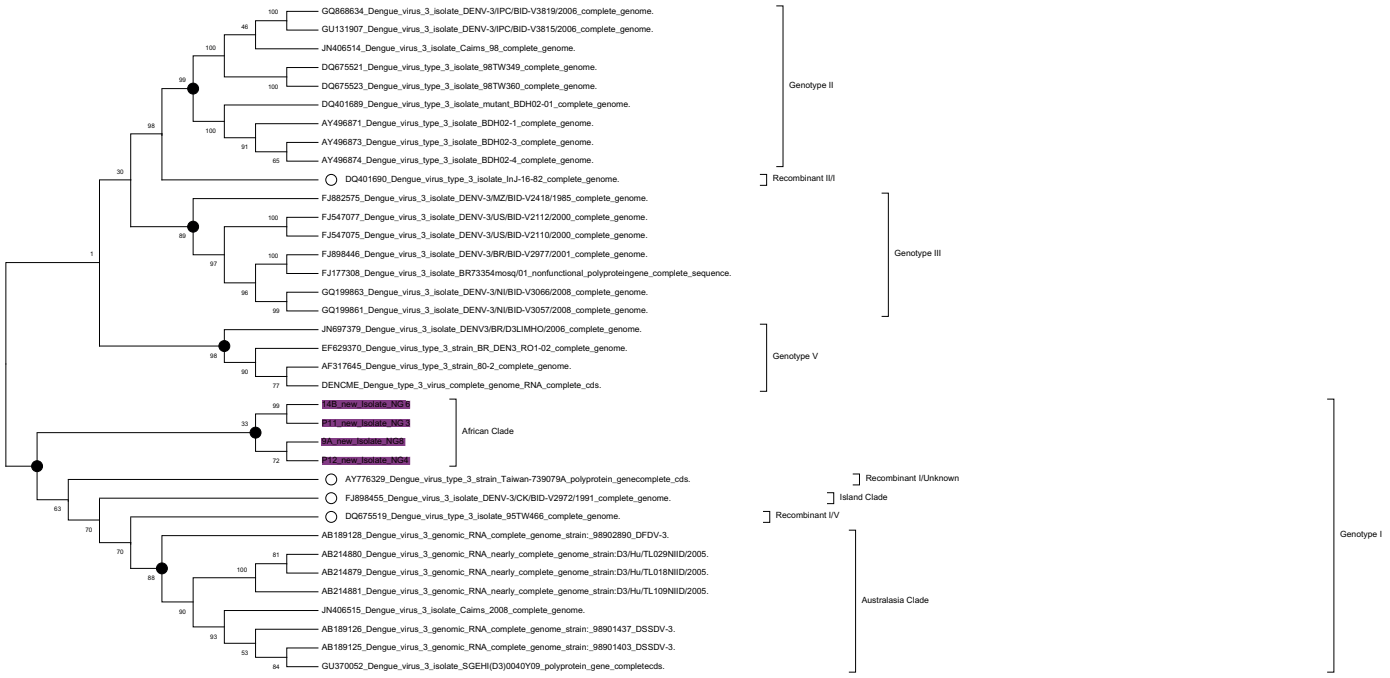


Fig. 3. Phylogenetic tree of the NS5 gene of DENV 3 genotypes isolated from Lagos, Nigeria.
The main genotypes are indicated using roman numerals at the node of the lineage. The countries of isolation of the strains are indicated next to the strain names. The serotypes circulating in Lagos, Nigeria are indicated by the sample ID, color and code-'new isolate NG'. Phylogenetic studies were conducted by using MEGA version 10. Genetic distances were calculated with the Tamura-Nei model at the nucleotide level. Phylogenetic tree was generated using the maximum likelihood method. The robustness of the tree was evaluated with 1000 bootstrap replicates. Due to the low numbers of alignable NS5 sequences collected from African countries, sequences from other regions were included in the phylogenetic analysis.

Table 3
Description of Dengue serotypes and genotypes with accompanying symptoms identified from febrile Nigerian patients.

S/N	ID	Serotype	Genotype	Symptoms	Accession number
1	2A_new_isolate_NG1	1	I	Rashes	MK045280
2	4A_new_isolate_NG7	1	I	Headache	MK045281
3	P9_new_isolate_NG2	1	I	Joint pain	MK045284
4	10A_new_isolate_NG9	1	Not sequenced[a]	Rashes	N/A
5	13B_new_isolate_NG10	1	Not sequenced[a]	Headache	N/A
6	P11_new_isolate_NG3	3	I	Rashes	MK045285
7	P12_new_isolate_NG4	3	I	Rashes	MK045286
8	14B_new_isolate_NG6	3	I	Joint pain	MK045283
9	9A_new_isolate_NG8	3	I	Joint pain	MK045282
10	11A_new_isolate_NG11	3	Not sequenced[a]	Headache	N/A
11	1A_new_isolate_NG5	3	Not sequenced[a]	Rashes	N/A

[a] These samples gave unreadable sequences.

database collected recently from African countries. Supplementary file 1 gives the raw data used for the analysis.

2.2. Recruitment of participants, sample collection and processing

Participants were recruited after obtaining their informed consent. Informed verbal and/or written consent was obtained from patients, parents and guardians who allowed their children to take part in the study. Those who were unable or unwilling to give a written consent were excluded. The patients were assured of confidentiality of their information. Participants were also subjected to the following criteria: patients with symptoms of fever ($> 37.5\,^{\circ}C$) as well as with more than or equal to one of the following symptoms: joint pain, rash, myalgia, headache, retro-ocular pain, abdominal pain and hemorrhagic manifestation based on 2009 WHO Dengue classification. One hundred and thirty participants referred by physicians, who met our study criteria as well as consented to participate, over a space of 5 months from April to August 2018 were included in the study. Five milliliters of whole blood was collected from each participant into bottles containing EDTA anticoagulant. Plasma was separated from each sample in the field, stored at $-20\,^{\circ}C$ and transported in a cold chain to the Department of Microbiology, University of Lagos where they were stored until analyzed. Samples that were positive by DENV RT-PCR were serotyped using typing primers while genotypic analysis were carried out by sequencing and phylogenetics of the NS5 gene sequence.

2.3. Eligibility/exclusion criteria

Those who were unable or unwilling to give a written consent were excluded.

2.4. PCR amplification and serotyping

Viral RNA was extracted using E.Z.N.A viral RNA kit following manufacturer's instructions. Dengue virus NS5 gene was amplified using duplex reverse transcription –PCR followed by nested PCR assay

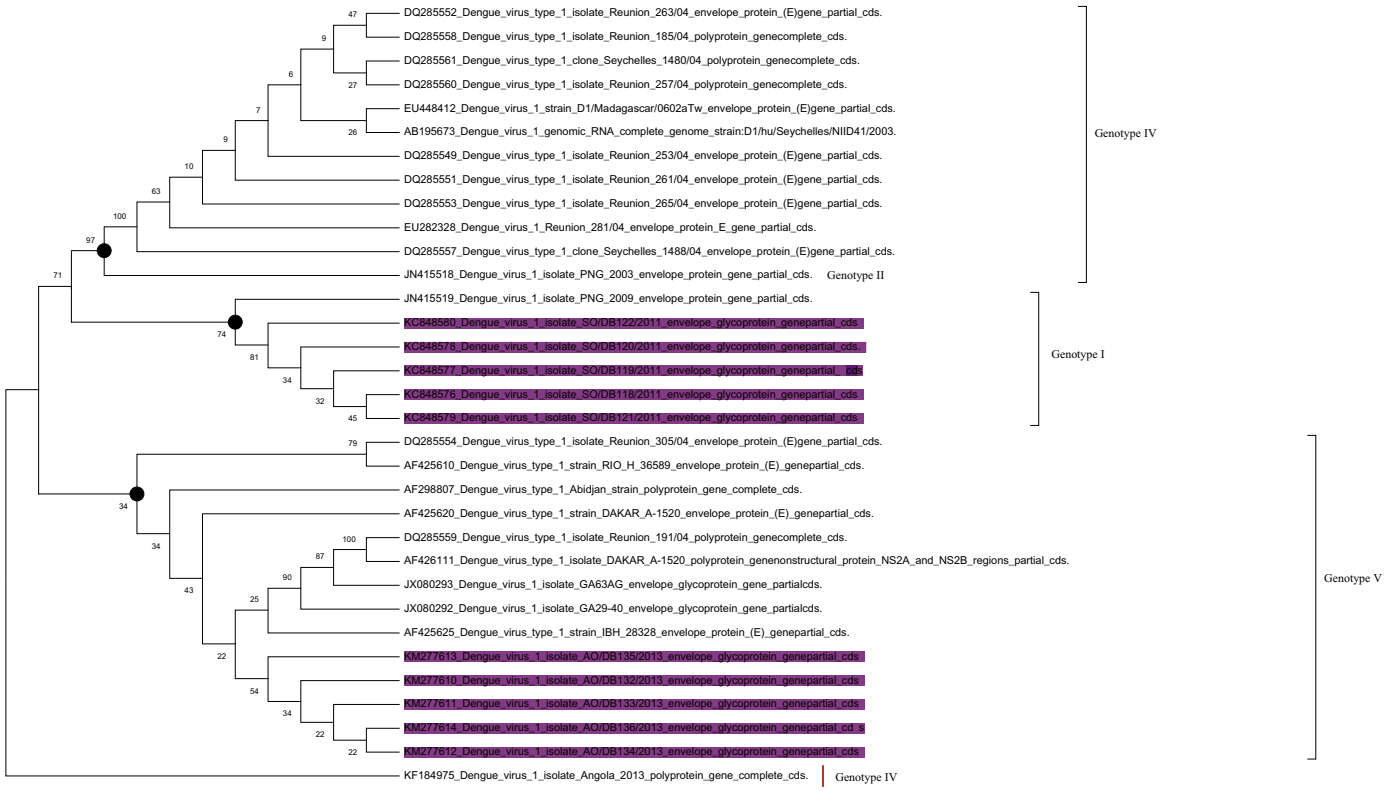


Fig. 4. Phylogenetic tree of previously unknown DENV 1 genotypes collected from African countries.
The main genotypes are indicated using roman numerals at the node of the lineage. The countries of isolation of the strains are indicated next to the strain names. The unknown DENV 1 genotypes previously identified in Africa are highlighted in color. Phylogenetic studies were conducted by using MEGA version 10. Genetic distances were calculated with the Tamura-Nei model at the nucleotide level. Phylogenetic tree was generated using the maximum likelihood method. The robustness of the tree was evaluated with 1000 bootstrap replicates. All sequences used for analysis were previously identified DENV genotypes known to circulate in African countries.

as previously described(de Morais Bronzoni et al., 2005). Briefly, reverse transcription was done using genus specific primer set – forward FG1 (TCAAGGAACTCCACACATGAGATGTACT) and reverse FG2 (GTGTCC CATCCTGCTGTGTCATCAGCATACA) which anneals to the NS5 gene of *Flavivirus*, producing amplicons of approximately 958 bp.

Multiplex nested PCR was then used to detect Dengue virus serotypes 1, 2 and 3. Due to closeness in amplicon sizes of Dengue virus serotypes 2 and 4, a different set up of conventional PCR was used to screen for Dengue virus serotype 4. Forward primer FG1 and specific inner primers used for Dengue virus serotypes detection are as shown in Table 1. Amplicons were detected and serotyped by gel electrophoresis.

2.5. Identification of genotypes and phylogenetics

Amplicons were shipped on ice to a commercial laboratory for Big Dye® Terminator cycle sequencing using FG1 and FG2 primers. The sequences were cleaned, edited and consensus sequencing generated using CLC Main Workbench v8 (Qiagen, Denmark). Genotypes were determined by phylogenetic analysis of the NS5 gene by comparing consensus sequence of each sample to reference sequences of corresponding serotypes. Due to the low numbers of alignable NS5 sequences collected from African countries, sequences from other regions were included in the phylogenetic analysis. Phylogenetic analyses were performed using MEGA software version 10. Alignment of sequences were performed using Clustal W algorithm.

Genetic distances were inferred using the Tamura-Nei model and a phylogenetic tree was generated using the maximum likelihood method. The robustness of the tree was evaluated with 1000 bootstrap replicates. All consensus nucleotide sequences obtained in this study were submitted to GenBank Database and assigned accession numbers MK045280-MK045286. Phylogenetics of submitted Dengue sequences collected from Africa with previously unknown genotypes were also performed using MEGA software version 10 as described above. In instances where available sequences collected from Africa in GenBank database was not sufficient for analysis, alignable sequences from other regions of the world were included for the analysis.

2.6. Temporal and spatial analysis of circulating dengue serotypes and genotypes in Africa

We implemented geographical distribution of each DENV genotype on the map of Africa in a user- specified time span. A summary sheet of the raw data used to generate the geographical distribution is also available in the supplementary file1.

2.7. Data management and statistical analysis

Analyses were performed using SPSS version 20. Categorical variables were compared using chi-square tests as applicable. Significance was set at $P < .05$.

3. Results

3.1. Patient characteristics and burden of Dengue virus infection in Lagos, Nigeria

One hundred and thirty consecutive patients with febrile illness were recruited for this study. There were no records of decline, persons that did not participate in the study were those that failed to meet our eligibility criteria which were people that were neither rational nor

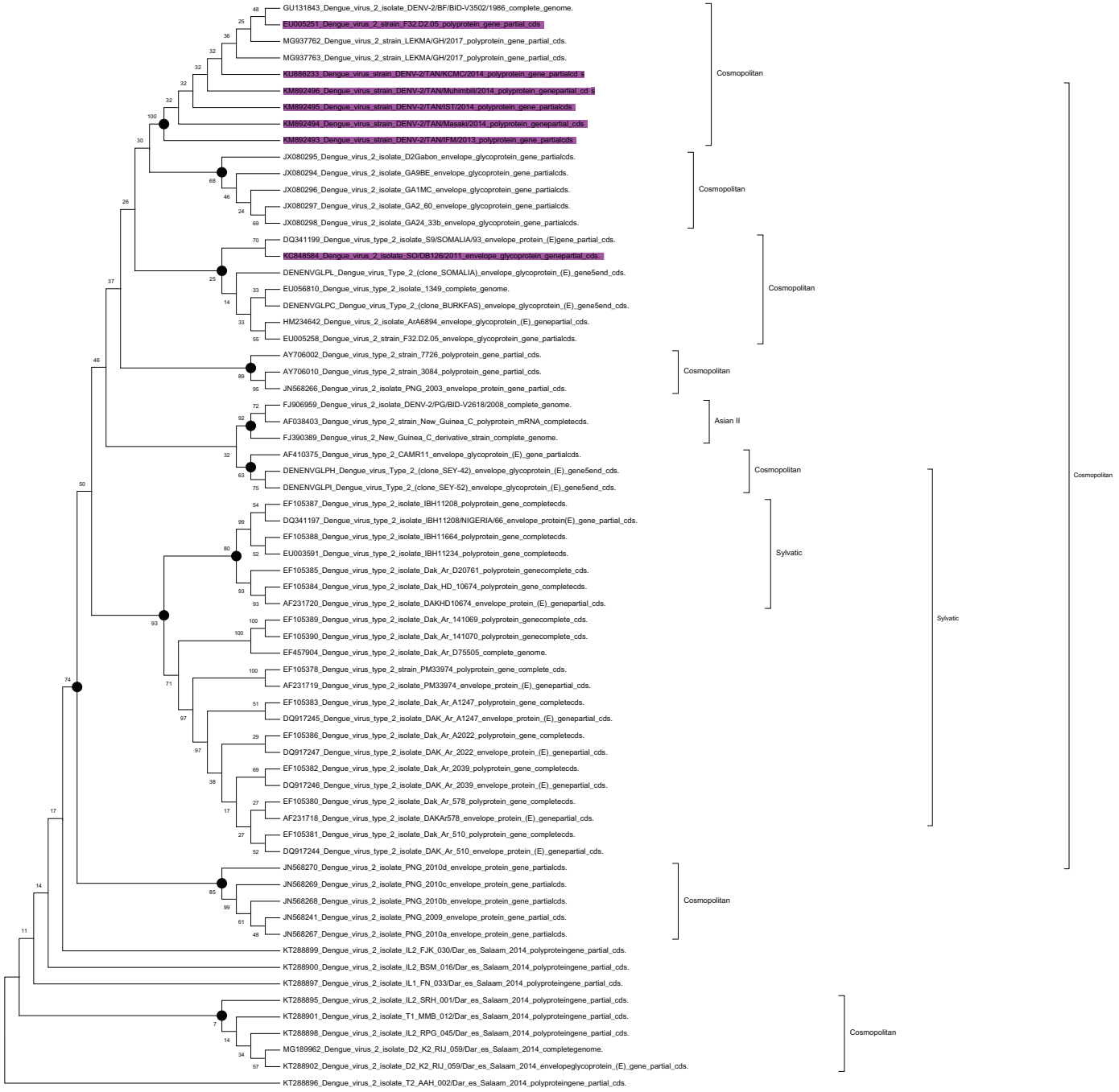


Fig. 5. Phylogenetic tree of previously unknown DENV 2 genotypes collected from African countries.
The main genotypes are indicated using roman numerals at the node of the lineage. The countries of isolation of the strains are indicated next to the strain names. The unknown DENV 2 genotypes previously identified in Africa are highlighted in color. Phylogenetic studies were conducted by using MEGA version 10. Genetic distances were calculated with the Tamura-Nei model at the nucleotide level. Phylogenetic tree was generated using the maximum likelihood method. The robustness of the tree was evaluated with 1000 bootstrap replicates. All sequences used for analysis were previously identified DENV genotypes known to circulate in African countries.

conscious to give informed consent. Seventy six (58.4%) of these individuals were female and the median age of the participants was 6 years (mean age: 13.32 years; range 1–60 years). Over 47% of the participants were aged less than or equal to 5 years. A total of 11 persons were diagnosed positive for dengue infection by RT-PCR, giving a prevalence of 8.5%.

The prevalence varied by sex, age group and location. As shown in Table 2, females had a significantly higher ($P = .033$) prevalence compared to males. Individuals presenting at Alimosho health center also had a higher prevalence (12.2%) compared to those presenting at Orile-Agege health center (3.6%), although the difference was not significant ($P = .081$). The vulnerable population, that is, children less than or equal to 5 years old (8.2%) and adults above 50 years (25%) had an higher prevalence than adolescents (6.9%) and the sexually active group (7.7%). Five (45.4%) of the individuals with Dengue virus infection presented with rashes while others had either headache or joint pains.

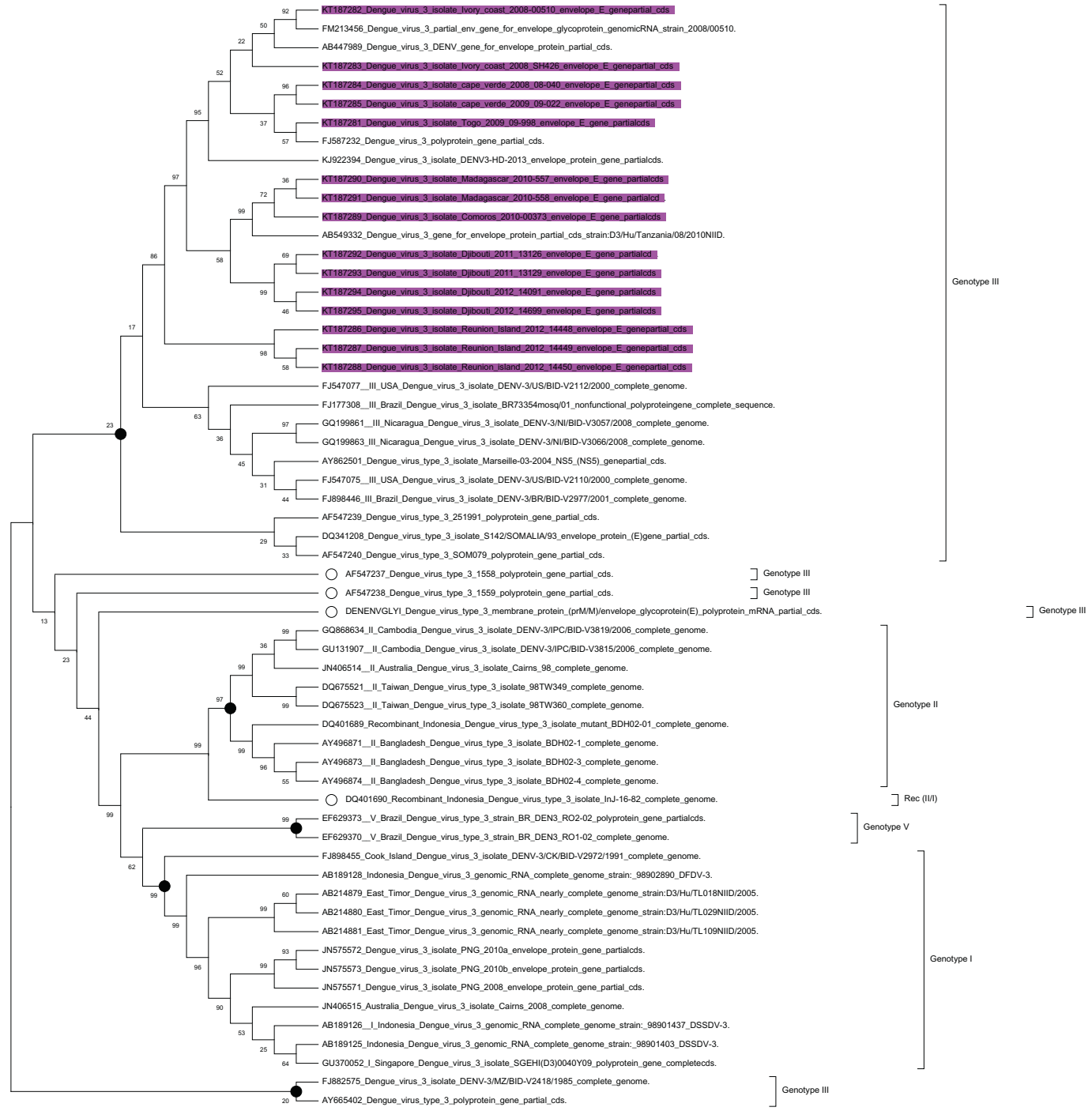


Fig. 6. Phylogenetic tree of previously unknown DENV 3 genotypes collected from African countries.
The main genotypes are indicated using roman numerals at the node of the lineage. The countries of isolation of the strains are indicated next to the strain names. The unknown DENV 3 genotypes previously identified in Africa are highlighted in color. Phylogenetic studies were conducted by using MEGA version 10. Genetic distances were calculated with the Tamura-Nei model at the nucleotide level. Phylogenetic tree was generated using the maximum likelihood method. The robustness of the tree was evaluated with 1000 bootstrap replicates. Due to the low numbers of alignable sequences collected from African countries, sequences from other regions were included in the phylogenetic analysis.

3.2. Dengue virus serotypes and phylogenetic analyses

The eleven isolates detected were successfully serotyped whereas seven of the 11 isolates sequenced were genotyped. Figs. 2 and 3 show the estimated phylogeny of the DENV 1 and 3 serotypes respectively, with respect to relevant sequences available in GenBank database. As shown in both figures, all DENV 1 serotypes were grouped into genotype I while DENV 3 were also grouped into genotype I. DENV 1 Nigerian strains were closely related to those from Cambodia. This is the first detection of DENV 1 genotype I in Africa. DENV 3 Nigerian strains were closely related to strains from Taiwan, Cook Island and Indonesia. This genotype was also detected for the first time in the continent. As shown in Table 3, DENV1 and 3 detected in this study was associated with rashes, headache and joint pains.

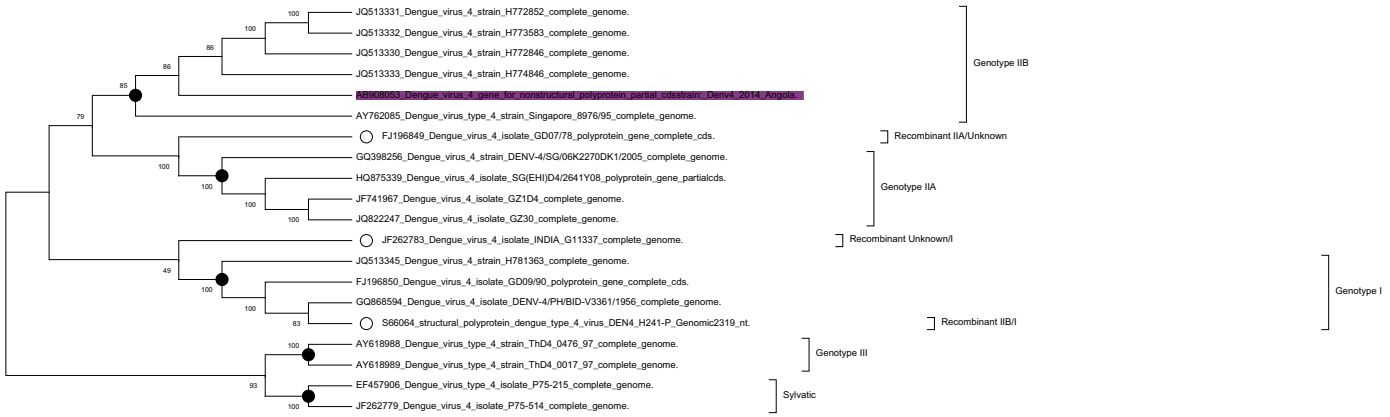


Fig. 7. Phylogenetic tree of previously unknown DENV 4 genotype collected from African countries.
The main genotypes are indicated using roman numerals at the node of the lineage. The countries of isolation of the strains are indicated next to the strain names. The unknown DENV 4 genotype previously identified in Angola is highlighted in color. Phylogenetic studies were conducted by using MEGA version 10. Genetic distances were calculated with the Tamura-Nei model at the nucleotide level. Phylogenetic tree was generated using the maximum likelihood method. The robustness of the tree was evaluated with 1000 bootstrap replicates. Due to the low numbers of alignable sequences collected from African countries, sequences from other regions were included in the phylogenetic analysis.

3.3. Phylogenetic analyses of previously unknown genotypes of DENV sequences collected from various regions in Africa

Figs. 4–7 show the estimated phylogeny of DENV 1-4 nucleotide sequences collected from various African countries. Serotypes with previously unknown genotypes are in bold fonts.

3.3.1. DENV 1 genotyping

As shown in Fig. 4, Dengue serotypes of Somalia origin with accession numbers KC848576-848580 were classified as genotype I. These sequences were closely associated with a sequence collected from Papua New Guinea. Similarly, Dengue serotypes collected from Angola (accession numbers KM277610-277614) were classified as genotype V. They were associated with sequences collected from Reunion, Senegal, Gabon and Nigeria.

3.3.2. DENV 2 genotyping

As shown in Fig. 5, all DENV-2 serotypes analyzed were grouped into Cosmopolitan genotype. Isolate EU005251 from Ghana was associated with other strains from BurkinaFaso, Ghana as well as with isolates KM892493-892496 and KU886233 from Tanzania. DENV 2 with accession number KC848584 from Somalia was closely related to other isolates from Somalia, BurkinaFaso and Ghana.

3.3.3. DENV 3 genotyping

As shown in Fig. 6, all DENV 3 sequences analyzed for genotypes were grouped into Genotype III. Isolate with accession number KT187282 from Ivory Coast was closely related to other strains from Ivory Coast including isolate KT187283. Other isolates from Ivory Coast, Cape Verde and Togo (accession numbers-KT187283-187285 and KT187281) were closely related. DENV 3 serotypes from Madagascar and Comoros (KT187289-291) were associated and related closely with another isolate from Tanzania. All DENV 3 sequences from Djibouti (KT187292-187295) and Reunion Island (KT187286-187288) also clustered together.

3.3.4. DENV 4 genotyping

Since isolate AB908053 from Angola is the only DENV4 identified in Africa, analysis were done with other strains around the world. The isolate was genotyped as group IIB and was closely related to isolates from Brazil and Singapore. Table 4 gives a summary of the assigned genotype of previously unknown Dengue serotypes circulating in the African continent.

3.4. Temporal and spatial analyses of circulating DENV genotypes in Africa since 1944

Spatial and temporal distribution of DENV genotypes across the various regions of the African continent are illustrated in Fig. 8A and B respectively. Genotype V have been the predominant DENV1 circulating in the continent since 1968. Recently, other genotypes such as I, II and IV have been detected. DENV 2, cosmopolitan genotype is the predominant circulating strain in the continent both in number and coverage. It appears as if the sylvatic genotype have been replaced with this genotype over the decades. DENV 3 genotype III have been the only genotype detected since 1985 across all the regions of the continent. The other genotype (I) was just detected for the first time in this study. DENV 4 is relatively unknown to the continent. A single genotype, IIB, detected for the first time in 2014 in Angola is the only identified genotype till date. No DENV genotype(s) have been identified in the northern and southern parts of Africa.

4. Discussion

The results of this study show that Dengue virus serotypes 1 (genotype I) and 3 (genotype I) are actively circulating in Lagos Nigeria. It was observed among our participants that Dengue fever is a major cause of hospital presentations especially among children under 5 years and older adults. Also, we found out in this study that over the past four decades there is a relative stability in the circulating DENV genotypes in the continent. DENV 1 genotype V, DENV 2 cosmopolitan genotype and DENV 3 genotype III has over the years been the predominant circulating Dengue strains in the continent. However, pockets of new genotypes seem to be emerging across the continent. DENV 4 genotype IIB was described recently in Angola while DENV 1 genotype I and DENV 3 genotype I were described in this study for the first time as circulating in the continent. It seems that Dengue serotypes circulating in the continent are skewed to those associated with outbreaks, severe infections and imported cases (Caron et al., 2013; Dias et al., 2019; Eldin et al., 2016; Faye et al., 2009; Goncalvez et al., 2002; Grange et al., 2014; Messer et al., 2003).

In this study, Dengue fever was detected in every age group studied except persons aged 15–24 years. This pattern is consistent with previous studies in the African continent that have investigated this relationship (Onoja et al., 2016; Oyero and Ayukekbong, 2014; Ridde et al., 2016). Older people are at a higher risk of Dengue virus re-exposures especially to a different serotype in hyper-endemic regions

Table 4
Genotype classification of previously unknown Dengue serotypes circulating in Africa.

Accession	Serotype	Country	Year	Assigned genotype	Reference
KC848576	1	Somalia	2013	I	Unpublished[a]
KC848577	1	Somalia	2013	I	Unpublished
KC848578	1	Somalia	2013	I	Unpublished
KC848579	1	Somalia	2013	I	Unpublished
KC848580	1	Somalia	2013	I	Unpublished
KM277610	1	Angola	2014	V	Unpublished
KM277611	1	Angola	2014	V	Unpublished
KM277612	1	Angola	2014	V	Unpublished
KM277613	1	Angola	2014	V	Unpublished
KM277614	1	Angola	2014	V	Unpublished
EU005251	2	Ghana	2007	Cosmopolitan	Unpublished
KC848584	2	Somalia	2013	Cosmopolitan	Unpublished
KM892493	2	Tanzania	2014	Cosmopolitan	Unpublished
KM892494	2	Tanzania	2014	Cosmopolitan	Unpublished
KM892495	2	Tanzania	2014	Cosmopolitan	Unpublished
KM892496	2	Tanzania	2014	Cosmopolitan	Unpublished
KU886233	2	Tanzania	2016	Cosmopolitan	Unpublished
AY862501	3	Senegal/ Guinea/Sierra Leone	2004	III	Unpublished
FM213456	3	Ivory Coast	2008	III	Unpublished
KJ922394	3	Togo/Benin/ Burkina Faso	2014	III	Unpublished
KT187281	3	Togo	2015	III	Unpublished
KT187282	3	Ivory Coast	2015	III	Unpublished
KT187283	3	Ivory Coast	2015	III	Unpublished
KT187284	3	Cape Verde	2015	III	Unpublished
KT187285	3	Cape Verde	2015	III	Unpublished
KT187286	3	Reunion Island	2015	III	Unpublished
KT187287	3	Reunion Island	2015	III	Unpublished
KT187288	3	Reunion Island	2015	III	Unpublished
KT187289	3	Comoros	2015	III	Unpublished
KT187290	3	Madagascar	2015	III	Unpublished
KT187291	3	Madagascar	2015	III	Unpublished
KT187292	3	Djibouti	2015	III	Unpublished
KT187293	3	Djibouti	2015	III	Unpublished
KT187294	3	Djibouti	2015	III	Unpublished
KT187295	3	Djibouti	2015	III	Unpublished
AB908053	4	Angola	2014	IIB	Parreira et al. (2014)

[a] These sequences were deposited directly into the GenBank database without any eventual publication where these sequences (or accession numbers) were cited.

(Cummings et al., 2009; Nisalak et al., 2003). It is interesting that young person seems to be immune from symptomatic Dengue fever. Further studies to identify the correlates of this protection are needed.

Findings from this study also showed that Dengue fever was significantly higher in female participants compared to their male counterparts. This may be due to the fact that more females are known to seek care in hospitals compared to males (Hunt et al., 2011). Also, females are known to be more immune competent which may lead to a higher proportion of dengue infection (Chakravarti et al., 2016). Furthermore, it was found that Dengue fever was associated with rashes, joint pain and headache in this study. No severe forms of Dengue was identified. The black gene pool have been shown to be resistant to severe Dengue infection (Halstead and Cohen, 2015; Sierra et al., 2017; Sierra et al., 2007).

Detection of Dengue virus serotypes 1 and 3 from persons with febrile illness re-affirms the view that these serotypes are actively circulating in Lagos State, Nigeria. All Dengue virus serotypes have been detected in the country although none of these studies used molecular methods (Baba et al., 2009; CAREY et al., 1971; Fagbami et al., 1977; Oyero and Ayukekbong, 2014). Temporal studies in Taiwan have established that co-circulation of DENV 1 and 3 in a region enable clades and genotypes replacements. Occurrence of multiple serotypes create pools for genetic recombination to take place (Cai et al., 2017).

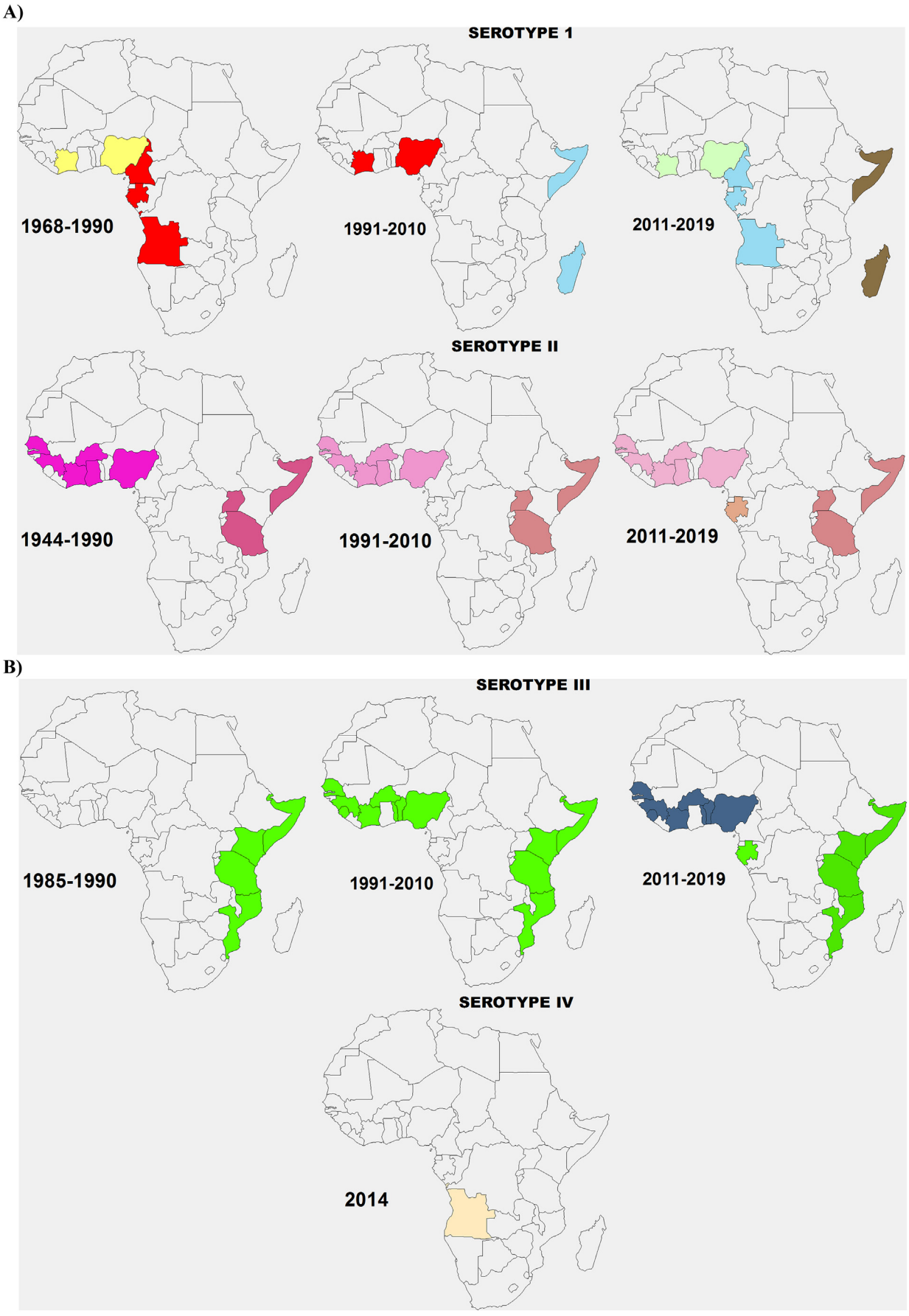
DENV1 and 3 have been previously detected in West African countries as well as in other parts of the continent (Domingo et al., 2011; Faye et al., 2009; Franco et al., 2010; Konongoi et al., 2016; Tarnagda et al., 2018; WHO, 2009). Prior to the detection of genotypes I of DENV 1 and 3 in this study, genotypes V for DENV 1 and III for DENV3 have been the predominant circulating strains in Africa. However, this has to be interpreted with caution as we have showed in this study that DENV 1 genotype I was circulating in Somalia by 2013. Most studies on Dengue infection in the country are restricted to outbreaks, imported cases and severe Dengue infections. This pattern ultimately skew data on Dengue viruses to those associated with severe infections whereas silently circulating genotypes are not identified. It is important that changes and shifts in circulating DENV genotypes are properly monitored as this phenomenon have been showed to be followed by major outbreaks (Jiang et al., 2019).

Over the years, DENV 2 Cosmopolitan genotype has been the predominant serotype/genotype in the continent, although the sylvatic genotype is still found to be circulating in West Africa especially Nigeria (Galula et al., 2014; Vasilakis et al., 2008b; Yamashita et al., 2016). Several studies have also documented the global distribution of DENV 2 Cosmopolitan genotype as well as the use of the strain for vaccine studies (Galula et al., 2014; Halstead, 2013; Kamau et al., 2019; Twiddy et al., 2002). For the West African continent and the African region as a whole, vaccine studies should include the sylvatic strain because the serotype is still circulating in the region and the possibility of re-emergence cannot be ruled out (Vasilakis et al., 2007). Due to the gross under-representation of Dengue sequences circulating in the African region in the GenBank database, it is difficult to conclusively say that the sylvatic strain of DENV 2 has been completely replaced by the Cosmopolitan genotype. Resistance of the sylvatic strain to vaccine candidates have been previously described (Galula et al., 2014).

DENV 3 genotype III has been the most predominant genotype of this serotype in Africa over the past three decades. The other genotype detected in the region is I genotype and was identified for the first time in this study to the best of our knowledge. DENV 3 genotype III is associated with disease outbreaks and severe dengue infections (Dias et al., 2019; Faye et al., 2014; Messer et al., 2003; Moi et al., 2010; Ninove et al., 2009). There is the possibility of silent circulation of other genotypes aside genotype III in the African continent especially among asymptomatic individuals and persons with mild infections just as was detected among febrile patients in this study. This suspicion is plausible since all previous detections of DENV 3 genotype III was due to its associations with disease outbreaks or severe dengue infections.

Very limited information exists on the molecular epidemiology of DENV4 serotypes in Africa. Only one sequence of this serotype is available in the GenBank database and it was isolated from a woman who visited Angola (Parreira et al., 2014). In this study, this DENV4 serotype was classified as genotype IIB. Genotype IIB has been shown to be easily transmissible and was responsible for over a million cases of Dengue in Brazil in the year 2013(Ortiz-Baez et al., 2019). There is presently scanty or no data on the circulating Dengue serotypes in North and South Africa (Amarasinghe et al., 2011). This calls for action as there is documented evidence of the mosquito vector in South Africa (Amarasinghe et al., 2011) while few studies have postulated the possibility of dengue infection in North Africa.

Weak surveillance systems and low awareness of Dengue infection among physicians in sub Saharan continent are major barriers to effective control of Dengue (Amoako et al., 2018; Stoler and Awandare, 2016). There is gross underrepresentation of Dengue serotypes circulating in Africa in the GenBank database compared to other regions. This calls for an intentional and coordinated surveillance of circulating DENV serotypes across the various regions of the continent. There is a need to develop robust human resources and infrastructure in Africa if



(caption on next page)

Fig. 8. (A) Spatial distribution of reported DENV 1 and 2 since 1944.
Darker-colored areas represent genotypes identified in the respective regions of Africa in the given period under consideration. The interpretation of the various colors are as named: DENV serotype 1- Yellow = V and I genotypes co-circulating, Red mars = V genotype only, Green = I genotype, Leather Brown = I, II and IV genotypes co circulating, Orange = V and IV genotypes co-circulating while Blue = IV and V co-circulating. DENV serotype 2- Orange = Sylvatic genotypes only, Coral = Cosmopolitan genotype only while Pink = Co-circulation of sylvatic and cosmopolitan genotypes. Intensity of color corresponds to a higher number of identified genotypes. (B) Spatial distribution of reported DENV 3 and 4 since 1985. Darker-colored areas represent genotypes identified in the respective regions of Africa in the given period under consideration. The interpretation of the various colors are as named: DENV serotype 3- Medium Apple = III genotype, Yellow = I genotype only while Larkspur Blur = III and I genotypes co-circulating in the region. DENV serotype 4- Colored area correspond to genotype IIB. Intensity of color corresponds to a higher number of identified genotypes.

effective and efficient control of Dengue and other similar viruses in the continent would be achieved(Munoz et al., 2015; Quick et al., 2016).

5. Conclusions

Taken together, our study supports the occurrence of Dengue in Lagos Nigeria. It also provides molecular evidence of active circulation of DENV1 (genotype I) and DENV 3 (genotype I) in the commercial capital of the country. This study also describes the spatial and temporal distribution of Dengue serotypes and genotypes in Africa. DENV 2 (cosmopolitan genotype) and DENV 3 (genotype III) are the predominant circulating strains in the continent while the prevalence of DENV 4 is relative low in the region. There is the need to describe Dengue genotypes circulating among asymptomatic individuals and persons with febrile illnesses in Africa as this will give a clearer picture of the diversity of Dengue genotypes circulating in the region.

Ethical approvals and consent to participate

Ethical approval for this research was obtained from the Institutional Review Board of Nigerian Institute of Medical Research (IRB /18/009) and the Lagos State Government Health Service Commission (LSHSC/2222/VOLIVB/283). Procedures were followed in accordance with the ethical standards of these committees and with the Helsinki Declaration of 1975, revised in 2000. All results were delinked from patient identifiers and anonymized. Participants were recruited after obtaining their informed consent. Informed verbal and written consent was obtained from patients, parents and guardians who allowed their children to take part in the study. The patients were assured of confidentiality of their information.

Consent for publication

Not applicable.

Availability of data and material

Sequence data that support the findings of this study have been deposited in GenBank with the accession codes: MK045280-MK045286.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

CIA, BAO and SAI designed the study. GOO and SAI executed the experiments. BAO and GOO performed data analysis. BAO wrote the first draft. CIA supervised the study and reviewed the first draft. All authors read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.103947.

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