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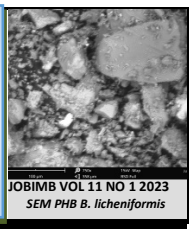

Seasonal Patterns and Genetic Variability of Aedes Mosquitoes in Some Selected Communities of Maiduguri Metropolitan Council, Borno State, Nigeria

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ABSTRACT

Aedes aegypti and *Aedes albopictus* mosquitoes spread dengue and yellow fever in Africa and worldwide. Rural-urban drift creates Aedes mosquito breeding sites through uncontrolled urbanization, inadequate urban infrastructure, lack of basic public health delivery, indiscriminate waste disposal, varying socioeconomic activities, and climatic changes. The above have happened in Maiduguri Metropolis since Boko Haram insurgencies began. This study will examine *Aedes aegypti*'s seasonal distribution, abundance, composition, and genetic variability in selected Maiduguri Metropolitan Council and Jere Local Government Council communities. From October 2016 to May 2017, CDC traps and vacuum aspirators collected adult mosquitoes from study sites and stored at – 80 °C. These were identified morphologically with taxonomy keys and stereomicroscopes, then molecularly with markers. Aedes mosquito population structure and genetic variability were determined using statistical software and molecular methods on samples. Hot-dry season has the highest relative abundance of this mosquito species in these study sites, while cold-dry season has the lowest. The molecular identification found *Aedes aegypti* in nine of twelve study locations but not *Aedes albopictus*. Some mosquitoes from the study locations had mutations due to carrying dengue virus or host population genes, but those without mutations showed a good phylogenetic relationship with Aedes aegypti mosquitoes from other countries, suggesting no genetic variability. The relative abundance of Aedes mosquitoes in Maiduguri Metropolitan Council increases the risk of dengue, zika, yellow fever, and chikungunya virus infections, and this mosquito species' seasonal distributions vary within and across seasons, but no genetic variability was found in the mosquitoes from the different locations used in this study.

INTRODUCTION

Aedes aegypti is a major vector of dengue and yellow fever viruses, the origin of which could be traced to be a wild, zoophilic species in sub-Saharan Africa, a location where considerable number of this species is still found [1]. But as time goes on, many populations of the species have been found to grow in human habitats and bite humans [1]. *Aedes aegypti* and *Aedes albopictus* are responsible for dengue fever virus transmission in tropical and sub-tropical areas such as Africa, including Borno State, Nigeria, the Americas, Asia, the Caribbean, and the Pacific [2-5]. It was reported by the World Health Organization (WHO) [6] and Fraga *et al*. [7] that about 2.5 billion people, comprising more than 40% of the world's population, are at risk of dengue

fever, and they also estimated about 50 million new cases of the disease.

Aedes aegypti is presently found throughout the tropics particularly in Maiduguri, Nigeria, where there is rapid human population growth due to migration of people from rural to urban areas as a result of insurgency in Borno State. This mosquito appears to be the major reason for the dramatic increase in the incidences of dengue fever. This domestic form was found to be responsible for the dengue outbreaks in Africa, particularly in West Africa (e.g. Senegal in 1990) [8]. Another specie of Aedes mosquito known as *Aedes albopictus* is mostly found in urban environment, rapidly grows, and takes advantage of human transportation, increasing contact between itself, humans and viruses [9]. It was originally thought to be an Asian forest mosquito but later discovered to be almost as domesticated as *Aedes aegypti.*

The two species of *Aedes* mosquitoes, *Aedes aegypti* and *Aedes albopictus,* have been found to be the principal vectors of dengue and yellow fever viruses the world over, which cause a wide range of illnesses varying from the asymptomatic (80%) or just a mild symptoms such as an uncomplicated fever [5,10,11]. In some cases they cause more severe illnesses (5%), and in a small proportion such cases are life threatening [5,10]. *Aedes aegypti* is mostly found in domestic areas, where they breed in and around human habitation. They feed on humans and lay their eggs in water-filled manmade containers (e.g. water storage jars and discarded containers). This species of mosquitoes and the diseases transmitted have therefore been on the increase in association with human population growth, economic development, increased mobility and uncontrolled urbanization, especially in Maiduguri Metropolitan Council (MMC) where there is massive movement of people from rural areas to the urban center to take refuge due to insurgency in Borno State [12-15].

Moreover, Nigeria has been linked to a danger of dengue fever outbreak, a disease transmitted by Aedes mosquitoes found within our surrounding environments and which happened to be in the same class with the dreaded and deadly Ebola Virus Disease [16]. There might be differences in the biting behavior, vector capacity and many other characteristics of high epidemiological significance, which are driven by genetic factors and may only be determined through molecular (DNA) markers [17-23, 7]. These DNA markers could be used to understand the relatedness of geographical populations and vector movements, which are helpful tools to analyze the risk associated with the transmission of diseases [24]. Genetic population structure as put forward by Balloux and Lugon-Moulin [25] usually results from "a combination of several contemporary and historical processes including dispersal ability of the species, mating patterns, environmental barrier to dispersal and demographic history".

Multi-markers have been previously used to carry out populations genetic studies on *Aedes aegypti* from different regions across the world [17-21, 26]. These studies have given new insights on the population structure and dispersion rates at both micro and macro geographic levels, clearly indicating that environmental, social factors and human interventions (Urbanization, controlled activities) affect the population structure of this vector [27]. The introduction of molecular tools such as karyotyping, restriction fragment length polymorphism (RFLP) and Polymerase chain reaction (PCR) has gradually improve the ability to resolve and identify species and subspecies down to the molecular level [28]. Random amplified polymorphism DNA (RAPD) and Restriction fragment length polymorphism (RFLP) appear to be the most commonly used molecular markers for the studies of genetic variations in *Aedes aegypti* [29,30].

Aedes aegypti showed both morphological and genetic variations and could therefore be considered as a heterogenous species. This heterogeneity seen in the population genetic structure of *Aedes aegypti* may be as a result of change in climatic conditions and the use of insecticides [31]. There have been various reports on the prevalence of dengue virus in Nigeria, for instance a prevalence of 0.5% for DENV- 1&2 and a high prevalence of DENV-3 antibodies in female patients (18.5%) compared to male patients (6.3%) with febrile illnesses attending University of Maiduguri Teaching Hospital, Borno State, Nigeria [32,33].

This study aimed to determine and assess the seasonal pattern in the distribution, abundance and composition of *Aedes aegypti* and its genetic variability in some selected communities of Maiduduguri Metropolitan Council in Borno State, North Eastern Nigeria. This study is imperative because the displacement of communities in Borno State due to the recent insurgencies has led to human population growth in the metropolitan center due to migration of people from rural to urban areas in search for refuge. The inadequate basic urban infrastructure and public health measures have created good and favorable breeding sites for the mosquitoes that transmit the virus [34]. The socio-economic activities of the people could have also contributed to the proliferation of the mosquitoes causing more harm to human health. In addition, climatic changes, ecological impact and the use of insecticides for public health and agricultural pest protection might have affected the population genetics of the mosquitoes but these are largely unreported, hence this study will fill the noticeable gaps and pave way for further studies.

MATERIALS AND METHODS

The study area

The study was conducted in Maiduguri, located in the semi-arid zone of Borno State, Nigeria with an area of about 69,436km^2 at latitude 10-30^0 N and longitude 12-15^{0}E. It lies within the savannah vegetation zone with low rainfall. Borno State is located in the North Eastern part of Nigeria. It is consist of twenty seven (27) Local Government Areas. It shares boundaries with Republic of Niger to the North, Chad Republic to the Northeast, Cameroun to the East, Adamawa and Yobe States to the South and West respectively. The main industrial center and capital city of Borno State is Maiduguri, the population of the entire state is estimated to be 4,151,193 [35]. The National Population Commission [35] reported that 64.37% of the populations are living in rural and 35.63% in urban areas. The major source of the state's economy is from agricultural and livestock farming. Up to 90% of the populations are farmers, while 10% hold white-collar jobs [35].

The region has a climate condition characterized by a dry season between November and April, a cold season from November to February, and a wet season between April to October. The hottest period has a temperature in the range of 31 to 44^{0}C and usually around April and/or May. The coldest period is from November to February and is usually characterized by a dusty harmmatan peak period in December and January. Annual rainfall ranges from 500-1000mm per annum, starting from June and ends in September in the North, and May to October in the southern parts [36, 37].

There are three vegetation zones in the state; the Sahel, Sudan savannah and the Northern Guinea Savannah vegetation zones. The latter is associated to the southern part of the state and is characterized by tall grass woodland savannah vegetation. The other two vegetation zones are found in the northern parts of the state and are characterized by shrub, thorn, bush and short grass savanna woodland. These zones are regarded as areas with high-risk of turning in to desert [38].

Determination of *Aedes* species distribution, abundance and composition

Periods and the locations of sample collection
Adult mosquitoes were collected from July to October 2016 (peak of rainy season), November 2016 to February, 2017 (cold dry-harmmatan season) and February to May, 2017 (hot dry season). The samples were obtained randomly from 12 selected geographical sites (Polo, Abaganaram, Adam Kolo, Zamamari, Dala, Kululuri, Bulumkutu,Gamboru Area, Baga road, Muna Chad Basin, Custom Area, Gwange), within Maiduguri Metropolitan Council and Jere local government area. The geographic coordinates (i.e. latitude and longitude) of each locality (site) was captured using GPS tracking device (Model: Garmin GPS 72H Marine Handheld).

Techniques for sample collection
The adult mosquitoes were collected using;
CDC light traps: A CDC light trap (Bio Quip Product Inc.) was used in each house on each night of trapping. The trap was suspended from the roof near a bed net and about 25cm above the ground in each of the houses.
The trap was operated twice weekly. Traps were switched on for eleven hours at 06:00pm local time and the people sleeping were asked to switch them off at 05:00 am after the neck of the bags used for the collection was tied. All the mosquitoes collected in the traps were removed from the catching bags and placed in bottles, which were labeled according to sites and dates of collection.

Vacuum aspirators: the mosquitoes were collected in both artificial and natural breeding places associated with human domicile using a vacuum aspirator and killed by freezing.

Storage of the samples
The samples were stored at -80^{0}C in Ultra Low Temperature Freezer (Model: Haier Biomedical), for DNA extraction.

Morphological identifications of *Aedes* species
The identification of the mosquito species was done by employing taxonomy identification [Hopkin, 1952[39] Highton, 1983[40] and Snell, 2005[41]] keys using Stereomicroscope (Model: Motic SMZ-140 series).

Confirmation of identification using molecular techniques
The identification was confirmed using molecular technique described by Hebert *et al*. [42] and Kumar *et al*. [43]**.** Phenol/chloroform method described by Rivero *et al*. [44] was employed to extract the DNA from individual whole mosquitoes. The **e**xtracted DNA was used for the amplification of the DNA fragment barcode (barcode) of ~700 nucleotides (nt) of mitochondrial gene cytochrome oxidase I with the MTNF / MTNR oligonucleotides as described by Hebert *et al*. [42] and Kumar *et al*. [43]. Each PCR mixture contained 1x NH_4SO_4 buffer, 1 mM each of the dNTP, 5 mM $MgCl_2$, 0.5 µM primers, 0.4µL of the Taq polymerase (Bioline, Thailand), 4µL DNA then making it to a final volume of 50 µL with molecular water (Gibco BRL). The amplification conditions of the Multigene thermocycler (Labnet, New Jersey) was set as follows: one cycle at 94 °C for 10 min; followed by 35 cycles at 95 °C for 60 s, 50 °C for 60 s and 72 °C for 60s respectively; then final extension at 72 °C for 5 min; and this was stored at
4 °C for preservation. The PCR product was visualized using UV transilluminator after it was run on 1% agarose gel and stained with GELSTAR® stain diluted 1/50.

Determination of *Aedes species* sample size for elucidation of genetic population structure
The sample size was calculated using the formula

$n = Z^2pq/d^2$ [25]

Where,
n=defined sample size
z= the standard normal deviation=1.96 for a normal distribution
p= the proportion of the target population estimated to have the desired characteristics.
The abundance and diversity of *Aedes* mosquitoes was estimated as 13.5 % (0.135) [25].
d=degree of accuracy desired, set at 0.05 i.e. 95% confidence interval.
q=1-p=1-0.135=0.87
$n=1.96^2x0.135x0.87/0.05^2=180$, approximately 200.

The inclusion criteria of this study are adult *Aedes* species and cross-seasonal collections while the exclusion criteria of this study are the pupae and larvae of *Aedes* species and the adult, pupae, and larvae of other mosquito species.

Elucidating the genetic variability and population structures
The process of elucidating the genetic variability and population structures involved extracting the DNA, amplification of the region of interest (ND4 gene), and sequencing the DNA. The results of which are analyzed using suitable statistical package. The procedures are described in detail below.

DNA extraction
The DNA was extracted from individual whole mosquitoes using a phenol/chloroform method described by Rivero *et al*. (2004). The DNA samples were rid of any contaminating RNA present by incubating each sample with 0.7 units of RNAse (Sigma, MO, USA) at 37^{0}C for 10 min. Separation of the DNA sample was achieved by running electrophoresis on 1% agarose gels and visualizing the DNA bands after staining with ethidium bromide solution as described by Coen *et al*. [45]. The samples were then stored at -80^{0}C.

Gene amplification
A 300-base pair (bp) region of the Nicotinamide Adenine Dinucleotide Dehydrogenase Subunits 4 (ND4) gene was amplified using the primers and reaction conditions described by Gorrochotegui *et al.* [18]. The Polymerase Chain Reaction (PCR) amplification was carried out in 50µl reaction volumes using 1µl of template DNA in a phenylthiocarbamide (PTC)-100 thermal Cycler (MJ Research, Inc., water town, MA). Negative control (a mixture containing all reagents except the template DNA) to detect any contamination was run along with the samples.

The PCR conditions followed that of Gorrochotegui *et al.* [18] with little modification involving the addition of Taq DNA polymerase to the mixture. The amplified product was visualized by electrophoresis in 2% agarose gels after being stained with ethidium bromide.

Sequencing of PCR products
The PCR primers designed by Herrera *et al*. [21] were used to sequence PCR products from 3-6 individuals of each haplotype. ABI PRISM Big Dye Terminator v 3.1 Cycle Sequencing Kit on an applied Bio System Model 310 Genetic Analyzer was used to perform the sequencing reaction.

Data analyses
The species distribution, abundance and composition of *Aedes* species was analyzed using student's t- test, and Least Significant Difference (L.S.D.) to show whether the species are even (richness) or not even [46]. Clustal 2.1 Omega software was used to analyze the multiple sequence alignment and Omega seven software was used to design the phylogenetic tree [47]. A cladogram was constructed among all collections using genetic distance matrices by means of unweighted pair-group method with arithmetic averaging analysis in the NEIGHBOR procedure of PHYLIP3.5C as described by Felsentein [48]. The phylogenetic tree was analyzed using the Maximum Likelihood method described by Tamura and Nei [49].

RESULTS

Species composition and abundance of *Aedes* mosquito populations in Maiduguri metropolis
The results presented in **Table 1**, showed the abundance and composition of *Aedes aegypti* in Maiduguri Metropolis and Jere Local Government Area. The results revealed that, Baga road (18.0%), had the highest abundance of *Aedes aegypti*, followed by Bulumkutu (16.5%), Dala (15.9%), Gwange (9.4%), Abaganaram (8.7%), Kululuri (8.1%), Muna Chad Basin and Custom area (5.8%), Polo (5.4%), Zamamari (3.6%), Adam Kolo (1.9%) and Gamboru area, had the lowest abundance of *Aedes aegypti*, with 0.9%, respectively.

Table 1. Species composition and abundance of *Aedes aegypti* mosquito populations in Maiduguri Metropolis.

Location	No. Collected	Abundance (%)
Polo	38	5.4
Abaganaram	61	8.7
Adam Kolo	14	1.9
Zamamari	25	3.6
Dala	112	15.9
Kululuri	57	8.1
Bulumkutu	116	16.5
Gamboru Area	6.0	0.9
Baga road	127	18.0
Muna Chad Basin	41.0	5.8
Custom Area	41.0	5.8
Gwange	66.0	9.4
TOTAL	704	100

Seasonal abundance of *Aedes aegypti* in Maiduguri Metropolis
The results presented in **Table 2**, showed the seasonal abundance of *Aedes aegypti* in Maiduguri Metropolis, which revealed that, highest abundance of *Aedes* aegypti were caught during cold dry season with 343, followed by rainy season with 209 *Aedes* aegypti, and 153 *Aedes aegypti* were caught during the hot dry season, respectively. The results also indicated that *Aedes aegypti* was moderately higher (P≤0.05) during the peak of rainy season from July to October 2016, with Abaganaram (48), having the highest abundance, followed by Gwange, (20.6%), Polo (16.7%), Dala and Bulumkutu (12.9%), Adam Kolo (6.7%), Gamboru area (2.4%) and the lowest during the rainy season was Gamboru (2.4%) and Zamamari (2.4%) area with five *Aedes* species, respectively. The results also indicated that during the hot-dry season there was a significant relatively higher abundance of *Aedes aegypti* in Kululuri with (37.3%) highest across the seasons, followed by Muna Chad Basin with (26.8%) *Aedes aegypti* during the hot dry season, Custom area (23.3%), Bulumkutu (9.1%) and Dala was relatively lower with (3.3%) of *Aedes aegypti* during the hot dry season respectively.

The results also revealed that the seasonal abundance of *Aedes aegypti* caught within and across the season are that the hot dry season had the highest percentage relative abundance of *Aedes aegypti,* with Kululuri having higher percentage of 37.3%, followed by Baga road with (37.1%) during cold dry season, Muna Chad Basin (26.8%) during hot dry season, Custom area (23,5%) hot dry season, Dala (23.4%) cold dry season, Abaganaram (22.9%) rainy season, Bulumkutu (21.9%) cold dry season, Gwange (20.6%), Polo (16.7%), Dala and Bulumkutu (12.9%) rainy season, Bulumkutu (9.1%) during hot dry season, Adam Kolo(6.7%) rainy season, Gwange (6.7%) cold dry season, Zamamari (5.8) cold dry season, Abaganaram (3.8%) cold dry season, Dala (3.3%), Gamboru & Custom area (2.4%) rainy season, Polo (0.9%) cold dry season, and Gamboru are(0.3%) during the cold dry season had the lowest abundance of *Aedes aegypti*.

Table 2. Seasonal abundance of *Aedes aegypti* in Maiduguri Metropolis.

Location	Rainy season n (%)	Cold-dry season n (%)	Hot-dry season n (%)
Polo	35(16.7)	3(0.9)	0.0(0.0)
Abaganaram	48(22.9)	13(3.8)	0.0(0.0)
Adam Kolo	14(6.7)	0.0(0.0	0.0(0.0)
Zamamari	5(2.4)	20(5.8)	0.0(0.0)
Dala	27(12.9)	80(23.4)	5(3.3)
Kululuri	0.0(0.0)	0.0(0.0)	57(37.3)
Bulumkutu	27(12.9)	75(21.9)	14(9.1)
Gamboru area	5(2.4)	1(0.3)	0.0(0.0)
Baga road	0.0(0.0)	127(37.1)	0.0(0.0)
Muna Chad Basin	0.0(0.0)	0.0(0.0)	41(26.8)
Custom area	5(2.4)	0.0(0.0	36(23.5)
Gwange	43(20.6)	23(6.7)	0.0(0.0)
Total	209(100)	342(100)	153(100)

Seasonal distribution of *Aedes aegypti* in Maiduguri Metropolis
The results in **Table 3**, showed the seasonal distribution of *Aedes aegypti* in Maiduguri Metropolitan. The mean and the standard error from the five (5) sites in each location was estimated using descriptive statistics and the significant difference at P (0.05) was obtained using the student t-test between and within the seasons. The results revealed that, in the seasonal distribution of *Aedes aegypti* in Polo area, there was significant difference between the rainy ($7.0\pm0.9_a$) and cold dry ($0.6\pm0.4_{a)}$ season, and between rainy ($7.0\pm0.9_a$) and hot dry ($0.0\pm0.0_a$) season, but there was no significant difference between cold ($0.6\pm0.4_b$) and hot dry ($0.0\pm0.0_b$) season in the distribution.

The results also indicated that, at 5% probability level, there was a significant difference across the seasonal distribution, since the calculated t is greater than the critical t at P= (0.05). The results showed that, in Abaganaram, there was no significant difference between the rainy ($9.6\pm3.0_a$) and cold dry ($0.0\pm0.0_{b)}$ season in the seasonal distribution of *Aedes aegypti*, but there was significant difference between rainy ($9.6\pm3.0_a$) and hot dry ($2.4\pm1.5_b$) season and between cold and hot dry season respectively. The results also revealed that, at 5% probability level, there was a significant difference across the seasonal

distribution, since the calculated t is greater than the critical t or tabulated t value.

In addition, the results revealed that, in Adam Kolo, there was no significant differences between the rainy ($2.8\pm1.5_b$) and cold dry ($0.0\pm0.0_b$) seasons, rainy ($2.8\pm1.5_b$) and hot dry ($0.0\pm0.0_b$) seasons and between the cold ($0.0\pm0.0_b$) and hot dry ($0.0\pm0.0_b$) season, with p-value of greater than 0.05. The results also indicated that, in Zamamari, there was significant difference between the cold ($3.6\pm1.4_a$) and hot dry ($0.0\pm0.0_a$) season in the distribution of *Aedes aegypti*, but there was no significant difference between the distribution during the rainy ($1.0\pm0.4_b$) and cold dry ($3.6\pm1.4_a$) season and between rainy ($1.0\pm0.4_b$) and hot dry ($0.0\pm0.0_a$) seasons respectively. The results also showed significant difference across the seasons at 5% probability level.

Moreover, in Dala ward, there was no significant difference between the rainy ($5.4\pm1.3_b$) and cold dry ($16\pm5.1_a$) season but there was significant difference between the rainy ($5.4\pm1.3_b$) and hot dry ($1.0\pm0.3_a$) season and between the cold ($16\pm5.1_a$) and hot dry ($1.0\pm0.3_a$) season respectively. The results also indicated that, there was significant difference across the seasonal distribution of *Aedes aegypti*, at 5% probability level. In Kululuri, the results showed that, there was no significant difference between the rainy ($0.0\pm0.0_b$) and cold dry ($0.0\pm0.0_b$) season, and between the rainy ($0.0\pm0.0_b$) and hot dry ($11.4\pm4.0_a$) season, as well as no significant difference between the cold dry ($0.0\pm0.0_b$) season and the hot dry ($11.4\pm4.0_a$) season, though the results revealed that, there was a significant difference across the seasonal distribution, at 5% probability level.

The results also revealed that, in Bulumkutu, there was no significant difference between the rainy ($5.4\pm1.7_b$) and cold dry ($15\pm3.3_a$) season and between the rainy ($5.4\pm1.7_b$) and hot dry ($2.8\pm0.9_b$) season, but there was significant difference between the cold ($15\pm3.3_a$) and hot ($2.8\pm0.9_b$) dry season. The results indicated that, there was significant difference across the seasons at 5% probability level (P=0.05). In Gamboru area, the results showed that, there was no significant difference between the rainy ($1.0\pm0.3_a$) and cold dry ($0.2\pm0.2_b$) season, and between the cold and hot dry season, but there was significant difference between the rainy ($1.0\pm0.3_a$) and hot dry ($0.0\pm0.0_b$) season respectively. The results also indicated that, there was significant difference across the seasonal distribution.

Table 3. Seasonal distribution of *Aedes aegypti* in Maiduguri Metropolis.

Location (N=5)	Rainy season Mean ± SE	Cold-dry season Mean ± SE	Hot-dry season Mean ± SE	P-Value (0.05)
Polo	$7.0\pm0.9_a$	$0.6\pm0.4_b$	$0.0\pm0.0_b$	0.000*
Abaganaram	$9.6\pm3.0_a$	$0.0\pm0.0_b$	$2.4\pm1.5_b$	0.012*
Adam Kolo	$2.8\pm1.5_b$	$0.0\pm0.0_b$	$0.0\pm0.0_b$	0.070 ≠
Zamamari	$1.0\pm0.4_b$	$3.6\pm1.4_a$	$0.0\pm0.0_a$	0.033*
Dala	$5.4\pm1.3_b$	$16\pm5.1_a$	$1.0\pm0.3_a$	0.013*
Kululuri	$0.0\pm0.0_b$	$0.0\pm0.0_b$	$11.4\pm4.0_a$	0.006*
Bulumkutu	$5.4\pm1.7_b$	$15\pm3.3_a$	$2.8\pm0.9_b$	0.005*
Gamboru area	$1.0\pm0.3_a$	$0.2\pm0.2_b$	$0.0\pm0.0_b$	0.016*
Baga road	$0.0\pm0.0_b$	$24.4\pm10.2_a$	$0.0\pm0.0_b$	0.014*
Muna Chad Basin	$0.0\pm0.0_b$	$0.0\pm0.0_b$	$8.2\pm2.8_a$	0.040*
Custom area	$1.4\pm0.6_b$	$0.0\pm0.0_b$	$7.4\pm3.2_a$	0.036*
Gwange	$8.6\pm2.9_a$	$4.6\pm1.3_b$	$0.0\pm0.0_b$	0.020*
Total	42.2	33.8	33.2	

a= significantly different, b= not significantly different, *= significant since the P-value is less than or equal to P (0.05), ≠ = not significant since the P-value is greater than P (0.05)
SE= Standard Error, N= Number of sites in each location which is equal to five (5).

Molecular Identification of *Aedes aegypti* in Maiduguri Metropolis

The result in **Table 3**, also revealed that, in Baga road, there was no significant difference between rainy ($0.0\pm0.0_b$) and cold dry ($24.4\pm10.2_a$) season and between rainy ($0.0\pm0.0_b$) and hot dry ($0.0\pm0.0_b$) season, but there was significant difference between the cold ($24.4\pm10.2_a$) and hot dry ($0.0\pm0.0_b$) season, though the results indicated that, there was significant difference across the seasonal distribution, at 5% probability level.

In Muna Chad basin and Custom area, the results indicated that, there was significant difference between and within the seasons, though revealed that, there was a significant difference across the seasonal distribution at 5% probability level, since the calculated t value is greater than the critical or tabulated t. The results also showed that, in Gwange, there was no significant difference between the rainy ($8.6\pm2.9_b$) and cold dry ($4.6\pm1.3_b$) season, but there was a significant difference between the rainy ($8.6\pm2.9_b$) and hot dry ($0.0\pm0.0_a$) season and between the cold ($4.6\pm1.3_b$) and hot dry ($0.0\pm0.0_a$) season respectively. The results also revealed that, there was a significant difference across the seasonal distribution at 5% probability level.

The results in **Fig. 1**, showed the molecular identification of *Aedes aegypti* in Maiduguri Metropolis by Polymerase Chain reaction (PCR) using microsatellite primer specific for *Aedes aegypti* (Forward: GCGTATYACCACCCGTAAGA, Reverse**:** GCGAGTGCARATCGATGAT). Two percent agarose gel of PCR thermal gradient of annealing temperature of 56^0C-58^0C from DNA of the whole mosquitoes from *Aedes aegypti*. Amplification gave approximately 300bp fragments from all amplified DNA template. Lane 2,4,6,7 and 8 has a stronger band around 300bp compare to lane 1, 10, 11 and 12, which also showed the band around 300bp. The other bands below 100bp are unspecific bands, which might be due to unspecific annealing of primers, caused by contaminant in the DNA of the sample.
The results indicated that, *Aedes aegypti*, was detected present at Polo, Abaganaram, Zamamari, Kululuri, Bulumkutu, Gamboru, Baga road, Muna Chad Basin, Custom area, Adam Kolo, Gwange, and Kululuri while, there was no *Aedes aegypti* detected at Dala.

The sequence results in **Fig. 2** showed that there were mutations in lane 2, 3, 4, 9, 10, 11 and 12. These might be that the mosquitoes were harboring the dengue virus, which could be due to the vector site or the host population being one of the contributing factors while lane 1, 6, 7, and 8, showed a good sequence pattern and have phylogenetic relationship with other *Aedes* species.

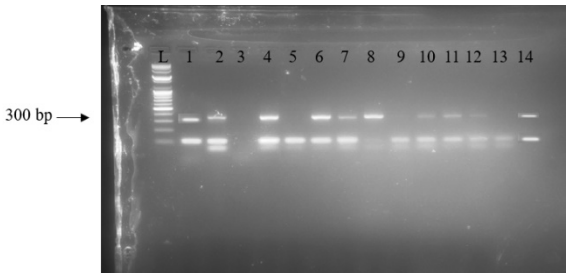

Fig. 1. Molecular identification of *Aedes aegypti* in Maiduguri Metropolis. L=1kb Ladder, lane 1 = Polo, lane2= Abaganaram, lane 3 = Adam Kolo, lane 4 = Zamamari, lane 5 = Dala, lane 6 = Kululuri, lane 7 = Bulumkutu, lane 8 = Gamboru Area, lane 9 =Baga road lane 10 = Muna Chad Basin, lane 11 = Custom Area, lane 12 = Gwange, 13 = Negative control, 14 = Positive control

Molecular phylogenetic analysis

The results in **Fig. 2** showed the evolutionary history as inferred using the Maximum Likelihood method based on the model described by Tamura and Nei [49]. The tree with the highest log likelihood (-1753.74) is indicated in **Fig. 2**. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The initial tree(s) used for the investigative search were automatically obtained by applying Neighbor-Join and Bio NJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, after which the topology having a superior log likelihood value was selected. The analysis carried out involved the use of 12 nucleotide sequences. The first+second+third+noncoding coding positions were all included whereas all positions that contain gaps and missing data were eliminated. The final data set contains a total of 167 positions. MEGA7 software was employed in carrying out all the evolutionary analysis [43].

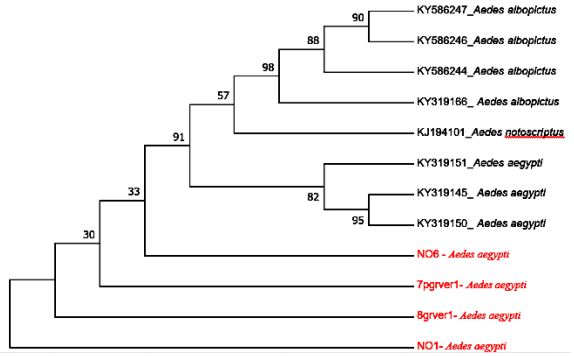

Fig. 2. Phylogenetic tree of *Aedes aegypti* from Maiduguri Metropolis.

DISCUSSION

Aedes aegypti and *Aedes albopictus* are responsible for dengue fever virus transmission in tropical and sub-tropical areas such as Africa, including Borno State, Nigeria, the Americas, Asia, the Caribbean, and the Pacific [4,5]. The results obtained from this study indicated that *Aedes aegypti* was caught in nine locations out of the twelve locations across the seasons. This is in agreement with the findings that *Aedes* mosquitoes are found widely distributed in Africa and could be important vectors for the transmission of dengue virus [50]. This might also support the fact that *Aedes aegypti* is common specie in the urban areas and at the same time the principal vector of dengue and yellow fever, being responsible for intensifying epidemics among the urban populations [51,52].

In addition, it has been reported that Nigeria is in danger of diseases transmitted by *Aedes aegypti* such as yellow fever and dengue fever due to the fact that this mosquito is very common in the country found in and around homes [16]. The results presented in **Table 1**, showed the abundance and composition of *Aedes aegypti* in Maiduguri Metropolis. The results revealed that Baga road (18.0%), had the highest abundance of *Aedes aegypti*, followed by Bulumkutu (16.5%), Dala(15.9%), Gwange (9.4%), Abaganaram (8.7%), Kululuri (8.1%), Muna Chad Basin and Custom area (5.8%), Polo (5.4%), Zamamari (3.6%), Adam Kolo(1.9%) and Gamboru area, had the lowest abundance of *Aedes aegypti*, with 0.9%, respectively. This could be because most of these areas with *Aedes aegypti* in highly relatively abundant are densely populated areas with no proper environmental sanitation, where people used drainages, gutters, flowerpots, doorpost etc. as their refuse dumps. This agrees with the findings of Mousson *et al*. [53] that *Aedes aegypti* is a cosmo-tropical species of mosquitoes associated mostly with the urban population, thus favoring densely populated areas but could also breed in rural areas in rare cases.

The current findings do not agree with previous reports, which showed the presence of *Aedes albopictus* in Central African Republic [54,55]. This is because no *Aedes albopictus* was caught in all the study locations across the seasons. The results presented in **Table 2**, showed the seasonal abundance of *Aedes aegypti* in Maiduguri Metropolis. These revealed that the highest abundance of *Aedes aegypti* was recorded during cold dry season with 343, followed by rainy season with 209, and 153 during hot dry season, respectively. The results also indicated that *Aedes aegypti* was moderately higher during the peak of rainy season from July to October 2016, with Abaganaram (48), having the highest abundance, followed by Gwange, (43), Polo (35), Dala and Bulumkutu (27), Adam Kolo Gamboru area (5) and the lowest during the rainy season was Gamboru and Zamamari area with 5 *Aede*s species each.

The results in **Table** 2, also indicated that during the hot-dry season there was a significant relatively higher abundance of *Aedes aegypti* in Kululuri with 57 highest across the seasons, followed by Muna Chad Basin with 41 *Aedes aegypti* during the hot dry season, Custom area (36), Bulumkutu (14) and Dala was relatively lower with 5 *Aedes aegypti*, during the hot dry season, respectively. These revealed that, the seasonal abundance of *Aedes aegypti* caught within and across the season is higher in hot dry season, which had the highest percentage relative abundance of *Aedes aegypti,* with Kululuri having higher percentage of 37.3%, followed Baga road with 37.1% during cold dry season, Muna Chad Basin (26.8%) during hot dry season, Custom area (23,5%) hot dry season, Dala (23.4%) cold dry season, Abaganaram (22.9%) rainy season, Bulumkutu (21.9%) cold dry season, Gwange (20.6%), Polo (16.7%), Dala and Bulumkutu (12.9%) rainy season, Bulumkutu (9.1%) during hot dry season, Adam Kolo(6.7%) rainy season, Gwange (6.7%) cold dry season, Zamamari (5.8) cold dry season, Abaganaram (3.8%) cold dry season, Dala (3.3%), Gamboru & Custom area (2.4%) rainy season, Polo (0.9%) cold dry season, and Gamboru are(0.3%) during the cold dry season had the lowest abundance of *Aedes aegypti*.

Campos *et al*. [56] reported their study carried out in Sao Paulo Brazil towards understanding the seasonal population dynamics and the genetic structure of *Aedes aegypti* that, despite the large differences in mosquito abundance it appears that the effective population size remained steady over the years and that *Aedes aegypti* is capable of maintaining an adequately active breeding population during dry season thus maintaining a steady genetic frequency. In addition, Akhtar and Ebi [57] reported that DEN virus transmission occurs throughout the year in the tropics but that in most countries the seasonal peaks occur during the months with high rainfall and humidity. Girmand Associates [37] reported that in Maiduguri the largest part of the yearly rainfall (over 90%) falls between the months of June and September. *Aedes aegypti* do not live at an altitude above 100m, which is colder, because they always desire a warm climate. These mosquitoes live in densely populated areas around houses occupied by humans where they lay and hatch their eggs [58].

Similarly, climatic factors may also play a major role in the multiplication of the disease agent and the transmission of the disease, thus expanding the geographical range of dengue fever [59]. It has been shown that the geographical range of mosquitoes increase with increase in the temperature of the region [60]. This

trend has been observed in many places; for instance, in Columbia where the attitudinal range of *Aedes aegypti* mosquito increased from 1500 m to 2200 m [61].

Johansson *et al.* [62] and CDC [58] found that, precipitation; temperature and humidity are very crucial to the survival, replication and growth of mosquitoes and can influence their presence and abundance. More also, the rate at which the virus can replicate and disseminate within the mosquito is favored by higher temperatures. Johansson *et al.* [62] also stated that mosquito density and dengue distribution cannot be explained by climatic variations alone but that abundant breeding sites and human shelters with plenty of food are some of the other factors that may be responsible for the prevalence of dengue fever virus. Hopp and Foley [60] have reported that high temperatures accelerate the development of mosquitoes and increase their abundance. Similarly, many researchers have studied the effects of climatic factors on the ecology of *Aedes aegypti* mosquito and observed that these factors significantly influence the development of the mosquito, mosquito density, breeding habitats, and the survival and development of the virus within the mosquito [63-69].

The results in **Table 3** showed the seasonal distribution of *Aedes aegypti* in Maiduguri Metropolitan. The results revealed that in the seasonal distribution of *Aedes aegypti* in Polo area, there was significant difference between the rainy ($7.0\pm0.9_a$) and cold dry ($0.6\pm0.4_a$) season, and between rainy ($7.0\pm0.9_a$) and hot dry ($0.0\pm0.0_a$) season, but there was no significant difference between cold ($0.6\pm0.4_b$) and hot dry ($0.0\pm0.0_b$) season in the distribution. The results also indicated that at 5% probability level, there was a significantly difference across the seasonal distribution, since the calculated t is greater than the critical t at P= (0.05). The results showed that, in Abaganaram, there was no significant difference between the rainy ($9.6\pm3.0_a$) and cold dry ($0.0\pm0.0_b$) season in the seasonal distribution of *Aedes aegypti*, but there was significant difference between rainy ($9.6\pm3.0_a$) and hot dry ($2.4\pm1.5_b$) season and between cold and hot dry season respectively. The results also revealed that at 5% probability level, there was a significant difference across the seasonal distribution, since the calculated t is greater than the critical t or tabulated t value.

In addition, the results revealed that, in Adam Kolo, there was no significant difference between the rainy ($2.8\pm1.5_b$) and cold dry ($0.0\pm0.0_b$) seasons, rainy ($2.8\pm1.5_b$) and hot dry ($0.0\pm0.0_b$) seasons and between the cold ($0.0\pm0.0_b$) and hot dry ($0.0\pm0.0_b$) season, with p-value of greater than 0.05. The results also indicated that in Zamamari, there was significant difference between the cold ($3.6\pm1.4_a$) and hot dry ($0.0\pm0.0_a$) season in the distribution of *Aedes aegypti*, but there was no significant difference between the distribution during the rainy ($1.0\pm0.4_b$) and cold dry ($3.6\pm1.4_a$) season and between rainy ($1.0\pm0.4_b$) and hot dry ($0.0\pm0.0_a$) seasons respectively. The results also showed significant difference across the seasons at 5% probability level.

Moreover, in Dala ward, there was no significant difference between the rainy ($5.4\pm1.3_b$) and cold dry ($16\pm5.1_a$) season but there was significant difference between the rainy ($5.4\pm1.3_b$) and hot dry ($1.0\pm0.3_a$) season and between the cold ($16\pm5.1_a$) and hot dry ($1.0\pm0.3_a$) season respectively. The results also indicated that, there was significant difference across the seasonal distribution of *Aedes aegypti*, at 5% probability level. In Kululuri, the results showed that, there was no significant difference between the rainy ($0.0\pm0.0_b$) and cold dry ($0.0\pm0.0_b$) season, and between the rainy ($0.0\pm0.0_b$) and hot dry ($11.4\pm4.0_a$) season, as well as no significant difference between the cold dry ($0.0\pm0.0_b$) season and the hot dry ($11.4\pm4.0_a$) season, though the results revealed that, there was a significant difference across the seasonal distribution, at 5% probability level.

The results also revealed that, in Bulumkutu, there was no significant difference between the rainy ($5.4\pm1.7_b$) and cold dry ($15\pm3.3_a$) season and between the rainy ($5.4\pm1.7_b$) and hot dry ($2.8\pm0.9_b$) season, but there was significant difference between the cold ($15\pm3.3_a$) and hot ($2.8\pm0.9_b$) dry season. The results indicated that, there was significant difference across the seasons at 5% probability level (P=0.05). In Gamboru area, the results showed that, there was no significant difference between the rainy ($1.0\pm0.3_a$) and cold dry ($0.2\pm0.2_b$) season, and between the cold and hot dry season, but there was significant difference between the rainy ($1.0\pm0.3_a$) and hot dry ($0.0\pm0.0_b$) season respectively. The results also indicated that, there was significant difference across the seasonal distribution. This is in agreement with the fact that, various researchers have shown that environmental conditions strongly control the density and distribution of *Aedes aegypti,* mosquito [60,65,70-74].

Depradine and Lovell [75] showed that increased in minimum temperature has stronger effect on mosquito density than the maximum temperature. McMichael *et al.* [61] reported that the favorable temperature for the growth is between 25 ˚C to 27 ˚C and that it should not exceed a temperature of 40 ˚C. Similarly, Baba and Talle [32] in their studies observed that the warmer the temperature the faster the infectivity of the mosquito as it may have a higher chance of infecting humans before it dies. They also noted that other factors such as the presence of the virus, adequate numbers of susceptible population, and the availability of mosquito vectors are as important as the environmental factors in the transmission of dengue virus. More also, they noted that there are differences in the climatic conditions of the different parts of Nigeria, which may result in variations in the relative abundance and distribution of this mosquito in the different regions of the country.

The effect of temperature on the survival rate of mosquitoes cannot be easily predicted [11]. Also, humidity has been shown to contribute to the transmission of dengue fever by influencing the activities and survival of the mosquito vector [76]. Several studies have indicated a strong association between the relative humidity with hatching and other activities of mosquitoes [60,63,77]. Hopp and Foley [60] discovered an increased in modulated egg and larvae indices during the summer months when the temperature and humidity are high. In addition, the survival rate for the mosquito appears to be greater at higher humidity [11]. Reiter *et al.* [11] showed that the survival and density of mosquitoes is positively influenced by relative humidity.

Johansnson *et al.* [62] revealed that differences in local climates account for the strong relationship between monthly changes in temperature and precipitation and monthly changes in dengue transmission. Furthermore, the change in climatic conditions and use of insecticide cause increase in the population genetic structure of *Aedes aegypti* [27,79, 31]. The results in **Fig.** 1, showed the molecular identification of *Aedes aegypti* in Maiduguri Metropolis by Polymerase Chain reaction (PCR). In these results *Aedes aegypti* was detected at Abaganaram, Zamamari, Kululuri, Bulumkutu, Gamboru, Baga road, Muna Chad Basin, Custom area and Gwange, while, *Aedes aegypti* was not detected at Polo, Adam Kolo and Dala, respectively. Two percent agarose gel of PCR thermal gradient of annealing temperature of 56 ˚C-58 ˚C from DNA of the whole mosquitoes

from *Aedes aegypti* was used. Amplification gave approximately 300bp fragments from all amplified DNA template. Lane 2,4,6,7 and 8 has a stronger band around 300 bp compared to lane 10, 11 and 12, which also showed the band around 300 bp. The other bands below 100 bp are unspecific bands, which might be due to unspecific annealing of primers caused by contaminants in the DNA of the sample. The sequence results showed that there were mutations in lane 2, 3, 4, 9, 10. 11 and 12. These might suggest that the mosquitoes are harboring the dengue virus, which could be due to the vector site and the host population being one of the contributing factors while lane 1, 6, 7, and 8 showed a good sequence pattern and have phylogenetic relationship with other *Aedes* species as shown in **Fig. 2**.

These indicated that those areas are more susceptible to be infected with dengue, as the vector *Aedes aegypti*, responsible for it transmission was detected in those wards or locations. This is also in conformity with the findings reported by WHO, 2017 [79] that *Aedes aegypti* mosquito is the major vector responsible for the transmission of dengue virus that causes dengue fever in humans. It is the infective female Aedes mosquito that is responsible for passing the virus to humans after biting, the virus being acquired by the mosquito while feeding on the blood meal of an infected individual. *Aedes aegypti*, a vector of dengue virus (DENV) and yellow fever virus (YFV), whose susceptibility to infection is genetically influenced [80].

Other species of Aedes could transmit dengue virus infection in urban settlements but to a lesser extent. In forests, however, other non-human primates are responsible for maintaining the dengue virus transmission cycle. *Aedes aegypti* is mostly found in a population of humans and usually breeds in artificial containers and used tyres. It is widely spread in Africa and some other parts of the world due to increasing international travel, urban drift and population explosion, suggesting that there is high risk of local transmission as is the tendency for extensive autochthonous disease spread [50]. This is in agreement with a report by Traore *et al*. [8] alleging that *Aedes aegypti* is found throughout the tropics. Hence by implication in Maiduguri, Nigeria, where there is rapid human population growth due to migration of people from rural to urban areas as a result of insurgency in Borno State.

CONCLUSION

The present study is the first to be carried out on genetic population structure of dengue mosquitoes (*Aedes* species) from the Northeast, Borno State of Nigeria. The study has shown that there is a high chance of exposure to the risk of Dengue, Zika, Yellow Fever, and Chikungunya virus infections in Maiduguri Metropolitan Council. It was also apparent that there were seasonal variations in the abundance and distribution of *Aedes aegypti* in the study locations. Mutations were discovered in the sequences of some of the *Aedes aegypti* mosquitoes whereas those with no mutations appeared to have a phylogenetic relationship with *Aedes* species from other countries.

REFERENCES

1. Brown JE, McBride CS, Johnson P, Ritchie S, Paupy C, and Powell J R. Worldwide patterns of genetic differentiation imply multiple 'domestications' of *Aedes aegypti*, a major vector of human diseases. Proc R Soc. 2011;278:2446–2454.
2. Pinheiro FP, and Corber SJ. Global situations of dengue and dengue hemorrhagic fever and its emergence in the Americas. WHO Stat Quest. 1997;50:161-169.
3. Fradin MS, and Day JF. Comparative efficacy of insect repellants against mosquito bite. N Engl J Med. 2002;347(1):13-18.
4. Gibbons RV, and Vaughn, DW. Dengue: An escalating problem. Biomed Med J. 2002;324:1563-1566.
5. World Health Organization (WHO). Dengue:Guidelines for Diagnosis, Treatment, Prevention and Control. Geneva World Health Organization and the Special Programme for Research and Training in Tropical Diseases, 2009.
6. WHO. Dengue hemorrhagic fever, diagnosis, treatment and control. 2nd ed. World Health Organization Switzerland. 1997.
7. Fraga, EC, Santos JMM, Maia JF. Enzymatic variability in *Aedes aegypti* (Diptera:Culicidae) populations from Manaus-AM, Brazil. Genet Mol Biol. 2003;26:181-187.
8. Traore LM, Zeller H, Monlun E, Mondo M, Hervy JP, Adam F, Digoutte JP. Dengue 2 outbreak in Southeastern Senegal during 1990:virus isolations from mosquitoes (Diptera:Culicidae). J Med Entomol. 1994;31:623-627.
9. Marie V, Mousson L, Kakatoarivony I, Villeret R, Rodmain F, Duchemin JB, Failloux AB. Population genetic structure and competence as a vector for dengue type 2 virus of *Aedes albopictus* from Madagascar. Am J Trop Med Hyg. 2001;65(5):491-497.
10. Whitehorn J, and Farrar J. "Dengue". British Med Bull. 2010;95:161–173.
11. Reiter P. Climate change and mosquito-borne disease. Environ Health Perspect. 2001;109:141-161.
12. Gubler DJ, and Clark GG. Dengue/dengue hemorrhagic fever:the emergence of a global health forum. Emerg Infect Dis J. 1995;1:55-57.
13. Gubler DJ. Dengue and Dengue Hemorrhagic Fever. Clin Microbiol Rev. 1998;11(3):480-496.
14. Guzman MG, and Kouri G. Dengue:an update. Lancet Infect Dis. 2002;2:33-42.
15. WHO. Mobilizing research to halt exponential growth of dengue. TDR News. 2007;77:8-11.
16. Muanya C. Mosquito-transmitted Zika virus may spread to Nigeria. Retrieved on 03 February, 2016.
17. Ayres CF, Romao TPA, Melo-Santos MAV, Furtado AF. Genetic diversity in Brazilian populations of *Aedes albopictus*. Mem Inst Oswaldo Cru. 2002;97:871-875.
18. Gorrochotegui-Escalante N, Gomez-Machorro C, Lozano-Fuentes S, Fernandez-Salas L, Munoz ML, Farlan-Ale JA, Garcia-Rejon J, Beaty BJ, Black WC IV. Breeding structure of *Aedes aegypti* populations in Mexico varies by region. Am J Trop Med Hyg. 2002;66:213-222.
19. Ravel S, Herve JP, Diarrassouba S, Kone A, Cuny G. Microsatellite markers for population genetic studies in *Aedes aegypti* (Diptera:Culicidae) from Cote d' Ivoire:evidence for a micro geographic genetic differentiation of mosquitoes from Bouake. Acta Trop. 2002;82:39-49.
20. Beebe NW, Whelan PI, Hurk AVD, Ritchie SA, Cooper RD. Genetic diversity of the dengue vector *Aedes aegypti* in Australia and implications for future surveillance and mainland incursion monitoring. Commun Dis Intell Q Rep. 2005;29:299-304.
21. Herrera F, Urdancta L, Rivero J, Zoghbi N, Ruiz J, Carrasquel G, Martinez JA, Pernalete M, Villeges P, Montoya A, Rubiopais Y, Rojas E. Population genetic structure of the dengue mosquito *Aedes aegypti* in Venezuela. Mem Inst Oswaldo Cru. 2006;101:625-633.
22. Bracco JE, Capurro ML, Lourenco-de-Oliveira R, Sallum MAM. Genetic variability of *Aedes aegypti* in the America using a mitochondrial gene:evidence of multiple introductions. Mem Inst Oswaldo Cru. 2007;2:573-580.
23. Tabachnick WJ. Evolutionary genetics and arthropod-borne disease: The yellow fever mosquito. Am Entomol.1991;37:14-24.
24. Ballinger-Crabtree ME, Black IV WC, Miller BR. Use of genetic polymorphisms detected by the random-amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) for differentiation and identification of *Aedes aegypti* subspecies and populations. Am J Trop Med and Hygiene. 1992;47:893-901.
25. Balloux F, and Lugon-Moulin N. The estimation of population differentiation with microsatellite markers. Mol Ecol. 2002;11:155-165.
26. Apostol BL, Black W C, Reiter P, Miler B. Population genetics with RAPD-PCR markers:the breeding structure of *Aedes aegypti* in Puerto Rico. Heredity.1996;76:325-334.
27. Huber K, Le Loan TH, Hoang TH, Ravel S, Rodhain F, and Failloux AB. Genetic differentiation of the dengue vector. *Aedes*

aegypti (Ho Chi Minh City, Vietnam) using microsatellite markers. Mol Ecol Notes. 2002;11:1629-1635.
28. Rinker CD, Pitts RJ, and Zwiebel LJ. Disease vectors in the era of next generation sequencing. Genome Biol. 2016;17:95.
29. Ayres CFJ, Melo-Santos MAV, Sole-Cava AM, Furcado AF. Genetic differentiation of *Aedes aegypti* (Diptera:Culicidae), the major dengue vector in Brazil. J Med Entomol. 2003;40:430-435.
30. Julio NB, Chiappero MB, Rossi HJ, Rondan Duenas JC, Gardenal CN. Genetic structure of *Aedes aegypti* in the city of Cordoba (Argentina), a recently reinfested area. Mem Inst Oswaldo Cru. 2009;104:626-631.
31. Scarpassa VM, Cardoza TB, and Cardoso Junior RP. Population genetics and phylogeography of *Aedes aegypti* (Diptera:Culicidae) from Brazil. Am J Trop Med Hygiene. 2008;78:895-903.
32. Baba MM, and Talle M. The Effect of Climate on Dengue Virus Infections in Nigeria. New York Sci J. 2011;4(1):28-33.
33. Idris AN, Baba MM, Thairu YI, and Bamidele O. Sero-prevalence of dengue type-3 Virus among patients with febrile illnesses attending a tertiary hospital in Maiduguri, Nigeria. Int J Med Med Sci. 2013;5 (12):560-563.
34. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler D J, Hunsperger E, Kroeger A, Margolis H S, Martínez E, Nathan M B, Pelegrino J L, Simmons C, Yoksan S, Peeling R W. (2010). "Dengue:A continuing global threat". Nature Rev Microbiol. 2010;8 (12):57–116.
35. NPC. National Population Commission. Nigeria Census Report 2006. *http://www.nigeriamasterweb.com/Nigeria06CensusFigs.html*.
36. Molta NB, Watila IM, Out TI, Oguche SO, Daniel HI, and Gadzama NM. (1995). Malaria in Nigeria:an update on its chemotherapy with chloroquine, pyrimethamine/sulphadoxine and pyrimethamine/sulphalene and the need for alternative antimalarial drugs. Res J Sci. 1995;1(2):59-64.
37. Gimand Associates. Environmental and human resources development consultant. Environmental impact assessment of Nigerian Bottling Company PLC. Coca-cola Maiduguri Plant 'A' Wastewater discharge project: Draft report. 2002;1-81.
38. Bukbuk DN, Dowall SD, Lewandowski K, Bosworth A, Baba SS, Varghese A, Watson RJ, Bell A, Atkinson B, Hewson R. Serological and Virological Evidence of Crimean Congo Haemorrhagic Fever Virus Circulation in the Human Population of Borno State, Northeastern Nigeria. PLoS Neglect Trop Dis. 2016;10 (12):5126.
39. Hopkin GHE. Mosquitoes of the Ethiopian Region. Larval Bionomics of Mosquitoes and Taxonomy of Culicine larvae. British Museum (National History). 1952;8:1-14.
40. Highton, R.B. Taxonomic keys for the identification of the Afrotropical mosquitoes. Highton April: Revised and prepared by RB. 1983;1-85.
41. Snell AE. Identification keys to larval and adult female mosquitoes (Diptera:Culicidae) of New Zealand, New Zealand J Zoology. 2005;32:2, 99-110.
42. Hebert PD, Cywinska A, Ball SL, and deWaard JR. Biological identifications through DNA barcodes. Proc. Biol. Sci.2003;270:313-321.
43. Kumar NP, Rajavel AR, Natarajan R, Jambulingam P. DNA barcodes can distinguish species of Indian mosquitoes (Diptera:*Culicidae*). J Med Entomol. 2007;44:1-7.
44. Rivero J, Urdaneta L, Zoghbi N, Pernalete M, Rubio-Palis Y, Herrera F. Optimization of extraction procedure for mosquito DNA suitable for PCR-based techniques. Int J Trop Insect Sci. 2004;24:266-269.
45. Coen ES, Strachan T, and Dover G. Dynamics of concerted evolution of ribosomal DNA and histonegene families in the melanogaster species subgroup of Drosophila. J Molecular Biology. 1982;15:17–35.
46. Begon M, Harper JL. and Townsend C R. Ecology:Individuals, Populations, and Communities, 3rd edition. Blackwell Science Ltd. Cambridge, MA. 1996.
47. Kumar S, Stecher G, and Tamura K. MEGA7:Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evolut. 2016;33:1870-187.
48. Felsentein J. PHYLIP, Phylogeny Inference Package. Version 3.5C. Seattle, WA, University of Washington. 1993.
49. Tamura, K. and Nei, M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evolut. 1993;10:512-526.
50. Amarasinghe A, Kuritsky JN, Letson GW, Margolis HS. Dengue virus infection in Africa. Emerg Infect Dis. 2011;**17**(8):1349–1354.
51. WHO. Yellow fever. Rapid field entomological assessment during yellow fever outbreaks in Africa. Methodological field approaches for scientists with a basic background in entomology. Australia:Biotext Pty Ltd;2014.
52. Huang YJS, Higgs S, Horne KME, Vanlandingham DL. (2014). Flavivirus-mosquito interactions. Viruses. 2014;6:4703–4730.
53. Mousson L, Dauga C, Garrigues T, Schaffner F, Vazeille M, Failloux AB. Phylogeography of *Aedes* (Stegomyia) *aegypti* (L.) and *Aedes* (Stegomyia) *albopictus* (Skuse) (Diptera:*Culicidae*) based on mitochondrial DNA variations. Genetic Resources.2005;86:1–11.
54. Coffinet T, Mourou JR, Pradines B, Toto JC, Jarjavai F, Amalvict R. First record of *Aedes albopictus* in Gabon. J Am Mosq Control Assoc. 2007;23:471-472.
55. Diallo M, Laganier R, Nangouma A. First record of *Ae. albopictus* (Skuse 1894), in Central African Republic. Trop Med Int Health. 2010;15:1185-1189.
56. Campos M, Spenassatto C, Macoris da Graca M, Paduan K, Pinto J, Ribolla P.. Seasonal population dynamics and the genetic structure of the mosquito vector *Aedes aegypti* in Sao Paulo, Brazil. Ecology and Evolution. Blackwell Publishing Ltd. 2012;2 (11):2794-2802. doi:10.1002/ece3.392.
57. Akhtar R, and Ebi K L. Working group II:Climate change;Impacts, adaptation and vulnerability. Chapter 9. Human Health. 2001.
58. Centers for Disease Control and Prevention (CDC). Dengue and Climate http://www.cdc.gov/dengue/entamologyEcology/climate html#az. 2010;Retrieved 16 February, 2017.
59. Ramchurn SK, Goorah SS. Letter to the editor:ongoing outbreak of dengue type 1 in the Autonomous Region of Madeira, Portugal. Euro Surveill. 2013;10;18(2):20351.
60. Hopp, M.J. and J.A. Foley. World wised fluctuations in dengue fever cases related to climate variability. Climate Res.2003;25:85-94.
61. McMichael AJ, Haines A, Sloof R, and Kovats S. Climate change and human health, World Health Organization, Geneva. 1996.
62. Johansson MA, Cummings DA, Glass GE. Multiyear climate variability and dengue–El Nino southern oscillation, weather, and dengue incidence in Puerto Rico, Mexico, and Thailand:a longitudinal data analysis. PLoS Med. 2009;6(11):e1000168.
63. Koopman JS, Prevots DR, Marin MA, Dantes HG, Aquino AML, Jr. Longini IM, and Amor JS. (1991). Determinants and predictors of dengue infection in Mexico. Am J Epidemiology. 1991;133:1168-1178.
64. Nicholls N. El Niño-Southern Oscillation and Vector-Borne Disease. Lancet. 1993;342:1284-1285.
65. Ram S, Khurana S, Kaushal V, Gupta R, and Khurana SB. Incidence of dengue fever in relation to climate factors in Ludhiana Punjab. Indian J Med Res. 1998;108:128-133.
66. Martens P, Kovats RS, Nijhof S, de Vries P, Livermore MTJ, Bradley DJ, Cox J, and McMichael AJ. Climate change and future populations at risk of malaria, Global Environmental Change. 1999;9:89-107.
67. Wilson K. Global warming and the spread of disease:the debate heats up. Trends in Ecology and Evolution. 2000;15:488.
68. Sutherst R. Global Change and Human Vulnerability to Vector-Borne Diseases, Clinical Microbiology Reviews. 2004;17:136-173.
69. Dibo MR, Chierotti AP, Ferrari MS, Mendonca AL, and Neto FC. Study of the relationship between *Aedes* (stegomyia*) aegypti* egg and adult densities, dengue fever and climate in Mirassol State of Sao Paulo Brazil. Mem Inst Oswaldo Cru. 2008;103:554-560.
70. Rueda LM, Patel KJ, Axtell, RC, Stinner RE. Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera:Culicidae). J Med Entomol.1990;27:892–898.
71. Focks DA, Haide DG, Daniels E, and Mount GA. Dynamic life **Table** model of *Aedes aegypti* (Diptera:*Culicida*e):analysis of the literature and model development. J Med Entomol.1993;30:1003-1017.

72. Promprou S, Jaroensutasinee M, and Jaroensutasinee K. Climatic factors affecting dengue haemorrhagic fever incidence in Southern Thailand. Dengue Bull.2005;29:41-48.
73. Favier C, Degallier N, Vilarinhos PTR, Carralho MSL, Yoshizawa MAC, and Knox MB. Effects of climate and different management strategies on *Aedes aegypti* breeding sites:a longitudinal survey in Brasilia (DF;Brazil).Trop Med Int Health. 2006;11:1104-1118.
74. Wu PC, Guo HR, Lung SC, Lin CY, and Su HJ. Weather as an effective predictor for occurrence of dengue fever in Taiwan. Acta Trop. 2007;103:50-57.
75. Depradine CA, and Lovell EH. Climatological variables and the incidence of Dengue fever in Barbados. Int J Environ Health Res. 2004;14:429-441.
76. Mofareh SAA. (2013). Dengue fever Outburst and its Relationship with Climatic Factors. World Appl Sci J. 2013;22 (4):506-515.
77. Rigau-Perez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ. & Vorndam AV. Dengue and dengue haemorrhagic fever. Lancet.1998;352:971–977.
78. Paupy C, Chantha N, Reynes JM, Failloux AB. Factors influencing the population structure of *Aedes aegypti* from the main cities in Cambodia. Heredity. 2005;95:144-147.
79. World Health Organization (WHO) updates fact sheet on Dengue and Severe Dengue. http://www.who.int/mediacentre/factsheets/fs117/en/. Retrieved on 6th April 2017.
80. Black IV W C, Bennett K E, Gorrochótegui-Escalante N, Barillas-Mury C V, Fernández-Salas I, de Lourdes Muñoz M, Farfán-Alé JA, Olson K E, and Beaty BJ. Flavivirus susceptibility in *Aedes aegypti*. Arch Med Res. 2002;33:379–388.