Assessment of Mosquito Diversity and *Plasmodium falciparum* in Female *Anopheles* Mosquito in Students' Hostels, Ahmadu Bello University Zaria, Kaduna State Nigeria

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Abstract

Mosquitoes are considered as the most important insect vectors of many infectious disease agents, whose presence pose a serious public health concern within localities where they are found especially in tertiary institution. The study was conducted to identify mosquito species and Plasmodium falciparum in female Anopheles mosquito in students' hostels of Ahmadu Bello University. Mosquitoes were caught once in a week for five months June to October (2018), by the used of test tube, chloroform and cotton wool. A total of 1341 mosquito vectors was identified, comprising four (4) species: Anopheles gambiae complex, Anopheles funestus, Culex quinquefasciatus and Aedes aegypti. The mosquitoes were dissected transversely between the first and third pairs of legs under the dissecting microscope The entire samples were air dried, fixed in methanol and were stained using Giemsa and thin blood film examined under the light microscope for identification of Plasmodium falciparum. The result in this study showed that Culex quinquefasciatus constitutes the most abundant with 950 (59%) followed by Anopheles gambiae 370, (22%), and An. funestus 15(0.9%) while Aedes aegypti 3(0.1%) had the least. Of the two species of anophelene mosquitoes encountered in this study only Anopheles gambiae was positive for Plasmodim falciparum. The result simply showed that efforts to control malaria especially in Zaria area should be a combination of existing methods which is usually referred to as integrated control.

Keywords: Mosquito, *Plasmodium falciparum, Anopheles,* Hostels

INTRODUCTION

Plasmodium falciparum is a protozoan parasite that causes an infectious disease known as malaria, and is the most severe strain of the malaria species responsible for malarial death

(CDC, 2015). This parasite is responsible for most of the complications (Black water fever, gastrointestinal disturbance, hyperpyrexia, and hypoglycemia in pregnant women) associated with human malaria (Bechemagbor, 2010). The other 3 species that cause malaria include: *P. vivax*, *P. ovale*, and *P. malariae.*

Malaria is considered to be one of the most severe infectious diseases worldwide, causing about half a million deaths every year, primarily in the developing world (WHO, 2016). More than two-thirds of malaria deaths globally is found in children under 5 years of age. About 3.2 billion people (almost half of the world's population) are at risk of malaria (WHO/UNICEF, 2015). Fifteen countries mainly in sub-Saharan Africa, accounted for 80 % of cases and 78% of deaths globally in 2015 (WHO/UNICEF, 2015). It is transmitted by *Plasmodium*-infected female *Anopheline* mosquitoes.

The disease (malaria) can be attributed almost entirely to some mosquito species namely: *Anopheles gambiae*, *An. arabiensis* and *An. funestus,* which are three of the most efficient malaria vectors in the world (Collins and Besansky, 1994). All live almost exclusively in close association with humans and feed on blood, primarily from humans (Collins and Besansky, 1994).

Mosquitoes belong to the order Diptera, Family Culicidae and Subfamily Culicinae. They are characterized by having long conspicuous needle-shaped mouthparts comprising the proboscis which, in the female, is used for sucking blood (W.H.O., 2014). There are about 3,100 known species of mosquitoes (Abubakar, 2014), which are separated into two great divisions - the Anophelines and Culicines. The Anophelines being the smaller division, but includes the vectors of human malaria and filariasis. The Culicines include vectors of viral and filarial diseases of man (Service, 1980).

Mosquitoes are unambiguously the most important vectors of infectious disease-causing agents that tremendously affect global health, with over half of the global human population at risk of exposure to mosquito-transmitted infections (Tolle, 2009) and more than 1 billion cases of such infections are reported each year (W.H.O., 2014).

Anopheles mosquitoes constitute various species with peculiar behaviour associated with their biting activities and malaria transmission (Oyewole *et al.*, 2007; Nwosu *et al.*, 2011). Large number of *Anopheles* species have been reported in Nigeria, but the world's most efficient vectors of human malaria belong to members of *Anopheles gambiae sensu lato* (*s.l.*) complex and *An. funestus* complexes (Oyewole *et al.,* 2007). *An. gambiae s.l.* is a complex of eight sibling species, which presently includes *An. gambiae* (*sensu stricto*) Giles; *An. arabiensis* Patton; *An. bwambae* White; *An. melas* Theobald; *An. merus* Dönitz; *An. quadriannulatus* Theobald; *An. amharicus* Hunt, Coetzee and Fettene and *An. comorensis* Brunhes, le Goff and Geoffroy (Obembe and Awopetu, 2014). These sibling species are morphologically identical, but genetically distinct. The two members of the *An. gambiae* complex most responsible for transmission of malaria, *An. gambiae s.s.* and *An. arabiensis*, are broadly sympatric, although the latter is more broadly distributed in arid regions (De Souza *et al.*, 2010; Levine*et al.*, 2004). The range and relative abundance of *An. arabiensis* and *An. gambiae s.s.* appear to be strongly influenced by climatological factors especially total annual precipitation (Onyabe and Conn, 2001). Tropical areas including Nigeria have the best combination of adequate rainfall and humidity allowing for breeding and survival of anopheline mosquitoes (Ayanda, 2009). Therefore, malaria could be the largest contributor to total disease burden and productivity losses resulting from major tropical diseases in the country. Demography

as well as the socio-economic and cultural characteristic of the population, the predominant vectors and parasites play vital role in the sustenance of malaria burden in the country. In Nigeria, malaria is endemic and stable, being a major cause of morbidity and mortality, resulting in 25% infant and 30% childhood mortality (FMH, 2013).

Prevention of malaria is best achieved by vector control, which today in Africa relies on the use of insecticides. Monitoring mosquito vector populations is an integral and essential component of most vector control programmes. Contemporary data on mosquito species composition, resistance to insecticides, and actual infection status of mosquito vectors are a prerequisite for GCeffective interventions (Vontas, 2016). Malaria control methods focus largely on impacting the vector population to reduce transmission. Interventions such as use of indoor residual spray (IRS) and long-lasting insecticidal nets, take advantage of species-specific vector behaviors that result in exposure to lethal insecticide (Bayoh *et al.,* 2014). Knowledge of mosquitoes diversity and abundance in students' hostel will provide information on the health implications and effective strategies against mosquito-borne diseases.

Furthermore, the understanding the transmission status of *Plasmodium* parasite by Anopheline mosquitoes in students' hostel will provide useful information to the health authorities on proper measures in case of malaria outbreak.

MATERIALS AND METHODS
Study area
The study was carried out in Ahmadu Bello University Zaria, Kaduna state. Zaria is situated at Latitude 11° 25′ North and Longitude 4° 27′ East of Nigeria. Zaria is located in the North Western part of Nigeria.

Collection of Mosquitoes
Two blocks were randomly selected from each of the male and female halls, Mosquitoes were collected indoors using the method of Mbah and Egidenda, 2007. The open end of a test tube measuring 10cm in length by 1.4cm in diameter was placed over the resting mosquito and slightly moved to enable the mosquito fly into the tube. A piece of cotton wool lightly soaked with chloroform was used to plug the mouth of the test tube. The dead mosquitoes were then transferred gently into a Petri dish, clearly labelled indicating the date and site of collection. The mosquitoes collected were then transported to the Entomology laboratory Department of Zoology, Ahmadu Bello University Zaria for identification.

Consecutive indoor collections of indoor resting mosquitoes were carried out in each location once in a week during the rainy seasons between June and October, 2018.

Morphological Identification
The mosquitoes were identified morphologically with the aid of a light microscope at x20 and x40 magnification using the identification keys of Gilles and Coetzee (1987) and Gillies and De-mellion (1968).

Dissection of Mosquitoes
The engorged female *Anopheles* mosquito was dissected transversely between the first and third pairs of legs under the dissecting microscope; Each abdomen was cut off using sterile tips. The entire samples were air dried, fixed in methanol and were stained for 15-30

minutes using Giemsa and thin blood film examined under the light microscope using ×100 objective lens as described by Cheesbrough, (2009).

Data Analyses

Two diversity indices, Shannon-Wieners (H), and Evenness (E), were used to determine the mosquitoes species diversity. Principal Component Analysis (PCA) Biplot was used to correlate the relationship of mosquitoes diversity, relative abundance, location and prevalence of *Plasmodium* parasite

RESULTS AND DISCUSSION

A total of 1341 mosquitoes were collected in 6 students' hostels from June to October 2018. They were classified into four genera and four species the highest number of individual mosquitoes were recorded from Akenzua (320) while the least was from Amina (161). Among the individual female mosquitoes caught in hostels. Akenzua 135(36.49%) had the highest number of *Anopheles gambiae* while Ribadu 22 (5.95%) had the least (Table 1). In ICSA, 7 (46.67%) *Anopheles funestus* which formed the highest collection of this species were recorded, followed by Akenzua and Suleiman where 4 (26.67%) were recorded. Akenzua 179 (19.67%) had the highest *Culex quinquefascitus* while Amina had the least 124 (13.05%). *Aedes aegypti* was recorded the highest in Akenzua 2 (66.67%) while Suleiman had the least 1 (33.33%). In terms of Shannon diversity Akenzua, ICSA/Ramat and Suleiman Hostel were recorded the moderate 1.05, 1.07 and 1.05 respectively while Amina, 0.85, Danfodio, 0.89 and Ribadu,0.77 showed lower species diversity. The evenness of species distribution of Akenzua, Amina, Danfodio, ICSA/Ramat hall, Ribadu and Suleiman were found to be 0.57, 0.78, 0.81, 0.73, 0.72 and 0.57 respectively.

**Table 1: Overall Species Distribution of Mosquitoes in Students Hostels Ahmadu Bello University, Zaria**

	Locations (Halls)					
Species	Akenzua	Amina	Danfodio	ICSA/Ramat	Ribadu	Suleiman
An. gambiae	135 (36.49)	33 (8.92)	30 (8.11)	88 (23.78)	22 (5.95)	62 (16.76)
An. funestus	4 (26.67)	0 (0.00)	0 (0.00)	7 (46.67)	0 (0.00)	4 (26.67)
Cx. quinquefasciatus	179 (18.34)	128 (13.05)	166 (17.01)	170 (17.42)	170 (17.89)	141 (14.45)
Ae. aegypti	2 (66.67)	0 (0.00)	0 (0.00)	0 (0.00)	0(0.00)	1 (33.33)
Abundance	320	161	196	264	192	208
Shannon_H	1.05	0.85	0.89	1.07	0.77	1.05
Evenness_e^H/S	0.57	0.78	0.81	0.73	0.72	0.57

A total of 810 blood-fed mosquitoes were caught in students hostels in Ahmadu Bello University, Zaria (Table 2). The distribution of individual female mosquitoes caught in the hostel were as follows: *Culex quinquefasciatus* were recorded the highest number of blood-fed 591(60.55) followed by *An. gambiae s.s* 210(56.75), *An. funestus* 8(53.33) while *Aedes aegypti* had the least 1(33.33). The number of unfed mosquitoes recorded indicate that *Culex quinquefasciatus* had the highest 359(36.78) while *Aedes aegypti* had the least 2(66.66).

Table 2: Species Distribution of Blood-fed and unfed Mosquitoes in Students hostels, A.B.U

Species	Total No. examined	Blood fed (%)	Unfed (%)
An. gambiae	370	210 (56.75)	160 (43.24)
An. funestus	15	8 (53.33)	7 (46.66)
Cx. quinquefasciatus	950	591(60.55)	359 (36.78)
Ae. aegypti	3	1 (33.33)	2 (66.66)
Total	1338	810(60.53)	528(39.46)

The Relative abundance of mosquitoes in students hostels is shown in (Fig.1) *Cx. quinquefasciatus* recorded the highest (59.12) followed by *An. gambiae s.s* (22.47), *An. funestus* (0.91) while *Ae.aegypti* had the least (0.18)
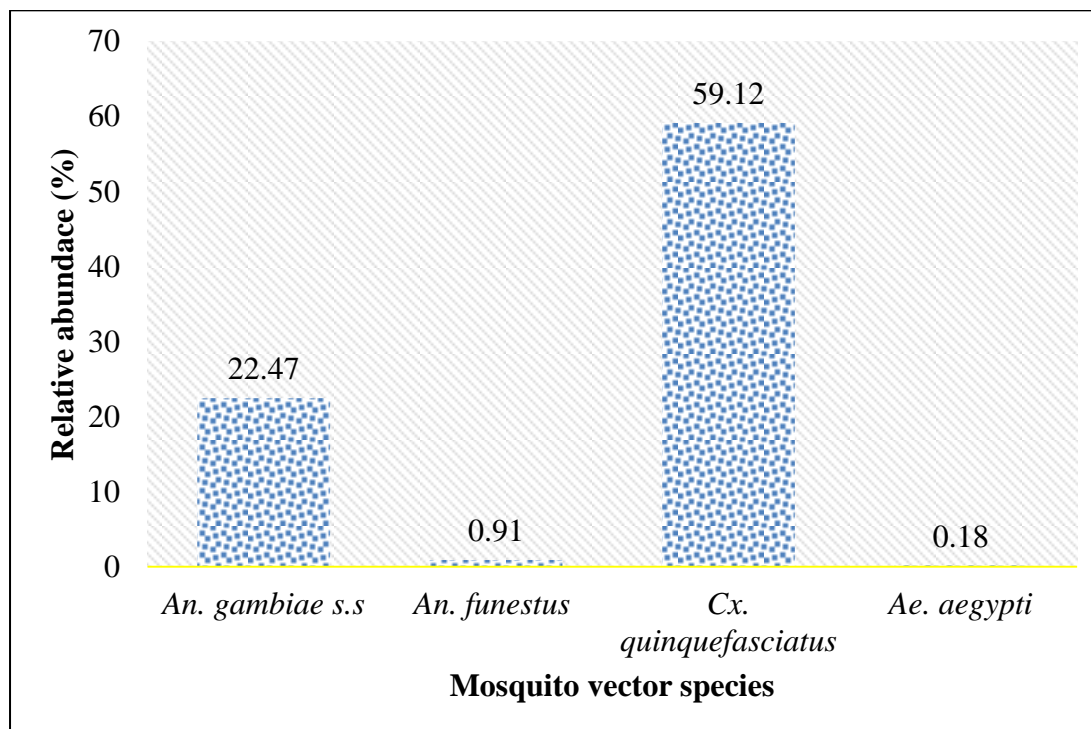
Figure 1: Relative Abundance of Mosquito Species in students hostels Ahmadu Bello   University

A total of 60 blood–fed female *Anopheles* mosquitoes were examined in this study. *Plasmodium falciparum* was encountered and ICSA/Ramat (7) had the highest number of infected mosquitoes while Danfodio had 2(28.54)

Table 3: Malaria parasite in blood-fed Mosquitoes

	No examined	Prevalence (%)
		P. falciparum
Akenzua	12	5 (41.67)
Amina	12	3 (25)
Danfodio	7	2 (28.57)
ICSA/Ramat	12	7 (58.33)
Ribadu	5	0 (0)
Suleiman	12	4 (33.33)
TOTAL	60	21 (35.00)

The first component had the highest possible variance (64.49). *An. gambiae, An. funestus, Ae.aegypti*, and *Cx. quinquefasciatus* were positively correlated with Akenzua, ICSA/Ramat and Suleima hostels, *Plasmodium falciparum* are associated with Suleiman hostel. The Evenness of species distribution were negatively correlated with Amina hostel, Danfodio hostel and Ribadu hostel (27.76) as seen in fiqure 2
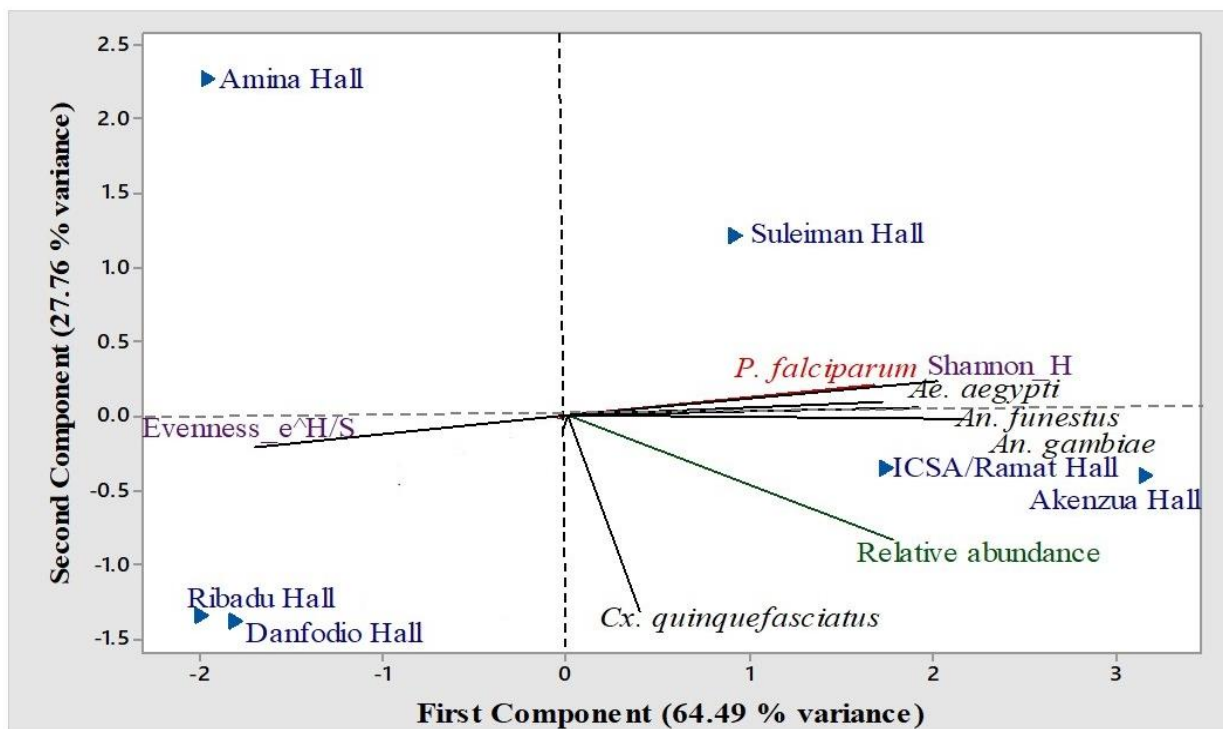

Figure 2: Principal component analysis (Biplots) of mosquito diversities, evenness, their relative abundance and plasmodium parasites detected in students' halls, Ahmadu Bello University, Zaria, Nigeria

DISCUSSION

The dominant species identified were *Culex quinquefasciatus,* and *Anopheles gambiae* collected in all the six student hostels while the others like *Anopheles funestus* were less frequent. *Aedes aegypti* were present only in Akenzua and Suleiman hostels. This could be as a result of the presence of collections of dirty water in sample locations which serve as their breeding grounds. Similarly, the surrounding bushes around the various residential hostels which include tufts of grass and other emergent vegetation which could serve as their resting places, this could be responsible for large number observed. These findings agree with the report of Michael (2014) who reported the presence of similar species of mosquitoes in Zaria dam in which *Culex quinquefasciatus* and *Anopheles gambiae* were the most dominant. This is in line with the study of Adeleke *et al.,* (2010) who recorded *Culex quinquefasciatus* to be the second predominant species of mosquitoes occurring in Abeokuta.

The proportion of *Anopheles gambiae* and *Culex quinquefasciatus* with blood were higher than the others namely : *An. funestus*, and *Ae. aegypty* .The high percentage of blood-fed mosquitoes clearly shows that the two genera are not just endophagic, but also endophilic as they were caught while resting indoors. This is not surprising as both species are anthropophilic. This finding was observed by Ebenezer *et al.,* (2012) during his similar study in some part of Bayelsa State. And Adeleke *et al.,* (2010) in Abeokuta. The preponderance of blood-fed mosquitoes over the unfed ones is a positive indication that man-biting mosquitoes are active in the halls with the subsequent transmission of the parasites they carry and resultant diseases being spread within the student population.

Culex quinquefasciatus, have the widest range of distribution and formed the dominant species in this study. This could be due to the fact that the *culex* species are known to breed in polluted water. Water storage containers and ground collection of dirty water were observed around the hostels which may increase the breeding ground.

Two species of *Anopheles* were caught: *Anopheles gambiae* and *Anopheles funestus*. *Anopheles gambiae* constitute the highest out of the two *Anopheles* species sampled which was also the second most abundant species caught during the sampling after *Culex quinquefasciatus*. This observation can be explained in the light of the fact that generally, *An. gambiae* complex are associated with human beings and therefore tend to enter the hostels and breed in water collections within the vicinity. Another reason could be associated with their feeding and resting habits where *An. gambiae* complex is known to be anthropophilic. *Anopheles funestus* was in relatively low proportion and this may be due to the fact that it breeds in more permanent large water bodies like rivers and lakes (Ndams, 2004). These findings agrees with the report of Ayanda (2009), who recorded more than half of *An. gambiae* and to a lesser extent *Anopheles funestus* which are commonly reported malaria vectors in Nigeria.

Aedes aegypti were in relatively low proportion in this study, and this may be due to the fact that it is a diurnal mosquito and has ubiquitous mode of feeding. *Aedes aegypti* is a well-known vector of yellow fever virus. The presence of these few specimens may not pose any danger now, but continuous monitoring of this environment for *Aedes* specimens is invaluable to forestall unexpected increase and possibility of yellow fever epidemic.

Parasites detected in blood-fed females *Anopheles gambiae* was *Plasmodium falciparum.* This is not surprising that they were found in mosquitoes blood samples from all the six students hostels because it has been the major malaria parasite in the area. These findings agree with the report of Igbeneghu *et al.* (2012) who reported *Plasmodium falciparum* as the dominant

species in Iwo community. Several studies reported high prevalence of *P. falciparum* in different parts of Nigeria. (Babamale and Ugbomoiko, 2016 and WHO, 2017).

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CONCLUSION
The study showed that a total of one thousand three hundred and fourty (1341) one mosquitoes were identified and these comprises of five species; *Cx. quinquefasciatus* (59.12%), *An. gambiae* (22.47%), *An. funestus*, (0.91%) and *Ae. aegypti* (0.18%). The results showed that majority of the mosquitoes species were fed and therefore control measures aimed at eliminating the breeding sites and reducing its contact with the students so as to minimize disease transmission among the student hostel. Strong emphasis should also be laid on the importance personal protection and environmental hygiene in the student' hostel.

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