SURVEILLANCE AND INSECTICIDE SUSCEPTIBILITY STATUS OF CULICINE MOSQUITOES IN SELECTED COMMUNITIES UTILIZING LONG-LASTING INSECTICIDAL NETS IN KWARA STATE, NIGERIA

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ABSTRACT

Vector control programs in Nigeria are mostly targeted towards reducing the burden of malaria with less emphasis placed on other debilitating vector borne diseases such as dengue, yellow fever and filariasis. This study assessed the indoor resting densities and insecticide susceptibility status of Culex and Aedes mosquitoes in selected communities utilizing long-lasting insecticidal nets (LLIN) in Kwara State, Nigeria. Pyrethrum spray collections of indoor resting Aedes and Culex mosquitoes were conducted in three communities while adults of both mosquito species reared from larval collections were exposed to pyrethroid, organochlorine, and carbamate insecticides following WHO procedure. Results showed that the higher indoor resting densities of Culex quinquefasciatus (2.5 – 3.4) collected were not significantly (p>0.05) different from the Aedes aegypti (0.3 – 1.3) in all the communities. Carbamate resistance (≤ 81 % post exposure mortalities) was observed in both Culex (Ilota and Amoyo) and Aedes populations (Gaa-Bolorunduro). Aedes in Gaa-Bolorunduro and Ilota were fully susceptible (100 % mortality) to permethrin and DDT while the Culex population showed differential susceptibility (64 – 100%) to pyrethroid and organochlorine insecticides tested. These findings show focal insecticide resistance requiring specific intervention in each community based on the evidence provided by this study. Pyrethroid resistance status of the Culex quinquefasciatus in these areas may result in access of the vectors to net occupants leading to lower LLIN utilization rates among users. Environmental management strategy in addition to the use of effective insecticide will be most probable for management of vector borne diseases in these localities.

Keywords: Insecticide resistance, *Aedes aegypti*, *Culex quinquefasciatus,* LLIN, Dengue, Lymphatic Filariasis, Kwara State, Nigeria

INTRODUCTION

Baseline studies on vector identification and insecticide susceptibility status are often conducted to guide malaria vector control programmes with less emphasis on non – malaria vector species such as *Aedes aegypti* and *Culex quinquefasciatus*. These often neglected species are responsible for the transmission of life-threatening diseases such as yellow fever, lymphatic filariasis (LF) and dengue fever. Vector control is the only means of tackling *Aedes* transmitted dengue fever which still has no vaccine or prophylaxis (Kamgang *et al.,* 2011; Marcombe *et al.,* 2012). The latent posture of the dengue virus in West Africa no longer holds as a result of increased number of outbreaks or case reports in countries like Mali, Cape Verde and Senegal (Franco *et al.,* 2010). There is an increasing evidence of active circulation of the dengue fever virus in various parts of Nigeria. Seroprevalence rates were 35 % in Ibadan, south west (Oyero and Ayukekbong, 2014) and 68 % in Maiduguri north eastern zone of the country (Baba *et al.,* 2013) while 31 % true incidence rates of the virus have also been reported among febrile children in Ilorin, Kwara State (Adedayo *et al.,* 2013). In addition to this, the burden of LF in Nigeria is the highest in Africa with approximately two-third of the country's population at risk and as many as 5 % of women and 50 % of men suffering from swollen limbs and genitals respectively in some communities (FMoH, 2014).

Earlier assertions of culicine mosquitoes' incompetence as LF disease vectors in West Africa (Lenhart *et al.,* 2007) has been refuted by recent reports of infective microfilaria in culicine (*Mansonia*) mosquitoes from Ghana (Ughasi *et al.,* 2012). Other factors such as low or inefficient drug distribution in Nigeria (FMoH, 2014), mass drug administration (MDA) failure in Ghana and Burkina Faso (Ughasi *et al.,* 2012) and the ability of the culicines to transmit low levels of microfilariae (Duerr *et al.,* 2005) have further led to a review of the WHO filariasis elimination strategy to include vector control (WHO, 2012). In Nigeria, both *Culex* and *Aedes* species are responsible for the transmission of LF, dengue and yellow fever (Okogun *et al.,* 2003). Yet, only few reports on *Culex*-human host interactions in urban areas (Oduola and Awe, 2006; Manyi *et al.,* 2014) are available. Studies on surveillance of adult *Aedes* and *Culex* mosquitoes within rural Nigerian communities are almost non-existent. Such studies are required to establish the level of interaction between these mosquitoes and humans in a bid to feed.

To date, frontline tools for effective vector control are still insecticide-based, challenged by the need to ensure continued susceptibility of the local vectors to the recommended insecticides. Moreover, Nigeria has recently launched a nationwide malaria and elephantiasis elimination co-implementation plan based on integrated distribution of LLINs and MDA (FMoH, 2014). Rapid scale up of LLINs in Nigeria over the years have been accompanied with several studies on insecticide resistance in *Anopheles* mosquito in different parts of the Country (Awolola *et al.,* 2007; Oduola *et al.,* 2010; Oduola *et al.,* 2012; Ibrahim *et al.,* 2013), to the neglect of the *Aedes* and *Culex* species. Only Ayorinde *et al.* (2015) and a few other available urban community reports investigated the susceptibility status of *Ae. aegypti* and *Cx. quinquefasciatus* to insecticides such as DDT and permethrin in Lagos and Zaria (Ndams *et al.,* 2006) with none considering rural communities. Also, the nationwide deployment of LLINs (FMoH, 2014) either alone or together with other insecticide based strategies (Obembe *et al.,* 2014a) used against malaria vectors is expected to exert some level of resistance selection pressure on both *Culex* and *Aedes* species in the rural communities. Third, insecticidal control of *Culex* and *Aedes* mosquitoes in the country localities has to be evidence-based to be effective.

This study assessed the susceptibility status of *Culex* and *Aedes* mosquitoes in three (two rural and one semi-urban) communities utilizing LLIN in Kwara State north central Nigeria, to three classes of insecticides recommended for vector control.

MATERIALS AND METHODS

Study Area: The study involved three communities located in two Local Governments Areas (LGA) of Kwara State, Nigeria. Two of the communities, Ilota (N 08° 28ˈ E 04° 38′) and Gaa-Bolorunduro (08° 27ˈ E 04° 38′) are rural and located within Ilorin South Local Government Areas while, the semi-urban Amoyo community (08° 42ˈ E 04° 62′) is located in Ifelodun LGA of the State. Gaa-Bolorunduro is inhabited by cattle-rearing nomadic Fulanis who also practice rice farming using water from Balogun Dam while, Amoyo and Ilota residents are mostly Yoruba farmers. Ilota is particularly known for the local production of Shea butter for industrial use. The Shea butter in Ilota and cassava processing points in Amoyo featured water-filled earthen pots (Figure 1) that had *Culex* and *Aedes* mosquito larvae during this study.

Figure 1: Shea butter processing pots, habitat of *Culex* and *Aedes* mosquito larvae in Ilota community, Kwara State, Nigeria

All the communities were chosen on the basis of widespread use of LLIN (at least 60 %) among the residents.

Ethics: Ethical approval for this study was obtained from the University of Ilorin Ethical Review Committee (UERC/ASN/195).

Mosquito Collection and Rearing: Adult endophilic mosquitoes were collected once a week for six consecutive weeks between the month of February and April 2013 in ten randomly selected rooms inhabited overnight, using pyrethrum spray catch method as described (WHO, 2003). The mosquitoes were examined for evidence of a blood meal., Indoor resting densities (IRD) of the mosquitoes were also determined as numbers of female mosquitoes collected divided by the number of rooms surveyed (WHO, 2003). *Culex* and *Aedes* larvae were collected using a dipper from the earlier described earthen pots and semi-permanent ground pools in the localities. Larval samples collected were transported and reared to adulthood in insectaries of the Department of Zoology, University of Ilorin.

Insecticide Susceptibility Tests: Insecticide susceptibility tests conducted at ambient temperature (26.00 ± 4.00°C) and relative humidity (71.00 ± 11.00 %), followed WHO procedures (WHO, 2013) in exposing four replicates of twenty (20) 2 – 3 day old blood-unfed female *Culex* and *Aedes* mosquito samples to pyrethroid (0.05 % deltamethrin, 0.75 % permethrin, 0.05% lambdacyhalothrin and 0.5% etofenprox), organochlorine (4 % DDT) and carbamate (0.1 % bendiocarb) using WHO diagnostic test kits. The *Culex* in Gaa-Bolorunduro and *Aedes* in Amoyo were not tested for insecticide susceptibility because of the low numbers of mosquito larvae encountered in the respective communities. Knock down rates of mosquitoes were recorded at 10 minutes interval for one hour and the final mortality noted after 24 hours. Adult mosquitoes tested for susceptibility and those collected indoors were preserved on silica for further morphological identification using standard key (Rueda, 2004).

Statistical Analysis: Differences in the indoor resting densities of female *Culex* and *Aedes* mosquitoes were subjected within and across the communities to statistical analysis using student's t-test to determine the levels of significance ($p<0.05$). Insecticide susceptibility of the mosquitoes tested was based on 98 – 100 % post exposure mortality (WHO, 2013) while knock down times (KDTs) were estimated using a log time probit model with SPSS version 16.0 for windows (SPSS Corporation USA). Chi-square test of homogeneity of proportion was used to determine the association between knock down and mortalities of the mosquitoes.

RESULTS

Mosquito Surveillance: All the *Culex* and *Aedes* mosquitoes collected were identified as *Cx. quinquefasciatus* and *Ae. aegypti* respectively. The highest numbers of adult *Culex* mosquito were found in Gaa-Bolorunduro while Ilota community had the highest numbers of *Aedes* mosquitoes. The average indoor resting densities (IRD) of *Culex* mosquitoes in the three communities; Amoyo (3.30 ± 4.19), Ilota (2.50 ± 4.10) and Gaa-Bolorunduro (3.40 ± 4.91) were higher than those of *Aedes;* Amoyo (0.30 ± 0.95), Ilota (1.30 ± 2.17) and Gaa-Bolorunduro (0.80 ± 2.06) (Table 1). Amoyo had the lowest number of *Aedes* mosquitoes. Similarly, Ilota community with the lowest number of *Culex* mosquitoes also had the highest number of *Aedes* (Table 1). Interestingly, the same Ilota community had the closest proportion of *Culex* (66.00 %) and

Aedes (34.00 %) mosquitoes. However, the highest difference between the percentage of *Culex* (92 %) and *Aedes* (8.00 %) samples was found in Amoyo community (Figure 2).

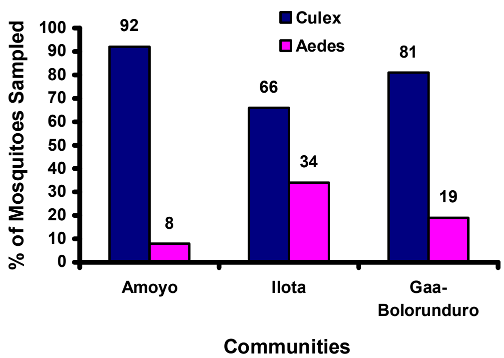

Figure 2: Percentages of *Culex* and *Aedes* mosquitoes in the three communities in Kwara State, Nigeria

Only the difference between the IRD of *Cx. quinquefasciatus* mosquitoes found in Amoyo and Ilota was significant (p = 0.003). Conversely, no significant differences (p>0.05) were observed between the IRD of *Ae. aegypti* in Amoyo and Ilota (p = 0.934), Amoyo and Gaa-bolorunduro (p = 0.659), and Ilota and Gaa-bolorunduro (p = 0.261). Likewise, the differences found between the IRD of adult *Cx. quinquefasciatus* and *Ae. aegypti* samples collected in Amoyo (p = 0.059), Ilota (p = 0.384) and Gaa-bolorunduro (p = 0.218) were not significant.

Blood-fed and Un-Fed Status of Mosquito

The abdominal status of the mosquitoes collected indoors showed that 19(58.00 %) of *Culex* mosquitoes sampled in Amoyo community were engorged with blood compared with lower blood feeding rates of mosquitoes in Ilota 7(28.00 %) and Gaa-Bolorunduro 6(18.00 %) communities (Table 2). The differences between blood-fed and blood-unfed *Culex* mosquitoes were not significant (p>0.05) in the three communities: Amoyo (p = 0.645), Ilota (p = 0.335) and Gaa-Bolorunduro (p = 0.337) (Table 3). While the entire adult *Aedes* mosquitoes sampled in Amoyo and Gaa-Bolorunduro were blood-unfed, the difference between the average number of blood-fed (0.33 ± 0.52) and blood-unfed (1.00 ± 1.27) *Aedes* mosquito in Ilota was not significant (p = 0.102) (Table 3). Overall, the difference between the blood-fed (5.34 ± 7.34) and blood-unfed (10.00 ± 13.79) *Culex* mosquito sampled in the three communities was not significant (p = 0.280) (Table 3).

Insecticide Susceptibility: Mortality results from exposures to different insecticides showed that *Culex* mosquitoes from Ilota were resistant to deltamethrin (93 % mortality), lambdacyhalothrin (95 % mortality) and etofenprox (96 %). These populations were however susceptible (100 % mortality) to permethrin. Similarly, resistance to deltamethrin (64 % mortality) and permethrin (79 %) insecticides were observed in the *Culex* mosquitoes from Amoyo community (Table 4). On the other hand, *Ae. aegypti* mosquitoes from Gaa-Bolorunduro community were susceptible to permethrin (Table 5). Moreover, *Culex* mosquitoes from Ilota were susceptible (99 %) to DDT as against those from Amoyo which were resistant (71 %) (Table 4). Mortalities from exposure of *Aedes* and *Culex* species to Bendiocarb showed that all the mosquito samples tested from the three communities were resistant (Tables 4 and 5).

DISCUSSION

This study evaluated the indoor resting densities and insecticide susceptibility of *Cx. quinquefasciatus* and *Ae. aegypti* in three communities utilizing LLIN in Kwara State, North Central Nigeria. Higher numbers of *Cx. quinquefasciatus* mosquitoes, which are known to rest indoors after biting in the night (Nwoke *et al.,* 2010) were found in the three communities compared to the day biting (Uttah *et al.,* 2013) *Ae. aegypti* samples. Yet, the total number of blood-fed *Culex* mosquitoes was about half of the blood-unfed probably due to the protective effect of the LLIN being utilized in these communities.

Adults of these mosquito species found resting in the rooms inhabited overnight by householders of the three communities show some level of endophily. However, the numbers of mosquitoes collected indoors in all the communities were too low to inform any reliable conclusion.

Table 1: Indoor resting densities of *Aedes* and *Culex* mosquitoes in the three communities in Kwara State, Nigeria

Mosquito	Community	Indoor Resting Densities (IRD) of Mosquitoes						Total	Mean IRD per room
		Weeks							
		1	2	3	4	5	6		
Culex	**Amoyo**	1(0.1)	0(0)	12(1.2)	2(0.2)	9(0.9)	9(0.9)	33(3.3)	3.3±4.19
	Ilota	0(0)	1(0.1)	9(0.9)	3(0.3)	5(0.5)	7(0.7)	25(2.5)	2.5±4.10
	Gaa-Bolorunduro	20(2.0)	6(0.6)	4(0.4)	1(0.1)	1(0.1)	2(0.2)	34(3.4)	3.4±4.91
Aedes	**Amoyo**	0(0)	0(0)	0(0)	1(0.1)	2(0.2)	0(0)	3(0.3)	0.3±0.95
	Ilota	0(0)	5(0.5)	0(0)	6(0.6)	0(0)	2(0.2)	13(1.3)	1.3±2.17
	Gaa-Bolorunduro	1(0.1)	1(0.1)	2(0.2)	3(0.3)	0(0)	1(0.1)	8(0.8)	0.8±2.06

Number in parenthesis = percentages

Table 2: Blood meal status of *Culex* and *Aedes* mosquitoes from the three communities in Kwara State, Nigeria

Communities	*Culex* N(%)			*Aedes* N(%)		
	Blood-fed	Blood-unfed	Total	Blood-fed	Blood-unfed	Total
Amoyo	19(58)	14(42)	33(100)	0(0)	3(100)	3(100)
Ilota	7(28)	18(72)	25(100)	4(31)	9(69)	13(100)
Gaa-Bolorunduro	6(18)	28(82)	34(100)	0(0)	8(100)	8(1000)

N = number of mosquitoes sampled

Table 3: Blood-fed and unfed *Culex* and *Aedes* mosquitoes from the three communities in Kwara State, Nigeria

Communities	*Culex* N (%)			*Aedes* N (%)		
	Blood-fed	unfed	*p*-value	Blood-fed	Unfed	*p*-value
Amoyo	3.17±3.87	2.33±2.58	0.645	0.00	0.5±0.84	-
Ilota	1.17±1.47	3.00±3.58	0.335	0.33±0.52	1.00±1.27	0.102
Gaa-Bolorunduro	1.00±2.00	4.67±7.63	0.337	0.00	1.33±1.03	-
Total	5.34±7.34	10.00±13.79	0.280	0.33±0.52	2.83±3.14	-

N = number of mosquitoes sampled

An interesting observation was the cohabitation of *Cx. quinquefasciatus* and *Ae. aegypti* mosquito larvae in the same Shea butter processing pots in one of the communities (Ilota) in this study. The two species rarely co-exist in the same breeding site (David *et al.,* 2012). Species cohabitation in this study could be due to scarcity of breeding sites in the late dry season when the study was conducted.

Insecticide resistance of *Cx. quinquefasciatus* mosquitoes in two of the localities, to different insecticides could be due to insecticide use either in the form of massively distributed pyrethroid LLINs in the state (Obembe *et al.,* 2014b) or for crop protection. The observation of cross-resistance between DDT and pyrethroids (deltamethrin and permethrin) in *Culex* mosquitoes from Amoyo may suggest the possible involvement of target site resistance mechanism while the absence of the same cross-resistance in *Culex* from Ilota populations suggests the involvement of metabolic resistance. This should be further analyzed to inform insecticide resistance management. Interestingly, both *Cx. quinquefaciatus* and *Ae. aegypti* mosquitoes were susceptible to permethrin and DDT in most of the sites, showing perhaps the similar mechanism responsible for both class of insecticide.

Table 4: Estimated KDT_{50}, KDT_{95} and mortality of *Culex quinquefasciatus* populations in two communities in Kwara State, Nigeria

Study Area	Class of Insecticide	Insecticide	N	24 hour % mortality	KDT_{50} (95% CL)	KDT_{95} (95% CL)	Status	X^2 (Significance)
Ilota	**Pyrethroids**	0.05% Deltamethrin	80	92.5	21.31 (19.24-24.60)	23.94 (21.27-28.38)	R	2.049 (p>0.05)
		0.05% Lambdacyhalothrin	80	95	25.81 (22.69-31.16)	29.76 (25.10-37.23)	R	15.45 (p<0.05)
		0.5% Etofenprox	80	96.25	26.63 (23.82-32.22)	29.53 (25.89-37.26)	R	32.22 (p<0.05)
		0.75% Permethrin	80	100	44.04 (41.08-48.13)	73.64 (63.05-97.79)	S	37.42 (p<0.05)
	Carbamate	0.1% Bendiocarb	80	81.25	-	-	R	-
	Organochlorine	4.0% DDT	80	98.75	-	-	S	-
Amoyo	**Pyrethroids**	0.05% Deltamethrin	80	63.75	25.4 (13.92-35.43)	47.65 (34.52-100.28)	R	14.89 (p<0.05)
		1.0% Permethrin	80	78.75	38.05 (26.51-46.10)	55.56 (45.91-69.56)	R	13.43 (p>0.05)
	Carbamate	0.1% Bendiocarb	80	77.50	-	-	R	-
	Organochlorine	4.0% DDT	80	71.25	a	b	R	-

N=numbers of mosquitoes exposed, X^2 values are for the test of fit of the log-time probit model used to estimate the KDT_{50} and KDT_{95}, KDT_{50} and KDT_{95} were not estimated for bendiocarb because they are not known to have knock down effects, a = not obtained after 60 minutes, b = not obtained after 60 minutes, sig. = Deviations significance, S = Susceptible, R = Resistance, - = not done

Table 5: Estimated KDT_{50}, KDT_{95} and mortality of the *Aedes aegypti* mosquito populations in two communities in Kwara State, Nigeria

Study Area	Class of Insecticide	Insecticide	N	24 hour % mortality	KDT_{50} (95% CL)	KDT_{95} (95% CL)	Status	X^2 (Significance)
Gaa-Bolorunduro	**Pyrethroid**	1.0% Permethrin	80	100	27.78 (23.69-33.89)	48.76 (40.70-54.98)	S	41.95 (P<0.05)
	Carbamate	0.1% Bendiocarb	80	50	-	-	R	-
Ilota	**Pyrethroid**	1.0% Permethrin	80	100	29.59 (25.88-34.79)	49.89 (42.01-56.76)	S	45.01 (P<0.05)
	Organochlorine	4.0% DDT	80	100	20.69 (8.96-28.91)	35.69 (24.75-56.78)	S	17.66 (P<0.05)

N=numbers of mosquitoes exposed, X2 values are for the test of fit of the log-time probit model used to estimate the KDT50 and KDT95, sig. = Deviations significance, S = Susceptible, R = Resistance, KDT50 and KDT95 were not estimated for Bendiocarb because they are not known to have knock down effects.

The only exception to this was the Amoyo *Culex* population which showed resistance to both insecticides. This is attributable to the possibility of more intense insecticide pressure against the mosquitoes in Amoyo, being the only semi-urban locality among the three communities. Similar results of adult *Cx. quinquefasciatus* and *Ae. aegypti* mosquito susceptibility to permethrin insecticide have also been reported in Zaria, North West Nigeria (Ndams *et al.,* 2006). Conversely, both *Culex* and *Aedes* mosquitoes from all the three communities were resistant to the carbamate (bendiocarb) probably due to resistance selection pressure from agricultural insecticides being used in these agrarian communities. Similar results of multiple pyrethroid and carbamate resistance found in the *Culex* in this study have been reported in *Anopheles* mosquitoes in Lagos Nigeria (Oduola *et al.,* 2012) while the results of full permethrin susceptibility in addition to bendiocarb resistance observed in the *Aedes* samples have also been found in *Anopheles* mosquitoes in Ibadan (Ibrahim *et al.,* 2013). The implication of the all-round bendiocarb resistance is that carbamates should not be considered as an alternative insecticide for non-pyrethroid based IRS in these areas. The main mosquito control strategy in Nigeria has been the massive distribution of free LLINs known to effectively reduce LF transmission in *Loa loa* co-endemic areas (Emukah *et al.,* 2009). However, reports have shown that *Culex* mosquitoes are usually not as responsive to LLIN intervention (Kulkarni *et al.,* 2007) as the *Anopheles* species. Chemical larviciding is not feasible in this cassava or Shea butter processing breeding sites due to contamination. Also, biolarvicides like oil will also not work for long because of the high level of organic (cassava or shea butter) content. The adoption of screens to cover the Shea butter and cassava pots may be considered at a community level to deny the culicine mosquitoes access to these domestic breeding sites.

Conclusion: Differential insecticide susceptibility amongst the three communities considered justifies the need for specific intervention based on the evidence provided by this study. Long-lasting insecticidal nets impregnated with permethrin will be useful against the *Culex* and *Aedes* mosquitoes in Ilota community where both species were susceptible to the insecticide. In addition, environmental management of the focal dry season mosquito breeding sites is required to control these vectors in the three communities.

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