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Susceptibility of *Anopheles gambiae* to Pyrethroids on Long Lasting Insecticide Treated Nets (LLINs) in Wukari, Taraba State, Nigeria

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Abstract

The use of Long Lasting Insecticide Nets is one of the strategies in malaria prevention and control. This study which was conducted within July to September 2014, comparatively determines the susceptibility of *Anopheles gambiae* to pyrethroids on Long Lasting Insecticide Treated Nets (LLINs) in Kinkiso, Mission and Wapan-ngaku areas of Wukari Local Government Area of Taraba State, Nigeria. LLINs were collected from households and primary health care clinics. A total of 450 laboratory bred female *Anopheles gambiae* mosquitoes were used in the test. A total of 12 used LLINs comprising 1, 2, 3 and 4 year old were sampled from each of the three communities and Bio-assayed. The larvae were collected from these communities and reared in the laboratory. The result of the susceptibility test showed that the mosquitoes were more susceptible to the pyrethroids in 0 - 2 years old nets than in 3 – 4 years. This could be as a result of the usage and the number of times the nets were washed leading to degradation of active ingredients. The highest knockdown rate was observed in Kinkiso (72.5 %) while Wapan-ngaku had the least (68.3 %). Within 24 hours, 100 % mortality was recorded Wapan-ngaku area. There was no significant difference in knock-down and mortality rate across the three communities. (χ^2cal=0.173, χ^2tab=9.49, df=4, $P>0.05$). Mean number of mosquitoes knocked-down after 3 minutes exposure among the communities and net ages showed that Kinkiso had the highest knock-down of 9.0 in one year old nets. The least was in Wapan-ngaku community with 4.3 knocked-down in 4 years old net (χ^2Cal= 0.903, χ^2tab=12.59, df=6, $P>0.05$). These indicate a gradual decrease in the efficacy of the chemical (pyrethroid) on the nets with age.
Keywords: *Anopheles gambiae*; Long lasting insecticide treated nets; Pyrethroids; Susceptibility.

1. Introduction

The mosquitoes are a family of small, midge-like flies: Although a few species are harmless or even useful to humanity, most are considered a nuisance because they feed on blood from living vertebrates, including humans. The females of many species of mosquitoes are blood-sucking pests. In feeding on blood, some of them transmit extremely harmful human and livestock diseases, such as malaria, yellow fever and filariasis [1].

Anopheles gambiae is the principal vector of malaria in Africa. According the latest WHO statistics, these parasitic diseases infects from 300 to 500 million persons per year in the world and kills more than a million and a half each year, mainly African children [2]. It is a complex of at least six morphologically indistinguishable species of mosquitoes in the genus *Anopheles*. This complex was recognised in the 1960s and includes the most important vectors of malaria in sub-Saharan Africa particularly of the most dangerous malaria parasite, *Plasmodium falciparum*. It is one of the most efficient malaria vectors known. This species complex consists of the following; *Anopheles arabiensis*, *Anopheles bwambae*, *Anopheles merus*, *Anopheles melas*, *Anopheles quadriannulatus* and *Anopheles gambiae* [3].

Insecticide-based control measures (e.g. indoor spraying with insecticides, bed nets) are the principal ways to kill mosquitoes that bite indoors. However, after prolonged exposure to an insecticide over several generations, mosquitoes, like other insects, may develop resistance, a capacity to survive contact with an insecticide. Since mosquitoes can have many generations per year, high levels of resistance can arise very quickly. Resistance of mosquitoes to some insecticides has been documented within a few years after the insecticides were introduced. Over 125 mosquito species have documented resistance to one or more insecticides. The development of resistance to insecticides used for indoor residual spraying was a major impediment during the Global Malaria Eradication Campaign. Judicious use of insecticides for mosquito control can limit the development and spread of resistance. However, use of insecticides in agriculture has often been implicated as contributing to resistance in mosquito populations [4].

One of the major recommendations from the meetings of the Roll Back Malaria (RBM) summit held in April, 2000, was that the private sector should take the lead in the production, procurement, promotion, use and sales of

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Insecticide Treated Nets (ITNs)/LLINs and public insecticides in Nigeria [5]. The policy encourages adoption of positive Net culture and development of appropriate mechanisms for monitoring and evaluation of ITNs/LLINs.

Taraba State has been involved in donor-founding integrated malaria control programmes involving mass distribution of free pyrethroid-treated bed-nets. There has not been effective monitoring and evaluation of insecticide decay/ or resistance to ascertain the effectiveness of the process. Therefore, the need to carry out the study to determine the effectiveness of the program in selected areas (Kinkiso, Mission quarters and Wapan-ngaku) of Wukari as this has not been carried out in these areas.

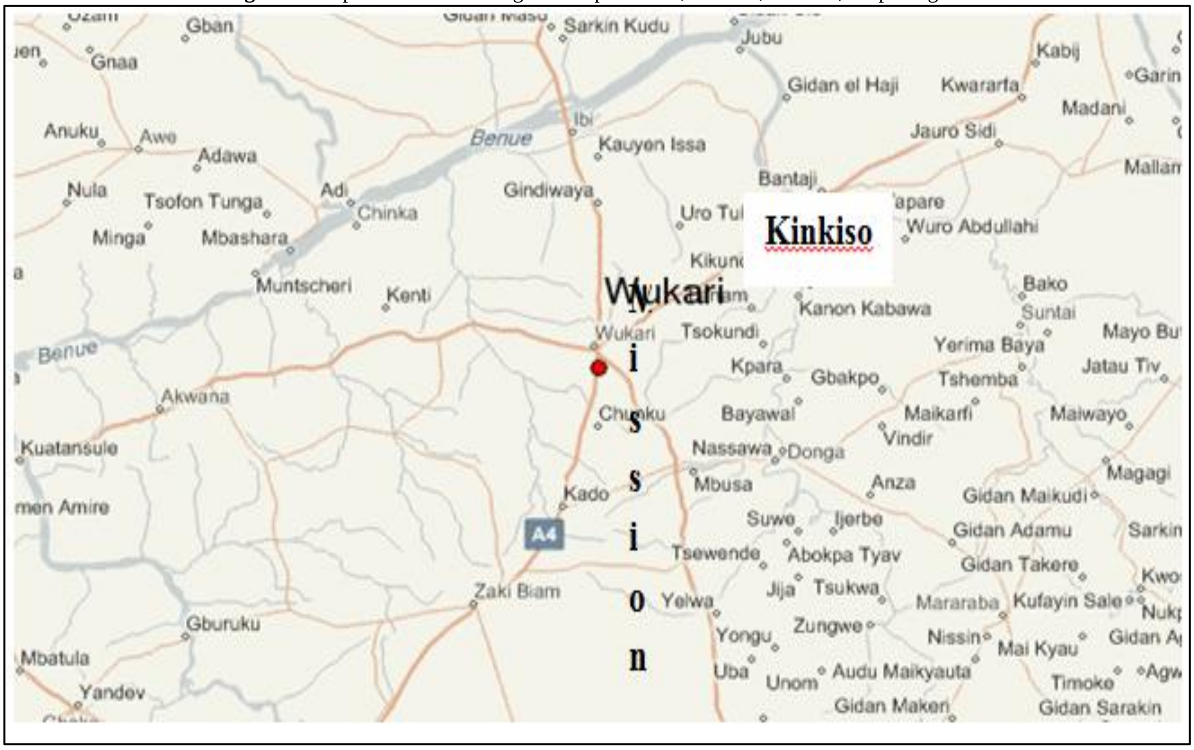
2. Materials and Methods

2.1. The Study Area

The study was conducted in three selected communities of Wukari Local Government Area of Taraba State. Taraba State is situated at 8°00′N 10°30′E 8.000°N 10.500°E . It covers a total of 54, 428sqkm land area and has a population of 241,546 as at the 2006 census [6] (Figures 1 and 2).

Figure-1. Map of Nigeria showing the map of Taraba State

Figure-2. Map of Wukari showing the sampled areas; Kinkiso, Mission, Wapan-ngaku



2.2. Insectary and Net Cage

An insectary was set up, where the immature stages (larvae and pupae) was reared to the adult stage. About nine insectaries were made for each community. A total of three cages were built, which were 32 x32cm. Each of the communities had one cage each where the insects were kept.

2.3. Larva Collection

It has been established that larvae of *Anopheles gambiae* lies parallel to the water. A dipper and collecting (Scooping spoon) spoon were used to collect the larvae from their natural breeding sites in the selected communities. Care was taken in other not to disturb the larvae. The larva and pupae collected were transferred gently into plastic containers that contain breeding site water and kept tightly to stop the water from spilling. Care was taken to allow air in the top- of the plastic container so that the larvae and pupae can breathe while they are being transferred to the insectary (small containers) that was kept in the cages. The larval stages were fed with yeast powder. The water in insectary was changed as soon as it is noticed to be polluted to avoid high mortality. Larval collection was within the months of July to September, 2014.

2.4. Identification of Mosquito

The adult stages that emerge were fed with sucrose solution; the solution was in cotton wool. The cotton wool was squeezed to drain out most of the solution. The squeezed cotton was placed on the cages at various locations. Emerging adult mosquitoes fed on the juice—like solution in the cottons.

The mosquitoes were stored over desiccated silica gel and later set apart in Petri dishes for identification. Identification was done using microscope and morphological keys of Gillet [7] and Gillies and Coetzee [8]. The female *Anopheles species* were then sorted out from the males on the basis of morphological features. The females were sucked out using an aspirator.

2.5. Net Collection

Collection of nets was in households and primary health care clinics (PHC). Used nets of one, two, three and four years old were collected from randomly selected households in the communities. The used nets were replaced with new ones. A total of twelve nets were collected from the households, four nets of different age each from the three communities.

2.6. Bioassay

2.6.1. Cone-test

Cone – test as recommended by the World Health Organization/ World Health Organization Pesticide Evaluation Schemes (WHO/WHOPES) was used to test the knock down (KD) effects of pyrethroid on the nets collected.

The knocked-down time for each mosquito were recorded after every 10 minutes interval for 60 minutes, the average KD were calculated using the KD of the three replicated cones of different nets age. After which their mortality were monitored and recorded for 24 hours. The experiment was repeated three times for each of the net for each the net age collected from households using the same number of mosquitoes. The Bioassays were carried out at room temperature. Equal numbers of mosquitoes were exposed to corresponding control nets (new nets).

2.7. Analysis of Data

The criteria for efficacy of at least >80% mortality or>90% knocked-down was used to evaluate the resistance/susceptibility status of the mosquitoes. Chi-square test was used in analysing the result using Statistical Package for Social Sciences (SPSS) version 18.

3. Results

3.1. Susceptibility Tests

Table 1 shows the average knock-down in relation with the net age during 60 minutes exposure across the three communities. Wapan and Kinkiso communities had the highest knocked-downs at 3 minutes with 9.0 knocked-downs which is for age one nets while the least knocked-down occurred in ages three and four which is between 3 minutes and 60 minutes. Before the end of 60 minutes all mosquitoes had been knockdown except those exposed to nets of age four. In Kinkiso and Wapan communities, knocked-down is highest at 3 minutes in nets of age one with 9.0 knocked-downs, the least is 4 years old nets at 50 minutes with 0.67 knocked-down. Mission community has highest knocked-down at one year with 8.0 knocked-downs at 3 minutes (χ^2Cal= 0.284, χ^2tab=21.03, df=12, $P<0.05$). There is no significant difference.

After 60 minutes exposure of mosquitoes to LLINs, the control had the highest knocked-down of 100% while those exposed to four years old LLINs had the least knocked-down of 97.78% (χ2cal=0.029, χ2tab=9.49 df=4, $P<0.05$) (Table 2). In table 3, the mosquitoes exhibited less than 75% knockdown to the net samples after 60 minutes. They however showed greater than 98% mortality after 24 hours post exposure in all the three communities. Kinkiso had the highest knocked-down rate with 72.5% while Wapan ngaku had the least knockdown rate with 68.3%. Within 24 hours, Wapan ngaku recorded 100% mortality. There was no significant difference in mortality across the three communities (χ2cal=0.173, χ2tab=9.49 df=4, $P<0.05$).

The mean number of mosquitoes knocked-down after 3 minutes exposure across the communities and net ages is shown in Table 4. Kinkiso and Wapan had the highest knocked-down of 9.0 in one year old nets. The least is in wapan community with 4.3 knocked-down in 4 years old net (χ^2Cal= 0.903, χ^2tab=12.59, df=6, P<0.05).

Table-1. Mean knocked-down of mosquitoes at each time interval in nets of different ages across the communities

Community	Net age (Years)	Number of mosquitoes knocked down at each time interval						
		3mins	10mins	20mins	30mins	40mins	50mins	60mins
Kinkiso	1	9.00	1.00	0.00	0.00	0.00	0.00	0.00
	2	7.00	1.00	1.00	0.33	0.33	0.00	0.00
	3	6.00	2.00	0.00	1.67	0.33	0.00	0.00
	4	5.00	1.67	0.00	1.33	0.00	0.67	0.00
Mission	1	8.00	0.00	1.00	0.00	0.33	0.00	0.00
	2	7.00	1.00	0.33	0.00	0.33	0.00	0.00
	3	7.00	0.00	0.00	0.33	0.00	0.67	0.33
	4	6.00	0.67	0.00	0.00	0.67	0.00	0.33
Wapan-ngaku	1	9.00	0.00	0.00	1.00	0.00	0.00	0.00
	2	7.00	0.00	0.33	0.00	0.67	0.00	0.00
	3	6.00	0.00	0.00	1.00	0.33	0.00	0.33
	4	4.33	1.67	0.00	0.00	0.00	0.00	0.67

χ^2cal=0.029, χ2tab=9.49, df=4, P>0.05. There is no significant difference.

Table-2. Mortality rate of 2-3 days old female *Anopheles gambiae* after 60 minutes across all net age at 24hours post exposure

Net age (Years)	Number (%) Exposed	Number (%) knocked down at 60 minutes	Number (%) Mortality at 24 hour
Control	90(100)	90(100)	90(100)
1	90(100)	90(100)	90(100)
2	90(100)	90(100)	90(100)
3	90(100)	89(98.89)	90(100)
4	90(100)	88(97.98)	88(97.78)
Mean	90(100)	89.4(99.33)	89.6(99.56)
SEM	±0.00	±1.20	±1.20

χ^2cal=0.029, χ2tab=9.49, df=4,P>0.05. There is no significant difference.

Table-3. Mortality rate of 2-3 days old non-blood fed female *Anopheles gambiae* mosquitoes after 60 minutes across the three communities at 24hours post exposure

Community	Number (%) Exposed	Number (%) knockdown (60mins)	Number (%) Mortality (24hrs)
Kinkiso	120(100)	87(72.5)	118(98.3)
Mission	120(100)	86(71.7)	119(99.7)
Wapan-ngaku	120(100)	82(68.3)	120(100)
Mean	120(100)	85(70.83)	119(99.3)
SEM	±0.00	±2.60	±1.70

χ^2cal =0.173, χ^2tab=9.49, df=4, P>0.05. There is no significant difference.

Table-4. Mean number knocked down after 3 minutes exposure across the communities and net ages.

Community	Net Age (Years)				
	Control (New Net)	1	2	3	4
Kinkiso	0.00	9.00	8.00	7.00	6.00
Mission	0.00	8.70	7.00	7.00	6.00
Wapan-ngaku	0.00	9.00	8.00	6.70	4.30
Total	0.00	26.70	23 .0	20.7	16.30
SEM	±0.00	±0.60	±1.90	±0.70	±2.40

χ^2Cal=0.903, χ^2tab=12.59,df=6, P>0.05.There is no significance difference, Number of mosquitoes per replicate = 10

4. Discussion

The work was done within the raining season; as a result there was not much difficulty in locating and collecting the *Anopheles* larvae. A large number was collected. The climatic and atmospheric conditions were very favorable for *Anopheles* larvae to thrive and breed successfully. Temperature is the most important factor affecting growth rates of larvae. There is specific temperature range in which development can occur [9]. Growth and development vary dramatically with the temperature fluctuations within this range. Ambient temperature can dramatically affect mosquito production. Larvae were reared at 25^{o}C to 27^{o}C, this is similar to the environment from which they are collected. Many were found under *Spirogyra* colonies, a very large percentage of the larvae metamorphosed into adult, this can attest to the fact that the climatic condition of Wukari is favorable.

World Health Organization [10], criteria for determining resistance or susceptibility state that; 98 to 100% mortality indicates susceptibility, less than 80% mortality suggests resistance while 80 to 97% mortality requires confirmation to resistance. Overall the mosquitoes record 100% mortality. There was no significant difference in the susceptibility of *Anopheles gambiae* complex in all the communities sampled. This confirms that the LLINs are established tools in malaria control and are very effective in killing and repelling *Anopheles gambiae* the African malaria parasites vector [11, 12].

The result of this study contrast with the work of Beach (2011) who found susceptibility greater than 78% but less than 100% in all the six geopolitical zones of Nigeria. They however recorded more than 97% mortality in two zones (Mangrove and Sudan savanna) and knockdown values of >80% was found in all the geopolitical zones. These corroborate the findings in this study where the least knockdown value was 68.3% recorded in Wapan community and the highest being 72.5% in Kinkiso community.

This result shows that there was susceptibility of mosquitoes to pyrethroid treated nets in all the three communities and across all net age. The knocked-down rate of mosquitoes between 3 minutes to 30 minutes of exposure to all the nets was high. However there was a noticeable decrease in the knocked-down rates in the nets of 3 years and 4 years, respectively, indicating that there is insecticidal decay in the net age. LLINs are designed to maintain their bio-efficacy against vector mosquitoes for at least 3 years under recommended conditions of use in the field [10]. This study shows that the performance of 3 years and 4 years old LLINs were poor as compared to 1 and 2 years old nets.

There was no significant difference in mortality of mosquitoes tested with LLINs from the three communities, there was no significant difference in mortality, there was also no significant difference in the Knocked-down rates and there was no significant difference in LLINs age. The highest mean of the mosquitoes was shown in the control which is the new nets. This shows that mosquitoes are more susceptible to nets of this age group than other age groups; this can be seen between the 3rd and 10th minutes. Mean mortality difference was progressive in nets of one and two years old at the 3rd ,10th ,20th and 30th minutes and terminated at age 3 nets. The resurgence of the mean mortality difference at the 50th and 60th minutes in age 4 nets may indicate a gradual decay process of the insecticide. Permethrin and Deltamethrin are among the World Health Organization Pesticide Evaluation Scheme (WHOPES) recommended pyrethroids, used in impregnating LLINs for malaria control World Health Organization [10], and most of the LLINs used in malaria intervention programmes in Nigeria are those treated with permethrin including those used in this study. The highest frequency of resistance in the work of Beach [13] was recorded in permethrin and previous studies have also reported decrease levels of susceptibility of *Anopheles gambiae* to permethrin Ansari, *et al.* [14]; Rafinejad, *et al.* [15]; Adeogun, *et al.* [16].

The impact of pyrethroid resistance on the bio-efficacy of the LLINs in this study is not certain, reduction in the efficacy of ITNs in pyrethroid resistance areas has been recently reported in the Benin Republic with higher Kdr frequency Corbel, *et al.* [17]; N'Guessan, *et al.* [18]. The result of this research did not provide evidence of pyrethroids resistance in *Anopheles gambiae*. There is need for constant surveillance of bio-efficacy of LLINs as they increase in age.

5. Conclusion

The knocked-down time (KDT) for nets of age one against *Anopheles gambiae* in all the three communities were found to be higher than those in other net ages suggesting that there is gradual decrease in chemical (pyrethroid) effect on the mosquitoes. Observation in this study did not provide evidence of pyrethroids resistance in the net ages but in the control, suggesting that the observed reduction in susceptibility in *Anopheles gambiae* to LLINs is due to age. These observations suggest the possibility of insecticide resistance in the nearest future hence, the need for continued surveillance.

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