PMI | Africa IRS (AIRS) Project

Indoor Residual Spraying (IRS 2) Task Order Six

AIRS NIGERIA
FINAL ENTOMOLOGY REPORT
FEBRUARY - DECEMBER 2016

Recommended Citation: AIRS Nigeria Final Entomology Report. November 2014 – December 2015, Africa Indoor Residual Spraying Project, Abt Associates Inc.

Contract: GHN-I-00-09-00013-00

Task Order: AID-OAA-TO-11-00039

Submitted to: United States Agency for International Development/PMI

Abt Associates Inc. | 4550 Montgomery Avenue | Suite 800 North
| Bethesda, Maryland 20814 | T. 301.347.5000 | F. 301.913.9061

AIRS NIGERIA
FINAL ENTOMOLOGY REPORT

FEBRUARY - DECEMBER 2016

CONTENT

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

CDC	Centers for Disease Control and Prevention
CDC LT	CDC Light Trap
ELISA	Enzyme-Linked Immunosorbent Assay
HLC	Human Landing Catch
IRD	Indoor Resting Density
IRS	Indoor Residual Spraying
ITN	Insecticide-treated Net
IVM	Integrated Vector Management
Kdr	Knock down resistance
LGA	Local Government Area
NMEP	National Malaria Elimination Program
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
WHO	World Health Organization

EXECUTIVE SUMMARY

Background

The President's Malaria Initiative (PMI) supported entomological surveillance in six sentinel sites namely Ebonyi , Oyo, Nasarawa, Akwa Ibom, Bauchi and Sokoto States from February – December 2016. AIRS Nigeria captured PMI entomological indicators in all sentinel sites and information collected from these sites are meant to support the National Malaria Elimination Program (NMEP) in making data-driven decisions for programming vector control activities. All teams across the six sentinel sites of Nigeria used PSC and Human - baited CDC light trap collections indoor and outdoor to sample mosquitoes and determine key entomological indicators. To measure insecticide resistance, all teams conducted WHO tube tests, CDC bottle bioassays, and insecticide resistance intensity assays. Molecular characterization included identification of *An. gambiae* M and S sibling species, *kdr* and metabolic resistance mechanisms.

Methods

Entomological surveillance activities were carried out using human- baited CDC light trap methods (placed indoors and outdoors) used in four houses for three nights per sentinel site to measure mosquito biting time. All teams also systematically sampled 32 houses per sentinel site per month using the PSC method to sample indoor-resting mosquitoes. Parity rates were determined by dissecting the ovaries from randomly selected female unfed *An. gambiae* **s.l.** specimens collected using human- baited CDC light traps. The use of Prokopac aspirator for indoor collections was also assessed. Molecular identification of *Anopheles* mosquitoes collected from the six vector surveillance sites were conducted using the Polymerase Chain Reaction (PCR). *Plasmodium* infection rate in the mosquito population was estimated through Enzyme-Linked Immunosorbent Assay (ELISA) tests for *Plasmodium falciparum*.

Insecticide susceptibility tests were carried out using the standard WHO protocol and CDC bottle bioassay to determine phenotypic resistance of *An. gambiae* s.l. to the four classes of WHOPES – approved IRS insecticides which include alphacypermethrin, deltamethrin, permethrin and lambda-cyhalothrin (all pyrethroids), bendiocarb and propoxur (carbamates), pirimiphos-methyl (organophosphate) and DDT (organochlorine). Resistance intensity was determined through intensity assays carried out following the Center for Disease Control and Prevention (CDC) protocol with three to four days old adult *Anopheles* mosquitoes using four different concentrations of deltamethrin and permethrin (x1, x2, x5 and x10). Resistance mechanism analysis was conducted to identify underlying resistance mechanism(s) and to

estimate the frequency of the knock down resistance (*kdr*) gene in the mosquito across all sentinel sites. Synergist test was conducted to investigate the plausible role of metabolic enzymes in insecticide detoxification in the resistant mosquito population from all sentinel sites. Synergist assay was done using piperonyl butoxide (PBO) an inhibitor of mixed function oxidase on Anopheles gambiae from each site. The presence of *kdr* mutation using allele-specific PCR diagnostic tests designed for the West African *kdr* mutation was used. The proportion of the molecular M and S form of Anopheles gambiae from samples collected at all sentinel sites were also determined using established protocols.

Results

Vector seasonality

Overall, significantly higher numbers of *An. gambiae* s.l. were collected indoors than outdoors across all sentinel sites. The primary vector across all six sentinel sites was *An. gambiae* s.l. The presence of *An. funestus* was also observed in two sentinel sites of Nassarawa and Ebonyi states. Other secondary vectors collected were *An. pharoensis* and *An. coustani*. Although overall, consistently higher numbers of indoor resting mosquitoes were observed in Sokoto and Ebonyi States as compared to the other sentinel sites, indoor biting peaks were observed to be highest in Sokoto.

Of the six sentinel sites the highest indoor collections from PSC were recorded in Sokoto in the Sahel/Sudan savannah and Ebonyi in the rainforest . Peak collections were recorded in the months of August (783) and September (497) with a mean IRD ranging from 0.1-24.5. In Ebonyi state located in the rainforest, peak collections from PSC were recorded in the months of March (612) and July (419) with mean IRD ranging from a peak of 19.1 in the month of March to 2.0 in the month of December. Nasarwa Eggon in the Guinea savannah recorded consistently higher numbers in March, April and July with mean IRD ranging from 1.3-12.8.

Insecticide resistance

Both WHO tube tests and CDC bottle bioassay methods were used to determine the susceptibility level of the vector population across the different ecological zones. Findings indicated that local mosquitoes (*An. gambiae* s.l.) were found to show resistance to DDT (organochlorine) across all six sentinel sites. *An. gambiae* s.l. was found also to be resistant to the pyrethroids lamdacyhalothrin, deltamethrin, and permethrin across most sites with the exception of alphacypermethrin and lambdacyhalothrin to which local mosquitoes showed susceptibility in Bauchi. In the carbamate class susceptibility to bendiocarb and propoxur was observed across all. Pirimiphos-methyl (organophosphate) showed susceptibility in all 4 local government areas of Bauchi state .

Resistance intensity assays

Resistance intensity assays showed variations in intensity across the six sentinel sites.

Molecular results

PCR analysis indicated a steadily increasing predominance of *Anopheles gambiae* s.s. with changing ecozone across areas of increasing rainfall from 48.2 percent in the arid/semi-arid Sahel increasing to 89 .3% in the Mangrove forest on the sea coast . *Anopheles arabiensis* was the other member of the complex identified by PCR across all the ecozones but absent at Ebonyi sentinel site. PCR analyses to determine the proportion of the Molecular M (*Anopheles coluzzii*) and S-form (*Anopheles gambiae s.s.*) at each site indicated that the molecular M and S form of *Anopheles gambiae* occurred in sympatry across all sentinel sites except Sokoto in the Sudan/Sahel savannah with the S form being predominant ranging from 70.73 -100 percent while the M form ranged between 2.65 -21.95 percent.

ELISA analysis for sporozoite infection indicated that infection rate was highest in Bauchi (5.74%) followed by Sokoto(5.40) and Akwa Ibo(5.3) Nassarawa (4.3) and Ebonyi (4.2). In Nasarawa sentinel site (4.3%) of the samples were positive for *Plasmodium* infection. *An. arabiensis* from three sentinel sites of Ebonyi, Nassarawa and Bauchi were infected with *P. falciparum*. None of the other sites recorded *An. arabiensis* infection.

Conclusions

A total of 66.02% of mosquitoes that were PCR positive were *An. gambiae* s.s. while *An. arabiensis* represented 16.22% across the six sentinel sites indicating a slight decrease in *An. gambiae* s.s. and a slight increase in the proportion of *An. arabiensis* and a possible extension of range from the arid areas of Nigeria. PCR analyses was conducted to determine the proportion of the molecular M and S-form at each site indicated that the molecular M and S form of *Anopheles gambiae* occurred in sympatry across all sites with the S form in predominance. The proportions representing 19.90% (M) and 75.31 % (S) across all sites. Insecticide resistance data shows very high pyrethroid resistance across most sentinel sites and susceptibility to the carbamates and organophosphates (pirimiphos-methyl) insecticides.

1. INTRODUCTION

In September 2014, Abt Associates was awarded a new contract to implement indoor residual spraying (IRS) under IQC IRS 2 Task Order Six (TO6) in up to 17 African countries. The Nigeria program is included to continue entomological activities started under the TO4 contract. In 2016, The PMI AIRS project re aligned its sentinel sites from the 2015 PMI-supported entomological surveillance in six sentinel sites namely Enugu, Lagos, Nasarawa, Plateau, Rivers and Sokoto States to sentinel sites in PMI-supported states of Akwa Ibom, Ebonyi, Bauchi, Nassarawa, Sokoto and Oyo. AIRS Nigeria captured PMI entomological indicators in all sentinel sites and information collected from these sites are meant to support the National Malaria Elimination Program (NMEP) in making data-driven decisions for programming vector control activities. This report provides information on the entomological monitoring activities completed between February –December 2016 .

The Africa Indoor Residual Spraying (AIRS) Nigeria program, funded by the President's Malaria Initiative (PMI) supported entomological surveillance in six sentinel sites across a geographic transection of all five ecological zones. The sites, in South West, South East, South, North West, and two in North Central geopolitical zones, were selected from 18 sites proposed by the National Malaria Elimination Program. The objectives for work in the sentinel sites were to:

- Identify malaria vectors in the sites, (using both morphological identification keys and molecular assays).
- Determine Sporozoite rates
- Establish vector density and seasonality,
- Monitor vector feeding period and time in the sentinel sites,
- Determine vector susceptibility and mechanism of resistance
- Determine intensity of resistance among local malaria vectors.

The PMI-AIRS project gathers key entomological indices relevant to the National Malaria Elimination program by :

- Identifying the malaria vector population
- Guiding optimal time and place to implement Vector Control
- Detecting behavior changes that would limit the efficacy of Vector Control
- Monitoring the entomological impact of Vector Control
- Detecting development of insecticide resistance and modes of action

1.1 INDOOR RESIDUAL SPRAYING

In an effort to scale up Indoor Residual Spraying (IRS) in Nigeria, the Federal Ministry of Health conceptualized the Public Private Partnership (PPP) as a strategy in December 2016. The FGN provided funds for its piloting in six selected states (one per geo-political zone of the country). These states are Nassaawa in North Central, Bauchi in North East, Jigawa in North West, Lagos in the South west, Rivers in South South and Anambra in South East. The baseline data showed the presence of *Anopheles* and other nuisance mosquitoes in the communities. The preliminary results showed that a total of 19,837 households were visited and covered, 30,759 structures covered, 70,218 rooms were sprayed and 130,061 persons were protected with IRS against malaria and possibly other mosquito borne diseases such as Lymphatic filariasis. The PPP strategy could be a possible method for sustainable IRS implementation in Nigeria if well planned and implemented.

2. MONITORING VECTOR BEHAVIOR AND DENSITY

2.1 OVERVIEW

Nigeria's National Malaria Elimination Program (NMEP) of the Federal Ministry of Health in collaboration with PMI - AIRS Nigeria, established malaria vector surveillance sentinel sites in six states (Figure 1 and Table 1). The sentinel sites are linked to universities/research institutions located in the same states. Some of the institutions were selected based on their proximity to the Drug Therapeutic and Efficacy Trial sentinel sites that the NMEP established in the early 1990s. They were also selected because they have the human capacity, facilities, and basic equipment for entomological work.

Each sentinel site had a team made up of a Principal Investigator and eight technicians, who carried out the surveillance work, including the determination of indoor resting densities (IRDs) with Pyrethrum Spray Catches (PSC), mosquito biting time and location (indoor/outdoor) using CDC light traps, and mosquito identification and preservation in Eppendorf tubes. Protocols for the mosquito collections are as approved in the 2016 work plan and described below.

Figure 1: Sentinel Sites Supported By PMI 2016 for Monitoring Mosquito Density and Behavior

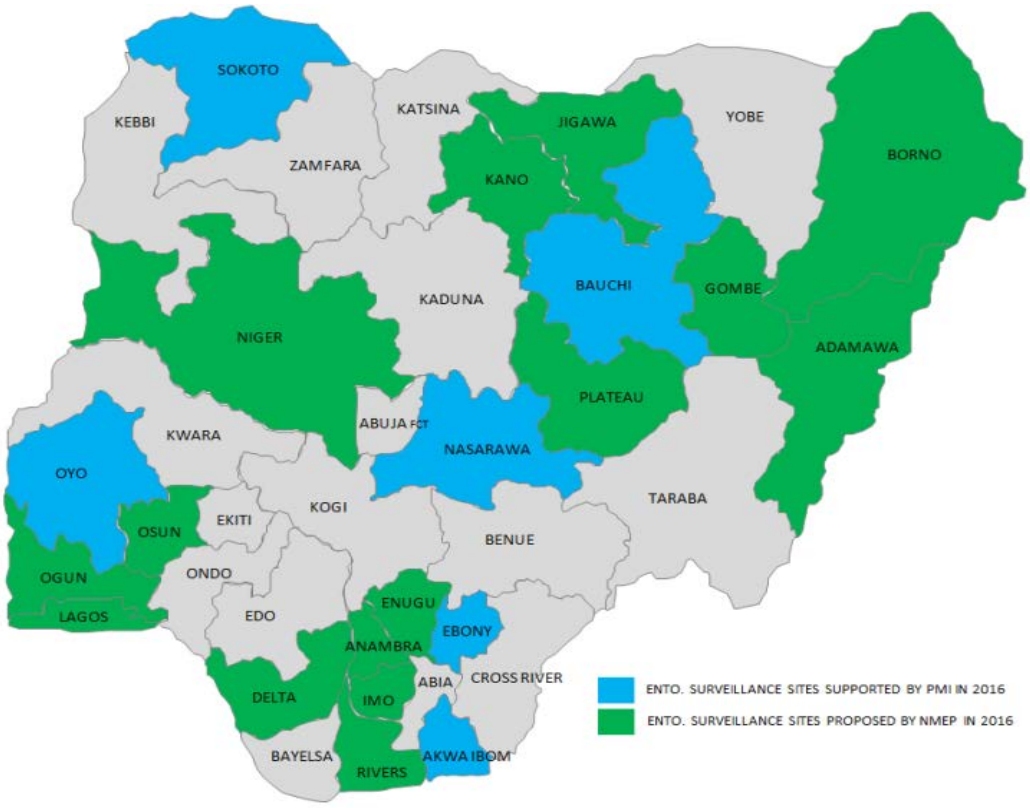


Table 1:Sentinel Sites Supported by PMI in 2016 and their affiliate institutions

S/N	States	Collaborating Institutions	Ento-Surveillance Sites (LGA)	Ecozones
1	Akwa Ibom	University of Uyo	Ibekwe Akpannya	Mangrove/Forest
2	Bauchi	Abubakar Tafawa Balewa University	Gwantar	Sahel Savannah
3	Ebonyi	Ebonyi State University	Umuaghara	Rain Forest
4	Nasarawa	Nasarawa States University	Lambaga Arikpa/Alagye	Guinea Savannah
5	Oyo	University of Ibadan	Olorisaoko	Forest Savannah
6	Sokoto	Usmanu Dandodiyo University	Rabah	Sudan/Sahel Savannah

3. COLLECTION METHODS

Bi- monthly collections were carried out in the various sentinel sites using PSCs and CDC Light trap methods. Prokopac aspirators were used in collecting adult indoor resting mosquitoes for resistance intensity assays. Anopheline larvae were collected using ladles. An assessment of the use of Prokopac aspirators in indoor resting collections .

3.1 CDC LIGHT TRAP COLLECTION

CDC light trap methods (baited traps, one placed indoors and one outdoors) were used in four houses for three nights each month per sentinel site to measure mosquito biting time and location following the methods of Yohannes and Boelee (2012).

3.2 PYRETHRUM SPRAY CATCHES

The teams systematically sampled 32 houses per sentinel site per month using the PSC method as described by the WHO (1975) to sample indoor-resting mosquitoes. All samples collected from the field were sent to the centrally located insectary at Nasarawa State University Keffi for further processing and analysis.

3.3 IDENTIFICATION OF MALARIA VECTORS

The *Anopheles* mosquitoes collected using human - baited CDC light traps and PSC were first identified to the species level morphologically (Gillies and De Meillon 1968; Gillet 1972; Gillies and Coetzee 1987; Kent, 2006). All *Anopheles* specimens that were not dissected were labeled and stored individually in Eppendorf tubes over silica gel for further processing. All samples collected from the field were sent to the centrally located insectary at Nasarawa State University Keffi.

3.4 DETERMINATION OF PARITY

Ovaries from randomly selected female unfed An. gambiae s.l. specimens captured by human baited CDC light traps were dissected to determine their physiological age and parity rate as

described by Gillies and Wilkes (1963) and WHO (2003). Parity was determined only in places where the technical expertise was confirmed (Nasarawa and Enugu sentinel sites). Parity was established by observing the degree of coiling of ovarian tracheoles (Detinova 1962, Detinova and Gillies 1964). The parity rate was obtained by determining the number of parous females and dividing by the total number of mosquitoes examined (WHO 2013).

3.5 PCR IDENTIFICATION OF MEMBERS OF THE *ANOPHELES GAMBIAE* COMPLEX

Overall a total of 2,418 *Anopheles* mosquito samples from six States: Sokoto, Nassarawa, Bauchi, Akwa Ibom, Ebonyi and Oyo collected from February to August 2016 were analysed using the Polymerase Chain Reaction (PCR) and ELISA for the detection of Plasmodium infection in mosquitoes .This mosquito samples comprised of 1768 specimens of the *Anopheles gambiae* complex. In addition, a total of 500 *Anopheles coustani* from Nassarawa sentinel site collected in 2015 and 2016 were analysed using ELISA for the detection of Plasmodium infection in mosquitoes . Of the *An. coustani* collected in 2016 a total of 241 *An. coustani* were collected from the month of March to August 2016 were analysed in addition to *An. coustani* collected in the year 2015 comprising of a total of 262 *An. coustani.* (90 *An. coustani* collected in the month of August 2015) and (172 *An. coustani* collected in the month of October 2015). All *An. coustani* were collected using outdoor CDC light-traps.

Anopheles gambiae s.l. mosquitoes collected from the six vector surveillance sites were analyzed for species identification using the Polymerase Chain Reaction (PCR). The samples were collected using pyrethrum spray collection and CDC light trap collection indoors or outdoors. This was a subset of all *An. gambiae s.l.* mosquitoes caught and represented approximately 10% of samples caught across the sentinel sites both indoors and outdoors. All members of the *Anopheles gambiae* complex were analyzed using a standard method. Extracted DNA was amplified using the *Anopheles gambiae* species-specific multiplex PCR (Scott *et al*.,1993).

3.5.1 PLASMODIUM SPOROZOITES ASSAY

To estimate *Plasmodium* infection rate in the mosquito population, Enzyme-Linked Immunosorbent Assay (ELISA) tests for *Plasmodium falciparum*, *Plasmodium ovale* and *Plasmodium malariae* were carried out on a proportion of mosquitoes collected from the field. Head and thorax of each female *Anopheles* mosquito was crushed in Phosphate Buffered Saline (PBS) and tested for the circumsporozoite antigen using an ELISA assay (Burkot *et al*., 1984).

4. RESULTS

4.1 ANOPHELINE SPECIES COLLECTED BY THE DIFFERENT COLLECTION METHODS

During the study period between February to December 2016, the study teams using baited CDC light traps and PSC sampling methods collected a total of 17,535 *Anopheles* mosquitoes from six sentinel sites. Detailed data are included in Annex A. The species composition of collected mosquitoes follows:

- 15,882 *An. gambiae s.l.*
- 88 *An. funestus*
- 861 *An. coustani/ziemanni*
- 43 *An. nili*
- 187 *An. pharoensis*
- 364 *An. moucheti*

A total of 15,882 (90.6 percent) were *An. gambiae s.l.* and 88(0.5 percent) were *An. funestus*. The difference between the two major vectors, *An. gambiae s.l.* and *An. funestus*, was statistically significant ($p<0.0001$). Other species caught were *An. coustani* 861(4.9 percent), *An. nili* 43 (0.2 percent), *An. pharoensis* 187(1.1 percent) and *An. moucheti* 364 (2.1 percent) and other species (0.6 percent). *An. gambiae s.l.* was common in all the six sites, while *An. coustani* was collected in Ebonyi, Nasarawa and Bauchi states, *An. moucheti* was collected from Ebonyi and Akwa Ibom site only. *An. pharoensis* was only collected from Nasarawa and Sokoto and Ebonyi (Annex A-1).

4.2 PYRETHRUM SPRAY CATCH

Between February to December 2016, The study teams using PSC sampling methods collected a total of 8,219 *Anopheles* mosquitoes. The highest Indoor Resting Density (IRD) of 24.5 was observed in the month of August in Sokoto. A high indoor IRD of 19.1 was also observed in Ebonyi state in the month of March. Lastly, the IRD ranged from 0.1-1.9 in Akwa Ibom where it was the lowest among all the sentinel sites.

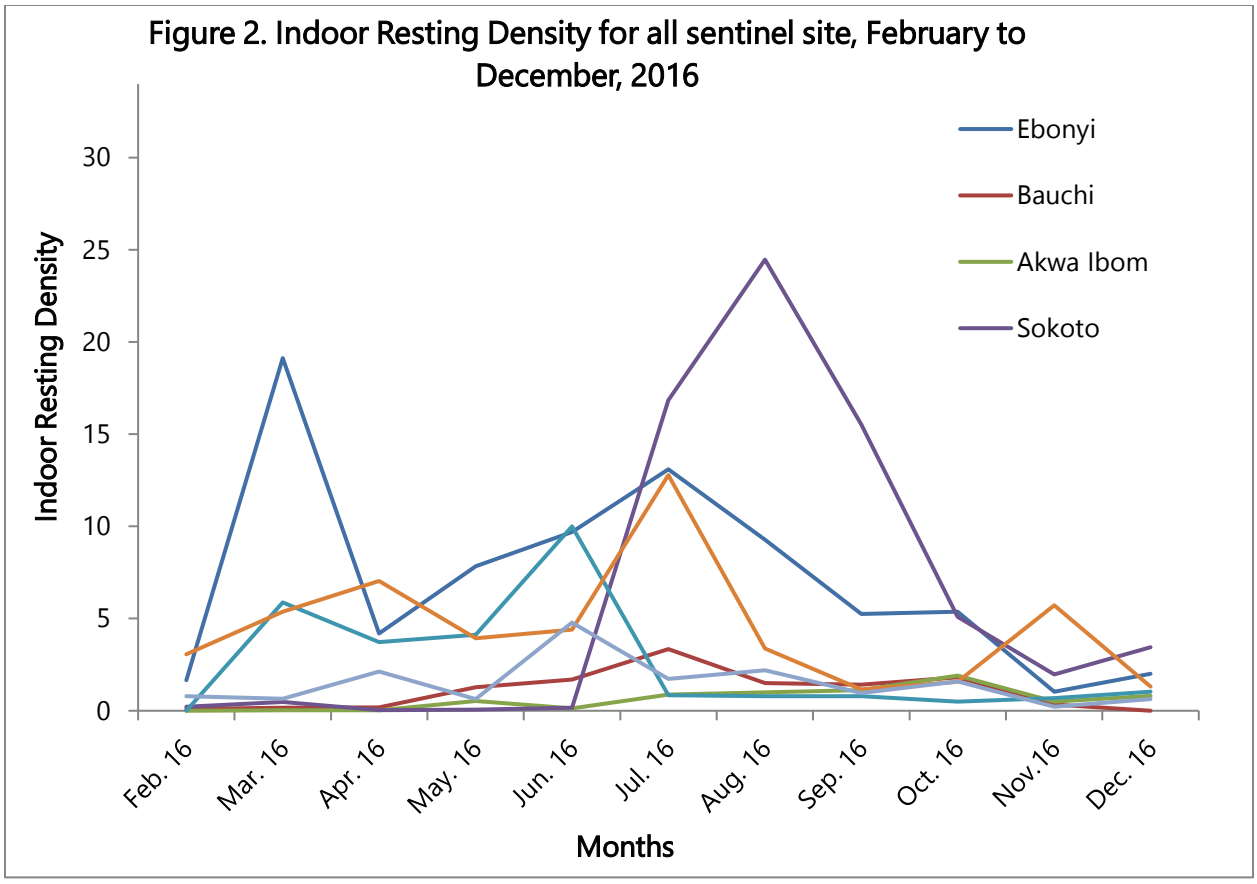


Figure 2: Indoor Resting density for all sentinel site, February to December, 2016

4.3 HUMAN - BAITED CDC LIGHT TRAP COLLECTIONS

Overall results indicated that higher proportions of *An. gambiae s.l.* were collected indoors than outdoors using CDC light trap method and the difference was statistically significant (χ^2 = 691.44 , df =1; p<0.0001) Table 5.

In Ebonyi 85.1 percent *Anopheles gambiae* s.l. were collected indoors while 14.9 percent *An. gambiae s.l.* were collected outdoors. Significantly higher number of mosquitoes were collected indoors than outdoors (χ^2 = 40.500, df =1; p<0.0001)

In Oyo State, 50.7% were collected indoors while 49.3% were collected outdoors, and no significant difference were observed between indoor and outdoor collections (p= 0.88).

In Akwa Ibom State, *An. gambaie s.l.* mosquitoes collected 67.1 percent indoors while 32.9 percent were collected outdoors .The difference between indoor and outdoor were significantly different (p=0.0061).
In Nassarawa state, *An. gambaie s.l.* were collected from two LGA of Doma and Nasarawa Eggon. Overall significantly higher numbers of *An. gambaie s.l.* were collected indoors 63.4 percent than outdoors 36.6 percent (p=0.0073).

In Sokoto higher numbers of *An. gambiae s.l.* were collected indoors 63.7% than 36.3 % outdoors using the human baited CDC light traps. Difference between mosquitoes collected indoors and outdoors is statistically significant(χ^2 =0.00062).

In Bauchi state higher numbers of *An. gambiae s.l.* were collected indoors 70.4 % than outdoors 29.6 % using the human baited CDC light traps. The difference between mosquitoes collected indoors and outdoors is statistically significant(χ^2 =0.00062)(refer to Table 2).

Table 2: Mosquito species caught using Human-baited CDC Light Trap from the various sentinel sites

Mosquito Species		*An. gambiae s.l.*	*An. funestus*	*An. nili*	*An. coustani*
Ebonyi	In	85.1%	70.0%		66.7%
	Out	14.9%	30%		33.3%
	P-Value	P<0.0001S	P<0.0001S		0.00067S
Bauchi	In	70.4%	60 %	80 %	51.9 %
	Out	29.6%	40 %	20 %	48.1 %
	P-Value	P<0.0001 S	0.045 S	P<0.0001 S*	0.70 NS
Nasarawa	In	63.4%	75%		46.9%
	Out	36.6%	25%		53.1%
	P-Value				
Oyo	In	50.7%			
	Out	49.3%			
	P-Value	0.88NS			
Akwa Ibom	In	67.1%	50 %		100%
	Out	32.9%	50 %		0.0%
	P-Value	0.00062S	1NS		P<0.0001 S
Sokoto	In	63.7%	40.9 %		
	Out	36.3%	59.1 %		
	P-Value	0.0061S	0.068NS**		

NB: *S= Significant; **NS= Not significant

4.4 BITING TIME AND LOCATION ACROSS SIX SENTINEL SITES

Overall results indicated that higher proportions of *An. gambiae s.l.* were biting indoors than outdoors using CDC light trap method.

In Ebonyi peak indoors biting activity was recorded at 1-2 am while peak outdoors biting was recorded at 4-5 am.

In Oyo State, both indoors and outdoors biting activity were close but peak indoor biting time was 12-1pm while peak outdoor biting time was 4-5 am (Figure 3) .

In Bauchi state the peak biting time indoors was 2-3 a.m while peak outdoor biting was 11-12 a.m and 2-3 a.m.
In Akwa Ibom State peak indoors biting was 1-2 a.m while outdoor biting 2-3 a.m (Figure 5).

In Sokoto peak indoors biting time was 1-2 a.m while outdoors was 4-5 a.m (Figure 6).

In Nassarawa state, *An. gambaie* s.l. biting times from the two LGAs of Doma and Nasarawa Eggon were slightly different. In Doma peak indoor time was 2-3 a.m while outdoors was 11-12 a.m while in Nassarawa eggon peak indoor time was 11-12a.m and outdoors was12 -1 a.m. (Figures 7 and 8).

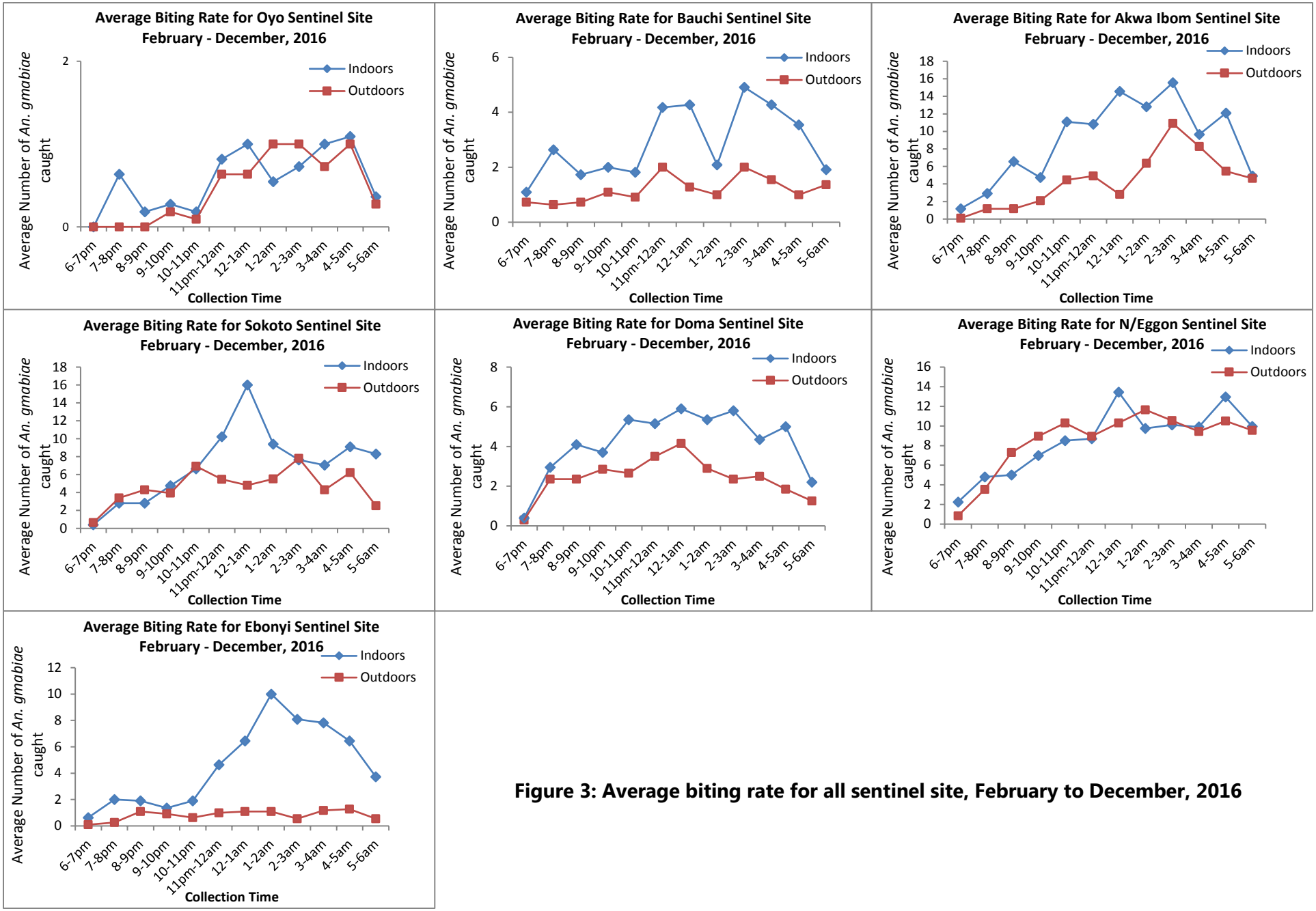


Figure 3: Average biting rate for all sentinel site, February to December, 2016

4.5 PCR IDENTIFICATION OF MEMBERS OF THE *ANOPHELES GAMBIAE* COMPLEX

PCR indicated the proportion of *An. gambiae* s.s. varied with eco zone from wet to dry areas but the same was not observed with *An. arabiensis* Figure 4 . The S form of *An. gambiae* was observed across ecozones and occurred in sympatry with the M form of *An. gambiae* in all zones except Sokoto in the Sudan/ Sahel savannah Figure 5. ELISA indicated higher infection rates in *An.gambiae* 4.50 -5.74 than *An. arabiensis* 3.49-4.80 and occurred in sympatry at all ecozones except in the mangrove forest Figure 4. Significantlyhigher human bloodmeals was observed in *An.gambiae* 49.56 -62.57 percent than *An. arabiensis* and varied according to the ecozones (Figure 6).

Ecozone	**Total No. Processed for PCR**	**No. +ve PCR for *An. gambiae* s.s. (%)**	***An. gambiae* s.s.**				***An. arabiensis***		
			No.CSP tested	**CSP +ve**	**SPR (%)**	**No. +ve PCR for *An.arabiensis***	**No. CSP tested**	**CSP +ve**	**SPR (%)**
Sudan/Sahel (Sokoto)	433	328(76%)	328	12	3.66	91(21%)	91	1	1.1
Guinea savannah (Nassarawa)	830	563(68%)	563	22	3.9	182(22%)	182	3	1.65
Mangrove	335	281(84%)	281	14	4.98	6(2%)	6	-	-
Rainforest Ebonyi	704	505(72%)	505	12	2.38	96(14%)	96	1	1.04
Forest Savannah	371	248(67%)	248	11	4.44	46(12%)	46	-	-
Sudan (savannah) Bauchi	239	120(50%)	120	5	4.16	48(20%)	48	1	2.08

Table 3: Total number of mosquitoes processed for PCR and ELISA from 6 ecozones of Nigeria

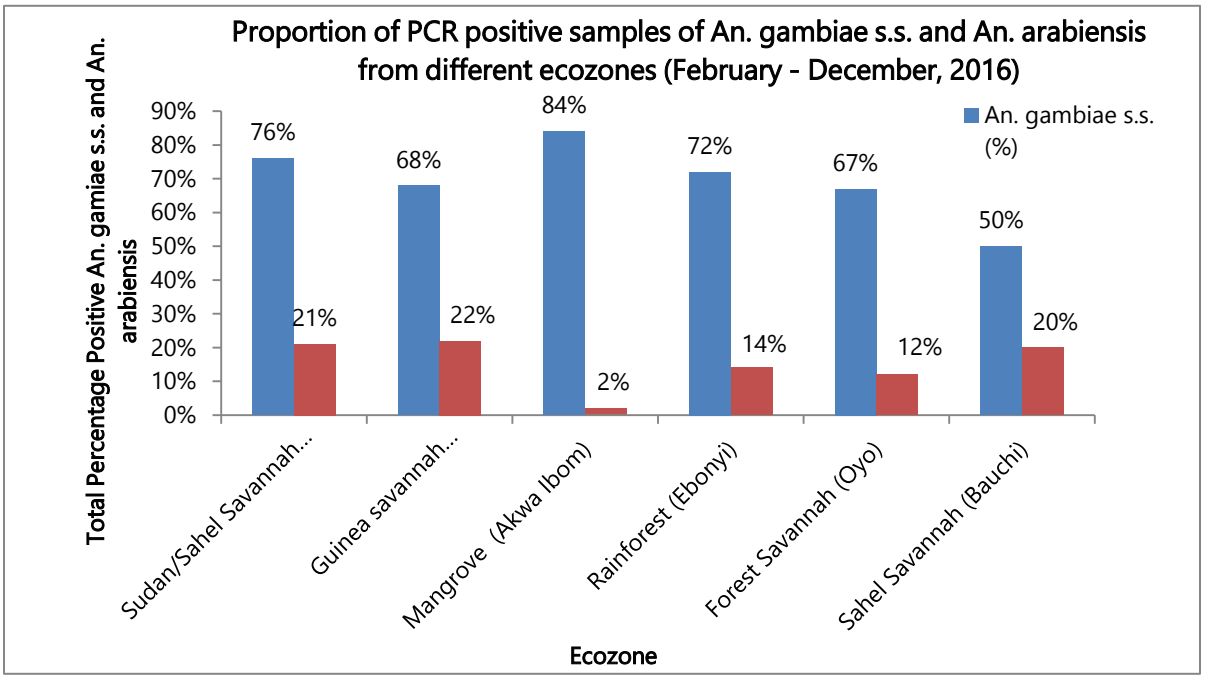


Figure 4: Proportion of PCR positive samples of *An. gambiae* s.s. and *An. arabiensis* from different ecozones (February - December, 2016)

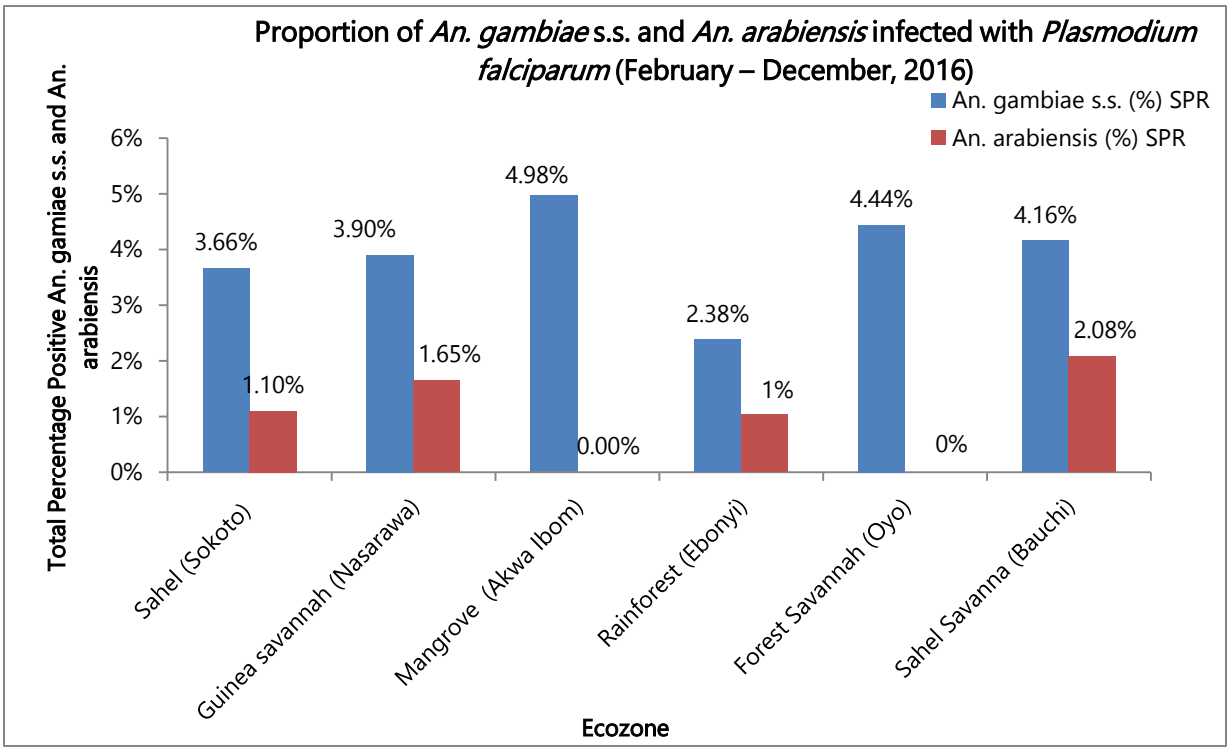


Figure 5: Proportion of *An. gambiae* s.s. and *An. arabiensis* infected with *Plasmodium falciparum* (February – December, 2016)

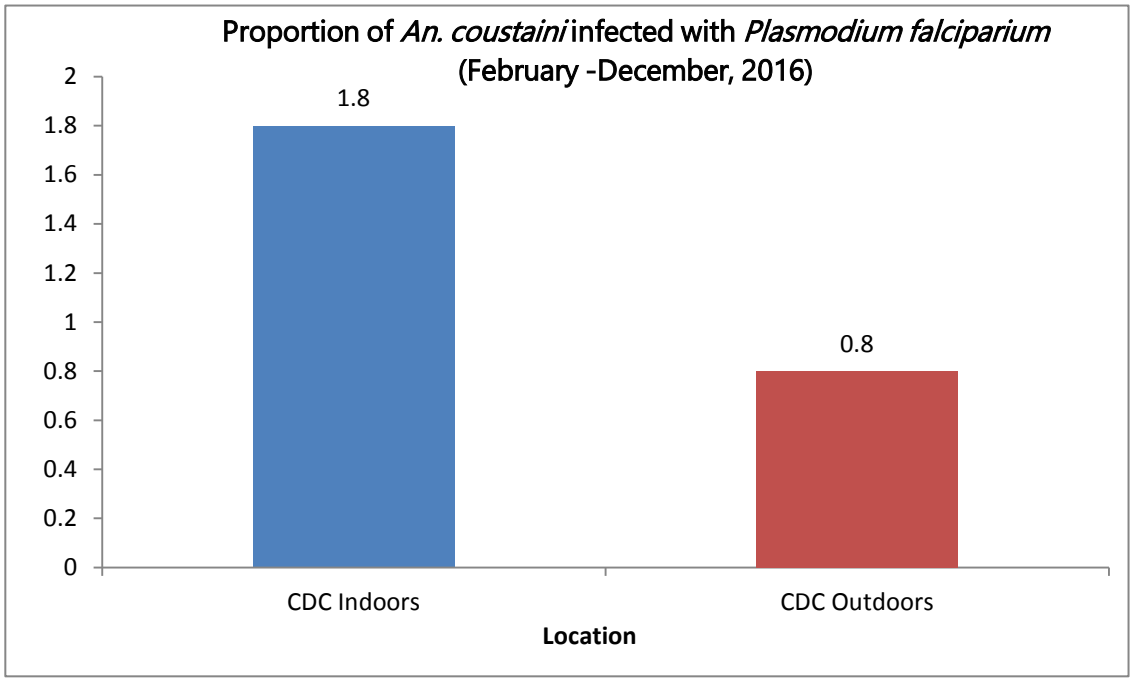


Figure 6: Proportion of *An. coustaini* infected with *Plasmodium falciparium* (February - December, 2016)

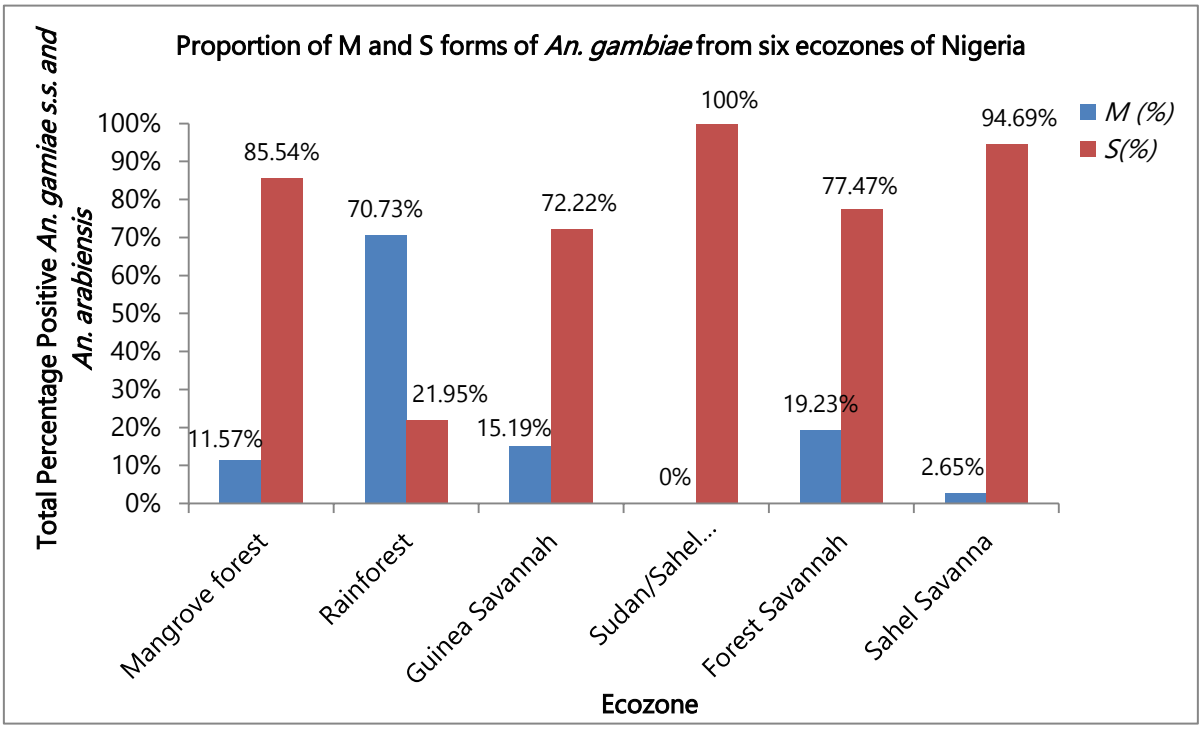


Figure 7: Proportion of M and S forms of *An. gambiae* from six ecozones of Nigeria

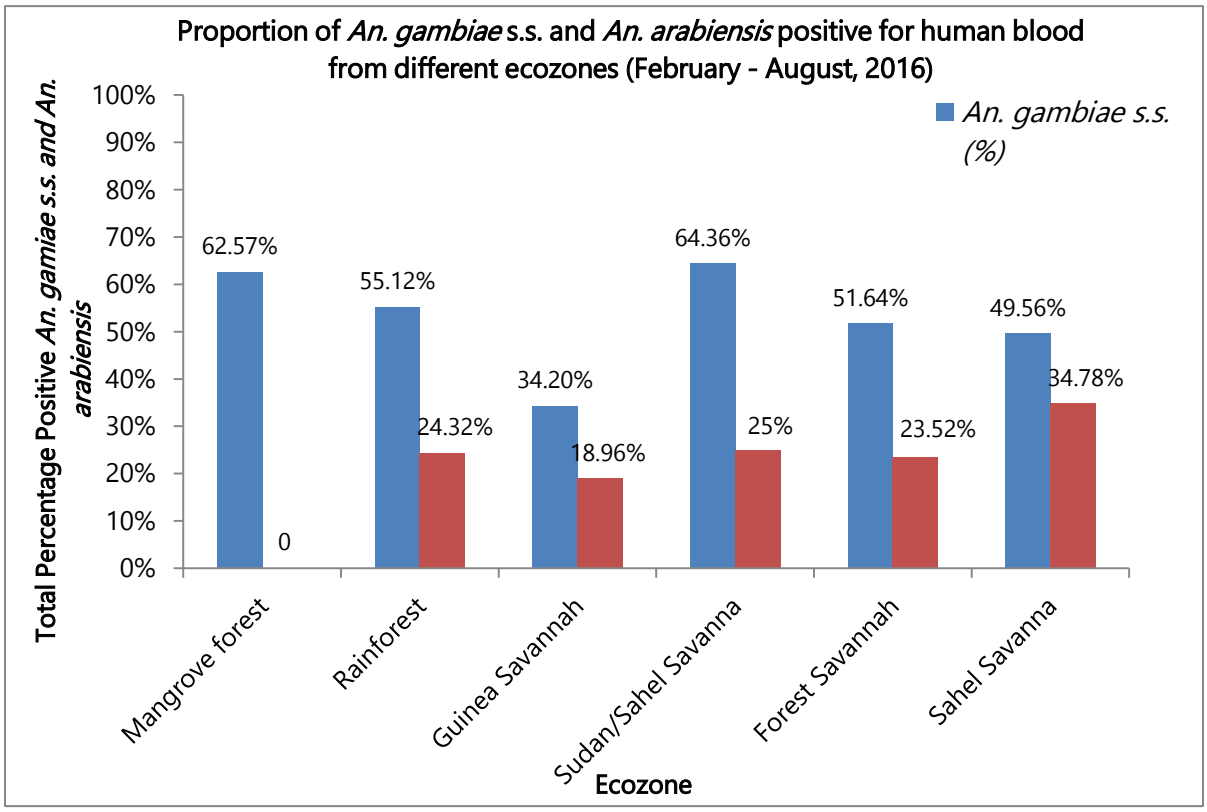


Figure 8: Proportion of *An. gambiae* s.s. and *An. arabiensis* positive for human blood from different ecozones (February - August, 2016)

Table **4**:Intensity of Malaria transmission (Entomological Inoculation Rates) across the six ecozones of Nigeria (EIR for each month and yearly EIR) for *An.gambiae* s.s

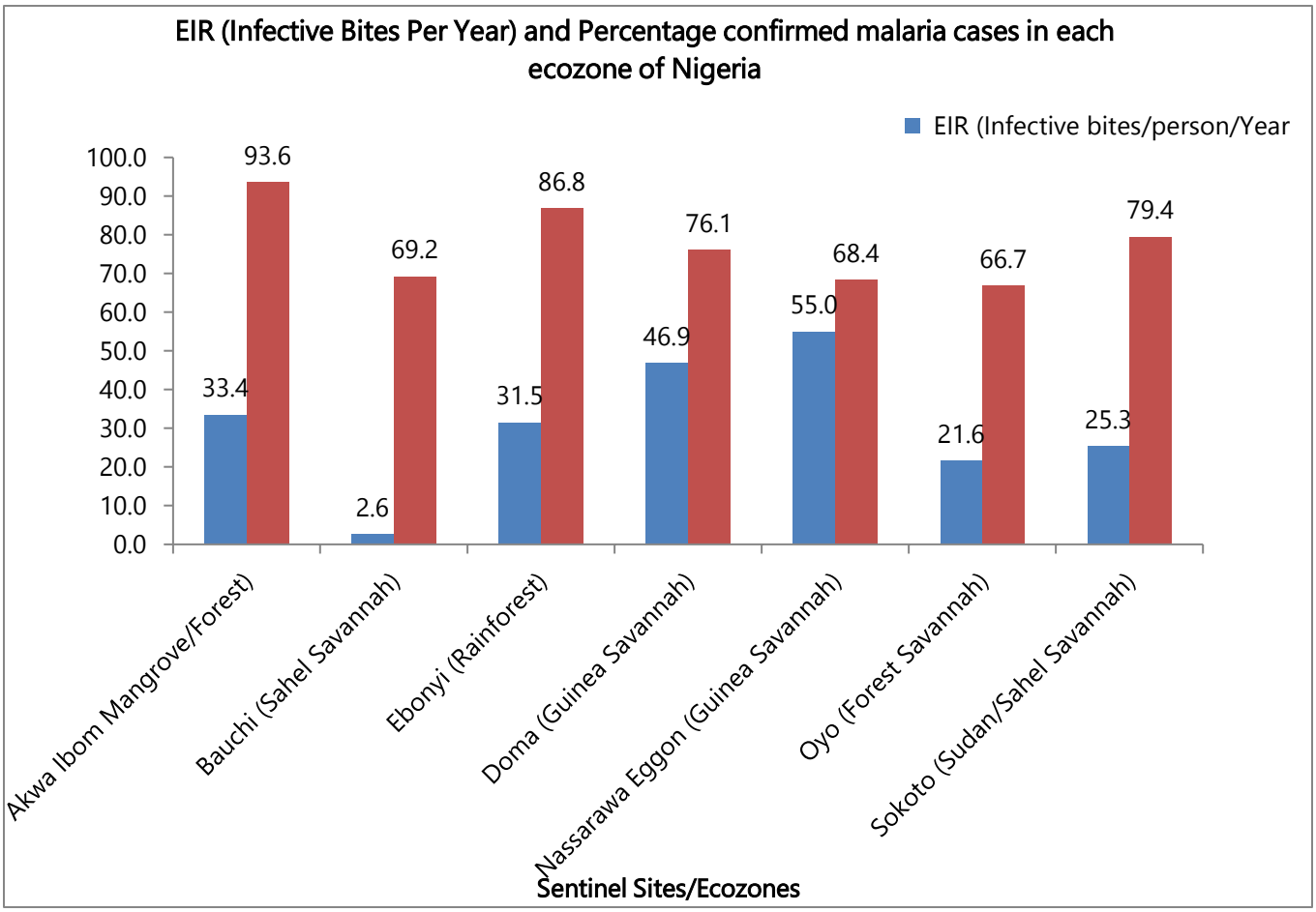
Month	Akwa Ibom (Mangrove/Forest)			Bauchi (Sahel Savannah)			Ebonyi (Rainforest)			Doma (Guinea Savannah)			Nassarwa Eggon (Guniea Savannah)			Oyo (Forest Savannah)			Sokoto (Sudan/Sahel Savannah)		
	MBR	Infectious bite/Person/Nig	**EIR/month**	MBR	Infectious bite/Person/Nig	**EIR/month**	MBR	Infectious bite/Person/Nig	**EIR/month**	MBR	Infectious bite/Person/Nig	**EIR/month**	MBR	Infectious bite/Person/Nig	**EIR/month**	MBR	Infectious bite/Person/Nig	**EIR/month**	MBR	Infectious bite/Person/Nig	**EIR/month**
Feb-16	0	0.0	**0.0**	0.2	0.0	**0.0**	1	0.0	**0.0**	1	0.4	**10.9**	1.8	0.7	**19.5**	0	0.0	**0.0**	0.1	0.0	**0.0**
Mar-16	0.2	0.0	**0.0**	0.2	0.1	**2.0**	1.9	0.1	**4.0**	0.9	0.2	**5.5**	1.7	0.3	**10.4**	2	0.5	**14.3**	0.5	0.1	**3.0**
Apr-16	0.02	0.0	**0.1**	0.1	0.0	**0.0**	1.4	0.1	**3.2**	2.4	0.3	**9.0**	2.1	0.3	**7.9**	1.5	0.0	**0.0**	0.1	0.0	**0.0**
May-16	1.31	0.1	**4.2**	0.2	0.0	**0.6**	2.2	0.2	**6.3**	1.2	0.1	**2.6**	1.1	0.1	**2.3**	1.5	0.0	**0.0**	0.1	0.0	**0.0**
Jun-16	0.17	0.0	**0.0**	0.4	0.0	**0.0**	2.9	0.0	**0.0**	3.8	0.0	**0.0**	1.3	0.0	**0.0**	2.6	0.2	**5.5**	0.6	0.1	**3.0**
Jul-16	0.93	0.1	**3.5**	0.5	0.0	**0.0**	3.9	0.2	**5.9**	2.6	0.0	**0.0**	4	0.0	**0.0**	0.5	0.0	**0.0**	2.1	0.1	**4.5**
Aug-16	8	0.8	**22.6**	0.3	0.0	**0.0**	3.1	0.0	**0.0**	1.8	0.2	**4.9**	1	0.1	**2.7**	0.5	0.0	**0.0**	4.1	0.3	**7.6**
Sep-16	5.14	0.0	**0.0**	0.2	0.0	**0.0**	1.6	0.2	**5.4**	1.3	0.2	**5.8**	0.6	0.1	**2.7**	0.5	0.0	**1.0**	1.8	0.2	**7.2**
Oct-16	10.2	0.0	**0.0**	0.2	0.0	**0.0**	1.5	0.2	**6.8**	1.5	0.2	**6.3**	0.7	0.1	**2.9**	0.3	0.0	**0.8**	1.2	0.0	**0.0**
Nov-16	1.23	0.1	**3.1**	0.1	0.0	**0.0**	0.6	0.0	**0.0**	0.8	0.1	**2.0**	2.6	0.2	**6.5**	0.6	0.0	**0.0**	0.7	0.0	**0.0**
Dec-16	0.25	0.0	**0.0**	0	0.0	**0.0**	0.7	0.0	**0.0**	0.7	0.0	**0.0**	1.7	0.0	**0.0**	0.7	0.0	**0.0**	1.4	0.0	**0.0**
Total	27.5	1.1	**33.4**	2.4	0.1	**2.6**	20.8	1.0	**31.5**	18	1.6	**46.9**	18.6	1.8	**55.0**	10.7	0.7	**21.6**	12.7	0.8	**25.3**

Table 5:Intensity of Malaria transmission (Entomological Inoculation Rates) across the six ecozones of Nigeria (EIR for each month and yearly EIR) for *An. Arabiensis*

Month	Akwa Ibom (Mangrove/Forest)			Bauchi (Sahel Savannah)			Ebonyi (Rainforest)			Doma (Guinea Savannah)			Nassarwa Eggon (Guniea Savannah)			Oyo (Forest Savannah)			Sokoto (Sudan/Sahel Savannah)		
	MBR	Infectious bite/Person/Nig	**EIR/month**	MBR	Infectious bite/Person/Nig	**EIR/month**	MBR	Infectious bite/Person/Nig	**EIR/month**	MBR	Infectious bite/Person/Nig	**EIR/month**	MBR	Infectious bite/Person/Nig	**EIR/month**	MBR	Infectious bite/Person/Nig	**EIR/month**	MBR	Infectious bite/Person/Nig	**EIR/month**
Feb-16	0.0	0.0	**0.0**	0.2	0.0	**0.0**	1.0	0.0	**0.0**	1.0	0.3	**9.0**	1.8	0.0	**0.0**	0.0	0.0	**0.0**	0.1	0.0	**0.0**
Mar-16	0.2	0.0	**0.0**	0.2	0.0	**0.0**	1.9	0.3	**9.0**	0.9	0.3	**9.0**	1.7	0.0	**0.0**	2.0	0.0	**0.0**	0.5	0.0	**0.0**
Apr-16	0.0	0.0	**0.0**	0.1	0.0	**0.0**	1.4	0.0	**0.0**	2.4	0.0	**0.0**	2.1	0.0	**0.0**	1.5	0.0	**0.0**	0.1	0.0	**0.0**
May-16	1.3	0.0	**0.0**	0.2	0.0	**0.0**	2.2	0.0	**0.0**	1.2	0.0	**0.0**	1.1	0.0	**0.0**	1.5	0.0	**0.0**	0.1	0.0	**0.0**
Jun-16	0.2	0.0	**0.0**	0.4	0.0	**0.0**	2.9	0.0	**0.0**	3.8	0.0	**0.0**	1.3	0.0	**0.0**	2.6	0.0	**0.0**	0.6	0.0	**0.0**
Jul-16	0.9	0.0	**0.0**	0.5	0.0	**0.0**	3.9	0.0	**0.0**	2.6	0.0	**0.0**	4.0	0.0	**0.0**	0.5	0.0	**0.0**	2.1	0.0	**0.0**
Aug-16	8.0	0.0	**0.0**	0.3	0.0	**0.0**	3.1	0.0	**0.0**	1.8	0.0	**0.0**	1.0	0.0	**0.0**	0.5	0.0	**0.0**	4.1	0.0	**0.0**
Sep-16	5.1	0.0	**0.0**	0.2	0.0	**0.0**	1.6	0.0	**0.0**	1.3	0.0	**0.0**	0.6	0.0	**0.0**	0.5	0.0	**0.0**	1.8	0.1	**3.0**
Oct-16	10.2	0.0	**0.0**	0.2	0.0	**0.0**	1.5	0.0	**0.0**	1.5	0.0	**0.0**	0.7	0.0	**0.0**	0.3	0.0	**0.0**	1.2	0.0	**0.0**
Nov-16	1.2	0.0	**0.0**	0.1	0.0	**0.0**	0.6	0.0	**0.0**	0.8	0.0	**0.0**	2.6	0.0	**0.0**	0.6	0.0	**0.0**	0.7	0.0	**0.0**
Dec-16	0.3	0.0	**0.0**	0.0	0.0	**0.0**	0.7	0.0	**0.0**	0.7	0.0	**0.0**	1.7	0.0	**0.0**	0.7	0.0	**0.0**	1.4	0.0	**0.0**
Total	27.5	0.0	**0.0**	2.4	0.0	**0.0**	20.8	0.3	**9.0**	18.0	0.6	**18.0**	18.6	0.0	**0.0**	10.7	0.0	**0.0**	12.7	0.1	**3.0**

Table 6:Percentage malaria cases and (Ento) malaria transmission intensity (EIR) indices across the six ecozones of Nigeria(Source HMIS 2017)

Months	Akwa Ibom (Mangrove/Forest)			Bauchi (Sahel Savannah)			Ebonyi (Rainforest)			Doma (Guinea Savannah)			Nassarawa Eggon (Guinea Savannah)			Oyo (Forest Savannah)			Sokoto (Sudan/Sahel Savannah)		
	MBR	EIR (Infectious bites/person/Month	Percentage of malaria cases	MBR	EIR (Infectious bites/person/Month	Percentage of malaria cases	MBR	EIR (Infectious bites/person/Month	Percentage of malaria cases	MBR	EIR (Infectious bites/person/Month	Percentage of malaria cases	MBR	EIR (Infectious bites/person/Month	Percentage of malaria cases	MBR	EIR (Infectious bites/person/Month	Percentage of malaria cases	MBR	EIR (Infectious bites/person/Month	Percentage of malaria cases
Feb-16	0.0	0.0	78.0	0.2	0.0	53.4	1.0	0.0	78.7	1.0	0.0	58.6	1.8	0.0	56.5	0.0	0.0	59.1	0.1	0.0	70.7
Mar-16	0.2	0.0	90.4	0.2	0.0	54.3	1.9	0.0	83.2	0.9	0.0	65.1	1.7	0.0	57.8	2.0	0.0	61.4	0.5	0.0	64.9
Apr-16	0.0	0.0	90.6	0.1	0.0	55.3	1.4	0.0	84.1	2.4	0.0	68.1	2.1	0.0	60.8	1.5	0.0	61.8	0.1	0.0	70.5
May-16	1.3	0.0	91.2	0.2	0.0	68.7	2.2	0.0	84.7	1.2	2.0	76.7	1.1	2.3	62.8	1.5	0.0	65.8	0.1	0.0	73.9
Jun-16	0.2	0.0	92.4	0.4	0.0	69.7	2.9	0.0	86.0	3.8	2.6	77.3	1.3	2.7	69.4	2.6	0.0	66.1	0.6	0.0	81.2
Jul-16	0.9	0.0	93.4	0.5	0.0	73.3	3.9	3.2	87.5	2.6	4.9	81.4	4.0	2.7	69.6	0.5	0.0	67.0	2.1	0.0	82.7
Aug-16	8.0	0.1	94.4	0.3	0.0	74.4	3.1	4.0	87.7	1.8	5.5	81.6	1.0	2.9	72.1	0.5	0.0	67.8	4.1	3.0	83.0
Sep-16	5.1	3.1	94.7	0.2	0.0	75.0	1.6	5.4	90.5	1.3	5.8	99.9	0.6	6.5	74.3	0.5	0.8	69.3	1.8	3.0	85.9
Oct-16	10.2	3.5	95.4	0.2	0.0	77.3	1.5	5.9	90.6	1.5	6.3	-	0.7	7.9	74.7	0.3	1.0	69.3	1.2	4.5	89.7
Nov-16	1.2	4.2	95.6	0.1	0.6	79.8	0.6	6.3	90.9	0.8	9.0	-	2.6	10.4	77.1	0.6	5.5	71.1	0.7	7.2	91.9
Dec-16	0.3	22.6	113.6	0.0	2.0	80.2	0.7	6.8	91.4	0.7	10.9	-	1.7	19.5	77.2	0.7	14.3	75.5	1.4	7.6	-
Total	2.5	33.4	93.6	0.2	2.6	69.2	1.9	31.5	86.8	1.6	46.9	76.1	1.7	55.0	68.4	1.0	21.6	66.7	1.2	25.3	79.4



[1]

Figure 9: EIR (Infective Bites Per Year) and Percentage confirmed malaria cases in each ecozone of Nigeria

[1] Note *The National Malaria Elimination Programme made available the percentage Malaria cases data for 2016 for this purposes of comparing with the AIRS Ento data for malaria risk stratification purpose for the six ecozones of Nigeria .

4.6 Insecticide Susceptibility And Mechanism Of Resistance

Both the WHO tube and CDC bottle bioassays test was aimed to determine the susceptibility level of the vector population across the different ecological zones. Insecticide susceptibility results indicated that DDT (organochlorine) was strongly resistant across all six sentinel sites. *An. gambiae s.l.* was found to show resistance to the pyrethroids in most of the sites. The vector is susceptible to carbamates in most of the sites. The low mortality observed for pirimiphos-methyl tests using the CDC bottle assays in some of the sentinel sites (Ebonyi, Nasarawa and Sokoto) could be due to the stability of insecticides used for the assays. All the teams will conduct the tests using formulated product of pirimiphos-methyl in the next work plan period.

Table 7: Test Results (Percent Mortality After 24 Hours) against *An. gambiae s.l.* using WHO Tube test and CDC Bottle Bioassay Methods at 30 Minutes Diagnostic Time (45 Minutes for DDT) for Akwa Ibom Sentinel Site

Class of Insecticides	Insecticides	WHO Tube Bioassay												CDC Bottle Bioassay									
		Ikot Ekpene			Itu			Mpat Enin			Oron			Ikot Ekpene			Itu			Mpat Enin			Oron		
		Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality
Pyrethroid	**Lambdacyhalothrin**	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	99	99% S	100	100	100 % S	100	99	99% S	100	99	99% S
Pyrethroid	**Permethrin**	100	96	96% PR	100	96	96% PR	100	97	97% PR	100	96	96% PR	100	98	98% S	100	99	99% S	135	98	72.6% R	100	98	98% S
Pyrethroid	**Deltamethrin**	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100% S	100	100	100 % S
Pyrethroid	**Alphacypermethrin**	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	97	97% PR	100	100	100 % S	100	100	100% S	100	99	99% S
Carbamate	**Bendiocarb**	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100% S	100	100	100 % S
Carbamate	**Propoxur**	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100% S	100	100	100 % S
Organo-phosphate	**Pirimiphos-methyl^**	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	105	100	95.2% PR	100	100	100 % S
Organo-chlorine	**DDT**	100	100	100 % S	100	97	97% PR	100	100	100 % S	100	96	96% PR	100	82	82% R	100	34	34% R	100	50	50% R	100	44	44% R

Table 8: Test Results (Percent Mortality After 24 Hours) against *An. gambiae s.l. using* WHO Tube tests and CDC Bottle Bioassay Methods at 30 Minutes Diagnostic Time (45 Minutes for DDT) for Bauchi Sentinel Site

Class of Insecticides	Insecticides	WHO Tube Bioassay												CDC Bottle Bioassay											
		Bauchi			Dass			Misau			Shira			Bauchi			Dass			Misau			Shira		
		Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality
Pyrethroid	**Lambdacyhalothrin**	100	93	93% PR	100	93	92% PR	100	96	96% PR	100	94	94% PR	100	98	98% S	100	97	97% PR	100	98	98% S	100	97	97% PR
Pyrethroid	**Permethrin**	100	84	84% R	100	86	85% R	100	87	87% R	100	85	85% R	100	94	94% PR	100	96	96% PR	100	99	99% S	100	98	98% S
Pyrethroid	**Deltamethrin**	100	94	94% PR	100	92	91% R	100	93	93% PR	100	92	92% PR	100	96	96% PR	100	96	96% PR	100	96	96% PR	100	97	97% PR
Pyrethroid	**Alphacypermethrin**	100	88	88% R	100	85	83% R	100	89	89% R	100	87	87% R	100	97	97% PR	100	97	97% PR	100	98	98% S	100	99	99% S
Carbamate	**Bendiocarb**	100	99	99% S	100	99	99% S	100	97	97% PR	100	98	98 % S	100	100	100% S	100	99	99% S	100	100	100% S	100	99	99% S
Carbamate	**Propoxur**	100	98	98% S	100	98	98% S	100	99	99% S	100	98	98% S	100	99	99% S	100	98	98% S	100	100	100% S	100	99	99% S
Organo-phosphate	**Pirimiphos-methyl^**	100	100	100% S	100	99	99% S	100	97	97% PR	100	98	98% S	100	100	100% S	100	100	100% S	100	99	99% S	100	98	98% S
Organo-chlorine	**DDT**	100	93	93% PR	100	89	88% R	100	90	90% PR	100	93	93% PR	100	100	100% S	100	-	-	100	-	-	100	-	-

Class of Insecticides	Insecticides	WHO Tube Bioassay				CDC Bottle Bioassay			
		Ezza North	Ikwo	Ohaozara	Ohaukwu	Ezza North	Ikwo	Ohaozara	Ohaukwu

Table 9: Test Results (Percent Mortality After 24 Hours) against *An. gambiae s.l.* ,WHO Tube tests and CDC Bottle Bioassay Methods at 30 Minutes Diagnostic Time (45 Minutes for DDT) for Ebonyi Sentinel Site

		Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality
Pyrethroid	**Lambdacyhalothrin**	100	77	77% R	100	-	-	100	-	-	100	-	-	100	93	93% PR	100	87	87% R	100	75	75% R	100	81	81% R
Pyrethroid	**Permethrin**	100	5	5% R	100	-	-	100	62	62% R	100	40	40% R	100	53	53% R	100	38	38% R	100	55	55% R	100	62	62% R
Pyrethroid	**Deltamethrin**	100	100	100 % S	100	92	92 % PR	100	-	-	100	-	-	100	74	74% R	100	80	80% R	100	91	91% PR	100	46	46% R
Pyrethroid	**Alphacypermethrin**	100	100	100 % S	100	-	-	100	-	-	100	-	-	100	92	92% PR	100	54	54% R	100	88	88% R	100	93	93% PR
Carbamate	**Bendiocarb**	100	100	100 % S	100	-	-	100	-	-	100	-	-	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S
Carbamate	**Propoxur**	100	100	100 % S	100	-	-	100	-	-	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S
Organo-phosphate	**Pirimiphos-methyl^**	100	90	90% PR	100	-	-	100	-	-	100	-	-	100	68	68% R	100	66	66% R	100	56	56% R	100	63	63% R
Organo-chlorine	**DDT**	100	30	30% R	100	-	-	100	-	-	100	-	-	100	45	45% R	100	77	77% R	100	58	58% R	100	82	82% R

Table 10: Test Results (Percent Mortality After 24 Hours) against *An. gambiae s.l.* using WHO Tube tests and CDC Bottle Bioassay Methods at 30 Minutes Diagnostic Time (45 Minutes for DDT) for Nasarawa Sentinel Site

Class of Insecticides	Insecticides	WHO Tube Bioassay												CDC Bottle Bioassay											
		Doma			Karu			Nasarawa			Nassarawa Eggon			Doma			Karu			Nasarawa			Nassarawa Eggon		
		Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality
Pyrethroid	**Lambdacyhalothrin**	100	30	30% R	100	100	100 % S	100	46	46% R	100	100	100 % S	100	100	100 % S	100	99	99% S	100	100	100 % S	100	100	100 % S
Pyrethroid	**Permethrin**	100	86	86% R	100	78	78% R	100	78	78% R	100	85	85% R	100	61	61% R	100	87	87% R	100	93	93% PR	102	99	97.1 % S
Pyrethroid	**Deltamethrin**	100	48	48% R	100	73	73% R	100	91	91% PR	100	94	94% PR	100	98	98% S	100	100	100 % S	100	100	100 % S	100	100	100 % S
Pyrethroid	**Alphacypermethrin**	100	81	81% R	100	80	80% R	100	97	97% PR	100	93	93% PR	101	100	99% S	100	100	100 % S	100	100	100 % S	100	100	100 % S
Carbamate	**Bendiocarb**	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	103	103	100 % S	100	100	100 % S	100	100	100 % S
Carbamate	**Propoxur**	100	99	99% S	100	100	100 % S	100	100	100 % S	100	99	99% S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S
Organo-phosphate	**Pirimiphos-methyl^**	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	99	99% S	101	29	29% R	101	78	77% R	100	41	41% R	100	24	24% R
Organo-chlorine	**DDT**	100	16	16% R	100	51	51% R	100	60	60% R	100	62	62% R	100	48	48% R	100	62	62% R	100	62	62% R	100	49	49% R

Table 11: Test Results (Percent Mortality After 24 Hours) against *An. gambiae s.l.* ,WHO Tube and CDC Bottle Bioassay Methods at 30 Minutes Diagnostic Time (45 Minutes for DDT) for Oyo Sentinel Site

Class of Insecticides	Insecticides	WHO Tube Bioassay												CDC Bottle Bioassay									
		Afijio			Akinyele			Egbeda			Oluyole			Afijio			Akinyele			Egbeda			Oluyole		
		Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality
Pyrethroid	**Lambdacyhalothrin**	100	96	96% PR	100	0	0% R	100	59	59% R	100	85	85% R	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S
Pyrethroid	**Permethrin**	100	50	50% R	100	78	78% R	100	67	67% R	100	99	99% S	100	96	96% PR	100	72	72% R	100	85	85% R	100	63	63% R
Pyrethroid	**Deltamethrin**	100	81	81% R	100	53	53% R	100	69	69% R	100	97	97% PR	100	100	100 % S	100	100	100 % S	100	99	99% S	100	100	100 % S
Pyrethroid	**Alphacypermethrin**	100	99	99% S	100	58	58% R	100	95	95% PR	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	91	91% PR	100	100	100 % S
Carbamate	**Bendiocarb**	100	100	100 % S	100	100	100 % S	100	93	93% PR	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S
Carbamate	**Propoxur**	100	91	91% PR	100	100	100 % S	100	99	99% S	100	99	99% S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S
Organo-phosphate	**Pirimiphos-methyl^**	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	99	99% S	100	96	96% PR	100	61	61% R	100	100	100 % S
Organo-chlorine	**DDT**	100	94	94% PR	100	10	10% R	100	57	57% R	100	4	4% R	100	74	74% R	100	0	0% R	100	44	44% R	100	0	0% R

Class of	Insecticides	WHO Tube Bioassay	CDC Bottle Bioassay

Table 12: Test Results (Percent Mortality After 24 Hours) against *An. gambiae s.l.* ,WHO Tube tests and CDC Bottle Bioassay Methods at 30 Minutes Diagnostic Time (45 Minutes for DDT) for Sokoto Sentinel Site

Insecticides		Bodingo			Rabah			Tambawal			Wamakko			Bodingo			Rabah			Tambawal			Wamakko		
		Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality	Total No. Tested	Total No. Dead	Percentage Mortality
Pyrethroid	**Lambdacyhalothrin**	100	70	70% R	100	66	66% R	100	71	71% R	100	76	76% R	100	70	70% R	100	-	-	100	-	-	100	-	-
Pyrethroid	**Permethrin**	100	55	55% R	100	55	55% R	100	69	69% R	100	100	100 % S	100	55	55% R	100	76	76 % R	100	52	52% R	100	99	99% S
Pyrethroid	**Deltamethrin**	100	72	72% R	100	88	88% R	100	87	87% R	100	62	62% R	100	72	72% R	100	77	77 % R	100	80	80% R	100	39	39% R
Pyrethroid	**Alphacypermethrin**	100	98	98% S	100	80	80% R	100	82	82% R	100	100	100 % S	100	98	98% S	100	93	93 % PR	100	99	99% S	100	100	100 % S
Carbamate	**Bendiocarb**	100	100	100 % S	100	83	83% R	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	-	-	100	-	-	100	-	-
Carbamate	**Propoxur**	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	100	100 % S	100	-	-	100	-	-	100	-	-
Organo-phosphate	**Pirimiphos-methyl^**	100	60	60% R	100	93	93% PR	100	100	100 % S	100	64	64% R	100	60	60% R	100	95	95 % PR	100	100	100 % S	100	60	60% R
Organo-chlorine	**DDT**	100	84	84% R	100	65	65% R	100	7	7% R	100	81	81% R	100	84	84% R	100	40	40 % R	100	20	20% R	100	54	54% R

4.7 INSECTICIDE RESISTANCE INTENSITY ASSAY RESULTS FROM THE SIX SENTINEL SITES.

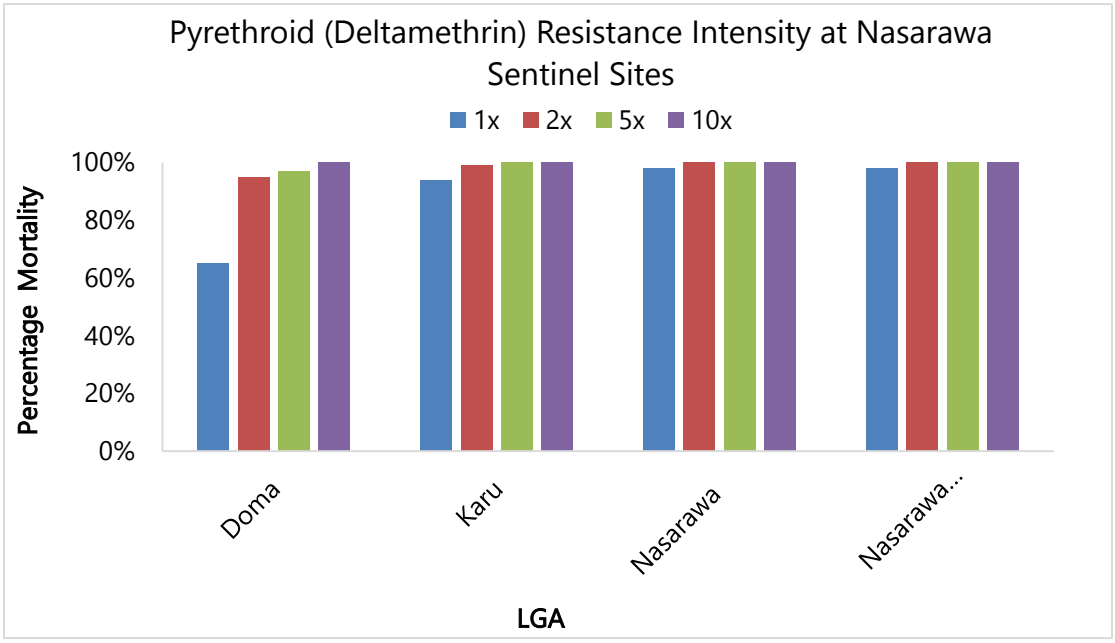


Figure 10: Pyrethroid (Deltamethrin) Resistance Intensity at Nasarawa Sentinel Sites

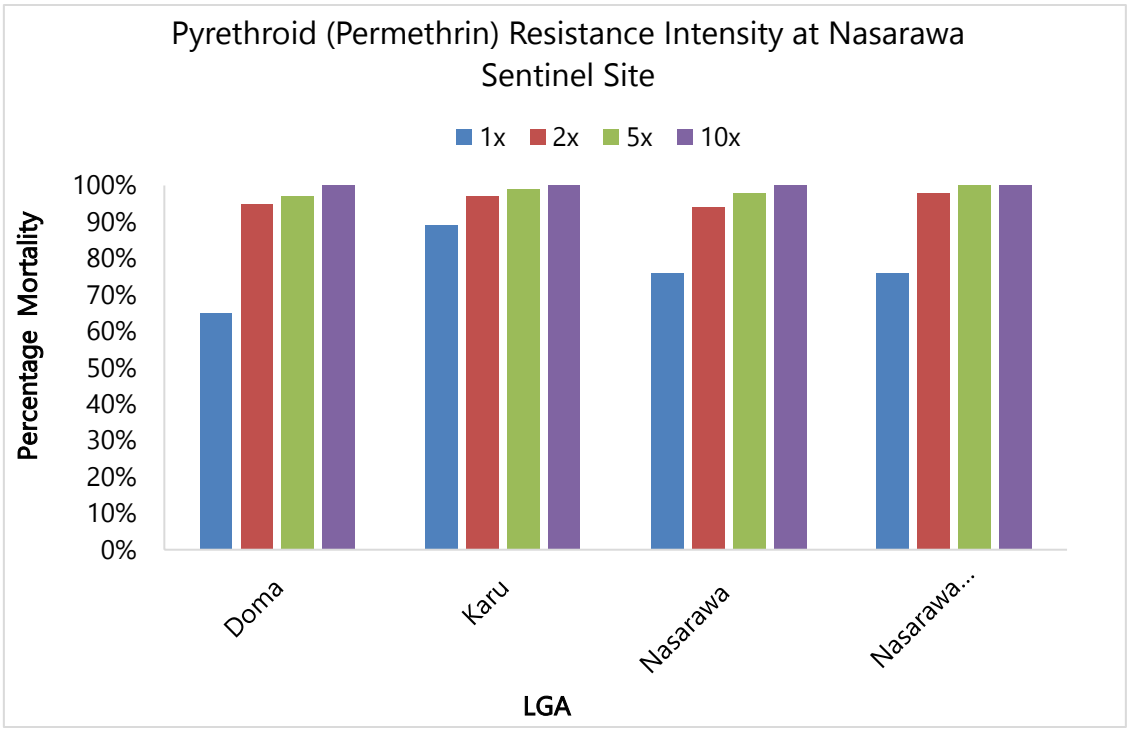


Figure 11: Pyrethroid (Permethrin) Resistance Intensity at Nasarawa Sentinel Site

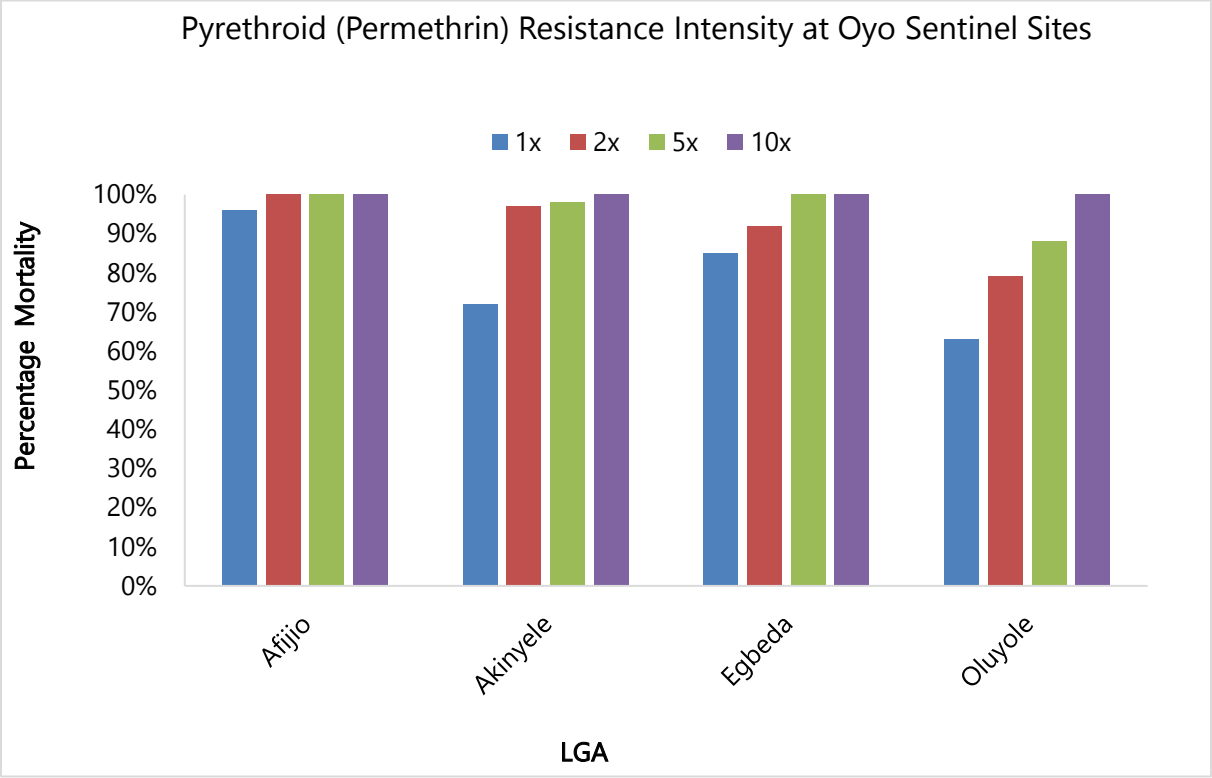


Figure 12: Pyrethroid (Permethrin) Resistance Intensity at Oyo Sentinel Sites

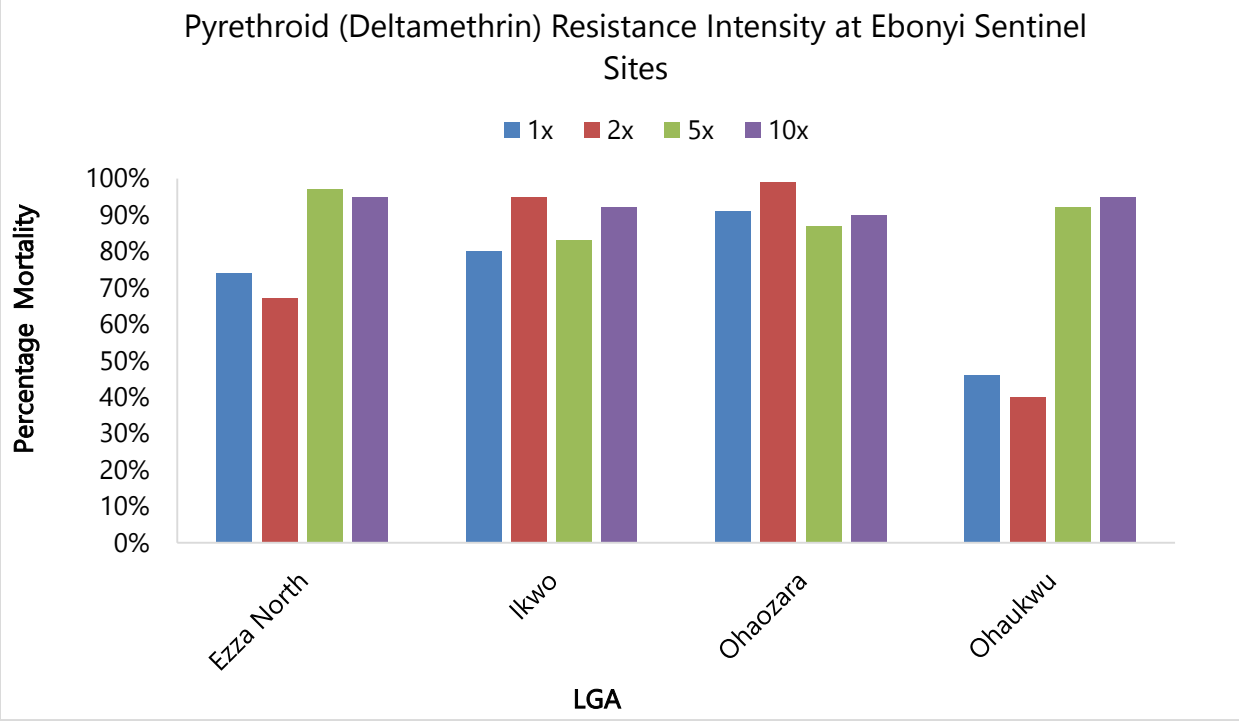


Figure 13: Pyrethroid (Deltamethrin) Resistance Intensity at Ebonyi Sentinel Sites

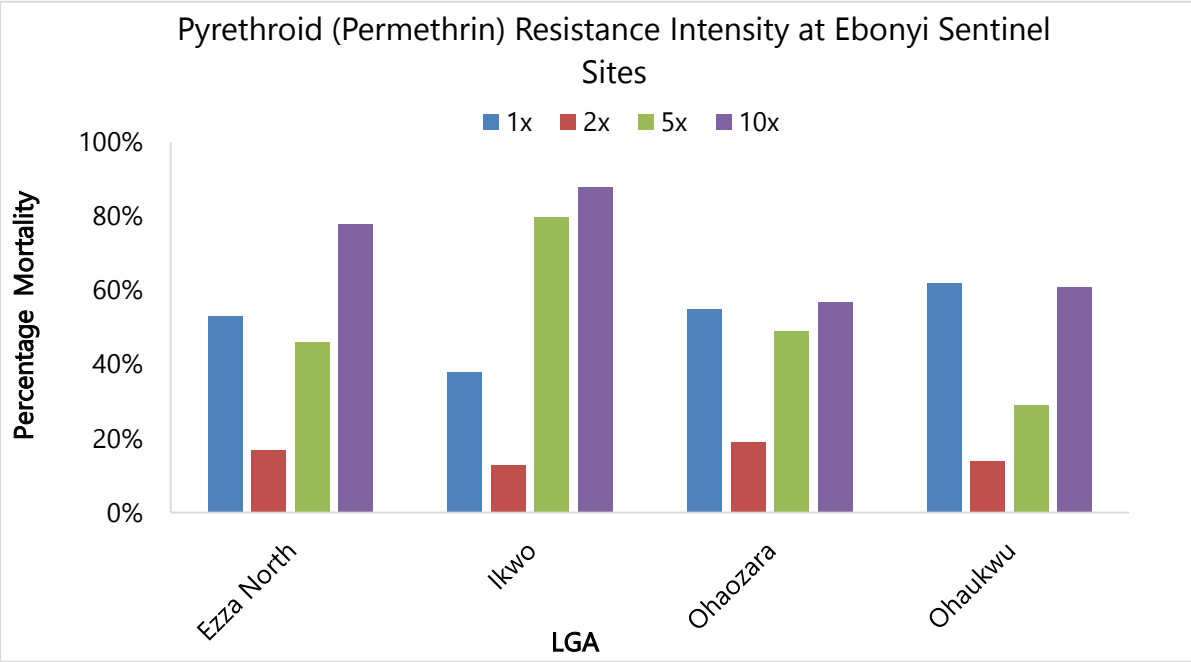


Figure 14: Pyrethroid (Permethrin) Resistance Intensity at Ebonyi Sentinel Sites

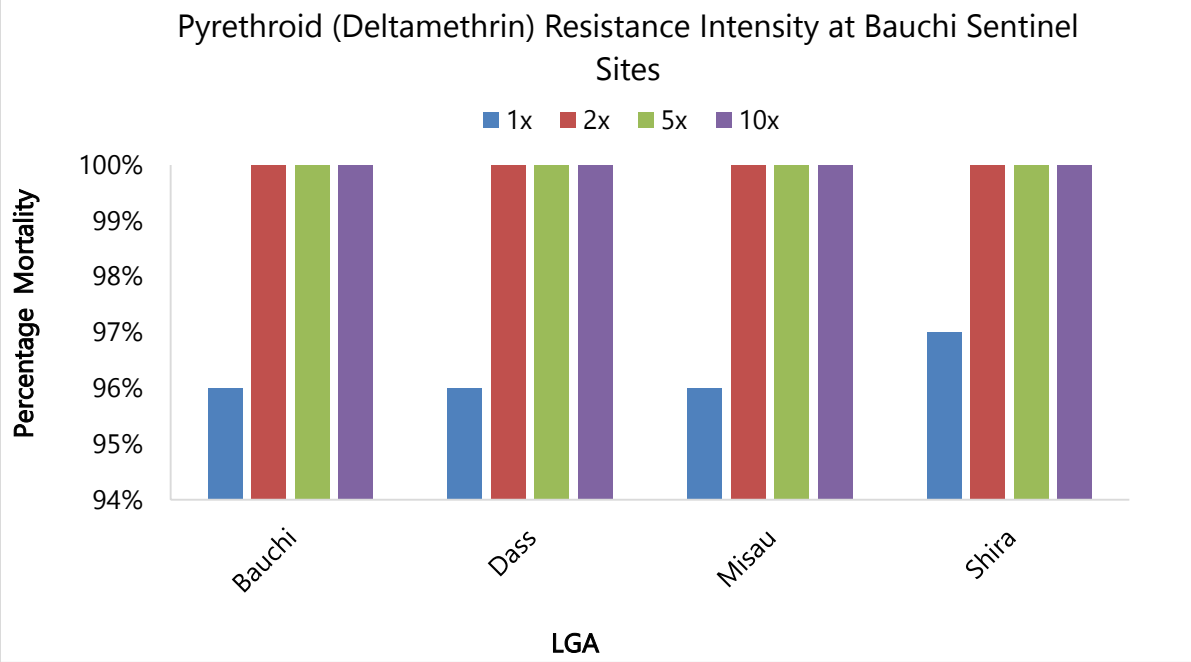


Figure 15: Pyrethroid (Deltamethrin) Resistance Intensity at Bauchi Sentinel Sites

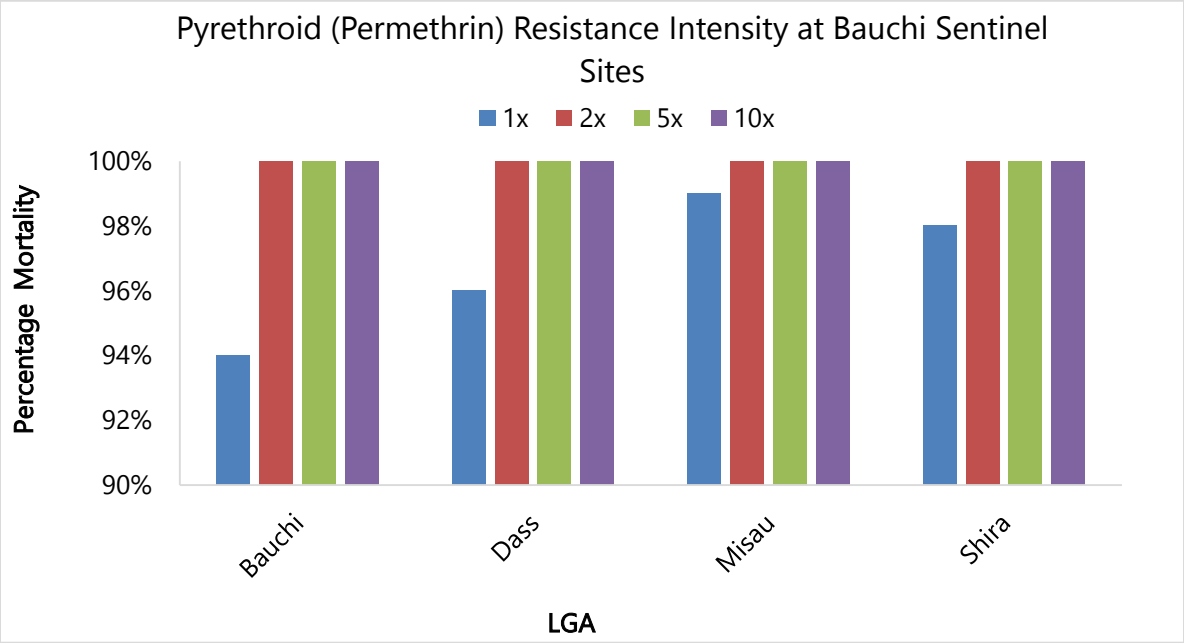


Figure 16: Pyrethroid (Permethrin) Resistance Intensity at Bauchi Sentinel Sites

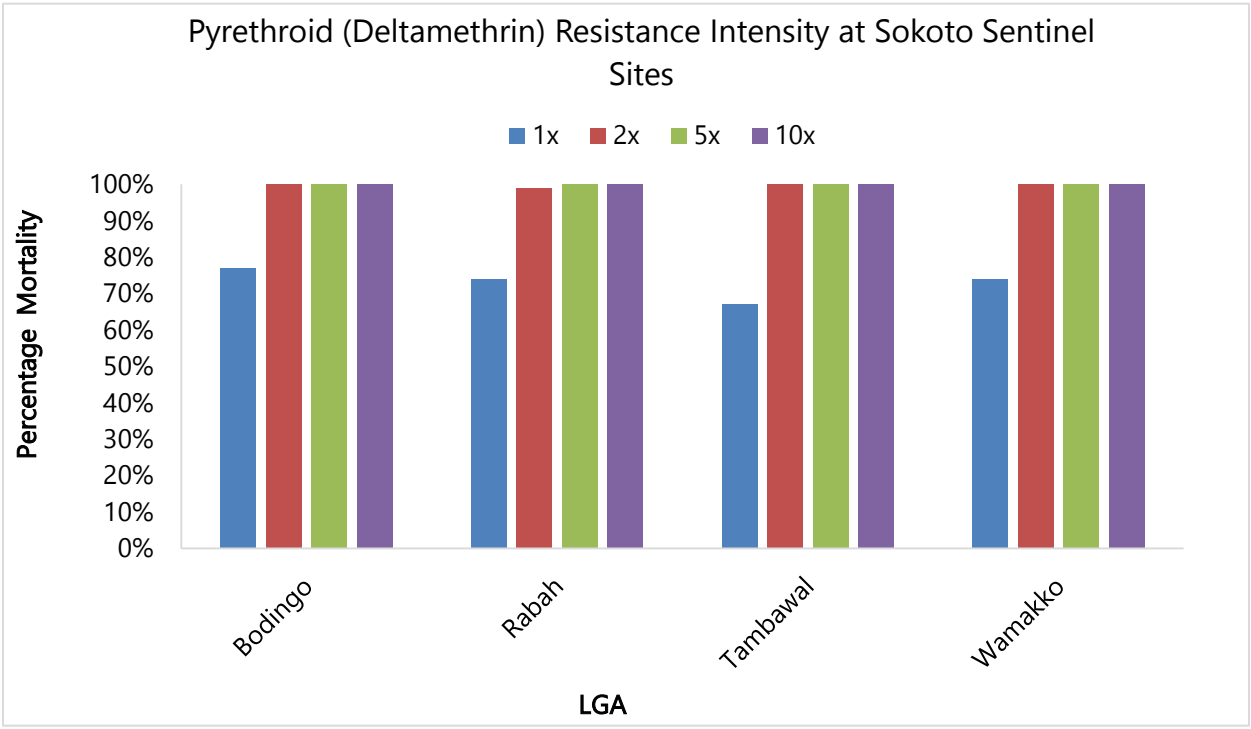


Figure 17: Pyrethroid (Deltamethrin) Resistance Intensity at Sokoto Sentinel Sites

4.8 KNOCK DOWN RESISTANCE ANALYSIS FOR THE SIX ECOZONES OF NIGERIA

The West and East African knock down resistance mutation was analysed in mosquitoes exposed to insecticides. Additional 40 mosquitoes randomly selected from the routine collection at each site were also tested. * Mosquito samples not exposed: selected from routine population survey.

Table 13:Frequency of the knock down resistance mutation at the Sokoto site

	Insecticide	No. Resistant	*Kdr* genotype in resistant mosquito				No. susceptible	*Kdr* genotype in susceptible mosquito			
			Kdr-w			*Kdr-e*		*Kdr-w*			*Kdr-e*
			RR	Rr	rr			RR	Rr	rr	
WHO Assay	Deltamerhin	100	65	24	10	0	25	0	14	11	0
	DDT	100	72	11	0	0	25	0	12	13	0
	Permetrin										
	Not exposed (n=40)*		0	32	8		-	-	-	-	0
CDC bottle Assay	Deltamerhin										
	DDT										
	Permetrin										

Table 14:Frequency of the knock down resistance mutation at the Ebonyi site

	Insecticide	No. Resistant	*Kdr* genotype in resistant mosquito				No. susceptible	*Kdr* genotype in susceptible mosquito			
			Kdr-w			*Kdr-e*		*Kdr-w*			*Kdr-e*
			RR	Rr	rr			RR	Rr	rr	
WHO Assay	Deltamerhin	100	78	22	0		25	0	8	17	0
	DDT	51	42	8	0		25	0	5	20	0
	Permetrin										
	Not exposed (n=40)		0	4	36		-	-	-	-	0
CDC bottle	Deltamerhin										
	DDT										

Assay	Permetrin										

Table 15: Frequency of the knock down resistance mutation at the Nassarawa site

	Insecticide	**No. Resistant**	***Kdr* genotype in resistant mosquito**				**No. susceptible**	***Kdr* genotype in susceptible mosquito**			
			Kdr-w			***Kdr-e***		***Kdr-w***			***Kdr-e***
			RR	**Rr**	**rr**			**RR**	**Rr**	**rr**	
WHO Assay	Deltamerhin	56	50	16	0	0	0	0	0	0	0
	DDT	116	98	18	0	0	0	0	0	0	0
	Permetrin										
	Not exposed (n=40)*		0	30	12	0	-	-	-	-	0
CDC bottle Assay	Deltamerhin	4	4	0	0	0	0	0	0	0	0
	DDT	74	62	12	0	0	0	0	0	0	0
	Permetrin	-	-	-	-	-	-	-	-	-	-

Table 16:Frequency of the knock down resistance mutation at the Bauchi site

	Insecticide	**No. Resistant**	***Kdr* genotype in resistant mosquito**				**No. susceptible**	***Kdr* genotype in susceptible mosquito**			
			Kdr-w			***Kdr-e***		***Kdr-w***			***Kdr-e***
			RR	**Rr**	**rr**			**RR**	**Rr**	**rr**	
WHO Assay	Deltamerhin	80	56	22	2	0	50	0	24	26	0
	DDT	36	30	6	0	0	25	0	10	15	0

	Permetrin										
	Not exposed (n=40)*		0	16	24	0	-	-	-	-	0
CDC bottle Assay	Deltamerhin				0	0	0	0	0	0	0
	DDT				0	0	0	0	0	0	0
	Permetrin	-	-	-	-	-	-	-	-	-	-

Table 17:Frequency of the knock down resistance mutation at the Akwa-Ibom site

	Insecticide	**No. Resistant**	***Kdr* genotype in resistant mosquito**				**No. susceptible**	***Kdr* genotype in susceptible mosquito**			
			Kdr-w			***Kdr-e***		***Kdr-w***			***Kdr-e***
			RR	**Rr**	**rr**			**RR**	**Rr**	**rr**	
WHO Assay	Deltamerhin	0	0	0	0	0	0	0	0	0	0
	DDT	78	48	30	0	0	25	0	11	14	0
	Permetrin	22	14	7	1	0	25	0	8	17	0
	Not exposed (n=40)*		0	25	15	0	-	-	-	-	0
CDC bottle Assay	Deltamerhin					0	0	0	0	0	0
	DDT					0	0	0	0	0	0
	Permetrin	-	-	-	-	-	-	-	-	-	-

Table 18:Frequency of the knock down resistance mutation at the Oyo site

	Insecticide	**No. Resistant**	***Kdr* genotype in resistant mosquito**				**No. susceptible**	***Kdr* genotype in susceptible mosquito**			
			Kdr-w			***Kdr-e***		***Kdr-w***			***Kdr-e***
			RR	**Rr**	**Rr**			**RR**	**Rr**	**rr**	
WHO	Deltamerhin	100	75	18	0	0	25	3	9	13	0

Assay	DDT	100			0	0	25	5	14	6	0
	Permetrin	-	-	-	-	-	-	-	-	-	-
	Not exposed (n=40)*		4	25	11	0	-	-	-	-	0
CDC bottle Assay	Deltamerhin	-	-	-	0	0	0	0	0	0	0
	DDT	-	-	-	0	0	0	0	0	0	0
	Permetrin	-	-	-	-	-	-	-	-	-	-

RR: Homozygous Resistant

Rr: Heterozygous **rr: Homozygous susceptible**

5. Discussion, Lessons Learned, and Challenges

PCR indicated the proportion of *An. gambiae* varied with eco zone from wet to dry areas but the same was not observed with *An. arabiensis*. *Anopheles gambiae* was found in all six ecological zones, and *An. arabiensis* were found across five out of the six. This finding agrees with Onate and Conn (2001) who compared to the distributions determined from samples of indoor-resting females reported over 20 years ago by Coluzzi *et al*. (1979). *An. arabiensis* was now prevalent in several localities in the Guinea savanna, an area where it was virtually absent over 20 years ago. The data suggest that *An. arabiensis* has extended its range from arid areas of the Sahel down to the Guinea savannah .

The S form of *An. gambiae* was observed across ecozones and occurred in sympatry with the M form of *An.gambiae* in all zones except Sokoto in the Sudan/ Sahel savannah .

Although the distribution of the molecular M and S forms is still being determined for much of West Africa and for Nigeria in particular, this study shows that the molecular S form is predominant and has as a wider distribution across Nigeria . PCR results indicate that the 'S' form was predominant and corroborates findings from Awolola *et al.* (2005; 2007).

ELISA indicated higher infection rates in *An. gambiae* 2.38 -4.98 and in *An. arabiensis* 1.04-2.08. Both occurred in sympatry at all ecozones except in the mangrove forest (Figure 12). Significantly higher human bloodmeals was observed in *An. gambiae* 49.56 -64 percent than *An. arabiensis* 18.96-34.78 and varied according to the ecozones.

This study incriminated *An.coustani* possibly for the first time in Nigeria as a malaria vector biting both indoors and outdoors .Though it is noteworthy that significantly higher numbers of *An.coustani* were collected outdoors. Recent studies have indicated that *An. coustani* is playing a major role in outdoor transmission (Mwangangi *et al*. 2013). Fornadel *et al. (*2011) had earlier observed an increased anthropophily in *An. coustani*..Effective malaria control programs should therefore include tools that target both indoor and outdoor transmission.

The highest percentage of confirmed malaria cases and sporozoite in malaria vectors was recorded in Akwa Ibom in the mangrove in addition to significantly higher numbers of infective bites per person per year. Entomological Inoculation Rates(EIR) across all ecozones ranged from 2.6 infective bites per person per year for *An. gambaie* s.s and 0.1 *An.arbiensis* to 55 infective bites per person per year in *An. gambiae* s.s and 1.6 in *An. arabiensis* the in the Guinea Savannah . A summary of

entomological inoculation rates (EIR) reported in 86 studies from Nigeria suggests that EIR for *A. gambiae s.l.* ranges from 18 to 145 infective bites per person per year .(RBM, 2008) and findings from this work indicates this trend . Beier *et al*. (1999) determined that there were no sites with less than 50% prevalence when the EIR exceeded 15 infective bites per year. Earlier studies have shown that annual entomological inoculation rates (EIRs) must be reduced to less than one to substantially reduce the prevalence of malaria infection (Shaukat *et al*. 2010). Pyrethroid and DDT resistance was recorded across all ecozones. No significant difference was observed between DDT and pyrethroids indicationg the possibility of cross resistance between DDT and pyrethroids across the ecozones .

6. CHALLENGES

1. Having to wait up to December for a comprehensive report of results from the surveillance which must be sent for molecular analysis,,.

2. Some sites could not complete the resistance tests in the 4 LGAs due mainly to the fact that most of the sites are new to the project as well as non availability of enough mosquitoes.

7. RECOMMENDATIONS

- Entomological surveillance should be scaled up by establishing malaria vector sentinel sites in all PMI – supported states in Nigeria.
- Entomological impact assessment of LLIN distribution in PMI supported states should be carried out to link epidemiological data with entomological data.
- More efforts should be made to assist the NMEP to develop IRM plan for Nigeria
- The PMI-funded insectary in Keffi could serve as a training center of sentinel site technicians across the country in basic surveillance techniques.
- Pirimiphos-methyl was found to show low mortality in some of the sentinel sites . This could be due to stability of insecticide used for the tests. It is important to use premeasured dosages prepared using actellic cs formulation be used in further tests as recommended by Bill Brogdon.
-
- Pyrethroid resistance does not seem to be high in intensity in most states -- This is an indication that resistance will probably not cause operational failure, LLINs are likely to still be efficacious to use.
- Sokoto has particularly poor indicators, may be worth additional study (bed net coverage and usage?) to see why this is a problem.
- Problems with species PCR not working in some sites: recommend checking with sites on following sample storage protocols properly, checking a subset of morphological IDs to make sure they are not misidentifying other species as An. gambiae.

BIBLIOGRAPHY

Africa Indoor Residual Spraying Nigeria (2014). *Entomological Summary Report II. March - October, 2014*. Nigeria. Africa Indoor Residual Spraying Project, Abt Associates Inc. 58pp.

Akogbeto, M., Yakoubou, S., (1999). Resistance of malaria vectors to pyrethroids used for impregnated bednets in Benin. West Afr. Bull. Soc. Pathol. Exot. **92:** 123–130.

Amajoh, C.N. (2012). Update on Malaria Control in Nigeria. Paper Presentation at the third Annual Malaria Control Programme Review Meeting, Atlanta, U.S.A., February, 2012.

Antonio-Nkondjio, C, Kerah, C.H., Simard, F., Awono-Ambene, P,L. (2006). Complexity of the malaria vectorial system in Cameroon: contribution of secondary vectors to malaria transmission. *Journal of Medical Entomology* **43:**1215–1221.

Antonio-Nkondjio, C., Ndo, C., Costantini, C., Awono-Ambene, P., Fontenille, D., Simard, F. (2009). Distribution and larval habitat characterization of *Anopheles nili* and *An. moucheti* along river networks in south Cameroon. *Acta Tropica* **112:**270-276.

Antonio-Nkondjio, C., Kerah Hinzoumbe, C., Simard, F., Awono-Ambene, P., Tchuinkam, T., Fontenille, D. (2006). Complexity of the malaria vectorial system in Cameroon: Contribution of secondary vectors to malaria transmission. *Journal of Medical Entomology* **43:**1215-122

Antonio-Nkondjio C., Simard F, (2013) : Highlights on *Anopheles nili* and *An. moucheti* Malaria Vectors. http://www.intechopen.com/books/anopheles-mosquitoes-new-insights-into-malaria-vectors/highlights-on-anopheles-nili-and-anopheles-moucheti-malaria-vectors-in-africa" accessed 4 March 2015.

Awolola, T.S, Oyewole, I.O., Koekemoer, L.L. and Coetzee, M. (2005). Identification of three members of the *Anopheles funestus* (diptera: Culicidae) group and their role in malaria transmission in two ecological zones in Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **99:**525–531.

Awolola, T.S, Bitsindou, P., Bagayoko, M,, Manga, L. (2007). Malaria Entomological Profile for Nigeria. WHO Document. 50p.

Awolola, T,S., Okwa, O, Hunt, R,, Ogunrinade, A., Coetzee, M. (2002): Dynamics of malaria vector populations in coastal Lagos, south-western Nigeria. *Annals of Tropical Medicine and Parasitology* **96:**75 - 82.

Awolola.T.S.,Oduola A.O, Oyewole I.O Obansa J.B.,Amajoh C.N.,Koekemoer L . L . , Coetzee .M. (2007) Dynamics of knockdown pyrethroid insecticide resistance alleles in a field population of *Anopheles gambiae s.s.* in southwestern. *Nigeria Journal of Vector Borne Disease* 44, 181–188

Ayanlade, A., Adeoye, N. O., & Babatimehin, O. (2010). Climate change /variability and malaria transmission in sub-Saharan Africa: A case of Nigeria. Report of the Royal Norwegian Society of sciences and Letters 250 anniversary Conference, June 21-24, 2010 Trondheim, Norway.[OnlineAvailable:http://climsec.prio.no/papers/Climate%20Changeariability%20and %20malaria%20transmission%20in%20Sub-Saharan%20Africa-%20A% 20case%20of%20Nigeria.pdf (July 12, 2011).

Beier, J.C., Perkins, P.V, Koros, J.K. (1990). Malaria sporozoite detection by dissection and ELISA to assess infectivity of Afrotropical *Anopheles* (Diptera: Culicidae). *Journal of Medical Entomology* **27:** 377-384.

Beier. J. C. (1998). Malaria parasite development in mosquitoes. Annu Rev Entomol .**43**: 519-543.

Besansky, N.J., Hill, C.A., Costantini, C. (2004). No accounting for taste: host preference in malaria vectors. *Trends in Parasitology*. **20***:* 249–251.

Bigoga, J.D., Manga, L., Titanji, V.P.K., Coetzee, M. (2007). Malaria vectors and transmission dynamics in coastal south-western Cameroon *Malaria Journal* , **6**:5

Brooke, B.D, Kloke, G., Hunt, R.H., Koekemoer, L.L, Temu, E.A, Taylor, M.E, Small, G., Hemingway, J., Coetzee, M.(2001): Bioassay and biochemical analyses of insecticide resistance in southern African *Anopheles funestus* (Diptera: Culicidae). *Bull Entomol Res*; **91**:265-272.

Bruce-Chwatt, L. J. (1986). Essential Malariology. London: William Heinemann Medical Books. 452pp.

Bruce – Chwatt, LJ. (1954). Malaria in Nigeria. Bull World Health Organ.;4:301-327.

Brogdon .B and Chan A.(2010). Guideline for Evaluating Insecticide Resistance in Vectors Using the CDC Bottle Bioassay 28 pp

Brogdon, W.G., McAllister, J.C. (1998). Simplification of adult mosquito bioassays through use of time-mortality determinations in glass bottles. *Journal of American Mosquito Control Association* **14(2):**159-164.

Brogdon W.G (2015) Resistance Intensity bottle assay A critical RDT for IRM,Adama, Ethiopia July 2015.

Carnevale, P, Le Goff, G, Toto, J.C,, Robert, V.(1992): *Anopheles nili* as the main vector of human malaria in villages of southern Cameroon. *Medical and Veterinary Entomology*, **6:**135-138.

Centers for Disease Control (2010). Methods in *Anopheles* Research. Second Edition/CDC Technical Report. 343.

Chandre, F., Darriet, F., Manguin, S., Brengues, C., Carnevale, P., Guillet, P., 1999. Pyrethroid cross-resistance spectrum among populations of *Anopheles gambiae* s.s. from Cote D'Ivoire. J. Am. Mosq. Cont. Assoc. **15**: 53–59.

Chandre, F., Darriet, F., Duchon, S., Finot, L., Manguin, S., Carnevale, P., Guillet, P., (2000). Modifications of pyrethroid effects associated with *kdr* mutation in *Anopheles gambiae*. Med. Vet. Entomol. 14: 81–88.

Coetzee, M., Craig M, le Sueur, D. (2000). Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitology today* (Personal ed) **16:**74–77.

Coetzee, M. (2004). Distribution of the African Malaria Vectors of the *Anopheles gambiae* complex. *American Journal of Tropical Medicine and Hygiene* **70(2):** 103-104.

Coetzee, M, Van Wyk, P, Boorman, M. Koekemoer, L.L., Hunt, R. H. (2006). Insecticide resistance in malaria vector mosquitoes in a gold mining town in Ghana and implications for malaria control. *Bulletin de la Société de pathologie exotique* **99(5):** 400-403.

Costantini, C., Sagnon, N., Ilboudo-Sanogo, E., Coluzzi, M. and Boccolini, D. (1999). Chromosomal and bionomic heterogeneities suggest incipient speciation in *Anopheles funestus* from Burkina Faso. *Parassitologia* **41:**595–611.

Dia, I,, Diop, T,, Rakotoarivony, I., Kengne, P., Fontenille, D. (2003): Bionomics of *Anopheles gambiae* Giles, *An. arabiensis* Patton *An. funestus* Giles and *An. nili* (Theobald) (Diptera: Culicidae) and Transmission of *Plasmodium falciparum* in a Sudano-Guinean Zone (Ngari, Senegal). *Journal of Medical Entomology* **40:**279-283.

Dabire, K.R., Baldet, T., Diabate, A., Dia, I., Costantini, C., Cohuet, A., Guiguemde, T.R., Fontenille, D. (2007). *Anopheles funestus* (Diptera: Culicidae) in a humid savannah area of western Burkina Faso: Bionomics, insecticide resistance status, and role in malaria transmission. *Journal of medical entomology* **44**:990–997.

Della Torre A, Fanello C, Akogbeto M, Dossou-yovo J, Favia G, Petrarca V, Coluzzi M. Molecular evidences of incipient speciation within *Anopheles gambiae s.s.* in West Africa. *Insect Mol Biol* 2001; *10:* 9–18.

De Meillon, B., Van, Eeden G., Coetzee, L., Coetze, M, Meiswinkel, R., du Toit, C.L.N., Hansford, C.F. Observations on a species of the *Anopheles funestus* subgroup, a suspected exophilic vector of malaria parasites in north-eastern Transvaal, South Africa. *Mosquito News* **37:**657–661.

Detinova, T.S. (1962). Age-grouping methods in Diptera of medical importance: with special reference to some vectors of malaria. World Health Organization (WHO) (Geneva, Switzerland) Monograph series **47:** 1-216.

Malaria Consortium RBM needs Assessment Nigeria Report 2008 99pp

Health Management Information software (HMIS), (2017). Powered by the District Health Information Software (DHIS 2), January 12, 2017.

ANNEX

ANNEX A1: Total Number of anophelines Caught in all Sentinel Sites, February – December, 2016

Mosquito Species	Nasarawa			Sokoto			Ebonyi			Akwa Ibom			Oyo			Bauchi			Total (In)	Total (Out)	Total (PSC)	Overall
	In	Out	PSC	In	Out	PSC	In	Out	PSC	In	Out	PSC	In	Out	PSC	In	Out	PSC				
An. gambiae s.l.	1834	1245	2112	1073	611	2152	606	106	2408	1175	576	210	75	73	793	379	159	295	5142	2770	7970	15882
An. funestus	13	4	0	9	13	0	7	3	32	1	1	0				3	2	0	33	23	32	88
An. coustani	347	399	0				2	1	14	1	0	0				14	13	70	364	413	84	861
An. nili	3	1	0				0	0	17							8	2	12	11	3	29	43
An. pharoensis	21	12	0	81	51	15	2	2	3										104	65	18	187
An. malculipalpis	1	5	0				5	1	10							2	16	0	8	22	10	40
An. moucheti							2	1	6	257	85	12				0	1	0	259	87	18	364
An. obscurus										0	1	0							0	1	0	1
An. pretoriensis													0	1	0	0	0	2	0	1	2	3
An. rufipes							1	0	3							0	0	3	1	0	6	7
An. squamosus							3	1	20							0	3	5	3	4	25	32
An. ziemanni																0	0	1	0	0	1	1
An. longipalpis													2	0	24				2	0	24	26
Total	2219	1666	2112	1163	675	2167	628	115	2513	1434	663	222	77	74	817	406	196	388	5927	3389	8219	17535

ANNEX A2:

State	Ecological Zone	gambiae indoor density	gambiae biting behavior	molecular identification	sporozoite rate	human blood index	pyrethroid resistance	pyrethroid resistance intensity	other notes
Akwa Ibom	Mangrove/Forest	0-2per house *	mainly indoors, peaking at midnight	*gambiae* s.s. (84%) *arabiensis* (2%)	*gambiae* – 4.98% *arabiensis* – 1.04 %	*gambiae* – 62.6% *arabiensis* 0 %	(72.6-100% mortality in WHO test)	Yet to be done	
Bauchi	Sahel Savannah	0-3 per house	mainly indoors, peaking at midnight	*gambiae* s.s. (50%) *arabiensis* (20%)	*gambiae* – 4.16% *arabiensis* – 2.08 %	*gambiae* – 49.56% *arabiensis* 34.78 %	(84-100% mortality in WHO test)	x1 resistance only	
Ebonyi	Rain Forest	0-19.1 per house (high)	mainly indoors, peaking at midnight	*gambiae* s.s. (72%) *arabiensis* (14%)	*gambiae* – 2.38% *arabiensis* – 1.04 %	*gambiae* – 55.12% *arabiensis* 24.32 %	(5-100% mortality in WHO test)	x1, x2 and x5 resistance	
Nasarawa	Guinea Savannah	1.2-13 per house (high)	mainly indoors, peaking at midnight	*gambiae* s.s. (68%) *arabiensis* (22%)	*gambiae* – 3.9% *arabiensis* – 1.65 %	*gambiae* – 34.20% *arabiensis* 18.96 %	(48-100% mortality in WHO test)	x1 resistance only	
Oyo	Forest Savannah	0-10 per house (high)	mainly indoors, peaking at midnight	*gambiae* s.s. (67%) *arabiensis* (22%)	*gambiae* – 4.44% *arabiensis* – 0 %	*gambiae* – 51.64% *arabiensis* 23.52%	moderate (50-100% mortality in WHO test)	x1, x2 and x5 resistance	
Sokoto	Sudan/Sahel Savanna	0-23 per house (high)	mainly indoors, peaking at midnight	*gambiae* s.s. (76%) *arabiensis* (21%)	*gambiae* – 3.66% *arabiensis* – 1.1 %	*gambiae* – 64% *arabiensis* – 25%	moderate (55-100% mortality in WHO test)	x1 resistance only	pirimiphos methyl resistance reported – needs confirmation

CDC light traps collected higher numbers than PSC in Akwa Ibom with proxy IRD estimates ranging from 0.1 to 13.5 .

**CDC light traps collected higher numbers than PSC in Akwa Ibom with proxy IRD estimates ranging from 0.1 to 13.5 .*