PMI | AFRICA IRS (AIRS) PROJECT
INDOOR RESIDUAL SPRAYING (IRS 2) TASK ORDER SIX

AIRS NIGERIA
FINAL ENTOMOLOGY REPORT
JANUARY - DECEMBER 2017

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ACRONYMS

AIRS	Africa Indoor Residual Spraying project
CDC	Centers for Disease Control and Prevention
CDC LT	CDC Light Trap
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
GPIRM	Global Plan for Insecticide Resistance Management
HBR	Human Bite Rate
IRD	Indoor Resting Density
IRS	Indoor Residual Spraying
Kdr	Knock Down Resistance
LGA	Local Government Area
LLIN	Long-Lasting Insecticidal Net
NMEP	National Malaria Elimination Program
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SPR	Sporozoite Rate
WHO	World Health Organization

EXECUTIVE SUMMARY

The AIRS Nigeria project team conducted entomological monitoring at six sentinel sites in Nigeria from January through December 2017. Pyrethrum spray catches and human-baited CDC light traps situated both indoors and outdoors were used to sample mosquitoes and determine the species composition of malaria vectors, indoor resting density, behavior, biting rates, longevity, and entomological inoculation rates (EIRs) at each site. Insecticide resistance frequency and intensity were also determined for *Anopheles gambiae* s.l. The underlying mechanisms of resistance were assessed using molecular methods and synergist assays.

Morphological identification of *Anopheles* mosquitoes indicated that *An. gambiae* s.l. is the dominant malaria vector (94 percent of the total *Anopheles* mosquitoes collected). Other species found included *An. funestus*, *An. coustani*, *An. nili*, *An. pharoensis*, *An. maculipalpis*, *An. moucheti*, *An. pretoriensis*, *An. squamosus,* and *An. longipalpis.* The occurrence and abundance of these species varied among sites. Among the sibling species of *An. gambiae* s.l., *An. gambiae* s.s. was predominant, followed by *An. coluzzii* and *An. arabiensis.*

The indoor resting density of *An. gambiae* s.l. was the highest in Sokoto, with bimodal peaks in May and September with 22 and 23 *An. gambiae* s.l. per house per day, respectively. The biting rate was also the highest at Sokoto with peak biting occurring indoors between 10 and 11 pm (19 bites/person/hour), and outdoors from 3 to 4 in the morning (18 bites/person/hour). Indoor biting rates were higher than outdoor rates at most sites, except at Oyo and Sokoto where similar levels of biting were recorded both indoors and outdoors. Early evening biting was low in all sites. Because most people are likely to be indoors during the peak biting hours observed in the rainy season at most of the sites, deployment of indoor residual spraying and long lasting insecticidal nets is likely to reduce malaria transmission.

An. gambiae s.l. was found to be resistant to DDT and pyrethroids at most of the sentinel sites. The vector was susceptible to carbamate and organophosphate insecticides at most sites. The intensity of pyrethroid resistance was high, at 10 times the diagnostic dosage in the rainforest (Ebonyi and Oyo) sites. Resistance among different pyrethroids varied within the class, with generally more intense resistance to permethrin observed. In most sampling stations (28) across the sites, piperonyl butoxide (PBO) synergist assays indicated that elevated oxidases are the only contributors to resistance. However, in Oyo and Ebonyi, additional resistance mechanisms are likely as PBO did not increase mortality to 100 percent. Resistance mitigation strategies—such as procuring and distributing non-permethrin, PBO, or next-generation long-lasted insecticidal nets (LLINs) during the next planned mass campaigns—may be required for Ebonyi and Oyo.

In all sites except Ebonyi, EIRs were higher for *An. gambiae* than *An. coluzzii* and *An. arabiensis.* Indoor EIR values for *An. gambiae* ranged from 1.2 infective bites per person per year in Sokoto to a peak of 23.9 infective bites per person per year in Akwa Ibom. For *An. coluzzii*, indoor EIR values ranged from 0.9 infective bites per person per year in Oyo to 11.4 infective bites per person year in Ebonyi. In all sites, outdoor EIRs were lower than those recorded indoors. This further suggests that indoor vector control interventions such as indoor residual spraying and LLINs are having an impact on malaria transmission in Nigeria.

I. INTRODUCTION

Africa bears 80 percent of the global malaria burden, with Nigeria alone representing 27 percent of the malaria burden on the continent. Nigeria loses close to 100,000 lives annually to malaria, and in 2017 spent N1.1 trillion–15 percent of the annual budget–on the disease (NMEP 2015; Awolola 2017). The Nigeria Federal Ministry of Health's National Malaria Elimination Plan (NMEP), in collaboration with the PMI Africa Indoor Residual Spraying (AIRS) project in Nigeria, established malaria vector surveillance sentinel sites in six states representing a variety of ecological zones (henceforth referred to as ecozones), with the staff capacity, facilities, and basic equipment for entomological monitoring. These sentinel sites were linked to local universities/research institutes in an effort to build sustainable entomology capacity and institutionalize surveillance activities.

We report here on entomological monitoring activities conducted between January and December 2017. Throughout this time, AIRS Nigeria collected data at six sentinel sites to:

- Identify the malaria vector species composition, seasonality, and density in different ecozones

- Inform the optimal time and place to implement vector control

- Determine vector feeding time and location

- Assess development of insecticide resistance and its intensity and mechanism

Data gathered, analyzed and presented here support NMEP data-driven decisions for programming vector control activities.

2. MONITORING VECTOR BEHAVIOR AND DENSITY

In 2017, PMI supported entomological monitoring at six sentinel sites—from this point forward referred to by the state in which they are located—representing the five major ecozones seen in Nigeria (Table 1 and Figure 1). At each sentinel site, a principal investigator and 10 technicians carried out the surveillance work according to PMI's 2015 Technical Guidance. The team determined indoor resting densities (IRDs) using pyrethrum spray catches (PSC), and mosquito biting time and location (indoor/outdoor) using Centers for Disease Control and Prevention Light Traps (CDC LTs). Mosquitoes were morphologically identified and preserved in desiccated form in Eppendorf tubes for subsequent analyses.

TABLE 1: SENTINEL SITES SUPPORTED BY PMI IN 2017 AND THEIR AFFILIATE INSTITUTIONS

Sentinel site location		Collaborating Institutions	Ecozone(s) represented
State	**Local Government Area**		
Akwa Ibom	Mpat-Enin	University of Uyo	Mangrove swamps, Rainforest
Bauchi	Gwantar	Abubakar Tafawa Balewa University	Sudan Savannah
Ebonyi	Ezza North	Ebonyi State University	Rainforest
Nasarawa	Nasarawa Eggon/Doma	Nasarawa State University	Guinea Savannah
Oyo	Olorisaoko	University of Ibadan	Rainforest, Guinea Savannah
Sokoto	Rabah	Usmanu Dandodiyo University	Sahel Savannah

FIGURE 1: GEOGRAPHIC DISTRIBUTION OF SENTINEL SITES SUPPORTED BY PMI/AIRS IN 2017

3. COLLECTION AND ANALYTICAL METHODS

The team collected adult mosquitoes monthly in all the sentinel sites using PSC and CDC light trap methods. The team collected anopheline larvae using ladles and reared them to adults for insecticide susceptibility tests.

3.1 CDC LIGHT TRAP COLLECTION

The teams used human-baited CDC LTs—one placed indoors and one outdoors—in four houses per sentinel site for three nights each month to measure mosquito biting time and location. The teams followed the methods of Yohannes and Boelee (2012).

3.2 PYRETHRUM SPRAY CATCHES

The team randomly sampled 32 houses per sentinel site per month using the PSC method (WHO 1975) to sample indoor-resting mosquitoes. The teams sent all samples collected from the field to the centrally-located insectary at Nasarawa State University Keffi for further processing and analyses.

3.3 IDENTIFICATION OF MALARIA VECTORS

The team morphologically identified at the species level *Anopheles* mosquitoes collected using human-baited CDC light traps and PSC (Gillies and De Meillon 1968; Gillet 1972; Gillies and Coetzee 1987; Kent 2006). The teams labeled all non-dissected *Anopheles* specimens and stored them individually over silica gel in Eppendorf tubes for further processing. The team sent all samples collected from the field to the centrally-located insectary at Nasarawa State University Keffi.

3.4 DETERMINATION OF PARITY

To determine physiological age and parity rate, the team dissected ovaries from randomly-selected, unfed female *An. gambiae* s.l. specimens captured with human-baited CDC LTs. The teams used methods as described by Gillies and Wilkes (1963) and the WHO (2003). Parity rate was determined by dividing the number of parous females—confirmed by observing the degree of coiling by the ovarian tracheoles (Detinova 1962, Detinova and Gillies 1964)—by the total number of mosquitoes examined (WHO 2013).

3.5 PCR IDENTIFICATION OF MEMBERS OF THE *AN. GAMBIAE* COMPLEX

The team identified the species of *An. gambiae* s.l. mosquitoes collected from the six sentinel sites using a polymerase chain reaction (PCR) carried out at the Nigeria Institute for Medical Research (NIMR), Yaba Lagos. PCR was conducted on approximately 10% of the total number of samples caught, including both those that had been caught indoors and outdoors by CDC LTs and by PSC. The team amplified extracted DNA using the *An. gambiae* species-specific multiplex PCR (Scott *et al.* 1993). Further PCR assays were then carried out to differentiate between *An. gambiae* and *An. coluzzii* (Fanello *et al.* 2002).

3.5.1 PLASMODIUM SPOROZOITES ASSAY

To estimate the *Plasmodium* infection rate in the mosquito population, the team also performed enzyme-linked immunosorbent assay (ELISA) tests at NIMR, Lagos on a proportion of mosquitoes collected from the field. The team crushed the head and thorax of each female *Anopheles* mosquito in phosphate-buffered saline and used an ELISA assay to test for the circumsporozoite antigen (Burkot *et al.* 1984).

4. RESULTS

4.1 ANOPHELES SPECIES COLLECTED BY VARIOUS METHODS

Between January and December 2017, the study teams collected 21,459 *Anopheles* mosquitoes from six sentinel sites. Out of the total collected, 93.7% (n=20,099) were identified as *An. gambiae* s.l., and 0.5% (n=98) were *An. funestus*. Other species caught included *An. coustani* (3.1% of the total, n=658), *An. moucheti* (1.9%, n=399), and *An. pharoensis* (0.7%, n=148). The percent of *An. maculipalpis*, *An. nili*, *An. pretoriensis*, *An. squamosus*, and *An. longipalpis* was each less than 0.1% of the total collected.

An. gambiae s.l. was found at all six sentinel sites, while teams collected *An. coustani* only at Ebonyi, Nasarawa, and Bauchi, *An. moucheti* only at Ebonyi and Akwa Ibom, and *An. pharoensis* only at Nasarawa, Sokoto, and Ebonyi. The number of each subspecies found at each site is detailed in Annex 1.

4.2 IRD OF *AN. GAMBIAE* S.L. CAUGHT USING PYRETHRUM SPRAY CATCH

Between January and December 2017, study teams used PSC sampling methods to collect 8,592 adult female *An. gambiae* s.l. mosquitoes in 32 houses per sentinel site over a period of three nights per month. Overall, the indoor resting density (IRD) across sites remained below 10 *An. gambiae* s.l. mosquitoes per house per day throughout the year. As Figure 2 shows, the two highest IRD values—21.8 and 23.1—both occurred in Sokoto, in May and August, respectively. Among the other five sites, Nasarawa (N/Eggon) saw the next highest IRD (8.5), which occurred in July. Akwa Ibom experienced the lowest overall IRD (range: 0.1-2.4).

FIGURE 2: *AN. GAMBIAE* S.L. INDOOR RESTING DENSITY (IRD) ACROSS SENTINEL SITES IN 2017
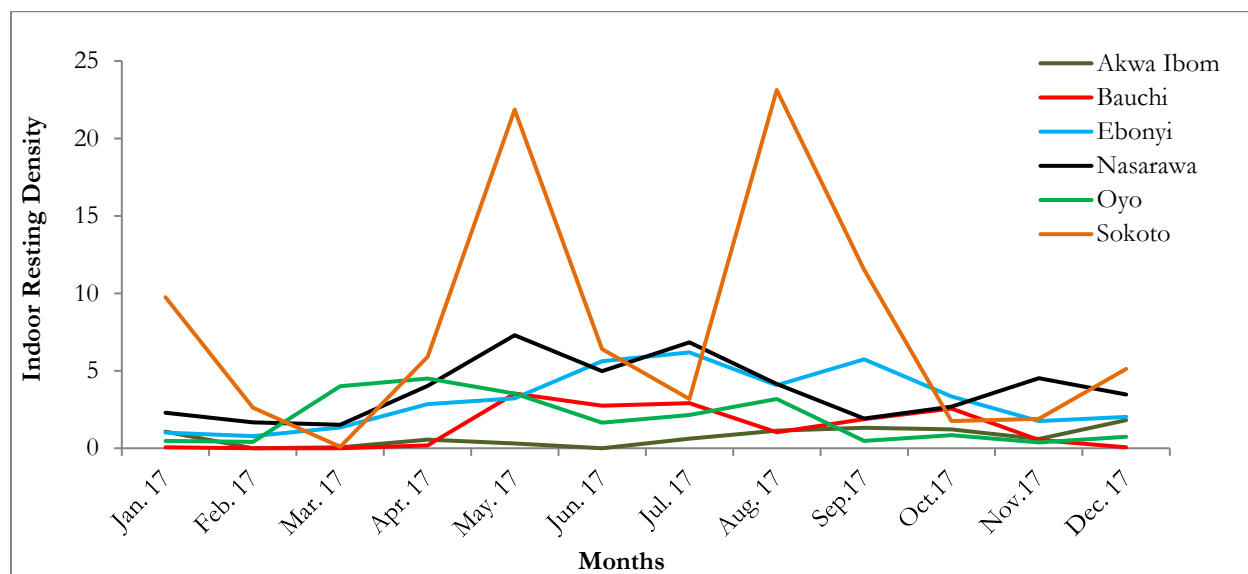
Note: IRD calculated as number of An. gambiae s.l. mosquitoes collected per house per day

4.3   HUMAN-BAITED CDC LIGHT-TRAP COLLECTION

Table 2 shows the percentage of *An. gambiae* s.l., *An. funestus, An. nili,* and *An. coustani* caught using CDC LTs indoors and outdoors at each site. Higher proportions of *An. gambiae* s.l. were collected indoors as compared to outdoors at four sites (Ebonyi, Bauchi, Nasarawa, and Akwa Ibom). At one site (Oyo), teams collected equal numbers of *An. gambiae* s.l. indoors and outdoors, while at Sokoto, the percentage collected outdoors slightly outweighed the proportion collected indoors (53.3% and 46.7%, respectively).

TABLE 2: MOSQUITO SPECIES CAUGHT USING CDC LTS, BY SENTINEL SITE

Sentinel Site	Location	*An. gambiae* s.l. n (%)	*An. funestus* n (%)	*An. nili* n (%)	*An. coustani* n (%)
Akwa Ibom	In	1020 (71)	0 (0)	0 (0)	0 (0)
	Out	426 (29)	0 (0)	0 (0)	0 (0)
Bauchi	In	810 (60)	2 (100)	4 (67)	39 (56)
	Out	531 (40)	0 (0)	2 (33)	31 (44)
Ebonyi	In	500 (78)	11 (85)	2 (67)	0 (0)
	Out	145 (22)	2 (15)	1 (33)	1 (100)
Nasarawa	In	2643 (57)	27 (53)	1 (14)	215 (37)
	Out	1993 (43)	24 (47)	86 (86)	368 (63)
Oyo	In	41 (51)	0 (0)	0 (0)	0 (0)
	Out	39 (49)	0 (0)	0 (0)	0 (0)
Sokoto	In	1560 (46)	0 (0)	0 (0)	0 (0)
	Out	1799 (54)	0 (0)	0 (0)	0 (0)

4.4   BITING TIME AND LOCATION ACROSS SITES

As shown in Figure 3, indoor biting rates were higher than outdoor biting rates in four of the six sites (Adwa Ibom, Bauchi, Ebonyi, and Nasarawa). Notable exceptions to this trend were Oyo, where indoor and outdoor biting rates remained equally low throughout, and in Sokoto, where outdoor biting rates exceeded indoor biting rates until about 2-3 a.m. Indoor biting mainly occurred between 10:00 p.m. and 6:00 a.m., with peak biting periods varying between 10-11 p.m. at Sokoto to 5-6 a.m. at Bauchi. Early evening biting was low across all sites. This result highlights the potential for significantly reducing malaria transmission in Nigeria with IRS and LLINs.

FIGURE 3: AVERAGE BITING RATES OF *AN. GAMBIAE* S.L. MOSQUITOES BY SITE, JANUARY TO DECEMBER, 2017
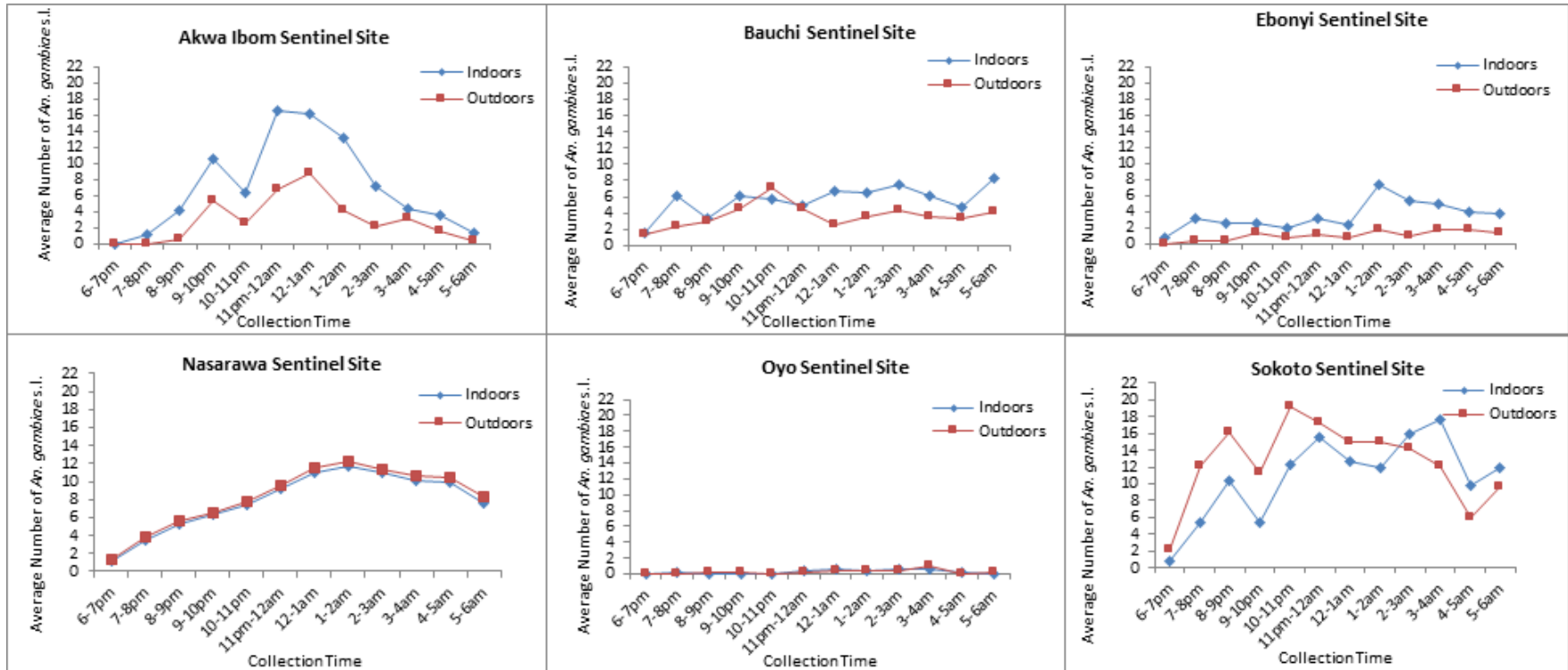
4.5 PCR IDENTIFICATION OF MEMBERS OF THE *AN. GAMBIAE* COMPLEX

PCR analysis based on Scott *et al.* 1993 indicated higher proportions of *An. gambiae* s.s. (66.7%) than *An. arabiensis* (9.53%) (p=0.0001) in all sites. *An. gambiae* s.s. and *An. arabiensis* were the members of *An. gambiae* s.l. identified by PCR in all sites.

As shown in Table 3, further PCR analyses based on Fanello *et al.* 2002 revealed that the proportion of *An. gambiae* and *An. coluzzii* varied across sites, although the two species occurred in sympatry in all sites. The proportion of *An. gambiae* ranged from 30.2 to 65.1 percent and was predominant in all sites except Ebonyi, where *An. coluzzii* was predominant. The highest proportion of *An. gambiae* from the CDC LT indoor collection occurred in Bauchi (64%; 95% CI 57.0-70.9), while the lowest was found in Ebonyi (29%; 95% CI: 21.9-36.9). On the other hand, *An. gambiae* collected via outdoor CDC LTs were found to be most abundant in Akwa Ibom (63%, 95% CI: 54.0-71.1), while the lowest proportion were found in Ebonyi (27%, 95% CI 17.1-38.1).

Similar to the indoor CDC LT results, the highest percentage of *An. gambiae* collected using PSC were found in Bauchi (69.10%, 95% CI: 60.1-77.1). The lowest percentage of *An. gambiae* was found in Ebonyi (32%, 95% CI: 22.8-39.9).

Across all three method types, Ebonyi saw the highest percentage of *An. coluzzii* collected, while the lowest percentage occurred in Sokoto.

TABLE 3: NUMBER AND PERCENTAGE OF *AN. COLUZZII, AN. GAMBIAE,* AND *AN. ARABIENSIS* ACROSS SITES

Sentinel Site	CDC LT Indoor				CDC LT Outdoor				PSC			
	Total tested	*An gambiae* n (%)	*An. coluzzii* n (%)	*An. arabiensis* n (%)	Total tested	*An gambiae* n (%)	*An. coluzzii* n (%)	*An. arabiensis* n (%)	Total tested	*An gambiae* n (%)	*An. coluzzii* n (%)	*An. arabiensis* n (%)
Akwa Ibom	165	97 (59)	17 (10)	3 (2)	132	83 (63)	7 (5)	8 (6)	110	52 (47)	18 (16)	2 (2)
Bauchi	115	74 (64)	9 (8)	4 (3)	97	59 (61)	11 (11)	15 (15)	123	85 (69)	12 (10)	2 (2)
Ebonyi	152	44 (29)	64 (42)	8 (5)	75	20 (27)	25 (33)	18 (24)	276	87 (32)	133 (48)	16 (6)
Nasarawa	203	122 (60)	17 (8)	20 (10)	202	98 (49)	22 (11)	20 (20)	218	135 (62)	18 (8)	17 (8)
Oyo	48	21 (44)	7 (15)	2 (4)	29	14 (48)	3 (10)	0	302	187 (62)	48 (16)	10 (3)
Sokoto	159	57 (36)	8 (5)	45 (28)	195	73 (37)	7 (4)	58 (30)	249	105 (42)	9 (4)	60 (24)

CDC LT = CDC Light Trap, PSC = Pyrethrum Spray Catch

4.6 *P. FALCIPARUM* SPOROZOITE ELISA RESULTS OF INDOOR AND OUTDOOR COLLECTION ACROSS SITES

ELISA analysis for *P. falciparum* sporozoite infection in *An. gambiae* s.s. indicated that the infection rate was highest in Nasarawa (9%), followed by Akwa Ibom (7%) and Oyo (7%). The lowest infection rate was in Bauchi (6%). *P. falciparum*-infected *An. arabiensis* were detected in five of the six sites. The exception was Bauchi.

As shown in Tables 4 and 5 and Figure 4, the team recorded higher *P. falciparum* sporozoite rates in *An. gambiae* than in either *An. coluzzii* or *An. arabiensis* in most sites. The exception was Ebonyi, where *An. coluzzii* had a higher sporozoite rate than *An. gambiae*. In specimens collected via indoor CDC LTs, sporozoite rates were higher for *An. arabiensis* than either *An. gambiae* or *An. coluzzii*. The highest sporozoite rate of *An. gambiae* collected using indoor CDC LTs occurred in Nasarawa (0.08, 95% CI: 0.05-0.12).

Results further indicated the presence of outdoor malaria transmission in three out the six sites, with sporozoite rates in *An. gambiae* ranging from 4 to 17 percent, rates for *An. arabiensis* ranging from 1 to 3 percent, and rates for *An. coluzzii* ranging from 0.5 to 3 percent. The highest sporozoite rate for *An. gambiae* collected using outdoor CDC LTs was from Oyo (0.17, 95% CI: 0.06-0.36). No *An. gambiae* collected outdoors in Ebonyi tested positive for *P. falciparum*.

An. coustani was also tested for *P. falciparum* and *P. malariae* sporozoite antigens (Table 5). Only those collected via indoor CDC LTs tested positive for *P. falciparum*. No positive results for *P. malariae* were received. *An. coustani* was the only species tested for *P. malariae*.

TABLE 4: NUMBER AND PERCENT OF *AN. GAMBIAE, AN. COLUZZII,* AND *AN. ARABIENSIS* COLLECTED USING HUMAN BAITED CDC-LT AND THE NUMBER THAT TESTED POSITIVE FOR *P. FALCIPARUM* ACROSS SITES

Sentinel Site	CDC LT Indoor							CDC LT Outdoor						
	Total tested	*An. gambiae* n (%)	*An. gambiae* (SPR) n (%)	*An. coluzzii* n (%)	*An. coluzzii* (SPR) n (%)	*An. arabiensis* n (%)	*An. arabiensis* (SPR) n (%)	Total tested	*An. gambiae* n (%)	*An. gambiae* (SPR) n (%)	*An. coluzzii* n (%)	*An. coluzzii* (SPR) n (%)	*An. arabiensis* n (%)	*An. arabiensis* (SPR) n (%)
Akwa Ibom	165	97 (59)	8 (5)	17 (10)	2 (1)	3 (2)	0	132	83 (63)	5 (4)	7 (5)	1 (1)	8 (6)	1 (1)
Bauchi	115	74 (64)	3 (3)	9 (8)	0	4 (3)	0	97	59 (61)	6 (6)	11 (11)	0	15 (15)	0
Ebonyi	152	44 (29)	2 (1.3)	64 (42)	3 (2)	8 (5)	15 (10)	75	20 (27)	0 (0)	25 (33)	5 (6.7)	18 (24)	2 (3)
Nasarawa	203	122 (60)	16 (8)	17 (8)	1 (0.4)	20 (10)	0 (0)	202	98 (49)	13 (6)	22 (11)	1 (0.5)	20 (20)	3 (1)
Oyo	48	21 (44)	3 (6)	7 (15)	1 (2)	2 (4)	0	29	14 (48)	5 (17)	3 (10)	1 (3)	0	1 (3)
Sokoto	159	57 (36)	4 (3)	8 (5)	3 (2)	45 (28)	1 (0.6)	195	73 (37)	7 (4)	7 (4)	0 (0)	58 (30)	3 (2)
Total	842	415 (49)	36 (4)	122 (14)	10 (1)	82 (10)	16 (2)	730	347 (48)	36 (5)	75 (10)	8 (1)	119 (16)	10 (1)

TABLE 5: NUMBER AND PERCENT OF *AN. GAMBIAE, AN. COLUZZII,* AND *AN. ARABIENSIS* COLLECTED USING PSC AND THE NUMBER THAT TESTED POSITIVE FOR *P. FALCIPARUM* ACROSS SITES

Sentinel Site	PSC						
	Total tested	*An. gambiae* n (%)	*An. gambiae* (SPR) n (%)	*An. coluzzii* n (%)	*An. coluzzii* (SPR) n (%)	*An. arabiensis* n (%)	*An. arabiensis* (SPR) n (%)
Akwa Ibom	110	52 (47)	9 (8)	18 (16)	5 (5)	2 (2)	0
Bauchi	123	85 (69)	9 (7)	12 (10)	2 (2)	2 (2)	0
Ebonyi	276	87 (32)	7 (3)	133 (48)	0	16 (6)	4 (2)
Nasarawa	218	135 (62)	20 (9)	18 (8)	3 (1)	17 (8)	1 (0.5)
Oyo	302	187 (62)	16 (5)	48 (16)	2 (1)	10 (3)	1 (0.3)
Sokoto	249	105 (42)	17 (16)	9 (4)	2 (2)	60 (24)	3 (3)
Total	1278	651 (51)	78 (6)	238 (19)	14 (1)	107 (8)	9 (1)

FIGURE 4: PERCENTAGE OF *AN. ARABIENSIS, AN. COLUZZII,* AND *AN. GAMBIAE* TESTING POSITIVE FOR *P. FALCIPARUM* SPOROZOITES ACROSS SITES
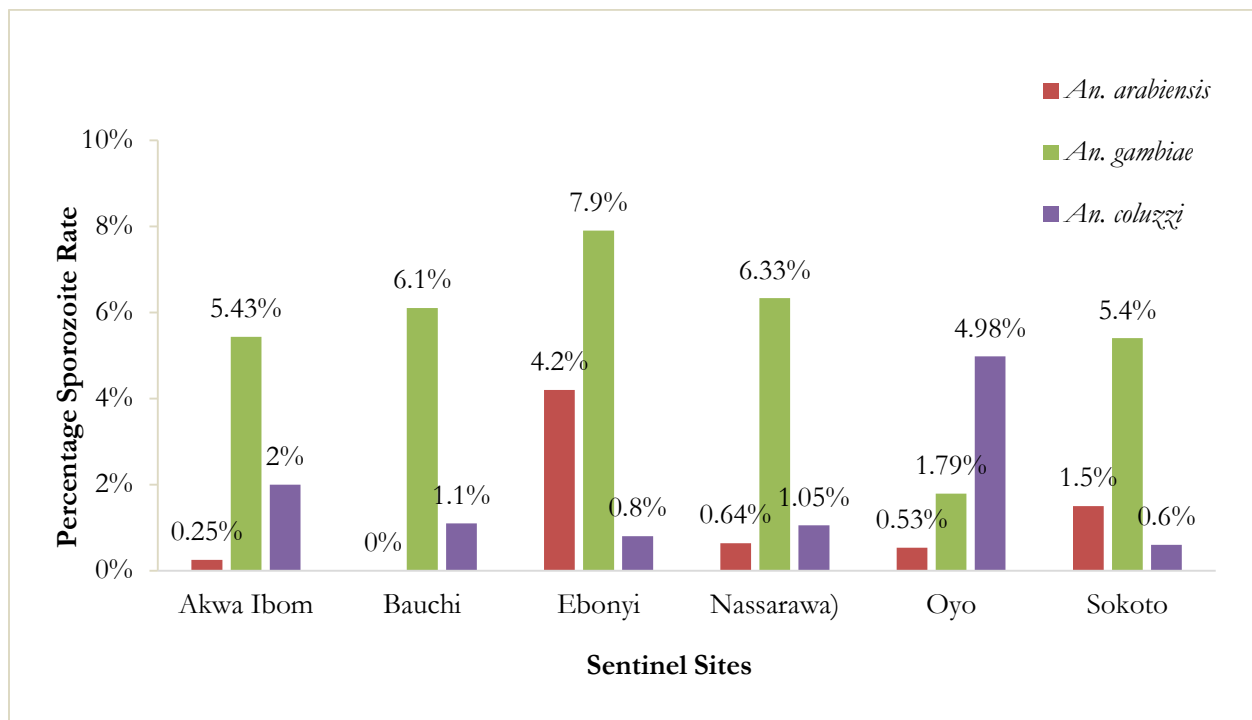
TABLE 6: SUMMARY OF NUMBER AND PERCENT OF *AN. COUSTANI* TESTING POSITIVE FOR *P. FALCIPARUM* AND *P. MALARIAE* SPOROZOITES BY COLLECTION METHOD

Collection Method	Total Collected	N (%) testing positive for *P. falciparum*	N (%) testing positive for *P. malariae*
PSC	13	0	0
CDC LT Indoors	145	2 (1.4)	0
CDC LT Outdoors	236	0	0
Total	394	2 (0.51)	0

4.7 ENTOMOLOGICAL INOCULATION RATES ACROSS SITES

As shown in Figure 5, indoor EIR values for *An. gambiae* s.l. ranged from 1.2 infective bites per person per year (Ib/p/yr) in Sokoto to 23.7 in Akwa Ibom and 23.9 Ib/p/yr in Nasarawa. In Oyo and Ebonyi, indoor EIR values for *An. coluzzii* ranged from 0.9 Ib/p/yr to 11.4 Ib/p/yr, respectively. The team recorded high indoor EIR among *An. coluzzii* only in Ebonyi (Figure 5), though this did not vary significantly from *An. gambiae* ($p=0.059$). For *An. arabiensis,* the highest indoor EIR (4.68 Ib/p/yr) occurred in Sokoto, followed by 4.55 Ib/p/yr in Nasarawa. Please refer to Annex 2 for additional information.

For outdoor EIR, *An. gambiae* remained the dominant malaria vector, with the highest EIR (19.17 Ib/p/yr) in the Sokoto and the lowest EIR (0.82 Ib/p/yr) in Ebonyi. For *An. coluzzii,* outdoor EIR ranged from 0.08 Ib/p/yr in Adwa Ibom to 0.74 ib/p/yr at Nasarawa. Outdoor EIR for *An. arabiensis* ranged from 2.93 ib/p/yr at Nasarawa to 0.16 ib/p/yr in Ebonyi and Adwa Ibom (Figure 5). Annex 3 provides more details.

FIGURE 5: ANNUAL EIRS OF *AN. GAMBIAE, AN. COLUZZII,* AND *AN. ARABIENSIS* ACROSS SITES
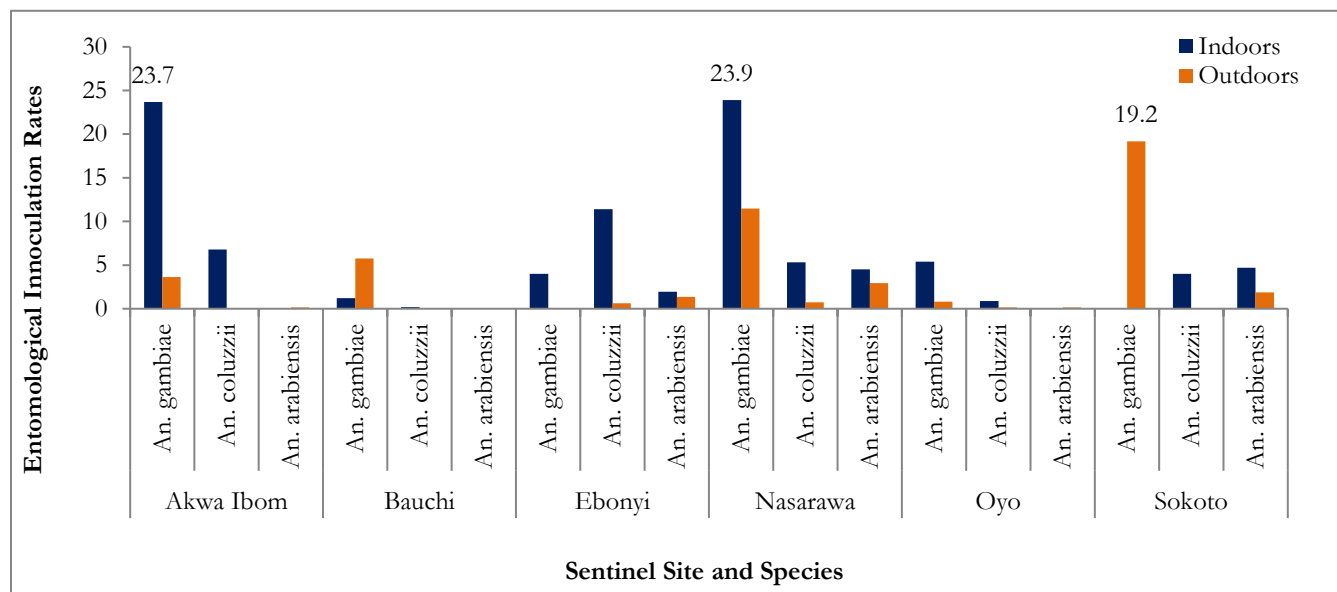
4.8　INSECTICIDE SUSCEPTIBILITY AND MECHANISMS OF RESISTANCE

Both WHO tube tests and CDC bottle bioassays were used to determine the susceptibility of vector populations at the different sites. Insecticide susceptibility test results indicated that vectors were strongly resistant to DDT (organochlorine) at all six sentinel sites. *An. gambiae* s.l. showed resistance to pyrethroids in most of the sites, with significant resistance in the rainforest/Guinea savannah (Oyo) and the Sahel (Sokoto). The vector is susceptible to carbamates and organophosphates in all ecozones, with 22 out of 24 sampling stations recording susceptibility for carbamates, and 21 out of 24 sampling stations recording susceptibility for organophosphates (Tables 7 and 8).

TABLE 7: WHO TUBE TEST METHOD RESULTS (PERCENT MORTALITY AFTER 24 HOURS) FOR *AN. GAMBIAE* S.L.

Class of Insecticides		Pyrethroid								Carbamate				Organo-phosphate		Organo-chlorine	
Insecticides		Lambda-cyhalothrin		Permethrin		Deltamethrin		Alpha-cypermethrin		Bendiocarb		Propoxur		Primiphos methyl		DDT	
Sentinel Site	Sampling Station	% Mortality	Status	% Mortality	Status	% Mortality	Status	% Mortality	Status	% Mortality	Status	% Mortality	Status	% Mortality	Status	% Mortality	Status
Akwa Ibom	Ikot Ekpene	100%	S	45%	R	42%	R	100%	S	100%	S	100%	S	100%	S	100%	S
	Itu	53%	R	42%	R	86%	R	51%	R	100%	S	100%	S	100%	S	61%	R
	Mpat Enin	96%	PR	72%	R	93%	PR	93%	PR	100%	S	100%	S	100%	S	72%	R
	Oron	84%	R	68%	R	80%	R	94%	PR	100%	S	100%	S	100%	S	76%	R
Bauchi	Bauchi	98%	S	78%	R	79%	R	92%	PR	100%	S	99%	S	99%	S	75%	R
	Dass	78%	R	93%	PR	88%	R	90%	PR	100%	S	100%	S	100%	S	85%	R
	Misau	77%	R	92%	PR	91%	PR	63%	R	100%	S	98%	S	99%	S	89%	R
	Shira	92%	PR	85%	R	89%	R	90%	PR	97%	PR	99%	S	97%	PR	78%	R
Ebonyi	Ezza North	86%	R	30%	R	95%	PR	81%	R	100%	S	100%	S	82%	R	94%	PR
	Ikwo	69%	R	63%	R	86%	R	90%	PR	100%	S	100%	S	98%	S	83%	R
	Ohaozara	87%	R	62%	R	91%	PR	23%	R	100%	S	100%	S	98%	S	80%	R
	Ohaukwu	64%	R	78%	R	81%	R	89%	R	100%	S	100%	S	100%	S	92%	PR
Nasarawa	Doma	73%	R	94%	PR	90%	PR	94%	PR	100%	S	98%	S	100%	S	56%	R
	Karu	98%	S	77%	R	89%	R	96%	PR	100%	S	98%	S	100%	S	67%	R
	Nasarawa	90%	PR	80%	R	95%	PR	96%	PR	99%	S	98%	S	100%	S	71%	R
	Nasarawa Eggon	98%	S	87%	R	92%	PR	96%	PR	100%	S	99%	S	100%	S	71%	R
Oyo	Afijio	16%	R	14%	R	53%	R	20%	R	93%	PR	98%	S	100%	S	14%	R
	Akinyele	26%	R	50%	R	60%	R	12%	R	100%	S	100%	S	100%	S	5%	R
	Egbeda	34%	R	6%	R	42%	R	11%	R	100%	S	95%	PR	100%	S	11%	R
	Oluyole	36%	R	92%	PR	36%	R	42%	R	100%	S	75%	R	100%	S	13%	R
Sokoto	Bodingo	77%	R	76%	R	76%	R	100%	S	100%	S	100%	S	73%	R	52%	R
	Rabah	69%	R	73%	R	83%	R	80%	R	87%	R	100%	S	90%	PR	0%	R
	Tambawal	79%	R	67%	R	84%	R	79%	R	100%	S	100%	S	100%	S	40%	R
	Wamakko	72%	R	100%	S	67%	R	100%	S	100%	S	100%	S	85%	R	73%	R

TABLE 8: CDC BOTTLE BIOASSAY TEST RESULTS FOR *AN. GAMBIAE* S.L.

| Class of Insecticides | | Pyrethroid | | | | | | | | Carbamate | | | | Organo-phosphate | | Organo-chlorine | |
| Insecticides | | Lambda-cyhalothrin | | Permethrin | | Deltamethrin | | Alpha-cypermethrin | | Bendiocarb | | Propoxur | | Primiphos methyl | | DDT | |
Sentinel Site	Sampling Station	% Mortality	Status	% Mortality	Status	% Mortality	Status	% Mortality	Status	% Mortality	Status	% Mortality	Status	% Mortality	Status	% Mortality	Status
Akwa Ibom	Ikot Ekpene	71%	R	68%	R	68%	R	59%	R	99%	S	99%	S	98%	S	69%	R
	Itu	87%	R	64%	R	64%	R	60%	R	99%	S	100%	S	98%	S	71%	R
	Mpat Enin	61%	R	66%	R	65%	R	58%	R	99%	S	100%	S	98%	S	71%	R
	Oron	65%	R	68%	R	62%	R	55%	R	100%	S	100%	S	98%	S	57%	R
Bauchi	Bauchi	99%	S	89%	R	97%	PR	98%	S	100%	S	100%	S	100%	S	93%	PR
	Dass	95%	PR	97%	PR	100%	S	95%	PR	100%	S	100%	S	100%	S	97%	PR
	Misau	100%	S	98%	S	99%	S	99%	S	100%	S	98%	S	100%	S	98%	S
	Shira	98%	S	93%	PR	98%	S	100%	S	95%	PR	99%	S	97%	PR	96%	PR
Ebonyi	Ezza North	82%	R	58%	R	80%	R	90%	PR	99%	S	100%	S	84%	R	53%	R
	Ikwo	80%	R	56%	R	89%	R	87%	R	100%	S	100%	S	81%	R	73%	R
	Ohaozara	88%	R	58%	R	82%	R	92%	PR	100%	S	100%	S	74%	R	62%	R
	Ohaukwu	91%	PR	54%	R	90%	PR	89%	R	100%	S	100%	S	14%	R	68%	R
Nasarawa	Doma	99%	S	69%	R	98%	S	98%	S	99%	S	100%	S	53%	R	57%	R
	Karu	100%	S	63%	R	98%	S	98%	S	99%	S	100%	S	50%	R	74%	R
	Nasarawa	99%	S	88%	R	100%	S	98%	S	100%	S	99%	S	63%	R	71%	R
	Nasarawa Eggon	98%	S	82%	R	99%	S	100%	S	100%	S	100%	S	56%	R	58%	R
Oyo	Afijio	87%	R	33%	R	100%	S	100%	S	100%	S	100%	S	97%	PR	33%	R
	Akinyele	84%	R	47%	R	100%	S	50%	R	100%	S	100%	S	100%	S	62%	R
	Egbeda	92%	PR	15%	R	100%	S	66%	R	100%	S	100%	S	36%	R	55%	R
	Oluyole	100%	S	46%	R	96%	PR	77%	R	100%	S	100%	S	100%	S	63%	R
Sokoto	Bodingo	42%	R	71%	R	80%	R	100%	S	99%	S	100%	S	61%	R	47%	R
	Rabah	73%	R	62%	R	82%	R	86%	R	90%	PR	100%	S	70%	R	48%	R
	Tambawal	74%	R	79%	R	78%	R	100%	S	100%	S	100%	S	100%	S	45%	R
	Wamakko	56%	R	99%	S	79%	R	100%	S	100%	S	100%	S	83%	R	66%	R

4.9   INSECTICIDE RESISTANCE INTENSITY

Intensity of resistance to deltamethrin by *An. gambiae* s.l. remained generally low (1-times the diagnostic dosage) in four out of six sentinel sites (Akwa Ibom, Bauchi, Nasarawa, and Sokoto) (Figures, 6, 7, 9 and 11). The team recorded permethrin resistance at 5- and 10-times the diagnostic doses at three out of four sampling stations in Oyo (Figure 10) and for deltamethrin at all four sampling stations in Ebonyi (Figure 8). The teams observed intensity of resistance to permethrin at 2x and 5x in Akwa Ibom and Nasarawa (Figure 6 and 9).

**FIGURE 6: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT AKWA IBOM**
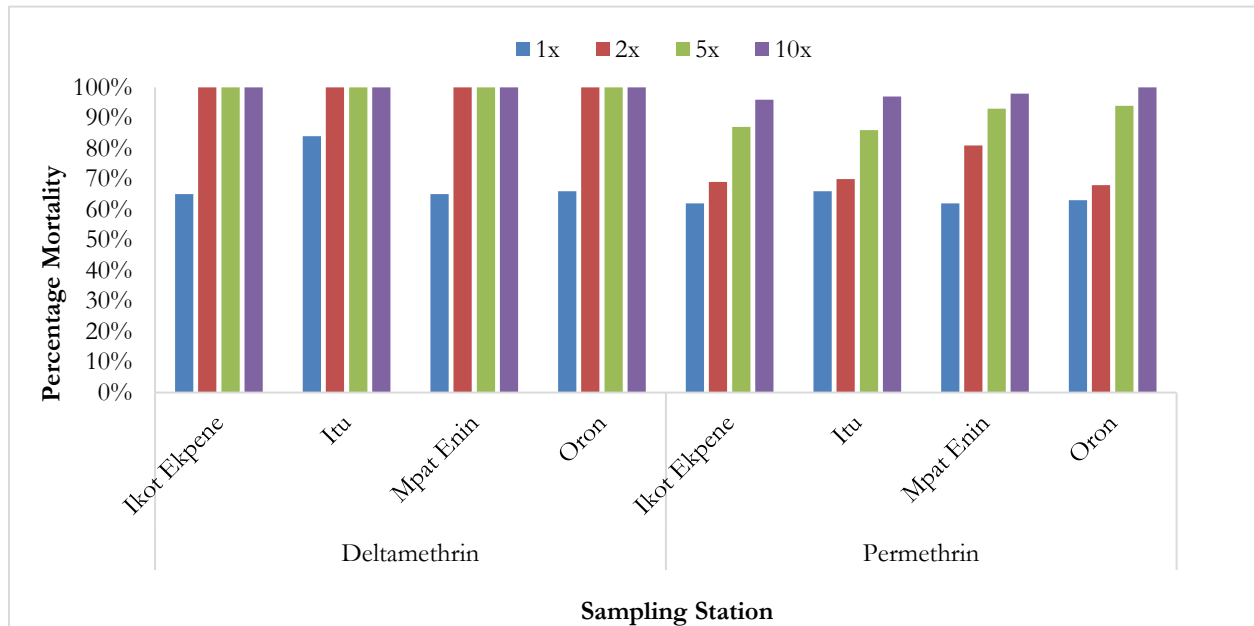
FIGURE 7: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT BAUCHI
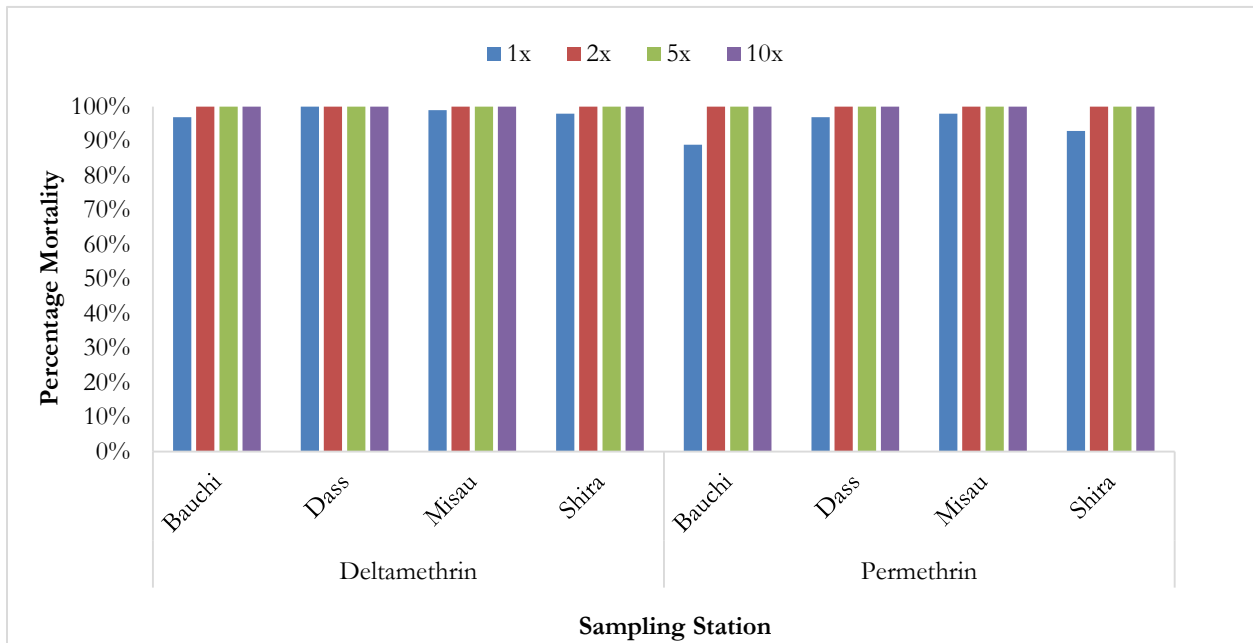
FIGURE 8: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT EBONYI
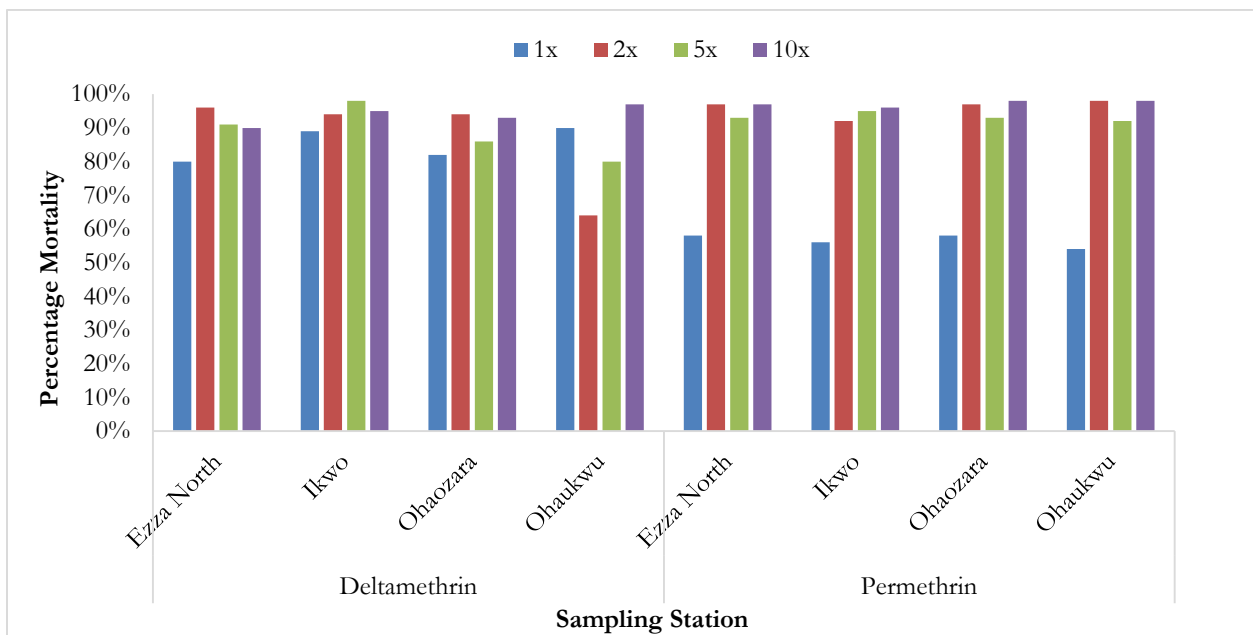
FIGURE 9: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT NASARAWA
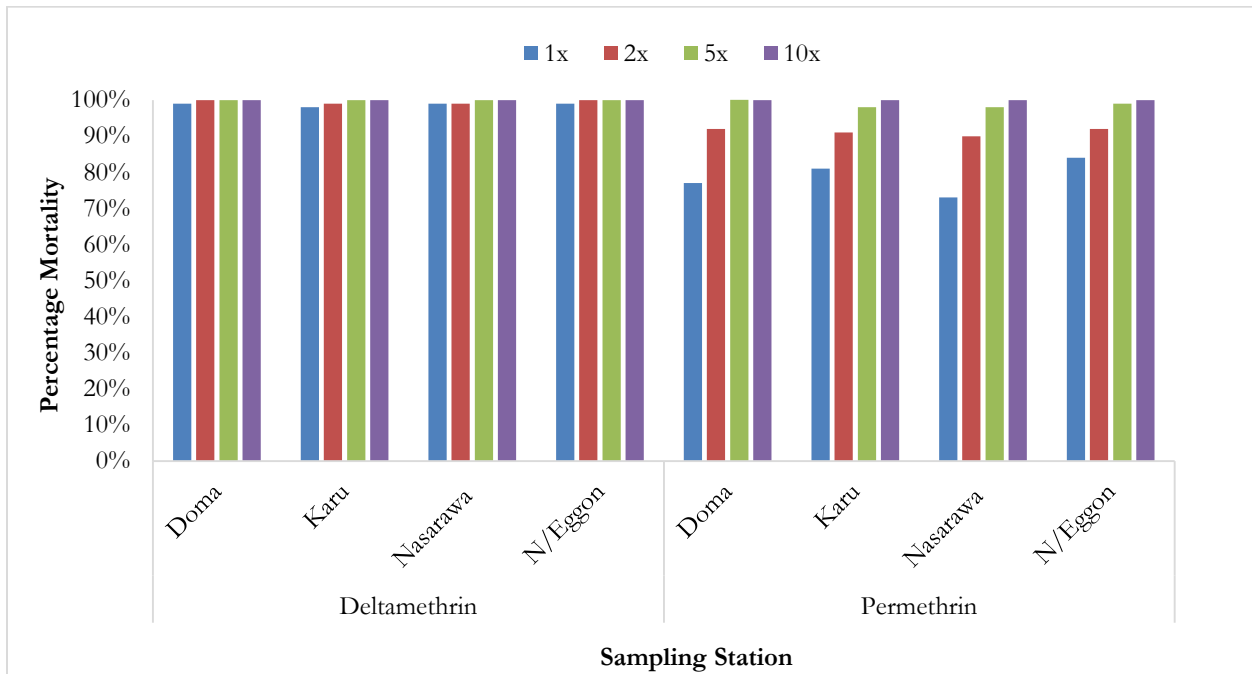
FIGURE 10: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT OYO
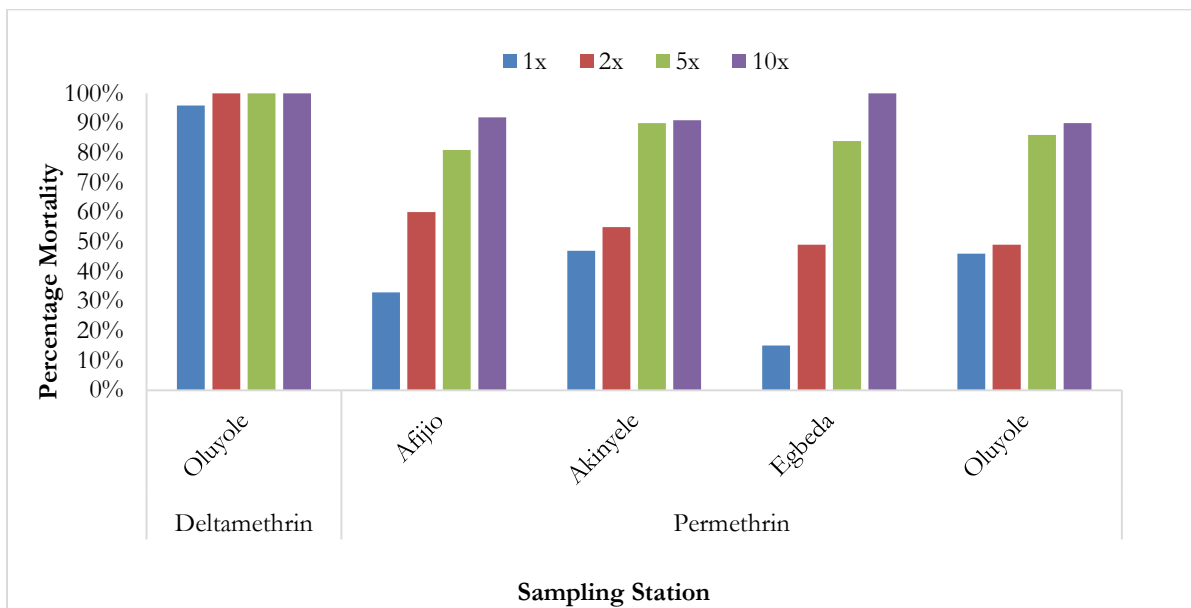
FIGURE 11: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT SOKOTO
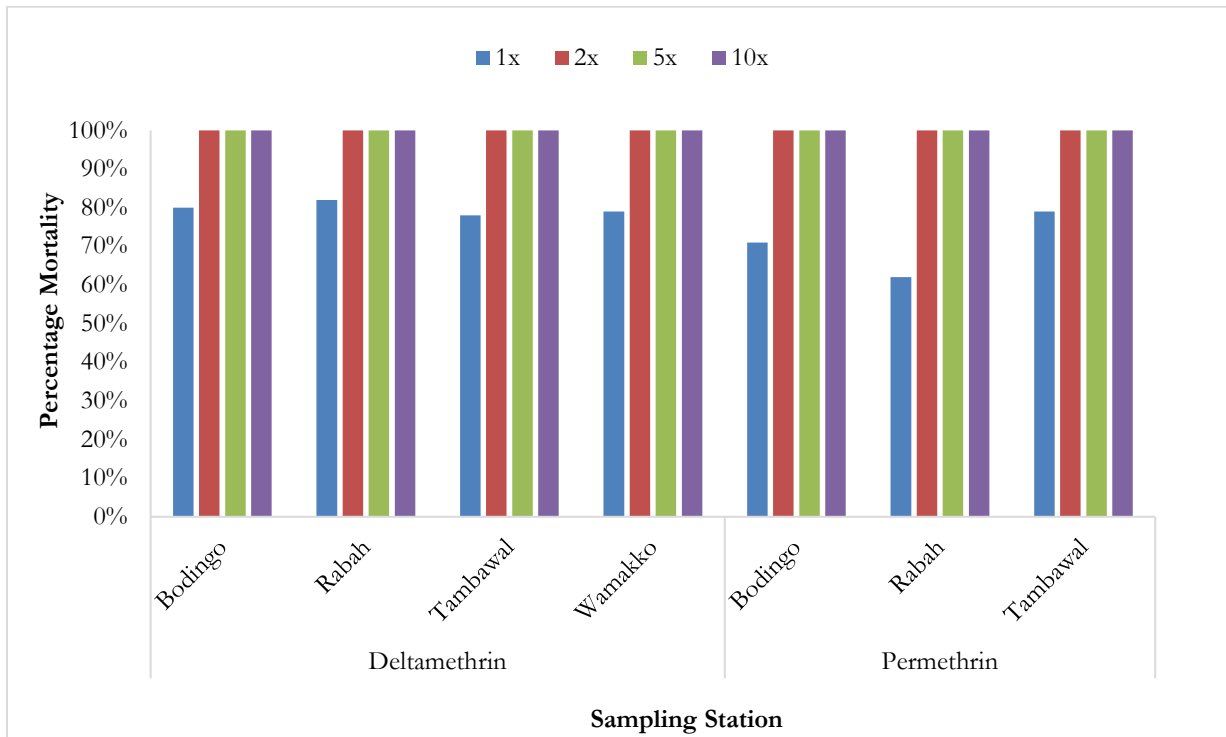
4.10 SYNERGIST ASSAYS

In five out of six sentinel sites, PBO fully restored pyrethroid susceptibility in synergist assays, indicating that elevated oxidases are the basis of resistance (Figures 12, 13, 14, 16, and 17). In Oyo, PBO did not fully restore pyrethroid susceptibility, suggesting other contributors to resistance (Figure 15). The teams did not carry out synergist assays using deltamethrin in three out of four sampling stations in Oyo because local mosquitoes showed susceptibility to deltamethrin in those stations using CDC bottle bioassays. However, synergist assays in the sampling station Oluyole indicated that PBO fully restored pyrethroid susceptibility when exposed with deltamethrin (Figure 16).

FIGURE 12: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM AKWA IBOM
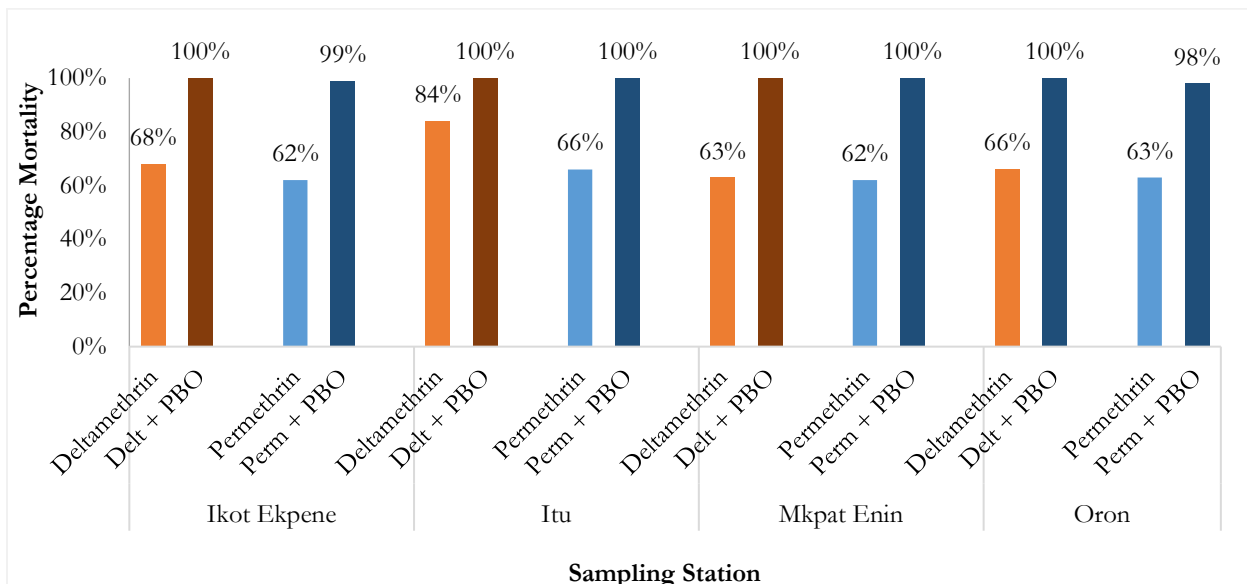
FIGURE 13: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. AT EBONYI
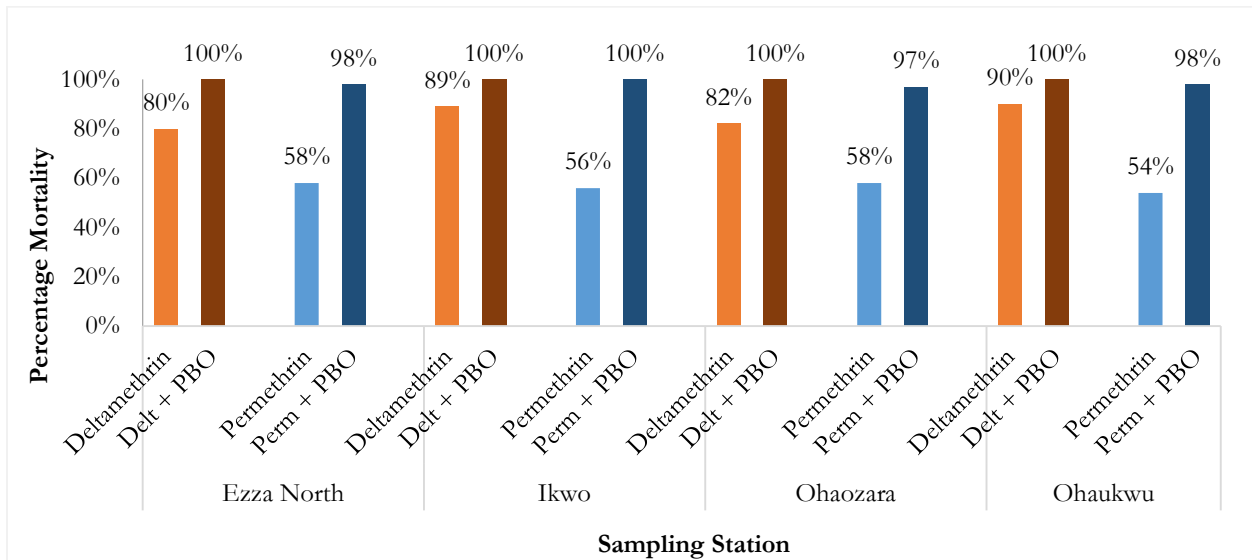
FIGURE 14: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. AT NASARAWA
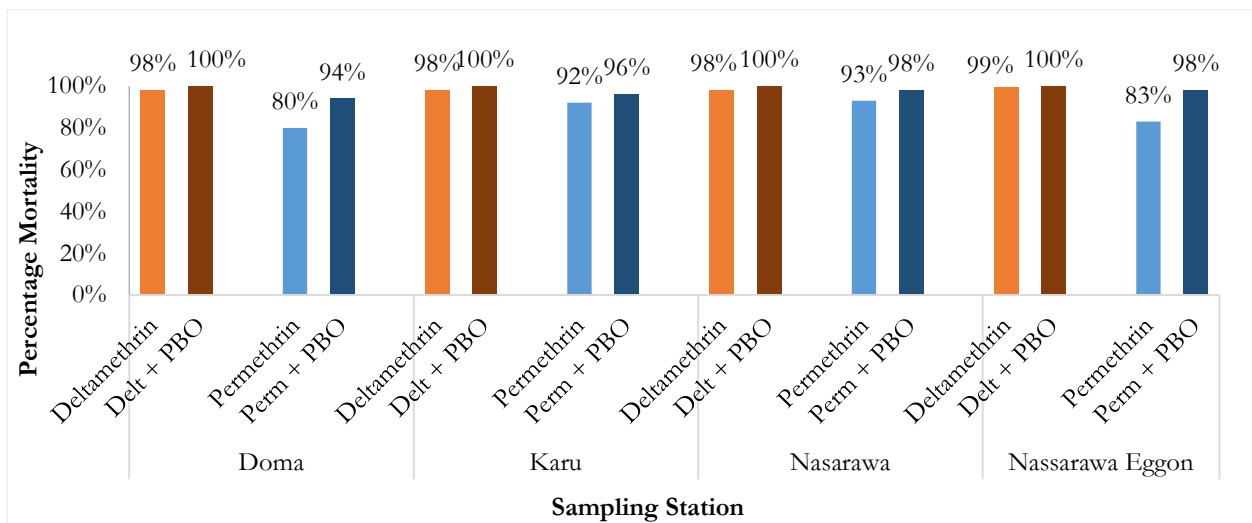
FIGURE 15: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. AT OYO
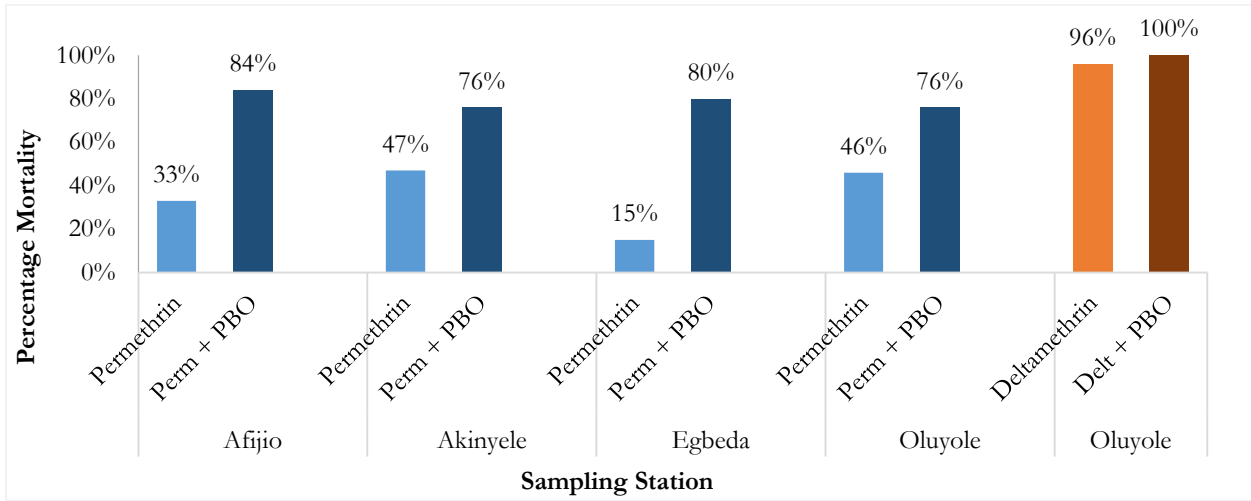
FIGURE 16: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. AT SOKOTO
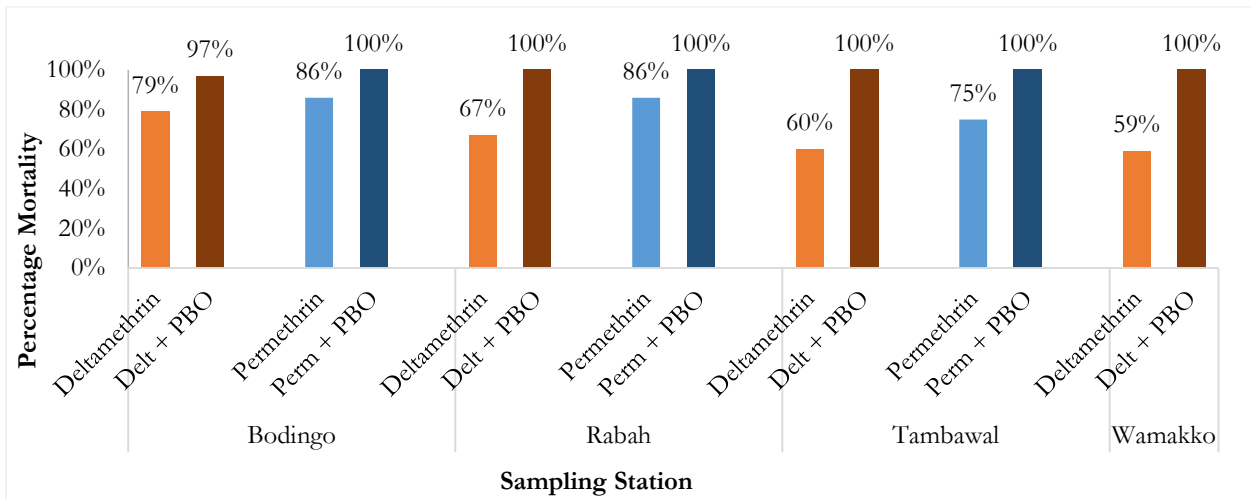
4.11 *KDR* GENE FREQUENCY IN *AN. GAMBIAE* S.L. EXPOSED TO DELTAMETHRIN AND DDT ACROSS SITES

Table 9 shows that, in all sites, frequency of the West *kdr* gene mutation (*kdr-w*) in both deltamethrin- and DDT-exposed *An. gambiae* s.l. mosquitoes was generally low, ranging from 0.13 in Nasarawa to 0.29 in Sokoto for deltamethrin-exposed *An. gambiae* s.l., and from 0.04 in the Bauchi to 0.23 in Sokoto in DDT-exposed *An. gambiae* s.l. The difference in gene frequency between the insecticides was not statistically significant in five out of six sites. PCR tests using primers designed for the East African *kdr* mutation (*kdr-e*) did not result in a positive case at any site.

TABLE 9: *KDR* GENE FREQUENCY ACROSS SITES

Sentinel Site	Ecozone(s) represented	*Kdr - w*			*Kdr - e*	
		Deltamethrin	DDT	P-Value	Deltamethrin	DDT
Akwa Ibom	Mangrove swamps, rainforest	0.21	0.16	0.35	0	0
Bauchi	Sudan Savannah	0.22	0.04	0.0001	0	0
Ebonyi	Rainforest	0.24	0.16	0.12	0	0
Nasarawa	Guinea Savannah	0.13	0.18	0.31	0	0
Oyo	Rainforest/Guinea Savannah	0.20	0.13	0.14	0	0
Sokoto	Sahel Savannah	0.29	0.23	0.33	0	0

5. DISCUSSION AND CONCLUSION

5.1 SPECIES COMPOSITION

PCR analysis based on Scott *et al.* 1993, indicated higher numbers of *An. gambiae* s.s. than *An. arabiensis* in all sites. *An. gambiae* s.s. and *An. arabiensis* were the members of the *An. gambiae* complex identified by PCR in all sites. Both species occur in sympatry, which confirms earlier observations by Gillies and Coetzee (1987); Coetzee *et al.*, (2000); Onyabe *at al.,* (2003) and Awolola *et al.* (2005).

ELISA analyses for sporozoite infection indicated that the infection rate was highest in the Guinea savannah of Nasarawa, followed by the mangrove Akwa Ibom. The teams recorded significantly lower sporozoite rates in *An. arabiensis*.

Further PCR analysis indicated that *An. coluzzii* and *An. gambiae* s.s. occurred in sympatry in all sites, with *An. gambiae* being predominant in five out the six sentinel sites. Higher numbers of *An. coluzzii* than *An. gambiae* were collected *in* Ebonyi—the same was true in 2016 (AIRS Report 2016).

5.2 BEHAVIOR/TRANSMISSION/EIR

Across sites, indoor biting occurred most frequently between 10 p.m. and 6 a.m. while peak biting hours of *An. gambiae* s.l. varied from site to site. Continued deployment of IRS and LLINs may lower malaria transmission in Nigeria. ELISA analysis of CDC LT outdoor samples for sporozoites also indicated that outdoor malaria transmission occurs in five out of the six sentinel sites (Ebonyi was the exception).

Although the teams observed lower malaria transmission outdoors across sites, there is a need for continued monitoring of behavior, species composition, and seasonality to see if changes in behavior (to outdoor and early feeding) occur. Such changes can compromise the efficacy of LLINs, which provide little protection against vectors that bite outdoors or in the early evening before people go to bed (Service 2012; PMI 2018). Routine baseline monitoring may also be relevant before mass LLINs campaigns. Following the trends after campaigns also is critical.

5.3 ENTOMOLOGICAL INOCULATION RATE

In all sites except Ebonyi, sporozoite rates and EIR values were higher for *An. gambiae* than *An. coluzzii* and *An. arabiensis*. EIR values for *An. gambiae* indoors ranged from 1.2 infective bites per person per year in Sokoto to a peak of 23.9 infective bites per person per year in Nasarawa. For *An. coluzzii*, EIR values ranged from 0.9 infective bites per person per year in Oyo to a peak of 11.4 infective bites per person year in Ebonyi. Overall, sites recorded lower outdoor EIRs than indoor EIRs. The most notable combination of outdoor EIR values for all three malaria vectors—*An. gambiae* (11.46 Ib/p/yr), *An. coluzzii* (0.74 Ib/p/yr), and *An. arabiensis* (2.93 Ib/p/yr)—occurred in Nasarawa. This state has the second highest malaria prevalence (32 percent) in Nigeria (NMEP 2015).

5.4 INSECTICIDE SUSCEPTIBILITY

The Global Plan for Insecticide Resistance Management (GPIRM), WHO Global Technical Strategy for Malaria 2016-2030, and Global Vector Control Response 2017-2030 all highlight insecticide resistance as a major obstacle to achieving malaria-control targets (WHO 2015; WHO 2017). Countries are therefore encouraged to implement pre-emptive IRM strategies against malaria vectors. According to the GPIRM, monitoring and management of resistant disease vectors is essential to limit the selection and spread of insecticide resistance and to maintain the effectiveness of vector control (Chanda 2016).

AIRS Nigeria recorded susceptibility to carbamates and organophosphates (Primiphos-methyl) in most sites. It is noteworthy that *An. gambiae* s.l. mosquitoes showed susceptibility to carbamates in 22 out of 24 sampling stations, and 21 out of 24 sampling stations recorded susceptibility to organophosphates. *An. gambiae* s.l. showed resistance to pyrethroids in most of the sites, with significant resistance to permethrin in the Rainforest/Guinea savannah (Oyo) and the rainforest (Ebonyi) in both permethrin and deltamethrin. The intensity of resistance to permethrin and deltamethrin varied within sites. A recent study in four ecozones of Nigeria indicated the magnitude of insecticide resistance in malaria mosquitoes (PMI AIRS Report 2016).

Between 2002 and 2017, pyrethroid resistance spread to 20 out of 36 states in Nigeria, including Abuja. Researchers have identified other causal factors of resistance, such as agricultural use of insecticides. But the significant increase in insecticide-based malaria vector control in the last 10 years has likely exerted significant insecticide selection pressure in the country (Awolola 2017). Further insecticide susceptibility results indicated strong vector resistance to DDT (organochlorine) in all six sentinel sites.

5.5   RESISTANCE INTENSITY AND MECHANISMS

To predict the operational impact of resistance more accurately, PMI and the WHO highly recommend implementation of resistance intensity bioassays (PMI 2018; WHO 2012; WHO 2016). They generate more operationally meaningful data for monitoring insecticide resistance in malaria vectors. The data will enable alignment with new IRM developments in the five key areas of the GPIRM (WHO 2012; WHO 2016). Confirmed levels of resistance at 5x and especially at 10x highlight a particularly urgent need to develop and implement an appropriate resistance management strategy (WHO GMP 2017a; WHO GMP 2017b).

Results from this investigation indicated that intensity of resistance to deltamethrin remained generally low (1x) in four out of six sentinel sites. However, there is an urgent need to focus on the three locations in Oyo where there was intense resistance to permethrin, and the four sampling stations in Ebonyi that recorded resistance to both permethrin and deltamethrin at five- and ten-times the diagnostic dosages. Additional sample sites in the vicinity of the original collection sites could help ascertain the size of the focus of resistance and further investigate whether resistance intensity correlates with control failure of pyrethroid LLINs at these sites. The data will inform PMI's efforts to focus additional interventions on those areas to mitigate resistance by deploying IRS with organophosphates or PBO LLINs (Brogdon 2015). Resistance studies in Guatemala showed a variation not only by presence or absence of resistance but also by the level of resistance and the mix of mechanisms responsible for resistance (Brogdon, 1988).

5.6   METABOLIC RESISTANCE MECHANISMS

Since metabolic resistance can have a strong impact on malaria vector-control efforts, PMI emphasizes monitoring mosquito phenotypes for physiological resistance (PMI 2018). In Oyo, PBO did not increase mortality to the range of 98-100% for permethrin, suggesting oxidases may not be the only mechanisms of resistance at work. This highlights an urgent need for further investigation in line with the PMI Guidelines (PMI 2015, 2018) and the WHO framework for a national plan for monitoring and management of insecticide resistance in malaria vectors (WHO 2017).

Although highly relevant to malaria control, not much is known about the relationship between insecticide resistance in *Anopheles* mosquitoes and the infection level of the *Plasmodium* species in Nigeria. As highlighted in earlier studies (Cohuet *et al.,* 2009), there is a need to measure resistance in the population of mosquitoes actively becoming infected with sporozoites or transmitting malaria (Brogdon 2015) at this particular site and the rainforest. This data could also assist in the justification for ITN and IRS overlap in certain areas with high pyrethroid resistance plus high transmission, areas with high transmission despite high net coverage, and areas where the use of IRS with a non pyrethroid could serve as a resistance management tool to preserve the effectiveness of pyrethroids on ITNS (PMI 2018).

5.7 *KDR* GENE IN THE FIVE ECOZONES

So far there is no convincing evidence that *kdr* alone produces operationally significant levels of pyrethroid resistance (Hemingway 2014). In its heterozygote state, *kdr* has a low association with failure of malaria vector-control measures (PMI, 2018). Results of *kdr* assays conducted by AIRS Nigeria indicate that the *kdr* gene is present at a lower frequency. Figures recorded by the team in this study were lower than those of Awolola *et al.* (2005; 2007). Awolola *et al.,* (2007) observed that the *kdr* frequencies in *An. coluzzii* and *An. gambiae* have not increased significantly since 2002 when *kdr* was first reported in Nigeria (Awolola et al., 2007).

Other works within the sub region indicate that generally the *kdr* gene is present at a lower frequency in Nigeria compared with other West African countries (Fanello *et al.,* 2003).

6. CHALLENGES

The team experienced delays in the completion of resistance work at remote sites. The rains do not commence early in the Sahel region (Sokoto) which can make it difficult to get enough mosquitoes for resistance tests. Moreover, substantial flooding during the rainy season in sites in the rainforest and mangrove swamp regions (Ebonyi, Oyo, and Akwa Ibom) poses a challenge in obtaining enough larvae to complete the resistance tests on time. One proposed solution for 2018 is for field teams to commence resistance tests as soon as the rainy season begins to allow the maximum amount of time to collect the mosquitoes and larvae needed across all sites.

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7. RECOMMENDATIONS

1. Resistance intensity assay results from Ebonyi and Oyo—high intensity resistance to permethrin and deltamethrin in Ebonyi, and to permethrin at five- and 10-times the diagnostic doses in Oyo—indicates a need for increased frequency of testing and an expansion in geographic range at both sites.

2. There is urgent need to implement the insecticide resistance management strategy developed for Nigeria.

3. Data suggest that the current trend of insecticide resistance may reduce the efficacy of LLINs. Strategic deployment of next-generation nets such as combination nets or PBO nets with proven efficacy to curb resistance in key problem areas such as the Ebonyi and Oyo sites should be considered.

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ANNEXES

ANNEX 1: NUMBER OF *ANOPHELES* CAUGHT, BY SPECIES, METHOD, AND SITE, FEBRUARY – DECEMBER, 2017

Mosquito Species	Akwa Ibom			Bauchi			Ebonyi			Nasarawa			Oyo			Sokoto			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.	1020	426	278	810	531	496	500	145	1214	2643	1993	2905	41	39	715	1560	1799	2984	6574	4933	8592	20099
An. funestus	0	0	0	2	0	0	11	2	32	27	24	0	0	0	0	0	0	0	40	26	32	98
An. coustani	0	0	0	39	31	4	0	1	0	215	368	0	0	0	0	0	0	0	254	400	4	658
An. nili	0	0	0	4	2	1	2	1	0	1	6	0	0	0	0	0	0	0	7	9	1	17
An. pharoensis	0	0	0	3	4	0	0	0	0	40	52	2	0	0	0	30	16	1	73	72	3	148
An. malculipalpis	0	0	0	2	2	0	0	2	0	12	10	0	0	0	0	0	0	0	14	14	0	28
An. moucheti	205	102	90	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	205	102	92	399
An. pretoriensis	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	2
An. squamosus	0	0	0	4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	4	1	1	6
An. longipalpis	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	1	3	4
Grand Total	1225	528	368	865	571	502	513	151	1248	2938	2453	2907	41	41	718	1590	1815	2985	7172	5559	8728	21459

In=Indoor CDC Light Trap, Out=Outdoor CDC Light Trap, PSC=Pyrethrum Spray Catch

ANNEX 2: INDOOR EIRS OF *An. gambiae*, *An. coluzzii*, AND *An. arabiensis* ACROSS SITES

Month	Akwa Ibom (Mangrove swamps, rainforest) EIR — An. gambiae	An. coluzzii	An. arabiensis	Bauchi (Sudan Savannah) EIR — An. gambiae	An. coluzzii	An. arabiensis	Ebonyi (Rainforest) EIR — An. gambiae	An. coluzzii	An. arabiensis	Oyo (Rainforest/ Guinea Savannah) EIR — An. gambiae	An. coluzzii	An. arabiensis	Sokoto (Sahel Savannah) EIR — An. gambiae	An. coluzzii	An. arabiensis	Nasarawa Doma (Guinea Savannah) EIR — An. gambiae	An. coluzzii	An. arabiensis	Nasarawa Eggon (Guinea Savannah) EIR — An. gambiae	An. coluzzii	An. arabiensis
Jan-17	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.5	0.00	0.4	0.4	0.00	3.5	3.5	0.00	0.9	0.9	0.00	1.8	1.8	0.00
Feb-17	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.6	0.00	1.9	0.5	0.00	3.0	0.0	0.00	1.0	0.0	0.00	0.5	0.0	0.00
Mar-17	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.5	0.06	0.0	0.0	0.00	0.1	0.0	0.03	0.0	0.0	0.00	0.0	0.0	0.00
Apr-17	0.0	0.0	0.00	0.0	0.0	0.00	0.0	1.0	0.00	0.0	0.0	0.05	0.5	0.5	0.00	0.7	0.0	0.00	2.1	0.0	0.00
May-17	0.0	0.0	0.00	0.3	0.0	0.00	0.9	0.5	0.00	0.0	0.0	0.00	3.1	0.0	0.00	2.5	2.5	0.00	2.2	2.2	0.00
Jun-17	0.0	0.0	0.00	0.2	0.0	0.00	0.0	2.8	0.00	0.0	0.0	0.00	0.8	0.0	0.00	5.5	0.0	0.00	3.5	0.0	0.00
Jul-17	0.0	0.0	0.00	0.6	0.0	0.00	1.5	1.5	0.00	1.2	0.0	0.00	2.2	0.0	4.40	1.5	0.0	0.00	1.9	0.0	0.00
Aug-17	2.7	2.7	0.00	0.1	0.1	0.00	0.8	0.8	1.53	0.9	0.0	0.00	1.8	0.0	0.00	2.4	1.2	0.00	2.6	1.3	0.00
Sep-17	21.0	0.0	0.00	0.0	0.0	0.00	0.0	1.2	0.00	0.0	0.0	0.00	0.8	0.0	0.00	1.4	0.0	0.00	1.6	0.0	0.00
Oct-17	0.0	3.3	0.00	0.0	0.2	0.00	0.0	0.8	0.34	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.0	4.55
Nov-17	0.0	0.0	0.00	0.0	0.0	0.00	0.4	0.4	0.03	0.6	0.0	0.00	0.3	0.0	0.26	0.6	0.0	0.00	2.0	0.0	0.00
Dec-17	0.0	0.9	0.00	0.0	0.0	0.00	0.4	0.8	0.00	0.4	0.0	0.00	1.2	0.0	0.00	3.3	0.0	0.00	5.8	0.0	0.00
Annual EIR	**23.7**	**6.8**	**0.00**	**1.2**	**0.2**	**0.00**	**4.0**	**11.4**	**1.96**	**5.4**	**0.9**	**0.05**	**17.2**	**4.0**	**4.68**	**19.8**	**4.6**	**0.00**	**23.9**	**5.3**	**4.55**

ANNEX 3: OUTDOOR EIRS OF *AN. GAMBIAE, AN. COLUZZII,* AND *AN. ARABIENSIS* ACROSS SITES

Sentinel Site	Month	HBR Outdoor	*An. gambiae*		*An. coluzzii*		*An. arabiensis*	
			SPR	EIR	SPR	EIR	SPR	EIR
Akwa Ibom	Jan-17	0.5	0	0	0	0	0	0
	Feb-17	1.3	0.06	0.08	0.06	0.08	0	0
	Mar-17	0.2	0	0	0	0	0	0
	Apr-17	0	0	0	0	0	0	0
	May-17	0.3	0	0	0	0	0	0
	Jun-17	1.8	0	0	0	0	0	0
	Jul-17	7.2	0	0	0	0	0	0
	Aug-17	4.8	0.1	0.48	0	0	0	0
	Sep-17	13.7	0	0	0	0	0	0
	Oct-17	2.3	0	0	0	0	0.07	0.16
	Nov-17	21.8	0.14	3.06	0	0	0	0
	Dec-17	17	0	0	0	0	0	0
	Annual EIR			**3.62**		**0.08**		**0.16**
Bauchi	Jan-17	0.2	0	0	0	0	0	0
	Feb-17	0	0	0	0	0	0	0
	Mar-17	0.2	0	0	0	0	0	0
	Apr-17	0	0	0	0	0	0	0
	May-17	4.2	0	0	0	0	0	0
	Jun-17	12.5	0.14	1.75	0	0	0	0
	Jul-17	36.2	0.05	1.81	0	0	0	0
	Aug-17	8.3	0.05	0.42	0	0	0	0
	Sep-17	7.3	0.05	0.37	0	0	0	0
	Oct-17	16.7	0.07	1.17	0	0	0	0
	Nov-17	2.3	0.11	0.26	0	0	0	0
	Dec-17	0.7	0	0	0	0	0	0
	Annual EIR			**5.77**		**0**		**0**
Ebonyi	Jan-17	0.3	0	0	0	0	0	0
	Feb-17	0.7	0	0	0.05	0.03	0	0
	Mar-17	0.8	0	0	0	0	0	0
	Apr-17	2.2	0	0	0.25	0.54	0	0
	May-17	2.2	0	0	0	0	0	0
	Jun-17	4.8	0	0	0	0	0	0
	Jul-17	3.7	0	0	0	0	0.33	1.21
	Aug-17	6.7	0	0	0	0	0	0
	Sep-17	2	0	0	0	0	0.08	0.16
	Oct-17	0.3	0	0	0.2	0.07	0	0
	Nov-17	0.2	0	0	0	0	0	0
	Dec-17	0.3	0	0	0	0	0	0
	Annual EIR			**0**		**0.64**		**1.37**

Sentinel Site	Month	HBR Outdoor	An. gambiae		An. coluzzii		An. arabiensis	
			SPR	EIR	SPR	EIR	SPR	EIR
Nasarawa	Jan-17	17.4	0.08	1.39	0	0	0	0
	Feb-17	10.6	0.15	1.59	0	0	0	0
	Mar-17	3.7	0.08	0.29	0	0	0	0
	Apr-17	23.3	0.15	3.49	0	0	0	0
	May-17	14.8	0	0	0.05	0.74	0	0
	Jun-17	19.1	0	0	0	0	0	0
	Jul-17	19.9	0.15	2.99	0	0	0	0
	Aug-17	14.1	0	0	0	0	0	0
	Sep-17	8.6	0.05	0.43	0	0	0	0
	Oct-17	9.8	0.13	1.28	0	0	0	0
	Nov-17	17	0	0	0	0	0.14	2.38
	Dec-17	7.8	0	0	0	0	0.07	0.55
	Annual EIR			**11.46**		**0.74**		**2.93**
Oyo	Jan-17	0	0	0	0	0	0	0
	Feb-17	0.5	0	0	0	0	0	0
	Mar-17	1	0	0	0	0	0	0
	Apr-17	3.2	0.26	0.82	0.05	0.16	0.05	0.16
	May-17	1	0	0	0	0	0	0
	Jun-17	0	0	0	0	0	0	0
	Jul-17	0.3	0	0	0	0	0	0
	Aug-17	0	0	0	0	0	0	0
	Sep-17	0.5	0	0	0	0	0	0
	Oct-17	0	0	0	0	0	0	0
	Nov-17	0	0	0	0	0	0	0
	Dec-17	0	0	0	0	0	0	0
	Annual EIR			**0.82**		**0.16**		**0.16**
Sokoto	Jan-17	10	0	0	0	0	0.08	0.8
	Feb-17	3	0	0	0	0	0.08	0.24
	Mar-17	0.8	0	0	0	0	0	0
	Apr-17	7.8	0.05	0.39	0	0	0	0
	May-17	134.5	0.1	13.45	0	0	0	0
	Jun-17	9.2	0.08	0.73	0	0	0	0
	Jul-17	59.7	0.05	2.98	0	0	0	0
	Aug-17	24.2	0.05	1.21	0	0	0	0
	Sep-17	11.8	0	0	0	0	0	0
	Oct-17	16.7	0	0	0	0	0	0
	Nov-17	12	0	0	0	0	0.07	0.84
	Dec-17	10.2	0.04	0.41	0	0	0	0
	Annual EIR			**19.17**		**0**		**1.88**