

Insecticides Susceptibility of Malaria Vectors in Okitipupa, Ondo State, Nigeria Using WHO Susceptibility Test

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Abstract: Pyrethroid insecticides are recommended for use in long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) due to their low mammalian toxicity and fast action. Currently pyrethroid resistance has been reported in western and eastern Africa, therefore monitoring resistance is important in all malaria endemic countries. The overall goal of this study is to monitor resistance levels in malaria vectors in Okitipupa. Susceptibility of malaria vectors to insecticides was determined in Ayeka, Idepe, Igbo-digo and Okitipupa districts of Okitipupa town using WHO test. Three sentinel sites from each district were selected and mosquitoes were collected from each sentinel site. The collected *Anopheles* mosquitoes were reared to adults in the insectary of the Department of Biological Sciences of Olusegun Agagu University of Science and Technology, Okitipupa. Two to five days old *An. gambiae* mosquitoes were assessed for resistance levels to Pirimiphos-methyl (0.25%), Lambda-cyhalothrin (0.05%), Bendiocarb (0.1%), Permethrin (0.75%) and Propoxur (0.1%). Knockdown time (KDT) was recorded up to 60 minutes and maintained for 24hrs post-exposure on 10 % glucose solution, after which mortality was recorded. The susceptibility test showed that mosquito mortality after 24 hrs for Pirimiphos-methyl was 25%, Lambda-cyhalothrin was 0%, Propoxur was 75%, Bendiocarb was 100% and Permethrin was 50%. This study revealed development of resistance to Pirimiphos-methyl, Permethrin and Lambda-cyhalothrin in *An. gambiae* s. l. across all sentinel sites. The implication of this was discussed and suggestion for monitoring was made before this problem becomes widespread in Okitipupa town.

Keywords: Pyrethroids, Insecticides, Susceptibility, Propoxur, Bendiocarb, Permethrin

1. Introduction

Global efforts in controlling malaria have produced remarkable results over the past decade. In 2010, for example, there were an estimated 219 million episodes of malaria (range 154–289 million) and an estimated 660, 000 deaths (range 490, 000–836, 000) [19]. It was also reported that an estimated 1.1 million malaria deaths were averted during the past decade, in which many lives were saved in ten countries with the highest malaria burden [19]. The recent decrease in the global malaria burden has been achieved through the scale-up of vector control interventions, especially, the use of insecticides for indoor residual spraying (IRS) and the use of treated mosquito bed nets and other materials [9].

Female *Anopheles* mosquito are known to transmit malaria fever. The most important vectors of malaria are members of *Anopheles gambiae* s. l. (complex), a group of

morphologically identical yet genetically and behaviourally distinct species that differ markedly in their ability to transmit the diseases. Members of the species complex include *Anopheles gambiae* s. s., *Anopheles arabiensis*, *Anopheles merus*, *Anopheles malas*, *Anopheles bwambe*, and *Anopheles quadrannulatus* [22]. *Anopheles* mosquitoes breed in areas with water bodies such as ponds, rivers, surface water, waste-waters, well, etc [15], these areas are suitable for the growth and development of various strains of mosquitoes and are available during rainy season, May to October and supplemented waste-water throughout the year which could also serve as breeding sites [10].

Although four classes of insecticide were recommended by World Health Organization for use against adult mosquitoes in public health programmes, in practice however, modern-day malaria vector control has become highly dependent on just one class of insecticide – the pyrethroids because of its

several advantages over other insecticides in terms of cost, safety (less toxic to mammals) and duration of residual action, excito-repellent properties, rapid rate of knock-down and killing effects [21] and has thus be recommended as the only option for net treatment. Currently, pyrethroids are used on all approved long-lasting insecticidal nets (LLINs) and are the basis of the vast majority of IRS programmes worldwide [18].

The use of only one class of insecticide has in a way given rise to fresh concerns that has to do with the problem of resistance to insecticides in malaria vectors. Following an increase in entomological surveillance in malaria-affected regions in recent years, sufficient data have now been collected to confirm the suspicions that the wide-scale use of insecticide-based malaria control strategies over the past decade has been associated with the development of resistance in several important vector species, including *Anopheles gambiae*, *An. funestus* and *An. Arabiensis* [14].

Pyrethroid resistance has been reported in malaria vector mosquito populations in 27 countries in sub-Saharan Africa [18]. Insecticide resistance in mosquitoes, especially in malaria endemic regions, has increased dramatically in the last decade [14]. The mechanisms responsible for the widespread levels of resistance have also been identified and are of two main types, those mediated by changes at the target site of the insecticide (e.g. *kdr* mutations) and those caused by increases in the rate of insecticide metabolism [5].

In an attempt to reduce the possibility of the development of resistance to insecticide and to also improve the success rates of interventions against disease vectors, an integrated approach to the control of vector-borne diseases has been promoted [2]. These combined interventions have been shown to be effective in significantly reducing malaria morbidity and mortality [13]. Eliminating breeding sites and targeting immature stages of mosquitoes have been found out to significantly impact the incidence and prevalence rates of mosquito-borne diseases [7].

The spread of resistance is generating concern and there has been increasing reports from different parts of Africa which suggest IRS and ITNs are losing their effectiveness due to increased resistance [12]. Therefore, to sustain ITNs and IRS, there must be a continued susceptibility of mosquitoes to the insecticides. It has been found out that using the WHO bio-assays in areas with high coverage of ITNs have detected a gradual decrease in susceptibility levels giving alert on the efficacy of ITNs and IRS with pyrethroids [23]. Malaria vector resistance to pyrethroid and other insecticides is a major threat to the gains achieved by the use of LLINs and IRS malaria control campaigns in Africa.

Insecticide resistance involves changes in one or more genes, leading to the reduction in insecticide sensitivity of an insect population. This is manifested in an insecticide's repeated failure to achieve the projected level of control when used following the recommendations for that species [19]. Apart from genetic, the changes leading to resistance may also be enzymatic [14]. Corbel *et al.* [3] indicated that insecticide resistance could happen due to selection pressure and increasing mutation rates.

WHO has a well-known test using insecticide impregnated filter papers that detects the presence of insecticide resistance phenotypes in mosquitoes. The Centres for Disease Control and Prevention (CDC) has also developed a method using insecticide-treated glass bottles. Either of these tests can now be used to measure the intensity of resistance and to also evaluate the involvement of metabolic mechanisms in resistance. The new methods using the WHO tube test are presented in the updated test procedures for insecticide resistance guidelines that were released in November 2016. In addition, biochemical and molecular tests have been developed to detect enzymes or mutations that are conferring insecticide resistance to the mosquito populations.

Malaria vector resistance to insecticides in Benin for example, was combated by two main mechanisms: alterations at site of action in the sodium channel via the *kdr* mutations and an increase of detoxification and or metabolism through high levels of multi-function oxidases (MFOs) and non-specific esterases (NSEs) [4, 1].

Recently, mass deployment of LLINs and IRS using insecticides remains the main hub of mosquito control strategies to eliminate malaria globally. However, rapid emergence and geographical spread of insecticide resistance among malaria vectors has threatens vector control programmes in many malaria endemic nations including Nigeria. Considering the importance of insecticides in malaria control, regular monitoring of insecticides susceptibility among *Anopheles* vectors is essential particularly in the regions where malaria remains a burden on the communities.

Knowledge of insecticide resistance levels is important to policy makers within the Ministry of Health, because, it is advantageous in early planning and development of resistance management strategies to safeguard the already existing chemical based vector control tools. Thus, this study evaluated the resistance/ susceptibility of *Anopheles gambiae* s. I populations in Okitipupa communities of Ondo State, Southwest Nigeria, to insecticides used in malaria vector control. The study monitored the insecticidal susceptibility status of *Anopheles* mosquito in four sentinel districts of Okitipupa, detects the emergence of resistant individuals in the mosquito population and recommended need for further monitoring of insecticide.

2. Materials and Methods

2.1. Study Area

The study was conducted within Okitipupa town in Okitipupa Local government Area (LGA) of Ondo State where malaria is a public health concern. The province covers an area of 803km² and a population of 233, 565 inhabitants at the 2006 census (Post office archive, 2009). The coastal region is largely hot and humid with two rainy seasons, the "long rains" from April to July, and the "short rains" between October and December. The town lies between 6° 30' 0" North, 4° 48' 0" East. The annual average

temperature is 27.0°C. It is the lowest average temperature of the whole year. Okitipupa is a town with a lot of agricultural activities. The districts of Ayeka, Idepe, Okitipupa and Igodan were selected for the study based on malaria vector species composition, malaria prevalence, and ecological differences. The selection criterion of this site was the presence and abundance of malaria vectors, densely populated areas, presence of palm oil refineries and numerous breeding sites along the existing river streams cutting across the town.

2.2. Mosquito Population

Unfed female mosquitoes aged 2 to 3 days (F1 generation) were used in the test because the physiological status of female mosquitoes such as blood fed, semi gravid or gravid have an effect on susceptibility to insecticide [16].

2.3. Larval Sampling

In order to increase the sample size of getting enough F1 generation to perform the susceptibility tests, larval sampling was done in the nearby breeding sites. Larval collection was done using standard dipping technique [15] by scooping in the habitats within the selected districts. The *Anopheles* larvae were collected from a wide range of breeding sites, representative of the diversity of the mosquito population in the study area, such as marshes, ponds, road puddles and river banks. In each location, larvae collection was performed in at least 3 breeding sites with an average of 40 larvae of all instars collected per breeding habitat and reared to adult in the Zoology laboratory. *Anopheles* larvae were separated from the culicines by the use of an aspirator and kept in a screen cage. The screen cages containing *Anopheline* larvae were kept in a cool environment of 27°C.

Determination of susceptibility of *Anopheles* mosquitoes to insecticides.

The larval and adult mosquitoes were reared in the insectaries to produce the first filial (F1) generation. The F1

generation was categorized into two groups: a test group (mosquitoes subjected to insecticide) and a control group (mosquitoes not subjected to insecticides).

2.4. Procedure and Condition of Susceptibility Testing

Susceptibility test was done using WHO standard guideline [21]. Twenty five not-fed female *Anopheles gambiae* mosquitoes aged 2 – 5 days were exposed to the diagnostic dosages of standard WHO insecticide papers. The mosquitoes were exposed to a dosage of 0.1% propoxur, 0.75% permethrin, 0.05% lambdacyhalothrin, 0.25% pirimiphosmethyl and 0.1% bendiocarb using the WHO susceptibility test kit to assess resistance level.

Number of mosquitoes knocked down during exposure time was recorded at 10 minute intervals for 1 hour. The knocked down mosquitoes were then transferred to holding tubes where 10% glucose was provided and held for 24 hours then mortality recorded.

Laboratory colony, that is, *An. Gambiae s. l.* was used as control test. This susceptibility test was conducted under 26 – 29°C and relative humidity of 74 – 82%.

2.5. Susceptibility Test

The mortality was recorded for the entire exposed field mosquitoes. When there is a ninety eight to a hundred percent mosquito mortality this indicates the population is susceptible, 80 – 97% suggests potential resistance that needs to be confirmed while less than 80% mortality suggests resistance. To determine insecticide resistance, the level of insecticide was scaled by using resistance ratios (RR) which translated as: Susceptible (RR=1), Possible resistance (PR= 2) and Resistance (RR>3) [16, 6].

2.6. Survival of the Mosquitoes

After recording mortality for 24 hours post exposure, all surviving and dead mosquitoes were disposed.

Table 1. Insecticides tested using WHO impregnated papers and their recommended diagnostic concentration.

CLASS OF INSECTICIDE	INSECTICIDE TYPE	DIAGNOSTIC CONCENTRATION (%)
Pyrethroid	Lambdacyhalothrin	0.05
	Permethrin	0.75
	Propoxur	1.0
Carbamate	Bendiocarb	0.1
Organophosphate	Pirimiphosphate	0.1

3. Results

The knockdown effect of five insecticides determined against *Anopheles gambiae* S. I over an exposure time period of 30 minutes, one hour and 24 hours is shown in Table 2.

Table 2. Total knockdown rates of *anopheles* mosquitoes to the insecticides after 30 minutes (a), 60 minutes (b) and 24 hours (c) exposure.

INSECTICIDES	OKITIPUPA			AYEKA			IGBODIGO			IDEPE		
	A	B	C	A	B	C	A	B	C	A	B	C
Bendiocarb	100	100	100	100	100	100	91	100	100	88	100	100
Propoxur	96	98	98	85	96	97	70	100	100	96	98	98
Permethrin	92	100	100	72	78	88	81	100	100	60	88	90

INSECTICIDES	OKITIPUPA			AYEKA			IGBODIGO			IDEPE		
	A	B	C	A	B	C	A	B	C	A	B	C
Lamba-cyhalothrin	35	63	81	24	74	81	20	74	82	22	44	81
Pirimiphos-methyl	27	77	91	23	89	99	28	82	94	91	39	97
Control	0	0	0	0	0	0	0	0	0	0	0	0

Table 3. Status of female *Anopheles* mosquitoes from the four sentinel sites to insecticides using WHO bioassay technique. Sites.

		Insecticides			
		% Mortality after 30 minutes	Status	% Mortality after 24 hours	Status
Ayeka	Propoxur	85	Resistant	97	Possible Resistant
	Permethrin	72	Resistant	88	Resistant
	Lambda- cyalothrin	24	Resistant	24	Resistant
	Pirimiphos-methyl	23	Resistant	99	Susceptible
	Bendiocarb	100	Susceptible	100	Susceptible
Idepe	Propoxur	96	Possible Resistant	98	Susceptible
	Permethrin	60	Resistant	90	Possible Resistant
	Lambda- cyalothrin	22	Resistant	81	Resistant
	Pirimiphos-methyl	39	Resistant	97	Possible Resistant
	Bendiocarb	88	Resistant	100	Resistant
Igbodigo	Propoxur	70	Resistant	100	Susceptible
	Permethrin	81	Resistant	100	Susceptible
	Lambda- cyalothrin	20	Resistant	82	Resistant
	Pirimiphos-methyl	28	Resistant	94	Possible Resistant
	Bendiocarb	100	Susceptible	100	Susceptible
Okitipupa	Propoxur	96	Possible Resistant	98	Susceptible
	Permethrin	92	Possible Resistant	100	Susceptible
	Lambda- cyalothrin	15	Resistant	81	Resistant
	Pirimiphos-methyl	27	Resistant	91	Possible Resistant
	Bendiocarb	100	Susceptible	100	Susceptible

A total number of 2000 *An-gambiae* mosquitoes were tested in this study to determine their susceptibility status against insecticides using WHO guidelines [20], the mortality ranging between 98-100% indicates susceptibility, 90-97% as tolerant for which further investigation is needed and less than 90% is considered resistant where pre-emptive action is required to manage the resistance against insecticides used for malaria vector control.

Based on these recommendations, *Anopheles gambiae* S. I populations from Ayeka community were resistant to propoxur, permethrin and lambda-cyhalothrin insecticides (81-97% 24hrs mortality), while they were susceptible to pirimiphos-methyl insecticide (99% 24hrs mortality) and bendiocarb with complete susceptibility (100% 24hrs mortality) using WHO susceptibility tests.

Regarding *Anopheles gambiae* S. I populations from Idepe community, they were resistant to permethrin, lambda-cyhalothrin and pirimiphos-methyl insecticides (81-97% 24hrs mortality), while they were susceptible to Propoxur and bendiocarb insecticides (98-100% 24hrs mortality) with WHO susceptibility test.

Anopheles gambiae S. I populations from Igbodigo community were resistant to pirimiphos-methyl and lambda-cyhalothrin insecticides (82-92% 24hrs mortality), while they were susceptible to propoxur, permethrin and bendiocarb insecticides (100% 24hrs mortality).

Anopheles gambiae S. I populations from Okitipupa community were susceptible to permethrin, propoxur and bendiocarb insecticides (98-100% 24hrs mortality), while they were resistance to pirimiphos-methyl and lambda-cyhalothrin insecticides (81-91% 24hrs mortality).

The highest percentage mortality from all the insecticides in this study was recorded in Bendiocarb (100%), while the lowest percentage mortality from all the insecticides in this study was recorded in Labda-cyhalothrin (81%). The level of resistance of *An. gambiae* s. l. to pyrethroids was very high at almost all study sites.

Knockdown rates (30minutes) of all anopheles mosquitoes from the four sentinel sites exposed to five selected insecticides showed 75% resistant status, 15% susceptibility status and 10% possible resistance status. Mortality rates (after 24hours) showed 40% resistant status, 50% susceptibility status and 10% possible resistance status. The mosquitoes were more susceptible to bendiocarb insecticide. There was significant difference in susceptibility status across the insecticides used where Bendiocarb was more effective while Lambda-cyhalothrin was less effective.

The results also showed that increase in time interval can lead to increase in susceptibility of Anopopheles mosquitoes exposed to insecticides because more susceptibility status was recorded at 24hours compared to at 30minutes.

4. Discussion

The results presented in this work showed that Propoxur insecticide had its highest mortality rate (100%) at 24hours in Igbodigo and its lowest mortality rate (97%) in Ayeka. Permethrin insecticide had a high mortality rate (100%) at 24hours in Igbodigo and Okitipupa and least mortality rate (88%) in Ayeka. The highest mortality rate recorded in lambda-cyhalothrin insecticide was (82%) at 24hours in Igbodigo and the lowest (81%) in Ayeka, Idepe and

Okitipupa sites. Pirimiphos-methyl insecticide has its highest mortality rate (99%) at 24hours in Ayeka and least mortality rate (91%) at Okitipupa.

Susceptibility was recorded in Igbodigo, Okitipupa, and Ayeka to Propoxur, Permethrin and Pirimiphos-methyl respectively. This was contrary to the result obtained from the findings of Knox *et al.* [8] who reported resistance to these insecticides but agrees with the work of Manokore *et al.* [9]. Mosquitoes were susceptible to Propoxur insecticide across all the sentinel sites, this may be as a result of this insecticide not being used previously, except for Ayeka sentinel site which showed possible resistance, the reason for this could not be determine in this study.

Mosquitoes were susceptible to Permethrin insecticide at Igbodigo and Okitipupa sites, this may be as a result of the community being an urban area where agricultural activities are not common, while mosquitoes from Ayeka and Idepe showed resistance and possible resistance respectively; this may be as a result of climate change, particularly the effects of temperature and the previous use of pyrethroids in this community, this was similar to the findings of the result obtained from the study of Munhenga *et al.* [11].

Mosquitoes were resistant to Lambda-cyhalothrin insecticide across the four sentinel sites, this may be as a result of the use of pyrethroids in this communities for agricultural purposes, this is in agreement with the result obtained from the findings of Knox *et al.* [8] while its contradicts the result obtained from findings of Manokore *et al.* [9].

Mosquitoes showed possible resistance in Pirimiphos-methyl insecticide across three sentinel sites except in Ayeka sentinel site which showed susceptibility to the insecticide.

Bendiocarb insecticide was effective in all sentinel sites this may be due to bendiocarb insecticide not being used regularly in this area except for Idepe sentinel site, which showed resistance this may be as a result of the previous use of this chemical in Idepe community and this was in agreement with the result obtained from the research work of Knox *et al.* [8].

In all insecticides used, mosquitoes were recorded to be susceptible to Propoxur insecticide across all the sentinel sites which was not significantly different in Ayeka and Okitipupa sites ($p>0.05$). Permethrin insecticide also showed susceptibility status in mosquitoes from Igbodigo and Okitipupa sites, while mosquitoes from Ayeka and Idepe showed resistance and possible resistance respectively and this showed significance difference in the statistical results. Mosquitoes were resistant to Lambda-cyhalothrin insecticide across the four sentinel sites and this showed no significant difference. Bendiocarb insecticide was also effective in all sentinel sites.

The level of resistance of *An. gambiae s. l.* to pyrethroids was very high at almost all study sites which was similar to the findings of Kofi *et al.* [24]. This resistance of malaria vector to pyrethroids within the study area, may be as a result of previous use of these insecticides in Internal Residual Spraying (IRS) intervention; use of pyrethroids based aerosols, use of coils, use of herbicides while the

susceptibility of malaria vector to permethrin within the study area may be as a result of non-use of these insecticides in Internal Residual Spraying (IRS) intervention.

5. Conclusion

From the results of this work, it can be deduced that the use of pyrethroids and lambda-cyhalothrin that showed highest resistance should be discontinued while the others like propoxur, bendiocarb and pirimiphos-methyl should be encouraged.

Sustainable insecticide resistance management strategy is important to avoid control failures when the resistant insecticides are used for IRS program. Thus, there is a need for periodic monitoring of insecticide resistance in malaria control programmes in the state, as it affects Insecticide Treated Nets (ITNs) and IRS interventions across Africa.

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