ASSESSMENT OF LEVEL OF SUSCEPTIBILITY OF *ANOPHELES GAMBIAE S.L* TO PUBLIC HEALTH INSECTICIDES IN A MALARIA VECTOR SENTINEL SITE, RIVERS STATE

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Abstract

The status of resistance was investigated in *Anopheles gambiae sensu lato* (Diptera: Culicidae) mosquitoes from Rivers State sentinel site to four classes of insecticides approved by World Health Organization for indoor residual spraying . This study was sponsored by USAID/PMI-AIRS, Project, Nigeria, and is among the studies commissioned by the NMEP to provide an update on the current status of resistance to the major insecticide classes in wild populations of *Anopheles gambiae sensu lato* (*s.l.*) in designated sentinel sites in Nigeria. *Anopheles* larvae were collected from malaria surveillance site (Oduoha -Emohua) in Rivers State, established by the National Malaria Elimination Programme.The mosquitoes were reared to adulthood in the insectary and identified using morphological keys. Two- to three-day old adult female mosquitoes were exposed to WHO diagnostic doses of 0.05% deltamethrin, 0.05% lambdacyhalothrin,0.05% alphacympermethrin, 0.05% permethrin, 0.13% bendiocarb, 0.05 % propuxur, 4.0%DDT and 0.25% premiphos-methyl. Standard WHO protocols, insecticide susceptibility test kits and treated papers were used for the determination of susceptibility. Knockdown every 10 min and mortality 24 h after post exposure were noted. All *Anopheles* mosquitoes tested belonged to the *Anopheles gambiae* complex. The vectors were susceptible to bendiocarb and alphacympermethrin with mortality rates of100% and 98% respectively. There was complete resistance to deltamethrin, lambdacyhalothrin, permethrin, propuxur, DDT and premiphos-methyl with mortality ranges of 25%-65%.The present study shows the effectiveness of bendiocarb and alphacympermethrin in malaria control with Indoor Residual Spraying. It also provides baseline information for monitoring the status of insecticide resistance in Rivers State.

Key words: *Anopheles gambiae*, susceptibility, insecticides, Rivers State

Background

Malaria is a life threatening disease in the tropics and accounts for much of the disease burden in the continent. In Nigeria, malaria is highly endemic and has claimed thousands of lives and caused massive economic losses. It is transmitted by female *Anopheles* mosquitoes. Malaria control is reliant on insecticides to control the mosquito vector. In view of this, World Health Organization (WHO) has approved certain classes of insecticides to be used in

their formulation namely, pyrethroids for both LLINs and IRS and organophosphates, carbamates and organochlorides for IRS only (Loroño-Pino *et al*., 2013).

Because of the intense selection pressure caused by agricultural practices (Diabate *et al.*, 2002) and the large-scale implementation of malaria vector control interventions (Corbel and Guessan, 2013). Resistance has been implicated in the reduced efficacy of vector control interventions such as IRS and LLIN [N'Guessan *et al*., 2007; Asidi *et al*., 2012) and malaria resurgence (Trape *et al*., 2011; McCann *et al*., 2014; Strode *et al*., 2014; Churcher *et al*., 2016; Ransome and Lissenden 2016).

Insecticide resistance in malaria vectors is a growing concern in many countries which requires immediate attention because of the limited chemical arsenal available for vector control. The Global malaria community is responding to the potential threat posed by emerging insecticide resistance; In May, 2012, WHO launched the Global plan for insecticide resistance management in malaria vectors (GPIRM), which includes planning and implementing National resistance management strategy (WHO, 2012). Nigeria as a nation has joined other African countries in adopting an insecticide resistance management plan. The plan involves the setting up of sentinel sites for the routine monitoring of insecticide resistance by renowned research institutions in the country.

The current extent and distribution of this resistance in many parts of the continent is unknown and yet such information is essential for the planning of effective malaria control interventions (Ranson *et al.*, 2009).

The mechanisms of insecticide resistance in mosquitoes are multiple and include behavioural and physiological changes leading to insecticide avoidance, reduced penetration, sequestration, target site modification (knock-down resistance or kdr mutation for pyrethroids

and DDT) and increased biodegradation [Liu, 2015;Hemingway *et al.,* 2000; David *et al.,* 2013).

To effectively utilize insecticides in malaria vector control, it is pertinent to know the levels of insecticide resistance in the main malaria vector. Comprehensive evaluation of insecticides resistance across different malaria-endemic areas will provide critically needed data on use of new IRS strategies as alternative malaria control tools for further reducing malaria incidence in Africa (Wanjala *et al.,* 2015).

Effective insecticide resistance management is highly essential in preventing resistance, regain susceptibility or delay the development of resistance in mosquitoes to support and improve public health (Baffour-Awuah *et al.,* 2016) Routine monitoring of resistance and detection of temporal changes in both prevalence and intensity of resistance are needed to guide malaria vector interventions and resistance management plan (Ranson and Lissenden, 2016).

At present, there are insufficient data about the susceptibility status of malaria vectors to Public Health insecticides in this region as compared to other areas of the country. This lack of data is a major constraint to effectively control malaria vectors in this region.

Thus the objective of this study was to provide an update on the current status of resistance to the major insecticide classes in African malaria vectors in Rivers State sentinel site.

Methods:

Study site

The study was conducted in Oduoha-Emuoha community. Emuoha is the headquarters of Emuoha local government Area of Rivers State and is one of the malaria endemic zones in the state. It is Located in the Niger Delta region of Nigeria and lies between: 0 4°52'44"N

06°51'40"E (see Fig 1). The environment is a typical tropical rain forest. The topography is flat and pockets of forest stream and fresh water bodies are found. The climate is characterized with two distinct seasons, the wet and dry seasons, the former taking place from April to October and dry season between November and March.

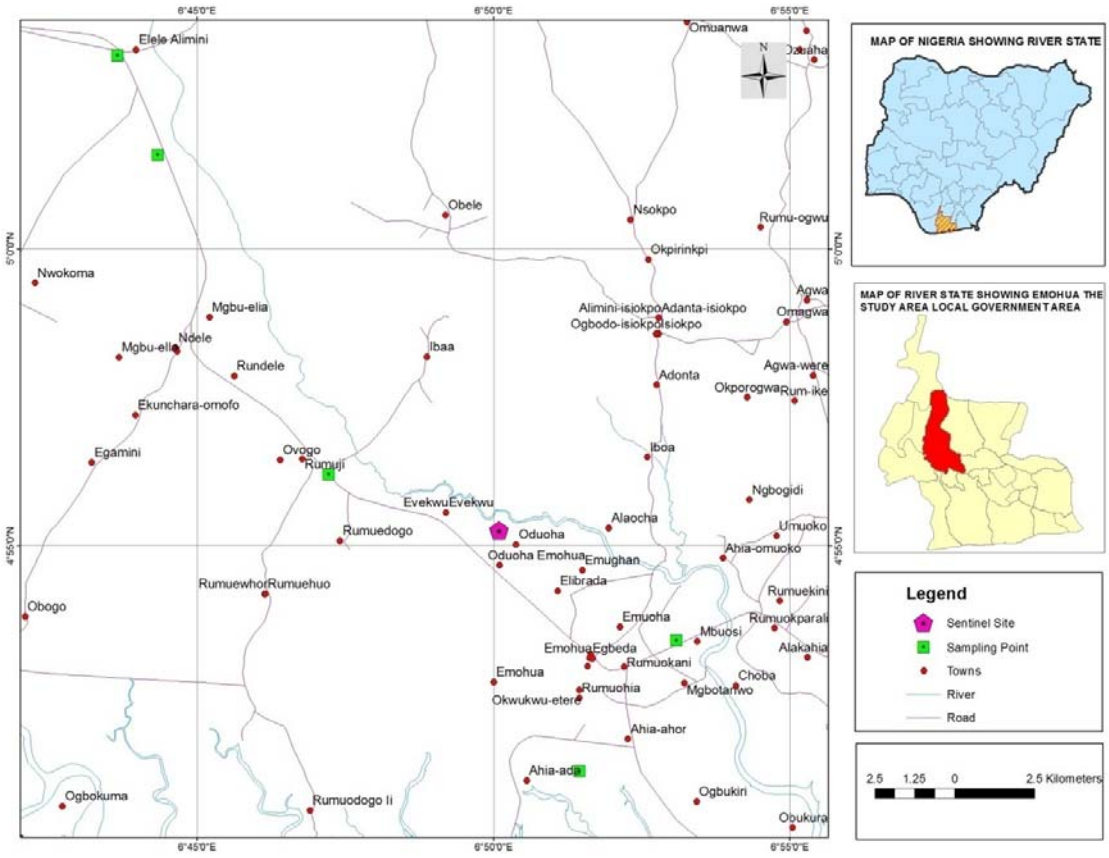

Fig. 1: Map of the study area showing the sampling locations

Larval Collection

Larvae were collected by searching different types of *Anopheles* larval breeding sites around the sentinel site, using a dipping method described by World Health Organization.

All the potential breeding sites were surveyed for *Anopheles* larvae. A white iron dipper (ladle) was used for larvae collection. The GPS locations of the larval collection sites including some characteristics of the breeding sites were recorded

The water containing the larvae from various areas was pooled together and transferred into 20L plastic container and transported to the insectary for identification and rearing.

The emerged adults were used for susceptibility test.

Rearing of Larvae

The larvae were reared in the Malaria Entomology Research laboratory, Rivers State University of Science and Technology, Port Harcourt under the ambient laboratory environmental condition. The female *Anopheles* larvae were sorted out and put in plastic containers containing rearing water. The containers were covered with nets fastened with rubber bands and placed in platform containing water to prevent crawling insects from invading the larvae. The larvae were fed with wheat powder mixed with grinded biscuits and monitored till about emergence.

Rearing of Adult Mosquitoes

The emerged adults were introduced into wooden rearing cages and fed with glucose solution. Two to three day old *Anopheles* female mosquitoes were aspirated out and used for susceptibility test.

Morphological Identifications of Mosquitoes

Female adult mosquitoes used for the susceptibility test were subjected to morphological identifications using the keys of Gillies and de meillon (Gillies and de Meillon, 1968) and Gillies and Coetzee (Gillies and Coetzee, 1987).

Susceptibility Tests

Insecticide susceptibility tests were carried out using the standard WHO protocol (WHO, 1998).

WHO Susceptibility Test Procedure

WHO Insecticide susceptibility test kits and impregnated papers were used for this test. Two to three day old non blood-fed adult female *Anopheles* mosquitoes collected around the sentinel site were tested. Batches of 25 mosquitoes were exposed to test papers impregnated with Permethrin (0.75%), Deltamethrin (0.05%), Alphacympermethrin (0.75%), Lambdacyhalothrin (0.05%), Propuxur (0.05%), Bendiocarb (0.13%) DDT (4.0%) and Premiphos-Methyl (0.25%).

Each insecticide was replicated 4 times. A total of 100 adult female *Anopheles* mosquitoes were tested for each insecticide. Two control experiments with the same batch (i.e 25 each) of mosquitoes from the site were carried out for each insecticide at the same time; in this case, the mosquitoes were exposed to untreated papers impregnated with mineral oils. Experimental set-up was placed on a platform surrounded with water to prevent crawling insects from eating up the mosquitoes. The knockdown effect of each insecticide was recorded every 10 minutes over the one hour exposure period. A mosquito is considered knocked down if it was unable to stand or fly in a coordinated way. After the exposure,

mosquitoes were then transferred to a recovery tube and provided with 10% glucose solution. Final mortality was recorded 24 hours post-exposure. A mosquito was classified as dead if it was immobile or unable to stand or fly in a coordinated way.

The mosquitoes used for the tests were preserved individually in Eppendorff tubes, labeled appropriately for identification and further analysis.

Data analysis

Knock-down time (KDT_{50} and KDT_{95}) along with slope and 95% confidence interval (CI) were determined using probit analysis software

The mortality of test sample was calculated by summing the number of dead mosquitoes across all four exposure replicates and expressing this as a percentage of the total of the total number of exposed mosquitoes.

Observed Mortality = $\frac{Total\ Number\ of\ Dead\ Mosquito}{Total\ sample\ size}\ x\ 100$

The susceptibility levels of the mosquitoes were evaluated on the basis of the WHO (2013) criteria of test mortality. Correction with Abbots formula was not used as the mortality in all the controls were below 5%.

Results

Knock- Down Effect

The knock down (KD) effect of the 8 insecticides tested over a 1– hour period is presented in Table 1.0 whereas the percentage knock -down effects is represented in Fig 2.0

The KD_{50} ranged from 9.3 to 80.9mins while the KD_{95} ranged from 16.6 to 149,8 minutes for all insecticides tested. The least knockdown effects were noticed in the vectors exposed to bendiocarb and alphacympermethrin insecticides treated papers with a rapid knock down effect on the malaria vectors within 10 minutes of exposure. The percentage knock down at

10mins of both insecticides were 36% and 64% respectively. Within 30 minutes exposure, both insecticides knocked down all the mosquitoes exposed to them. Similarly, the KDT_{50} of both insecticides were 15.4 and 9.0 minutes respectively while the KDT_{95} were 32.4 and 19.6 minutes respectively.

The knock down effects of the remaining insecticides were slow. Only deltamethrin recorded 84% knockdown after 60 min exposure to the insecticide. The remaining insecticides apart from propoxur (51% at 60min) could not knock down half of the population of the vectors within the1 hour period. Primiphos-methyl and lambdacyhalothrin had % KD_{1h} of 46% and 36% respectively. Permethrin and DDT had the least % KD_{1h} (14% and 22% respectively) and higher KD_{50} of 83.8 and 80.9 minutes respectively. The KD_{95} for both insecticides were 176.6 minutes (permethrin) and 170.4 minutes (DDT). The KD_{50} for the remaining insecticides; Primiphos- methyl, Propoxur, Lambdacyhalothrin and Deltamethrin ranged from 41.9-61.7minutes while the KD_{95} ranged from 83.3-130minutes.

Table 1.0: Toxicity and knock- down time of *Anopheles gambiae*(s.l) exposed to public health insecticides

Insecticides	% KD_{1h}	N	KDT_{50} (95%CI)	KDT_{95} (95% C1)
DDT	22	100	80.9(67.4-100.7)	170.4 (131.4-248.9)
Bendiocarb	100	100	15.4(12.8-18.0)	32.4 (27.1-40.9)
Propoxur	51	100	61.7(53.8-72.6)	130.1 (104.6-179.7)
Primiphos-methly	46	100	58.8(51.2-68.4)	123.9 (101.0-167.2)
Lambdacyhalothrin	36	100	59.7(51.0-69.6)	125.7(103.5-165.5)
Alphacympermethrin	100	100	9.0(7.4-11.3)	19.6 (16.1-25.0)
Deltamethrin	84	100	41.9 (36.9-47.4)	88.3 (74,3-113.6)
Permethrin	14	100	83.8(68.6-1O5.3)	176.6 (135.9-256.3)

KDT- knock down time in minutes; N-no of mosquitoes exposed; CI-Confidence interval; SD- Standard deviation

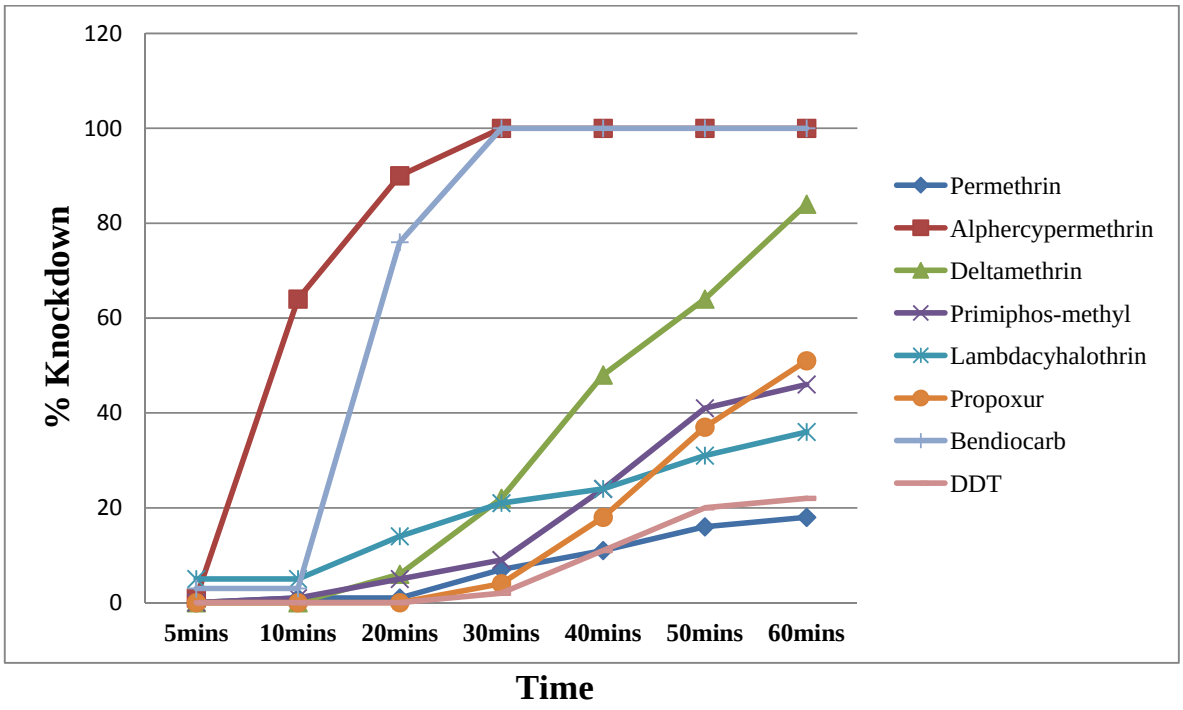

Fig 2: Percentage (%) knockdown of *Anopheles gambiae s.l* exposed to IRS insecticides

Percentage Mortality

Percentage mortalities after the 24 hour post exposure period are presented in Tables 1. In all cases the control mortality was less than 5%, therefore Abott's formula was not applied. The result revealed that bendiocarb and alphacympermethrin caused 100% and 98% mortalities respectively on the vectors. The carbamate (propoxur) and organophosphate (primiphos-methyl) recorded 65% and 59% mortality respectively. None of the remaining insecticides, lambdacyhalothrin (48%), deltamethrin (40%), DDT (37%), and permethrin (25%) recorded 50% mortality after the 24hrs post exposure periods.

Table 1: Susceptibility status of Anopheles gambiae s.l after 24 hr post exposure period

Insecticides	**Total no tested**	**Total no dead**	**Percentage mortality**	**Total no. Tested (control)**	**Total No. Dead (control)**	**Percentage Mortality**	**Susceptibility status**
Lambdacyhalothrin	100	48	*48	50	2	4	Resistant
Bendiocarb	100	100	***100	50	0	0	**Susceptible**
Primiphos-methyl	100	59	*59	50	0	0	Resistant
DDT	100	37	*37	50	1	2	Resistant
Permethrin	100	25	25	50	1	2	Resistant
Deltamethrin	100	40	*40	50	0	0	Resistant
Alphacympermethrin	100	98	***98	50	0	0	**Susceptible**
Propoxur	100	65	*65	50	1	2	Resistant

**<90% =Resistance ** 98% -100% = Susceptible ***90% -97% =Possible Resistance*

Discussions

Anopheles gambiae s.s is the most predominantly encountered species in the study area. The result demonstrated that the field population of *Anopheles gambiae s.l* from Rivers State sentinel site are highly susceptible to the carbamate, bendiocarb and resistant to the pyrethroids, (deltamethrin, lambdacyhaloathrin and permethrin), organochlorine (DDT), carbamate (propoxur), and organophosphate (primiphos-methyl). The vectors also showed some level of susceptibility to alphacympermethrin.

Complete susceptibility to bendiocarb has been recorded in Nigeria (Okorie *et al.,* 2015;Ebere and Nwakanma, 2016; Nwankwo *et al.,* 2017) and other African countries (Aikpon, 2013). Reports from Equatorial Guinea, Namibia, Mozambique, Mexico, Benin and India have also shown good performance of bendiocarb as an indoor residual spraying treatment against mosquito vectors (Akogbeto *et al.,* 2010).

Resistance of An.gambiae s.l to multiple classes of insecticides has been reported elsewhere in Nigeria (Oduola *et al*., 2010; 2012;Riveron *et al*., 2015).

The level of insecticide resistance reported in the present study portends great danger to continue use of long-lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) in this area.

High level of resistance found in both DDTand pyrethroids In this study is consistent with earlier findings. Cross-resistance between DDT and the pyrethroids have been reported in An.gambiae (Corbel and N'Guessan, 2013;Protopopoff *et al.,* 2013, Luisa *et al.,* 2013, Nwankwo *et al.,* 2017). Awolola *et al.,* 2014 confirmed resistance of Anopheles mosquitoes to DDT and the Pyrethroids (deltamethrin, permethrin and lambdacyhalothrin) in Southwest, Nigeria.

The observed DDT and pyrethroids resistance in this zone is not surprising given the numerous reports of insecticides resistance in other African countries (Chandre *et al.,* 1999; Hargeaves *et al.,* 2000). DDT and pyrethroid insecticides are used for IRS, LLINs and for personal protection including controlling crop pests in agriculture. Although no IRS has been carried out in the sentinel site, but the mass deployment of LLINs in the area coupled with the applications of local insecticides of unknown chemical composition and normal household insecticides may have contributed to the build-up of insecticide resistance in the local mosquito populations. This continuous exposure of mosquitoes to pyrethroids as well as other commonly used insecticides adds up to mosquitoes becoming strongly resistant to them (Loroño-Pino *et al.,* 2013).

The high DDT resistance observed is amazing and might indicate that DDT pressure is still available despite the fact that DDT is not used anymore for the control of malaria (Van Bortel *et al.,* 2008). This portends danger and calls for more concern and proper monitoring of the chemicals used for pest control.

The relatively higher level of KD_{50} and KD_{95} recorded for both DDT and permethrin indicate the existence of kdr mutations in the population of the vectors as suggested by Chandre *et al*. (1999). The comparably lowKDT_{50}values recorded for other insecticides also suggest the presence of other mechanisms of resistance in the populations (Adeogun *et al*., 2017).

Resistance of mosquitoes to insecticides usually arises through one of two mechanisms, or a combination of the two; metabolic resistance due to increased production of detoxifying enzymes and target site resistance due to mutations in the sodium channels, acetylcholinestrase or GABA receptor (Hemingway *et al.,* 2004; Verhaeghen *et al.,* 2010).

Variation in insecticide resistance mainly depends upon the type of insecticide and frequency of use. Excessive and unwanted usage of insecticides not only increases vector resistance, but also results in cross resistance to other insecticides (Tikar *et al.,* 2011). Although various

mechanisms of insecticide resistance in insects such as metabolic resistance (i.e. esterases, monooxigenase or glutathione-s-transferase), resistance due to reduced penetration or behavioural resistance are reported in several vectors, generally it is governed by either involvement of metabolic mechanisms or alterations at target sites. Revealing the mechanism of resistance is equally important to that of monitoring resistance in mosquito vectors.

Effective insecticide resistance management is highly essential in preventing resistance, regain susceptibility or delay the development of resistance in mosquitoes to support and improve public health (Baffour-Awuah *et al.,* 2016).

Detection of resistance suggests a change in insecticide class as part of a proactive resistance management programme and in line with the principles of the global plan for insecticide resistance management. In addition, updating the general public on the level of the resistance status of malaria vectors locally and exploiting such information fully is a must for improved programmatic decision-making, and to ensure continued impact of implemented vector control interventions on malaria morbidity and mortality.

Conclusions:

The findings of this study indicate that the populations of *Anopheles gambiae s.l* around the Rivers State sentinel site have developed resistance to a broad range of insecticides including pyrethroids, primiphos-methyl (organophosphate), DDT (organochlorine). The vectors are susceptible to the, bendiocarb (carbamate) and alphacympermethrin (Pyrethroid). Our finding on the complete susceptibility to carbamate in malaria vectors suggests that bendiocarb insecticides are a potentially effective insecticide for IRS. This finding has critical implications in guiding malaria vector control in Rivers State.

This study provides baseline information for monitoring the status of insecticide resistance in Rivers State.

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