INSECTICIDE SUSCEPTIBILITY PROFILE OF MALARIA VECTOR FROM CRUDE OIL IMPACTED COASTAL AREA OF EASTERN OBOLO LGA, AKWA IBOM STATE, NIGERIA

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ABSTRACT

The capacity of malaria vectors to transmit *Plasmodium* parasites and their vulnerability to vector control measures vary by mosquito species and are influenced by local environmental factors**.** This study evaluated the susceptibility of *Anopheles* mosquito populations from four study sites in Eastern Obolo L.G.A, Akwa Ibom State to Permethrin(0.75%), Lambdacyhalothrin (0.5%), alphacypermethrin (0.75%) and Deltamethrin (0.5%) insecticides. The mosquitoes were obtained as aquatic forms from the study sites and reared under laboratory conditions to adults. The adults were subjected to WHO susceptibility bioassays following standard procedure. Across the study sites, the ranges of mean mortality in groups treated with deltamethrin (23.75 ± 0.50 – 24.25 ± 0.50), lambdacyhalothrin (23.25 ± 0.50 - 24.00 ± 0.00) and alphacypermethrin (22.75 ± 0.05 -23.00 ± 0.82), were significantly ($p < 0.05$) higher than that observed in the permethrin (3.75 ± 0.50 - 17.75 ± 0.50) treated groups. This implies that malaria vectors were resistant to permethrin. Vector Populations in all the study sites showed marginal susceptibility to deltamethrin, lambdacyhalothrin and alphacypermethrin (mortality rate: 90 % - 97 %). KDT_{50} and KDT_{95} estimated for each insecticide using a log-time probit model revealed that knockdown was more rapid for deltamethrin (KDT_{50} = 33.93 - 47.48 min; KDT_{95} = 76.07 - 144.39 min), lambdacyhalothrin (KDT_{50} = 33.01 - 44.01 min; KDT_{95} = 74.63 - 148.31 min) and alphacypermethrin (KDT_{50} = 38.92 - 47.53 min; KDT_{95} = 95.32 - 166.93 min) than for permethrin (KDT_{50} = 37.28 - 587.83 min; KDT_{95} = 174.14 - 2391.91 min). Morphological identification of all the mosquitoes used for the tests revealed populations of only *Anopheles gambiae*s.l. Emergence of focal points with insecticide resistance and marginal susceptibility in this study gives serious concern especially with the scale-up in the distribution of pyrethriod treated nets to these areas. This may increase selection pressures due to over-exposure and compromise vector control interventions employing these insecticides.

INTRODUCTION

The spread of insecticide resistance genes in populations of malaria vectors across Africa may jeopardize vector based malaria control programmes, which essentially rely on the use of insecticide-treated materials such as Long Lasting Insecticidal Nets (LLINs) or indoor house spraying (Awolola, 2017). Resistance to organophosphates, organochlorines, carbamates and Pyrethroid class of insecticides has been widely found in different parts of Africa (Elissa *et al*., 1993 and Hemingway *et al*., 2002). In Nigeria, the first case of pyrethroids resistance in *Anopheles* was reported by Awolola *et al.* (2002) and Awolola (2017) in mosquito populations from western Nigeria; Since then, reports of pyrethroid resistance in the country has spread covering 20 out of the 36 states (Awolola, 2017) including some areas in Akwa Ibom State.

Tropical Africa harbors environments that are conducive to the prevalence of malaria transmissions. Most African countries contain an abundance of surface water, warm climate and humid conditions. All these provide good breeding sites for mosquitoes, conducive

atmosphere for their successful development and facilitate longevity of the adult mosquitoes. Poor quality houses that offer little or no protection from mosquito bites facilitate interaction between mosquitoes and humans (Grove-Kopec *et al,* 2006). Eastern Obolo LGA of AkwaIbom State, Nigeria is an area that has abundance of surface water and is constantly challenged with incidences of crude oil contamination of water bodies coupled with poor quality houses that offer little or no protection from mosquito bites.

WHO (2013) had earlier reported that the Niger Delta (despite the oil spillage experience) is an established malaria endemic region. Malaria Vector Control (MVC) in Nigeria is achieved through Indoor Residual Spray (IRS) and Long Lasting Insecticidal Nets (LLINs); and it currently relies on a number of WHOPES- approved insecticides including the pyrethroids. Pyrethroid insecticides are the only recommended class of insecticides for the treatment of bed nets and the others are applied for IRS. The vulnerability of malaria vectors to control measures is very focal and local (Hemingway and Brogdon, 1998), varying by mosquito species and being influenced by local environmental factors. It is advocated that before an evidenced based policy direction is designed for the implementation of integrated vector management in malaria control, insecticide resistance monitoring must be given a very wide spread within a geographical area (Hemingway *et al*., 2002 and Awolola 2017). Unfortunately, data on pyrethroid resistance profile are still very limited, highly insufficient and difficult to consolidate (Awolola, 2017) and for this reason, designing evidence based malaria vector control policies by the National Malaria Elimination Programme (NMEP) of the Federal Government of Nigeria may be a mirage.There is lacuna in information on the species profile of the malaria vectors and their pyrethroid resistance status in Eastern Obolo LGA of Akwa Ibom State. This study was therefore designed to address these problems.

MATERIALS AND METHOD

Study Areas/ Sites

The study area (Eastern Obolo Local Government Area) chosen for this work is a crude oil rich area that has been recently exposed to crude oil pollution. The area is located in the Niger Delta fringe between Imo and Qua Iboe Rivers estuaries and lies between latitudes 4° 28`/ 4° 53`; longitudes 7° 50`/ 7° 55` East. It is bounded in the north by Mkpat Enin the north- east by Onna, the west by Ikot Abasi, the southeast by Ibeno Local Government Areas and in the south by the Atlantic Ocean. lt has a total land mass of 117,008 km^2 with an estimated shoreline of about 184 km. A total of thirty two villages are gazetted for Eastern Obolo (Ministry of Economic Development, AKS, 2016).

With the aid of local guards, a pre-survey of the study areas was conducted. Villages notorious for high population of mosquitoes were identified and considered as study sites. These were Emereoke I, Emereoke II, Emeremen and Agansa villages. All the study sites were located in the mangrove swamp eco-zones where malaria transmission by the vectors is more prevalent between March and November (Ayanlade *et al*., 2010). The sites are characterized by thick bushes, swamps, forest, and clustered residential buildings; water logs were also vastly distributed. All these presented good breeding ground(s) for mosquitoes.

Larvae Collections and Laboratory Rearing of Mosquitoes

The sites were surveyed to locate the breeding sites, including, muddy water, edges of rivers, swamps, shallowly dug wells, ponds, small pools of stagnant water, and irrigated vegetable farms *Anopheles* larvae were identified from their horizontal position on the surface of water. Other species identified by their angular position were also carefully collected with a 350 ml dipper and transferred into 5000 ml plastic containers which were loosely capped to allow aeration and then transported to the USAID/PMI/VECTORLINK Malaria Research Laboratory/ Insectary in the Department of Animal and Environmental Biology, University of Uyo for rearing. The method described by Kabula *et al*., (2011) was employed. The development of the larvae was monitored regularly and all pupated larvae were collected into shallow plastic cups/small beakers using Pasteur pipettes, and then placed in appropriately labeled cages for

adult emergence (. The larvae were fed with cabin biscuit and yeast while the adults were fed with ten percent (10 %) sugar solution.

Insecticide Susceptibility Bioassay Tests

The test was carried out according to standard protocol (WHO, 2016). WHO insecticide susceptibility test kits with insecticide-impregnated papers were used. Specially designed plastic tubes were lined with insecticide impregnated papers including permethrin (0.75 percent), deltamethrin (0.05 percent), alphacypermethrin (0.75 percent) and lambdacyhalothrin (0.05 percent) papers were prepared for the assay. First, at least 150 non-blood-fed active adult female *anopheles* mosquitoes of 2-5 days-old were aspirated (in batches) from a mosquito cage into six holding tubes (prepared by lining the tube with clean sheets of white paper, 12 x 15 cm in dimension) to give six replicate samples of at least 25 mosquitoes per tube. The mosquitoes were allowed in the holding tube for one hour period of acclimatization. The content of four of the holding tubes were then transferred to four exposure tubes lined with a particular insecticide thereby forcing exposure to the insecticide for one hour (Brogdon, 1989). Contents of the two holding tubes left were also transferred into two tubes labeled 'control experiments'. In this case, the mosquitoes were exposed to untreated papers impregnated with mineral oils for one hour also.

During the exposure period, knock-down (KD) rates were recorded after 10, 15 20, 30, 40, 50 and 60 minutes as previously described by Bilali, *et al.,* (2012), WHO (2016) and Niang *et al.*, (2016). At the end of exposure period, the mosquitoes were transferred back to the holding tubes and kept there for 24 hrs. During this period, they were fed with 10 % of sugar solution. The temperature and humidity were recorded and maintained at 27 ^{o}C ± 2 ^{o}C and 75 % ± 10 %, respectively (WHO, 2016). At the end of the 24 hrs post-exposure period, the number of dead mosquitoes was recorded. Each round of the test (conducted per site) involved four replicates each of the four different insecticides and two replicates for the control experiment. This implies that one set of control experiment was used for each round of the test involving four different insecticides. At least four hundred and fifty (450) non-blood-fed active adult female anopheles mosquitoes of 2-5 days-old were randomly sampled per site and subjected to this test at each round. The same procedure was repeated for all the four (4) study sites selected within the study area.

In the end, the mosquitoes used for the tests were preserved individually in Eppendorf tubes containing silica gel and labeled appropriately for identification. The status of mosquito samples tested with the WHO tube test were determined after twenty four hours (24 hrs) holding period according to the latest WHO criteria (WHO, 2016).

Identification of Anopheles Mosquitoes

All the mosquitoes were morphologically identified using morphological keys (Gillies and de Meillon, 1968; Gillies and Coetzee, 1987; Kent, 2006) and dissecting microscope (Olympus, USA).

RESULTS

Susceptibility of female *Anopheles* mosquito populations to pyrethriod insecticides.

Across the study sites, mean mortality in groups treated with deltamethrin (23.75 ± 0.50 – 24.25 ± 0.50), lambdacyhalothrin (23.25 ± 0.50 - 24.00 ± 0.00) and alphacypermethrin (22.75 ± 0.05 -23.00 ± 0.82), were significantly ($p < 0.05$) higher than that observed in the permethrin (3.75 ± 0.50 - 17.75 ± 0.50). The results have shown that malaria vectors were resistant to permethrin. Vector Populations in all the study sites showed marginal susceptibility to deltamethrin, lambdacyhalothrin and alphacypermethrin (mortality rate: 90 % - 97 %). The results of knockdown assessment determined over a one-hour exposure period of female *Anopheles* mosquitoes to pyrethriod impregnated insecticide papers are presented (Figure 1 - 4) have shown that knockdown was more rapid for deltamethrin, lambdacyhalothrin and alphacypermethrin than for permethrin. It was observed that all the mosquito populations, but those of Emereoke 2 study site had over 90% knock-down within the one hour of exposure to

deltamethrin and lambdacyhalothrin. KDT_{50} and KDT_{95} estimated for each insecticide using a log-time probit model revealed that knockdown was more rapid for deltamethrin (KDT_{50} = 33.93 - 47.48 min; KDT_{95} = 76.07 - 144.39 min), lambdacyhalothrin (KDT_{50} = 33.01 - 44.01 min; KDT_{95} = 74.63 - 148.31 min) and alphacypermethrin (KDT_{50} = 38.92 - 47.53 min; KDT_{95} = 95.32 - 166.93 min) than for permethrin (KDT_{50} = 37.28 - 587.83 min; KDT_{95} = 174.14 - 2391.91 min).

Morphological identity of the prevalent malaria vectors from the surveyed study sites in Eastern Obolo L.G.A, Akwa Ibom state

A total of 1800 mosquitoes from the four study sites used for susceptibility bioassay were morphologically identified as female *Anopheles gambiae*s l.

DISCUSSION

An effective site specific strategic plan is informed by a comprehensive monitoring and evaluation of resistance of malaria vectors to insecticides. This will ensure the success of malaria vector control efforts and malaria elimination in Africa (WHO, 2010). The President's Malaria Initiative (PMI) has supported this approach in Nigeria, focusing on areas where insecticide based vector control measures (IRS and LLINs) have been deployed (WHO, 2013). Understanding the diversity of malaria vectors at local and regional levels is of utmost importance. According to Jan (2016), not all female *Anopheles* mosquito species are equal in vectorial capacity and susceptibility to chemical agents in their environment. In this study, morphological analysis of the preserved mosquito samples used susceptibility bioassay showed populations of only *Anopheles gambiae*s.l. This malaria vector has been reported to be the principal vector of malaria in Sub-Saharan Africa (Gillies and Coetzee, 1987; Samdi *et al*., 2006). Although *Anopheles funestus* and *Anopheles gambiaes* have been established as major malaria vectors in Nigeria (Molineaux and Gramiccia, 1980), the present study incriminated only *A .gambiae*s.l as the transmitters of malaria in the study sites.

Susceptibility assay has revealed high levels of resistance to permethrin in the field populations of *A. gambiae.* . Deltamethrin, lambda-cyhalthrin and alpha-cypermethrin were not spared as the populations of *A.gambiae* tested exhibited only marginal susceptibility indicating resistance against the insecticides. The high level of resistance recorded in this study is strongly corroborated by the trend in median knockdown time (KDT_{50}) observed in the study area. The type II pyrethroids including deltamethrin, alphacypermethrin, and lambdacyhalothrin insecticides were more effective on the malaria vectors than the type I pyrethriod,permethrin. Bloomquist (1996) reported that the type II pyrethroids have a cyano group at the α-benzylic position (the α-carbon of the 3-phenoxybenzyl alcohol) and cause a pronounced convulsive phase that results in better kill because depolarization of the nerve axons and terminals is irreversible. In addition, the differing toxicity effects have been explained by the fact that the duration of modified sodium currents by type I compounds lasts only tens or hundreds of milliseconds, whilst those of type II compounds last for several seconds or longer (Bloomquist, 1996). Higher KDT_{50} values in the field population have been suggested to give an early indication of the involvement of *kdr* mechanism of resistance (Chandre *et al.,* 1999a, b). Generally, these results are consistent with those of other studies conducted (Adasi *et al.*, 2000; Adeniran, 2002; Achonduh, 2005 and Kahindi, 2005), where *A. gambiae*s.l has been reported to be resistant to pyrethroids and DDT.

The development of resistance has also been linked to an increase in the activities of detoxification enzymes in mosquito populations in oil polluted breeding habitat (Imam and Yusuf, 2015). Therefore the impact of the environment in the present study findings cannot be ruled out. Mosquitoes in Eastern Obolo LGA, an area with frequent cases of crude oil contamination may readily developed detoxification enzyme machinery with abnormally high activities that allowed the aquatic stages of the mosquitoes to tolerate and thrive in this area.

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Table 1: Susceptibility of female anopheles mosquito population from Emereoke 1, Eastern Obolo L.G.A, AkwaIbom State, Nigeria to pyrethriod insecticides

Insecticide Papers	No. exposed	No of replicates	Mean mortality ± SD	Total mortality (%)	Status
PY control	50	2	1.0 ± 0.00^{a}	4	
thrin (0.75 %)	100	4	4.25 ± 0.50^{a}	22	Resistant
Deltamethrin (0.05 %)	100	4	$24.25 \pm 0.50^{a,b}$	97	Suspected resistance
Alphacypermethrin (0.75 %)	100	4	$22.75 \pm 0.50^{a,b,c}$	91	Suspected resistance
Lamdacyahalothrin (0.05 %)	100	4	$24.00 \pm 0.00^{a,b,d}$	96	Suspected resistance

Number of mosquitoes per replicate = 25

a = $p \leq 0.05$ (comparing insecticide test groups with the PY control), b = $p \leq 0.05$ (comparing other insecticide test groups with Permethrin)

c = $p \leq 0.05$ (comparing Alphacypermethrininsecticide test group with Deltamethrin insecticide test group),

d = $p \leq 0.05$ (comparing Lamdacyahalothrin insecticide test group with the Alphacypermethrin insecticide test group).

Table2: Susceptibility of female anopheles mosquito population from Emereoke 2, Eastern Obolo L.G.A, Akwa Ibom State, Nigeria to pyrethriod insecticides

Insecticide Papers	No. exposed	No of replicates	Mean mortality ± SD	Total mortality (%)	Status
PY control	50	2	0.00 ± 0.00^{a}	0	
Permethrin (0.75 %)	100	4	3.75 ± 0.50^{a}	15	Resistant
Deltamethrin (0.05 %)	100	4	$23.75 \pm 0.50^{a,b}$	95	Suspected resistance
Lamdacyahalothrin (0.05 %)	100	4	$23.25 \pm 0.50^{a,b}$	93	Suspected resistance
Alphacypermethrin (0.75 %)	100	4	$22.75 \pm 0.50^{a,b}$	91	Suspected resistance

Number of mosquitoes per replicate = 25

a = $p \leq 0.05$ (comparing insecticide test groups with the PY control) b = $p \leq 0.05$ (comparing other insecticide test groups with Permethrin)

Table 3:Susceptibility of female *anopheles* mosquito population from Emeremen, Eastern Obolo L.G.A, AkwaIbom state, Nigeria to pyrethriod insecticides

Insecticide Papers	No. exposed	No of replicates	Mean mortality ± SD	Total mortality (%)	Status
PY control	50	2	0.50 ± 0.71^{a}	2	
Permethrin (0.75 %)	100	4	4.25 ± 0.50^{a}	17	Resistant
Deltamethrin (0.05 %)	100	4	$23.75 \pm 0.50^{a,b}$	95	Suspected resistance
Lamdacyahalothrin (0.05 %)	100	4	$23.75 \pm 0.50^{a,b}$	95	Suspected resistance
Alphacypermethrin (0.75 %)	100	4	$23.00 \pm 0.82^{a,b}$	92	Suspected resistance

Number of mosquitoes per replicate = 25

a = $p \leq 0.05$ (comparing insecticide test groups with the PY control). b = $p \leq 0.05$ (comparing other insecticide test groups with Permethrin).

Table 4: Susceptibility of female anopheles mosquito population from Agansa, Eastern Obolo L.G.A, Akwa Ibom state, Nigeria to pyrethriod insecticides.

Insecticide Papers	No. exposed	No of replicates	Mean mortality ± SD	Total mortality (%)	Status
PY control	50	2	0.50 ± 0.71	0	
Permethrin (0.75 %)	100	4	17.75 ± 0.50[a]	19	Resistant
Deltamethrin (0.05 %)	100	4	24.50 ± 0.58[a,b]	97	Suspected resistance
Lamdacyahalothrin (0.05 %)	100	4	24.50 ± 1.00[a,b]	95	Suspected resistance
Alphacypermethrin (0.75 %)	100	4	23.75 ± 0.50[a,b]	93	Suspected resistance

Number of mosquitoes per replicate = 25
a = $p \leq 0.05$ (comparing insecticide test groups with the PY control)
b = $p \leq 0.05$ (comparing other insecticide test groups with the Permethrin)

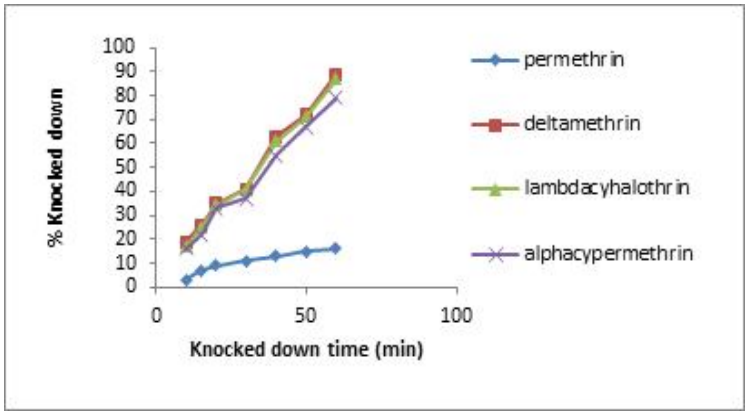

Figure 1: Knockdown rate of female *Anopheles gambiae*s.l. mosquitoes from Agansa exposed to four different pyrethroid treated papers.

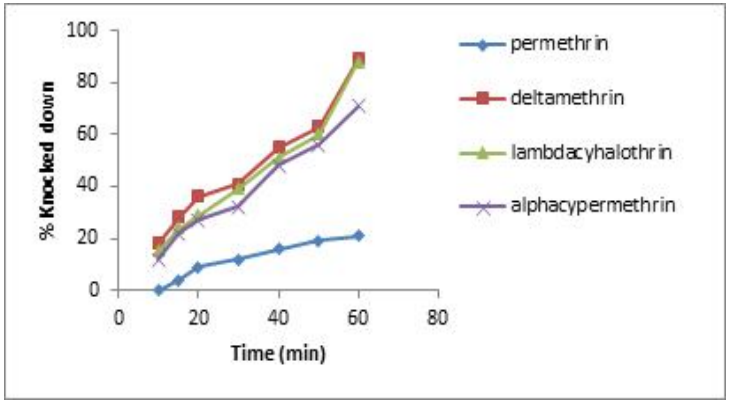

Figure **2:** Knockdown rate of female *Anopheles gambiae*s.l. mosquitoes from Emereoke 1 exposed to four different pyrethroid treated papers.

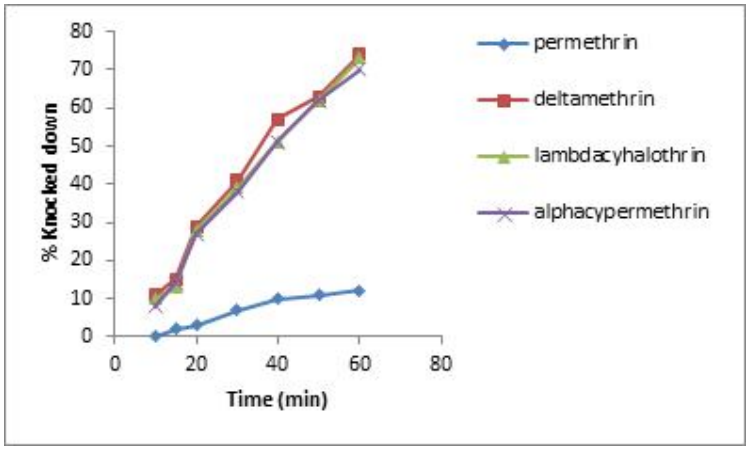

Figure 3**:** Knockdown rate of female *Anopheles gambiae*s.l. mosquitoes from Emereoke 2 exposed to four different pyrethroid treated papers

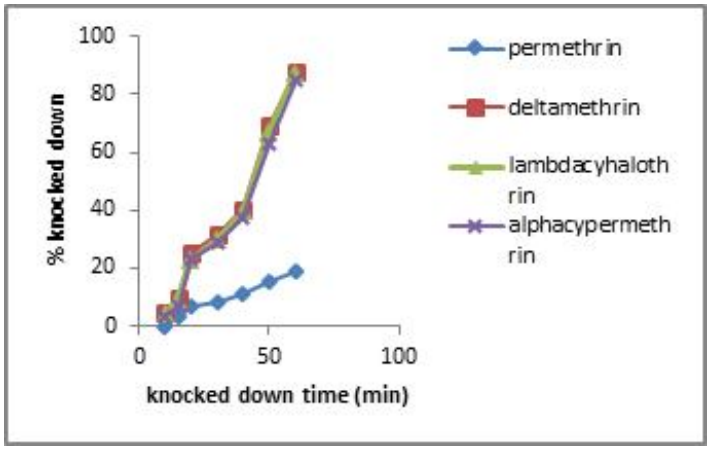

Figure 4:Knockdown rate of female *Anopheles gambiae*s.l. mosquitoes from Emeremen exposed to four different pyrethroid treated papers.

Table 5:Knockdown times (KDTs) for *Anopheles gambiae*s.l. in the four different study sites after exposure to different insecticides

Study Sites	Number exposed	Knocked down time (min)							
		Permethrin		Deltamethrin		Lamdacyhalothrin		Alphacypermethrin	
		KDT_{50} (95 % CL)	KDT_{95} (95 % CL)	KDT_{50} (95 % CL)	KDT_{95} (95 % CL)	KDT_{50} (95 % CL)	KDT_{95} (95 % CL)	KDT_{50} (95 % CL)	KDT_{95} (95 % CL)
Emereoke 1	100	159.082 (105.715 - 352.116)	1453.672 (562.670- 9718.763)	29.745 (22.505 -40.594)	168.324 (92.381- 769.784)	32.834 (25.290- 45.546)	167.740 (93.488- 722.777)	40.097 (35.632- 46.233)	257.348 (177.837- 442.982)
Emereoke 2	100	257.748 (140.674 - 1124.529)	2391.910 (678.716 - 54461.889)	35.024 (31.855- 38.872)	162.543 (125.472- 232.273)	37.077 (33.685- 41.305)	170.803 (131.012- 246.565)	38.022 (34.530- 42.417)	173.869 (133.090- 251.896)
Emeremen	100	192.354 (119.62- 519.585)	1712.162 (601.355 - 20129.238)	36.430 (29.572- 47.326)	114.992 (76.442- 282.514)	37.510 (30.979- 47.687)	114.690 (78.227- 254.810)	39.302 (32.346- 50.809)	119.786 (80.465- 283.233)
Agansa	100	579.282 (202.848- 17439.825)	34482.514 (2951.93 - 114506912.1)	25.118 (20.219- 30.684)	90.226 (63.258- 332.270)	27.273 (22.098- 33.588)	124.172 (81.449- 282.553)	29.415 (24.691- 35.360)	144.103 (96.117- 296.767)

KDT_{50}: knockdown time for 50% mosquitoes; KDT_{95}: knockdown time for 95% mosquitoes; CL: Confidence limi

This enzyme system may have conferred a metabolic resistance mechanism which helped the adult population of the vectors to resist the toxic effects of the pyrthriods used in the bioassay. The results suggest that the population of mosquitoes in the study sites may have developed resistance to insecticides or are selectively being primed to develop resistance to insecticides. Previous studies by Boyer, *et al*., (2006), Suwanchaichinda and Brattsen, (2001) and; Suwanchaichinda and Brattsen, (2002) have shown that exposure to environmental xenobiotics may enhance the development of insecticides resistance by several insect species. In addition, other studies (Hemingway *et al*., 2002; Poupardin, *et al*., 2008; Namountougou, *et al*., 2012) have also established a correlation between increase in tolerance to insecticides in many insects and induction of detoxification enzymes in mosquitoes exposed to environmental xenobiotics.

LLIN was deployed to Eastern Obolo for usage in protection against mosquitoes since 2010; and scale-up of the net distribution in these areas has been witnessed every four years (4) including the recent one done in 2018. This implies that pyrethriod treated nets have been in use till date in in the study area. Sustained susceptibility of malaria vectors is necessary for successful malaria control with insecticide treated nets. Emergence of focal points with insecticide resistance gives serious concern especially with the scale-up in distribution of pyrethriod treated nets to these areas. This is because such scale-up may increase selection pressures due to over-exposure Previous studies have revealed that the use of LLINs could enhance insecticide resistance in *Anopheles* mosquitoes (Kabula, *et al*., 2011; Umar, *et al*., 2014) to pyrethriods. The possibility of LLINs inducing changes in the adult mosquito populations thus contributing to the resistance status recorded in this study may not be ruled out.

CONCLUSION

Given the growing threat of insecticide resistance, it is essential that up-to-date data on the magnitude and distribution of insecticide resistance be collected. Currently in Nigeria, PMI supports resistance monitoring in six sentinel sites of which Akwa Ibom State is one. The areas covered by PMI project in AkwaIbom State included Mkpat Enin, Oron, Itu and Ikot Ekpene L.G.As. This study was conducted to expand resistance monitoring to the oil impacted coastal area of Eastern Obolo L.G.A of the State. The present study presents for the first time, baseline data on the susceptibility status of *Anopheles* mosquitoes from Eastern Obolo L.G.A, Akwa Ibom State, South-south Nigeria to pyrthriods commonly used for bed nets treatment. This will guide in planning site specific integrated vector management program.

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