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Phenotypic Physiognomies and Malaria Vectors KDT to Certain Insecticides in Some Populations of Anambra State, Southeast Nigeria

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ABSTRACT

Background: *Malaria which is a disease that is transferred by disease-ridden female Anopheles mosquitoes remains a foremost unrestricted health problem in Nigeria particularly and the world at large. Obviously, innumerable classes of insecticides have frolicked substantial starring role in the management and elimination of malaria by confronting the malaria vectors. Regrettably, the achievement of the use of insecticides in the effective and efficient control of malaria vectors is endangered by the development of resistance to the insecticides in malaria-endemic areas such as Anambra State, Southeast part of Nigeria. The current study was hence aimed at determining the phenotypic physiognomies and subsequent knockdown times (KDT) of the vectors to certain insecticides in some communities of Anambra State, Southeast Nigeria whose results will be used for effective management and control.*

Methods: *Mosquitoe larval samples were collected from diverse habitats using the dipper method and reared to adults for susceptibility test using the test-kits recommended by World Health Organization (WHO). During the exposure of the vectors to insecticides, records were taken of the number of mosquitoes knocked down intermittently after 10, 15, 20, 30, 40, 50 60 minutes and 24 hours post exposure. One-way analysis of variance (ANOVA) and log-probit regression analysis were used to calculate the knockdown times ($KDT_{50,}KDT_{90,}$and KDT_{95}) whereas multiple comparisons of mortality and knockdown rate were done using the least significant difference (LSD) test.*

Results: *The result disclosed that Anopheles gambiae with a prevalence of (54.2%) was the leading vector, tailed by Anopheles coluzzii which recoreded (45.8%) in the survey area. Percentage mortality of Anopheles gambiae s.l. after 24 hours was 100% in Malathion (Organophosphate) and Bendiocarb (Carbamate) in all the study areas. Mortality rate in Permethrin (Pyrethroid) ranged from 62%-65% and for Deltamethrin (Pyrethroid) was 54%-63% across the study areas. In Awka South LGA, knockdown times ($KDT_{50,}KDT_{90,}$and KDT_{95}) were: 170 minutes, 396.5 minutes, and 501 minutes, respectively. While in Awka North LGA, the ($KDT_{50,}KDT_{90,}$and KDT_{95}) were: 199 minutes, 478 minutes and 602 minutes, whereas in Njikoka LGA, the ($KDT_{50,}KDT_{90}$and KDT_{95}) were: 186 minutes, 436.5 minutes and 562.3 minutes, respectively.*

Conclusion: *High knockdown times to Malathion and Bendiocarb insecticides was repeatedly detected in the study areas. This is a pointer that the use of Pyrethroids is efficient for insecticidal control of malaria vectors. Nevertheless, low knockdown times to Deltamethrin and Permethrin is a plank in the fight against the vectors. To that effect, there is an imperative requirement for implementation of resistance management approaches alongside insecticide resistance monitoring in the State to avert the increasing resistance rate as was detected in the study.*

Keywords: *Anambra State, communities, knockdown times, malaria vectors, Nigeria*

Introduction

The malaria issues in Nigeria particularly and Africa at large has triggered terrific health dares to the general public where their vectors transmits diseases to humans especially pregnant women and children which are the most susceptible group because of their little or no resistance to the parasitic disease [1]. In the combat to both manage and except malaria and its resultant vectors, some groups of insecticides such as organochloride, organophosphate, carbamate, and pyrethroid have been ratified for Indoor Residual Spray (IRS), nonetheless only pyrethroids are currently certified in Insecticide Treated Nets (ITNs) usage. The reason for this was because of their squat mammalian poisonousness and extraordinary insecticidal effectiveness as reported by [2]. Over time, this presence of resistance has triggered series of insecticide changes, ranging from DDT to pirimiphosmethyl, lambda-cyhalothrin, dieldrin, malathion, propoxurand deltamethrin to the current time [3].

It is on record that malaria vector-human contact has been reduced by the availability and usage ofI TNs and IRS [4], nevertheless, insects have development both behavioral change and physiological resistance leading to selection pressure [5]. In all of these *Anopheles gambiae* sensu lato (*s.l.*) and *Anopheles funestus s.l.* which are the major two African malaria vector groups most effectively embattled by ITNs have equally widespread insecticide resistance. Insecticide resistance incorporates either physiological, biochemical, molecular and behavioral mechanisms as reported by [6]. Physiological resistance and other multiple mechanisms like the one which are caused by knockdown resistance (*kdr)* mutations in the *para*-type sodium channel gene are ways through which mosquitoes evade pyrethroids and other insecticides [7]. Development of resistance to both DDT and pyrethroids have been established in Nigeria and other Afro-tropical countries of the world owing to that reason, this would definitely have substantial consequences for the success of vector survilliance, monitoring and other interventions that is currently ongoing [8]. Hence, the stout need that field populations of Anopheline mosquitoes be monitored by the development of suitable apparatuses. This strategy will definitely benefit the target populace by eliminating malaria in the environment. Regretably, the control of malaria vectors will always be dragged behind by resistance to insecticides and there is urgent need to improve understanding of the underlying mechanisms of resistance to manage and curtail the situation [9]. The fight to ascertain resistance genes and DNA makers have been subdued by target-site resistance and behavioral changes or thickening of the cuticle. Whereas candidate gene and quantitative trait locus studies to solve this problem is not only anticipated but achiavable. Although DDT and pyrethroids which are insecticides commonly used for malaria control share a common target site, [10] but then again knockdown resistance (kdr) mutations in this *para* voltage-gated sodium channel can consequently confer cross-resistance to both pyrethroids, DDT and any other insecticides on that particular group as observed by [11, 12]. The enhancement of monitoring and programmatic policymaking in *Anopheles* mosquito studies can make a positive swing toward the potential identification of resistance markers [13]. Regardless of various malaria interpositions which include but not limited to the following; IRS, distributions of LLINs, and prompt and active treatment with antimalarials which have been rolled out by the World Health Organization in its functioning regions, malaria still remains is still a leading challenge in the health sector which requires uninterrupted studies in the struggle to both manage and eradicate the disease and its concurrent vectors. This is urgent and critical especially now that we are close to the pre-elimination stage of the disease.

Materials and Methods

Study area

Malaria Vectors investigation was undertaken in three local government areas of Central Senatorial Zone of Anambra State, Southeast Nigeria namely: Akamanator and Amaezike communities of Mgbakwu in Awka North L.G.A., Umuanum and Ezeawulu communities of Nibo in Awka South L.G.A., and then Ifite and Umu Agidi communities of Enugwu Agidi in Njikoka L.G.A. The State is located between Latitude 6˚ 12'45.68''N to Latitude 6˚ 22'45.68''N and Longitude 7° 04'19.16''E to Longitude 7° 15'19.16''E of Greenwich. It has an average daily temperature of 26.8 °C / 80.2 °F as recorded by [14]. Still, Awka South LGA is located between Lat. 6˚ 10' North to Lat. 6˚ 23' N of the equator and Long. 7° 04' E to 7° 44' E of Greenwich, Awka North is located between Lat. 6° 15' N to 6° 35' N of equator and Long. 7°10' E to 7°30' E of Greenwich while Njikoka LGA is lying between Lat. 06° 20' 58 '' N to 06° 21' 00'' N and Long. 06° 12' 55''E to 06° 52' 55''E of Greenwich. The predictable population of 2020 for Anambra State is 6,182,924 as postulated by [15]. Anambra State experiences a rainy season which falls (between April and October) and the dry season which cascades (between November and March) as its seasonal climatic condition. The areas experience a petite spell of harmattan between November and January when the atmosphere is generally misty which is a period of cold weather [16]. The State has 11,450mm and above as its total annual rainfall for the six to seven months of the rainy season although the mean annual temperature ranges between 30.0–36.0 °C as reported by (Microsoft Encarta, 2009). Even though the English language is widely enunciated throughout the State as a secondary language, the indigenous Anambra State people are Igbo-speaking folks as their official language [14]. They cultivate yams, cassava, corn (maize), palm oil and kernels and other agricultural products which are both consumed and equally traded. This is possible because the soil in the State with its good vegetation sustains agricultural activities as reported by [14]. The current research covered the two main seasons of the year and was undertaken between September 2021 to August 2022 in Anambra State Southeast Nigeria.

Study design

The study design used for the current research was a completely randomized design (CRD) which comprised four treatments each of which was replicated four times with a control treatment on the laboratory bench. Each replicate was tagged as T1, T2, T3, and T4 whereas the control was tagged C1 and C2.

Mosquito larvae collection

The collection of Larval were implemented on a monthly basis for three successive days from 9:00am to 12:00pm for twelve months of the year, it covered both rainy and dry seasons. Diverse water bodies were critically examined like catchment pits, gutters, puddles, tyres, streams and river bodies, tyre tracks, containers, crab holes, excavations, hoof prints and rice fields for mosquitoes, especially malaria vectors. To ensure that each site in the areas were explored in the course of sourcing for mosquito larvae, ladles and pipettes were used during the study. Uneven debris that was collected alongside the larvae during the survey like sticks and plant leaves were carefully handpicked and thrown away. A mesh size sieve of 0.55mm was engaged during the study to distinguish the larvae from other debris. The reason for this usage was to ensure a comprehensive collection of mosquito populations of all possible vectors breeding in the study areas. This was achieved by embarking on wide-ranging larval sampling at the course of the investigation [17].

Rearing of mosquito larvae

A labelled container provided was used to keep all mosquito larvae collected. This was retained in cork plastic containers and labeled according to the site of collection. The time and date of the collection were also noted before they were reared to adult stages. A provision of yeast in 500 ml larval bowls was made which was covered with a transparent net was used to fed the larvae.

When the adult emerged, an aspirator was used to re-assigned the mosquitoes to cages. These newly emerged adults were fed with a 10% sugar solution saturated in cotton wool which was dropped on top of the mosquitoes cage.

World Health Organization (WHO) tube insecticide resistance bioassay tests

Insecticide susceptibility resistance bioassay tests were carried out using WHO-impregnated/control papers test-kits and standard techniques that were delivered by Universiti Sains Malaysia (USM), Penang, Malaysia. As described by [18], four (4) replicates of 3-5 day-old 25 non-blood-fed adult female mosquitoes were used for the study. The assays were carried out in consignments by exposing the adults of *An. gambiae*s from all sites in the study areas to impregnated papers which were achieved with a recommended concentration of a given insecticide in the assay tubes. although, for the control, paper without insecticide that was treated with paraffin oil was used. 0.75% Permethrin, 0.05% Deltamethrin, 0.1% Bendiocarb and 5% Malathion were the following insecticides that was used for the survey. Each replicate of 25 mosquitoes in each tube were exposed to the insecticide-impregnated filter paper for the total duration of time recommended. A total of 150 female non-blood-fed mosquitoes were exposed for each insecticide (using four replicates). (100 mosquitoes for the test tubes and 50 mosquitoes for the controls, using two replicates of 25 mosquitoes each). As the exposure period lasts, the number of mosquitoes knocked down were recorded after 10, 15, 20, 30, 40, 50, 60 minutes and 24 hours subsequently. After the experimental time lapses, the mosquitoes were subsequently transferred into the holding tube and fed with glucose solution via a pad of cotton wool soaked in 10% glucose solution. This solution is placed on the mesh-screen end of the holding tubes while the time taken to achieve 50%, 90%, and 95% of population knockdown ($KDT_{50,}$ $KDT_{90,}$ and KDT_{95}) were taken. Further evaluation was done using log-probit analysis. The mortality rate was determined after 24 hours post-exposure by counting the number of dead and alive mosquitoes on each tube. For an adult mosquito to be considered to be alive, it must be able to fly willy-nilly of the number of legs remaining. Whereas any other knocked-down mosquito, whether or not it has lost a leg or wing, was considered and counted as dead. The condition for mosquitoes to be classified as either dead or knocked down was that they were immobile or even unable to stand or finally take off.

NB: Abbott's formula was used in the study to precise the observed mortality when the mortality in the control is sandwiched between 5–20% as described by [19].

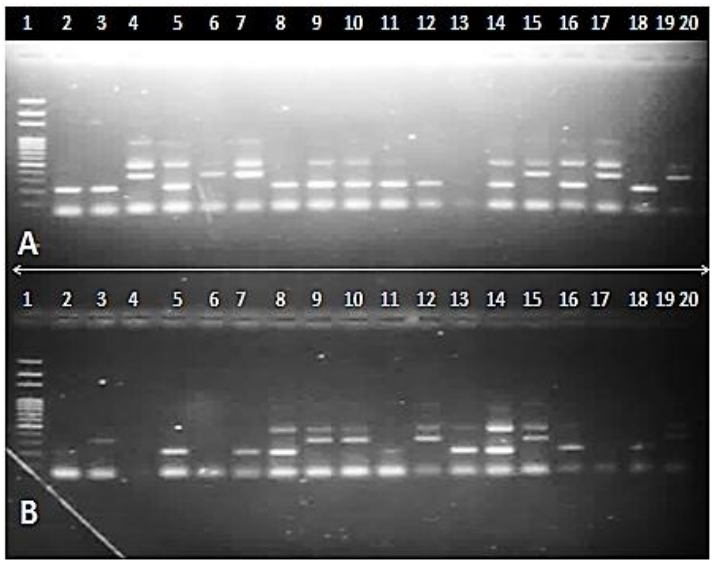
Analysis of data

Records acquired from the survey were precised and scrutinized using comparative and descriptive statistics.

Also to compare the differences between means, one-way analysis of variance (ANOVA) was used whereas the least significant difference (LSD) test was used to compare the level of significance at 0.05% level of significance. Abbot Formula was used for the correction of mortality while Log-probit regression analysis was used to determine the $KDT_{50,}$ KDT_{90} and KDT_{95}.

Results

Plate 1: The characteristic PCR plate of Agarose gel electrophoresis species identification for Anopheles gambiae s. l. complex in the study area



Agarose gel electrophoresis species identification for *Anopheles gambiae s. l.* complex.

(A): Lane 1 standsfor ladder of DNA whereas Lanes 18, 12, 8, 3 and 2 were products of *An. coluzzii* and then Lanes 17, 16, 15, 14, 11, 10, 9, 7, 6, 5 and 4 were products of *An. gambiae* whereas Lanes 20, 19 and 13 were unamplified and thus unidentified.

(B): The Lane 1 stands for ladder of DNA. Lanes 16, 11, 7, and 5 were products of *An. coluzzii* while Lanes 15, 14, 13, 12, 10, 9, and 8 were products of *An. gambiae* whereas Lanes 20, 19, 18, 17, 6, 4, 3 and 2 were unamplified and thus unidentified as shown in Plate 1.

The Mean percentage knockdown time of *An. gambiae s. l.* that was exposed to different insecticides at varying time intervals in Awka South Local Government Area of Anambra State

In Awka South LGA, the KDT_{50} time engaged for 50% of the mosquitoes tested to be knocked down, the KDT_{90} time engaged for 90% of the tested mosquitoes to be knocked down, and KDT_{95} time taken for 95% of the tested mosquitoes to be knocked down were detected: The means of four replicates (±s.e), df=3, Pv=0.011, Means of Eight replicates was (±s.e), df=7, Pv=0.021 as shown in Table 1.

Table 1. The Mean percentage knockdown time of An. gambiae s. l. that was exposed to different insecticides at varying time intervals in Awka South Local Government Area of Anambra State

Insecticides/ Concentrations	Exposure Period (Time)										
	10mins	15mins	20mins	30mins	40mins	50mins	60mins	24hrs	Mean (±s.e)	% Mortality	Probit
Delthamethrin 0.05%	0.00	0.00	0.00	0.75	3.75	8.50	10.50	15.50	4.87±2.10	62.00	5.24
Permethrin 0.75%	0.25	0.25	0.25	0.25	0.50	0.50	0.75	16.25	2.37±1.98	65.00	5.33
Malathion 5.0%	0.00	0.25	3.25	10.00	22.25	25.00	25.00	25.00	13.84±4.11	100.00	8.12
Bendiocarb 0.1%	0.00	0.00	9.00	24.75	25.00	25.00	25.00	25.00	16.71±4.13	100.00	8.12
Control	0.00	0.00	0.00	0.00	0.00	0.75	1.00	1.25	0.25±1.12	5.00	
Mean (±s.e)	0.63±0.63	0.13±0.07	3.13±2.09	8.94±5.13	12.88±6.27	14.62±6.18	15.31±5.94	20.43±2.64			
% Mortality	0.25	0.50	12.5	35.75	51.5	59.0	61.25	81.25			
Probit	-3.35	-3.32	3.58	4.54	4.97	5.02	5.32	5.85			

The means of four replicates (±s.e), df=3, Pv=0.011, Means of eight replicates was (±s.e), df=7, Pv=0.021

The Mean percentage knockdown time of *An. gambiae s. l.* that was exposed to different insecticides at varying time intervals in Awka North Local Government Area of Anambra State

In Awka North LGA, the KDT_{50}time taken for 50% of the tested mosquitoes to be knocked down, KDT_{90} time taken for 90% of the mosquitoes tested to be knocked down, and KDT_{95} time taken for 95% of the mosquitoes tested to be knocked down. Means of (4) Four Replicates (±s.e), df=3, Pv=0.002, Means of eight (8) replicates (±s.e), df=7, Pv=0.106 as shown in Table 2.

Table 2. Mean percentage knockdown time of An. gambiae s. l. exposed to different insecticides at varying time intervals in Awka North Local Government Area of Anambra State

Insecticides Concentrations	Exposure Period (Time)										
	10mins	15mins	20mins	30mins	40mins	50mins	60mins	24hrs	Mean (±s.e)	% Mortality	Probit
Delthamethrin 0.05%	0.00	0.00	0.25	1.50	2.00	2.50	2.75	13.50	2.81±1.57	54.00	5.03
Permethrin 0.75%	0.00	0.50	0.75	1.00	1.00	1.00	1.00	5.50	1.34±0.61	22.00	4.08
Malathion 5.0%	0.00	0.00	3.00	17.25	24.75	25.00	25.00	25.00	15.00±4.21	100.00	8.12
Bendiocarb 0.1%	0.00	0.25	8.75	19.50	25.00	25.00	25.00	25.00	16.06±3.99	100.00	8.12
Control	0.00	0.00	0.00	0.00	0.00	0.75	1.00	1.25	0.25±1.12	5.00	
Mean (±s.e)	0.00±0.00	0.18±0.12	3.18±1.95	9.81±4.97	13.19±6.75	13.38±6.72	13.43±6.68	17.25±4.76			
% Mortality	0.00	0.75	12.75	39.25	52.75	53.5	53.75	69.00			
Probit	-3.38	-3.30	3.60	4.64	5.00	5.02	5.03	5.45			

Means of Four Replicates (±s.e), df=3, Pv=0.002, Means of eight replicates (±s.e), df=7, Pv=0.106

Mean percentage knockdown time of *An. gambiae s. l.* exposed to different insecticides at varying time intervals in Njikoka Local Government Area of Anambra State

In Njikoka LGA, the KDT_{50} time taken for 50% of the mosquitoes tested to be knocked down,KDT_{90} time taken for 90% of the mosquitoes tested to be knocked down, KDT_{95} time taken for 95% of the mosquitoes tested to be knocked down were observed. Means of Four (4) Replicates (±s.e), df=3, Pv=0.001, Means of eight (8) replicates (±s.e), df=7, Pv=0.142 as shown in Table 3.

Table 3. Mean percentage knockdown time of An. gambiae s. l. exposed to different insecticides at varying time intervals in Njikoka Local Government Area of Anambra State

Insecticides Concentrations	Exposure Period (Time)										
	10mins	15mins	20mins	30mins	40mins	50mins	60mins	24hrs	Mean (±s.e)	% Mortality	Probit
Delthamethrin 0.05%	0.00	0.00	0.00	0.50	0.75	0.75	1.00	15.75	2.34±1.92	63.00	5.28
Permethrin 0.75%	0.00	1.25	1.50	1.75	1.75	1.75	2.50	10.50	2.62±1.15	42.00	4.71
Malathion 5.0%	0.00	0.00	17.25	23.25	25.00	25.00	25.00	25.00	17.56±3.94	100.00	8.12
Bendiocarb 0.1%	0.00	1.00	11.25	15.75	23.50	25.00	25.00	25.00	15.81±3.79	100.00	8.12
Control	0.00	0.00	0.00	0.00	0.00	0.75	1.00	1.25	0.25±1.12	5.00	
Mean (±s.e)	0.00 ± 0.00	0.56±0.33	7.50±4.09	10.69±5.67	12.75±6.65	13.12±6.86	13.37±6.72	19.06±3.59			
% Mortality	0.00	2.25	30.00	41.25	51.00	52.5	53.5	76.25			
Probit	-3.36	-3.08	4.36	4.69	4.95	5.00	5.02	5.67			

Means of Four Replicates (±s.e), df=3, Pv=0.001, Means of eight replicates (±s.e), df=7, Pv=0.142

Discussion

Considering the species identification of malaria vectors in three Local Government Areas of Anambra State, Southeast Nigeria under study, the total number of two thousand, eight hundred and seventy (2,870) malaria vectors gathered together in the study was relatively low compared to the mosquitoes trapped by [20] in Papua-New Guinea where female *Anopheles* mosquitoes of seven different species totalling the number of 7,146 which includes: *Anopheles hinesorum*, *Anopheles koliensis*, *Anopheles longirostris*, *Anopheles bancroftii*, *Anopheles farautis.s*, *Anopheles farauti* and *Anopheles punctulatus s.s* were collected, including 2,611 (36.5%) blood-fed and 4,535 (63.5%) unfed mosquitoes. The high number of malaria vectors in the slated study could probably be because mosquitoes were sampled daily (consistent assessment) from dawn to dusk in five villages for four years (2012 to 2016) whereas the contemporary survey sampled only three communities within twelve calendar months. The seemingly low abundance of malaria vectors in the present study may be connected with the mean diurnal temperature range that increases the species sensitivity to probably changes in climates, leading to low presence of *Anopheles* species as also reported by the studies done by [21 and 22].

Molecular identification of malaria vectors collected from the three Local Government Areas comprised of 54.2% of *Anopheles gambiae* and 45.8% of *Anopheles coluzzii* even though there was no significant difference. The outcome was in agreement with previous studies done by [23, 24, 25 and 26] where the model results showed that *An. gambiae*, *Anopheles coluzzii* and *An. arabiensis* are widespread across all ecological zones in Nigeria including Anambra State where the current study was undertaken. Study findings also acclaimed that diverse malaria vectors breeds concurrently with each other in all the LGAs surveyed and these species co-exist in sympatric relationships as also reported by the survey of [27]. Coincidentally, this report is probably the second-time concurrent breeding for the two sibling species (*Anopheles gambiae* and *Anopheles coluzzii*) has been reported in enormous measure synchronized study in the Eastern part of Nigeria after the research reported by [28].

The malaria vector knockdown times in the existing study showed that the mean percentage knockdown of *An. gambiae s.l* exposed to different insecticides at varying time intervals in Awka South LGA Anambra State as follows: delthamethrin (0.05%) was 62%, permethrin (0.75%) was 65%, whereas malathion (5.0%) and Bendiocarb (0.1%) both recorded 100% with KDT50, KDT90, and KDT95 of 170 minutes, 396.5 minutes and 501 minutes respectively. Also the mean percentage knockdown of *An. gambiae s. l.* exposed to different insecticides at varying time intervals in Awka North LGA Anambra State recorded 54% for delthamethrin (0.05%), 22% for permethrin, whereas malathion (5.0%) and bendiocarb (0.1%) both recorded 100% with KDT50, KDT90 and KDT95 of 199 minutes, 478 minutes and 602 minutes respectively. Whereas the mean percentage knockdown of *An. gambiae s.l.* exposed to different types of insecticides at varying time intervals in Njikoka LGA Anambra State were as follows: delthamethrin (0.05%) was 63%, permethrin was 42%, whereas malathion (5.0%) and Bendiocarb (0.1%) both recorded 100% with KDT50, KDT90 and KDT95 of 186 minutes, 436.5 minutes and 562.3 minutes. In this study, the mean percentage knockdown of *An. gambiae s.l.* exposed to different insecticides at varying time intervals in all the LGAs showed that malaria vectors were fully susceptible to bendiocarb alongside with malathion. Outcomes of this current study substantiated by means of the findings in Amansea, a community in Awka North LGA of Anambra State where malaria vectors were only susceptible to bendiocarb which falls into the group of carbamates as reported by [29]. It's recorded with substantiated data that between the year (2014 and 2015), the populations of malaria vectors showed the greatest resistance against DDT and later pyrethroids [30] and by the next year 2016, evidence-based data on resistance to at least one insecticide was reported from more than 80% of malaria-endemic countries which Nigeria is included. This is a serious threat to the continued efficacy and potency of key malaria management strategies. This probably may be due to excessive use of the pyrethroid group of insecticide before now as reported by [31]. Also widely distribution of LLINs in South-East Nigeria for more than ten years now couple with the information that the pyrethroids group of insecticides are the only group of insecticides used in LLINs may possibly to have goaded the condition. Transversely the three LGAs, Malathion (5%) and Bendiocarb (0.1%) performed better than other insecticides used in the present survey. This possibly will not be contemplative of the global presentation of the diverse insecticides in the pyrethroid class since Deltamethrin existence as a type II pyrethroid comprises an alpha-cyano group

permitting it to wield an enhancedcarnage consequence on insects as also postulated by[32]. Nonetheless, the comparative impression of dwindled mosquito vulnerability to pyrethroids on vectorial capability remains unidentified in the study areas largely due to a paucity of field data which other further studies have to address.

Conclusion and recommendations

Although extensive *Anopheles* species resistance to pyrethroid has been reported in this study, there is unquestionably a foremost need for timely management of mosquitoes vectors particularly as it edges the challenge readily accessible to accomplish the objective of scheming the diseases mosquitoes cause. The results on the Insecticide Resistance across these three LGAs revealed that *An. gambiae s. l.* populations were fully susceptible to malathion which is an organophosphate and bendiocarb which is a carbamate but resistant to the pyrethroids and permethrin and this may thwart the impact and continued effectiveness of pyrethroids insecticide in malaria vector control as time progresses. Also, the annotations in the present study showed an extensive resistance to one or more of the classes of insecticides used in public health in the LGAs in Anambra State, Nigeria. It is thereby observed that insecticide resistance will likely have weighty operational impact in the LGAs if no pre-emptive action is taken in Anambra State predominantly and Nigeria at large.

Whereas the world wide obligation to eradicate malaria by 2030 necessitates immediate hard work that includes but not limited to the establishment of development of a combination of operative and effectual vector control strategies and interpositions in parasite control. Henceforward, a crucial necessity for unceasing and stretched susceptibility/insecticide resistance monitoring in the 3 LGAs particularly is not only immenent but urgent. This will assist in gaining a larger and purer impression of the condition, and how to tackle it. It is of great importance that new insecticides with extraordinary susceptibility status be made to assist in the management and reduction of the bearing of resistance in malaria vectors as observed in the current study.

Author contributions: OVE, EOO and DEN wrote the first draft and edited the manuscript, EOO and CIN developed the protocol, UCU and IEO oversaw study implementation and monitoring. EOO and DEN did the experimental design and molecular characterization of the malaria vector samples.

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Competing interest: The writers assert that no struggles of interest exist amongst them.

Ethical approval and informed consent

We approve that all procedures used in the study were undertaken in harmony with the appropriate procedures, protocols and regulations as accepted by the committee on ethics and human research. Research informed consent was sought and gotten while ethical approval was also acquired from the office of the Director, Nnamdi Azikiwe University that is in ofcharge of Human Research Ethics Committee with study Research Reference Number: NAU/HREC/2S/02/12/2023/05. The study partakers were properly organized and study objectives were explained to them by a sensitization rally before the commencement of the research. Participation in the survey was voluntary and participants had the liberty to withdraw from the study.

Data availability: The data used for this study are available upon reasonable request.

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