International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 23-02-2022, Accepted: 09-03-2022, Published: 25-03-2022
Volume 7, Issue 3, 2022, Page No. 133-137

Insecticide resistance status of *Anopheles gambiae* s.l in Amechi Idodo, a rural community in Enugu State, Nigeria

Nwabor E A[1, 3], Aribodor D N[1], Nwangwu U[2]
[1] Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
[2] National Arbovirus and Vector Research Centre, Enugu, Enugu State, Nigeria
[3] Department of Parasitology and Entomology, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria

Abstract
A study of the insecticide resistance status of *Anopheles gambiae* complex in Amechi Idodo, a rural community in Enugu State, was conducted from October, 2018 to November, 2020. The sampling of mosquitoes was done in all four villages (Eziobodo, Ohuani, Eziama and Obunagu) that make up Amechi Idodo community. *Anopheles* larvae were collected from diverse natural breeding sites including puddles, ditches rice fields, among others, with the aid of ladles, dippers and pipettes. They were reared to adults in the laboratory. Susceptibility of *Anopheles gambiae* to insecticides (DDT 4%, Pirimiphos-methyl 0.25%, Deltamethrin 0.05% and Bendiocarb 0.1%) was assessed using the WHO susceptibility test protocol. Susceptibility test showed that Bendiocarb recorded the highest mortality rate 98.75%, while DDT recorded the least 5.0%. Deltamethrin and pirimiphos-methyl recorded 8.75% and 10.0%, respectively. *Anopheles gambiae* was susceptible to only Bendiocarb. On the contrary, they were highly resistant to DDT, deltamethrin and pirimiphos-methyl. There is need for adequate insecticide resistance management to restrain the growing threat of insecticide resistance in Amechi Idodo.

Keywords: vectors, anopheles gambiae s.l, larvae, insecticide, resistance, bendiocarb, DDT, deltamethrin

Introduction

Anopheles mosquitoes are efficient vectors of malaria parasites. The vectors also constitute biting nuisance to humans (Aribodor, 2012) [1]. The frequency of malaria disease is related to the abundance of the vectors and malaria vectors are characterized by their biological diversity (WHO, 2015). In recent times, insecticide resistance is prevalent among malaria vectors in Africa and is expected to menace the success of malaria control (Salihu and Sanni, 2013) [25]. In Democratic Republic of Congo, malaria vectors resistance to pyrethroids insecticides was recorded (Wat'senga *et al*, 2018) [28]. In Zambia, Chinula *et al*, (2018) [4] reported that the effectiveness of long-lasting insecticidal-treated nets (LLINs) and indoor residual spraying (IRS) for malaria control is threatened by resistance to commonly used pyrethroid insecticides. Thomsen *et al*., (2017) [27] reported that there is a shift in *Anopheles farauti* biting times to early morning several years after mass pyrethroid-based LLIN distribution in Papua New Guinea. In Benin Republic and Senegal, changes in biting behaviour were also recorded for *An. funestus* following the same pyrethroid-based LLIN universal coverage campaigns (Moiroux *et al*., 2012; Sougoufara *et al*., 2014) [26, 16]. Mosquitoes resistance to pyrethroids insecticides has become the latest threat to eradicating malaria in Nigeria, where thousands of people die each year from the killer disease (WHO, 2020). PMI (2021) reported a pervasive pyrethroid resistance across Nigeria, while Chukwuekezie *et al.* (2020) [5] reported malaria vectors resistance to deltamethrin and DDT in five South-East states of Nigeria.

One of the measures for control of mosquitoes that transmit malaria parasites is the interruption of disease transmission by killing or preventing mosquitoes from biting man and animals (Egunyomi *et al*, 2010). Therefore, insecticide-based vector control still represents the most crucial and affordable method to ameliorate the malaria burden, by interrupting the transmission cycle (Zofou *et al*, 2014), although insecticide-resistance frequency and intensity have increased dramatically in malaria vector populations (Karunamoorthi, 2013). This study determined the insecticide resistance status of malaria vectors in Amechi Idodo community to the four major classes of insecticides approved by the World Health Organization.

Materials and Methods

The study area was Amechi Idodo, a rural community in Nkanu East Local Government Area of Enugu State, South-eastern Nigeria. Amechi Idodo Community is situated between Longitude 7^0 39'E and Latitude 6^0 20'N (wikipedia.org). Amechi Idodo is in the tropical rainforest zone of Nigeria and experiences two distinct seasons (Wet and dry seasons). The monsoon winds from the Atlantic Ocean creates eight months of heavy tropical rains between April and November and is followed by four months of dryness (December - March) due to North eastern dry wind from the Sahara Desert.

There is also a short period of cold and dryness (Harmattan) at the beginning of the dry season. This is particularly windy and dusty and starts in late December or in the early part of January. The community is about 155m above sea level. Amechi Idodo community has a population of 12,397 inhabitants (NPC, 2006) and an estimated population of 16, 406 in 2017.

Amechi Idodo Community is made up of four villages, namely, Eziobodo, Ohuani, Eziama and Obunagu. The community is still a rural area as the local council that was formed around 1983 has not advanced the area very much in terms of basic amenities like electricity, hospitals, modern housing and schools. The inhabitants of the area are mainly farmers, civil servants and traders. The agricultural produce of the area include rice, cassava, yam, maize, palm produce, plantain and coconuts. Their method of cultivation which is heaps and ridge making makes room for collection of water in the ridge furrows and spaces between sand heaps thereby creating breeding sites for mosquitoes especially during the rainy season. Also, some of the villages have streams. Each of these streams has freshwater swamps that serve as mosquito breeding sites. Prior to this study, there has been an effort to control malaria vectors in Amechi Idodo community through government intervention programmes by distribution of LLINs in the last decade.

Study Design

The study is a field survey of larvae and pupae of *Anopheles* mosquitoes reared to adults in the laboratory and eventually exposed to different classes of insecticides. Randomized controlled trial was employed with communities as units of study. The study was conducted from October, 2018 to November, 2020.

This is a cross-sectional, observational study. Larval sampling was embarked upon across the four villages that make up the community. This was done in quick succession, such that enough larvae were collected from the four villages, within the shortest possible time for the tests. All the collections were gathered in rearing bowls, reared to adults and utilized for the test once they were of the standard age. The WHO test procedure was utilized for all the tests.

Advocacy visits and Community Sensitization

Advocacy visits to community leaders including the traditional ruler to elicit the cooperation of the community members was done. This was facilitated with an introductory letter from the Head of the Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka. The entire community was also sensitized through meetings organized with the aid of the community leaders, as the project objectives and the methodologies were explained to them. Their oral consent was obtained for the study.

Selection of sampling Area

Larval sampling was carried out in Eziobodo, Ohuani, Eziama and Obunagu villages of Amechi Idodo community. This was for proper representation of all parts of the community.

Collection and Rearing of Larval stages of Mosquitoes

The breeding habitats of the mosquitoes were determined through collection of mosquito larval stages from standing water pools as described by (Onyido *et al*., 2016). *Anopheles* larvae were collected using the standard dipping and scooping method as described by Onyido *et al.* (2016). Larvae were aggregated in well-labelled plastic containers and transported to the Entomology Laboratory of National Arbovirus and Vectors Research Centre (NAVRC), Enugu, where they were reared to adults for susceptibility test. The larvae were reared at room temperature and fed with poultry feed. Once they pupated, pupae were transferred into plastic cups using a disposable pipette. The cups and their contents were introduced into labelled cages for adult emergence. All adults were fed on 10% sugar solution.

Morphological Identification of Collected Mosquitoes

The mosquitoes subjected to test were eventually identified using the morphological keys of Gillies and de Meillon (1968), Gillet (1972), Gillies and Coetzee, (1987).

Insecticide Susceptibility Assays

The test for insecticide susceptibility status of mosquitoes was carried out as described by WHO, 2013.

According to the guidelines 98-100% mortality range indicates susceptible mosquito population, 90-97% mortality range indicates suspected resistance. Mortality of below 90% indicates existence of resistance. Female *Anopheles* mosquitoes reared in the laboratory were held for one hour in resting tubes and were supplied with 10% glucose solution before testing. The test was performed using WHO test kit and methods for measuring mosquito susceptibility (WHO, 2013). Non-blood-fed 2–5-day old females were exposed to one insecticide from each of the four major classes of insecticides. Insecticides used were 4% DDT (organochlorine), 0.1% Bendiocarb (carbamate), 0.05% Deltamethrin (pyrethroids) and 0.25% pirimiphos-methyl (organophosphate). A total of 80 mosquitoes were tested for each insecticide. This was done in four replicates of 20 mosquitoes each. For each test, two controls of 20 mosquitoes were exposed to the control/untreated papers.

The mosquitoes knocked down after 10, 15, 20, 30, 40, 50, 60 minutes were recorded. After the exposure, all knockdown and surviving mosquitoes were transferred to the holding tubes. They were fed with 10% glucose solution and allowed for a 24-hour recovery period. Mosquitoes were recorded as either dead (susceptible) or alive (resistant) after the recovery period (24 hours).

Data Analysis

Data collected from the study were summarized and later analysed using the Statistical Package for Social Sciences (SPSS) version 23 for analysis of variance (ANOVA) at 5% significant level. The knockdown times for 50% and 95% of the tested mosquitoes (KDT_{50} and KDT_{95}) were analyzed using probit analysis. The mortality of test samples was calculated by summing the number of dead mosquitoes after 24 hours across all four replicates and expressing this as a percentage of the total number of exposed mosquitoes.

$$\text{Observed mortality} = \frac{\text{Total number of dead mosquitoes} \times 100}{\text{Total sample size}}$$

Experiments that had more than 5% death in control were corrected using the Abbott's formular (WHO, 1998).

$$\text{Abbott's formular} = \frac{(\text{\% observed mortality- \% control mortality}) \times 100}{(100 \text{ -\% control mortality})}$$

Results

Bendiocarb recorded the highest mortality rate, 98.75%, while DDT recorded the least, 5.0%. Deltamethrin and Pirimiphos-methyl recorded 8.75% and 10.0%, respectively. Thus, the population of *Anopheles* mosquitoes tested were susceptible to only Bendiocarb. They were resistant to DDT, Deltamethrin and Pirimiphos-methyl (Table 1).

Table 1: Efficacy of insecticides and resistance status of *Anopheles gambiae s.l* after 24hours recovery period in Amechi Idodo community, Enugu State, Nigeria

Insecticide	Treatment Concentration (%)	No. of mosquitoes tested	Percentage Mortality (%)	Resistance Status
Bendiocarb	0.1	80	98.75	Susceptible
DDT	4.0	80	5.0	Resistance
Deltamethrin	0.05	80	8.75	Resistance
Pirimiphos-methyl	0.25%	80	10.0	Resistance

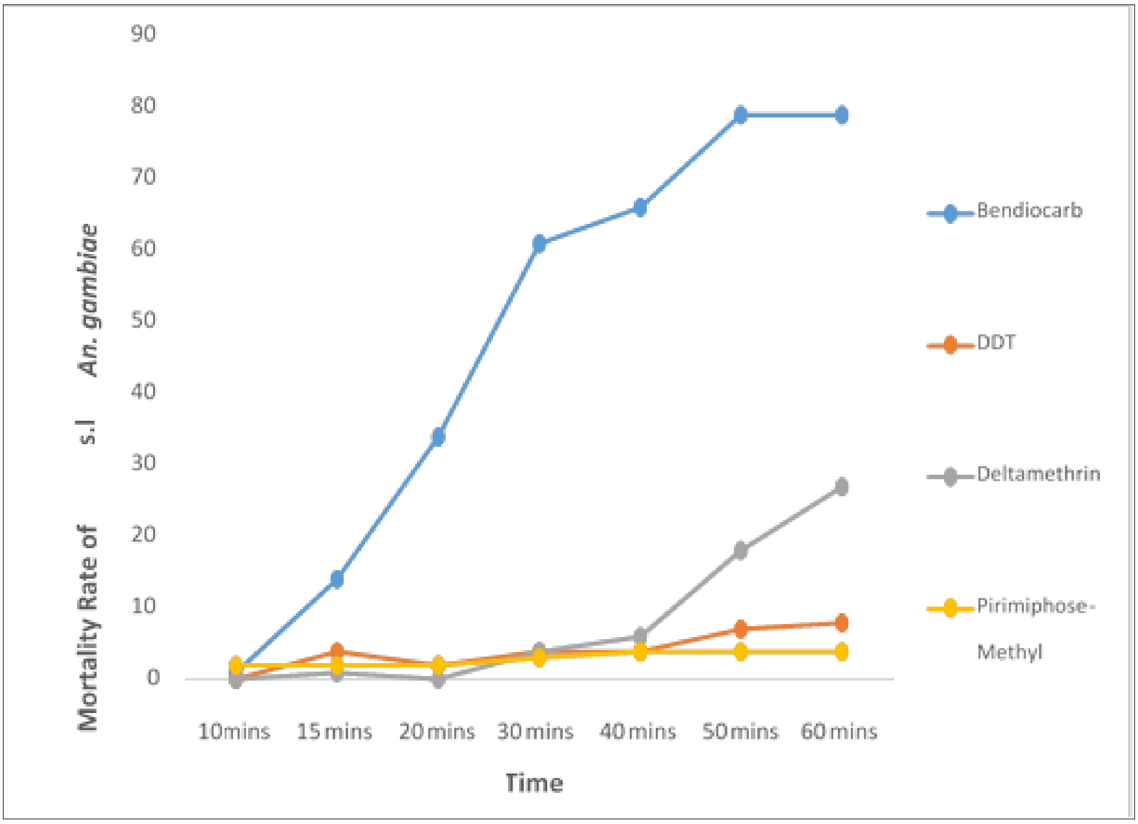

Fig 1: Insecticide Knockdown effect in relation to time on malaria vectors in Amechi Idodo community, Enugu State, Nigeria

Morphological Identification of the Mosquitoes

All mosquitoes subjected to the bioassay were identified to be members of *Anopheles gambiae* complex.

Discussion

All the *Anopheles* mosquito larvae were collected in one type of breeding site - ground water pool/puddles. Different studies have also reported the collection of *Anopheles* species from ground water pools (Onyido *et al*., 2011; Irikannu *et al*., 2015; Egwu *et al*, 2018; Chukwuekezie *et al*., 2020, Egbuche *et al*., 2020) [5]. *Anopheles* mosquitoes breed prolifically in the study area, in puddles. This is in line with the findings of Mbanugo and Okpalaononuju (2003,) [15] which noted that the preponderance of mosquitoes in a study area was due to prevailing mosquito habitats. This study found different populations of *Anopheles gambiae* complex susceptible to bendiocarb only. On the contrary, they were resistant to DDT, Deltamethrin and the pirimiphos-methyl. This is in line with the results of Chukwuekezie *et al*., (2020) [5], who reported similar finding in five Southeast states of Nigeria and Ikpo *et al*. (2021) [12], who recorded 100% mortality rate with bendiocarb in Awgu, Enugu State. However, it contrasts the finding of PMI (2021), which reported susceptibility of malaria vectors to pirimiphos-methyl across several states in Nigeria. In another part of Africa, Pwalia *et al.* (2019), reported resistance to bendiocarb and susceptibility to Pirimiphos-methyl in their study in Accra, Ghana. Mosquitoes from this study reveals the exclusive presence of *Anopheles gambiae* complex from larval survey. This is not amazing, as members of the *Anopheles gambiae* complex are mostly found in ground water pools in most areas of Africa. This agrees with the findings of Nkya *et al*. (2014) [18], in Tanzania; Chouaibou *et al*. (2016) in Côte d'Ivoire; Osse *et al*. (2019), in Cotonou; and several studies in Nigeria (Okorie *et al*., (2015); Nwankwo *et al*. 2017; Awolola *et al*., (2018); Chukwuekezie *et al*., 2020) [5].

Conclusion

This study has revealed that bendiocarb is the only insecticide to which the populations of *An. gambiae* complex collected from Amechi Idodo community are susceptible. This poses huge threat to failure of intervention programmes which heavily relies on pyrethroid-based insecticides. The threat is underscored by the fact that the only control intervention in the community is use of pyrethroid-only insecticide treated nets (ITNs). Hence, it is recommended that insecticide resistance management practices/interventions should be put in place immediately. This will be achieved through evidence-based interventions that will determine the nature of second generation ITNs or insecticides that may be used for indoor residual spraying (IRS). Once insecticide resistance is properly managed, this existential threat would have been restrained in Amechi Idodo.

References

1. Aribodor DN. Mosquitoes, Malaria and Man: A Compendium. Rex Charles and Patrick Ltd, Nimo, Anambra State, Nigeria,2012:1-66.
2. Awolola TS, Adeogun AO, lakiigbe AK, Oyeniyi T, Olukosi YA, Okoh H. Pyrethroids resistance intensity and resistance mechanisms in Anopheles gambiae from malaria vector surveillance sites in Nigeria. PLoS One,2018:13:e0205230.
3. Chouaïbou MS, Fodjo BK, Fokou G, Allassane OF, Koudou BG, David JP. Influence of the agrochemicals used for rice and vegetable cultivation on insecticide resistance in malaria vectors in southern Côte d'Ivoire. Malaria Journal,2016:15:426.
4. Chinula D, Chadwick HS, Pascalina C, Busiku H, Reuben Z, Lisa R, *et al*. Wash-resistance of pirimiphos-methyl insecticide treatments of window screens and eave baffles for killing indoor-feeding malaria vector mosquitoes: an experimental hut trial, South East of Zambia, *Malaria Journal,2018:*17:164.
5. Chukwuekezie O, Nwosu E, Nwangwu U, Dogunro F, Onwude C, Agashi N, *et al*. Resistance status of *Anopheles gambiae* (s.l.) to four commonly used insecticides for malaria vector control in South-East Nigeria. Parasite Vectors,2020:13:152-10
6. Egbuche CM, Onyido AE, Umeanaeto PU, Nwankwo EN, Omah IF, Ukonze CB, *et al*. *Anopheles* species composition and some climatic factors that influence their survival and population abundance in Anambra East LGA, Anambra State, Nigeria. Nigeria Journal of Parasitology,2020:41(2):240-250.
7. Egunyomi A, Gbadamosi IT, Osiname KO. Comparative Effectiveness of Ethnobotanical Mosquito Repellents used in Ibadan. Nigeria. Journal of Applied Biosciences,2010:36:2383-8.
8. Egwu O, Ohaeri CC, Amaechi EC, Ehisianya CN. Distribution and abundance of mosquito larvae in Ohafia, Abia State, Nigeria. *Cuadernos de* Investigación UNED,2018:10(2):379-385
9. Gillies MT, de Meillon B. The *Anophelinae* of Africa South of the Sahara. Publ South African Inst Med Res,1968:54:1-343.
10. Gillet JD. Common African Mosquitoes and their Medical Importance. William Heinemann Medical Books Limited, London,1972:36:11.
11. Gillies MT, Coetzee BA. Supplementary to *Anophelinae* of Africa, South of Sahara (Afro- Tropical Region), *South Africa Institute of Medical Research*,1987:55:1-143.
12. Ikpo AU, Nwaorgu OC, Irikannu KC, Aniefuna CO, Anumba JU, Ezemuoka LC. Insecticide resistance and target site mutations (G119S ace-1 and L1014F kdr) in *Anopheles gambiae s.l.* in Enugu state, Southeastern Nigeria. Journal of Entomology and Zoology Studies,2021:9(4):111-117.

13. Irikannu KC, Chukwuekezie OC. Malaria and man-biting mosquitoes in tropical Africa. Lambert Academic Publishing, Deutschland Germany, 2015. ISBN: 978-3-659-76718-0. 14
14. Karunamoorthi K, Sabesan S. Insecticide resistance in insect vectors of disease with speciareference to mosquitoes: a potential threat to global public health. Health Scope,2013:2(1):4-18.
15. Mbanugo JI, Okpalaononuju CN. Surveillance of mosquito vectors in some Habitats of Awka Metropolis, Anambra State Nigeria. The Nigerian Journal of Parasitology,2003:24:185-190.
16. Moiroux N, Gomez MB, Pennetier C, Elanga E, Djenontin A, Chandre F. Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting insecticidal nets in Benin. *Journal of Infectious Disease,2012:*206:1622-9.
17. NPC-National Population Commission. Population Census of Nigeria. Population distribution in Local Government Areas by Sex and Number of Households, 2006.
18. Nkya TE, Akhouayri I, Poupardin R, Batengana B, Mosha F, Magesa S. Insecticide resistance mechanisms associated with different environments in the malaria vector *Anopheles gambiae*: a case study in Tanzania. Malaria Journal,2014:13:28-32
19. Nwankwo EN, Okorie PN, Acha CT, Okonkwo OE, Nwangwu UC, Ezihe EK. Insecticide resistance in *Anopheles gambiae* s.l. mosquitoes in Awka, Anambra State, Southeast Nigeria. Journal of Mosquito Resistance,2017:7:32-37.
20. Onyido AE, Agbata VO, Umeanaeto PN, Obiukwu MO. Ecology of Malaria Vectors in a Rainforest Sub-Urban Community of Nigeria. *African Research Review*,2011:5(2):293-305.
21. Okorie PN, Ademowo, OG, Irving H, Kelly-Hope LA, Wondji CS. Insecticide susceptibility of *Anopheles coluzzii* and *Anopheles gambiae* mosquitoes in Ibadan, Southwest Nigeria. Medical Vet nary Entomology,2015:29:44-50.
22. Onyido AE, Ezeani AC, Irikannu KC, Umeaneto PU, Egbuche CM, Chikezie FM, *et al*. Anthropophilic mosquito species prevalence in Nibo community, Awka South Local Government Area, Anambra State, Southeastern Nigeria. Ewemen Journal of Epidemiology and Clinical Medicine,2016:2(1):14-20.
23. PMI Vector Link Project. The PMI VectorLink Nigeria Annual Entomology Report, October 2019–September 2020. Rockville, MD. VectorLink, Abt Associates Inc, 2021.
24. Pwalia1 R, Joannides J, Iddrisu A, Addae C, Acquah-Baidoo D, Obuobi D, *et al*. High insecticide resistance intensity of *Anopheles gambiae* (*s.l.*) and low efficacy of pyrethroid LLINs in Accra, Ghana. Parasites Vectors,2019:12:299-301
25. Salihu OM, Sanni NA. Malaria Burden and the Effectiveness of Malaria Control Measures in Nigeria: A Case Study of Asa Local Government Area of Kwara State. Journal of Economics and Sustainable Development,2013:4(3):295-308.
26. Sougoufara S, Diedhiou SM, Doucoure S, Diagne N, Sembene PM, Harry M. Biting by *Anopheles funestus* in broad daylight after use of long lasting insecticidal nets: a new challenge to malaria elimination. Malaria Journal,2014:13:125-133
27. Thomsen EK, Koimbu G, Pulford J, Jamea-Maiasa S, Ura Y, Keven JB. Mosquito behaviour change after distribution of bednets results in decreased protection against malaria exposure. Journal of Infectious Disease,2017:215:790-798.
28. Wat'senga F, Emile ZM, Andre L, Roger M, Tania M, Neil L, *et al*. Nationwide insecticide resistance status and biting behaviour of malaria vector species in the Democratic Republic of Congo. Malaria Journal,2018:17:129-138
29. WHO. Insecticide Resistance Deepens Malaria Crisis in Nigeria, 2009.
30. WHO. World Health Organisation recommended insecticides for indoor residual spraying against malaria vectors, 2013. Available online at: http://www.who.int/whopes/Insecticides_IRS_Malaria_25_Oct_2013.pdf Accessed on 28/3/2018.
31. WHO. World Malaria Report. World Health Organisation. Geneva, 2015.
32. World Malaria Report. Geneva: World Health Organization, 2018, 32–59. https://www.who.int/malaria/publications/world-malaria-report-2018/en/. Accessed 11 Mar 2020.
33. World Health Organisation. World malaria report: 20 years of global progress and challenges, 2020. Available at https://www.who.int/publications/i/item/9789240015791 Accessed on 13/08/2021.
34. Zofou D, Nyasa RB, Nsagha DS, Ntie-Kang F, Meriki HD, Assob JC. Control of malaria and other vector-borne protozoan diseases in the tropics: enduring challenges despite considerable progress and achievements. Infectious Diseases of Poverty,2014:3(1):10-186.