CYTOCHROME P450 IS IMPLICATED IN PERMETHRIN RESISTANCE IN *ANOPHELES COLUZZII* POPULATIONS FROM DELTA STATE, NIGERIA

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ABSTRACT

Insecticide resistance is one of the major impediments threatening the control of malaria vector populations in Nigeria. The failure of WHO recommended control, especially with permethrin used for insecticide treated nets (ITNs) and indoor spray could lead to increase morbidities and mortalities, if left unattended to. This calls for sound management approach achievable through adequate knowledge of spread and mechanism of resistance. Hence, this study evaluated resistance status and involvement of metabolic resistance in Anopheles coluzzii populations from Delta State to permethrin. Anopheline larvae were collected from three Local Government Areas (LGA) and reared to adult stage at Entomology Laboratory, Delta State University, Abraka. Adult female mosquitoes aged 3 – 5 days were exposed to 0.75% permethrin using WHO bioassay method. Also, cohort were exposed to PBO and further exposed to permethrin. Exposed mosquitoes were identified molecularly. All identified mosquitoes were An. coluzzii. Exposure of An. coluzzii populations from Ika South, Ndokwa East and Ethiope West Local Government Area to permethrin recorded 62, 74 and 83 % mortality respectively. Mortalities in synergist assay were 99, 99 and 100 % respectively. Differences in mortality between permethrin and PBO/permethrin-exposed population were significant ($p<0.0001$). Knockdown Time (KDT_{50} and KDT_{95}) was lowest in Ethiope West LGA. Considering increase in mortality value of the populations with exposure to PBO, this study presents evidence for involvement of cytochrome P450 in development of resistance in permethrin-resistant Anopheles populations from Delta State.

Keywords: *Anopheles coluzzii*, Delta State, Cytochrome P450, Permethrin, Resistance status

INTRODUCTION

Malaria remains a serious public health challenge in Nigeria (WHO, 2020). The biting activities of the female *Anopheles* mosquito transmit the protozoan parasite; *Plasmodium* which causes the disease (Smith and Jacobs-Lorena, 2010). In Nigeria, three key species of *Anopheles* including *An. gambiae* (*An. gambiae s.s. An. coluzzii* and *An. arabiensis*) *An. funestus* and *An. melas* are responsible for transmission of the disease (Oyewole *et al.*, 2010; Okorie *et al.,* 2011; Okorie *et al.,* 2015), however Amaechi *et al.* (2018) documented members of *An. gambiae* complex as the most prominent transmitter of disease.

An. coluzzii mosquitoes has only one hybrid; the M form from the eco-diversity of the *An. gambiae* complex (Niang *et al.,* 2014). The immature stages of these mosquitoes are

commonly found in puddles, ponds, ditches and other potential sites (Ojianwuna *et al.,* 2021a). These mosquitoes are distributed in urban, semi-urban and rural communities in endemic areas (WHO, 2020). Host-seeking behaviour of the *An. coluzzii* mosquito is probably linked to the parasites they transmit to human population that cause malaria. WHO recommended control approaches which involves the use of insecticides (Fang *et al.,* 2019). Resistance of *An. coluzzii* has been reported in Nigeria (Okorie *et al.,* 2015; Muhammad *et al.,* 2021).

Approximately, there are more than 200 million humans at risk of malaria, most of which occur in Sub-Saharan Africa resulting in deaths that exceed 400,000, with attendant cost of treatment reaching 35 dollars per individual case and 2000 dollars (USD) in health care facilities (WHO, 2020). World Health Organization reported very high infection rate in most African countries and this has led to poor quality of life in the continent (WHO, 2020). In 2019, more than half of the infected cases reported globally occurred in six African countries, where 25 % was reported in Nigeria (WHO, 2019).

In the quest to reduce the spread of malaria, different interventions have been deployed to these affected areas in Africa, however, the use of insecticides and treated nets remain the two most recommended and effective methods used in the region (Omotayo *et al.*, 2021). These two strategies either singly or in combination have led to considerable reduction in the incidence of the diseases between 2010 and 2016 (WHO, 2016). However, the development of resistance in major malaria vectors as a result of overuse of these recommended insecticides has been an enormous challenge in sub-Saharan Africa (WHO, 2019).

Insecticide resistance (IR) to the four classes of insecticide recommended for mosquito control continues to thrive in major malaria vectors occurring in almost all countries in sub-Saharan Africa (WHO, 2019). IR has spread across the continent and has potentially jeopardized ongoing efforts committed to control malaria. The presence of resistant genes and metabolic enzymes are two main causes of resistance development in Africa (Munita and Arias, 2016). IR through knockdown resistance development in Africa (Munita and Arias, 2016). Development of insecticide resistance through especially knockdown resistance gene (*Kdr*) have been recorded in different *Anopheles* species in Nigeria (Awolola *et al.,* 2007; Oduola *et al.,* 2012) and it has been known to aid resistance development to pyrethroids and organochlorine insecticides (Fang *et al.,* 2019). Likewise, up-regulation of metabolic enzymes especially cytochrome P450 have also been a major route to resistance development in malaria vectors (Adedeji *et al.,* 2020). Involvements of metabolic enzymes in resistance development in malaria vectors have been recorded in other studies from sub-Saharan Africa (Fezeu *et al.,* 2007; Rajman *et al.*, 2017).

Management of IR has been said to be one of the focal points in control of malaria in recent years, however, this cannot be done except with sound knowledge of the mechanism involved in the development of resistance in these vectors (Nkya *et al.,* 2014).

Understanding the two major strategies through which resistance is achieved requires thorough investigation. It will provide good foundation for implementation of policies for management of IR. Not only can sound understanding of resistance mechanism be important in the introduction of bed nets treated with pyrethroids but with a combination of pyrethroids and synergist such as piperonyl butoxide (PBO). Exposure of mosquitoes to PBO increases the efficacy of pyrethroid by leading to increased mortality in mosquito populations where metabolic enzymes are involved in resistance development (Dadzie *et al.,* 2017).

Studies on efficacy and resistance mechanism of *Anopheles* spp., to selected pyrethroids have been reported in other parts of Nigeria (Djouaka *et al.,* 2016; Awolola *et al.,* 2018; Chukwuekezie *et al.*, 2020; Muhammad *et al.,* 2021; Ojianwuna *et al.*, 2021b), but in Delta State, Nigeria, this information is limited, therefore, the study was conducted to determine the level of pyrethroid resistance in *An. coluzzii* populations from some localities in Delta State. Likewise, the impact of metabolic enzymes; cytochrome P450 in development of

resistance in the resistant populations was also studied.

MATERIALS and METHODS

Study Area: Three Local Government Areas (LGAs) in Delta State were mapped out for this study. Delta State is one of the six states in the South-South region of Nigeria (Figure 1).

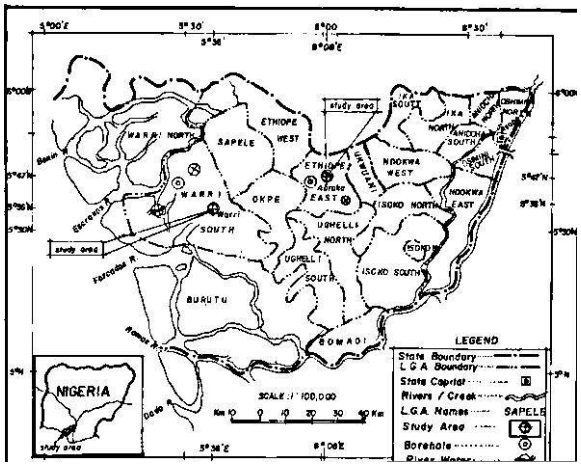

Figure 1: Map of Delta State showing study locations labeled study area (Nwankwo and Ogagarue, 2011)

The state is an oil producing state in Nigeria with a good number of its communities found in the creeks. The state is found in an ecozone with more than two-third of the year experiencing heavy downpour of rainfall. Three communities were selected from each of the LGAs for mosquito larvae collection, global positioning system (GPS) of the sample communities and nature of habitats as shown in Table 1.

Mosquito Collection, Breeding and Morphological Identification: Larvae and pupae of the *Anopheles* mosquitoes were collected in early hours of the morning from temporary breeding sites including tire marks, puddles and ponds in the sampled communities. The immature stages were carefully transferred to a transparent plastic bowls and transported to the Insectary at the Entomology Laboratory, Department of Animal and Environmental Biology, Delta State University, Abraka, Delta State, Nigeria. Larvae were transferred into larval holding trays and properly covered with nets and reared under standard conditions (Temperature: 28 ± 3°C and Relative humidity: 78 ± 3 %). Emerged adult mosquitoes were then fed with 10 % glucose solution. All mosquitoes were morphologically identified with the aid of a dissecting microscope using the keys described by Gillies and Coetzee (1987). Morphological identification was done using the anterior and dorso-ventral appendages as special reference.

Susceptibility Bioassay: Prior to exposure to permethrin insecticide female adult mosquitoes were sorted from males. Only females *An. coluzzii* mosquitoes were used for the bioassay test following WHO procedures (WHO, 2016). Twenty-five female mosquitoes in four replicates were exposed to permethrin. Permethrin (0.75 %) impregnated paper was introduced into four exposure tubes and fastened into position by a wired clip. Adult female *An. coluzzii* mosquitoes were carefully introduced into the four holding tubes with an aspirator and allowed to acclimatize for ten minutes. After which the mosquitoes were transferred into the exposure tubes. The setup was monitored and the number of mosquitoes knocked down at intervals of 10, 15, 20, 30, 40, 50 and 60 minutes recorded. After 60 minutes, survived mosquitoes were carefully transferred back to holding tubes and fed with glucose solution. The setup was kept for 24 hours after which mortality was read.

Cytochrome P450 Activity Bioassay: Cytochrome P450 activity assay was conducted in line with WHO standard protocol (WHO, 2016) by using piperonyl butoxide (PBO) to suppressed the activities of cytochrome P450 enzymes. Twenty-five female mosquitoes in four replicates were exposed to 5 % PBO impregnated papers for one hour before they were then transferred into exposure tubes containing 0.75 % permethrin impregnated paper for another one hour. Two control tubes were run in parallel at the time of testing. Records of mosquito knockdown were taken at 10, 15, 20, 30, 40, 50 and 60 minutes. After 60 minutes, survived mosquitoes were carefully transferred back to the holding tubes and kept

Table 1: Nature of habitat and global positioning system (GPS) of sampled communities in Delta State, Nigeria

Local Government	Villages	Longitude (0E)	Latitude (0N)	Nature of Habitat
Ika South	Abavo	6.18	6.12	Puddle
	Agbor-Alidima	6.14	6.18	Tyre-mark
	Agbor-Obi	6.19	6.25	Puddle
Ethiope West	Mosogar	5.73	5.87	Puddle
	Edjeba	5.73	5.54	Tyre-mark
	Oghara	6.10	5.59	Puddle
Ndokwa East	Aboh	6.52	5.54	Ditch
	Okpai	6.57	5.70	Tyre-mark
	Ashaka	6.39	5.63	Puddle

for 24 hours during which they were fed with 10 % glucose solution. Final mortality readings were taken after 24 hours.

Data Analysis: Knockdown and mortality data for *Anopheles* populations exposed to permethrin and PBO + permethrin were analysed using XL Stat version 2020. One-way analysis of variance (ANOVA) test was used to compare mean knock down and significance set at $p<0.05$. Resistance status was determined in line with WHO protocol (WHO, 2016). Probit analysis was used to compute KDT_{50} and $KDT_{95.}$ The time of knockdown at 50 and 95 % confidence limit were compared between LGAs and level of significance was set at $p<0.05$.

RESULTS

Species Identification: One hundred and two (102) mosquito samples were selected at random and morphologically identified as *An. gambiae* s.l. Further molecular analysis confirmed 62 amplified samples as *An. coluzzii*.

Susceptibility Status of *Anopheles coluzzii* Exposed to Permethrin and PBO + Permethrin: Susceptibility of female *An. coluzzii* exposed to permethrin and PBO + permethrin across the three LGAs is shown in Table 2. Twenty-four hours mortality recorded in populations exposed to permethrin from the three study site ranges between 62 and 83 %. Mortality recorded at 24 hours was lowest in Ika South LGA compared to other LGAs. Total mortality (100 %) was recorded when *Anopheles* population from Ethiope West LGA was first exposed to PBO before exposure to permethrin. Similarly, synergist assay on *An. coluzzii* populations from the other LGAs increased mortality from 62 to 95 % and 74 to 92 % for Ika South and Ndokwa East LGA respectively.

Knockdown Time of *Anopheles coluzzii* Exposed to Permethrin and PBO + Permethrin: Percentage knockdown was highest among population from Ethiope West LGA followed by Ndokwa East LGA and lowest in Ika South LGA (Figure 2). The difference between knock down time of *Anopheles* mosquitoes exposed to permethrin and PBO + permethrin across the three LGAs was significant ($F = 56.23$, $p<0.0001$). Knockdown times of *An. coluzzii* to permethrin and PBO + permethrin are shown in Table 3. Values for KDT_{50} ranged from 37.06 to 87.06 minutes, while KDT_{95} for the three populations ranged between 162.40 to 531.03 minutes. More so, prior exposure to PBO before exposure to permethrin reduced the values for KDT_{50} to range between 32.65 to 45.14 minutes and KDT_{95} to between 139.39 to 244.04 minutes (Table 3).

DISCUSSION

Malaria disease has prevailed for over a century in sub-Saharan Africa due to the ineffectiveness of insecticides recommended for use. This ineffectiveness leading to mosquito resistance is a major challenge affecting malaria control in Africa. *Anopheles* mosquito resistance to virtually all classes of insecticides has been recorded in all regions in Africa (WHO, 2019).

Table 2: Susceptibility status of *Anopheles coluzzii* exposed to permethrin and PBO + permethrin

Study Location	Insecticide	N	24 hours mortality (%)	Status
Ika South	Permethrin	100	62.00	Resistance
	Permethrin + PBO	100	95.00	Suspected resistance
Ethiope West	Permethrin	100	83.00	Resistance
	Permethrin + PBO	92	100.00	Susceptible
Ndokwa East	Permethrin	100	74.00	Resistance
	Permethrin + PBO	86	92.00	Suspected resistance

Note: *≥98 = susceptibility, 90 – 97 % = suspected resistance, ≤90 = resistance*

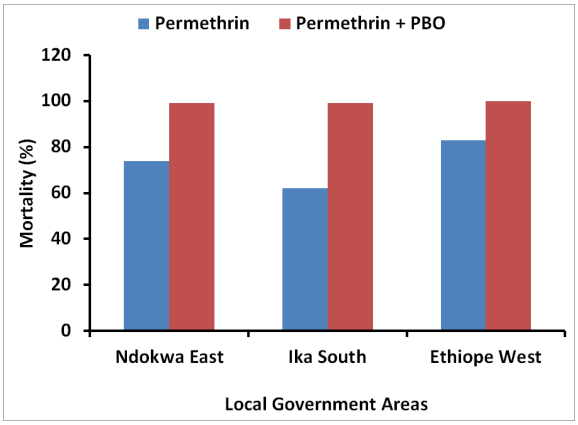

Figure 2: Percentage mortality of *Anopheles coluzzii* exposed to permethrin and PBO + permethrin in Delta State, Nigeria

This mandating the need to properly monitor insecticide resistance in malaria vectors, which is pertinent considering that the incidence of disease is high in this region. Thus, this study was conducted to assess the susceptibility status of *An. coluzzii* from Ika South, Ndokwa East and Ethiope West LGAs in Delta State, Nigeria to permethrin and PBO + permethrin.

Result from the study showed that *An. coluzzii* mosquitoes were resistant to permethrin in the three selected LGAs of Delta State, Nigeria. The resistance of mosquitoes to permethrin has been documented by other researchers in Nigeria (Ndams *et al.*, 2006; Awolola *et al.,* 2018). This study documented the resistance of *An. coluzzii* population from Delta state, Nigeria to permethrin. Species resistance to permethrin is of major concern to malaria vector control because permethrin is one of the major insecticides recommended for treatment of Long-Lasting Bed Nets (WHO, 2017). More so, it is the major components employed in treatment of PermaNet 2.0 which is the type of insecticide bed nets distributed for free in Delta State few years ago and the most common type of treated nets within the country (NMEP, 2013; NMEP, NPopC, NBS and ICF International, 2016). Also, permethrin constitute a major fraction of the pyrethroids that is continuously been used for indoor residual spray against malaria vectors in Nigeria (NMEP, 2013; 2017). Since selection pressure has led to the increasing release of insecticides into the environment, behavioural patterns of species to permethrin leading to resistance has recorded in this study and can be attributed to excessive use of permethrin occasioned by its use in treatment of LLINs as well as in IRS in the study area. Also, excessive use of aerosols by individual households in control of mosquitoes in Nigeria has been documented by Omotayo *et al.* (2021) and their continual use in large quantities without management may inflict harm to the environment.

The impact of metabolic enzymes in development of resistance in the three populations was also determined. Pre-exposure of the populations to PBO before exposure to permethrin resulted in increased mortality in the three populations. Piperonyl butoxide is a known synergist that suppresses the activities of cytochrome P450 enzymes. The effectiveness of PBO as a synergist with permethrin has been documented in other studies in Nigeria (Muhammad *et al.,* 2021; Ojianwuna *et al.,* 2021b). Not only did the pre-exposure to PBO in the present study increase mortality, but raised the mortality to a level in which the populations can be considered to be susceptible. The susceptibility seen with pre-exposure to PBO suggests strong evidence of metabolic resistance to permethrin in *An. coluzzii* population from the Delta State.

Table 3: Knock down time of *Anopheles coluzzii* exposed to permethrin and PBO + permethrin

Study location	Insecticide	N	KDT50 (95 % CI) (min)	KDT95 (95 % CI) (min)
Ika South	Permethrin	100	87.06 (70.22-121.55)	531.03 (306.05-1311.17)
	PBO + Permethrin	100	45.14 (40.21- 52.10)	244.04 (173.39- 400.26)
Ndokwa East	Permethrin	100	63.92 (56.09- 76.35)	254.51 (181.93- 418.67)
	PBO + Permethrin	92	32.65 (29.86- 35.09)	139.39 (110.69- 190.58)
Ethiope West	Permethrin	100	37.06 (33.76-41.13)	162.40 (126.05- 230.17)
	PBO + Permethrin	86	37.39 (34.60- 40.67)	139.39 (101.23- 156.42)

N = Total number of mosquitoes exposed; KDT = Knockdown time at 95 % confidence interval (Lower limit – Upper limit) presented in minutes

The knock down time for 50 % (KDT_{50}) of *An. coluzzii* populations from the 3 study sites ranged from 37.06 to 87.06 minutes while KDT_{95} ranged from 162.40 to 531.63 minutes. Likewise, KDT_{50} for the *An. coluzzii* populations when pre-exposed to PBO ranged from 32.65 to 45.14 minutes while KDT_{95} ranged from 139.4 to 244.0 minutes. Similar to the finding of this present study, Awolola *et al*. (2018) observed that the knock down time for 50 and 95 % of *Anopheles* mosquitoes were lower when pre-exposed to PBO. Lower knock down time with pre-exposure to PBO support use of PBO + permethrin in treatment of new insecti treated nets (ITNs). This will go a long way in preserving the effectiveness of permethrin treated nets especially in areas where metabolic resistance is the determinant conferring resistance to permethrin.

The development of resistance in vectors of malaria and the resulting spread is a challenge that requires urgent attention. Management of this challenge should be a focal point of every vector control programme as failure to address this may lead to total failure of the 2 most common interventions that have been helpful in reducing the burden of malaria in Africa.

Conclusion: The result of this study has shown that *An. coluzzii* populations from Ika South, Ndokwa East and Ethiope West LGAs in Delta State are resistant to permethrin, thus raising questions as regards the use of permethrin treated nets in these areas. However, pre-exposure of same *Anopheles* populations to PBO resulted in susceptibility of the populations, thereby pointing to metabolic resistance as the major factor leading to resistance within the population. Thus, the use of ITNs treated with a combination of PBO and permethrin will be a good choice for malaria vector control in Ika South, Ndokwa East and Ethiope West LGAs, Delta State, Nigeria.

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REFERENCES

ADEDEJI, E. O., OGUNLANA, O. O., FATUMO, S., BEDER, T., AJAMMA, Y., KOENIG, R. and ADEBIYI, E. (2020). *Anopheles* metabolic proteins in malaria transmission, prevention and control: a review. *Parasites and Vectors,* 13: 465. https://doi.org/10.1186/s13071-020-04342-5.

AMAECHI, E. C., UKPAI, O. M., OHAERI, C. C., EJIKE, U. B., IROLE-EZE, O. P., EGWU, O. and NWADIKE, C. C. (2018). Distribution and seasonal abundance of Anopheline mosquitoes and their association with rainfall around irrigation and non-irrigation areas in Nigeria. *Cuadernos de Investigación UNED*, 10(2): 267 – 272.

AWOLOLA, T. S., ADEOGUN, A., OLAKIIGBE, A. K., OYENIYI, T., OLUKOSI, Y. A., OKOH, H., AROWOLO, T., AKILA, J., ODUOLA, A. and AMAJOH, C. N. (2018). Pyrethroids resistance intensity and resistance mechanisms in *Anopheles gambiae* from malaria vector

surveillance sites in Nigeria. *PLoS One*, 13(12): e0205230. https://doi.org/10.1371/journal.pone.0205230

AWOLOLA, T. S., ODUOLA, A. O., OYEWOLE, I. O., OBANSA, J. B., AMAJOH, C. N., KOEKEMOER, L. L. and COETZEE, M. (2007). Dynamics of knockdown pyrethroid insecticide resistance alleles in a field population of *Anopheles gambiae* ss in southwestern Nigeria. *Journal of Vector Borne Diseases*, 44(3): 181 – 188.

CHUKWUEKEZIE, O., NWOSU, E., NWANGWU, U., DOGUNRO, F., ONWUDE, C., AGASHI, N., EZIHE, E., ANIOKE, C., ANOKWU, S., ELOY, E., ATTAH, P., ORIZU, F., EWO, S., OKORONKWO A., JOSEPH, A., IKEAKOR I., HARUNA, S. and GNANGUENON, V. (2020). Resistance status of *Anopheles gambiae* (s.l) to four commonly used insecticides for malaria vector control in South-East Nigeria. *Parasites and Vectors,* 13: 152. https://doi.org/10.1186/s13071-020-04027-z

DADZIE, S. K., CHABI, J., ASAFU-ADJAYE, A., OWUSU-AKROFI, O., BAFFOE-WILMOT, A., MALM, K., BART-PLANGE, C., COLEMAN, S., APPAWU, M. A. and BOAKYE, D. A. (2017). Evaluation of piperonyl butoxide in enhancing the efficacy of pyrethroid insecticides against resistant *Anopheles gambiae* sl in Ghana. *Malaria Journal*, 16(1): 342. https://doi.org/10.1186/s12936-017-1960-3

DJOUAKA, R. J., ATOYEBI, S. M., TCHIGOSSOU, G. M., RIVERON, J. M., IRVING, H., AKOTON, R., KUSIMO, M. O., BAKARE, A. A. and WONDJI, C. S. (2016). Evidence of a multiple insecticide resistance in the malaria vector *Anopheles funestus* in South West Nigeria. *Malaria Journal*, 15(1): 565. https://doi.org/10.1186/s12936-016-1615-9

FANG, Y., SHI, W. Q., WU, J. T., LI, Y. Y., XUE, J. B. and ZHANG, Y. (2019). Resistance to pyrethroid and organophosphate insecticides, and the geographical distribution and polymorphisms of target-site mutations in voltage-gated sodium channel and acetylcholinesterase 1 genes in *Anopheles sinensis* populations in Shanghai, China. *Parasites and Vectors*, 12(1): 396. https://doi.org/10.1186/s13071-019-3657-7

FEZEU, L., BALKAU, B., KENGNE, A. P., SOBNGWI, E. and MBANYA, J. C. (2007). Metabolic syndrome in a sub-Saharan African setting: central obesity may be the key determinant. *Atherosclerosis*, 193(1): 70 – 76.

GILLIES, M. T. and COETZEE, M. A. (1987). A supplement to the anophelinae of Africa South of the Sahara (Afrotropical Region). *Publication of South African Institute of Medical Research*, 55: 1 – 143.

MUHAMMAD, A., IBRAHIM, S. S., MUKHTAR, M. M., IRVING, H., ABAJUE, M. C., EDITH, N.M., DA'U, S. S., PAINE, M. J. and WONDJI, C. S. (2021). High pyrethroid /DDT resistance in major malaria vector *Anopheles coluzzii* from Niger-Delta of Nigeria is probably driven by metabolic resistance mechanisms. *PLoS One*, 16(3): e0247944. https://doi.org/10.1371/journal.pone.0247944

MUNITA, J. M. and ARIAS, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology Spectrum*, 4(2): https://dx.doi.org/10.1128%2Fmicrobiolspec.VMBF-0016-2015

NDAMS, I. S., LAILA, K. M. and TUKUR, Z. (2006). Susceptibility of some species of mosquitoes to permethrin pyrethroid in Zaria Nigeria. *Science World Journal*, 1(1): 15 – 19.

NIANG, E. H. A., KONATE, L., DIALLO, M., FAYE, O. and DIA, I. (2014). Reproductive isolation among sympatric molecular forms of *An. gambiae* from inland areas of South-Eastern Senegal. *PLoS One,* 9(8): e104622. http://doi.org/10.1371/journal.pone.0104622

NKYA, T. E., AKHOUAYRI, I., POUPARDIN, R., BATENGANA, B., MOSHA, F., MAGESA, S., KISINZA, W. and DAVID, J. P. (2014). Insecticide resistance mechanisms

associated with different environments in the malaria vector *Anopheles gambiae*: a case study in Tanzania. *Malaria Journal*, 13(1): 28. https://doi.org/10.1186/1475-2875-13-28

NMEP (2013). *National Malaria Strategic Plan 2014 - 2020*. National Malaria Elimination Programme (NMEP), Federal Ministry of Health, Abuja, Nigeria. https://www.health.gov.ng/doc/NMEP-Strategic-Plan.pdf Accessed August 27, 2021.

NMEP (2017). *National Malaria Operations Research Agenda*. National Malaria Elimination Programme (NMEP), Federal Ministry of Health, Abuja, Nigeria. https://www.health.gov.ng/doc/NMORA-PDF-VERSION.pdf Accessed August 27, 2021.

NMEP, NPOPC, NBS and ICF INTERNATIONAL (2016). *Nigeria Malaria Indicator Survey 2015*. National Malaria Elimination Programme (NMEP), National Population Commission (NPopC), National Bureau of Statistics (NBS) and ICF International, Abuja, Nigeria, and Rockville, Maryland, USA. https://dhsprogram.com/pubs/pdf/MIS20/MIS20.pdf

NWANKWO, C. N. and OGAGARUE, D. O. (2011). Effects of gas flaring on surface and ground waters in Delta State Nigeria. *Journal of Geology and Mining Research*, 3(5): 131 – 136.

ODUOLA, A. O., OTUBANJO, J. H., OLOJEDE, J. B., OYEWOLE, I. O. and AWOLOLA, T. S. (2012). Malaria transmission risk indices of three *Anopheles* species in selected rural communities in Oyo State South-West Nigeria. *International Journal of Tropical Medicine* 7(1): 42 – 48.

OJIANWUNA, C. C., ENWEMIWE, V. N. and EKEAZU, C. N. (2021a). Abundance and distribution of anopheles mosquito in relation to physicochemical properties in delta state, Nigeria. *Fudma Journal of Sciences*, 5(3): 274 – 280.

OJIANWUNA, C. C., ENWEMIWE, V. N. and OSSAI, P. I. (2021). The use of deltamethrin, permethrin, their combination and synergist on female *Anopheles* mosquito in Isoko South, Delta State. *Nigerian Journal of Science and Environment,* 19(1): 39 – 50.

OKORIE, P. N., ADEMOWO, O. G., IRVING, H., KELLY-HOPE, L. A. and WONDJI, C. S. (2015). Insecticide susceptibility of *Anopheles coluzzii* and *Anopheles gambiae* mosquitoes in Ibadan, Southwest Nigeria. *Medical and Veterinary Entomology*, 29(1): 44 – 50.

OKORIE, P. N., MCKENZIE, F. E., ADEMOWO, O. G., BOCKARIE, M. and KELLY-HOPE, L. (2011). Nigeria *Anopheles* vector database: an overview of 100 years' research. *PloS One*, 6(12): e28347. https://doi.org/10.1371/journal.pone.0028347

OMOTAYO, A. I., ANDE, A. T., ODUOLA, A. O., OLAKIIGBE, A. K., GHAZALI, A. K., ADENEYE, A. and AWOLOLA, S. T. (2021). Community knowledge, attitude and practices on malaria vector control strategies in Lagos State, South-West Nigeria. *Journal of Medical Entomology*, 58(3): 1280 – 1286.

OYEWOLE, I. O., IBIDAPO, C. A., OKWA, O. O., ODUOLA, A. O., ADEOYE, G. O., OKOH, H. I. and AWOLOLA, T. S. (2010). Species composition and role of *Anopheles* mosquitoes in malaria transmission along Badagry axis of Lagos Lagoon, Lagos, Nigeria. *International Journal of Insect Science*, 2: 51 – 57.

RAJMAN, I., KNAPP, L., MORGAN, T. and MASIMIREMBWA, C. (2017). African genetic diversity: implications for cytochrome P450-mediated drug metabolism and drug development. *EBioMedicine*, 17: 67 – 74.

SMITH, R. C. and JACOBS-LORENA, M. (2010). Plasmodium–mosquito interactions: a tale of roadblocks and detours. *Advances in Insect Physiology*, 39: 119 – 149.

WHO (2016). *Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes.* World Health Organization (WHO), Geneva, Switzerland. https://apps.who.int/iris/bitstream/handle/10665/

250677/9789241511575-eng.pdf Accessed February 2, 2021

WHO (2017). *World Malaria Report 2017*. World Health Organization (WHO), Geneva, Switzerland. https://www.mmv.org/sites/default/files/uploads/docs/publications/WMR2017.pdf Accessed February 2, 2021.

WHO (2019). *World Malaria Report 2019.* World Health Organization (WHO), Geneva, Switzerland. https://www.who.int/publications/i/item/9789241565721 Accessed February 2, 2021.

WHO (2020). *World Malaria Report 2020*. World Health Organization (WHO), Geneva, Switzerland. https://www.who.int/publications/i/item/9789240015791 Accessed February 2, 2021.