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Research Article Malaria Vector Abundance and Insecticides Susceptibility Status in Gidan-Yero Village, Sokoto State, Nigeria

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Abstract

Background and Objective: Malaria vector control interventions have been ongoing in Nigeria for several decades. The interventions are however undermined by the emergence and spread of insecticide resistance. This study investigated the malaria vector abundance, insecticide susceptibility status at Gidan-Yero village. **Materials and Methods:** Malaria vector abundance was determined monthly by morphological identification. The insecticides susceptibility status of *An. gambiae s.l.* was determined using the Centre for Disease Control and Prevention (CDC) bottle bioassay. Resistance intensity was investigated at ×1, ×2 and ×5 of the diagnostic concentrations and scored at 30 min. A synergy test to determine the mechanism of resistance was performed using piperonyl butoxide (PBO). **Results:** *Anopheles gambiae s.l.* was found to be more abundant in August. The vector was found to be susceptible to all the insecticides with exception of alphacypermethrin and permethrin, the resistance intensity was at 2× and 5× concentration, respectively. The resistance to the two pyrethroids was however reverted with the application of PBO. **Conclusion:** *Anopheles gambiae s.l.* was abundant and susceptible to bendiocarb, deltamethrin and pirimiphos-methyl but resistant to alphacypermethrin and permethrin. Susceptibility monitoring guided the Ministry of Health's decision to rotate between IRS insecticide classes.

Key words: Anopheles gambiae s.l., Gidan-Yero, vector abundance, susceptibility status, insecticide resistance, synergistic bioassay, intensity bioassay

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

MATERIALS AND METHODS

Malaria remains a major global health challenge, a cause of morbidity and mortality in the world and an important health problem in Nigeria and Africa¹. In 2018 and 2019, Sub-Saharan Africa accounted for 93 and 91% of global malaria episodes, respectively, with the highest proportion of 25% occurring in Nigeria, while Congo^{2,3}.

Indeed, despite the interventions, the African region continues to carry a disproportionately high share of the global malaria burden⁴. Many vectors play an important role in malaria transmission across Africa, notably Anopheles gambiaes.s., An. coluzzii), An. arabiensis and An. funestus s.s.⁵ In their battle against malaria, most African countries rely heavily on two vector control interventions namely: Longlasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Resistance to LLIN exposure increases mosquito survival, which may lead to rising malaria incidence and fatality in Africa⁶. It has been estimated that since 2000 more than 670 million cases of malaria have been averted by combining IRS and LLINs with case management and community education⁷. Recent global efforts have been made to control and eliminate malaria leading to a significant reduction in malaria cases and mortality in Africa by 42 and 66%, respectively.

The emergence and spread of insecticide resistance are undermining/threatening the effectiveness of malaria vector control interventions⁸. It has been shown that insecticide resistance is spreading particularly in malaria-prone countries⁷. The situation is also complicated by the extensive use of pyrethroids in agriculture, which constitute an additional selection pressure on malaria vectors, for example via insecticide-contaminated groundwater that pervade mosquito larval habitats⁹.

With the calls by WHO for all countries to develop and implement insecticide resistance management strategies in their malaria control programme to curb the spread of resistance as well as preserve the effectiveness of insecticide-treated nets¹⁰, it is imperative to better understand the distribution of resistance, its mechanisms and impact on the effectiveness of control interventions and malaria transmission. This paper presents the findings of a study on the abundance and insecticide susceptibility status of *An. gambiae s.l.*, to alphacypermethrin, bendiocarb, deltamethrin, permethrin and pirimiphos-methyl in Gidan-Yero village.

Study area: The study was carried out in Gidan-Yero village of Wamakko, Local Government Area, Sokoto State located on the longitude 13.12131N and latitude 5.19466E. The people of the area are mostly farmers and a few fishermen. The horticultural activities include millet, guinea corn and rice cultivation. The research was conducted between June and September, 2019.

Mosquito larval collection: Mosquito larvae were collected in June through September, 2019 from different larval habitats using standard dippers (350 mL) into white plastic containers and brought to a malaria entomology laboratory of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. The larvae were fed with a yeast table grounded with biscuits and reared to adults. All mosquito larvae were collected between 8 and 10 am. The emerging adult mosquitoes were transferred into cages and fed with a 10% sugar solution from cotton wool.

Susceptibility tests: Both larval collection and bioassay testing were conducted between June-September, 2019. The CDC bottle bioassay¹¹ was used in testing the susceptibility status of the malaria vector¹¹. Four replicates of twenty-five 3-5 days old adult mosquitoes were added to the CDC bioassay bottle coated with diagnostic concentration deltamethrin. This was repeated for permethrin, alphacypermethrin, bendiocarb and pirimiphos-methyl. For each test, another bioassay bottle coated with acetone only containing 25 adult mosquitoes was used as a control. Mosquitoes were considered dead when they could no longer stand, were immobile and slid along the curvature of the test bottle¹². Mortality was recorded every 15-30 and extended to 60 min for pirimiphos-methyl. All mosquitoes that die within the diagnostic time when exposed to insecticide-coated bottles are susceptible to a tested insecticide. Test mosquitoes surviving beyond the diagnostic time threshold are assumed to have some degree of resistance. Interpretation of CDC bioassay results at a diagnostic time is that <95% mortality indicates resistance.

Resistance intensity tests: Insecticide resistance intensity testing of Anopheles mosquitoes to permethrin and alphacypermethrin was performed by exposing them to CDC bottles coated with the diagnostic dosages and subsequently to doses of $2 \times$ and $5 \times$ of the diagnostic dosages.

Synergistic bioassay tests: Insecticide resistance mechanisms were investigated with a synergist, Piperonyl Butoxide (PBO), to the two insecticides that were resistant. Resistance mechanisms were determined by pre-exposing the mosquitoes to the oxidase inhibitor PBO at 100 µg/bottle for 1 hr before performing CDC bottle bioassay with bottles coated with diagnostic doses of alphacypermethrin and permethrin for 30 min.

Mosquito identification: All mosquitoes including exposed and unexposed were identified to species based on morphological characteristics¹³.

Statistical analysis: The mortality data were analyzed and expressed in terms of percentage and compared using Chi-square tests. The output provided estimated marginal means of mortality, 95% confidence intervals, standard errors and p-values.

RESULTS

Morphological identification: A total of 8918 mosquitoes both exposed and non-exposed were morphologically identified as *An. gambiae s.l.* and was more abundant in August (31.22%) and less in abundance in June (19.76%). *An. gambiae s.l.*, abundance in July and September was 27.30 and 21.72%, respectively (Fig. 1).

Susceptibility status: Mosquitoes were found to be fully susceptible to bendiocarb, deltamethrin and pirimiphosmethyl but resistant to alphacypermethrin and permethrin at a mortality rate of 87 and 68%, respectively (Fig. 2). The intensity of resistance is shown in Fig. 3. Resistance intensity of alphacypermethrin was reverted at $2\times$ concentration, while that of permethrin extended to $5\times$ concentration. The result for the synergistic assay is as shown in Fig. 4. Pre-exposure of *An. gambiae s.l.*, to PBO for 1 hr before exposure to discriminating doses of alphacypermethrin and permethrincoated CDC bottles restored the efficacy of these two insecticides that were initially resistant to $1\times$ concentration.

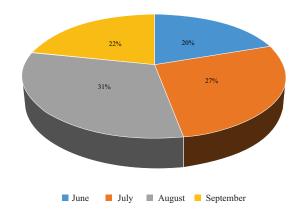


Fig. 1: Monthly abundance of *An. gambiae s.l.*, in Gidan-Yero village

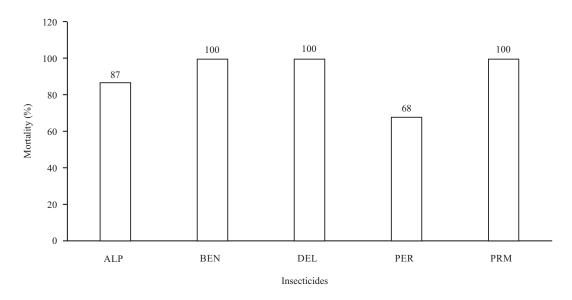


Fig. 2: Insecticides susceptibility status for malaria vector in Gidan-Yero village

Alp: Alphacypermethrin, Ben: Bendiocarb, Del: Deltamethrin, Per: Permethrin and Prm: Pirimiphos-methyl

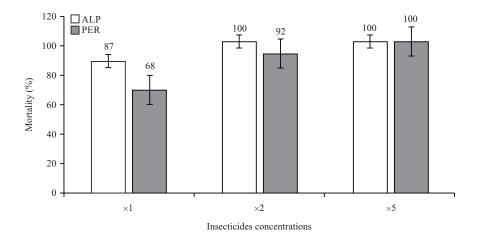


Fig. 3: Intensity of resistance of *An. gambiae s.l.*, exposed to alphacypermethrin and permethrin at the different concentrations at Gidan-Yero village

Alp: Alphacypermethrin and Per: Permethrin

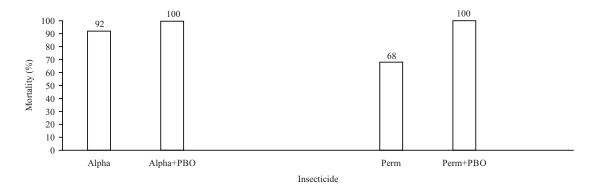


Fig. 4: Synergistic bioassay of *An. gambiae s.l.*, exposure to alphacypermethrin and permethrin with PBO at Gidan-Yero village Alp: Alphacypermethrin, Ben: Bendiocarb, Del: Deltamethrin, Per: Permethrin and Prm: Pirimiphos-methyl

DISCUSSION

Monitoring *Anopheles* spp., vectors for susceptibility to insecticides is recommended by WHO in all countries that use LLINs or indoor residual spraying for malaria control⁵. In Nigeria, integrated malaria control interventions (artemisinin-based combination therapy (ACT), LLINs and targeted IRS) have been in use since 2006. This has contributed to a significant reduction in clinical malaria cases in the country. However, the gains made were not much because of reduced efficacy due to the development of resistance¹⁴. Nigeria achieved universal coverage with LLINs in 2011 but the major challenge is to maintain this coverage and ensure effective usage of the mosquito nets, especially after it was recently reported from Rwanda that LLIN effectiveness lasts less than 3 years due to the rapid loss of insecticidal activity and physical deterioration in the field¹⁵. LLIN deterioration

problems were also shown in recent findings from Senegal where damaged nets provided less protection to malaria compared to intact ones¹⁶.

However, continuous exposure of mosquitoes to treated nets and the extensive use of pyrethroids in agriculture contributed to the development and emergence of insecticide resitance¹⁷.

Anopheles gambiae s.l. was the only malaria vector found in the study site, from June through September. This finding is in support of the report that An. gambiae s.l., abound in Gwagwalada, Abuja, Nigeria¹⁸ and western Kenya¹⁹ with a higher preponderance.

Mosquitoes were more abundant in August followed by July. This finding further confirmed the earlier report²⁰. However, the finding was in disagreement with the reported²¹ increase in abundance of *An. gambiae s.l.*, in September.

As in our study, in south-eastern Côte d'Ivoire, An. gambiae were found to be resistant to permethrin and alphacypermethrin but largely susceptible to deltamethrin²². An. gambiae s.l., were found to be fully susceptible to bendiocarb, deltamethrin and pirimiphos-methyl. This finding is in support of the previous reports from the south, central and east coasts of Madagascar²³. The full susceptibility of bendiocarb and resistance to alphacypermethrin and permethrin observed in this study, was in concordance with the results of Gari et al.20 but, contrary to our findings deltamethrin was resistant in Ethiopia¹² in northern Uganda mosquitoes were fully susceptible to also reported bendiocarb but resistant to deltamethrin and permethrin. As in this study, Rokotoson et al.²³ also reported resistance to alphacypermethrin, which was reverted after exposure to PBO. Permethrin efficacy was also restored in our study. This finding is however in contrast to the results of Okia et al.12, who reported partial abolition of permethrin resistance after exposure of mosquitoes to PBO. Resistance intensity in this study was reverted after exposure to $2\times$ and $5\times$ concentrations for alphacypermethrin and permethrin, respectively. This finding contrasts the report that An. gambiae s.l., exposed to permethrin survived $5 \times$ and $10 \times$ diagnostic concentrations¹². The study had its limitations being confined only to Gidan-Yaro village and did not take into cognisance the implication of the agrochemicals mostly used within the area to the development of resistance. This study for the first time reports the insecticide susceptibility status and malaria vector abundance in the study area. The results indicated that the pyrethroids alone are no longer effective in controlling the Anopheles gambiae s.l. The implication of this is that continuous usage of pyrethroids or pyrethroids based LLINs will only expose the populace to more mosquito bites and hence higher malaria infection. The importance of these findings is that the information provided would form the basis for detailed investigation on the effectiveness/durability of the nets and reason(s) for the development of insecticide resistance and its mechanisms. A combination of several methods should be considered as an effective and alternative way for vector control. It may also serve as a guide to the inhabitants of the community and the State Ministry of Health in planning and execution of its policies in vector monitoring, evaluation and control.

CONCLUSION

The results of the study show that resistance to pyrethroids is ever-increasing and spreading globally. To curb further spread of pyrethroid resistance and to preserve the

effectiveness of LLINs, an insecticide resistance management strategy shall switch to the usage of bendiocarb and pirimiphos-methyl for malaria vector control. There is also the need to implement a rotational strategy of insecticide usage every few years.

SIGNIFICANCE STATEMENT

The study discovers for the first time, the malaria vector abundance and insecticides resistance in the area. It also highlighted the fact that pyrethroids alone are no longer effective in controlling malaria vectors. The study will form the basis for detailed research on the effectiveness/durability of the nets and the mechanism of resistance in the area. It may also guide the community and State Ministry of Health in proper planning and execution of policies on vector control.

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