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Susceptibility Status of Malaria Vectors to Pyrethroids in Southern Gombe, Northeastern Nigeria

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ABSTRACT

The study was conducted to assess the susceptibility of malaria vectors to pyrethroids in four communities from Billiri and Balanga LGAs of Southern Gombe. Four pyrethroid insecticides approved by World Health Organization (WHO) for vector control were used. *Anopheles* larvae were collected from Kentengereng, Awai, Putoki and Talasse communities and reared to adults. Two to three- days old, non-engorged female mosquitoes were exposed to the discriminating dosages of 0.05% alphacypermethrin, 0.05% lambdacyhalothrin, 0.05% deltamethrin and 0.75% permethrin susceptibility test kits. Knockdown effect was recorded every 10 min and mortality scored 24 h post-exposure. The mosquito species were morphologically identified. The study revealed that the malaria vectors were resistant to lambdacyhalothrin (mortality rate between 3% and 74%) except in Talasse where resistance is suspected with mortality upto 97%. Mosquitoes subjected to deltamethrin in Kentengereng were suspected to be resistant with mortality rate of 96% while in other communities they were resistant. Also, resistant to alphacypermethrin and permethrin were observed in all the communities. This study revealed a great diversity of malaria vectors and variation in status of susceptibility between the species. This calls for a continued monitoring of resistance status of these mosquitoes for informed policy decision on malaria vector control.

Keywords: Susceptibility, pyrethroids, Gombe, *Anopheles* mosquitoes, malaria

INTRODUCTION

Malaria is a disease caused by *Plasmodium* parasites and poses a global health problem (Cowman, et al., 2016). In 84 countries with endemic malaria, there was an increase in cases in 2021; the majority of these cases were in the African Region. Reports of malaria cases from 29 countries ranged up to 96%. African countries accounted for over 95% of all cases worldwide in 2021, with 234 million cases. And Nigeria accounted for 27% of all cases globally, bearing the highest percentage than any other nation (WHO, 2022). Nigeria have upto 97% of its population at risk of malaria (PMI, 2022). Up to 60% of all outpatient visits to healthcare facilities and 30% of all hospital admissions in Nigeria are due to malaria. According to estimates, malaria is to be blamed for 11% of maternal deaths, 30% of child deaths, and 25% of infant mortality. Estimates indicate that this illness costs Nigeria's economy ₦132 billion yearly in missed wages as a result of medical bills, preventative measures, wasted labour, etc. (NMEP & FMoH, 2014). Gombe state have a malaria prevalence of 30% (NMCP, 2020).

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Anopheles mosquitoes are known to transmit malaria parasites. Recent studies have shown that the members of the *Anopheles gambiae* sl are well distributed in Nigeria as a result of their adaptive radiation and in essence their survival to environmental factors including factors relating to selection pressure (Adeogun et al., 2023). *Anopheles* mosquito species have diverse geographic distribution, and they can be divided into primary vectors, those with the greatest influence on the development of malaria in a specific area or alternate vectors, which have secondary significance in the epidemiology of the illness. When primary vector populations are decreased or eliminated, the secondary vectors are commonly ignored, despite the fact that research has shown that they may still transmit malaria (Antonio-Nkondjio et al., 2006, Braack et al., 2020, and Lobo et al., 2015). Controlling vectors is essential for lowering the prevalence of malaria.

Vector control is still the most often employed preventive measure and has historically resulted in the biggest decreases in disease burden for conditions for which therapies are available, such as malaria (WHO., 2022). The most popular and efficient malaria vector control strategies are chemical-based. They are deployed through indoor residual spraying (IRS), insecticide-treated nets (ITNs), and additional interventions like larviciding and space spraying (PMI, 2022).

The most commonly used insecticides are the pyrethroids. Resistance to insecticide classes commonly used in these interventions has emerged in malaria vector populations throughout the world. Of particular concern is pyrethroid resistance widely reported in Nigeria (Awolola et al., 2005, Awolola et al., 2007, Olayemi et al., 2011, Umar et al., 2014, Awolola et al., 2018, Olatunbosun-Oduola et al., 2019, Idowu et al., 2020 and Chukwuekezie et al., 2020). This is a great challenge in vector control because this insecticide class is used in all WHO-recommended Insecticide Treated Nets (ITNs) and is also used for Indoor Residual Spray (IRS) in many countries (WHO, 2018).

Insecticide resistance monitoring is an integral part of vector control and is aimed at understanding the susceptibility or otherwise of the vectors to insecticides (PMI, 2022). There is paucity of data in Gombe state on the status of insecticide resistance profile. Hence the necessity to undertake this resistance monitoring to provide data in order to inform policy in vector control interventions in Gombe state and Nigeria.

MATERIALS AND METHODS

Study Area

Four Southern Gombe communities, two each from Balanga and Billiri LGA were used for the study. Gombe State has a land mass of 20,265km^2 and is situated in the northeastern region of Nigeria. The dry and the wet seasons of the state last from November through March and April through October, respectively. The landscape is a representation of the Sudan Savanna, with wide-open grassland that dries out during the dry season. The states of Yobe to the north, Adamawa and Taraba to the south, Borno State to the east, and Bauchi State to the west encircle Gombe State. The study communities and their GPS coordinates were Awai (Lat. 9.9027600, Long. 11.2148930), Kentengereng (Lat. 9.8833850, Long. 11.2284320), Talasse (Lat. 9.9737370, Long. 11.6698450), and Putoki (Lat. 9.8390870, Long. 11.6777870) (Figure 1).

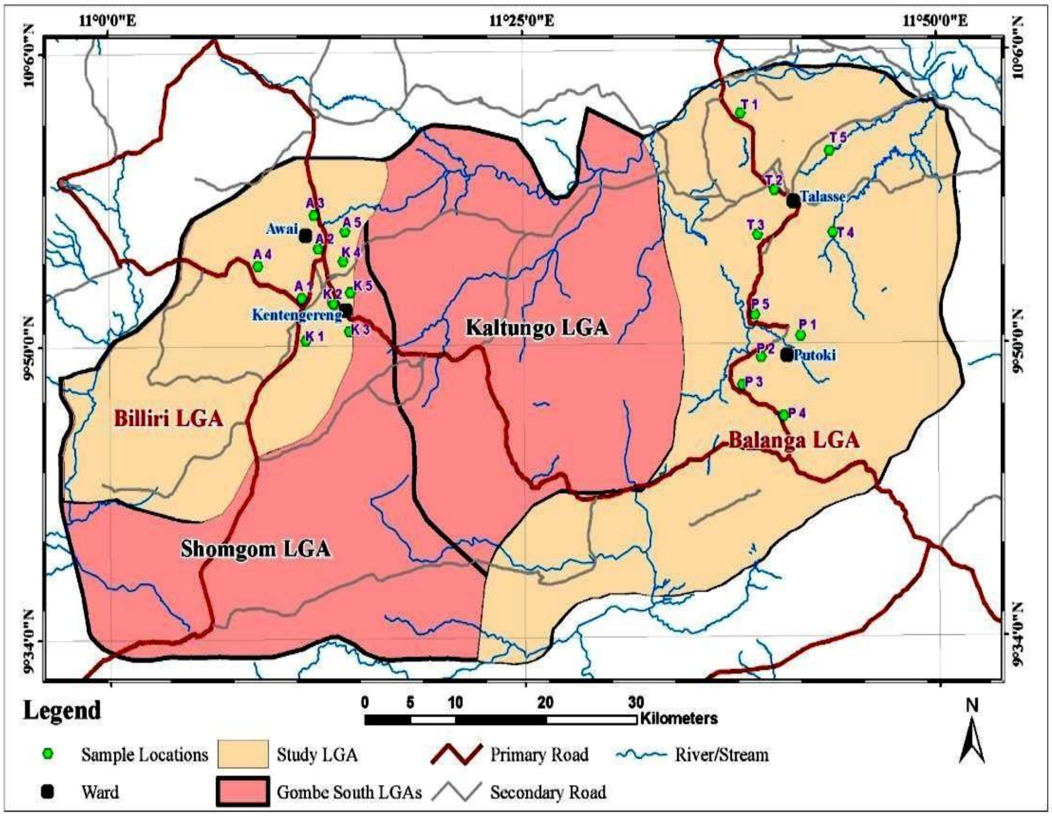

Figure 1: Map of Southern Gombe showing selected LGA's and study communities

Larval Collection and Rearing

In each of the four study communities, mosquito larvae were collected between April and December 2022 from natural breeding areas like ponds, ditches, streams, rice fields, and brick pits. Using the Global Positioning System (GPS), the study communities' and breeding locations' coordinates were established. Small, transparent containers were used to transport larvae to the Insect laboratory, Gombe state university. The larvae were then transferred to plastic trays that were 5 cm x 27 cm x 36 cm, and each tray was then covered with nets that were suitably tagged and labelled to represent the transfer and the depth of the larvae (about 2 cm) (World Health Organization, 2016). The larvae were fed 200mg of groundfish meal and housed in trays at 25-28°C and 76 ±5% relative humidity.

Susceptibility Test of Adult Female Mosquitoes

The WHO test kits for adult mosquitoes were used to conduct the susceptibility tests. Female *Anopheles* mosquitoes that had been given glucose but not blood-fed were separated and used in the experiment. 25 mature female *Anopheles* mosquitoes were exposed to test papers four times each and left to stand for an hour. The amount of mosquitoes that were knocked down was counted after 10 minutes, 15 minutes, 20 minutes, and then every ten minutes after that up to 1 hour. A 10% glucose solution was supplied to the mosquitoes in the holding tubes after they had been exposed, and they were subsequently placed on the mesh-screen end of the holding tubes. After 24 hours following exposure, mortality were recorded (World Health Organization, 2016). A mortality in the range of 98-100% indicates susceptibility. Mortality between 98% and 90% suggests the existence of resistance (confirmation required) and mortality below 90% confirms the existence of resistant genes in the population.

Data analysis

Statistical Package for Social Sciences (SPSS) was used for the data analysis. The percentage observed mortality was calculated and the analysis of variance between species and between communities were performed.

RESULTS AND DISCUSSION

The result indicated that the *Anopheles* mosquitoes tested with Lambdacyhalothrin from all the study communities were resistant except in one community. The 24 hours mortality for Kentengereng, Awai and Putoki were 59%, 67% and 3% respectively. Resistance to Lambdacyhalothrin was suspected in Talasse with mortality rate of 97%. The mosquitoes in Talasse subjected to this insecticide are *An. pretoriensis* and they tend to be more susceptible than *An. gambiae* sl. The findings of (Al-Koleeby et al., 2020) show agreement with this finding where they reported resistance of Lambdacyhalothrin to *An. arabiensis*. The mortality rate of the mosquitoes tested with Alphacypermethrin from Kentengereng, Awai, Putoki and Talasse were 79%, 53%, 75% and 89% respectively. Soma et al. (2020) also reported susceptibility of *An. gambiae* sl to Alphacypermethrin with less than 15% mortality. The wide variation in mortality rate observed compared to this study could be because of the diversity of *Anopheles* species reported in this study.

Mortality rate of *Anopheles* mosquitoes subjected to Deltamethrin was 96% in Kentengereng suggesting the existence of resistance but requiring confirmation. On the other hand, the mosquitoes from the three other communities were resistant to Deltamethrin, Awai (34%), Putoki (74%) and Talasse (3%) (Figure 2)

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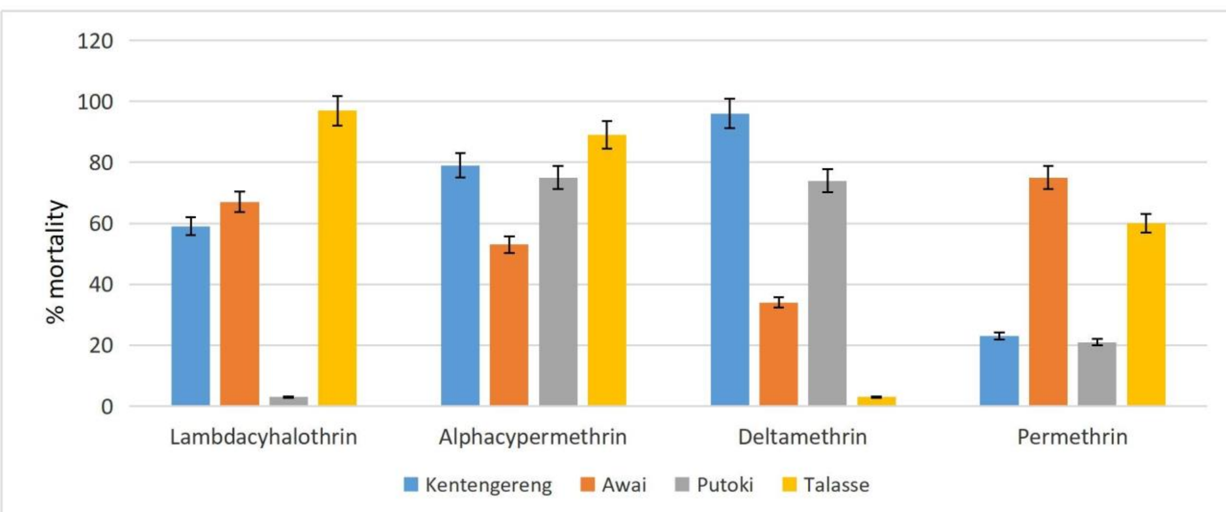

Figure 2: Susceptibility status of Malaria vectors from four communities of Gombe South

Another study by (Muhammad et al., 2021) showed similarity with the result in Awai, one of the communities of this study but showed variation with the other three communities where the mortality rate was higher. The variation could be as a result of the diversity of *Anopheles* species used in this study. It seems species such as *An. pretoriensis* and *An. maculipalpis* which are the dominant species encountered in this study are more susceptible than the *An. gambiae* sl. In the same vein (Adeleke et al., 2018) recorded post exposure mortality rate between 40-70% in the mosquitoes from Osun state exposed to deltamethrin. Ibrahim et al. (2013) reported 98.8% susceptibility to deltamethrin in Ojoo community of Ibadan, Nigeria. This is in variance to most communities in the present study. Mosquitoes from all the four communities were susceptible to permethrin with Awai recording the highest mortality of 75% followed by Talasse 60%, then Kentengereng and Putoki recorded 23% and 21% respectively. The wide difference observed is possibly because of the diversity in the species composition of the *Anopheles* mosquito species subjected to the test. *An. gambiae* sl tend to be more resistant than the other secondary vectors. Resistance to permethrin was equally reported by (Ibrahim et al., 2013) though with higher mortality rate compared to the findings of this present work. There was no significance difference in susceptibility insecticides between communities (P = 0.8392) and also between the pyrethroids tested (P = 0.7015).

Overall, out of the 1600 *Anopheles* mosquitoes identified, 777(48.6%) were *An. pretoriensis* and 443(27.7%) were *An. gambiae sl.* Other species were *An. maculipalpis* 15 5(9.7%), *An. rufipes* 128(8.0%), *An. coustani* 7(0.4%) and *An. pharoensis* 6(0.3%) (Table 1).

Table 1: Species composition of the mosquitoes from Billiri and Balanga LGAs

LGA's	Number Identified (N)	Species composition N(%)						
		An. gambiae sl N(%)	*An. pretoriensis* N(%)	*An. maculipalpis* N(%)	*An. rufipes* N(%)	*An. coustani* N(%)	*An. pharoensis* N(%)	Unidentified N(%)
Billiri	800	262(32.8)	284(35.5)	93(11.6)	98(12.2)	5(0.6)	4(0.5)	54(6.8)
Balanga	800	181(22.6)	493(61.6)	62(7.8)	30(3.8)	2(0.2)	2(0.2)	30(3.8)
Total	1600	443(27.7)	777(48.6)	155(9.7)	128(8.0)	7(0.4)	6(0.3)	84(5.3)

An. pretoriensis were the dominant species in both Billiri and Balanga LGA's constituting 284(35.5%) and 493(61.6%) respectively. Also, in Billiri, *An. gambiae* sl numbered 262(32.8) whereas in Balanga they accounted for 181(22.6%) (Table 2).

Table 2: Species composition of the mosquitoes from the study Communities in the LGA's

LGA's	Communities	Number Identified (N)	Species composition N(%)						
			An. gambiae sl N(%)	*An. pretoriensis* N(%)	*An. maculipalpis* N(%)	*An. rufipes* N(%)	*An. coustani* N(%)	*An. pharoensis* N(%)	Unidentified N(%)
Billiri	Kentengereng	400	131(32.8)	148(37.0)	32(8.0)	64(16.0)	0(0.00)	3(0.7)	22(5.5)
	Awai	400	131(32.8)	136(34.0)	61(15.3)	34(8.5)	5(1.2)	1(0.2)	32(8.0)
Balanga	Putoki	400	162(40.5)	182(45.5)	14(3.5)	11(2.8)	2(0.5)	2(0.5)	27(6.7)
	Talasse	400	19(4.8)	311(77.8)	48(12)	19(4.7)	0(0.00)	0(0.00)	3(0.7)
	Total	1600	443(27.7)	777(48.6)	155(9.7)	128(8.0)	7(0.4)	6(0.3)	84(5.3)

There is no significant difference between the LGA's mosquito species (P = 0.9999). Significant difference was observed between the species of mosquitoes (P = 0.0097). The result is at variance with previous studies (Yoriyo et al., 2014, Garba et al., 2017 and Wahedi et al., 2020) where they reported dominance of *An. gambiae* sl. in their findings.

Also, samples were taken in Gombe south from the same LGAs by (Olatunbosun-Oduola et al., 2019) and a varying results were discovered. Despite the fact that the geographical points are not the same, it is believed that this study's wider geographic scope might have contributed to the species' diversity variation observed. It is obvious that secondary malaria vectors are found in those communities. The presence of these vectors have implications for both insecticide usage and other vector control interventions.

CONCLUSION

There is a widespread of pyrethroids resistance in all the study area. The secondary vectors are more susceptible to the pyrethroids tested than *An. gambiae sl.* This poses a great threat to the efficiency of Insecticide Treated Nets and Indoor Residual Spray. It is critical to conduct routine surveillance of malaria vectors as well as monitoring their resistance status to insecticides. It is obvious that alternative vector control measures is necessary in order to sustain gains made in malaria control interventions.

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