



MALARIA VECTORS RESISTANCE TO COMMONLY USED INSECTICIDES IN THE CONTROL OF MALARIA IN BICHI, NORTHERN NIGERIA

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ABSTRACT

This study was carried out in 2015 to assess the level of resistance of sibling species of Anopheles gambiae complex the principal malaria vector from Bichi in Kano state to three classes of insecticides; (DDT, Permethrin and Bendiocarb) approved by World Health Organization (WHO) for vector control with the aim of obtaining baseline susceptibility and resistance data by evaluating the potency of brands of insecticides used against the mosquito vector in the area where the disease is prevalent. An. gambiae larvae collected from ecologically contrasting breeding sites were reared to adults in the laboratory. Adults from the F1 progeny were assayed for resistance using the WHO adult insecticide susceptibility bioassay protocol. The mosquitoes were exposed to DDT, permethrin and bendiocarb belonging to 3 classes of insecticides. The individual sibling species were identified molecularly and the molecular resistance mechanisms characterized by SINE and kdr PCR methods respectively. The An. gambiae populations tested revealed high level of resistance to DDT and permethrin but less resistant to bendiocarb. Analysis of the genetic composition of the vector population revealed predominance of An. coluzzii (> 77%). L1014F and L1014S kdr mutations correlated to insecticide resistance phenotype expression. This study indicates differential distribution of the resistant genotype of Anopheles malaria vector between ecologically different habitats in the area. This insecticide resistance represents a threat to vector control tool and should be monitored. The information obtained could be useful in decision and planning making for vector control programs in the region.

Key words—Anopheles coluzzii, Anopheles gambiae, Insecticides, Malaria control, resistance, Susceptibility

INTRODUCTION

Anopheles gambiae Giles is one of the most important malaria vectors in Africa. Malaria kills more people than HIV/AIDS or any other killer disease. Malaria is endemic throughout Nigeria accounting for 25% of infant mortality (Federal Ministry of Health, 2005). Malaria impedes on economic development not only by causing premature death but also through lost/diminished productivity, enormous medical cost and population growth. Malaria vector control programs in Africa rely heavily on the use of pesticides for insecticide-treated nets (ITNs)/long-lasting insecticide-treated nets (LLINs) and for indoor residual spraying (IRS) (Kabula *et al.* 2014). The two primary methods of vector control; Indoor Residual Spraying (IRS) and the distribution of Long-Lasting Insecticide-Treated Nets (LLINs) have been demonstrated to reduce the number of malaria cases when properly used against insecticide susceptible mosquito populations (Wondji *et al.* 2012). The use of both strategies has substantially increased over the years since 2000 in many

malaria endemic countries with increased donor funding to achieve Roll Back Malaria targets aimed at the elimination of malaria. The overall ownership of the nets has increased steadily from 8% in 2008 to 42% in 2010, so also the proportion vulnerable group sleeping under net increased from 6% to 29% in the same period (Kolade *et al.* 2013). The development and rapid spread of insecticides resistance to major malaria vectors across the African countries has posed a threat to effectiveness of these different mosquito control measures (Kolade *et al.*, 2013). This resistance is mainly associated with either reduced target site sensitivity arising from a single point mutation in the sodium channel gene, or increased metabolic detoxification of insecticides. The knockdown resistance (kdr) characterized by changing either a Leucine residue to a Phenylalanine; West African mutation (L1014F) or a Leucine to Serine; East African mutation (L1014S) have being identified in *An. gambiae* and confers knockdown resistance (kdr) to DDT and pyrethroid insecticides.

The metabolic resistance involves increased detoxification through enzymatic activities of the large enzyme families' esterases, glutathione S-transferases and monooxygenases.

Information on the susceptibility of principal malaria vectors to common insecticide used in public health and the underlying mechanism are crucial. This information will adequately inform control programs of the most suitable insecticide to use and facilitate the design of appropriate resistance management strategies. However, recently (Ibrahim *et al.* 2014; and Habibu *et al.*, 2017) have both reported high frequency of *kdr* L1014F associated with pyrethroid resistance in Auyo. In this study, we provided additional information on the differential distribution of the genotypic resistance on the agricultural and residential vector populations, established their species and resistance status to Permethrin, DDT and Bendiocarb and detected the *kdr*-mutations resistance markers.

MATERIALS AND METHODS

Study sites

The study area is located within the Sudan savannah zone of West Africa about 840 kilometres from the edge of the Sahara desert (Ibrahim *et al.*, 2014). Kano sees on average about 690 mm (27.2 in) of precipitation per year. The study localities in Bichi are characterized by high insecticides usage for agricultural or personal protection (1) Bichi residential (12^o13' N, 8^o15' E), an urban area with high population and a major commercial centre outside Kano metropolis, (2) Bichi agricultural (12^o7' N, 8^o14' E), an area of intensive agriculture throughout the year. Farmers in Bichi agricultural sites use a wide range of pesticides and herbicides to protect their crops. These pesticides marketed under different trade names belong to all the chemical classes including organophosphates, organochlorine, pyrethroids and carbamates.

Mosquito larval collection, processing, and rearing

Larval collections were carried out at the peak of the rainy season in 2015. Larvae and pupae of *An. gambiae s.l.* were collected from natural breeding sites using scoops from the two sampling sites. Larvae were transported to the insectary at Bayero University Kano. Larvae and pupae were maintained under standard insectary condition 25-28 °C and ~70-80% humidity (Das, *et al.*, 2007) supplied with

baker's yeast daily. The larvae were reared in light plastic containers covered with fine mesh mosquito nets and reared in the same water from which they were sampled to maintain the impact of the environmental xenobiotics present in the breeding habitat. The adults that emerged were transferred into cages from the breeding containers.

Insecticide susceptibility tests

Mosquitoes were assessed using World Health Organization (1998) discriminating dosages with three insecticides: 0.75% Permethrin, 0.1% Bendiocarb or 4% DDT. The numbers of knockdown mosquitoes for each insecticide were recorded at every 10 minutes during the 1h exposure period. After exposure, mosquitoes were kept in observatory/recovery tube and supplied with a 10% sugar solution and allowed a 24 h recovery period. Mosquitoes exposed to untreated filter papers served as Controls. The mortality was recorded after 24 h, and the susceptibility status of the population graded according to WHO recommended protocol (WHO, 2013c). Dead and surviving mosquitoes from assessments were used for molecular analysis.

Mosquito species identification and PCR detection of knock-down resistance (*kdr*) alleles in DNA extracted from a proportion of the dead and surviving mosquito specimens from insecticide treatments were subjected to the SINE PCR (Santolamazza *et al.*, 2008) for species and molecular form identification and also assayed for the presence of knockdown resistance (*kdr*) to detect the West African (L1014F) and East African (L1014S) *kdr* alleles based on TaqMan assay described by (Bass *et al.*, 2007) using a Max3000P Real-Time PCR (QPCR) system (Stratagene).

Data Analysis

SPSS 22 statistical software was used to analyze the relationships. Coefficients of correlation were calculated between the frequency of different mutations, and the corresponding mortality rates between the three sampling sites.

RESULTS AND DISCUSSION

The results of the insecticides bioassays performed on adults *An. gambiae s.l.* are shown in Figure1. Using the WHO (2013c) definitions of resistance the two populations from the Sudan savannah (Bichi) in northern Nigeria revealed high resistance to DDT and Permethrin with low resistance to Bendiocarb.

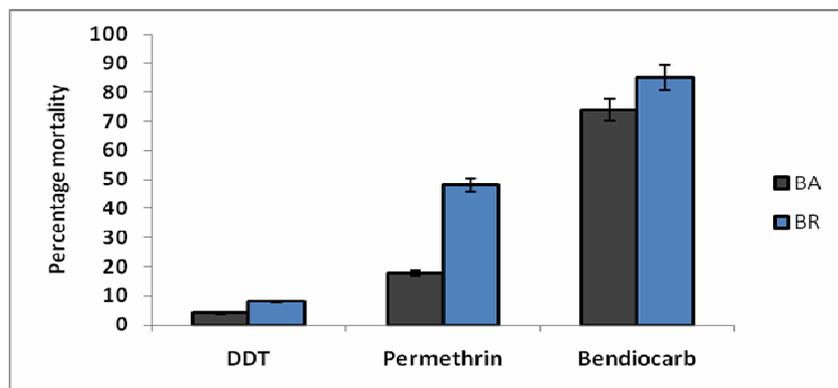


Figure 1 Mortality rate of *An. gambiae s.l.* 24 h post exposure. Mosquitoes were exposed to 4% DDT, 0.75% Permethrin and 0.1% Bendiocarb in WHO susceptibility test at: Bichi agricultural (BA) and Bichi residential (BR) sites.

Table 1 Species and molecular form distribution of *An. gambiae s.l.* from the two different sampling sites (in Bichi northern Nigeria) using SINE based assay

Site	Molecular form of <i>An. gambiae</i>							
	<i>An. arabiensis</i>		M		M/S		S	
	N	%	N	%	N	%	N	%
BA	46	23.0	150	75.0	4	2.0	0	0.0
BR	36	18.0	163	81.5	1	0.5	0	0.0
Total	82	20.5	313	78.3	5.0	1.2	0	0.0

BA = Bichi agricultural; BR = Bichi residential

Table 2 Distribution of *kdr*-West (L1014F) mutation in *An. coluzzii* and *An. arabiensis* mosquitoes

Site	<i>Anopheles coluzzii</i>						<i>Anopheles arabiensis</i>					
	Genotype count			Allelic frequency			Genotype		count			
	N	FF	FL	LL	F	L	N	FF	FL	LL	F	L
BA	50	20	15	15	0.700	0.300	10	2	3	5	0.500	0.500
BR	45	11	12	22	0.511	0.489	15	1	4	10	0.313	0.667

FF, FL and LL are the three possible genotypes, where F represents the resistant L1014F allele and L represents the susceptible wild-type allele, N is number of particular species either *An. coluzzii* or *An. arabiensis*

Table 3 Distribution of *kdr*-East (L1014S) mutation in *An. coluzzii* and *An. arabiensis* mosquitoes

Site	<i>Anopheles coluzzii</i>						<i>Anopheles arabiensis</i>					
	Genotype count			Allelic frequency			Genotype		count			
	N	SS	SL	LL	S	L	N	SS	SL	LL	S	L
BA	50	10	14	26	0.480	0.520	10	1	2	7	0.300	0.700
BR	45	5	10	30	0.333	0.667	15	0	2	13	0.133	0.867

SS, SL and LL are the three possible genotypes, where S represents the resistant L1014S allele and L represents the susceptible wild-type allele, N is number of particular species either *An. coluzzii* or *An. arabiensis*

The mosquitoes tested from all the sampling sites showed a high resistance. Similarly, the results of the probit model showed that bendiocarb was the only insecticide that showed significant effects on the mortality of *An. gambiae s.l.* population exposed to these insecticides in all the study sites with P-values of $P < 0.001$ in all the study sites. However, mosquitoes from agricultural sites (zone A) recorded higher insecticide resistance when compared to those from residential sites (zone B). These high levels of resistance are probably related to extensive pesticide use in the region. Decrease in mortality rates to DDT and Permethrin was associated to a significant increase in the KDT_{50} observed which were higher in agricultural sites were probably due to the involvement of *kdr* (Bigoga *et al.* 2014). The higher Knockdown time observed in this study, for DDT and permethrin is consistent with the findings of (Chandre *et al.*, 1999, Ibrahim *et al.*, 2014, and Alhassan, *et al.*, 2015) indicating the involvement of *kdr* mechanism of resistance. This is connected to the fact that the pyrethroid (permethrin) and organochlorine insecticides (DDT) shares same mechanism of action by targeting the voltage-gated sodium channel on the insects' neurons. However, the high level of resistance to Permethrin and DDT could also be associated with increased activities of the metabolic enzymes GSTs and Esterases which have been detected previously in populations of *An. gambiae* from Auyo resistant to these insecticides (Alhassan *et al.* 2015). The carbamate (Bendiocarb) on the other hand, targets the acetylcholinesterase gene (Awolola *et al.*, 2007). DDT and Permethrin resistance pattern observed in the *An. coluzzii* populations are similar to previously reported cases of DDT and Permethrin resistance in the north-western, north-central and south-western Nigeria (Ibrahim *et al.*, 2014, Alhassan *et al.* 2015, and Awolola *et al.* 2007). Selection for pyrethroid resistance in *An. gambiae* has been associated with the use of agricultural pesticides but not with DDT because of restricted use of DDT since it was banned (Sadasivaiah *et al.*, 2007, Adedayo *et al.*, 2010). But this was not the case in the present study, where some farmers confirmed the continued usage of DDT as pesticides and herbicides. This also suggests possible uncontrolled and illegal usage of DDT or other unspecified and unbranded locally made pesticides. The massive use of pesticides in agricultural settings has been well documented as a factor contributing to the emergence of resistance in

Anopheles populations (Akogbeto *et al.*, 2006). The sustained usage of these pesticides in the study sites may also have maintained a selection pressure for DDT resistance. The DDT and permethrin high resistance recorded in the residential areas were most likely due to increased use of household insecticides by high ITNs and IRS coverage or recurrent space spraying interventions.

The present study reveals the co-occurrence of L1014S and L1014F mutations coupled with high insecticide resistance in the two Anopheline populations belonging to agricultural and residential settings in northwest Nigeria suggesting the spreading of the L1014S mutation gene across Africa.

The high allelic frequencies recorded in the residential sites could be due to the increased use of pyrethroids for ITNs and IRS in public health as indicated in previous studies (Dykes *et al.* 2015; Li *et al.* 2015). The co-occurrence of the L1014S and L1014F mutations has already been reported in *An. gambiae s.l.* from both East and West Africa such as Tanzania and Nigeria (Kabula *et al.* 2014, and Ibrahim *et al.* 2014). The frequency of the L1014F mutation was positively correlated with resistance ($P = 0.04$) thus the higher the frequency of the L1014F mutation the higher the level of resistance to DDT and permethrin. The results also show a weak relationship between the frequency of L1014F and L1014S mutations ($P = 0.772$). This study also reports the co-occurrence of East form of *kdr* and West form of *kdr* in *An. coluzzii* and *An. arabiensis* mosquitoes in a higher frequency than previously reported (Ibrahim *et al.* 2014). The low but increased frequency of the East *kdr* indicates that its selection in this region is recent and is increasing. No S-form was recorded from the field collections which could be explained by the previous observation made by (Coluzzii, 1984) that the M-form is predominant in this type of ecological setting with irrigation systems providing ideal breeding site. Increased usage of insecticides for agricultural purposes and /or widespread of LLINs and repeated use of pyrethroids in IRS in the region could explain the high frequency of the *kdr* mutations.

Previous studies from this region and across some African countries have reported full susceptibility to the carbamate such as bendiocarb and malathion in *An. gambiae* mosquitoes (Ibrahim *et al.*, 2014) thus suggesting the use of this class of insecticide as an alternative to pyrethroids and DDT in IRS.

On the contrary, in this study *An. gambiae s.l.* mosquitoes tested against bendiocarb show from suspected resistance to weak resistance indicating that the *An. gambiae s.l.* mosquito population in this region has started to develop resistance against carbamate; however, further investigation is needed to establish the resistance status of the *An. gambiae* to carbamate.

Conclusion

The high insecticides resistance observed in *An. gambiae s.l.* populations as well as high frequency of L1014F and L1014S kdr mutation in *An. coluzzii* and *An. arabiensis* in this study particularly for pyrethroids, may compromise the efficacy of LLINs and IRS on which most African countries rely on to reduce malaria transmission. The findings from this work could help and guide the malaria control programme of Nigeria particularly in the choice of

insecticide.

Recommendation

It is recommended that, there should be inter-sectoral collaboration between pest management and vector control in agricultural and public health departments respectively towards monitoring and managing the impact of the growing market and usage of agricultural pesticides on the emergence of vector resistance and also to embark on public enlightenment on the danger and repercussions of the abuse of chemical insecticides, pesticides and herbicides both in the residential and agricultural settings towards sustainable resistance management strategies. Based on the findings of this study and also of previous studies by, Corbel *et al.*, 2007, Akogbeto *et al.*, 2010 and Ibrahim *et al.*, 2014, there is a need to look into alternatives to pyrethroid insecticides to manage resistance.

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