Susceptibility Status Of Anopheles Gambiae Complex To Insecticides Commonly Used For Malaria Control In Northern Nigeria

Habibu U. Abdu, Yayo A. Manu, Yusuf Y. Deeni

Abstract: this study was carried out to assess the level of susceptibility or resistance of sibling species of Anopheles gambiae complex from Auyo in Jigawa state between 2013 and 2014 to three classes of insecticides approved by World Health Organization (WHO) for vector control. A. 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 approved by WHOPES for malaria vector control. 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 were highly resistant to DDT and permethrin insecticides but less resistant to bendiocarb. Analysis of the genetic composition of the vector population revealed preponderance 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. The information could be useful in decision and planning making for vector control programs in the region.

Key words: Anopheles coluzzii, Anopheles gambiae, Insecticides resistance, kdr, Malaria control, Nigeria, Susceptibility

1. INTRODUCTION

Anopheles gambiae Giles is one of the most important malaria vectors in Africa [1] where the crusade towards effective malaria control involves the use insecticides for Long lasting insecticide treated nets (LLINs) and Indoor residual spray (IRS) as reported earlier [2, 3]. Nigeria has recently scaled up its malaria control programme through free mass distribution of LLINs and IRS [4]. The overall ownership of the nets has increased steadily from 8% in 2008 to 42% in 2010, so also the proportion of vulnerable group sleeping under net increased from 6% to 29% in the same period [5]. In 2009 4,137,464 LLINs were freely distributed in Kano state [6] while in Jigawa state between 2009 and 2015 approximately, five million LLINs were distributed [7]. IRS has been piloted in seven states of Nigeria including Jigawa state under the World Bank Malaria Control Booster Program. The work of [3] highlighted four major classes of chemical insecticides (i.e. pyrethroids, organochlorines, organophosphates, and carbamates) as the gold standard for malaria vector control strategies. However, pyrethroids are the only class of insecticide currently recommended for use on ITNs/LLINs or IRS by WHO, due to safety and cost effectiveness [8]. Nevertheless, bendiocarb and dichlorodiphenyltrichloroethane (DDT) are also used in some areas for IRS [9]. The malaria vectors have evolved resistance to the chemicals developed to control them [10].

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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 [5, 9]. This resistance is mainly associated with either reduced target site sensitivity arising from a single point mutation in the sodium channel gene [11], or increased metabolic detoxification of insecticides [13, 14]. The knockdown resistance (kdr) characterized by changing either a Leucine residue to a Phenylalanine; West African mutation (L1014F) [11], or a Leucine to Serine; East African mutation (L1014S) [12] 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 [13, 14]. 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 [24] have reported high frequency of kdr L1014F associated with pyrethroid resistance in the same area. 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.

2. MATERIALS AND METHODS

Study sites

The study area falls within the Sudan savannah vegetation region with pockets of Guinea Savannah in some areas of the state especially the southern zone. Farmers, in Auyo agricultural sites use a large number of insecticides 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. The study localities comprise (1) Auyo residential area ($12^0$20' N, $9^0$56' E), an area use for residential purposes

and located near big market for the vegetables and cereals produced in the irrigation scheme, (2) Auyo agricultural 1 ($12^0$18' N, $9^0$56 E') and (3) Auyo agricultural 2 ($12^0$21' N, $9^0$59' E), localities with intensive farming known for their history of irrigation activities in which rice and other vegetables are produced.

Mosquito larval collection, processing, and rearing

Larval collections were carried out at the peak of the rainy season between 2013 and 2014. Immature stages of An. gambiae s.l. were collected from the field as larvae and pupae using scoops from the three sampling sites. Larvae were transported to the insectary at Bayero University Kano. The larvae kept in light plastic containers were fed with baker's yeast and reared in the same water from which they were collected to maintain the impact of the environmental xenobiotics present in the breeding habitat. The larvae were maintained under standard insectary condition (25-28 ^oC and ~70-80% humidity, with a 12 h day/night cycle) [15]. The 4^{th} instar larvae and pupae were picked and transferred into cages to emerge while some were transferred into 1.5ml Eppendorf tubes containing RNA later (RLT) and stored for further molecular work. Upon emergence, the adults' mosquitoes were morphologically identified according to morphological identification keys [16, 17].The adults An. gambiae were maintained on 10% sugar solution until they were used for insecticide susceptibility tests.

Insecticide susceptibility / resistance bioassay tests

Insecticides susceptibility tests were carried out on 2-3 days old non-blood-fed adult An. gambiae mosquitoes using WHO insecticide susceptibility test-kits and standard protocol for adults [18]. The Bioassay test kits and papers impregnated with diagnostic doses of insecticides were obtained from the Vector Control and Research Unit, University Sains Malaysia (Penang, Malaysia). A batch of 20-25 (2-3 days) old adults mosquitoes were exposed to tubes containing papers impregnated with either 0.75% Permethrin, 0.1% Bendiocarb or 4% DDT. For each insecticide tested, 100 mosquitoes in four replicates and a control of 25 mosquitoes were used.

Mosquito species and molecular forms identification

An. gambiae s.l. complex and An. gambiae s.s. were identified to sibling species and molecular forms according to the SINE PCR method [19]. The PCR diagnostic approach proposed is based on the specific and irreversible insertion of a 230 bp transposable element (SINE200) in the M-form (and its absence in S-form), thus allowing an unambiguous, simple and straightforward recognition of M and S forms [19]. Table 1 shows the specific primer sequences, the reaction mixture and the condition for the SINE PCR.

Table 1 *Reaction set up and Primers for performing SINE PCR reaction*

	Primer sequence (5' – 3')	**Quantity(µl)**
Sterile water		9.0
10µM FP	TCG CCT TAG ACC TTG CGT TA	1.0
10µM RP	CGC TTC AAG AAT TCG AGA TAC	1.0
$MgCl_2$ 25µM		0.5
Taq DNA polymerase		12.5
DNA template 18ng/µl		1.0
Total		**25.0**

The reaction was set up as shown in table 1 in PCR tubes. PCR was carried out in Thermal cycler S1000™ Bio-Rad System. The PCR cycles comprise an initial step of 5 min at 94^oC to activate the DNA polymerase. Then followed by 35 cycles, each consisting of 30 s denaturation at 94^oC, 30 s annealing at 54^oC and 30 s extension at 72 ^oC, the final cycle products are extended for 10 min at 72^oC and 4^oC hold. The amplicons were then analyzed as described by Santolamazza et al., 2008 on 1.5% agarose gels stained with Gel red, with low and high molecular weight bands corresponding to fragments containing or lacking the targeted SINE200, respectively.

Detection of knock-down resistance alleles in An. gambiae

A proportion of the dead and surviving mosquito specimen from insecticide treatments were assayed for the presence of knockdown resistance (kdr) by PCR to detect the West African (L1014F) and East African (L1014S) kdr alleles based on TaqMan assay [20]. The detection of East kdr (L1014S) and West kdr (L1014F) mutations was performed using TaqMan PCR diagnostic assays described in [20] and a Max3000P Real-Time PCR (QPCR) system (Stratagene). According to [20] the assay is a PCR method employing oligonucleotide probes that are dual labeled with a fluorescent reporter dye and a quencher molecule. Amplification of the probe-specific product causes cleavage of the probe, generating an increase in reporter fluorescence as the reporter dye is released away from the quencher. The primer name, sequence and concentration used in the PCR reaction are shown in table 2. Cleavage of allele-specific probes can be detected in a single PCR by using different reporter dyes [21].

Table 2 *Primers used for detection of kdr alleles*

Primer name	Sequence (5'-3')	Concentration (nM)
Kdr-forward	CATTTTTCTTGGCCACTGTAGTGAT	800
Kdr-reverse	CGATCTTGGTCCATGTTAATTTGCA	800
MGB probe WT	(JOE) CTTACGACTAAATTTC (TAM)	200
MGB probe kdr-W	(6-FAM) ACGACAAAATTTC (TAM)	200
MGB probe kdr-E	(6-FAM) ACGACTGAATTTC (TAM)	200

Data Analysis / Interpretation of results

To investigate significant differences in the effect of the different insecticides on the mortality and Knockdown of An. gambiae s.l. from 3 study sites, the probit and mixed effect probit models were used in R statistical packages to account for the repeated measures of knockdown over time. Interaction effects between insecticide treatments and areas were first investigated, and if significant, the impact of the insecticide treatment was analyzed in each area individually. Statistical significance was determined in reference to DDT for all insecticides tested. In this study, Abbott's formula was not used for the correction of mortality because the mortality rates in all control tubes were less than 5% [22]. Mean percentage mortality was determined across all mosquitoes tested for a particular insecticide in a given site. WHO criteria [23] for discriminating individuals for susceptibility /resistance status were applied: mortality rate between 98% - 100% indicate full susceptibility; mortality rate between 90% - 97% suspected resistance requires further investigation and mortality rates < 90% indicate the population is resistant to the tested insecticides. To assess whether the frequency of different kdr alleles in An. gambiae s.l. was related to resistance 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.

3. RESULTS

The results of the insecticides bioassays performed on adults An. gambiae s.l. are shown in Figures 1 and 2. Using the WHO (2013c) definitions of resistance the two populations from the Sudan savannah (Auyo) in northern Nigeria revealed high resistance to DDT and Permethrin with low resistance to Bendiocarb. In zone A comprising AA1, and AA2 results of the bioassays performed in 2013(Figure 1) showed very low mortalities with DDT and Permethrin that ranged between 7% to 16% and 29% to 35% indicating high levels of resistance to the insecticides respectively. Survival after exposure to Bendiocarb, ranged between 81% to 95%. Populations of An. gambiae s.l. from zone B (AR) were also highly resistant to DDT and Permethrin. The mortality with DDT was 32% while for Permethrin 20% was recorded. Exposure to Bendiocarb resulted in 83% mortality (Figure 1). In 2014 in zone A, a similar pattern of mortality with DDT, Permethrin and Bendiocarb was observed. Mortality with DDT in AA1 decreased from 16% to 14%. Mortality with Permethrin in AA1 and AA2 also decreased from 38% to 26% and 23% to 10% respectively and for Bendiocarb decrease from 95% to 90% was at AA1 while for AA2 increase from 81% to 84% was recorded. DDT mortality in zone B in 2014 further revealed high resistance in these An. gambiae populations and was not much different from that recorded in 2013 (Figure 2). Similarly change in mortality with Permethrin from 20% to 25% was observed in zone B between 2013 and 2014. Furthermore, exposure of An. gambiae mosquitoes to Bendiocarb in 2014 resulted in higher mortality 88% compared to 83% in 2013. The results of the mixed effect probit model showed that only treatment with Bendiocarb in zone A and B in 2013 and 2014 had significant effects on knockdowns of An. gambiae s.l. population exposed to these insecticides with P-values ($P<0.001$). The probit model analysis also showed that Bendiocarb was the only insecticide that showed significant effects on mortality with P-values of $P< 0.001$ in all the study sites except in 2013 at AA2 where the p-value was ($P>0.05$). However, treatment with Permethrin showed varied levels of significant effects on both knockdowns and mortalities of An. gambiae s.l. compared to the baseline treatment with DDT. Significant effect was observed on knockdown and mortality in 2014 at AA1 with P values ($P<0.001$) and ($P<0.01$) respectively.

Mosquito species and molecular forms identification

A total of 570 An. gambiae complex mosquitoes from Auyo that survived exposure to DDT, Permethrin and Bendiocarb and 140 dead after the exposure were identified to species and molecular forms by SINE PCR according to [19] (Table 3). Among the An. gambiae s.l. population, 77.6% were An. coluzzii (formerly M form) 15.6% were An. arabiensis, 6.6% and 0.2% were hybrid (M/S) and An. gambiae s.s. (formerly S form) respectively. Figure 3 shows the diagnostic bands for each of the species and the molecular forms. Figure 4 is the Map of the study area showing the distribution of 1014F and 1014S in An. gambiae population in the 3 study sites in Nigeria.

Genotyping of knockdown resistance (kdr) mutations

A total of 180 identified mosquitoes from Auyo which were exposed to DDT and Permethrin were genotyped for the presence of East kdr (L1014S) and West kdr (L1014F) mutations. Of these 118 were homozygous for the susceptible wild-type, 18 were homozygous for L1014S and 47 were heterozygous for L1014S. When genotyped for L1014F, 98 were homozygous for the susceptible wild-type, 39 were homozygous for L1014F and 43 were heterozygous for L1014F as shown in Tables 4 and 5. The L1014S mutation was detected in both An. coluzzii and An. arabiensis. The L1014S mutation in agricultural setting was recorded at allelic frequencies of 40.0%, and 55.3% (AA1 & AA2) in An. coluzzii; and 20.0%, 30.8%, in An. arabiensis respectively. The L1014S mutation was in residential setting recorded at allelic frequencies of 27.5% in An. coluzzii; and 0.0% in An. arabiensis. Similarly the L1014F mutation was detected in both An. coluzzii and An. arabiensis. The L1014F mutation was detected at allelic frequencies of 48.9% and 65.9% in agricultural setting (AA1& AA2) in An. coluzzii; and; 20.0%, & 61.6% in An. arabiensis. While in the residential setting (AR) the frequencies were; 42.5% in An. coluzzii; and 5.0% in An. arabiensis. Although the two kdr mutations occurred in both

An. coluzzii and An. arabiensis, the L1014F was much associated with An. coluzzii. A significant positive correlation (P<0.05) between the frequency of the L1014F point mutation and resistance to DDT and Permethrin was observed. However, a weak or non-significant correlation (P>0.05) between the frequency of the L1014S point mutation and resistance was also found. L1014S and L1014F mutations co-occurred in both agricultural and residential settings with high frequencies. However, the frequencies of the two mutations were greater in the agricultural settings than in the residential settings.

Table 3 *Species and molecular form distribution of An. gambiae s.l. from the three sampling sites using SINE based assay*

Site/ Year	Molecular form of An. gambiae							
	An. arabiensis		M		M/S		S	
	N	%	N	%	N	%	N	%
AA1								
2013	36	30.0	64	53.0	20	17.0	0	0.0
2014	4	4.2	80	81.6	12	12.3	2	2.0
AA2								
2013	16	13.8	100	86.2	0	0.0	0	0.0
2014	8	9.1	80	90.9	0	0.0	0	0.0
AR								
2013	24	16.2	116	78.4	8	5.4	0	0.0
2014	23	16.4	110	78.6	7	5.0	0	0.0
TOTAL	**111**	**15.6**	**550**	**77.6**	**47**	**6.6**	**2**	**0. 2**

AA1= Auyo agricultural 1; AA1= Auyo agricultural 2; AR = Auyo residential;

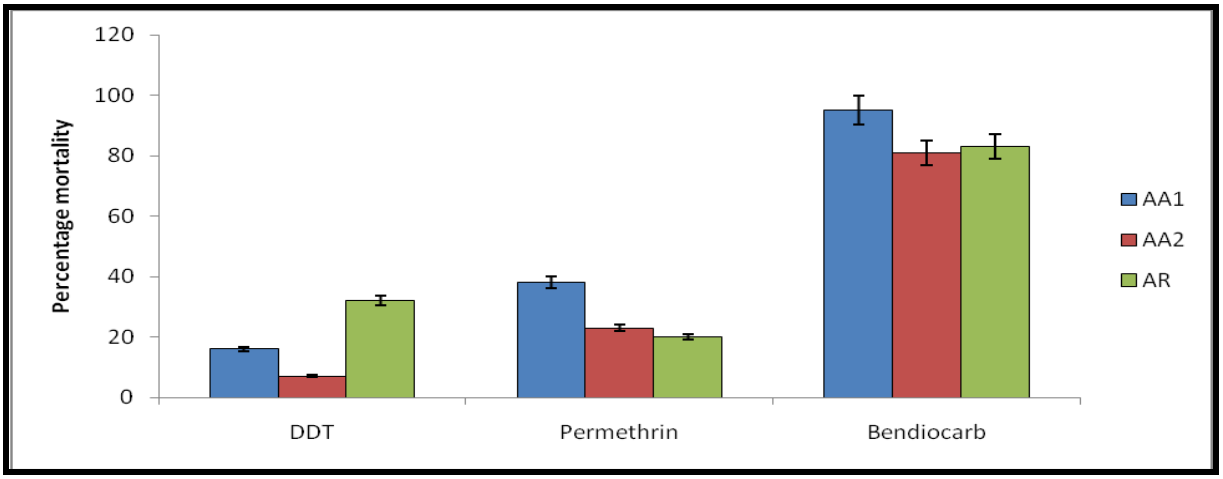

Figure 1 *Mortality rate of An. gambiae s.l. 24 h post exposure in Nigeria collected in 2013 rainy season. Mosquitoes Were exposed to 4% DDT, 0.75% permethrin and 0.1% bendiocarb in WHO susceptibility test at: Auyo agricultural 1(AA1); Auyo Agricultural 2 (AA2); Auyo Residential (AR) sites*

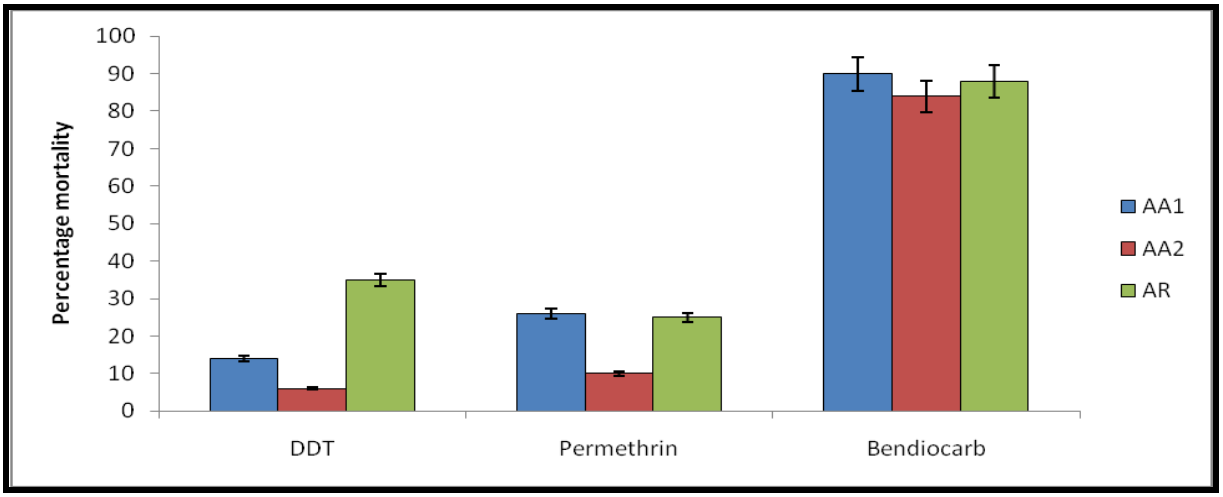

Figure 2 *Mortality rate of An. gambiae s.l. 24 h post exposure in Nigeria collected in 2014 rainy season. Mosquitoes Were exposed to 4% DDT, 0.75% permethrin and 0.1% bendiocarb in WHO susceptibility test at: Auyo agricultural 1(AA1); Auyo Agricultural 2 (AA2); Auyo Residential (AR) sites*

Table 4 *Distribution of kdr-East (L1014S) mutation in An. coluzzii and An. arabiensis mosquitoes*

	Anopheles coluzzii						Anopheles arabiensis					
	Genotype count			Allelic frequency			Genotype count			Allelic frequency		
Site	N	SS	SL	LL	S	L	N	SS	SL	LL	S	L
AA1	45	5	13	27	0.400	0.600	15	0	3	12	0.200	0.800
AA2	47	11	15	21	0.553	0.447	13	0	4	9	0.308	0.692
AR	40	2	9	29	0.275	0.725	20	0	0	20	0.000	1.000

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

Table 5 *Distribution of kdr-West (L1014F) mutation in An. coluzzii and An. arabiensis mosquitoes*

	Anopheles coluzzii						Anopheles arabiensis					
	Genotype count			Allelic frequency			Genotype count			Allelic frequency		
Site	N	FF	FL	LL	F	L	N	FF	FL	LL	F	L
AA1	45	10	12	23	0.489	0.511	15	0	3	12	0.200	0.800
AA2	47	20	11	16	0.659	0.341	13	2	6	5	0.616	0.384
AR	40	7	10	23	0.425	0.575	20	0	1	19	0.050	0.950

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

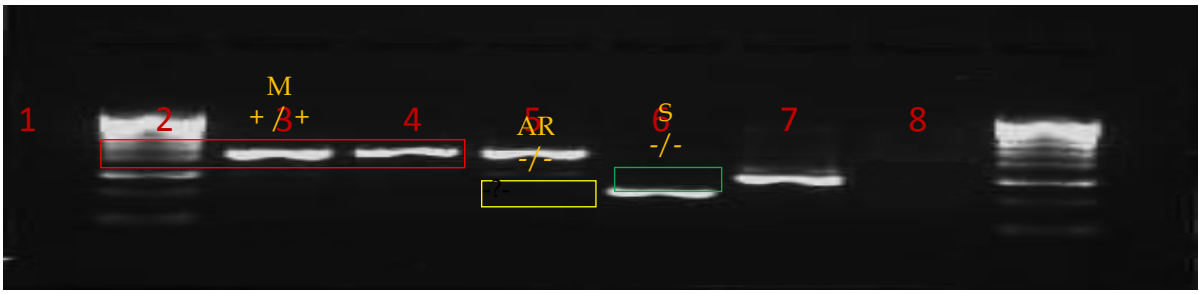

Figure 3 *Diagnostic PCR based on S200 X6.1 in Anopheles gambiae s.l. indicating the presence (+) or absence (-) of Insertion in Anopheles gambiae complex; M = An. gambiae M form; AR = An. arabiensis; S = An. gambiae S form. Ladder = 100 bp (Bioline hyper ladder IV). Lanes 1 and 8 = 1 kb (100 bp) molecular Weight ladder; Lanes 2, 3 and 4 = M-molecular form (479 bp), lane 5 = An. arabiensis (223 bp) lane 6 = S Molecular form (279 bp).*

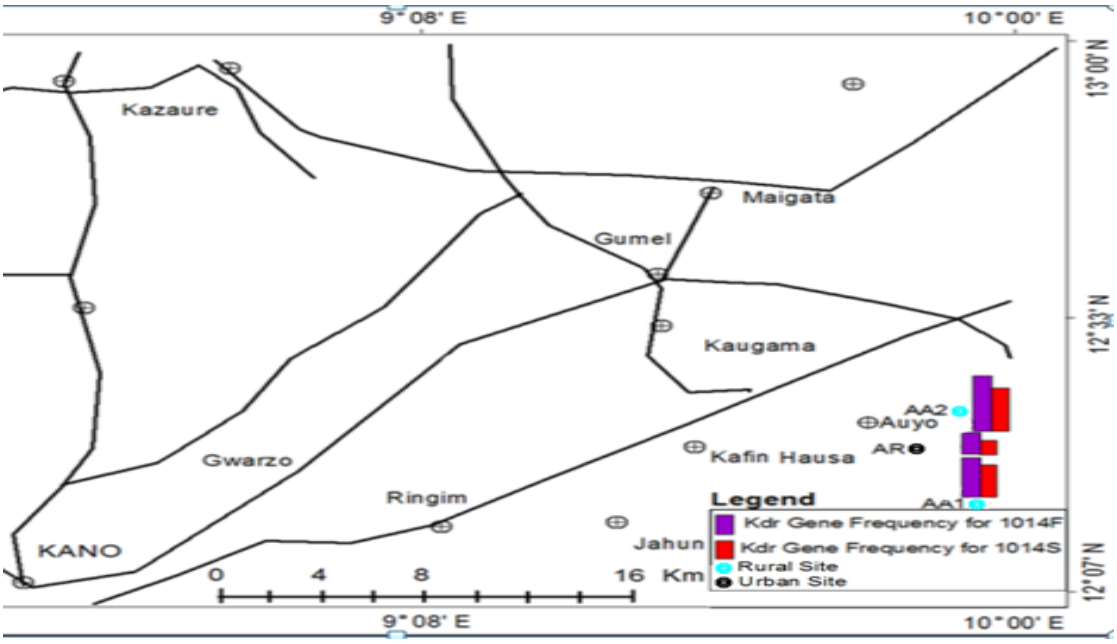

Figure 4 *Map of the study area in Nigeria showing the distribution of 1014F and 1014S in An. gambiae population.*

4. DISCUSSION

The study investigated the species composition, susceptibility status the presence and frequency distribution of east (L1014S) and west (L1014F) African kdr alleles in the An. gambiae s.l. complex population in the study sites-. This is because different members of the same species complex do not necessarily share the same resistance mechanisms, and nor do they necessarily exhibit the same insecticide resistance patterns [23]. An. coluzzii was predominant over An. arabiensis during this study. This supports previous observations that An. gambiae s.s. could be dominant in the Sudan savannah ecological zone compared to An. arabiensis that was spread across Sudan, Sahel and northern Guinea savannah ecological zones [24]. However, further collections are required to allow for the establishment of the malaria vector species distribution in this region of Nigeria. The mosquitoes tested from all the sampling sites showed a high resistance. 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. These different levels of insecticide susceptibility may reflect differential insecticide selection pressure exerted on the field populations of the mosquito fauna. Decrease in mortality rates to DDT and Permethrin observed which were higher in agricultural sites were probably due to the involvement of kdr [25]. The higher Knockdown time observed in this study, for DDT and permethrin is consistent with the findings of [24, 26], 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 [27]. 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 in this area resistant to these insecticides [28]. The carbamate (Bendiocarb) on the other hand, targets the acetylcholinesterase gene [30]. 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 [24, 28, 30, and 31]. The massive use of pesticides in agricultural settings has been well documented as a factor contributing to the emergence of resistance in Anopheles populations [32]. As reported by Akogbeto, some populations of An. gambiae laid their eggs in breeding sites containing insecticides residues. Larvae from these eggs are subjected to selection pressure leading to multiple insecticides resistance. 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 [33]. 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 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 [34]. Resistance to both DDT and Permethrin slightly increased in the An. gambiae populations in 2014 compared to 2013. This indicates evidence of continued accumulation of xenobiotics compounds in the environment. Our findings suggest that DDT and permethrin may not be effective for controlling An. gambiae s.l. in these sampling sites. Efficient Vector control would require a different insecticide (s) or different concentrations of these insecticides. 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. Agricultural activities have an effect on kdr allele's distribution compared to non–agricultural activities in the residential settings. 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 [35, 36]. The L1014F mutation appears to be the most significant mutation in both An. coluzzii and An. arabiensis in northwest Nigeria at present, however there exist the possibility that other mechanisms were also present and acted to confer resistance. 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 Kenya, Tanzania, Burkina Faso and Nigeria [3, 12, and 24]. 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 [24]. The low but increased frequency of the East kdr indicates that its selection in this region is recent and is increasing. On the other hand, the high frequency of the West kdr observed in the An. coluzzii is in agreement with the observation of [24] but contradict the observations in the south-western Nigeria [30] where the kdr mutation frequency was high in An. gambiae s.s. (formerly S-form) and low in An. coluzzii (once M-form). The very small number of the S-form (two) recorded from the field collections could be explained by the previous observation made by [37] 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 [24, 38] 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

5. CONCLUSION

The findings presented here provide evidence for Bendiocarb, DDT and Permethrin resistance as well as high frequency of L1014F kdr mutation in An. coluzzii and An. arabiensis and low frequency of L1014S kdr mutation in An. coluzzii and An. arabiensis. The high insecticides resistance observed in An. gambiae s.l. populations 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. These findings could help and guide the malaria control programme of Nigeria particularly in the choice of insecticide.

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