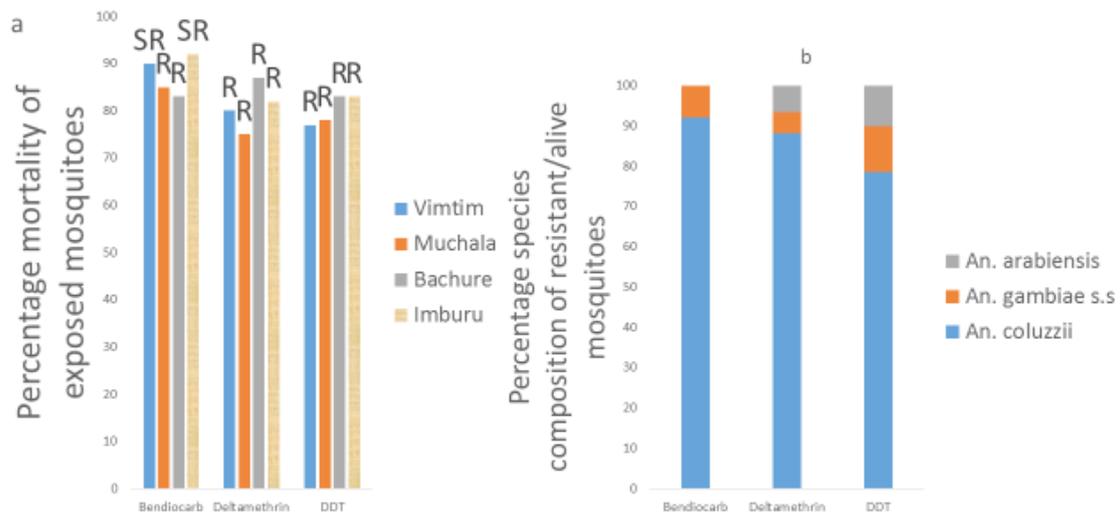


Bendiocarb resistance and, *kdr* associated deltamethrin and DDT resistance in *Anopheles gambiae s.l.* populations from North Eastern Adamawa State, Nigeria

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(a) Resistance-R, suspected resistance-SR status of *An. gambiae s.l.* in four sites in Adamawa, Nigeria.

(b) Sibling species composition of *An. gambiae s.l.* mosquitoes alive after insecticide exposures.

Highlights

- *An. gambiae s.l.* from Adamawa, Nigeria was resistant to bendiocarb, deltamethrin & DDT
- Mosquitoes alive after insecticide exposures were predominantly *An. coluzzii*
- There was 28% *kdr* allele occurrence in deltamethrin & DDT tolerant mosquitoes assayed
- All the *kdr* positive mosquitoes assayed were identified as *An. coluzzii*

RESEARCH ARTICLE

Bendiocarb resistance and, *kdr* associated deltamethrin and DDT resistance in *Anopheles gambiae s.l.* populations from North Eastern Adamawa State, Nigeria

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Received: 08/05/2020; Accepted: 16/01/2021

Abstract: Timely and consistent insecticide resistance monitoring efforts are required for early planning of management strategies. Here, we present the first report on bendiocarb, deltamethrin and dichlorodiphenyltrichloroethane (DDT) insecticide resistance in *Anopheles gambiae s.l.* populations from Adamawa, North Eastern Nigeria. Mosquitoes reared from larval collections were exposed to DDT (4%), bendiocarb (0.1%) and deltamethrin (0.05%) insecticides using standard WHO test kits and protocols. Species-specific Polymerase Chain Reaction (PCR) and PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) assays were used to determine the sibling species composition of the exposed mosquitoes while allele-specific PCR was used for *kdr* genotyping in the mosquitoes that survived after insecticide exposure. Mosquito populations from all the four study sites in Vintim, Imburu, Muchala and Bachure were resistant ($\leq 87\%$ mortality) to DDT and deltamethrin insecticides. Mosquito populations exposed to bendiocarb showed suspected resistance in Vintim and Imburu study sites and confirmed resistance in Muchala and Bachure sites respectively. Mosquitoes alive after deltamethrin, DDT and bendiocarb exposures were identified predominantly as *An. coluzzii* species (78.5 - 92%) compared to *An. gambiae s.s.* (5.3 - 11%) and *An. arabiensis* (0 - 10.1%). All the 22 *kdr* positive mosquitoes collected were identified as *An. coluzzii*. Percentage occurrence of *kdr* alleles in the mosquito populations alive after deltamethrin and DDT exposures were 30% and 25% respectively. *An. coluzzii* ($\geq 63\%$) was the predominant species identified in each study site compared to *An. gambiae sensu stricto (s.s.)* ($\leq 20\%$) and *An. arabiensis* ($\leq 17.4\%$) species. The results of *kdr* associated pyrethroid resistance is a potential threat to the effectiveness of the currently deployed deltamethrin-impregnated bed-net campaigns in Adamawa state. Evidence of bendiocarb carbamate resistance also implies potential ineffectiveness of future bendiocarb Indoor Residual Spraying. The involvement of metabolic resistance mechanisms in the mosquitoes from these study sites should be investigated for proper insecticide resistance management.

Keywords: *Anopheles gambiae s.l.* mosquitoes; insecticide resistance; *kdr*; malaria vector control; Nigeria.

INTRODUCTION

Vector control is a major component of the global strategy

for malaria prevention, control and elimination. Current best practices regarding malaria vector control involves the use of long-lasting insecticide-treated bed-nets (LLIN) and indoor residual spraying (IRS) of recommended long-acting insecticides. In Nigeria, where 25% of the global malaria burden is experienced (WHO, 2019), preliminary trials on improved IRS-based tools such as insecticide-treated durable wall-lining have been conducted in some rural communities (Obembe *et al.*, 2018a; Obembe *et al.*, 2019). However, the main focus of the Nigerian National Malaria strategic Plan (NMEP, 2014) entails the expansion of universal access to insecticide-treated materials through mass distribution of LLINs, significant scaling up of IRS, and expansion of larval source management (larviciding and environmental management). Between May 2009 and November 2013, the Nigerian government, with support from several partners, distributed over 60 million mosquito nets across the country (NMEP, 2014).

Insecticide resistance in the malaria vectors have been attributed to the increased deployment of these insecticide-based tools for malaria vector control (Toé *et al.*, 2014). Indeed, evidence-based concerns are currently being expressed concerning the tendency of insecticide resistance in the malaria vectors to slow, halt or reverse the gains made in malaria vector control (Ochomo *et al.*, 2013; Toé *et al.*, 2014). Therefore, current knowledge on the development of insecticide resistance in the local malaria vector population is required for the design and effective implementation of appropriate control measures. Pyrethroids is still a major class of insecticide recommended by WHO for LLIN impregnation [<http://www.who.int/whopes/en/>]. Since LLIN serves currently as the primary means of malaria prevention, timely and regular investigations of pyrethroids insecticide resistance and the mechanisms conferring the resistance in local malaria vector populations are imperative for the management of insecticides resistance detected. Resistance to pyrethroids/ DDT is conferred by two main physiological mechanisms including metabolic resistance and target site insensitivity (Hemingway *et al.*, 2004; Karunaratne *et al.*, 2018). Members of the *Anopheles gambiae* Giles species complex represents the major vectors of malaria in sub-Saharan Africa. According to

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Coetzee (2020), three of the nine sibling species that make up the members of the *Anopheles gambiae* Giles species complex currently include *An. gambiae arabiensis* (Patton, 1905), *An. gambiae coluzzii* (Coetzee & Wilkerson, 2013) and *An. gambiae s.s* (Giles, 1902). These morphologically indistinguishable sibling species exhibit diverse ecology and behaviour and their identification is of importance in setting up the most appropriate control interventions. Pyrethroid target site resistance mechanism within these members of the *Anopheles gambiae* species complex is conferred by knock down resistance (*kdr*) gene mutations including the substitutions of leucine to phenylalanine (L1014F) identified as *kdr*-west (Martinez-Torres *et al.*, 1998) and leucine to serine (L1014S) designated *kdr*-East Africa (Ranson *et al.*, 2000).

Data on malaria vector resistance in Nigeria (Awolola *et al.*, 2005; Oduola *et al.*, 2010; Oduola *et al.*, 2012; Okorie *et al.*, 2015; Adeogun *et al.*, 2017) have mostly been from the South Western part of the Country. Availability of extensive malaria entomological data set in the South West may have contributed to the implementation of control response and the report of lower malaria prevalence rates (32.1%) in this region as compared to those of other zones in the country (NMEP, 2016). According to the malaria prevalence results reported in the latest Nigerian National Malaria Indicator Survey (NMEP, 2016), the North East had the third highest malaria prevalence rates (42.8%) in Nigeria after North West (58.3%) and North Central (50.7%) zones. However, apart from Bauchi (Umar *et al.* 2014) and Gombe (Oduola *et al.*, 2019) states, malaria vector resistance data required to guide and subsequently assess the impact of suitable control measures on local vector population is scarcely available in the North East region. In fact, Okorie *et al.* (2011) earlier reported that the proportion of malaria vector studies carried out between year 2000 and 2010 was lowest in the North East followed by North West, South

South, South East and the North Central geo-political zones of Nigeria. Adamawa state currently has the highest malaria prevalence rate (55.5%) among the six states in the North Eastern region of Nigeria (NMEP, 2016). The State represents one of the largest states in Nigeria having flood plains with many rivers passing through especially during the rainy season (Adewumi, 2019). Data on susceptibility/resistance status of local *Anopheles* mosquito populations to insecticides such as deltamethrin (the most common insecticide used for LLIN impregnation), carbamate (potential alternative to pyrethroids IRS) and DDT (which could induce cross-resistance to deltamethrin) could serve as useful information for malaria vector control. This study presents a first report on carbamate, deltamethrin and DDT resistance in identified *An. gambiae* species complex mosquito populations from four study sites in Adamawa state, North Eastern Nigeria.

MATERIALS AND METHODS

Study area and mosquito collection

Adamawa State is located in North Eastern Nigeria, and lies between latitude 9° 19' 60.00"N and longitude 12° 29' 59.99"E. It covers a land area of 38,741 km² and shares boundaries with Taraba state in the south and west, Gombe state in its North West, Borno state to the North and Cameroon Republic along its Eastern border (Adebayo and Tukur, 1999). Adamawa has a tropical climate with a mean monthly temperature ranging from 26.7 °C to 27.8 °C and rainfall of 700 mm to 1600 mm (Adebayo and Umar, 1999). The state is blessed with floodplains otherwise known as fadama lands, characterized by the availability and accessibility to both open surface and underground water (Umar *et al.*, 2012). Water as a physical resource is adequate in Adamawa state due to the presence of River Benue and hundreds of other perennial streams and rivers used for fishing and irrigated agriculture in several parts of

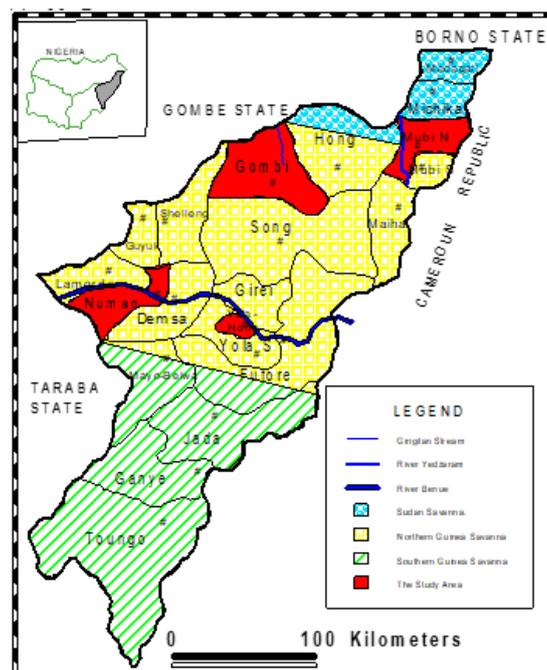


Figure 1: Map of Adamawa State showing the study locations.

the state (Adebayo and Umar, 1999). Four study sites from four different Local Government Areas (LGA); Muchala in Gombi LGA (10°33'50"N, 13°23'00"E), Vimtim in Mubi-North (10°23'50"N, 13°21'00"E), Bachure in Yola North (09°16'50"N, 12°24'50"E) and Imburu in Numan LGA (09°30'00"N, 11.5°20'00"E) (Figure 1) were selected from the state based on their proximities to major river tributaries. *Anopheles* larvae were collected from natural breeding sites such as ground pools, puddles, marshes, swamps and rice fields in each study site from July to October, 2017. *Anopheles* mosquito larvae and pupae collected together with water from the study sites with the aid of a dipper were transported to the insectary at Adamawa State University where the samples were reared to adulthood. Newly emerged adult *Anopheles* mosquitoes were maintained on 10% sugar solution.

Insecticide susceptibility assay

World Health Organization (WHO) test papers containing diagnostic concentrations of pyrethroid deltamethrin (0.05%), organochlorine DDT (4%) and carbamate bendiocarb (0.1%) insecticides were used for the tests following WHO standard procedures and test kits (WHO, 2013). Hundred female *Anopheles* mosquito samples (four replicates of 25 non-blood fed 2 - 3 day old) were exposed to the test papers of each insecticide for 1 h. Control experiments were conducted alongside with two replicates of 20 - 25 mosquitoes exposed to the control papers. Temperature and relative humidity conditions recorded during the susceptibility tests in the laboratory were 26° to 29 °C and 74% to 83% respectively. Exposed mosquitoes were transferred into holding tubes, supplied with 10% sugar solution and given a recovery period of 24 h after exposure (WHO, 2013). Dead and alive mosquitoes were separately preserved in Eppendorf tubes (one mosquito per tube) containing silica gel for subsequent molecular analysis at the Molecular Entomology and Vector Control Research Laboratory of The Nigerian Institute of Medical Research.

Identification of sibling species and knockdown resistance (*kdr*) mutation in *Anopheles* mosquitoes

Exposed mosquitoes were identified morphologically (Gillies and Coetzee, 1987) and a subset of the identified *Anopheles gambiae* species complex samples were further characterized into sibling species using PCR (Scott *et al.*, 1993) and PCR-RFLP (Favia *et al.*, 1997) standard procedures. Species specific primers (Primer sequence 5' to 3'): Universal (GTGTGCCCTTCTCGATGT), *An. gambiae* s.s (CTGGTTTGGTCGGCACGTTT), *An. merus* and *An. melas* (TGACCAACCCACTCCCTTGA), *An. arabiensis* (AAGTGTCCCTTCTCCATCCTA) and *An. quadriannulatus* (CAGACCAAGATGGTTAGTAT) designed from the DNA sequence of the intergenic spacer region of *An. gambiae* s.l. were used for the identification (Scott *et al.*, 1993). Polymerase chain reaction amplification was carried out with an initial denaturation step at 95 °C for 5 min, followed by 30 cycles each consisting of 30 seconds denaturation at 95 °C, 30 seconds annealing at 50 °C and 30 seconds elongation at 72 °C. The final elongation was carried

out at 72 °C for 5 minutes. The PCR product was digested using *Heamophilus haemolyticus* (Hha1) restriction enzyme to further identify the *Anopheles gambiae* s.s as *An. coluzzii* or *An. gambiae*. The digestion was carried out at 37 °C for 6 hours in a thermal cycler (Favia *et al.*, 1997). The PCR products were electrophoresed through ethidium bromide-stained 1.5% agarose gel and visualized under the UV light in a gel documentation machine.

The sibling species characterization was done on all the surviving mosquitoes and a subset of the dead mosquitoes per site in order to determine sibling species composition of *Anopheles gambiae* s.l. mosquitoes in each of the study sites within the LGAs considered. Sixty nine (53 alive, 16 dead) samples from Vimtim study site, 70 (62 alive, 8 dead) samples from Muchala, 86 (47 alive and 39 dead) from Bachure and 92 (43 alive and 49 dead) samples from Imburu were identified with PCR.

Fourty deltamethrin survivor mosquitoes (10 from each site) and 40 DDT survivor mosquitoes (10 from each site) were genotyped for *kdr* mutation after Genomic DNA extraction. Genomic DNA extraction was conducted following the protocol of Collins *et al.* (1987) while *kdr*-west L1014F mutation genotyping was done using the standard allele specific PCR designed for the West African *kdr* mutation (Martinez-Torres *et al.*, 1998). The primers; Agd1 (ATAGATTCCCCGACCATG), Agd2 (AGACAAGGATGATGAACC), Agd3 (AATTTGCATTACTTACGACA), and Agd4 (CTGTAGTGATAGGAAATTTA) were used for the *kdr* genotyping. The PCR conditions consists of 94 °C for 5 min (denaturation phase), 40 cycles of 94 °C for 1 min, 48 °C for 2 min, and 72 °C for 2 min (hybridization and extension phase), and a final extension phase of 72 °C for 10 min (Martinez-Torres *et al.*, 1998). The PCR-*Kdr* products were run on 2% agarose gel and photographed under UV light in a gel documentation machine.

Data analysis

Proportions of mosquitoes dead after the 24 h post-exposure period was used to calculate percentage mortality. Susceptibility or resistance status of exposed mosquito populations were determined using WHO (2013) criteria as follows: 98% to 100% mosquito mortality indicated susceptibility, 90 - 97% signified suspected or moderate resistance while less than 90% mosquito mortality implied confirmed resistance. None of the control mortalities was up to 5%. There was no need to correct insecticide induced percentage mortalities with Abbott's formula.

Ethical statement

The study was conducted after obtaining certified ethical approval S/MOH/81/T.II/330 from the State Ministry of Health Yola, Adamawa State, Nigeria.

RESULTS

Mosquito mortality rates and insecticide susceptibility status

Susceptibility status of *Anopheles gambiae* complex mosquito populations from the four different communities in Adamawa State is presented in Table 1. Mortalities from exposures to bendiocarb (83 - 92%) was higher than deltamethrin (75 - 87%) and DDT (77 - 83%) for each mosquito population except in Bachure community where lower mortality from bendiocarb (83%) was observed compared to deltamethrin (87%).

Sibling species composition of *Anopheles gambiae* complex from the study locations

The results of sibling species composition of *An. gambiae* complex mosquitoes collected in all the communities are detailed in Tables 2 and 3. A large proportion of deltamethrin (88.1%) and DDT (78.5%) surviving mosquitoes were identified as *An. coluzzii* species. Similarly, 65.2% and 76.2% of the deltamethrin and DDT susceptible mosquitoes were identified as *An. coluzzii* (Table 2). Conversely, the proportion of bendiocarb susceptible mosquitoes identified as *An. coluzzii* was very low (8.3%) compared to the bendiocarb resistant (92%) *An. coluzzii* (Table 2). Altogether, 85.4% of all the 205 resistant mosquitoes and 57.1% of the 112 dead/susceptible mosquitoes analyzed were *An. coluzzii* (Table 2). However, lower proportions 5.3%, 11.4% and 8% of *An. gambiae* s.s mosquitoes survived deltamethrin, DDT and bendiocarb exposures respectively compared to respective higher proportions of *An. gambiae* s.s 34.8%, 23.8% and 62.5% that were susceptible to the same insecticides. Consequently, there were higher proportions (36.6%) of susceptible *An. gambiae* s.s mosquitoes compared to resistant (8.3%) ones (Table

2). Proportions of susceptible and resistant mosquitoes identified as *An. arabiensis* were the same (6.3%). All the 22 *kdr* positive *An. gambiae* s.l mosquitoes collected were identified as *An. coluzzii* (Table 3). Overall, samples of *Anopheles arabiensis* species were not found in Vimtim and Muchala communities. Likewise, the *Anopheles arabiensis* species had the lowest occurrence in both Bachure (4.6%) and Imburu (17.4%) communities (Table 3). The occurrence of *An. gambiae* s.s was significantly lower compared to the *An. coluzzii* occurrence in all the study sites. This clearly demonstrated the preponderance of *An. coluzzii* ($\geq 80\%$) than *An. gambiae* s.s ($\leq 20\%$) in Vimtim and Muchala and *An. coluzzii* predominance ($\geq 63\%$) over *An. gambiae* s.s ($\leq 19.6\%$) and *An. arabiensis* ($\leq 17.4\%$) in Bachure and Imburu sites (Table 3). Diagnostic gel electrophoresis images for the three sibling species identified are shown in Figures 2 and 3.

Table 1: Insecticide susceptibility status of *Anopheles gambiae* s.l populations from study communities in Adamawa State (n = 100 per insecticide per site)

Insecticide	LGA	Study sites	Mortality (%)	Status
0.1% Bendiocarb	Mubi-North	Vimtim	90	Suspected Resistance
		Gombi	85	Resistant
	Yola-North	Bachure	83	Resistant
		Numan	Imburu	92
0.05% Deltamethrin	Mubi-North	Vimtim	80	Resistant
		Gombi	Muchala	75
	Yola-North	Bachure	87	Resistant
		Numan	Imburu	82
4% DDT	Mubi-North	Vimtim	77	Resistant
		Gombi	Muchala	78
	Yola-North	Bachure	83	Resistant
		Numan	Imburu	83

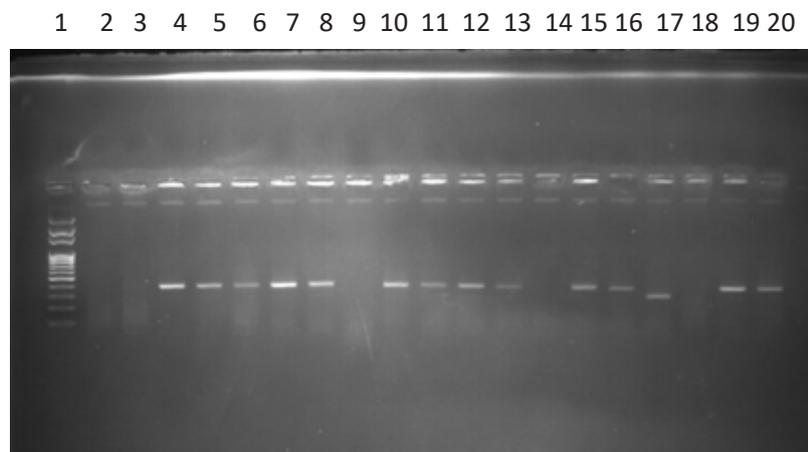
Table 2: Sibling species composition of resistant and susceptible *An. gambiae* s.l mosquitoes exposed to different insecticides

Status	Insecticides	Study sites	No of samples (%)	<i>An. coluzzii</i> N (%)	<i>An. gambiae</i> s.s N (%)	<i>An. arabiensis</i> N (%)
Resistant/ Alive	Deltamethrin	Vimtim	20	19	1	0
		Muchala	25	23	2	0
		Bachure	13	13	0	0
		Imburu	18	12	1	5
			76 (100)	67 (88.1)	4 (5.3)	5 (6.6)
	DDT	Vimtim	23	21	2	0
		Muchala	22	18	4	0
		Bachure	17	16	1	0
		Imburu	17	7	2	8
		79 (100)	62 (78.5)	9 (11.4)	8 (10.1)	
	Bendiocarb	Vimtim	10	9	1	0
		Muchala	15	13	2	0
		Bachure	17	16	1	0
		Imburu	8	8	0	0
			50 (100)	46 (92.0)	4 (8.0)	0 (0)
		Total Alive	205 (100)	175(85.4)	17 (8.3)	13 (6.3)
Susceptible/ Dead	Deltamethrin	Vimtim	8	4	4	0
		Muchala	3	2	1	0
		Bachure	15	13	2	0
		Imburu	20	11	9	0
			46 (100)	30 (65.2)	16 (34.8)	0 (0)
	DDT	Vimtim	4	4	0	0
		Muchala	3	0	3	0
		Bachure	15	9	6	0
		Imburu	20	19	1	0
		42 (100)	32 (76.2)	10 (23.8)	0 (0)	
	Bendiocarb	Vimtim	4	0	4	0
		Muchala	2	0	2	0
		Bachure	9	1	4	4
		Imburu	9	1	5	3
		24 (100)	2 (8.3)	15 (62.5)	7 (29.2)	
		Total Dead	112 (100)	64 (57.1)	41 (36.6)	7 (6.3)
	Grand Total	317 (100)	239 (75.4)	58 (18.3)	20 (6.3)	

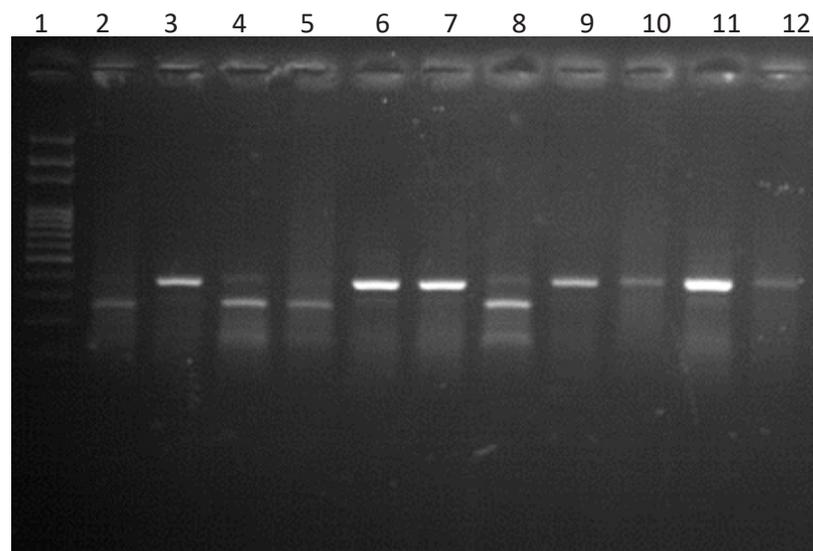
N- Number of samples.

Table 3: Presence of L1014F *kdr* mutation and the composition of sibling species in *An. gambiae* s.l. mosquitoes recorded from each study location

Study sites	No of <i>kdr</i> (RR+RS) positive samples found	Sibling species composition of <i>kdr</i> (RR+RS) positive <i>An. gambiae</i> s.l. samples analyzed			Sibling species composition of all <i>An. gambiae</i> s.l. mosquitoes analyzed			Total No of samples analyzed
		N (%)			N (%)			
		<i>An. coluzzii</i>	<i>An. gambiae</i> s.s	<i>An. arabiensis</i>	<i>An. coluzzii</i>	<i>An. gambiae</i> s.s	<i>An. arabiensis</i>	
Vimtim	7	7 (100)	0 (0)	0 (0)	57 (82.6)	12 (17.4)	0 (0)	69
Muchala	0	0 (0)	0 (0)	0 (0)	56 (80)	14 (20)	0 (0)	70
Bachure	7	7 (100)	0 (0)	0 (0)	68 (79.1)	14 (16.3)	4 (4.6)	86
Imburu	8	8 (100)	0 (0)	0 (0)	58 (63.0)	18 (19.6)	16 (17.4)	92
Total	22	22 (100)	0 (0)	0 (0)	239 (75.4)	58 (18.3)	20 (6.3)	317 (100)

**Figure 2:** Gel Electrophoresis of PCR products of *An. gambiae* and *An. arabiensis* mosquito samples.

Lane 1: 100 base pair (bp) DNA ladder/marker. Lanes 4 - 8, 10 - 13, 15 - 16, 19 - 20: *An. gambiae* showing 390bp band. Lanes 17: *An. arabiensis* showing 315bp band. Lanes 2, 3, 9, 14, 18: non-amplified samples*. *non-amplified samples were re-assayed.

**Figure 3:** Gel electrophoresis of PCR-RFLP products of *An. gambiae* s.s and *An. coluzzii* mosquito samples.

Lane 1: 100 base pair (bp) DNA ladder/marker. Lanes 2, 4, 5, and 8: *An. gambiae* s.s showing 257bp band. Lanes 3, 6 - 7, 9 - 12: *An. coluzzii* showing 367bp band.

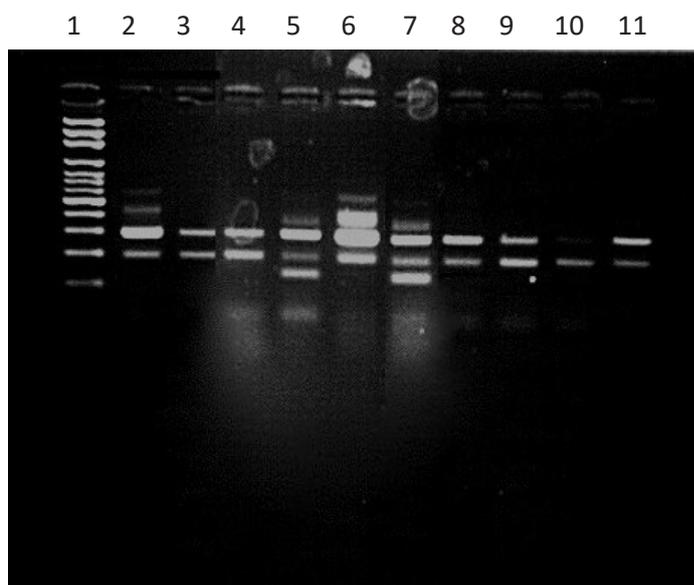


Figure 4: Gel Electrophoresis of PCR products to detect *kdr* L1014F mutation Lane 1: 100 base pair (bp) DNA ladder/marker. Lanes 2, 3, 4, 6, 8, 11: homozygous resistant (RR) samples showing 293bp and 195bp bands Lanes 5 and 7: heterozygous resistant (RS) samples showing 293bp, 195bp and 137bp bands Key: 293bp band is common to both susceptible and resistant specimens. 137bp band indicates susceptible (S) sample and 195bp band indicates resistant (R) *kdr* positive sample. Presence of all 3 bands in a single specimen indicates heterozygosity (RS).

Table 4: Percentage occurrence of knockdown resistance (*kdr*) L1014F mutation in *Anopheles* populations alive after exposures to deltamethrin or DDT

Insecticides	Study sites	No of mosquitoes alive after exposure	No of alive samples genotyped for <i>kdr</i>	<i>kdr</i> mutation genotypes			Percentage occurrence of resistant (RR+RS) alleles N (%)
				SS	RS	RR	
Deltamethrin	Vimtim	20	10	6	0	4	
	Muchala	25	10	10	0	0	
	Bachure	13	10	6	2	2	
	Imburu	18	10	6	1	3	
Total		76	40	28	3	9	12 (30)
DDT	Vimtim	23	10	7	0	3	
	Muchala	22	10	10	0	0	
	Bachure	17	10	7	0	3	
	Imburu	17	10	6	0	4	
Total		79	40	30	0	10	10 (25)
Grand Total		155	80	58	3	19	22 (27.5)

SS = homozygous susceptible, RS = heterozygous resistant, RR = homozygous resistant.

Occurrence of the *kdr* alleles in resistant *Anopheles* populations

The three *kdr* genotypes were identified by the characteristic 293bp band common to both susceptible and resistant specimens, a 137bp band associated with susceptible (SS) specimens and a 195bp *kdr* band (R). Presence of all three bands in a single specimen indicates heterozygosity (RS) (Figure 4).

Occurrences of *kdr* L1014F alleles in the DDT and

deltamethrin survivor mosquito populations were low (<50%). Percentage occurrences of knock down resistant alleles (RR+RS) among the mosquito populations alive after deltamethrin and DDT exposures were 30% and 25% respectively (Table 4). Taken together, 27.5% (22/80) of both DDT and deltamethrin survivor mosquito samples combined were identified as carrying the *kdr* (RR+RS) alleles which confers target site resistance on the mosquitoes (Table 4).

DISCUSSION

This study presents the first report on insecticide resistance status and *kdr* L1014F resistance mechanism in *An. gambiae* complex mosquito populations from Adamawa State, North eastern Nigeria. The DDT, deltamethrin and bendiocarb resistance results obtained are an important contribution to the current malaria vector surveillance and resistance monitoring efforts of the National Malaria Elimination Programme. Specifically, confirmed deltamethrin and suspected bendiocarb resistance similar to those obtained in Vimtim and Imburu sites in this study have been reported in Bauchi State, Northeastern Nigeria (Umar *et al.*, 2014). Equally, confirmed DDT, deltamethrin and bendiocarb resistance observed in Muchala and Bachure have also been noted in Lagos (Oduola *et al.*, 2012) southwest and Kano (Abdu *et al.*, 2017; Ibrahim *et al.*, 2019; Ononamadu *et al.*, 2020) Northwest Nigeria. Deltamethrin and bendiocarb resistance observed in this study could be attributed to intense use of insecticide-based tools such as LLIN for malaria vector control coupled with insecticide deployment in irrigated cereal and vegetable cultivation carried out by farmers in these areas. Such agricultural insecticide linked *Anopheles* mosquito resistance to carbamate have been reported in other areas in Africa (Yadouleton *et al.*, 2010).

All the *kdr* positive mosquitoes analyzed were identified as *An. coluzzii*. This could have contributed to the higher proportions of deltamethrin and DDT resistant *An. coluzzii* mosquitoes found compared to the *An. gambiae s.s* species. In contrast, higher proportions of deltamethrin and DDT susceptible *An. gambiae s.s* mosquitoes compared to resistant ones may be associated with the non-identification of *kdr* positive mosquitoes among the *An. gambiae s.s* species. The fact that 92% of the bendiocarb resistant mosquitoes and a large proportion ($\geq 65.2\%$) of deltamethrin and DDT exposed mosquitoes (susceptible or resistant) were identified as *An. coluzzii* showed the preponderance of this species in the study sites. Exception to the preponderance of *An. coluzzii* species (8.3%) was only found among the bendiocarb susceptible mosquitoes. This observation, coupled with high percentage (92%) of *An. coluzzii* species found among the bendiocarb surviving mosquitoes, call for the investigation of carbamate resistance mechanisms in the *An. coluzzii* mosquitoes available in these study sites.

Molecular and biochemical studies are required to identify the active mechanisms responsible for confirmed bendiocarb carbamate resistance observed in this study so as to guide the implementation of effective management decisions. Such studies should also investigate the possible operation of additional metabolic resistance mechanisms in the deltamethrin and DDT resistant mosquito populations. Evidence of suspected and confirmed bendiocarb carbamate resistance implies that the Indoor Residual Spraying of bendiocarb carbamate insecticide may not be an effective addition to the current mosquito net campaign intervention in Adamawa State.

The low *kdr* frequencies in the DDT (25%) and deltamethrin (30%) resistant mosquitoes in this study was associated with 77 - 83% DDT and 75 - 87% deltamethrin

induced mortalities obtained. Comparable trend of low *kdr* frequencies (19 - 25.6%) associated with 81% deltamethrin induced *An. gambiae s.l* mosquito mortality have been reported in Southwest Nigeria (Okorie *et al.*, 2015). Nevertheless, higher *kdr* frequencies 49 - 95.8% conferring lower DDT (0.83 - 56.9%) and deltamethrin (1 - 63%) induced *An. gambiae s.l* mortalities have been reported in some other parts of Nigeria (Abdu *et al.*, 2017; Ibrahim *et al.*, 2019; Ononamadu *et al.*, 2020). Some of these studies implicated metabolic resistance as an additional resistance mechanism conferring phenotypic *An. gambiae s.l* resistance to deltamethrin and DDT. Alone, the presence of *kdr* mechanism was initially associated with low level of phenotypic pyrethroids resistance having little or no operational effect on the efficacy of LLIN (Darriet *et al.*, 2000; Asidi *et al.*, 2005). The complacency and increased insecticide selection pressure that followed the earlier observed low level of *kdr* resistance led to the evolution of more potent resistance mechanisms yielding mosquitoes that are now insensitive to standard LLIN and IRS pyrethroids formulations (Ochomo *et al.*, 2013; Toé *et al.*, 2014). In Jigawa State, Northwestern Nigeria, initial moderate *An. coluzzii* deltamethrin resistance (78.4% mortality) noted between 2009 and 2011 (Ibrahim *et al.*, 2014) had escalated (1% mortality) in the recent studies conducted in 2017 (Ibrahim *et al.*, 2019). To avoid this situation in Adamawa state, continuous resistance monitoring and management efforts should be made to reduce and possibly reverse the moderate level of resistance observed in this study.

Confirmation of deltamethrin resistance in the *Anopheles gambiae s.l* populations from all the study sites in this study should be of particular concern especially since 7 out of the 13 WHO recommended LLIN brands available are deltamethrin impregnated (WHO, 2012). Four are alphacypermethrin impregnated while the remaining 2 brands are permethrin infused (WHO, 2012). *Anopheles gambiae s.l* mosquito populations from Bauchi, another state in north eastern Nigeria, were resistant to other pyrethroids insecticides but susceptible to alphacypermethrin. Further studies on the susceptibility status of *Anopheles* mosquitoes in Adamawa state to other pyrethroids such as alphacypermethrin should be conducted. Also, the involvement of metabolic mechanisms should be investigated for proper management of the resistance detected in Adamawa state as the wait for novel interventions continues.

Anopheles arabiensis species is known to prefer arid conditions available in the savannah areas of Nigeria (Onyabe and Conn, 2001). The predominance of *An. gambiae s.s* (Awolola *et al.*, 2005; Obembe *et al.*, 2018b; Oduola *et al.*, 2019) or *An. arabiensis* (Onyabe and Conn, 2001; Yoriyo *et al.*, 2014; Oduola *et al.*, 2016) mosquito species have been reported in some savannah areas of Nigeria. Adamawa State also belongs to the Savanna area of Nigeria. However, the result of this study in Adamawa shows the prevalence of *An. coluzzii* species probably because of the peculiarity of Adamawa State as a flood plain area with several perennial streams and rivers (Adebayo and Umar, 1999) providing suitable relatively

permanent breeding sites preferred by the predominant *An. coluzzii* mosquito species (Djègbè et al., 2018). Apart from investigations on the involvement of metabolic resistance mechanisms, further studies on continuous monitoring of insecticide resistance in these areas should consider *kdr* analysis for all the resistant samples to generate a more robust data on this aspect. Development of *kdr* type mutations other than L1014F and their contribution towards pyrethroid and DDT resistance should also be investigated. Results from these studies will assist in the implementation of suitable resistance management strategies required to ensure effective insecticide-based malaria vector control.

CONCLUSIONS

Knockdown resistance (*kdr*L1014F) associated deltamethrin pyrethroids and DDT organochlorine resistance were detected for the first time and identified as a potential threat to the current deployment of deltamethrin impregnated bed-nets through mass LLIN campaigns in Adamawa state, Nigeria. Evidences of suspected and confirmed bendiocarb carbamate resistance were also found implying the potential ineffectiveness of the implementation of bendiocarb IRS as an addition to the current LLIN campaign interventions in the State. The predominance of indoor-loving *An. coluzzii* mosquito sibling species in this study is predictive of the suitability of LLIN as an appropriate malaria vector control strategy in the study area. However, further studies on the involvement of metabolic resistance mechanisms that could contribute to the ineffectiveness of such deployed LLIN should be conducted for the implementation of effective resistance management strategies. Susceptibility of these mosquito populations to other promising pyrethroids insecticides such as alphacypermethrin should be tested to determine the possibility of switching to the use of bed-nets impregnated with such alternative.

ACKNOWLEDGEMENTS

We are grateful to the field assistants and community residents that assisted in the location and collection of mosquitoes from the natural breeding sites.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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