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Original Article

Multiple Insecticide Resistance in *Anopheles arabiensis* Patton in Khartoum State, Sudan, with High Pyrethroid Resistance Associated with Knockdown Resistant (*kdr*) Gene

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Abstract

Introduction: Insecticide resistance is one of the major challenges in vector control programs around the globe. This study investigated the insecticide resistance and *kdr* mutation in the *Anopheles arabiensis* malaria vector in Khartoum State, Sudan.

Methods: Entomological cross-sectional surveys were carried out at four urban and suburban sites in Khartoum State. Four insecticides were tested for World Health Organization (WHO) susceptibility, and *kdr* frequencies were estimated using two allele-specific PCR assays.

Results: WHO bioassay tests revealed that DDT, malathion, and permethrin showed high resistance in both urban and suburban sites. There is no significant difference in mortality rates between urban and suburban sites ($P>0.05$), with the exception of DDT, where mosquitoes from urban sites showed more susceptibility [64 (51.23-76.77)] than those from suburban areas [53.5 (69.73-95.27)]. In general, all populations from the four sites showed faster KDT50% to bendiocarb and permethrin than to malathion and DDT insecticides. Generalized linear model analysis revealed that insecticide type, site type, and their interaction were determinant factors in mortality rate. A high to moderate frequency of the West African *kdr* mutation (L1014F) was observed in urban and suburban sites, and the association between the presence of the *kdr* mutation and resistance phenotype was strong for permethrin and DDT (OR>7 in the allelic test).

Conclusion: This study showed the susceptibility status of the malaria vector *A. arabiensis* to four insecticides belonging to different classes in urban and suburban sites. This provides important knowledge that helps vector surveillance and control programs. Additionally, more research is necessary to explore the impact of pyrethroid resistance, particularly in bednets, and other resistance mechanisms in this malaria vector.

Keywords: *Anopheles arabiensis, kdr* mutation, Insecticide resistance

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Introduction

Malaria is a major health concern, particularly in the African continent; it has been estimated that about 219 million cases of malaria occur annually worldwide. The World Health Organization (WHO) African Area accounted for 92% of malaria cases, with fifteen nations in sub-Saharan Africa bearing the burden (1). Malaria control had different approaches, particularly in African countries, including immunological, social, traditionally used methods, and vector control methods (2).

Although malaria prevention has greatly progressed over the last two decades, particularly through vector control interventions, insecticide resistance poses a significant risk to intervention efficacy. The WHO recommended continuous monitoring of insecticide resistance, which would help in resistance management and could decrease malaria incidence and mortality (3). Long-lasting insecticide nets (LLINs) and indoor residual spraying (IRS) play an important role in reducing malaria incidence in malaria-endemic areas in Sub-Saharan Africa. Malaria vector control strategies in Sudan focus basically on two approaches: a) IRS and b) LLIN (4). The use of IRS and LLIN proved to be the most powerful and broadly applied vector control interventions over many years; combining LLIN and IRS is an effective control measure practiced in Sudan, resulting in a reduction of malaria incidence in highly endemic areas in eastern and central Sudan. Any loss of efficiency in these control procedures could negatively affect disease incidence and mortality (5).

Anopheles arabiensis is considered a major malaria

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vector all over Sudan and the solely known responsible vector in Khartoum State (6). Insecticide resistance in the *A. arabiensis* and *Anopheles gambiae* complexes has been reported in different African countries. Many countries in sub-Saharan Africa reported pyrethroid resistance; its low mammalian toxicity and high insecticidal activity make it one of the most used LLINs, and it is a highly recommended class of insecticide by WHO for LLINs. The reliance on one class of insecticide in LLINs makes pyrethroid resistance a critical threat to malaria control. Although recent studies have innovated new LLINs that contain the pyrethroid synergist piperonyl butoxide (PBO), a huge number of LLINs have been applied as a control measure in Africa, and the new non-pyrethroids products have not yet been used (7).

Anopheles arabiensis has been shown to be resistant to permethrin and DDT in eastern and central Sudan. A high frequency of the West African *kdr* allele (L1014F) has been reported, whereas the East African *kdr* (L1014S) allele has not been detected in Sudan (8). High levels of resistance to DDT, malathion, dieldrin, and permethrin were also demonstrated in Gezira state and Central Sudan (9,10). Multiple studies have documented the presence of pesticide resistance in populations of An. arabiensis in various regions of Sudan, specifically in central Sudan and Khartoum State (11). For several years, a study in six urban and suburban sites in Khartoum State revealed the impact of agricultural pesticide use on enhancing the development of insecticide resistance (12). Another study in Gezira State reported that *A. arabiensis* is fully susceptible to bendiocarb and fenitrothion. However, the L1014F *kdr* allele was significantly associated with resistance to pyrethroids and DDT (13). In Tunisia, other vectors, not malaria transmitters, also developed resistance to permethrin in agricultural areas (14); on the other hand, high pyrethroid resistance was also reported among pests in African countries (15).

Control measures in Africa and Sudan depend mainly on LLINs and the need for continuous monitoring of insecticide resistance, especially in the *A. arabiensis* population in Sudan is vital to the effectiveness of vector control programs. This study, carried out in urban and suburban study sites in Khartoum State, the capital of Sudan, aimed to explore the status of insecticide resistance in the major malaria vector *A. arabiensis* populations to the different classes of insecticides used in public health and estimate the frequency of *kdr* mutations between urban and suburban sites, aiming to provide data that can help guide and improve the malaria control programs in Khartoum State.

Methods

Study Area

Khartoum State lies in a poor savannah region characterized by a short rainy season (July to September), a winter season (October to March), and a summer season (April to July). The total area of the state is 28 000 km^2, divided by the River Nile into three greater administrative areas: Khartoum North, Khartoum, and Omdurman. The state is almost a semidesert region; the vegetation is mostly along the Nile River, with most of the population in suburban areas.

Entomological cross-sectional surveys were carried out in Khartoum State in four sites classified as urban or suburban, according to topography, agriculture, socio-economic activities, physical expansion of cities, and dissemination of socio-economic and cultural patterns. The urban sites were Kafouri and Wad-elbakhet, and the suburban sites were Al-mahalab and Al-salha (Figure 1).

Mosquito collection and rearing

Anopheles larvae were collected from their natural breeding sites, such as animal hoof prints and pools formed by leakage from pipes, ponds, and puddles, from February to June 2014 using WHO standard dippers (350 mL) and then transferred to the insectary. *Anopheles* mosquito larvae were reared to adults in the insectarium and identified to their species using a taxonomic key (16). They were kept at optimum conditions (temperature of 25 ± 2 °C and relative humidity of 70%-80%) and fed with a 10% sucrose solution until they were used.

Insecticide Susceptibility Test

Four insecticides belonging to the four classes recommended by the WHO were tested using WHO susceptibility tests. Four replicate cohorts of sugar-fed adult females of known age (24-48 hours post-emergence), each replicate consisting of 25 female mosquitoes (17). They were exposed to papers impregnated with WHO-recommended concentrations (V/W) of 0.75% permethrin, 4% DDT, 5% malathion, and 0.1% bendiocarb. The control group was exposed to oil-treated control papers (without insecticide), and each insecticide group test was done separately. Mosquitoes were exposed to the insecticide papers for 60 minutes. Knockdown was recorded after 10, 15, 20, 30, 40, 50, and 60 minutes of exposure. The knockdown and alive mosquitoes were transferred to a clean holding tube with 10% sucrose in a cotton piece, and mortality was observed 24 hours post-exposure.

Molecular Identification and kdr Mutation Detection

DNA was extracted from each female mosquito separately using the Livak extraction method; the extracted DNA was re-suspended in TE buffer and kept in a -20 °C freezer until used (18). The morphologically identified *Anopheles gambiae (s.l.)* mosquitoes were molecularly identified to their species using the five species-specific primers for the *A. gambiae* complex designed by Scot et al (Table 1). *Anopheles arabiensis* positive control was used in each PCR

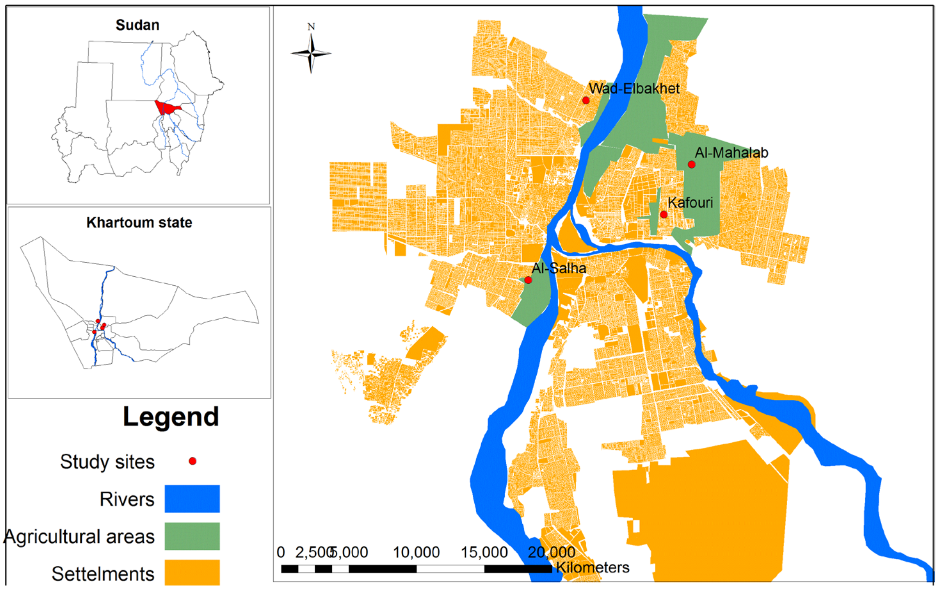

Figure 1. Map Showing the Location of the Study Area in Khartoum State, Sudan, Including Urban Sites (Kafouri, Wad-elbakhet) and Suburban Sites (Al-Mahalab, Al-Salha)

Table 1. *Anopheles gambiae* Complex Primers

Primer	Primer Sequence (5' to 3')	(T_m)(°C)	Expected Amplified DNA Size (bp)
UN	GTG TGC CCC TTC CTC GAT GT	58.3	468
GA	CTG GTT TGG TCG GCA CGT TT	59.3	390
ME	TGA CCA ACC CAC TCC CTT GA	57.2	464
AR	AAG TGT CCT TCT CCA TCC TA	47.4	315
QU	CAG ACC AAG ATG GTT AGT AT	42.7	153

*UN primer anneals to the same position of the rDNA of all the five species, GA anneals specifically to *A. gambiae*, ME anneals to both *A. merus* and *An. melas*, AR anneals to *A. arabiensis* and QD anneals to *An. quadriannulatus*.

Table 2. The *kdr* Primers for Resistant and Susceptible Alleles

Primer	Sequence
*Agd1[a]	5´ ATA GAT TCC CCG ACC ATG 3´
*Agd2 [a]	5´ AGA CAA GGA TGA TGA ACC 3´
*Agd3 [a]	5´ AAT TTG CAT TAC TTA CGA CA 3´
*Agd4 [a]	5´ CTG TAG TGA TAG GAA ATT TA 3´
**Agd5 [b]	5´ TTT GCA TTA CTT ACG ACT G 3´

[a] Martinez-Torres et al (20); [b] Ranson et al (5).

reaction, which was obtained from a susceptible colony maintained at the Department of Medical Entomology in the National Public Health Laboratory. Forty samples (20 dead and 20 alive) per insecticide were used at each site. The *kdr* genotypes were determined using two allele-specific PCR assays followed by a diagnostic PCR (19). The West African mutation (L1014F) in leucine-phenylalanine and the East African mutation (L1014S) in leucine-serine were detected using the PCR developed by (20). Two separate master mixes were prepared to detect the resistant alleles (heterozygous and homozygous states) and the susceptible alleles. Each master mix contained 1.25 µL of 10 X buffer (100 mM Tris-HCl, ph 8.3, 500 mM KCl), 0.75 µL of 25 mM $MgCl_2$, 1.25 µL of 2.5 mM dNTPs, and 0.1 µL of Taq DNA polymerase. For the detection of West African *kdr*, 1.5 µL of Agd1 primer (Table 2) and 3 µL of Agd3 (Table 2) primers were added to each 25 µL PCR reaction mix. Agd3 was substituted with 3 µL Agd5 (Table 2) primer for the East African *kdr* detection. For the detection of the susceptible alleles, 1.5 µL Agd2 (Table 2) and 3 µL Agd4 primers were used for each 25 µL PCR mix. The volume was made up to 25 µL by adding distilled water. The condition was 35 cycles consisting of 95 °C for 1 minute, 48 °C for 1 minute and 72 °C for 1.5 minutes, and a final extension step at 72 °C for 10 minutes. The amplified fragments were separated using 1.5% agarose gel stained with ethidium bromide and visualized under UV light.

Statistical Analysis

Sudan boundary and water maps were downloaded from the Mapcruzin site (21). The coordinates of the collection sites were collected using a geographical positioning system (GPS). Then, a study area map was generated using the ARC View Geographical Information System (GIS) (ESRI 2011). The resistance status of mosquito samples was determined according to the WHO test procedures . Consequently, a mortality rate of ≥ 98% was considered susceptible, 90%-97% was considered suspected/potential resistance, and < 90% was considered resistant; 50% and 95% knockdown times (minutes) (KDT_{50} and KDT_{95}) were computed using probit analysis for the first hour mortality time. Generalized linear models with a Poisson distribution and a log-linear link function were used to assess the effect of site, site type, insecticide, and their interaction on bioassay mortalities after 24 hours. The test was also conducted independently for each insecticide for sites and site types as factors. A chi-square test was used to determine whether observed genotype frequencies are consistent with Hardy-Weinberg equilibrium predictions or not. The association between the presence (yes/no) of the *kdr* genotype and resistance phenotype (resistance/susceptibility) was tested for each insecticide using logistic regression. Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 20.

Results

Bioassay Results

A total of 1600 *Anopheles* female adult mosquitoes were Bioassayed; all populations at urban and suburban sites were considered resistant to DDT, malathion, and permethrin. Mortality rates in urban and suburban areas were found not significant in all insecticides except DDT, which showed significant mortalities of 64 % (51.23-76.77) and 42% (29.73-55.27) in urban and suburban sites, respectively. Malathion was also highly resistant, with mortalities at 68% (55.23-80.77) in urban areas and 53.5% (40.73-63.27) in suburban sites. Bendiocarb revealed lower resistance percentages compared to other tested insecticides among the insecticides tested, with 82.5% (69.73-95.27) in urban and 89.5% (76.73-102.27) in suburban sites. The pyrethroids showed mortality of 56.5% (43.73-69.27) and 50.5% (37.73-63.27) in urban and suburban areas, respectively (Table 3).

Knockdown Time Thresholds

The KDT_{50} and KDT_{95} were calculated over one hour for bendiocarb, DDT, malathion, and permethrin for each site, as shown in Table 1. The knockdown time for *An. arabiensis* from the Wad-elbakhet site showed the lowest KDT50 and KDT95 for bendiocarb and malathion (22.58, 31.78 min) and (45.16, 108.13 min) respectively (Table 4).

GLM analysis indicated that insecticides, sites, and their interactions were determinant factors in mortality rates ($P<0.01$), while the site type (urban vs. suburban) was not ($P>0.01$) (Table 5). Furthermore, the GLM test was estimated for each insecticide separately. Sites and site types and their interactions were considered as factors, while mortality rates were the dependent variables (Table 5). Excluding permethrin, mortality rates of all insecticides vary significantly, according to sites ($P<0.001$). Mortality rates of DDT and malathion varied significantly by site types ($P<0.0001$), while mortality rates of bendiocarb and permethrin were not ($P>0.01$) (Table 5).

Table 3. Bioassays Mortality Means of Urban and Suburban Sites

Insecticides	Mean mortality percentages* (95% CI)		*P* Value
	Urban	Suburban	
Bendiocarb	82.5 (69.73- 95.27)	89.5 (76.73- 102.27)	>0.05
DDT	64 (51.23- 76.77)	42.5 (29.73- 55.27)	<0.05
Malathion	68 (55.23- 80.77)	53.5 (40.73- 66.27)	>0.05
Permethrin	56.5 (43.73- 69.27)	50.5 (37.73- 63.27)	>0.05

* Mean mortality%: means mortality rate after 24 hours of exposure to site type in the two urban areas and the two suburban sites.

Kdr Allele Frequency

The West African *kdr* mutation (L1014F) was observed in all study populations (Table 6), (while the East African *kdr* mutation (L1014S) was absent. A total of 320 samples were screened for the *kdr* mutation. The majority (46.56%) of the screened *An. Arabiensis* individuals were susceptible (SS or LL), 5% were homozygous resistant (RR or FF), and 48.43% were heterozygous (RS or LF) for the L1014F-*kdr* allele (Figure 2).

The association between survivorship (resistant phenotype) in permethrin and DDT insecticides and the existence of the 1014F-*kdr* allele were measured by odds ratios, and a strong correlation was observed; besides, urban and suburban areas vary significantly in the *kdr* frequencies. Genotypic odds ratios (ORs) are shown in (Table 6).

Discussion

Although malaria cases have decreased by 20 million cases since 2010, the burden has increased by about 3.5 million cases, with more than 430 000 deaths each year in African countries .

The LLINs and the IRS are the cornerstones of the malaria control program in Africa and Sudan; since the year 2000, the reduction of malaria cases globally has been attributed to the mass scale-up of LLINs and the IRS. In the years 2013-2014, 13 million LLINs were distributed, covering 92% of the country’s households. Subsequently, the evolution of insecticide resistance caused a major concern, threatening the effectiveness of vector control in Sudan (22). This study investigated the insecticide resistance status of *A. arabiensis* in four sites in Khartoum State, classified as urban and suburban. KD_{50} and KD_{95} for four insecticides were determined, and the *kdr* frequencies were estimated. The bioassay results showed high resistance to three of the four tested insecticides (DDT, malathion, and permethrin). Bendiocarb insecticide resistance was observed in both urban and suburban sites. *Anopheles arabiensis* showed resistance to carbamates class in urban and suburban agricultural areas in Khartoum State but was reported to be susceptible in eastern Sudan, central Sudan, and Khartoum (23,24). Although bendiocarb-resistant populations were previously detected in agricultural areas that use bendiocarb heavily, this study revealed that *An. arabiensis* showed resistance in both urban nonagricultural areas and suburban agricultural areas. This may be attributed to the adaptation of *A. arabiensis* larvae developed in different polluted breeding sites in different areas of Khartoum, which might enhance tolerance to the insecticides. This attribution, supported by a study in Khartoum, indicated that polluted breeding

Table 4. Knockdown Mortality Time (minutes) in *Anopheles arabiensis* in the 4 Study Sites

Study Area	Insecticide	Resistance Status	KDT$_{50}$ (95% CI)	KDT$_{95}$ (95% CI)
Al-mahlab (Suburban)	0.1% Bendiocarb	PR	37.83 (36.21 - 39.46)	62.19 (58.05 - 67.88)
	4% DDT	R	74.43 (65.64 - 91.35)	179.64 (132.75 - 304.05)
	5% Malathion	R	78.15 (64.79 - 116.64)	196.53(127.28 - 540.88)
	0.75% Permethrin	R	30.36 (28.00 - 32.97)	109.98(91.44 - 140.16)
Al-salha (Suburban)	0.1% Bendiocarb	R	35.88 (33.53 - 38.44)	77.10 (68.13 - 90.77)
	4% DDT	R	63.22 (58.48 - 70.87)	122.95 (101.25 - 168.56)
	5% Malathion	R	44.26 (39.93 - 50.05)	112.94 (89.28 - 164.82)
	0.75% Permethrin	R	30.67 (28.22 - 33.27)	71.32 (62.00 - 86.12)
Kafouri (Suburban)	0.1% Bendiocarb	R	31.99 (28.84 - 35.43)	80.35 (66.99 - 104.68)
	4% DDT	R	58.90 (55.14 - 64.47)	112.40 (94.94 - 146.39)
	5% Malathion	R	68.09 (56.92 - 93.82)	213.67 (137.15 - 523.76)
	0.75% Permethrin	R	34.55 (31.39 - 38.26)	109.64 (88.52 - 148.51)
Wad-elbakhet (Urban)	0.1% Bendiocarb	S	22.58 (20.75 - 24.46)	45.16 (40.05 - 52.99)
	4% DDT	R	42.99 (39.05 - 48.11)	118.97 (94.97 - 166.62)
	5% Malathion	R	31.78 (27.83 - 36.49)	108.13 (82.07 - 167.42)
	0.75% Permethrin	R	29.32 (27.25 - 31.54)	90.22 (77.66 - 109.30)

Note: R (Resistant), PR (Potentially Resistant) and S (Susceptible).
Number of tested mosquitoes per insecticide per site = 100

Table 5. The Effects of Site Type (Urban or Suburban) and Site (Nested Within Site Type) on Bioassay Mortality for Each Insecticide

Model factor	Bendiocarb	DDT	Malathion	Permethrin
Sites	*P* < 001	*P* < 001	*P* < 001	NS
Site types	NS	*P* < 001	NS	NS
Site X Site types	*P* < 001	*P* < 001	*P* < 001	NS

NS, not significant.

Table 6. Frequencies of L1014F Alleles Detected in Susceptible and Resistant Mosquitoes of *Anopheles arabiensis* Exposed to DDT and Permethrin in Urban and Suburban Areas

Insecticide	Area	Phenotype	LL	LF	FF	F	L	**P* Value	OR (95% CI)	P.value for OR
Permethrin	Urban	Susceptible	34	6	0	0.08	0.92	> 0.05	6.8 (2.6-17.9)	< 0.001
		Resistant	0	39	1	0.51	0.49	< 0.001		
	Suburban	Susceptible	38	2	0	0.03	0.97	> 0.05	24.2 (5.4-107.9)	< 0.001
		Resistant	0	33	7	0.59	0.41	< 0.001		
DDT	Urban	Susceptible	38	2	0	0.03	0.97	> 0.05	22.2 (5.01-98.559)	< 0.001
		Resistant	0	36	4	0.55	0.45	< 0.001		
	Suburban	Susceptible	39	1	0	0.01	0.99	> 0.05	44.4 (5.8-340.03)	< 0.001
		Resistant	0	36	4	0.55	0.45	< 0.001		

L = Leucine (wild-type allele); F = Phenylalanine (*kdr* allele); OR = odds ratio.
**P* value from χ2 test of allelic association.
The frequencies were calculated for each insecticide and mosquito status (resistant/susceptible) after exposure.

sites might directly affect mosquito susceptibility to insecticides (25), or it might be attributed to the intensive use of carbamates as agricultural pesticides in urban and suburban areas. However, carbamates are not yet used in public health vector control in the Khartoum area. The resistance of bendiocarb in urban (mean 82.5%) and suburban (mean 89.5%) areas is consistent with a previous study carried out in Khartoum State that observed 60%-80% mortality in *A. arabiensis* mosquito populations in Khartoum. The same study revealed that the heavy use of bendiocarb and malathion insecticides in agriculture might reduce the effectiveness of these insecticides in malaria control. Furthermore, the study showed *A. arabiensis* resistance to malathion, permethrin, and DDT (26). Several studies have reported malathion resistance in Sudan, and they have attributed its resistance to its continuous use in both public health and agricultural activities . Although DDT was banned decades ago, it was obtained from illegal markets and used in agriculture (27). This study reported the lowest rate of mortalities

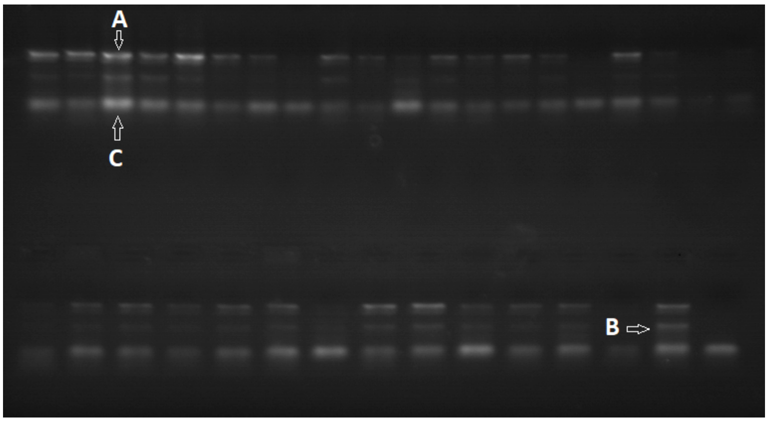

Figure 2. Gel Electrophoresis Showing the Resistant and Susceptible Alleles in *Anopheles arabiensis* From Khrtoum/Sudan. A = 293 bp, a fragment of the IIS6 segment containing the *kdr*; B = 195 bp, a fragment of *kdr* west African mutation resistance allele; C = 137 bp, a fragment of susceptible allele. Note: Samples with the three bands are considered heterozygous resistance

in DDT, 42.5% (29.73-55.27), permethrin 50.5% (37.73-63.27), and malathion 53.5% (40.73-66.27) in suburban areas. Although resistance to permethrin and DDT was reported years ago in Khartoum, it was with high mortality percentages of about 95% and 92% for permethrin and DDT, respectively, in suburban areas in Khartoum State. This study is in contrast with our results, which revealed that suburban mosquitoes were more resistant than urban mosquitoes. DDT was the only insecticide in our study that showed a significant difference between urban and suburban areas.

Insecticides can be considered a very strong predictor of mortality (a very high wald chi-square value). Currently, only four insecticide classes are available for vector control programs, and pyrethroids are the only class recommended by the WHO for use on LLINs.

The quickest knockdown time in Wad elbakheit was obtained by bendiocarb ($KDT_{50} = 22.58$), while the slowest knockdown time was obtained by DDT ($KDT_{50} = 42.99$). These results follow the study of (28), which showed that the bendiocarb has the fastest knockdown time (KDT 50 = 18.2) and the slowest showed by DDT ($KDT_{50} = 35.4$); the DDT slow killing of *Anopheles* mosquitoes was reported from other African countries (29). Although the 100% mortality shown by bendiocarb in Wad elbakheit and the increased KDT50 indicate the development of resistance to this insecticide, according to (30), KDT is an early indicator of developing resistance.

The *Kdr* western mutation (L1014F) was detected in all urban and suburban sites, whereas the eastern mutation (L1014S) was absent. The absence of the eastern *kdr* mutation (L1014S) has been confirmed by many studies in Khartoum and central Sudan. However, a survey carried out in Kassala, eastern Sudan, near the Ethiopian border (560 km from Khartoum) reported an eastern *kdr* mutation. The strong association between the presence of the *kdr* allele and susceptibility/resistance phenotype is consistent with, while others, in contrast, revealed the absence or weak association of the *kdr* with DDT and pyrethroid resistance (31). Our *kdr* frequency aligns with the study conducted by others which found no significant difference in kdr frequency between urban and suburban areas, except for the presence of DDT. The cross-resistance between chlorines and pyrethroids might be a reason for increasing the frequency of *kdr* and the resistance phenotype of pyrethroids, especially in suburban areas. Recently, a study of knockdown resistance (*kdr*) in *A. arabiensis* in the Galabat area of eastern Sudan found that a relationship between *kdr* frequency and malaria incidence was not apparent (32).

The continued deterioration in the susceptibility status of different insecticides, especially those used for ITNs/LLINs, IRS, or larval control, besides the 1014F kdr mutation increasing in malaria vectors, is a real threat to mosquito control campaigns, particularly in some African countries (33). Consequently, monitoring and updating knowledge about the susceptibility status of the currently used and recommended insecticides is vital to ensuring the quality and success of vector control, especially for the shift between alternative insecticides. Accordingly, continuous studies on the susceptibility status of *A. arabiensis* and its implications for vector control are crucial for optimal surveillance and control.

Conclusion

Our results confirmed resistance to all four classes of insecticides in urban and suburban sites; these findings are of significant concern, especially for the National Malaria Control Program (NMCP). The speed of insecticide resistance development in Bendiocarb, one of the best choices for the IRS in Sudan, should get more attention from decision-makers. We also recommend an investigation of the effect of pyrethroid resistance on the LLINs in the state. Finally, collaboration between agriculture authorities and vector control authorities would be necessary for the management of insecticide-resistant malaria vectors in agricultural areas.

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Writing–review & editing: Yagoob Garedaghi, Sara A. Abuelmaali.

Competing Interests

The authors declare that they have no competing interests.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Ethical Approval

Ethical approval was not needed for mosquito larvae collection. The permission to participate in the study by the farmers was sought and granted by the same.

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Not applicable.

References

1. World Health Organization (WHO). World Malaria Report 2018. WHO; 2018. Available from: http://www.who.int/iris/handle/10665/275867.
2. Adzu B, Haruna AK, Salawu OA, Katsayal UD, Njan A. In vivo antiplasmodial activity of ZS-2A: a fraction from chloroform extract of *Zizyphus spina-christi* root bark against *Plasmodium berghei berghei* in mice. Int J Biol Chem Sci. 2007;1(3):281-6. doi: 10.4314/ijbcs.v1i3.39714.
3. Garedaghi Y, Firouzivand Y, Hassanzadeh Khanmiri H, Shabestari Asl A. A review of the most important antiparasitic compounds effective on human fascioliasis from the past until now. Curr Drug Ther. 2023;18(5):365-76. doi: 10.2174/1574885518666230403111528 .
4. Seidahmed OM, Abdelmajed MA, Mustafa MS, Mnzava AP. Insecticide susceptibility status of the malaria vector *Anopheles arabiensis* in Khartoum city, Sudan: differences between urban and periurban areas. East Mediterr Health J. 2012;18(7):769-76. doi: 10.26719/2012.18.7.776.
5. Ranson H, Lissenden N. Insecticide resistance in African *Anopheles* mosquitoes: a worsening situation that needs urgent action to maintain malaria control. Trends Parasitol. 2016;32(3):187-96. doi: 10.1016/j.pt.2015.11.010.
6. Strode C, Donegan S, Garner P, Enayati AA, Hemingway J. The impact of pyrethroid resistance on the efficacy of insecticide-treated bed nets against African anopheline mosquitoes: systematic review and meta-analysis. PLoS Med. 2014;11(3):e1001619. doi: 10.1371/journal.pmed.1001619.
7. Kleinschmidt I, Mnzava AP, Kafy HT, Mbogo C, Bashir AI, Bigoga J, et al. Design of a study to determine the impact of insecticide resistance on malaria vector control: a multi-country investigation. Malar J. 2015;14:282. doi: 10.1186/s12936-015-0782-4.
8. Himeidan YE, Muzamil HM, Jones CM, Ranson H. Extensive permethrin and DDT resistance in *Anopheles arabiensis* from eastern and central Sudan. Parasit Vectors. 2011;4:154. doi: 10.1186/1756-3305-4-154.
9. Himeidan YE, Chen H, Chandre F, Donnelly MJ, Yan G. Short report: permethrin and DDT resistance in the malaria vector *Anopheles arabiensis* from eastern Sudan. Am J Trop Med Hyg. 2007;77(6):1066-8.
10. Ismail SM, Nugud AD, Jamal AE, Abdalmagid MA, Bashir AI, Toto HK. Insecticide resistance in *Anopheles arabiensis* Patton in White Nile state during the dry season. Sudan J Public Health. 2012;7(4):136-41 .
11. Hariri D, Garedaghi Y. Comparison of therapeutic effects of hydroalcoholic extract of Asafoetida with metronidazole in mice infected with Giardia lamblia. Journal of Zoonotic Diseases 2024;8(1):452-9. doi: 10.22034/jzd.2024.17396.
12. Abuelmaali SA, Elaagip AH, Basheer MA, Frah EA, Ahmed FT, Elhaj HF, et al. Impacts of agricultural practices on insecticide resistance in the malaria vector *Anopheles arabiensis* in Khartoum state, Sudan. PLoS One. 2013;8(11):e80549. doi: 10.1371/journal.pone.0080549.
13. Abdalla H, Wilding CS, Nardini L, Pignatelli P, Koekemoer LL, Ranson H, et al. Insecticide resistance in *Anopheles arabiensis* in Sudan: temporal trends and underlying mechanisms. Parasit Vectors. 2014;7:213. doi: 10.1186/1756-3305-7-213.
14. Tabbabi A, Daaboub J, Laamari A, Ben-Cheikh R, Feriani M, Boubaker C, et al. Impacts of agricultural practices on pyrethroid resistance in *Culex pipiens pipiens*, an important vector of human diseases, from Tunisia. Trop Biomed. 2019;36(2):542-9.
15. Sene SO, Tendeng E, Diatte M, Sylla S, Labou B, Diallo AW, et al. Insecticide resistance in field populations of the tomato fruitworm, *Helicoverpa armigera*, from Senegal. Int J Biol Chem Sci. 2020;14(1):181-91. doi: 10.4314/ijbcs.v14i1.15.
16. Gillies MT, Coetzee M. A Supplement to the Anophelinae of Africa South of the Sahara (Afrotropical Region). Johannesburg: South African Institute for Medical Research; 1987. Publications of the South African Institute for Medical Research no. 55.
17. World Health Organization (WHO). Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes. WHO Library Cataloguing-in-Publication Data; 2013.
18. Livak KJ. Organization and mapping of a sequence on the Drosophila melanogaster X and Y chromosomes that is transcribed during spermatogenesis. Genetics. 1984;107(4):611-34. doi: 10.1093/genetics/107.4.611.
19. Scott JA, Brogdon WG, Collins FH. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am J Trop Med Hyg. 1993;49(4):520-9. doi: 10.4269/ajtmh.1993.49.520.
20. Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Bergé JB, Devonshire AL, et al. Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae* s.s. Insect Mol Biol. 1998;7(2):179-

84. doi: 10.1046/j.1365-2583.1998.72062.x.

21. MapCruzin. 2018. Sudan, country, city and place-gis-shapefiles. Available from: https://mapcruzin.com/free-sudan-country-city-place-gis-shapefiles.htm. Accessed July 15, 2018.
22. Kafy HT, Ismail BA, Mnzava AP, Lines J, Abdin MS, Eltaher JS, et al. Impact of insecticide resistance in *Anopheles arabiensis* on malaria incidence and prevalence in Sudan and the costs of mitigation. Proc Natl Acad Sci U S A. 2017;114(52):E11267-75. doi: 10.1073/pnas.1713814114.
23. Ismail BA, Kafy HT, Sulieman JE, Subramaniam K, Thomas B, Mnzava A, et al. Temporal and spatial trends in insecticide resistance in *Anopheles arabiensis* in Sudan: outcomes from an evaluation of implications of insecticide resistance for malaria vector control. Parasit Vectors. 2018;11(1):122. doi: 10.1186/s13071-018-2732-9.
24. Mohammed TS, Ahmed KA, Zain HM, Abdelateef AG, Gibreel YA, Abdalmajed MA, et al. Susceptibility status of the malaria vector *Anopheles arabiensis* to insecticides in Khartoum state, Sudan. Sudan J Med Sci. 2015;10(2):39-52.
25. Azrag RS, Mohammed BH. *Anopheles arabiensis* in Sudan: a noticeable tolerance to urban polluted larval habitats associated with resistance to temephos. Malar J. 2018;17(1):204. doi: 10.1186/s12936-018-2350-1.
26. Bimenya GS, Mugisha PS, Okwi AL, Habarulema M, Lugemwa M. Does resistance of *Anopheles* mosquitoes to knock-out effect of DDT deter the ability of the chemical to control malaria in Uganda? Int J Biol Chem Sci. 2010;4(3):657-68. doi: 10.4314/ijbcs.v4i3.60481.
27. Oya C. Decent Work Indicators for Agriculture and Rural Areas. Conceptual Issues, Data Collection Challenges and Possible Areas for Improvement. ESS Working Paper No. ESS 15-10. FAO; 2015. Available from: http://www.fao.org/3/a-i5060e.pdf.
28. Abdalla H, Matambo TS, Koekemoer LL, Mnzava AP, Hunt RH, Coetzee M. Insecticide susceptibility and vector status of natural populations of *Anopheles arabiensis* from Sudan. Trans R Soc Trop Med Hyg. 2008;102(3):263-71. doi: 10.1016/j.trstmh.2007.10.008.
29. Ahadji-Dabla KM, Ngoagouni C, Dery BD, Apétogbo YG, Ketoh GK, Glitho IA. Spatio-seasonal distribution of *Anopheles gambiae* sensu lato and dynamics of the voltage gate sodium channel knock down resistance mutation (Vgsc1014F) in the city of Lomé, Togo. Int J Biol Chem Sci. 2019;13(3):1654-68. doi: 10.4314/ijbcs.v13i3.36.
30. Santiago-Figueroa I, Lara-Bueno A, González-Garduño R, Mendoza-de Gives P, Delgado-Núñez EJ, Maldonado-Simán ED, et al. Anthelmintic evaluation of four fodder tree extracts against the nematode *Haemonchus contortus* under in vitro conditions. Rev Mex Cienc Pecu. 2023;14(4):855-73. doi: 10.22319/rmcp.v14i4.6339.
31. Khakpour M, Garedaghi Y. Molecular differentiation of sheep and cattle isolates of fasciola hepatica using RAPD-PCR. Archives of Razi Institute. 2012;67(2):109-115. doi: 10.22092/ari.2016.103894.
32. Jannati R, Tavakoli Pasand S, Garedaghi Y. Evaluation of different techniques in laboratory diagnosis of intestinal amoebiasis. Int J Med Parasitol Epidemiol Sci. 2024;5(1):16-23. doi: 10.34172/ijmpes.3129.
33. Stevens ER, Aldridge A, Degbey Y, Pignandi A, Dorkenoo MA, Hugelen-Padin J. Evaluation of the 2011 long-lasting, insecticide-treated net distribution for universal coverage in Togo. Malar J. 2013;12:162. doi: 10.1186/1475-2875-12-162.