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Spatial and Temporal Variation in Susceptibility Status of *Anopheles arabiensis* the Malaria Vector to Insecticides in Khartoum State, Sudan

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Abstract:

Spatial and temporal variation in the susceptibility status of Anopheles arabiensis to five insecticides were investigated during 2011-2013 in nine sentinel sites in Khartoum State. Field-collected larvae and pupae Anopheles in three seasons (cold dry, hot dry and wet seasons) were reared to adult stages. Standard WHO diagnostic bioassay for 4% DDT, 5% malathion, 0.1% propoxur, 0.75% permethrin and 0.05% deltamethrin were used to test the susceptibility

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of adults An. arabiensis. A total of 6275 females of An. arabiensis were exposed to the aforementioned insecticides. Resistances in An. arabiensis to all insecticides were recorded in most of the sites investigated. Most of the populations of An. arabiensis were resistant to malathion, DDT and pyrethroids (permethrin and deltamethrin). The results of overall revealed a clear difference pattern between the seasons in the susceptibility status of An. arabiensis exposed to 4% DDT and 0.1% propoxur. However, the seasonal variations in susceptibility status in An. arabiensis from the sentinel sites did not follow clear trends. In contrast, a significant difference were observed between the seasons in mortality of An. arabiensis from the urban and periurban areas exposed to 4% DDT and 5% malathion respectively. The current study provides useful information on the distribution and seasonal variations in the susceptibility status in An. arabiensis populations from Khartoum State. The mortality rate in An. arabiensis exposed to these insecticides has increased in the wet season which can be explained by the selective insecticide pressures from the agricultural and malaria control programme activities.

Key words: *Anopheles arabiensis*; Insecticide susceptibility; Malaria vector control

Introduction:

Malaria vector control programmes in Sub-Saharan African rely heavily on both insecticide-treated nets (ITNs)/long-lasting insecticidal nets (LLINs) and/or indoor residual spraying (IRS) [(1)]. As a result of the increase in the deployment of these interventions, a major reduction in disease burden has been observed in several Africa countries [(2-4)]. Therefore, the ITNs/LLINs and IRS are considered as the main component of malaria control, due to their ease of implementation and cost effectiveness [(5)]. However, the use of ITNs/LLINs and IRS depend on vector susceptibility to the insecticides used. According to the World Health Organization, pyrethroids are the only group of insecticides currently approved for ITNs/LLINs and the four classes (Pyrethroids, organochlorine, carbamate and organophosphate) are recommended to be used for IRS [6].

In Sudan, malaria causes high morbidity and mortality. It was estimated 9 million cases and 44,000 deaths due to malaria infection in 2002 [7]. In contrast, in Khartoum State in the central Sudan, malaria accounts for 300000 cases and five hundred deaths every year [8]. The disease is mainly transmitted by *Anopheles arabiensis* [9]. The main vector control interventions in Sudan include the use of ITNs/LLINs, and IRS [6] especially in the irrigated scheme areas. In Khartoum State, the expansion of both urban and peri-urban resulted in a massive increase in anopheline larval habitats in across the State [10]. Therefore, the main malaria vector control in this state depend on Larval Source Management (i.e. chemical larviciding of water larval habitats by Temephos® EC50 and environmental management) and insecticide space spraying during the emergency situation [11].

The use of ITNs/LLINs and IRS have resulted in development of resistance to different classes of insecticides in malaria vectors throughout the Sub-Saharan African [12,13]. Currently, insecticide resistance particular, to pyrethroids in the major African vectors *Anopheles gambiae* s.l. has become widespread [13,14]. Likewise, insecticide resistance in *An. arabiensis* in Sudan has been reported from different regions in the country [15-17]. This includes mainly resistance to the dichlorodiphenyltrichloroethane (DDT) [15], the organophosphate malathion [16], the carbamates propoxur and to various pyrethroids [15-17]. The development and spread of insecticide resistance especially to pyrethroids in these vectors represents a threat for malaria control programmes in many African countries. Because of importance of insecticides in malaria vector control and to prevent decline of their efficiency especially pyrethroids, continuous monitoring the susceptibility of vectors to these insecticide classes is of a great value for resistance management.

Two mechanisms of insecticide resistance in mosquitoes are known. These are; 1. metabolic resistance and 2. Target site insensitivity (18). The Metabolic resistance to carbamates and organophosphates insecticides results from the over expression or amplification of genes coding for three large enzyme families: the cytochrome P450 monooxygenases (CYP450s), carboxyl/cholinesterases (CCEs), andglutathione-S transferases (GSTs) (19). In contrast the target site insensitivity to DDT and pyrethroids is a result of an amino acid substitution in the domain II in the voltage-gated sodium channel (VGSC) of neuronal membranes (20,21). In *An. gambiae* s.s. (hereafter named as *A. gambiae*), point mutations occur at different nucleotides of the same amino acid residue 1014 of the VGSC have been described, resulting in either a phenylalanine (L1014F) or serine (L1014S) substitution (22,23). Currently, both of these kdr mutations have been found in several widely dispersed populations of *An. gambiae* and *An. arabiensis* in different African countries (15,24,25).

In the current study, the distribution resistant strains and the seasonal variation in susceptibility status of *An. arabiensis* from nine sentinel sites in Khartoum State to five insecticides were investigated.

Materials and methods:

Study area:

The current study was carried out in Khartoum State which is located in central Sudan (15°.10'-16°.30' N and 31°.35'-34°.20' E) (Fig. 1). The State occupies approximately about 28 000 km^2. The confluence of the Blue and the White Niles divided the

State into three administrative areas; Khartoum, Khartoum North and Omdurman areas.

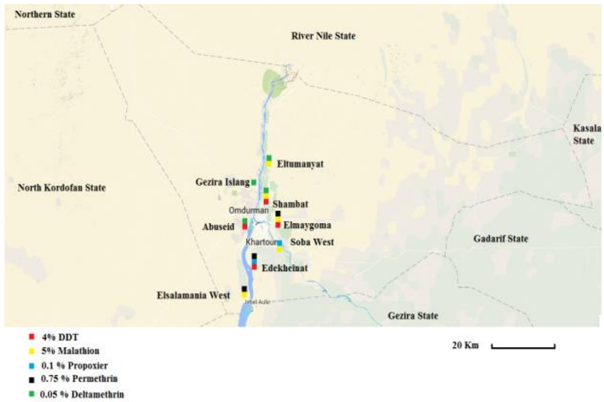

Figure 1: A map of Khartoum State shows sentinel sites and the insecticides resistant-strains of *Anopheles arabiensis.*

Study sites:

Mosquito larvae and pupae were collected form nine sentinel sites set by the Khartoum Malaria Free Project (KMFP), Khartoum State, Ministry of Health for the routine larval surveys and monitoring the insecticides susceptibility status for control of *An. arabiensis* the main malaria vector in the state. These sentinel sites are categorized as urban and periurban areas. The urban area were; Arkaweet (15° 32' 52.7964" N, 32° 33' 58.7298" E), Shambat (15° 39' 39.4446" N,32° 31' 25.683" E), Abuseid (15° 34' 20.7942" N, 32° 30' 32.6154" E) and; the periurban areas were Soba West (15° 31' 12.954" N, 32° 40' 51.5028" E), Edekheinat (15° 26' 9.042" N, 32° 28' 41.4768"), Elmaygoma (15° 18' 12.654" N, 32° 35' 43.7496" E), Eltumanyat (15° 57' 41.8392" N, 32° 33' 55.9908" E), Elsalamania West (15° 18' 12.654" N, 32° 28' 13.5294" E) and Al-Gizera Islang (15° 53' 2.9544" N, 32° 32' 6.3738").

Anopheline mosquito collection, rearing and identification:

Anopheline mosquitoes were collected as larvae and pupae between March 2011 and February 2013 from larval habitats in nine sentinel sites in Khartoum State. Anopheline mosquitoes were collected from these sites three times a year to coincide with the seasons of cold dry (November –February), hot dry (March – June) and wet or rainy (July – October) seasons. The collected larvae and pupae were then transported to the Khartoum Malaria Free Project (KMFP) insectary and reared to the adult stage to be used for WHO bioassay tests.

The emerged adult *Anopheles* mosquitoes from the colony-reared mosquitoes were morphologically identified [(26)]. Furthermore, a sub-samples (n = 900) of the morphologically identified *Anopheles* mosquitoes were randomly selected and analyzed by PCR for species identification [(27)].

PCR product amplicons were resolved in 1.5% agarose gel containing ethidium bromide and 100 bp DNA molecular weight ladder were used as a marker.

WHO-bioassay tests:

The emerged adult *Anopheles* from the colony-reared mosquitoes and identified morphologically as *An. gambiae* s.l. and by PCR as *An. arabiensis* were tested against five insecticides with discriminating doses; 4% DDT (organochlorine), 5% malathion (organophosphate), 0.1% propoxur (carbamate) and, 0.75% permethrin and 0.05% deltamethrin (pyrethroids). For these assays, only a non-blood fed, 3–5 day old adult females were used and the insecticide bioassay tests were conducted according to WHO standard procedures [(28)] using impregnated papers provided by KMFP. Furthermore, populations of *An. arabiensis* from each sentinel site collected in different seasons were tested against the aforementioned insecticides.

Data analysis:

Data obtained from the WHO-bioassay tests, the data were analyzed by SPSS software Version 20 and EXCEL software. The resistance/susceptibility status of the tested mosquitoes from each sentinel site for each insecticide in different seasons was determined according WHO criteria [28]: mortality rate ≥98% = susceptible, 90-97% = suspected/potential resistance, and <90% = resistant (tolerant). Abbott's formula was not required for correction of mortality results because percentage of mortality in control groups was less than 5% to all diagnostic concentrations. Chi-square test was used to compare the differences in percentages of mortalities between the different seasons in each sentinel site as well in the urban and periurban areas.

Furthermore, data on the resistance strains of *An. arabiensis* for insecticides used were overlaid on a map of Khartoum State.

Ethical considerations:

No ethical approval was required because the study was conducted under the supervision of the KMFP and Integrated Vector Management Unit (IVM), Federal Ministry of Health, Sudan as a part of their routine surveys.

Results:

WHO-susceptibility tests for populations of *Anopheles arabiensis* from Khartoum State:

Anopheline mosquitoes:

All anopheline specimens collected from different sentinel sites were identified morphologically as *An. gambiae* s.l. In addition, species identification of the specimens revealed only the presence of *An. arabiensis* in all sentinel sites investigated.

Descriptive Data:

A total of 6275 adult *An. arabiensis* reared from larvae collected from the nine sentinel sites were exposed to the aforementioned insecticides (550 - 1900 per insecticide). Of these 2125, 925 and 3175 specimens were tested in the cold dry, hot dry and wet seasons respectively. Three of insecticides; 4% DDT, 0.1% propoxur and 5% malathion were used to test populations of *An. arabiensis* collected in the three different seasons. In contrast, 0.75% permethrin were used to test mosquitoes collected in only wet season whereas, 0.05% deltamethrin was used for those collected in the cold and hot dry seasons.

Results of WHO-bioassay:

Distribution of resistance strains of *An. arabiensis* to insecticides:

The results of WHO-bioassay tests revealed that populations of *An. arabiensis* three sentinel sites (Edekheinat, Shambat and Elmaygoma) were resistant to three out of the five tested insecticides. In contrast, *An. arabiensis* from Soba west, Eltumanyat, Abuseid and Elsalamania West sites were resistant to two insecticides. Furthermore, *An. arabiensis* from Al-Gezira Islang site was resistant to only one insecticide whereas those from Arkaweet site were not resistant to any of the five insecticides used.

The results also showed that, the 4% DDT-resistant *An. arabiensis* were recorded in four sites; Edekheinat, Shambat, Elmaygoma and Abuseid. In contrast, *An. arabiensis* resistant to 5% malathion were recorded in Soba West, Shambat, Elmaygoma, Eltumanyat and Elsalamania West sites. However, *An. arabiensis* from only two sites; Soba West and

Edekheinat sites were found resistant to 0.1% propoxur. Permethrin-resistant *An. arabiensis* were recorded in four sites (Edekheinat, Elmaygoma and Elsalamania West). Likewise, four populations of *An. arabiensis* from Shambat, Eltumanyat, Abuseid and Al-Gizera Islang were resistant to 0.05% deltamethrin.

Seasonal variation in *Anopheles arabiensis* susceptibility status:

The overall results on variation in insecticide susceptibility status of *An. arabiensis* from Khartoum State in different seasons are shown in table 1. Specimens of *An. arabiensis* collected from the nine sentinel sites in the cold dry, hot dry and wet seasons were tested against the aforementioned insecticides. Significant differences in the mortality of *An. arabiensis* between the three seasons were observed only for 4% DDT (χ^2 =8.905, df = 2, P = 0.012) and 0.1% propoxur (χ^2 =7.96, df = 2, P = 0.019). However, *An. arabiensis* was resistance to 4% DDT during both cold and hot dry season (87 ±1.80 and 89 ±2.80 respectively), and suspected resistance in wet season. In contrast, this species was suspected resistance to 5% malathion in the cold dry season (90 ±2.80) and resistant in both other seasons (63 ± 12.0 and 86 ±2.00 respectively).

The results of analysis revealed that there were significant differences in mean mortality between the seasons in the mean mortality due to 4% DDT in *An. arabiensis* from Edekheinat (χ^2= 9.439, df = 2, P = 0.009) and Shambat (χ^2= 7.424, df = 2, P = 0.024) sites. However, slight variation in susceptibility status in different season was observed in *An. arabiensis* from Soba west also exposed to 4% DDT. Although, *An. arabiensis* from Elmaygoma site was resistance to 5% malathion during the three seasons, a significant difference was observed among specimens tested in different seasons (χ^2= 6.783, df = 2, P = 0.034).

The results also showed that, there is a significant difference between the three seasons in mortality rate *An. arabiensis* from the urban area exposed to DDT 4% (χ^2= 9.419, df = 2, P = 0.009) and periurban area due to malathion 5% (χ^2= 7.808, df = 2, P = 0.02) (figure 2 and 3) Although, the differences in mortality in *An. arabiensis* from urban area due to 4% DDT was not significant (P > 0.05), this species was resistant (84 ± 2.69) to this insecticide during the cold dry season and suspected/resistance in both dry hot and wet seasons (91 ± 2.48 and 91 ± 3.13 respectively). Similarly, *An. arabiensis* from urban area was resistant to 5% malathion in the cold dry season (78 ± 6.81) and suspected/resistance in hot dry and wet seasons (95 ± 1.50 and 95 ± 1.81 respectively).

Table 1: Mean ± SE mortality in *Anopheles arabiensis* from the three administrative areas in Khartoum State exposed to insecticides during 2011 - 2013.

Insecticide used	Cold dry season		Hot dry season		Wet season	
	No tested (Repl)	Mean± SE	No tested (Repl)	Mean± SE	No tested (Repl)	Mean± SE
DDT 4%	625 (25)	87 ±1.80	425 (17)	95 ±1.47	650 (26)	89 ±2.80
Malathion 5%	700 (28)	83 ±2.67	300 (12)	86 ±4.04	900 (36)	84 ±1.75
Propoxur 0.1%	400 (16)	90 ±2.80	200 (8)	63 ±12.0	450 (18)	86 ±2.00
Permethrin 0.75%	**ND**	**ND**	**ND**	**ND**	550 (22)	87.35±1.80
Deltamethrin 0.05%	400 (16)	93 ±2.23	**ND**	**ND**	625 (25)	95 ±1.82

Note; Repl = Replicates; ND = Not done

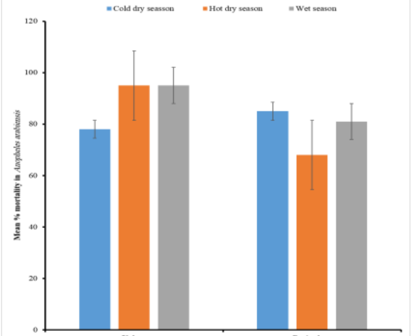

Figure 2: Mean percentage mortality in *Anopheles arabiensis* from urban and periurban areas in Khartoum State exposed to 4% DDT in different seasons during 2011 – 2013.

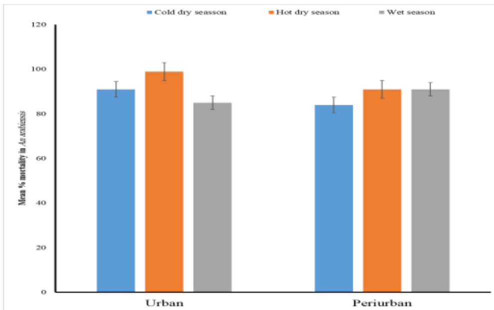

Figure 3: Mean percentage mortality in *Anopheles arabiensis* from urban and periurban areas in Khartoum State exposed to 5% malathion in different seasons during 2011 – 2013.

Discussion:

Control of malaria vectors remains a subject of interest to many scientists because malaria is threatening the life of millions of people especially in Africa (28). However, insecticide resistance is a growing problem affecting the sustainability of any malaria vector control programme. The resistance managements are needed so as to delay or prevent insecticide resistance in these vectors. An important part in the resistance management strategies is identifying the resistance strains and mechanisms involved. Therefore, this study was designed to obtain baseline data on distribution and seasonal variation of *An. arabiensis* susceptibility/resistance strains across Khartoum State. Such studies will help our understanding to set appropriate control strategies against malaria and other mosquito borne-diseases in Sudan and other endemic areas.

In Africa, there have been efforts to map the insecticide resistance of the main malaria vectors at nation or continental scale (29,24). Such information's are necessary to monitor, detect and manage insecticide resistance in mosquito vectors. In this study, resistance to DDT, malathion, propoxur, permethrin and deltamethrin were observed in populations of *An. arabiensis* from most of sentinel sites representing both urban and periurban areas. Nevertheless, the resistances to these insecticides are not uniformly distributed among the populations of *An. arabiensis* from different nine sentinel sites. For example, DDT-resistant strains of *An. arabiensis* were observed in only four of the aforementioned sites. These sites were urban areas; Shambat and Abuseid sites, periurban areas; Edekheinat Shambat (urban area) and Elmaygoma (periurban area) in and Abuseid site. Although, insecticide resistance has been reported in the main malaria vectors worldwide, it is however not uniformly distributed among vector species and can greatly differ from one village, province, country, region and continent to another. Unfortunately, the highest levels of insecticide resistance were reported in Africa where malaria burden is still the highest in the world [30]. The number of studies examining insecticide susceptibility and resistance mechanisms in *Anopheles* malaria vectors in African countries is growing rapidly. In Sudan, several previous and current studies to determine the susceptibility status of *An. arabiensis* to different agricultural pesticides and public health insecticides has been published [15,16,31,32]. Studies elucidated the distribution of resistance and susceptible strains of *An. arabiensis* to insecticides different parts of studies areas these include Kassala[15] and Gedarif States [32] in eastern Sudan, Gezira[16] and White Nile States [15] in Central Sudan and Khartoum State [15,32,33]. Likewise, mapping and distribution of insecticide resistance in malaria vectors in different African countries have been interviewed and published [13,24,29].

In this study, the variation in mortality rates in *An. arabiensis* due to insecticides between the three different seasons (dry cold, dry hot and wet) showed some differences especially for 4% DDT, 5% malathion and 0.1% propoxur. For example, in Khartoum State, 4% DDT and propoxur resistance was high with mortality rates of 87% and 83% respectively during the cold dry season. During the hot dry season, the mortality rates increased to 95% and 86% for 4% DDT and 0.1% propoxur respectively. The same trend is observed for 4% DDT in populations of Edekheinat and Shambat sites. However, for 5% malathion variation of mortality rates in *An. arabiensis* from Elmaygoma sites was higher in hot dry season. Although, there was variations in mortality rates in population of *An. arabiensis* from administrative areas or different land use (urban and periurban areas) due to 4% DDT and 5% malathion between the season, these differences did not follow fixed trends. However, seasonal variations in susceptibility of *An. arabiensis* to insecticides have been previously reported from Gizera States in Sudan (16). Likewise such variations have been observed in susceptibility of *Anopheles* mosquitoes to insecticides in different African countries (13,14,34).

Conclusion:

In conclusion, the results revealed that insecticide resistant strains *An. arabiensis* occurred in all investigated sentinel sites in Khartoum State. In addition, a clear difference was observed between the seasons in overall mean susceptibility status in *An. arabiensis* from the sentinel sites as well from urban and periurban areas.

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