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Short Report: Permethrin and DDT Resistance in the Malaria Vector *Anopheles arabiensis* from Eastern Sudan

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Abstract. Assessment of resistance to DDT and permethrin insecticides and molecular detection of knockdown resistance (*kdr*) alleles were conducted in three populations of *Anopheles arabiensis* from eastern Sudan. Bioassay mortalities ranged from 96.9% to 99.6% for 4% DDT and from 98.4% to 100% for 1% permethrin. The L1014F and L1014S alleles were detected in 25 of 498 mosquitoes. The overall *kdr* frequencies ranged from 7.0% in the area where insecticide-treated nets were used to 3.0% in the area with agricultural insecticide use. The presence of the *kdr* alleles in *An. arabiensis* in Sudan emphasizes the need to develop appropriate resistance monitoring and management strategies for *An. arabiensis*.

In Africa, resistance to pyrethroid insecticide in malaria vector mosquitoes may become a major problem for malaria interventions because pyrethroids are the mainstay of vector control strategies.[1] The first knockdown resistance (*kdr*) allele observed in *Anopheles gambiae* is caused by a leucine-phenylalanine substitution at position 1014 of the sodium channel gene.[2] This allele, termed L1014F, is widely spread in the S molecular form of *An. gambiae s.s.* in western Africa and has recently been observed in eastern Africa.[3] In the M form, it is thought to have arisen through introgression from the S form, but its occurrence is new and independent in *An. arabiensis*.[4,5] Another *kdr* allele, a serine replacement (L1014S) at the same position, was initially identified in eastern Africa and has been found in parts of central Africa.[6,7] This L1014S allele was observed recently in *An. arabiensis* from Kenya and Uganda, and the L1014F allele was observed at low frequency in *An. arabiensis* populations from Tanzania.[8–10] Apart from the detection of the L1014F allele in a laboratory colony from central Sudan,[11] all the previous observations showed that the *kdr* alleles were in a heterozygous state, but none was correlated with resistance phenotypes in *An. arabiensis*. We report the presence of both *kdr* alleles and their association with resistance phenotypes in three population samples of *An. arabiensis* from eastern Sudan.

The field collections were conducted in the cool dry season during November and December 2005. Anopheline larvae were collected from multiple larval habitats in each of the three following localities with different patterns of insecticide usage: 1) New Halfa (35°20′E, 15°34′N), an area where agricultural insecticides were used mainly for cotton pests, and indoor residual spraying (IRS) was the main method used for malaria vector control; 2) El-Girba, (35°57′E, 14°58′N), an area adjacent to the cotton area where IRS and larvicides were the major vector control methods; and 3) Kassala (36°26′E, 15°23′N), a horticultural area located along the valley of the El-Gash River, where larvicides and insecticide-treated nets (ITNs) were used for vector control.[12] DDT was used extensively for vector control and in agriculture in the 1960s when the New Halfa agricultural scheme was established. By the early 1980s, organophosphates and pyrethroids had replaced DDT.[13] The two classes of insecticides are the main compounds used currently for control of both the malaria vector and agricultural pests in the study area.

The field-collected anopheline larvae were transferred into an insectary, reared to adults, and identified to species by morphologic characteristics.[14] The insecticide bioassays for 4% DDT and 1% permethrin were performed on non-blood fed, female 1–3-day old adults using World Health Organization test tubes and protocols.[15] The mortalities at 24 hours post-exposure and the 50% and 90% knockdown time thresholds (KDT_{50} and KDT_{90}) are shown in Table 1. The mortalities ranged from 96.9% to 99.6% for 4% DDT and from 98.4% to 100% for 1% permethrin. For both insecticides, there was no significant difference in the mortalities among the three populations (For DDT, $\chi^2 = 3.83$, degrees of freedom [df] = 2, $P = 0.15$ for DDT and $\chi^2 = 2.38$, df = 2, $P = 0.30$ for permethrin). The 100% mortality against permethrin was found only in the cotton growing area of New Halfa. The DDT mortality in this area was similar to that observed by Himiedan and others,[16] which indicated no detectable in-

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TABLE 1

Bioassay mortalities and 50% and 90% knockdown time (in minutes) (KDT_{50} and KDT_{90}) of female *Anopheles arabiensis* in three populations from eastern Sudan

Insecticide	Population	No.	Mortalities %	KDT_{50} (95% confidence interval)	KDT_{90} (95% confidence interval)
4% DDT	New Halfa	245	97.6	16.2 (10.3–25.7)	33.4 (14.9–46.7)
	El-Girba	160	96.9	17.9 (14.4–21.2)	41.0 (33.3–57.3)
	Kassala	240	99.6	18.6 (6.4–30.0)	39.8 (14.9–72.5)
1% Permethrin	New Halfa	280	100	8.1 (7.3–8.8)	13.5 (12.9–14.4)
	El-Girba	160	99.4	9.5 (3.0–33.4)	16.2 (4.7–55.4)
	Kassala	240	98.4	9.4 (8.8–10.0)	15.0 (14.2–16.0)

TABLE 2

Frequencies of knockdown resistance (*kdr*) alleles, and *kdr* genotypes in relation to phenotypes determined by the permethrin-DDT resistance bioassay in three *Anopheles arabiensis* populations from eastern Sudan*

	Bioassay phenotype‡					
	New Halfa		El-Girba		Kassala	
kdr genotype†	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible
S S	0.75 (1)	99.25 (133)	1.80 (3)	98.20 (164)	4.65 (8)	95.35 (164)
S Rw	50.00 (2)	50.00 (2)	37.50 (3)	62.50 (5)	44.44 (4)	55.56 (5)
S Re	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	100.00 (4)
kdr allele frequency	2.90 (4)		4.57 (8)		7.03 (13)	

* Numbers in parentheses are the individuals of each geno-phenotype.
† S = susceptible allele; Rw = L1014F *kdr* allele; Re = L1014S *kdr* allele.
‡ Samples phenotyped as resistance or suseptible refers to permethrin and DDT bioassays results: the survived mosquitoes were termed resistant whereas those that died were susceptible.

crease in mosquito resistance over six years, the period between the two observations. However, the KDT_{50} and KDT_{90} estimated in the present study for both insecticides did not differ from those shown for a susceptible population of *An. arabiensis* in central Kenya.[17] The population of *An. arabiensis* in the New Halfa area showed no evidence of selection pressure from the pyrethroid insecticides used for control of cotton pests.

DNA was extracted individually from the bioassay-tested *An. arabiensis* mosquitoes, which were identified by the ribosomal DNA–polymerase chain reaction method.[18,19] *Anopheles arabiensis* was the only member of *An. gambiae* complex found in the study area, a finding that is consistent with previous cytogenetic results.[20] The L1014F and L1014S *kdr* alleles were screened in the three populations (n = 498 individuals) using a modified diagnostic method of Tripet and others[21] on a Li-Cor 4300 DNA Analyzer (Li-Cor, Lincoln, NE). A total of 25 individuals had *kdr* alleles, all as heterozygotes. The results were confirmed by DNA sequencing of ~300-baspair fragments amplified by the primers AgD_1 and AgD_2.[2]

The observed *kdr*-allele frequencies are shown in Table 2. The difference in the *kdr* allele frequencies was not significant among the three populations (7.0%, 4.6%, and 2.9% in Kassala, El-Girba, and New Halfa populations, respectively, $\chi^2 = 4.23$, df = 2, $P = 0.12$). Among the 25 *kdr* alleles observed in the three populations, 21 alleles (84.0%) had the L1014F mutation (western African *kdr* allele type) (Table 2). Four alleles (16.0%) had the L1014S mutation (eastern Africa *kdr* allele type), and the L1014S mutation was restricted in the Kassala population. Among 21 individuals with the resistance phenotype in the permethrin-DDT bioassays, 9 (42.9%) had *kdr* alleles. However, only 3.4% (16 of 477) of the bioassay-susceptible individuals had the *kdr* allele. This result showed a positive association between *kdr* allele frequency and bioassay-resistance phenotype in *An. arabiensis* ($\chi^2 = 55.4$, df = 1, $P < 0.001$).

The DDT resistance in *An. arabiensis* in Sudan was initially reported in our study area in the early 1970s.[22] The L1014F *kdr* frequency may be a consequence of DDT use in the 1960s. Recent pyrethroid-based vector control may have also selected for increased *kdr* frequency.[23] For example, a frequency of 7% was observed in Kassala where coverage of ITNs distributed by the National Malaria Control Program after the devastating floods in 2003 was high. Although the present study found low *kdr* allele frequencies in *An. arabiensis* in Sudan, there is concern about the spread of pyrethroid resistance because ITNs are now being used intensively and indoor residual spraying of DDT is being considered by the Ministry of Health of Sudan. Appearance of *kdr* alleles in *An. arabiensis* populations from Kenya,[8] Uganda,[9] Tanzania,[10] and Sudan emphasize the need to develop appropriate resistance monitoring and management strategies in *An. arabiensis*.

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