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Insecticide resistance in malaria vectors in central Sudan

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Anopheles arabiensis from Sudan: insecticide resistance and the West African *kdr* mutation

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Introduction

Malaria is by far the leading cause of morbidity and mortality in Sudan with 7.5 million cases and 35, 000 deaths recorded annually (Malik *et al*., 2006). *Anopheles arabiensis* is the only known malaria vector mosquito in northern and central Sudan. In the southern parts of Sudan, in addition to *Anopheles arabiensis* other vector species such as *An. gambiae* and *An. funestus* play an important role in the transmission of malaria. Chemical insecticides are the chief means of controlling the malaria vectors, mainly through indoor residual house spraying and recently with the use of insecticide-treated bednets (ITNs). Control is handicapped, however, by resistance to insecticides which is a growing problem in many African countries.

In Central Sudan, *An. arabiensis* has been reported resistant to several insecticides BHC, DDT and malathion (Haridi, 1972; Hemingway, 1983). In 2004 resistance to the pyrethroid permethrin was detected (unpublished data). Mechanisms conferring resistance to insecticides are either metabolic, i.e. detoxification or sequestration of the insecticides, or mutations which reduce sensitivity in the target sites of voltage gated sodium and chloride channel ions blocking the insecticidal activity. One target site mechanism, known as *kdr,* has been shown to confer resistance to pyrethroids and DDT in *An. gambiae* in West Africa (Martinez-Torres *et al.,* 1998) and another, in East Africa (Ranson *et al*., 2000).

To understand the current status and mechanisms of resistance, a study was initiated to identify the malaria vectors in Central Sudan, determine their susceptibility to the different classes of insecticides and the frequency and identity of *kdr* mutations in these populations.

Methods

Indoor resting wild mosquitoes were collected from 13 sites in Central Sudan from August 2005 to January 2006. The WHO standard assays were carried out on *An. gambiae* complex mosquitoes to determine insecticide susceptibility to pyrethroids (permethrin), organochlorines (DDT), organophosphates (malathion) and carbamates (bendiocarb). Mosquitoes were identified to species level using the species specific polymerase chain reaction assay (PCR) of Scott *et al.* (1993). The *kdr* mutation frequency was determined by assaying susceptible and resistant bioassay specimens (DDT/Permethrin) using the two-step PCR method described by Martinez-Torres *et al.* (1998) and Ranson *et al.* (2000).

Results

89% of 900 samples were identified as *An. arabiensis* (Fig. 1). The remainder did not amplify. The WHO susceptibility test results showed 100% mortality on bendiocarb; 54.6 - 94.2% on permethrin; 55.4 - 99.1% on DDT and 76.8 - 100% on malathion (Table 1).
The *kdr* analysis revealed the presence of the West African *kdr* mutation (Leu → Phe) in both bioassay susceptible and resistant specimens (Fig. 2). The East African *kdr* mutation (Leu → Ser) was not detected in these samples

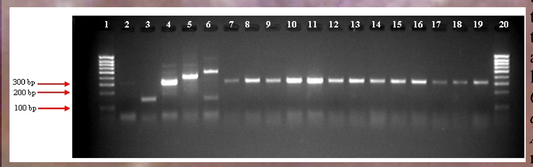

Fig. 1: Amplified fragments using the species-specific PCR assay for the identification of members of the *An. gambiae* complex. Lane 1 and 20: 1 kb molecular markers; lane 2: negative control; lanes 3 to 6 positive controls (*An. quadriannulatus*, *An. arabiensis*, *An. gambiae* and *An. merus* respectively); Lanes 7 to 19: wild caught *An. Arabiensis*.

Locality	Mortality %							
	Permethrin 0.75%		DDT 4%		Malathion 5%		Bendiocarb 0.1%	
	n	%	n	%	n	%	n	%
Elmaslamia	121	72.7	120	59.2	111	89.2	121	100
Tabat	121	76.03	82	84.1	82	76.8	125	100
El Hoosh	121	54.6	112	55.4	114	78.1	136	100
El Khoor	114	78.1	116	90.5	110	97.3	114	100
Medani	113	66.4	113	66.4	99	86.7	100	100
Mobi	119	72.3	146	83.6	80	86.3	127	100
Rofaa	105	85.7	88	83	100	97	126	100
Wad Rawa	116	58.6	120	60.8	126	89.7	129	100
El Managil	109	89.9	82	95.1	98	90.8	123	100
Sennar	102	92.2	107	93.5	97	100	107	100
El Suki	102	84	103	95.2	116	80.2	126	100
Singa	111	72	108	64.8	108	91.7	109	100
El Booster	103	94.2	111	99.1	100	99	100	100

Table 1: WHO standard susceptibility mortality results on 1-3 days old females of *An. arabiensis* from 13 different localities in Central Sudan. The values in red indicated resistance.

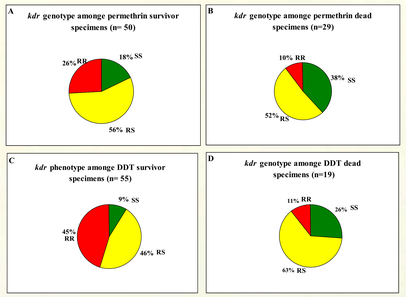

Fig 2: Survivors and dead mosquitoes from the WHO standard susceptibility tests (Table 1) were further analysed for the *kdr* mutation frequency. Figure 2A and B show *kdr* genotypes of permethrin survivor and dead mosquitoes, respectively. Figure 2C and D show *kdr* genotypes of DDT survivors and dead mosquitoes, respectively. In all cases the heterozygous (RS) genotype was in the majority.

Discussion

This study confirmed that transmission of malaria in this area of Sudan is by *An. arabiensis* as the only member of the *An. gambiae* complex present. The WHO susceptibility test results showed that *An. arabiensis* populations from central Sudan are fully susceptible to bendiocarb but show multiple resistance to permethrin, DDT and malathion.

The presence of the West African *kdr* mutation in both susceptible and resistant specimens showed no correlation between the resistance phenotype and the bioassay results, strongly suggesting that additional mechanisms are responsible for these high levels of resistance detected in this population. The lack of correlation between the *kdr* mutation and resistance phenotypes found in this study is contrary to a large body of evidence on the *An. gambiae* in West Africa (WHO, 2006). Although four other reports of the *kdr* mutation in *An. arabiensis* have been reported recently (Diabaté *et al.,* 2004; Stump *et al*., 2004; Verhaeghen *et al*., 2006; Kulkarni *et al*., 2006), all were from either with one or two specimens and none were correlated with bioassay data. This is the first report of high *kdr* frequencies in an *An. arabiensis* population that do not correlate with resistance phenotypes to either the pyrethroid permethrin or the organochlorine DDT. The lack of correlation has however been found in various mosquito species such as *Culex quinquefasciatus*, *C. Pipiens* and *An. gambiae* (Liu *et al*., 2006). Further, the lack of correlation between the presence of the Leu → Phe *kdr* mutation and the resistance phenotype in these populations undermines the use of the two step PCR assay to determine susceptibility.

These findings have serious implications for the malaria control programmes in Gezira and Sennar states of Sudan since they depend largely on the use of pyrethroid insecticides for indoor residual spraying and for insecticide-treated bednets. At present only pyrethroids are used in the treating of bednets. It is not known, however, how this finding will affect the impact of ITNs since kill is not the only method of protection with this strategy. For indoor residual spraying, bendiocarb (carbamates) could be used as a possible alternative. Issues of cost and appropriate application by spray teams would have to be taken into consideration. Furthermore, the rotational use of insecticides should be part of a resistance management strategy.

Acknowledgments
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