Insecticide resistance in the malaria vector *Anopheles arabiensis* in Mamfene, KwaZulu-Natal

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The control of malaria vector mosquitoes in South Africa's affected provinces is primarily based on indoor spraying of long-lasting residual insecticides. The primary vectors in South Africa are *Anopheles arabiensis* and *An. funestus*. South Africa's National Malaria Control Programme has adopted a malaria elimination agenda and has scaled up vector control activities accordingly. However, despite these plans, local transmission continues and is most likely because of outdoor feeding by populations of *An. arabiensis*. An outdoor *Anopheles* surveillance system has been set up in three sections of the Mamfene district in northern KwaZulu-Natal in order to assess the extent of outdoor resting *An. arabiensis* in Mamfene and to assess the current insecticide susceptibility status of this population. According to WHO criteria, the *An. arabiensis* samples tested showed evidence of resistance to deltamethrin (pyrethroid), DDT (organochlorine) and bendiocarb (carbamate), and full susceptibility to the organophosphates pirimiphos-methyl and fenitrothion. Pre-exposure to piperonyl butoxide completely nullified the deltamethrin resistance otherwise evident in these samples, supporting previous studies implicating monooxygenase-based detoxification as the primary mechanism of pyrethroid resistance. The data presented here affirm the presence of pyrethroid and DDT resistance previously detected in this population and also indicate the comparatively recent emergence of resistance to the carbamate insecticide bendiocarb. These data show that special attention and commitment needs to be given to the principles of insecticide resistance management as well as to investigations into alternative control techniques designed to target outdoor-resting *An. arabiensis* in northern KwaZulu-Natal.

Introduction

The control of malaria vector mosquitoes in South Africa's affected provinces is primarily based on indoor spraying of long-lasting residual insecticides.[1] The indoor residual spraying (IRS) method has been the mainstay of malaria vector control in South Africa since the late 1940s and has remained effective owing to carefully co-ordinated IRS programmes in South Africa's Limpopo, Mpumalanga and KwaZulu-Natal Provinces.[2]

Only *Anopheles* mosquitoes can transmit human malaria parasites and the primary vectors in South Africa are *Anopheles arabiensis* and *An. funestus*.[1] Of these, *An. funestus* is almost entirely anthropophilic (human biting), endophagic (indoor feeding) and endophilic (indoor resting).[3] These characteristics make this species especially susceptible to control by IRS, assuming that the insecticide employed for this purpose is effective against the target *An. funestus* population. Control by IRS means that the mosquitoes must retain complete or near complete susceptibility to the insecticide class being used, which can only be ascertained by regular monitoring and surveillance. The malaria epidemic experienced in South Africa during the period 1996–2000 was largely the result of the development of resistance to pyrethroid insecticides in populations of this species in northern KwaZulu-Natal and Mpumalanga which led to vector control failure.[1,2] Control was re-established using a mosaic resistance management system which was later drafted into a World Health Organization (WHO) document –- the Global Plan for Insecticide Resistance Management (GPIRM).[4] South Africa currently subscribes to the principles outlined in GPIRM as part of its malaria elimination agenda.[5] However, despite these plans and the scaling up of vector control activities in South Africa, local transmission continues, most likely because of outdoor transmission by populations of *An. arabiensis*. Unlike *An. funestus*, *An. arabiensis* has evolved substantial behavioural plasticity and will feed and rest indoors and outdoors, and will feed on humans as well as livestock, especially bovines.[3] *Anopheles arabiensis* is therefore substantially less susceptible to control by IRS.

Recently, a project was launched to assess the feasibility of the sterile insect technique for malaria vector control in South Africa, with special emphasis on controlling outdoor transmission by *An. arabiensis*.[6] As part of the baseline survey linked to this project, an outdoor *Anopheles* surveillance system has been set up in three sections of the Mamfene district in northern KwaZulu-Natal. This surveillance system has enabled recent assessments of insecticide resistance in outdoor-resting *An. arabiensis* in Mamfene as a follow-up to the discovery of pyrethroid resistance in this region in 2005.[7]

Methods

In order to assess the current insecticide susceptibility status of outdoor resting *An. arabiensis* in Mamfene, adult *Anopheles* mosquitoes were collected from outdoor-placed ceramic pots[8] and modified plastic buckets deployed in 20 households in Mamfene Sections 2, 8 and 9 during March and April 2015. These collections were transported live to the Botha De Meillon insectary facility at the National Institute for Communicable Diseases (NICD) in Johannesburg. Blood-fed female specimens were individually placed in egg-laying vials so that eggs could be harvested and reared by family. All wild-caught female individuals, including those that produced eggs, were identified to species group using morphological keys[9,10] and to species by standard PCR[11]. A total of 35 families identified as *An. arabiensis* was pooled and the F1 progeny were reared to adults under standard insectary conditions of 25 °C and 80% relative humidity.[12]

Samples of 2–5-day-old female adult F1 progeny were assessed for their susceptibility to diagnostic concentrations of a range of insecticides according to the standard WHO bioassay method.[13] Controls included samples of 2–4-day-old F1 male adults exposed to untreated papers. In addition, a subset of samples was used to assess the effect of pre-exposure to the insecticide synergist piperonyl butoxide (PBO) on the expression of pyrethroid resistance according to a method previously described by Brooke et al.[14]

Results and discussion

According to WHO criteria,[13] the F1 *An. arabiensis* samples tested showed evidence of resistance to deltamethrin (pyrethroid), DDT (organochlorine) and bendiocarb (carbamate), and full susceptibility to the organophosphates pirimiphos-methyl and fenitrothion (Table 1). Pre-exposure to PBO completely nullified the deltamethrin resistance otherwise evident in these samples (paired sample *t*-test: d.f. = 1, $t = 15.65$, $p = 0.04$) (Table 2).

The first assessments of resistance in *An. arabiensis* at Mamfene were conducted in 1996 and no resistance phenotypes were recorded.[15] However, subsequent samples collected in 2002 indicated the emergence of resistance to DDT[16] which was again recorded in 2005 together with the first indication of pyrethroid resistance[7]. The 2015 data presented here affirm the presence of pyrethroid and DDT resistance in this population, albeit at a low frequency, and also indicate the comparatively recent emergence of resistance to the carbamate insecticide bendiocarb. The PBO exposure data support previous analyses implicating monooxygenase-mediated detoxification as the primary mode of pyrethroid resistance in *An. arabiensis* at Mamfene,[7,17,18] because PBO enhances insecticide toxicity by providing an alternative substrate for monooxygenases. PBO pre-exposure assays can therefore be used to elucidate monooxygenase-based resistance mechanisms.

Conclusion

South Africa's 1996 to 2000 malaria epidemic illustrates the effect that a single insecticide resistance phenotype (pyrethroid resistance in *An. funestus*) can have on an IRS-based vector control programme.[2,19,20] The occurrence of multiple vector species carrying multiple resistance mechanisms coupled to ongoing outdoor transmission in northern KwaZulu-Natal means that special attention and commitment needs to be given to the principles of insecticide resistance management as outlined in the GPIRM document[4] as well as to investigations into alternative control techniques designed to target outdoor-resting *An. arabiensis*.

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Authors' contributions

B.D.B. assisted with data analysis and interpretation and produced the manuscript. L.R., N.V. and O.R.W. assisted with specimen collection, species identification and experimental procedures. M.L.K. and G.M. assisted with the experimental procedures and data analysis. E.R. assisted with data interpretation and the drafting of the manuscript. L.L.K. conceived the project, assisted with experimental procedures, data analysis and interpretation and the drafting of the manuscript. All authors read and approved the final draft of the manuscript.

Table 1: Mean percentage mortalities recorded for samples of 2–5-day-old F1 *Anopheles arabiensis* female adults following exposure to listed insecticides by class. Mortalities were recorded 24 h post exposure. The number of replicates, sample size (*n*) and standard error (s.e.) are given for each insecticide treatment. Samples by treatment are categorised as resistant (R) or susceptible (S) according to standard criteria.[13] The control was exposure of 2–4-day-old F1 *Anopheles arabiensis* male adults to untreated papers.

Insecticide (concentration)	Insecticide class	Number of replicates	*n*	Mean % mortality	s.e.	Resistance or susceptibility
Deltamethrin (0.05%)	Pyrethroid	8	191	87.21	4.63	R
DDT (4%)	Organochlorine	7	140	83.85	7.32	R
Bendiocarb (0.1%)	Carbamate	7	145	94.1	2.8	R
Pirimiphos-methyl (0.25%)	Organophosphate	2	45	100	–	S
Fenitrothion (1%)	Organophosphate	2	44	100	–	S
Control	–	10	234	2.74	1.6	–

All exposures were of 1 h duration except for fenitrothion for which there was a 2-h exposure

Table 2: Mean percentage mortalities recorded for samples of 2–6-day-old F1 *Anopheles arabiensis* male adults (M) or female adults (F) following exposure to either deltamethrin (0.05%), piperonyl butoxide (PBO) (4%), deltamethrin + PBO or untreated control papers. Mortalities were recorded 24 h post exposure. The number of replicates, sample size (*n*) and standard error (s.e.) are given for each treatment. Mortalities following exposure to PBO and control papers were negligible. There is a significant difference in mean mortality between the deltamethrin and deltamethrin + PBO treatments 24 h post exposure (paired sample *t*-test: d.f.=1, $t=15.65$, $p=0.04$).

Treatment	Sex	Number of replicates	*n*	Mean % mortality	s.e.
Deltamethrin	F	2	47	78.64	1.37
PBO	M	2	46	6.44	1.89
Deltamethrin + PBO	F	2	49	100	–
Control	M	4	90	5.49	3.35

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