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DATES:
Received: 15 July 2021
Revised: 16 Sep. 2021
Accepted: 05 Nov. 2021
Published: 29 Mar. 2022

HOW TO CITE:
Munhenga G, Oliver SV, Lobb LN, Mazarire TT, Sekgele W, Mashatola T, et al. Malaria risk and receptivity: Continuing development of insecticide resistance in the major malaria vector *Anopheles arabiensis* in northern KwaZulu-Natal, South Africa. S Afr J Sci. 2022;118(3/4), Art. #11755. https://doi.org/10.17159/sajs.2022/11755

ARTICLE INCLUDES:
☒ Peer review
☐ Supplementary material

DATA AVAILABILITY:
☐ Open data set
☒ All data included
☐ On request from author(s)
☐ Not available
☐ Not applicable

EDITORS:
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KEYWORDS:
malaria, vector control, risk and receptivity, malaria elimination

Malaria risk and receptivity: Continuing development of insecticide resistance in the major malaria vector *Anopheles arabiensis* in northern KwaZulu-Natal, South Africa

Malaria incidence in South Africa is highest in the three endemic provinces: KwaZulu-Natal, Mpumalanga and Limpopo. The contribution to malaria transmission by several mosquito species, variation in their resting behaviours and low levels of insecticide resistance makes it necessary to periodically monitor *Anopheles* species assemblages and resistance phenotypes in vector populations. The aim of this study was therefore to assess *Anopheles* species assemblage in northern KwaZulu-Natal and to collect insecticide susceptibility data for *An. arabiensis*, the primary vector of malaria in that province. *Anopheles* specimens were collected from Mamfene, Jozini, northern KwaZulu-Natal from November 2019 to April 2021. Progeny of wild-collected *An. arabiensis* females were used for standard insecticide susceptibility tests and synergist bioassays. *Anopheles arabiensis* contributed 85.6% (n=11 062) of the total catches. Samples for subsequent insecticide susceptibility bioassays were selected from 212 *An. arabiensis* families. These showed low-level resistance to DDT, permethrin, deltamethrin, and bendiocarb, as well as full susceptibility to pirimiphos-methyl. Synergist bioassays using piperonyl butoxide and triphenyl phosphate suggest oxygenase-based pyrethroid and esterase-mediated sequestration of bendiocarb. These low levels of resistance are unlikely to be operationally significant at present. It is concluded that northern KwaZulu-Natal Province remains receptive to malaria transmission despite ongoing control and elimination interventions. This is due to the perennial presence of the major vector *An. arabiensis* and other secondary vector species. The continued detection of low-frequency insecticide resistance phenotypes in *An. arabiensis* is cause for concern and requires periodic monitoring for changes in resistance frequency and intensity.

Significance:

- Insecticide resistance in the major malaria vector *Anopheles arabiensis* in northern KwaZulu-Natal Province is cause for concern in terms of resistance management and ongoing vector control leading toward malaria elimination.
- Despite ongoing control interventions, northern KwaZulu-Natal remains receptive to malaria owing to the perennial presence of several *Anopheles* vector species.

Introduction

South Africa's malaria-endemic provinces are KwaZulu-Natal, Mpumalanga, Limpopo and, to a far lesser extent, the North West. The incidence of locally acquired malaria is generally highest in those regions bordering southern Mozambique, eSwatini, Zimbabwe, and Botswana. Malaria vector control in the context of scaling up toward elimination is conducted annually in affected districts/municipalities in all of these provinces with the exception of the North West Province (as the incidence is extremely low). The primary methods of control include indoor residual spraying (IRS) of specially formulated insecticides, and larval source management.[1]

The human malarias are transmitted by *Anopheles* mosquitoes. To date, five *Anopheles* species have been directly implicated in the transmission of the malarial parasite *Plasmodium falciparum* in South Africa; these are the major vectors *Anopheles funestus* Giles, and *An. arabiensis* Patton, and the secondary vectors *Anopheles merus*, *Anopheles vaneedeni* and *Anopheles parensis*.[2-5] Populations of *An. arabiensis*[3,6,7], *An. merus*[3,8] and *An. parensis*[5,9,10] may include indoor- and outdoor-resting components; female *An. funestus* have a strong but not exclusive tendency to rest indoors[2,9,10] and *An. vaneedeni* tend to rest outdoors[11]. By targeting indoor-resting *Anopheles* mosquitoes, IRS-based vector control has reduced malaria incidence in South Africa to a point where elimination (i.e. zero locally acquired malaria cases) is a feasible prospect.[12] Yet despite the pro-active implementation of vector control/elimination operations year-on-year, local transmission persists at low levels in several districts and municipalities across the endemic provinces. This persistence can be attributed to several factors, one of which is the occurrence of outdoor-resting vector mosquitoes that are far less vulnerable to IRS. Another critical factor is the development of resistance to insecticides.

High-intensity resistance to pyrethroid insecticides was first recorded in southern African populations of *An. funestus* in 1999.[13] This phenotype caused substantial control failure in South Africa during the malaria epidemic of 1996–2000. The re-introduction of DDT for malaria vector control in South Africa in 2000, played a crucial role in substantially reducing incidence because the pyrethroid-resistant *An. funestus* populations retained full susceptibility to DDT.[14,15] Current control operations in South Africa include the concurrent use of deltamethrin (pyrethroid) and DDT in a mosaic approach designed to manage insecticide resistance in *An. funestus* and maintain

FUNDING:
Bill and Melinda Gates Foundation (OPP1210314); International Atomic Energy Agency Technical Cooperation Programme (SAF 5014/5017, research contract 19099); South African National Research Foundation (119765, 107428); Department of Science and Innovation Health Innovation Scheme; National Health Laboratory Service Research Trust

control efficacy. This resistance management strategy is, however, under constant review because low-level resistance to pyrethroid, DDT, and carbamate insecticides has since been recorded in *An. arabiensis* populations in northern KwaZulu-Natal Province.[16] Although these phenotypes have been detected in *An. arabiensis*, they have been of low intensity and frequency and are therefore not considered to be operationally significant at present.[17]

The contribution to malaria transmission by several vector species and variation in their resting behaviours makes it necessary to periodically monitor *Anopheles* species assemblages in endemic areas, especially in terms of malaria risk and receptivity. Additionally, and given that low-level resistance is likely to increase in intensity and frequency under selection pressure imposed by insecticide use, it is necessary to periodically monitor for resistance phenotypes in vector populations. The aim of this study was therefore to assess *Anopheles* species assemblage in northern KwaZulu-Natal Province and to collect insecticide susceptibility data for *An. arabiensis*, the primary vector of malaria there.

Materials and methods

Anopheles mosquito specimens were collected from Mamfene in the Jozini municipality of northern KwaZulu-Natal Province. Collections were made from three sites: Section 2 (S 27°24'14.2"; E 32°12'41.8"), Section 8 (S 27°27'34.3"; E 32°10'43.7"), and Section 9 (S 27°23'50.5"; E 32°12'20.1"). Collections took place from November 2019 to April 2021. Adult female mosquitoes were collected from permanently stationed clay pots. A total of 56 clay pots were deployed up to and including 12 October 2020, following which 116 were deployed in Sections 2 (n=39), 8 (n=37), and 9 (n=40) (Figure 1). Each pot was sampled twice per week (i.e. 8 times/month) during the surveillance period. Mosquitoes were also sampled from disused vehicle tyres (n=6) and drums (n=1) from Section 9, modified plastic buckets from Section 2 (n=3), carbon dioxide baited net traps on two occasions in each of the three sections, and direct aspiration of mosquitoes resting at cattle kraals, in a few instances.

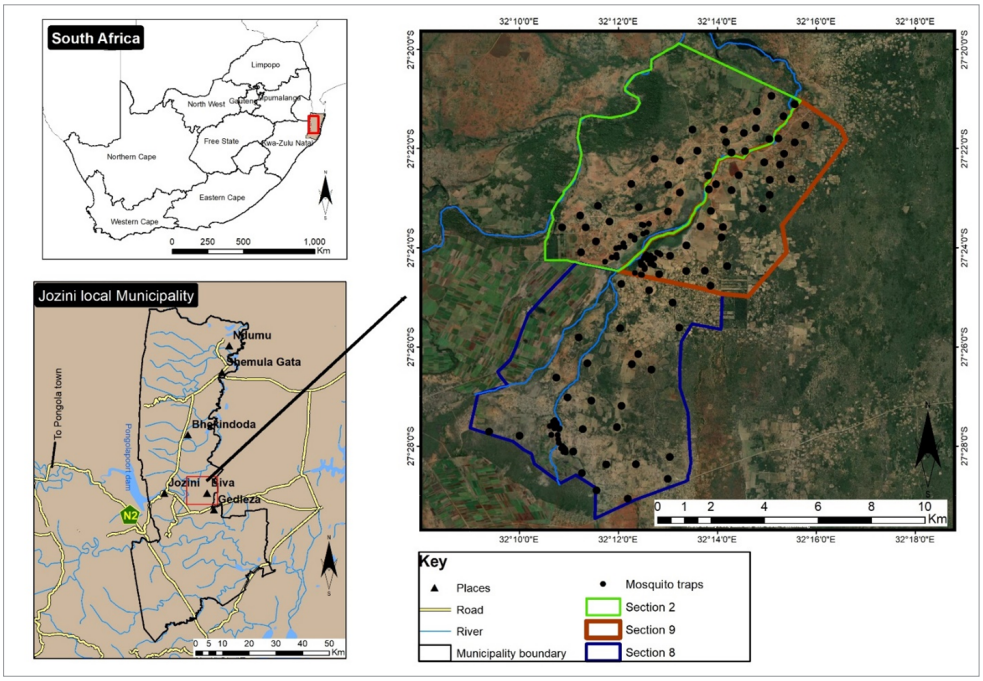

Figure 1: Map of Jozini municipality, northern KwaZulu–Natal Province, South Africa, showing sampling points from which anopheline mosquitoes were collected monthly between November 2019 and April 2021.

Specimens were identified to species, species complex or group in the field using dichotomous keys.[11] Live female mosquitoes identified as members of the *An. gambiae* complex were used to establish isofemale lines in the Botha De Meillon insectary, National Institute for Communicable Diseases, Johannesburg. After the first egg batch, the female adult was killed and preserved for subsequent identification by a standardised multiplex polymerase chain reaction.[18] Based on these species identifications, *An. arabiensis* F_1 larvae were pooled according to collection site. Larvae were reared according to standard procedures[19], i.e. rearing at 25 °C (±2 °C) and 80% relative humidity (±5%) with a 12:12 hour photoperiod and 30-min dawn/dusk cycles. The F_1 adults were maintained with ad libitum access to 10% sucrose until used for insecticide susceptibility assays.[20] These were performed on non-blood fed adults aged 3–5 days. F_1 *An. arabiensis* adults were assayed against 4% DDT, 0.75% permethrin, 0.05% deltamethrin, 0.1% bendiocarb, and 0.25% pirimiphos-methyl.[20]

Synergist bioassays were performed using synergist-impregnated papers that were produced in-house. Papers were impregnated with 4% piperonyl butoxide (PBO), a cytochrome P450 synergist, or 20% triphenyl phosphate (TPP), a general esterase synergist (Sigma Aldrich St. Louis, MO, USA). Treatment samples were exposed to one of the synergists for 60 min followed by exposure to an insecticide for 60 min (either 0.75% permethrin or 0.1% bendiocarb) according to the World Health Organization (WHO) standardised protocol.[20] Control samples were exposed to insecticide only, or to untreated papers. Adults were allowed access to 10% sucrose, ad libitum, and mortality was scored 24-h post-exposure.

Table 1: *Anopheles* mosquitoes sampled from Mamfene, northern KwaZulu-Natal, South Africa, between November 2019 and April 2021, stratified by section, year of collection, season and species

	Year of collection and total number of anophelines collected, N (relative abundance %/variable)			Total (% of total collected)
	Nov – Dec 2019	Jan – Dec 2020	Jan – April 2021	
Section of collection				
Section 2	94 (5.7%)	1282 (78.3%)	262 (16.0%)	**1638 (12.7%)**
Section 8	141 (7.9%)	1334 (75.0%)	303 (17.1%)	**1778 (13.8%)**
Section 9	497 (5.2%)	6848 (72.1%)	2158 (22.7%)	**9503 (73.5%)**
Total	**732 (5.7%)**	**9464 (73.3%)**	**2723 (21.1%)**	**12919**
Collection method used				
Carbon dioxide baited tent	0	33 (94.3%)	2 (5.8%)	**35 (0.3%)**
Clay pot	408 (5.4%)	5534 (73.4%)	1594 (21.2%)	**7536 (58.3%)**
Miscellaneous	50 (7.0%)	487 (68.0%)	179 (25.0%)	**716 (5.5%)**
Modified buckets	30 (9.4%)	276 (86.2%)	14 (4.4%)	**320 (2.5%)**
Disused tyres	244 (5.7%)	3134 (72.7%)	934 (21.6%)	**4312 (33.4%)**
Season of collection				
Autumn	0	2367 (65.0%)	1273 (35.0%)	**3640 (28.2%)**
Spring	576 (17.8%)	2651 (82.2%)	0	**3227(25.0%)**
Summer	156 (4.1%)	2208 (57.9%)	1450 (38.0%)	**3814 (29.5%)**
Winter	0	2238 (100%)	0	**2238 (17.3%)**
Species collected				
An. arabiensis	570 (5.2%)	8559 (77.4%)	1933 (17.4%)	**11062 (85.6%)**
An. coustani	4 (7.8%)	36 (70.6%)	11 (21.6%)	**51 (4.0%)**
An. demeilloni	0	9 (100%)	0	**9 (0.1%)**
An. leesoni	1 (5.8%)	8 (47.1%)	8 (47.1%)	**17 (0.1%)**
An. maculipalpis	0	9 (69.2%)	4 (30.8%)	**13 (0.1%)**
An. marshallii group	12 (25.0%)	36 (75.0%)	0	**48 (0.4%)**
An. merus	9 (7.1%)	102 (80.3%)	16 (12.6%)	**127 (1.0%)**
An. parensis	1 (0.2%)	81 (13.0%)	539 (86.8%)	**621 (4.8%)**
An. pharoensis	0	9 (75.0%)	3 (25.0%)	**12 (0.1%)**
An. pretoriensis	5 (12.5%)	33 (82.5%)	2 (5.0%)	**40 (0.3%)**
An. quadriannulatus	1 (50.0%)	1 (50.0%)	0	**2 (0.01%)**
An. rivulorum	0	19 (24.4%)	59 (75.6%)	**78 (0.6%)**
An. rufipes	18 (7.9%)	181 (79.4%)	29 (12.7%)	**228 (1.8%)**
An. squamosus	0	1 (50.0%)	1 (50.0%)	**2 (0.01%)**
An. vaneedeni	3 (3.3%)	43 (47.3%)	45 (49.4%)	**91 (0.7%)**
An. ziemanni	0	1 (100%)	0	**1 (0.01%)**
Not identified to species	108 (20.9%)	336 (65.0%)	73 (14.1%)	**517 (4.0%)**

Data were tested for normality using a Shapiro–Wilk test.[21] As the data were not normally distributed, a Kruskal–Wallis one-way analysis of variance (ANOVA) was used to determine differences between final mortality means.[22] For two-sample tests, a Mann–Whitney U-test was performed.[23]

Ethical approval

The Faculty of Health Sciences Research Ethics Committee of the University of the Witwatersrand (CR 20200218-10/ AREC-101210-002) and KwaZulu-Natal Health Research and Knowledge Management (KZ_202003_016) granted ethical approval. All household owners gave verbal consent to sample mosquitoes from their households.

Results

Species assemblage

In total, 12 919 anophelines were collected during the sampling period. Of these, 5.6% (n=732) were collected between November and December 2019, 73.3% (n=9464) were collected in 2020, and 21.1% (n=2723) were collected between January and April 2021 (Table 1). Most specimens (73.6%; n=9503) were collected from Section 9, while Section 2 was the least productive (n=1638). The largest number of mosquitoes was collected in summer (29.5%, n=3814) and the least in the winter months (17.3%, n=2238). Stratification of mosquito collections by method shows that clay pots (58.3%; n=7536) were the most productive, most likely because they were used more intensively than the other methods, and carbon dioxide baited net traps were the least productive (10.3%, n=35).

In total, 16 *Anopheles* species were collected over the sampling period. These included three members from the *An. gambiae* complex (*An. arabiensis*, *An. merus,* and *An. quadriannulatus*), two members from the *An. funestus* subgroup (*An. vaneedeni* and *An. parensis*), and one member each from the *An. minimus* subgroup (*An. leesoni*) and the *An. rivulorum* subgroup (*An. rivulorum* s.s). Stratification of species collected by section showed that some species were limited in their geographical range – *An. maculipalpis* was limited to Sections 2 and 9 while *An. quadriannulatus* was exclusively sampled from Section 9, and *An. squamosus* and *An. ziemanni* were limited to Sections 2 and 8, respectively. A total of 521 specimens could not be identified to species.

Anopheles arabiensis was the predominant species collected, contributing 85.6% (n=11 062) of the total. The population density of *An. arabiensis* (number caught/trap/month) shows a cyclical pattern with no discernible trend (Figure 2). Overall, Section 9 had the highest mean *An. arabiensis* density of 3.1/trap/month compared to that of Section 2 (1.1/trap/month) and Section 8 (0.9/trap/month). There were major peaks in *An. arabiensis* density in January 2020 (8.5 mosquitoes/trap/month), May and June 2020 (8.1 mosquitoes/trap/month) and September 2020 (5.1 mosquitoes/trap/month), all of which occurred in Section 9. The lowest mean number of mosquitoes caught per trap occurred in January 2021. No collections were conducted during April 2020 owing to COVID-19 restrictions.

Insecticide susceptibility tests

A total of 212 *An. Arabiensis* families were used for WHO susceptibility studies. According to the standardised method of interpreting insecticide susceptibility data[20], female and male *An. Arabiensis* F_1 samples showed signs of resistance to DDT, deltamethrin, permethrin and bendiocarb, and full susceptibility to pirimiphos-methyl. These results were consistent across all three sections of Mamfene (Table 2 and Figure 3). There was no significant difference in DDT-induced mortality (Kruskal–Wallis ANOVA: p=0.63, $F_{(2,37)}$=0.47, X^2=0.97), deltamethrin-induced mortality (p=0.64, $F_{(2,35)}$=0.45, X^2=4.47) or bendiocarb-induced mortality (p=0.10, $F_{(2,33)}$=2.43, X^2=4.47) between the Mamfene sections. There was, however, a significant difference in permethrin-induced mortality (p<0.01, $F_{(2,25)}$=6.34, X^2=8.89), with Section 2 showing the lowest mortality, followed by Sections 9 and 8 (Figure 3).

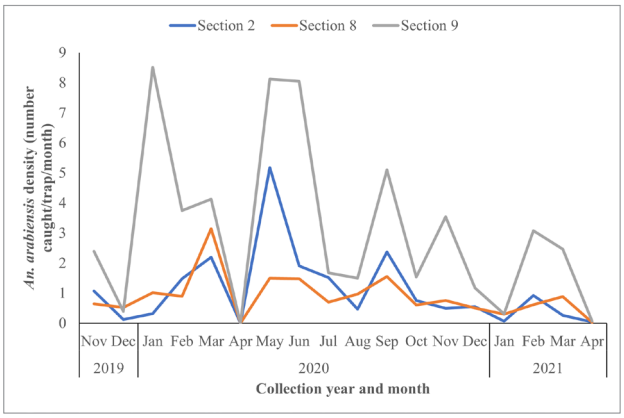

Figure 2: Mean number of *Anopheles arabiensis* collected per clay pot/month from Mamfene, northern KwaZulu-Natal, South Africa, November 2019 to April 2021, stratified by section.

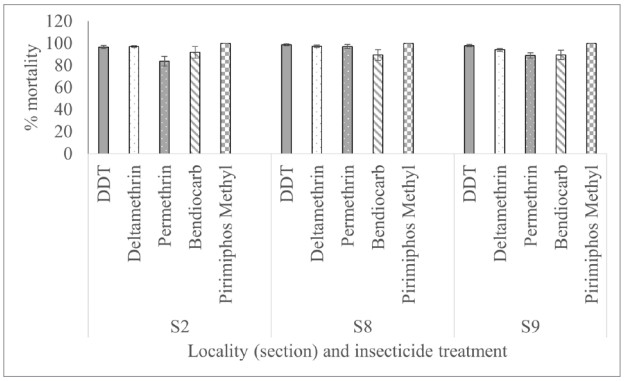

Figure 3: Insecticide susceptibilities of F1 *Anopheles arabiensis* derived from wild-collected material from Mamfene, northern KwaZulu-Natal Province, South Africa, 2019–2021. Mean mortalities (%) and standard errors are shown for each insecticide by Mamfene section (S2, S8, and S9).

Pre-exposure to the P450 synergist PBO caused a significant increase in permethrin-induced mortality in Sections 2 and 9, but not in bendiocarb-induced mortality in any of the sections. Pre-exposure to the general esterase synergist TPP also caused a significant increase in permethrin-induced mortality in all sections, and in bendiocarb-induced mortality in Sections 8 and 9 (Table 3). Median permethrin-induced mortality was significantly higher after PBO treatment (Mann–Whitney U=3, p=0.01, two-tailed) as well as after TPP treatment (Mann–Whitney U=0, p<0.01, two-tailed). PBO treatment did not result in a significant difference in bendiocarb-induced mortality (Mann–Whitney U=6, p=0.24, two-tailed). TPP treatment did, however, result in a significant increase in bendiocarb-induced mortality (Mann–Whitney U=2, p=0.03, two-tailed).

Discussion

Malaria vector surveillance in an elimination setting is specifically designed to collect information on a set of essential indicators – the most important being susceptibility to insecticides in those *Anopheles* populations implicated in disease transmission. Also important, therefore, are data on *Anopheles* species assemblages that can be used to assess malaria risk and receptivity, and to indicate which populations need to be prioritised for insecticide susceptibility assessments. This study presents a comprehensive survey of anopheline mosquitoes in northern KwaZulu-Natal Province and the most recent data on insecticide resistance in *An. arabiensis*, the primary vector of malaria there.

During the sampling period, 16 *Anopheles* species were collected. This level of diversity is comparable to a similar cross-seasonal anopheline survey conducted in the northern Kruger National Park where 9

Anopheles species were collected[24], and in the Limpopo Province where 20 species were collected[25]. *Anopheles arabiensis* was the most abundant member of the *An. gambiae* complex while *An. parensis* predominated in collections of the *An. funestus* group. The high density of *An. arabiensis* observed in this survey tallies with previous studies conducted between 2014 and 2015.[3] However, there was a notable difference in seasonal distribution between this study and a previous survey. The data presented here show higher numbers of *An. arabiensis* sampled during the winter months compared to the previous survey.[3] This could be due to uninterrupted mosquito surveillance throughout the year, although surveillance was scaled down during winter and no sampling was conducted in April 2020 due to COVID-19 restrictions.

Table 2: Combined insecticide susceptibilities of male and female F_1 *Anopheles arabiensis* derived from wild-collected material from three sections of Mamfene, northern KwaZulu-Natal Province, South Africa, 2019–2021. Mean percentage mortalities (%), standard errors (s.e.) and sample sizes (*n*) are given by sex.

Insecticide (class)	Number tested		24-h post-exposure % mortality ± s.e.		Susceptibility level
	Females	Males	Females	Males	
Pirimiphos-methyl (TP)	103	105	100 ± 0	100 ± 0	S
DDT (OC)	102	103	96.38 ± 1.55	98.07 ± 1.11	R
Deltamethrin (PYII)	121	113	92.6 ± 0.48	98.61 ± 0.39	R
Permethrin (PYI)	101	106	80.93 ± 4.33	94.52 ± 2.28	R
Bendiocarb (Carb)	117	114	72.1 ± 3.83	73.79 ± 6.21	R

TP, triphosphate; OC, organochlorine; PYII, pyrethroid class II; PYI, pyrethroid class I; Carb, carbamate; R, resistant; S, susceptible

Table 3: Insecticide (permethrin and bendiocarb) susceptibilities of F1 *Anopheles arabiensis* with or without pre-exposure to the P450 synergist piperonyl butoxide (PBO) or the general esterase synergist triphenyl phosphate (TPP). Mean mortalities (%), standard errors (s.e.) and sample sizes (*n*) are given by Mamfene section (S2, S8, and S9), northern KwaZulu-Natal Province, South Africa, 2019–2021

Treatment	S2	S8	S9
	% mortality ± s.e. (*n*)	% mortality± s.e. (*n*)	% mortality ± s.e. (*n*)
Permethrin	81.5 ± 1.76 (106)	95.24 ± 2.58 (112)	82.25 ± 1.82 (143)
Permethrin+PBO	98.96 ± 1.04 (100)	95.9 ± 1.36 (128)	96.36 ± 3.64 (122)
Permethrin+TPP	92.61 ± 3.08 (86)	98.68 ± 1.32 (91)	100 ± 0 (126)
Bendiocarb	91.88 ± 8.54 (113)	89.38 ± 0.53 (107)	84.67 ± 1.54 (130)
Bendiocarb+PBO	95.35 ± 2.69 (96)	95.48 ± 1.87 (93)	90.51 ± 9.48 (103)
Bendiocarb+TPP	100 ± 0 (81)	100 ± 0 (100)	96,74 ± 3.26 (101)

An interesting observation was the difference in trap productivity. Clay pots collected relatively high numbers of mosquitoes, re-emphasising their effectiveness as an *Anopheles* collection method. It is also notable that despite having access to only six disused tyres, these tyres collected over a third of the total collection, showing their potential as a sampling tool.

The perennial presence of the major vector *An. arabiensis* in northern KwaZulu-Natal indicates a high level of risk and receptivity to malaria. This receptivity is reinforced by the presence of secondary vectors such as *An. vaneedeni*, *An. parensis,* and *An. merus*, as well as several other *Anopheles* species that may also contribute to transmission, although none of the other species listed here have been directly implicated in malaria transmission in South Africa. Despite this high level of receptivity, malaria incidence in northern KwaZulu-Natal Province is currently very low, because of a scarcity of *Plasmodium* parasites for transmission, as a result of the IRS-based vector control programme and a well-developed case management system that includes active case detection in response to incidences of local transmission.

The continued presence of *An. arabiensis* in northern KwaZulu-Natal Province, despite a long history of IRS, may be attributable to the variable resting and feeding behaviours recorded for this species.[6,7,26] Female *An. arabiensis* will take blood meals from humans, livestock animals (especially cattle), and game animals such as buffalo. An important indicator of variability is also rooted in the methods used to collect samples of this species. Although *An. arabiensis* has been collected indoors (and outdoors) at other localities such as Tanzania[26], Ethiopia[6] and Malawi[7], all of the *Plasmodium*-infective *An. arabiensis* specimens collected in South Africa to date were found in outdoor-placed traps.[3,4] We do not know whether these specimens acquired their human blood meals indoors or outdoors, but their inclination to rest outdoors presumably made them substantially less susceptible to the insecticide deposits on sprayed walls indoors. Anecdotal evidence gathered over the last decade and based on periodic indoor searches in northern KwaZulu-Natal Province, shows that the IRS programme is particularly effective at controlling indoor-resting *Anopheles* mosquitoes because they are seldom, if ever, found inside sprayed houses in northern KwaZulu-Natal.

Evidence of ongoing resistance to several classes of insecticides in *An. arabiensis* in northern KwaZulu-Natal Province is of concern. The frequencies of resistance are, however, low. Previous analysis shows that the pyrethroid-resistant phenotypes inherent in this population are of low intensity and are, therefore, highly unlikely to be operationally significant.[17,20] These data also importantly show full susceptibility to pirimiphos-methyl, an insecticide that, along with DDT, is also indicated for use against pyrethroid-resistant *An. funestus* in southern Africa.[27-30]

An assessment of resistance mechanisms can yield important information on where cross-resistances between insecticide classes are likely, and on how quickly resistance might develop to high levels in an affected vector population under selection pressure. The synergist data given here suggests that cytochrome P450s (oxygenases) and general esterases are at least partially responsible for the pyrethroid- and carbamate-resistant phenotypes, although these data need to be interpreted with caution. This is because of the low-resistance frequencies recorded and the fact that enzyme synergists will always enhance the toxicity of insecticides, even in non-resistant mosquitoes. Nevertheless, resistance mechanisms based on enzyme-mediated detoxification have the potential to reach high levels of intensity that can lead to control failure. This includes the high-intensity pyrethroid resistance in *An. funestus* that has previously undermined vector control in South Africa and Mozambique.[2,13,16,31] Pyrethroid resistance in southern African populations of *An. funestus* is primarily based on P450 metabolism[32,33], bolstered by increased production of glutathione-S-transferases that likely protect against the oxidative damage caused by pyrethroid insecticides[34], and thickened cuticles that reduce the rate of insecticide absorption[35].

Conclusion

The northern regions of KwaZulu-Natal Province remain receptive to malaria transmission despite ongoing control and elimination interventions. This receptivity is due to the perennial presence of the major vector *An. arabiensis* and other secondary vector species whose populations include outdoor-resting components that are less susceptible to control by indoor residual spraying. The continued detection of low-frequency insecticide resistance phenotypes in *An. arabiensis* is cause for concern, and it is recommended that populations of this and other vector species be periodically monitored for changes in resistance frequency and intensity going forward.

Acknowledgements

We acknowledge the KwaZulu-Natal Department of Health for their valuable support during fieldwork, and National Institute for Communicable Diseases Vector Control Laboratory staff and students who assisted with diagnostic work and mosquito rearing. The Jozini community is thanked for giving access to their households during mosquito sampling. This study was supported through a Bill and Melinda Gates Foundation grant (grant no. OPP1210314) and partly funded by the International Atomic Energy Agency under their Technical Cooperation Programme (SAF 5014/5017) and Research Contract No. 19099, South African National Research Foundation grants (grant no. 119765 and 107428) awarded to G.M., the Department of Science and Innovation Health Innovation Scheme, and a National Health Laboratory Service Research Trust award to B.D.B.

Competing interests

We have no competing interests to declare.

Authors' contributions

G.M. conceived and supervised the study, contributed to data analysis and reviewed the first and subsequent drafts of the manuscript. S.V.O. designed and performed the laboratory component of the study, analysed the data, and reviewed all manuscript versions. L.N.L. and Y.D.M. coordinated laboratory activities and reviewed the final draft of the manuscript. T.T.M. participated in field data collection, mapped study sites and provided comments in the final version of the manuscript. N.M. and D.M.D. led field data collection and provided comments in the final version of the manuscript. M.Z., F.M., B.D.L., J.Z., A.B. and A.M. generated laboratory-based data. M.K. participated in field activities and provided comments on the final version of the manuscript. B.D.B. drafted the manuscript and critically revised the final draft. All authors read and approved the manuscript.

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