

Insecticide susceptibility status of *Anopheles culicifacies* to bendiocarb and deltamethrin in a sub-district of Chhattisgarh state, India

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Short Report

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Abstract

Objective: This study assessed the susceptibility status of the malaria vector *Anopheles culicifacies* within the Keshkal sub-district, Chhattisgarh, India. *Anopheles culicifacies* were collected in 23 sub-centres (~2000-3000 inhabitants/ sub-centre) of Keshkal sub-district in 2013- 2014. Adult female *An. culicifacies* were exposed to the World Health Organization (WHO) recommended diagnostic doses of deltamethrin (0.05%) and bendiocarb (0.1%) for one hour. Mortality was recorded after 24 hours and interpreted with respect to WHO criteria.

Results: Mosquito collections from only three of 23 clusters were scored as susceptible to deltamethrin, and all clusters except one were recorded as resistant to bendiocarb. The observation to a high frequency of resistance to the carbamate, bendiocarb, is unexpected as carbamates are not used in the study area in public health.

Introduction

Anopheles culicifacies (Diptera: Culicidae) are responsible for transmitting more than two-thirds of the total malaria cases reported in India¹ and is the primary malaria vector in Chhattisgarh state, India². Indoor residual spraying (IRS) of insecticides was the major component of India's malaria control programme since the early fifties and is now being supplemented by distributing insecticide-treated bed nets (ITNs). A variety of chemicals have been deployed for IRS, initially Dichloro-diphenyl trichloroethane (DDT) before malathion, hexachlorocyclohexane (HCH) and later synthetic pyrethroids³. The continuous use of insecticides in public health and agriculture has resulted in reports of insecticide resistance in *An. culicifacies* in different states of India⁴. Pyrethroid resistance is widespread in Tamil Nadu⁵; Gujarat⁶, Madhya Pradesh⁷, Odisha⁸, and Andhra Pradesh⁹ including the study area alpha-cypermethrin has been sprayed for more than a decade². Chhattisgarh state has the highest proportion of deaths (17%) due to malaria and contributes 12% of malaria cases in the country¹⁰. Assessment of mosquitoes' resistance status is essential for assessing the efficacy of different insecticide-based vector control interventions, such as IRS and long-lasting insecticidal nets (LLINs). The present study was a part of a multi-country WHO-coordinated study of the Implications of Insecticide Resistance (IIR study), to assess the impact of insecticide resistance in malaria vectors on the efficacy of LLINs and IRS^{11,12}. As part of this study, insecticide susceptibility status to diagnostic dosages of deltamethrin and bendiocarb were determined in tribal and rural sub-district Keshkal, Chhattisgarh State, India.

Methods

A one-year baseline study was conducted in 2013-2014 in the Keshkal block, (20.0847° N, 81.5867° E; Total population ≈100,000 and 632 m avg. elevation) in the southern region of Chhattisgarh. The area has a tropical climate with dry (Jan-May), monsoon (June – Sept), and winter (Oct-Dec) seasons. The average temperature ranges between 11 and 35°C with average relative humidity (RH) of around 70 % and 1500 mm of average annual rainfall.

Keshkal has a community health centre (CHC), four primary health centres (PHC), namely Bahigaon, Dhanora, Keshkal and Singhanpur comprising 28 sub-centres. Four Plasmodium parasite species, i.e. *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*, are present. The annual parasite incidence (API) of Keshkal CHC varied from 3 to 5 during the years 2010-2013) and contributed nearly 2% of the total annual malaria cases recorded. Chhattisgarh¹³. More recent data shows this trend remains with the slide positivity rate (SPR) for these areas being 3.1% while SPR of the state was 2.68 %¹⁴. There are 105 villages (henceforth clusters) within the study area, of which 80 were selected for the IIR project study based on logistics and security¹¹. The major ecotypes are hilly, low land, riverine and plain areas, with forty-five per cent of the state covered with dense forest. Among the 80 villages, 23 villages were selected for conducting insecticide susceptibility tests depending on the ecotypes and availability of mosquitoes. Adult susceptibility was determined using WHO diagnostic dosages of bendiocarb and deltamethrin by exposing field-collected mixed-age females or three-five day-old F₁ progeny of field-collected mosquitoes in a WHO tube test¹³. Insecticide impregnated papers were procured from Vector Control Research Unit, University Sains Malaysia, Penang, Malaysia.

For each test, four-five replicates of 20-25 female mosquitoes were exposed to insecticide-impregnated test paper for one hour. Mosquitoes were transferred into holding tubes; provided with 10% glucose-soaked cotton pads and maintained at standard temperature (27 ± 2 °C) and relative humidity (80%). After one hour and 24 hours, mortality was scored. Abbott's correction was made if the mortality in the control replicates was between five and 20%¹⁶. The criteria for characterizing the resistance/susceptibility status are, > 98% mortality- Susceptible; 91–97% mortality- possible resistance; and < 90 % mortality- Resistance¹⁵. The data were analyzed using a Kruskal-Wallis test for the differences in susceptibility status among the sub-centres' of four PHCs.

Results

The susceptibility status of *An. culicifacies* mosquitoes collected from 23 clusters to deltamethrin (0.05%) and bendiocarb 0.1% is shown in Table one. The results suggest that pyrethroids' resistance is less common than carbamates in the study area despite prolonged use of pyrethroid based IRS. *Anopheles. culicifacies* populations from only five clusters were resistant to deltamethrin, with three clusters scored as susceptible. The remaining 15 clusters showed intermediate levels of mortality that require further verification. In contrast, *An. culicifacies* populations from 22/23 clusters were resistant to bendiocarb with verification of status required for the remaining cluster. However, contrast results were observed next year, rendering the species completely susceptible and the exact reason for the contrasting result could not be ascertained. We grouped clusters by PHC and applied a Kruskal-Wallis ranking test to investigate whether there were significant differences in the susceptibility of populations to either deltamethrin ($P>0.05\%$; df 3; n=23) or bendiocarb ($p > 0.5$; df 3; n=23) between administrative boundaries

Discussion

The present study showed for the first time widespread resistance in *An. culicifacies* to the carbamate bendiocarb (0.1%) in the Keshkal block, Chhattisgarh, India. Carbamates are never used in the public health programme, and resistance is likely to have been driven by their use in agriculture. In the area, paddy is the major crop and the most commonly used insecticide class for plat protection is carbamates, followed by organophosphates, pyrethroids and neonicotinoids. In a recent report by Bhatt *et al.* (unpublished) resistance to bendiocarb has also been observed in southern districts including the present study district Kondagaon (Kanker, Kondagaon and Narayanpur) and northern districts (Raipur and Rajnandgaon).

Similarly, the development of resistance in *An. culicifacies* in Andhra Pradesh¹⁷ to Malathion is also thought to be due to agricultural selection and not due to public health sprays. However, next year the species reported complete susceptibility to bendiocarb and indicated instability of bendiocarb resistance. Later, it led to a significant conclusion that the resistance was dynamic and varied between the clusters¹⁸.

Conversely, only five of 23 clusters reported resistance to the pyrethroid deltamethrin (0.05%). Earlier studies from Chhattisgarh have also reported resistance to deltamethrin (0.05%)¹⁹. The species reported 88 % survivorship to DDT (n= 1273), 57% to Malathion (n=389), and 24% to alpha-cypermethrin (n=628).The use of alpha-cypermethrin for ten years has resulted in selection for resistance; it does not confer cross-resistance to deltamethrin.

In conclusion, despite long-term IRS with alpha delta susceptibility is high, LLINs based upon delta are likely to be effective. Widespread use of bendiocarb appears to have removed one of the few active ingredients we have at our disposal before it was deployed.

Declarations

Ethics approval and consent to participate

This study was undertaken as a part of a WHO-coordinated multi-country project and ethical clearance was obtained from the Institutional Ethics Committee of National Institute of Malaria Research (ICMR), New Delhi (ECR/NIMR/EC/2010/75).

Consent for publication

Not applicable

Availability of data and materials

"The dataset(s) supporting the conclusions of this article is(are) included within the article (and its additional file(s))."

Competing interests

None

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Author's contributions

DKS, KR, and RMB, designed the study. DKS, MKC, KR, and RMB drafted the manuscript. DKS, SA, CN and MKC have done the literature review and study protocol preparation. CN, SA, AM, and DKS supervised and collected the data. MKC, and MJD analysed the data. KR, RMB, DKS, SU, MKC, and MJD reviewed and helped write the manuscript. All authors made intellectual input to the study. All authors read and approved the final manuscript.

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Tables

Table 1: Insecticide susceptibility status of *An. culicifacies* to deltamethrin (0.05%) and bendiocarb (0.1%) in the study clusters of the Keshkal sub-district.

Sl.No	PHC	Sub-centres	Per cent mortality Deltamethrin (0.05%)	Mortality Range in test replicates		Per cent mortality Bendiocarb (0.1%)	Mortality Range in test replicates	
				Minimum	Maximum		Minimum	Maximum
1	Bahigaon	Anwarabhata	97	90	100	60	49	71
2		Arandi	96	92	100	63	50	85
3		Khetarpal	93	83	96	89	76	94
4		Nayanar	89	67	100	80	75	83
5		Pipra	92	83	99	71	42	95
6	Dhanora	Badekholi	97	89	100	69	49	87
7		Binjhe	91	77	99	74	72	77
8		Eragaon	88	85	92	67	52	82
9		Kararmeta	97	93	100	68	55	78
10		Korkoti	90	65	100	90	85	93
11		Sawala	94	84	100	61	49	71
12	Keshkal	Borgaon	85	65	95	81	74	87
13		Jamgaon	95	91	98	75	64	88
14		Khokhameta	94	93	96	74	42	86
15		Murvand	93	81	99	60	27	83
16		Salebath	100	99	100	78	65	89
17		Surdongar	93	90	96	98	95	100
18	Singanpur	Adenga	99	99	99	46	30	61
19		Anwari	88	80	100	53	36	75
20		Garawandi	96	90	99	75	64	91
21		Neerachindli	97	92	99	86	79	92
22		Singanpur	86	80	90	82	72	87
23		Toskapal	98	97	100	72	47	95

Values in boldface are categorized as resistant as per the WHO criterion.

Figures

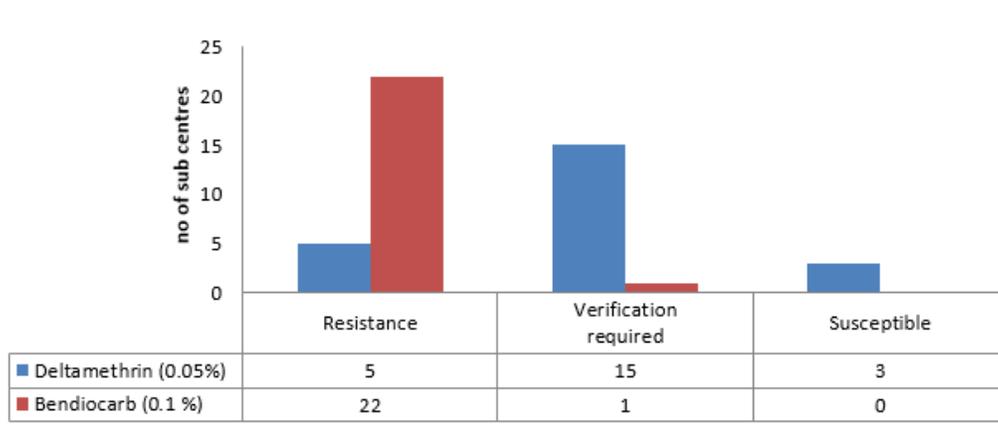


Figure 1

Susceptibility status of *Anopheles culicifacies* to deltamethrin (0.05%) and bendiocarb (0.1%) in study clusters of Keshkal sub-district, Chhattisgarh state, India