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Insecticide resistance status in *Anopheles culicifacies* in Madhya Pradesh, central India

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Malaria is one of the most common vector-borne diseases widespread in tropical and subtropical regions[1]. Vector control constitutes an important aspect of vector borne disease control programme. Use of insecticides for vector control is an effective strategy but is also associated with the development of insecticide resistance in the target disease vectors and is one of the reasons for failures of disease control in many countries including India. Earlier, indoor residual spraying (IRS) with insecticides was the key component of malaria control and was responsible for spectacular reduction in disease incidence during the early 20th century[2–5]. Still IRS and insecticide-treated materials are in use as adulticides in vector control[6]. Several studies have been done on the susceptibility status of adult mosquitoes in different locations and on different species in India[7–12].

Anopheles culicifacies (Diptera: Culicidae) is a principal malaria vector in rural and peri-urban areas and contributes about 65% of malaria cases in India[13]. To date, in India, DDT (organochlorine), malathion (organophosphorus) and deltamethrin, cyfluthrin, alpha-cypermethrin and lambda-cyhalothrin (synthetic pyrethroids) are the commonly used insecticides for vector control in public health. Resistance to DDT in *An. culicifacies* is reported widespread throughout the country[14–16], and also to malathion in the states of Maharashtra, Gujarat, Tamil Nadu and Uttar Pradesh[17–20] and there are few reports of decreased susceptibility to synthetic pyrethroids in various parts of the country[21–23] including Chhattisgarh (Bhatt *et al*. unpublished data). Recently, surveys were conducted in the year 2009–10 in 9 tribal districts of Madhya Pradesh to assess the susceptibility status in *An. culicifacies* to DDT, malathion and deltamethrin.

Madhya Pradesh is the largest state in the country (308,252 km^2) having 50 districts with forest cover of about 31% with intense perennial malaria transmission in these areas. The state has about 60 million population with 12 million tribal population. The main vector control intervention is IRS and in some places insecticide treated nets (ITN) and long-lasting insecticidal nets are also in use. The major malaria vector in this state is *An. culicifacies*.

The insecticide susceptibility of *An. culicifacies* to DDT, malathion and deltamethrin was tested during August and September, 2009 in Balaghat, Betul, Chhindwara, Dindori, Guna, Jhabua, Mandla, Shadol and Sidhi districts using standard WHO adult susceptibility kit and methods[24]. The districts selected for assessment of susceptibility to insecticides in *An. culicifacies* have almost similar ecotype, vector prevalence and employ same vector control strategies. For adult insecticide susceptibility test, insecticide impregnated papers were procured from Vector Control Research Unit, University Sans Malaysia, Malaysia. Female mosquitoes were collected from the study villages of the district in the early hours using a mouth aspirator and torch light[25]. The mosquitoes collected from field were held in a cloth cage and transported to base laboratory with a wet towel around the cage. Mosquitoes were identified to species based on morphological characters using a standard key. Mosquitoes were exposed to different insecticides, DDT, malathion and deltamethrin for 1 h. At least 3–4 test replicate exposures to the given insecticide and respective controls were run simultaneously. After exposure to diagnostic dosages of the given insecticides for one hour, mosquitoes were held for 24 h holding period at 27 ± 2ºC temperature and 70–80% relative humidity. Mortality was determined by scoring the dead and alive mosquitoes and results were expressed as percent mortality. In experiments, where mortality in control replicates was between 5 and 20%, the mortality was corrected using Abbott's formula[26] and tests with >20% control mortality were discarded. Based on the observed percent corrected mortality as per the WHO criteria[24] , the data are presented in three classes namely susceptible–mortality ≥98%, verification required (tolerance)–mortality between 81% and 97%, and resistant –mortality ≤80%.

The results of the present study are given in Table 1. *An. culicifacies* was found resistant to DDT in all the dis-

Table 1. Susceptibility status of *Anopheles culicifacies* to DDT, malathion and deltamethrin in nine districts of Madhya Pradesh

District	% Mortality		
	Insecticide		
	DDT 4%	Malathion 5%	Deltamethrin 0.05%
Shahdol	8.8 (180) R	77.8 (180) R	93.8 (180) VR
Sidhi	7.5*(360) R	78.8 (360) R	94.1 (360) VR
Dindori	12.8 (180) R	80 (180) R	71.6 (180) R
Mandla	13.3 (180) R	78.3 (180) R	76.6 (180) R
Balaghat	6.7 (120) R	84 (150) VR	92 (150) VR
Betul	12.4 (225) R	72.4 (225) R	83.1 (225) VR
Chhindwara	9.2 (315) R	74.9 (315) R	85.9* (315) VR
Guna	26.6 (300) R	100 (300) S	100 (270) S
Jhabua	6.6 (240) R	65.4 (240) R	87 (240) VR

*Figures in parentheses indicate number of mosquitoes tested; S–Susceptible (mortality ≥ 98%); R—Resistant (mortality ≤80%); VR—Tolerant (mortality 81–97%).

Table 2. Susceptibility status of *Anopheles culicifacies* to DDT, malathion and deltamethrin in three different insecticide spray areas of Madhya Pradesh

Villages with different insecticide sprays	Insecticide tested	% Mortality
Group-A		
SP sprayed since last 5–10 yr	DDT 4%	11.2 (780) R
	Malathion 5%	73.4 (780) R
	Deltamethrin 0.05%	77.4 (780) R
Group-B		
DDT spray	DDT 4%	16.2 (690) R
	Malathion 5%	87.5 (720) VR
	Deltamethrin 0.05%	96.5 (690) VR
Group-C		
Without insecticide IRS since last 5–10 yr	DDT 4%	5.9* (630) R
	Malathion 5%	77.4 (630) R
	Deltamethrin 0.05%	94.7 (630) VR

*Figures in parentheses indicate number of mosquitoes tested. Meaning of abbreviations as in Table 1.

tricts surveyed and to malathion in Sidhi, Shadol, Mandla, Betul, Chhindwara and Jhabua districts and was tolerant to malathion in Districts Dindori and Balaghat and the species was susceptible in Guna district. While to deltamethrin, the species registered resistance in Districts Mandla and Dindori, tolerant in Districts Sidhi, Shadol, Balaghat, Betul, Chhindwara and Jhabua but the species was still susceptible in Guna district. The observed mortality in different districts varied from 0 to 28.3% for DDT, 57.1 to 100% for malathion and 64.3 to 100% for deltamethrin (Table 1). The results indicated that the species was highly resistant to DDT in all the districts while to malathion the species was moderately resistant and was mostly tolerant to deltamethrin.

Further stratification of the group of villages in the study districts was made based on spray history in the last 5–10 yr as Group A (IRS with pyrethroids), Group B (IRS with DDT), and Group C (without insecticide IRS) (Table 2).

It may be stated that DDT is being sprayed in these areas since the inception of National Malaria Control activities in early-1950s. Decreased mortality in *An. culicifacies* to deltamethrin was found in Group A area that received deltamethrin IRS in the last 5–10 yr (Table 2). All the areas have shown resistance to DDT and also to malathion, however, in areas without deltamethrin spray the species registered higher mortality to deltamethrin in the range of 94.7 to 96.4%. Malathion was also not sprayed regularly in these areas and the observed levels of resistance to malathion could be due to selection by its use in agriculture/forestry in the absence of its use in public health sprays. Similar observations were made earlier in district Chhindwara, Madhya Pradesh on the presence of resistance to malathion in *An. culicifacies* (Raghavendra unpublished). Surprisingly, in the present study, *An. culicifacies* has shown resistance to DDT in all the areas irrespective of its withdrawal from IRS and replaced with other insecticide or no insecticide spray. This could be due to increased genetic stability of DDT resistance gene as was observed in *An. culicifacies* in Surat. A study carried out in Surat in 2005 and 2006, wherein pyrethroid IRS was discontinued earlier since 2002 resulted in reversion of pyrethroid resistance to susceptibility from 60% in 2001 to > 96% in 2005 and 2006 but, the species was still reporting resistance to DDT and malathion even after their withdrawal from IRS respectively for 30 and 9 yr. The relatively low levels of reversion against DDT and malathion in Surat could be due to increased genetic stability of DDT and malathion resistance in the populations[27].

In conclusion, the present data indicated development of resistance to pyrethroids in *An. culicifacies* is of great concern to Indian malaria control programme as it is in extensive use in public health programmes for IRS and for impregnation of bed nets. There is a need for regular monitoring to assess insecticide susceptibility to formulate effective vector control strategies.

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