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## Insecticide resistance in malaria vector *Anopheles culicifacies* in some tribal districts of Chhattisgarh, India

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**Insecticide resistance in *Anopheles culicifacies*, the major vector of malaria in tribal areas of Chhattisgarh, was determined using standard WHO susceptibility tests. Majority of the districts in Chhattisgarh are highly malaria-prone and under the Enhanced Malaria Control Project (EMCP), where synthetic pyrethroids are used for indoor spraying against *An. culicifacies*. Resistance/susceptibility of *An. culicifacies* to DDT (4%), malathion (5%) and deltamethrin (0.05%) was studied in a few villages of Kanker and Jagdalpur, Mahasamund and Raigarh Districts. The corrected mortality varied from 13.8 to 25% to DDT, 59.3 to 87.9% to malathion and 89.4 to 92.5% to deltamethrin. The tests also revealed a higher knock-down time for *An. culicifacies* collected from the Kanker and Jagdalpur Districts where synthetic pyrethroid is being sprayed, indicating incipient resistance to synthetic pyrethroid in this vector species.**

**Keywords:** *Anopheles culicifacies*, insecticides, malaria vector, resistance.

*ANOPHELES CULICIFACIES* is the major malaria vector of rural and peri-urban plain areas in India and contributes to the transmission of about 65% of the total malaria cases<sup>1</sup>. The main target of the National Vector Borne Disease Control Programme (NVBDCP), formerly known as National Anti Malaria Programme (NAMPP) of India, is to control malaria transmitted by *An. culicifacies* in rural areas by Indoor Residual Spraying (IRS) of insecticides. At present, DDT (organochlorine), malathion (organophosphorus), and deltamethrin, lamdacyhalothrin, cyfluthrin, etc. (synthetic pyrethroids) are the commonly used insecticides in India for IRS. However, due to the constant use of DDT during the past five decades, resistance in *An. culicifacies* is now widespread throughout the country<sup>2–4</sup>, except in some areas where DDT is still in use, as it is still an effective tool to control *An. fluviatilis*-transmitted *Plasmodium falciparum*-malaria<sup>5</sup>. During the sixties, HCH (another organochlorine insecticide) was also used for IRS in areas where DDT resistance was detected. How-

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ever, after 1997, HCH has been completely banned for IRS in India. In areas where resistance to DDT and HCH is reported, malathion is used as an alternative for IRS. However, resistance to malathion in *An. culicifacies* has also become widespread in Maharashtra, Gujarat, Tamil Nadu and Uttar Pradesh<sup>6-9</sup>.

Due to the triple resistance problem, synthetic pyrethroids were introduced for IRS in the eighties in India, and were found effective in triple insecticide resistant areas of Maharashtra and Gujarat<sup>7</sup>. Therefore, synthetic pyrethroids were recommended by NAMP under EMCP. However, there are now reports of reduced susceptibility to synthetic pyrethroids in different parts of India<sup>10-12</sup>. Therefore to rationalize IRS and also to save the useful life of synthetic pyrethroids, periodic monitoring of resistance in vector mosquitoes is required on priority.

Chhattisgarh has a total population of about 23 million and about 44% of the area is covered with forests. Malaria is a public health problem in Chhattisgarh. The annual parasite incidence (API) during 2000–02 was high (>10), and of the total cases of malaria reported, about 70% was caused by *P. falciparum*. Due to this, 13 out of 16 districts have been covered under EMCP since 1998. Synthetic pyrethroids are used for IRS in these districts to control the major malaria vector, *An. culicifacies*.

The susceptibility of *An. culicifacies* to DDT, malathion and deltamethrin was tested during September and October 2002 in four districts, viz. Kanker (81.32 long. and 20.15 lat.), Jagdalpur (82.04 long. and 19.05 lat.), Mahasamund (82.15 long. and 21.15 lat.) and Raigarh (83.26 long. and 21.54 lat.), using standard WHO procedure (WHO, unpublished). However, for deltamethrin 0.05% papers were used (WHO, unpublished). In the Kanker and Jagdalpur districts, where the proportion of *P. falciparum* is high, a synthetic pyrethroid (K-othrine) is used for IRS, whereas in Mahasamund and Raigarh districts DDT is still being used. Vector collections were carried out from two villages each in the Kanker (Mankeshwari of PHC Dhaneli Kanhar and Kalgaon of PHC Antagarh), Jagdalpur (Keshloor of Tokapal PHC and Tirathgarh of Darbha PHC) and Raigarh (Raikera of Gharghora PHC and Amapali of Lailunga PHC) districts. Due to low vector densities in Mahasamund district, collections were made from three villages (Kurmadi of Basana PHC, and Junwani and Ghupali of Bagbahra PHC). Indoor resting blood-fed adult female mosquitoes were collected in the early morning hours (6–8 a.m.) using a mouth aspirator and torch light<sup>13</sup>. The mosquitoes collected were held in a caged cloth and transported to a makeshift laboratory with suitable temperature and relative humidity during the tests.

Tests for DDT could not be performed in Jagdalpur district due to fewer mosquitoes and time constraints. At least two replicates each for experiment and control were kept for each diagnostic concentration of the insecticide. After 1 h exposure, the mosquitoes were transferred to holding tubes and kept for 24 h for recovery in a card-

board box designed to keep the mosquitoes in a confined humid environment maintained to simulate laboratory conditions. Mortality was determined by scoring the dead and living mosquitoes. In tests where control mortality was between 5 and 20%, corrected per cent mortalities were calculated and tests with >20% control mortality were discarded<sup>14</sup>.

In addition to susceptibility tests against diagnostic concentration for mosquitoes exposed for 1 h, knock-down times 50 and 90% (KDT<sub>50</sub> and KDT<sub>90</sub>) against deltamethrin were also determined for *An. culicifacies* in the Kanker and Jagdalpur districts. The number of mosquitoes exposed to 0.05% deltamethrin papers and knocked down after 5, 10, 15, 30, 45, 60 min was recorded, till the last mosquito was knocked down, and KDT<sub>50</sub> and KDT<sub>90</sub> were calculated using log–time and probit–knock-down regression models.

Table 1 shows the total number of *An. culicifacies* exposed to diagnostic dosages of DDT, malathion and deltamethrin and the per cent corrected mortality. The mortality varied from 13.8 to 25.0% for DDT, 59.3 to 87.9% for malathion and 89.4 to 92.5% for deltamethrin in the districts studied. Table 2 gives KDT<sub>50</sub> and KDT<sub>90</sub> values for *An. culicifacies* exposed to 0.05% deltamethrin. KDT<sub>50</sub> and KDT<sub>90</sub> were 40.19 and 41.8 min and 110.3 and 111.3 min respectively, in Kanker and Jagdalpur districts, which receive synthetic pyrethroids for IRS, and 29.14 and 37.77 min and 72 and 123.6 min respectively, in the Raigarh and Mahasamund districts, where DDT is still used for IRS. In an earlier study, KDT<sub>50</sub> and KDT<sub>90</sub> for the susceptible strain of *An. culicifacies* were reported to be 9.9–16.8 and 20.3–22.5 min. The higher KDT<sub>50</sub> and KDT<sub>90</sub> values observed in the present study indicate the development of incipient resistance by *An. culicifacies* to deltamethrin in all the four districts of Chhattisgarh. The present study also revealed high degree of resistance in *An. culicifacies* to DDT in all the three districts of the state, while resistance to malathion was intermediate in two out of four districts. According to WHO criteria, mortality <80% is considered to be resistant, whereas according to the NVBDCP in India, mortality <40% is classified as resistant. Hence in two out of four districts, where the mortality ranged between 40 and 80%, it was considered intermediate resistant. Resistance in *An. culicifacies* to DDT has been reported from different parts of India<sup>2-5,7,9</sup>. Though *An. culicifacies* was susceptible to malathion in Haryana, varying degree of resistance to the same insecticide has been reported from Maharashtra<sup>6</sup>, Gujarat<sup>8</sup> and Uttar Pradesh<sup>9</sup>. In Maharashtra and Gujarat, *An. culicifacies* was resistant to both DDT and malathion, but susceptible to synthetic pyrethroid (cyfluthrin)<sup>8</sup>. In Uttar Pradesh<sup>4,9</sup>, *An. culicifacies* was found susceptible to deltamethrin. In the present study, reduced susceptibility to deltamethrin was recorded in all the four districts, even though synthetic pyrethroids were sprayed in only two out of four districts. This reduced susceptibility to synthetic pyre-

**Table 1.** Susceptibility status of *Anopheles culicifacies* to DDT, malathion and deltamethrin in four districts of Chhattisgarh

District	Insecticide tested	No. of mosquitoes exposed	Corrected mortality (%)
Kanker	DDT 4%	40	22.9
Mahasamund	DDT 4%	78	25.0
Raigarh	DDT 4%	79	13.8
Kanker	Malathion 5%	43	74.1
Jagdalpur	Malathion 5%	40	83.8
Mahasamund	Malathion 5%	36	87.9
Raigarh	Malathion 5%	65	59.3
Kanker	Deltamethrin 0.05%	45	89.4
Jagdalpur	Deltamethrin 0.05%	46	90.4
Mahasamund	Deltamethrin 0.05%	40	89.5
Raigarh	Deltamethrin 0.05%	40	92.5

Note: Tests for DDT were not done in Jagdalpur District.

**Table 2.** Time–mortality response of *Anopheles culicifacies* against deltamethrin 0.05% impregnated papers in four districts of Chhattisgarh

District	No. exposed (% corrected mortality)	Knock-down time (min)			
		KT <sub>50</sub>	KT <sub>90</sub>	$\chi^2(df)$	Regression equation
Kanker	45 (89.4)	40.19	110.34	0.1927(3)	y = 2.9186x + 03180
Jagdalpur	46 (90.4)	41.88	111.38	1.0155(3)	y = 3.0135x + 0.1119
Mahasamund	40 (89.5)	37.77	123.62	0.774(3)	y = 2.4003x + 1.21
Raigarh	40 (92.5)	29.14	72.02	3.22 (3)	y = 3.0762x + 0.5079

throids in *An. culicifacies* could be due to either cross-resistance between DDT and the synthetic pyrethroid caused by the *Kdr* (Knock-down resistance) gene, as reported in some Anophelines spp.<sup>15</sup> or it could be due to the use of synthetic pyrethroids in agriculture. However, cross-resistance between DDT and synthetic pyrethroids due to *Kdr* has not been confirmed in *An. culicifacies* as yet. Further studies are therefore required to investigate the cause of reduced susceptibility in *An. culicifacies* to deltamethrin, even in those areas of Chhattisgarh, where synthetic pyrethroids are not in use for IRS.

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