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A note on the insecticide susceptibility status of principal malaria vector *Anopheles culicifacies* in four states of India

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ABSTRACT

Background & objectives: The major malaria vector, *Anopheles culicifacies* Giles is reported to contribute ~ 65% of the malaria cases in India. This species developed resistance to DDT and later to HCH, malathion and also to pyrethroids in some states due to their use in the national malaria control programme. In the present study, insecticide susceptibility of this species was monitored in four states of India.

Methods: To determine insecticide susceptibility status of the major malaria vector *An. culicifacies*, adult mosquitoes were collected from different localities of 32 tribal districts in the states of Andhra Pradesh, Odisha, Jharkhand and West Bengal during October/November 2009–10. Mosquitoes were collected from stratified ecotypes comprising a group of districts in West Bengal and individual districts in three other states. Mosquitoes were exposed to papers treated with WHO diagnostic dose: 4% DDT, 5% malathion and 0.05% deltamethrin following the WHO tube method.

Results: Results provided the susceptibility status of *An. culicifacies* to different insecticides used in the public health programme in 32 districts in four states. *An. culicifacies* was found resistant to DDT (mortality range 0–36%) in all the 32 districts; to malathion it was resistant in 14 districts, verification required in 10 districts and susceptible in eight districts (mortality range 32.2–100%). It was resistant to deltamethrin in four districts, verification required in 11 districts and susceptible in 17 districts (mortality range 43.3–100%).

Interpretation & conclusion: Development of widespread resistance to insecticides used in public health sprays for vector control including to pyrethroids in *An. culicifacies* in the surveyed districts is of great concern for the malaria control programme as the major interventions for vector control are heavily reliant on chemical insecticides, mainly synthetic pyrethroids used both for indoor residual spraying and for long-lasting insecticidal nets. Thus, there is a need to periodically monitor and update the susceptibility status of malaria vector(s) to suggest alternative vector control strategies for effective disease management.

Key words *Anopheles culicifacies;* India; insecticide resistance; vector control

INTRODUCTION

Since early 19th century, chemical insecticides based vector control methods are in practice globally in the vector control programmes[1]. Presently, among the available vector control interventions, indoor residual spraying (IRS) of insecticides and long-lasting insecticidal nets/materials (LNs/LMs) are considered effective for limiting malaria transmission in disease control programmes[2]. Currently, 12 insecticides are recommended by WHO for IRS to control mosquito vectors[3], while in India, DDT (organochlorine), malathion (organophosphate) and deltamethrin, cyfluthrin, alpha-cypermethrin and lambda-cyhalothrin (pyrethroids) are in use in the vector control programme. Pyrethroids were introduced in India in mid-nineties for IRS and later for impregnation of mosquito nets. Presently, pyrethroid impregnated long-lasting insecticidal nets are being used in India and is poised for further scale up in future. The strategy of sequential end point replacement of insecticides practiced in the vector control programme in India has resulted in multiple-insecticide-resistance in *An. culicifacies* (Diptera: Culicidae). *An. culicifacies* is the major malaria vector that contributes about 65% of the total malaria cases reported in India[4]. In India, widespread resistance in *An. culicifacies* to DDT has been reported[5–7]. Resistance to malathion has been reported from some states[8–11], while there are few reports of decreased susceptibility to synthetic pyrethroids in few areas[12–14] and in some districts of Chhattisgarh[15] and Madhya Pradesh[16].

In the present investigation, we present the results of insecticide susceptibility status of *An. culicifacies* in 32 tribal districts of four states, *viz.* Andhra Pradesh, Odisha, Jharkhand and West Bengal against commonly used insecticides in public health.

MATERIAL & METHODS

Study area

The study was conducted in 32 districts of four states, Andhra Pradesh, Odisha, Jharkhand and West Bengal. These districts were suggested by National Vector Borne Disease Control Programme (NVBDCP) that are forested areas inhabited predominantly by tribals and are reported to be endemic for malaria. Surveys were carried out in individual districts or in groups of two districts, and the districts in the groups had similar ecotypes. Mosquitoes were collected from two groups of two districts each in West Bengal, while in Odisha of the 19 districts, 15 were surveyed individually and four in groups of two districts each. While in Andhra Pradesh and Jharkhand states, surveys were carried out individually in the suggested districts.

Susceptibility tests

Adult insecticide susceptibility tests were conducted following WHO standard method[17] by exposing field-collected mixed age population of mosquitoes to DDT (4%), malathion (5%), deltamethrin (0.05%) and their respective control papers. The tests were carried out during October/November 2009–10. Mosquitoes were collected during the early morning hours using a mouth aspirator and torch light[18]. The mosquitoes collected from field were held in a cloth cage with a wet towel around the cage and brought to the base laboratory. The species were identified based on morphological characters using a standard identification key for anophelines[19]. Mosquitoes were exposed for 1 h followed by 24 h holding period at 27 ± 2°C temperature and 70–80% relative humidity using a carton lined with moist towel/filter papers at the bottom. At least 3–4 test replicates were used in the tests against the given insecticide and two replicates to respective control papers. Mortality was determined by scoring the dead and alive mosquitoes at the end of 24 h holding period and results were expressed as percent mortality. Test mortality was corrected by applying Abbott's formula[20], when control mortality was recorded between 5 and 20%, while tests with >20% control mortality were discarded. Based on the mortality, the data were categorized as susceptible: >98% mortality; verification required: 81–97% mortality; and resistant: <80% mortality.

RESULTS

Adult mosquitoes were collected from different localities as described above for the present investigation. However, in some tests, due to less availability of mosquitoes, the number of mosquitoes exposed to insecticide was very low. The results of the susceptibility tests are given in Table 1. *An. culicifacies* was found resistant to DDT in all the 32 districts surveyed.

To malathion, it was resistant in 14 districts, verification required category in 10 districts and susceptible in eight districts. Resistance to malathion was detected in all the five districts of Andhra Pradesh and nine districts of Odisha. To malathion the species was verification required category in two districts of Jharkhand (Gumla and East Singhbhum), four districts of Odisha (Nuapada, Rayagada, Jagatsinghpur and Ganjam) and in all the four districts of West Bengal. *An. culicifacies* was found susceptible to malathion in two districts of Jharkhand (Ranchi and West Singhbhum) and six districts of Odisha (Mayurbhanj, Sambalpur, Dhenkanal, Sonepur, Keonjhar and Angul).

To deltamethrin, it was found resistant in four districts, verification required category in 11 districts and susceptible in 17 districts. *An. culicifacies* was resistant to deltamethrin in four of the five districts surveyed in Andhra Pradesh except in Vizianagaram, while in Odisha, the species was verification required category in districts, Angul, Bolangir, Nuapada, Rayagada, Jharsuguda, Sundargarh, Kalahandi, Phulbani, Mayurbhanj and Sambalpur, and susceptible to deltamethrin in Keonjhar, Dhenkanal, Sonepur, Gajapati, Cuttack, Jagatsinghpur, Ganjam, Khurda and Baragarh districts of Odisha and all the districts of Jharkhand and West Bengal.

DISCUSSION

There are about 125 mosquito species with documented resistance to one or more insecticides[21]. Development of insecticide resistance in malaria vectors is one of the serious limitation for effective vector control for programmes that rely on chemical insecticides. Lack of new insecticide molecules with new chemistries is becoming a serious impediment for effective management of existing insecticide resistance in malaria vectors. In the present study, *An. culicifacies* was found resistant to DDT in all the 32 districts surveyed in Andhra Pradesh, Odisha, Jharkhand and West Bengal. These results are not different from the results reported so far in other studies[5–7].

An. culicifacies was reported susceptible to malathion

Table 1. Insecticide susceptibility status of *An. culicifacies* in four states of India

State	District	DDT (4%)		Malathion (5%)		Deltamethrin (0.05%)	
		% Mortality (n)	Status	% Mortality (n)	Status	% Mortality (n)	Status
Andhra Pradesh	East Godavari	36.6 (60)	R	80.0 (60)	R	70.0 (60)	R
	Khammam	23.3 (60)	R	63.3 (60)	R	43.3 (60)	R
	Srikakulam	0.0 (21)	R	44.4 (135)	R	77.7 (72)	R
	Visakhapatnam	6.6 (15)	R	46.6 (15)	R	73.6 (19)	R
	Vizianagaram	0.0 (17)	R	32.2 (62)	R	93.3 (15)	VR
Jharkhand	Gumla	26.3 (227)	R	96.9 (152)	VR	99.0 (191)	S
	Ranchi	10.4 (320)	R	98.1 (170)	S	98.1 (210)	S
	East Singhbhum	23.7 (140)	R	95.1 (110)	VR	100.0 (120)	S
	West Singhbhum	15.8 (180)	R	98.0 (170)	S	100.0 (160)	S
Odisha	Angul	9.7 (80)	R	100.0 (40)	S	96.3 (30)	VR
	Keonjhar	11.1 (40)	R	100.0 (30)	S	100.0 (20)	S
	Bolangir	7.8 (502)	R	74.4 (511)	R	96.0 (494)	VR
	Nuapada	3.3 (60)	R	93.8 (49)	VR	88.1 (59)	VR
	Dhenkanal and Sonepur	9.3 (30)	R	100.0 (20)	S	100.0 (20)	S
	Gajapati	12.6 (300)	R	70.3 (280)	R	98.0 (280)	S
	Rayagada	23.1 (272)	R	90.6 (278)	VR	89.2 (270)	VR
	Jharsuguda	12.6 (260)	R	40.0 (240)	R	96.7 (240)	VR
	Sundargarh	25.9 (280)	R	70.7 (260)	R	95.1 (260)	VR
	Kalahandi	11.8 (76)	R	78.3 (120)	R	81.6 (120)	VR
	Phulbani	6.4 (93)	R	59.1 (98)	R	93.7 (96)	VR
	Mayurbhanj and Sambalpur	14.8 (30)	R	100.0 (20)	S	96.3 (27)	VR
	Cuttack	20.0 (100)	R	74.0 (90)	R	100.0 (90)	S
	Jagatsinghpur	23.0 (100)	R	85.5 (80)	VR	100.0 (90)	S
	Ganjam	18.4 (30)	R	85.0 (20)	VR	100.0 (30)	S
	Khurda	20.0 (20)	R	80.0 (20)	R	100.0 (30)	S
	Baragarh	12.5 (300)	R	72.3 (280)	R	98.8 (340)	S
West Bengal	Bankura and Midnapur	3.3 (60)	R	88.3 (60)	VR	100.0 (40)	S
	Purulia and Birbhum	6.6 (75)	R	90.8 (65)	VR	100.0 (45)	S

Susceptibility status as per WHO criteria: Resistant (R) = <80% mortality; Verification required (VR) = 81–97% mortality; Susceptible (S) = 98–100% mortality; n = No. of mosquitoes exposed.

in villages of Haryana state[22], in Madhya Pradesh[16] and also in the present study in some districts of Jharkhand and Odisha. The species was reported in verification required category to malathion in Gumla and East Singhbhum districts of Jharkhand as reported earlier[23]. Widespread resistance to malathion was earlier reported from Maharashtra[8], Gujarat[10, 24], Uttar Pradesh[11], Madhya Pradesh[16], Andhra Pradesh[24] and Odisha (RK Hazra, RMRC, Bhubaneswar personal communication).

There are few reports of decreased susceptibility to synthetic pyrethroids in various parts of the country[12–14]. In the present study, *An. culicifacies* was found susceptible to deltamethrin in Jharkhand, West Bengal and some districts of Odisha, namely Keonjhar, Dhenkanal, Sonepur, Gajapati, Cuttack, Jagatsinghpur, Ganjam, Khurda and Baragarh. The species was reported susceptible to synthetic pyrethroids in some states like Uttar Pradesh, Maharashtra and Odisha[7, 11, 25], Gumla district of Jharkhand[23], Dantewada district of Chhattisgarh[15] and Guna district of Madhya Pradesh[16]. *An. culicifacies* was reported in verification required category in Kanker, Bilaspur, Korea and Korba districts of Chhattisgarh[15, 26], Sidhi, Shahdol, Balaghat, Betul, Chhindwara and Jhabua districts of Madhya Pradesh[16]. However, to deltamethrin, the species was reported resistant in Jagdalpur, Raipur, Dhamtari and Raigarh districts of Chhattisgarh[26], Mandla and Dindori districts of Madhya Pradesh[16] and in all the five districts of Andhra Pradesh in the present survey. Reported deltamethrin-resistance in some districts in the absence of reported use of pyrethroids in the malaria con-

trol programme needs further investigations and may be contemplated to its use in agriculture[15].

Results of this study in 32 districts in four states indicated variable levels of resistance to insecticides used in public health in this important vector. The study gains importance especially in view of the reported deltamethrin-resistance in this vector in some areas. Use of deltamethrin or other pyrethroids still remain a choice for vector control till an effective alternative insecticide is available for vector control for management of insecticide resistant vectors.

CONCLUSION

Indoor residual spraying of insecticides is the most preferred vector control option to manage vector borne diseases. Present study in 32 districts surveyed in four states indicated variable levels of insecticide-resistance to malathion and deltamethrin but resistance to DDT in *An. culicifacies*. Since, the introduction of pyrethroids in the 1980s, no new adulticide has been approved for vector control by the World Health Organization. Importantly, development of pyrethroid resistance in *An. culicifacies*, the major malaria vector in India is of concern as pyrethroids are presently the only viable choice for vector control. To overcome this, concern and for better insecticide resistance management strategies, there is a need for regular monitoring of insecticide resistance in malaria vectors.

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REFERENCES

1. Breman JG. The ears of the hippopotamus: Manifestations, determinants and estimates of the malaria burden. *Am J Trop Med Hyg* 2001; *64:* 1–11.
2. Raghavendra K, Barik TK, Reddy BPN, Sharma P, Dash AP. Malaria vector control: From past to future. *Parasitol Res* 2011; *108:* 757–79.
3. *Pesticides and their application for the control of vectors and pests of public health importance,* VI edn. Geneva: World Health Organization (WHO/CDS/NTD/WHOPES/GCDPP/2006.1). Available from: *http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_WHOPES_GCDPP_2006.1_eng.pdf*
4. Sharma VP. Fighting malaria in India. *Curr Sci* 1998; *75:* 1127–40.
5. Sharma VP, Chandrahas RK, Ansari MA, Srivastava PK, Razdan RK, Batra CP, *et al*. Impact of DDT and HCH spraying on malaria transmission in villages with DDT and HCH resistant *Anopheles culicifacies. Indian J Malariol* 1986; *23*(1): 27–38.
6. Sharma SN, Shukla RP, Raghavendra K. Susceptibility status of *An. fluviatilis* and *An. culicifacies* to DDT, deltamethrin and lambda-cyhalothrin in District Nainital, Uttar Pradesh. *Indian J Malariol* 1999; *36:* 90–3.
7. Gunasekaran K, Sahu SS, Jambulingam P, Das PK. DDT Indoor residual spray, still an effective tool to control *Anopheles fluviatilis*-transmitted *Plasmodium falciparum* malaria in India. *Trop Med Int Health* 2005; *10:* 160–8.
8. Vittal M, Bhate MR. Bioassay tests on the effectiveness of malathion spraying in Aurangabad town, Maharashtra. *Indian J Malariol* 1981; *18:* 124–5.
9. Deobhankar RK, Pelkar ND. Magnitude of DDT resistance in *Anopheles culicifacies* in Maharashtra state. *J Commun Dis* 1990; *22:* 77.
10. Yadav RL, Krishna Rao C, Biswas H. Field trial of cyfluthrin as an effective and safe insecticide for control of malaria vectors in triple insecticide resistant area. *J Commun Dis* 1996; *28:* 287–98.
11. Shukla RP, Sharma SN, Bhatt SK. Malaria outbreak in Bhojpur PHC of District Moradabad, Uttar Pradesh. *J Commun Dis* 2002; *34:* 118–23.
12. Mittal PK, Adak T, Singh OP, Raghavendra K, Subbarao SK. Reduced susceptibility to deltamethrin in *Anopheles culicifacies sensu lato* in District Ramanathapuram in Tamil Nadu: Selection of a pyrethroid resistant strain. *Curr Sci* 2002; *82:* 185–8.
13. Singh OP, Raghavendra K, Nanda N, Mittal PK, Subbarao SK. Pyrethroid resistance in *Anopheles culicifacies* in Surat district of Gujarat, west India. *Curr Sci* 2002; *82:* 547–50.
14. Sharma SK, Upadhyay AK, Haque MA, Singh OP, Adak T, Subbarao SK. Insecticide susceptibility status of malaria vectors in some hyper-endemic tribal districts of Orissa.*Curr Sci* 2004; *87:* 1722–6.
15. Bhatt RM, Sharma SN, Barik TK, Raghavendra K. Status of insecticide resistance in malaria vector, *Anopheles culicifacies* in Chhattisgarh state, India. *J Vector Borne Dis* 2012; *49:* 36–38.
16. Mishra AK, Chand, SK, Barik TK, Dua VK, Raghavendra K. Insecticide resistance status in *Anopheles culicifacies* in Madhya Pradesh, central India, *J Vector Borne Dis* 2012; *49:* 39–41.
17. *Test procedures for insecticide resistance monitoring in malaria vectors. Bio-efficacy and persistence of insecticides on treated surfaces.* Report of the WHO informal consultation. Geneva: World Health Organization 1998. WHO/CDC/MAL/98.12. p. 46
18. *Manual of practical entomology in malaria, vector bionomics and organization of antimalaria activities,* pt II. Geneva : WHO Offset Publication 1975; p. 191.
19. Wattal BL, Kalra NL. Region-wise pictorial keys to the female Indian *Anopheles*. Rev edn. *Bull Nat Soc India Malar Mosq Borne Dis* 1967; *92:* 85–138.
20. Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 1925; *18:* 265–7.
21. *Malaria vector control*. Atlanta: Centers for Disease Control and Prevention. Available from: *http://www.cdc.gov/malaria/about/biology/mosquitoes*.
22. Sharma VP, Uprety HC, Nanda N, Raina VK, Parida SK, Gupta VK. Impact of DDT spraying on malaria transmission in villages with resistant *Anopheles culicifacies*. *Indian J Malariol* 1982; *19*(1): 5–12.
23. Singh RK, Dhiman RC, Mittal PK, Das MK. Susceptibility of malaria vectors to insecticides in Gumla district, Jharkhand state,

India. *J Vector Borne Dis* 2010; *47:* 116–8.
24. Raghavendra K, Vasantha K, Subbarao SK, Pillai MKK, Sharma VP. Resistance in *Anopheles culicifacies* sibling species B and C to malathion in Andhra Pradesh and Gujarat states, India, *J Am Mosq Control Assoc* 1991; *7:* 255–9.
25. Dhiman RC, Shahi B, Sharma SN, Khargiwarkar VN, Subbarao SK. Persistence of malaria transmission in a tribal area in Maharashtra. *Curr Sci* 2005; *88*(3): 754–8.
26. Sharma SN, Shukla RP, Mittal PK, Adak T, Subbarao SK. Insecticide resistance in malaria vector *Anopheles culicifacies* in some tribal districts of Chhattisgarh, India. *Curr Sci* 2007; *92:* 1280–2.

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