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Response of malaria vectors to conventional insecticides in the southern districts of Odisha State, India

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***Background & objectives*: Updating information on response (susceptible / resistant status) of vectors to the insecticides in use is essential to formulate and introduce appropriate resistance management strategy. Therefore, a study was undertaken in the 10 southern districts of Odisha State, which are endemic for *Plasmodium falciparum* malaria, to determine the insecticide susceptibility/ resistance status of *Anopheles fluviatilis* and *An. culicifacies*, the vectors of malaria.**

***Methods*: Mosquitoes were collected during September 2010 - February 2012 from 60 randomly selected villages in the 10 districts and blood-fed females were exposed to the diagnostic dosage of DDT (4.0%), malathion (5.0%) and deltamethrin (0.05%) for one hour. Mortality was recorded at 24 h after the exposure. The test mortality was corrected to the control mortality.**

***Results*: *An. fluviatilis* was susceptible to the three insecticides tested while, *An. culicifacies* was resistant to DDT and malathion in all the 10 districts except in two, where its response against malathion was under 'verification required' category. Against deltamethrin, *An. culicifacies* was susceptible in two districts; while in the other eight districts its response was under 'verification required' category.**

***Interpretation & conclusions*: Since *An. fluviatilis* the vector species primarily associated with transmission of malaria, was still susceptible to DDT, indoor residual spraying with DDT could be continued in the 10 districts. Also, in view of the large scale implementation of long lasting insecticidal nets and the signs of development of resistance in *An. culicifacies* to deltamethrin, response of the vectors to synthetic pyrethroids needs to be periodically monitored.**

Key words *Anopheles culicifacies* - *Anopheles fluviatilis* - India - insecticide resistance - insecticide susceptibility - Odisha

Odisha State in India is afflicted with high incidence of malaria, predominantly of *Plasmodium falciparum* infection, for the last many years, and during 2010 it contributed to around 45 per cent of the total *P. falciparum* cases recorded in the country. Deaths due to cerebral malaria caused by this *Plasmodium* species are high and the data for 2005 to 2010 showed that more than 20 per cent (n = 1417) of the total deaths due to malaria in India (n = 6947) occurred in the State, although Odisha State represents only about 4 per cent of the population of the country[1]. Of the total 30 districts of Odisha State, the 10 southern districts:

Rayagada, Nowrangpur, Kalahandi, Nuapada, Bolangir, Kandhamal, Gajapati, Ganjam, Malkangiri and Koraput have seriously been affected by malaria contributing more than 70 per cent of the total positive cases and more than 64 per cent of the total malaria deaths in the State during 2011[2]. These highly malarious districts have been under the influence of two vectors, *viz. Anopheles fluviatilis* and *An. culicifacies*[3,4]. Despite various control measures, malaria continues to be a major public health problem with high morbidity and mortality even today. Currently, indoor residual spraying with DDT or synthetic pyrethroids (deltamethrin) has been carried out as the major vector control measure. In addition, long lasting synthetic pyrethroid (deltamethrin) treated mosquito nets (LLINs) have been distributed in these districts.

Development of resistance by malaria vectors to insecticides has been reported from various parts of India. DDT resistance in Odisha State in *An. culicifacies* was first reported during 1966[5]. Subsequently, there were reports of DDT and HCH resistance in this species from Koraput during 1990 and 1995[6,7] and from Sundargarh district during 1991[8]. Sharma *et al*[3] studied the susceptibility status of *An. fluviatilis* and *An. culicifacies* in eight northern and western districts of Odisha State. However, there is no information on the susceptibility status of the malaria vectors, particularly of *An. fluviatilis,* in the other highly malarious districts of the State. Since, such information is essential to formulate appropriate vector control strategy or to redesign the ongoing strategy in these districts, a study was undertaken to assess the current status of insecticide susceptibility of *An. fluviatilis* and *An. culicifacies* to DDT and deltamethrin, the insecticides in use in malaria control programme and also to malathion for cross resistance, if any, in the 10 southern districts of Odisha State.

Material & Methods

Study area: The study was carried out in the 10 southern districts of Odisha State during September 2010 - February 2012. Most of the districts are hilly and forested. Dry summer (March-June), wet rainy (July-September) and dry winter (October-February) are the three prevailing seasons. The districts have been hyper-endemic for malaria for many decades. *P. falciparum* is the predominant species, contributing to >90 per cent of the total malaria cases[2]. Malaria incidence peaks during two seasons, one during July to September and the other during November to December. *An. fluviatilis* has been incriminated as the major malaria vector[4]. Streams and terraced paddy fields are the major breeding habitats of *An. fluviatilis*[9]. *An. culicifacies,* which is a secondary vector, prevalent during summer and rainy seasons[4], prefers to breed in riverbed pools, terraced paddy fields and ponds[9].

There are altogether 115 community health centres (CHCs) in the 10 districts. Among these, 20 CHCs, two from each district, were randomly selected. In each CHC, three villages were selected randomly for collection of the vector mosquitoes to determine their susceptibility status. The study protocol was approved by the Ethical Committee of Vector Control Research Centre (VCRC), Puducherry, India.

Mosquito collections and susceptibility test: The required number of female mosquitoes of *An. fluviatilis* and *An. culicifacies* were collected from cattle sheds and human dwellings in the morning hours using mouth aspirator and flash light in the selected villages. Susceptibility tests were performed on the wild-caught blood-fed females using WHO kits[10]. The field-collected mosquitoes were provided with 10 per cent glucose solution soaked in cotton pads and brought to the camp laboratory in 1ft[5] mosquito cages wrapped with a wet towel. The temperature and relative humidity in the camp laboratory was maintained at 25 ± 2 °C and 70-85 per cent, respectively. Insecticide impregnated papers of DDT 4 per cent, malathion 5 per cent and deltamethrin 0.05 per cent were obtained from the University Sains Malaysia, Penang, Malaysia. Female mosquitoes were exposed for one hour in 3 to 4 replicates, each replicate with 15 to 20 mosquitoes, to the diagnostic dosage of the insecticides. Parallel controls for comparison were maintained. Number knocked down was recorded after one hour exposure and after the exposure the mosquitoes were maintained for 24 h with glucose food at the same temperature and relative humidity. Mortality was scored after 24 h holding period and if the control mortality remained within 5-20 per cent, the test mortality was corrected using Abbott's formula and expressed as corrected per cent mortality[11]. In case, the control mortality is >20 per cent, the tests were discarded[11]. According to the WHO criteria[10], a corrected mortality of >98 per cent is 'susceptible', <80 per cent is 'resistant' and 80-98 per cent is 'verification required'.

Results

The corrected mortality of *An. fluviatilis* on exposure to DDT 4 per cent, malathion 5 per cent and deltamethrin 0.05 per cent are given in Table I. Adequate number (minimum of 100) of *An. fluviatilis* could not

Table I. Response of *An. fluviatilis* to DDT, malathion and deltamethrin in the 10 southern districts of Odisha State

District	DDT 4%						Malathion 5%						Deltamethrin 0.05%					
	Number exposed		Number dead		CM (%)	Response	Number exposed		Number dead		CM (%)	Response	Number exposed		Number dead		CM (%)	Response
	T	C	T	C			T	C	T	C			T	C	T	C		
Rayagada	60	37	60	2	100	S	57	26	57	1	100	S	58	40	58	2	100	S
Nawarangapur	32	16	32	0	100	S	20	10	20	0	100	S	24	12	24	0	100	S
Kalahandi	79	41	79	1	100	S	62	31	62	2	100	S	79	41	79	1	100	S
Nuapada	54	27	54	1	100	S	54	27	54	1	100	S	58	29	58	0	100	S
Bolangir	56	27	56	0	100	S	54	27	54	1	100	S	54	27	54	0	100	S
Kandhamal	60	34	60	0	100	S	64	28	64	0	100	S	63	32	63	1	100	S
Gajapati	62	31	62	2	100	S	61	28	61	0	100	S	57	31	57	0	100	S
Ganjam	50	29	50	0	100	S	52	23	52	0	100	S	52	27	52	1	100	S
Malkangiri	67	32	67	1	100	S	44	33	44	1	100	S	56	41	56	0	100	S
Koraput	55	22	55	1	100	S	66	25	66	0	100	S	55	22	55	0	100	S

T, test; C, control; CM, corrected mortality; S, susceptible; R, resistant; VR, verification required

Table II. Response of *An. culicifacies* to DDT, malathion and deltamethrin in the 10 southern districts of Odisha State

District	DDT 4%						Malathion 5%						Deltamethrin 0.05%					
	Number exposed		Number dead		CM (%)	Response	Number exposed		Number dead		CM (%)	Response	Number exposed		Number dead		CM (%)	Response
	T	C	T	C			T	C	T	C			T	C	T	C		
Rayagada	102	38	17	0	16.7	R	105	44	T	C	77.6	R	108	49	T	C	89.8	VR
Nawarangapur	109	57	15	0	13.8	R	126	63	82	1	63.5	R	114	53	97	0	96.5	VR
Kalahandi	105	58	15	0	14.3	R	105	55	80	0	86.7	VR	104	67	110	0	81.7	VR
Nuapada	107	60	16	0	15.0	R	98	51	91	0	67.3	R	89	50	85	0	100.0	S
Bolangir	106	45	13	0	12.3	R	105	46	66	0	80.0	R	104	48	89	0	94.2	VR
Kandhamal	105	59	10	0	9.5	R	109	61	84	0	77.6	R	109	61	98	0	96.3	VR
Gajapati	103	44	16	0	15.5	R	105	46	85	1	83.8	VR	105	46	105	0	82.9	VR
Ganjam	102	53	15	0	14.7	R	101	47	88	0	70.3	R	104	50	87	0	95.2	VR
Malkangiri	105	59	16	0	15.2	R	110	69	71	0	75.5	R	109	77	99	0	86.2	VR
Koraput	111	37	15	0	13.5	R	111	37	83	0	76.6	R	123	71	94	0	98.4	S

T, test; C, control; CM, corrected mortality; S, susceptible; R, resistant; VR, verification required

be exposed to each of the three insecticides, because of its relatively lower density in the study area. The results indicated that *An. fluviatilis* was susceptible to DDT, malathion and deltamethrin in all the 10 southern districts.

Tables II summarizes the susceptibility status of *An. culicifacies* to DDT, malathion and deltamethrin. The corrected mortality of this species ranged between 9.5 and 16.7 per cent against DDT 4, 63.5 and 86.7 per cent against malathion 5 and 81.7 per cent and 100 per cent against deltamethrin 0.05 per cent. The results thus showed that *An. culicifacies* was resistant to DDT and malathion in all the 10 districts except in Kalahandi and Gajapati, where the response of this species against malathion was under 'verification required' category and further monitoring at periodical intervals could confirm the susceptibility/ resistance status of this species against malathion in these districts. To deltamethrin, *An. culicifacies* was susceptible in two districts *viz.* Nuapada and Koraput, while, in the remaining eight districts, its status was under 'verification required' category. Overall, the results indicated that *An. culicifacies* developed resistance to DDT and malathion, and showed an increased tolerance to deltamethrin, as the corrected mortality of this species fell under 'verification required' category in most of the districts.

Discussion

Chemical control of vectors continues to be the mainstay of the malaria control programme in India. Monitoring vector susceptibility to the insecticides at regular interval has become imperative to ensure judicious and effective use of insecticides in the control programme. The present findings highlight the current status of insecticide susceptibility / resistance status of malaria vectors in the southern districts of Odisha State, which are predominantly inhabited by tribes and hyperendemic for *P. falciparum* malaria. The primary role of *An. fluviatilis* and the secondary importance of *An. culicifacies* in transmission of malaria in the study area were established during nineties[3,4]. The earlier studies have reported that *An. fluviatilis* was susceptible to DDT, malathion and deltamethrin in Koraput[6,7] and Sundergarh district[8] of Odisha State. Sharma *et al*[3] also reported its susceptibility to the three insecticides in five other districts of Odisha State including Kalahandi and Phulabani (presently called as Kandhamal) which are among the 10 southern districts covered by the current study. However, resistance to DDT in this vector species has been reported from Puri and Balasore districts of Odisha[12]. Kumari *et al*[12] reported development of resistance by this species to DDT in 11 districts from eight States in India. Recently, DDT resistance in *An. fluviatilis* has been reported from Jharkhand[13,14]. In the current study, *An. fluviatilis* was found to be still susceptible to DDT, malathion and deltamethrin in all the 10 southern districts of Odisha. Further, *An. fluviatilis* is mainly distributed in hill-top, foot-hill and forested villages in India and its preferential breeding habitat is streams[15], which are less likely to be exposed to agriculture pesticides.

An. culicifacies was resistant to DDT and malathion in all the 10 southern districts except in two, where its mortality against malathion fell under 'verification required' category. To deltamethrin, this species was found susceptible in two districts while in the other eight districts, its response was under 'verification required' category indicating that this species was tending to develop resistance to deltamethrin. The earlier studies in Koraput district showed that *An. culicifacies* was resistant to DDT but susceptible to malathion 5 per cent and deltamethrin 0.025 per cent[6,7]. Subsequent studies carried out during 2004 in eight districts of Odisha State including the five districts covered in the current study reported that *An. culicifacies* was resistant to DDT in all the eight districts, to malathion in four districts (Mayurbhanj, Bolangir, Nuapada and Kalahandi) and was showing signs of development of multiple resistance to DDT, malathion and deltamethrin in three districts (Bolangir, Nuapada and Kalahandi)[3].

Double or triple resistance to DDT, dieldrin and malathion was reported in *An. culicifacies* from 30 districts of Maharashtra[16]. Malathion resistance in this species was first observed in the adjoining State of Gujarat[17]. Subsequent report of resistance to malathion came from Andhra Pradesh[18]. In Dhanora taluka of Gadchiroli district in Maharashtra, *An. culicifacies* was resistant to DDT, but found susceptible to malathion and deltamethrin[19]. In Murumgaon PHC area of Gadchiroli district in Maharastra *An. culicifacies* was found resistant only to DDT, while it was tolerant to malathion and deltamethrin[20]. There are also reports of *An. culicifacies* showing resistance to synthetic pyrethroids in Tamil Nadu and Gujarat[21,22], indicating the possibility of widespread resistance to other related compounds of this group. Synthetic pyrethroids are the potent insecticide most commonly used for indoor residual spraying, space spraying and for impregnating

bednets under vector control programme. Synthetic pyrethroids are highly effective, if optimally applied, but development of resistance to these insecticides reduces their impact[23]. Although, *An. culicifacies* is only a secondary vector in the study area, the sign of development of resistance by this species to deltamethrin may pose certain amount of threat to the ongoing vector control programme. Therefore, regular monitoring is required for early detection of development of resistance by this species to deltamethrin and for assessing its epidemiological impact. The density of *An. fluviatilis* in the study area was low and, therefore, as per the WHO criteria[10], the minimum number of 100 mosquitoes of this species could not be exposed to the insecticides in each district due to non-availability of adequate number in the field.

Two important conclusions could be derived from the current study. The first one is that *An. fluviatilis,* the major vector of malaria in the study area was susceptible to DDT and synthetic pyrethroids, the presently used insecticides; while DDT has been used for indoor residual spraying, synthetic pyrethroids are used both for indoor residual spraying and impregnating bednets under public health programme in these districts. Therefore, indoor residual spraying with DDT could be continued but by ensuring adequate coverage and quality through strengthening the advance information system. Also, compliance of bednet use by the community should be enhanced. While advocating use of bednets, it is necessary to insist upon regular use and to focus malaria prevention as the benefit of using nets, because, people who used bed nets as protection against mosquito bites were more likely not to use these when mosquitoes were few than those who used bed nets for malaria protection[24,25].The second conclusion is that in view of resistance developed by *An. culicifacies* to DDT and malathion and of its 'verification required' status to deltamethrin, monitoring susceptibility of this species to synthetic pyrethroids, which are currently being used in the malaria control programme, is essential for a rationalized use of insecticides for vector control.

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