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Triple insecticide resistance in *Anopheles culicifacies*: a practical impediment for malaria control in Odisha State, India

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***Background & objectives*: In Odisha State, the control of malaria vectors has become dependent on synthetic pyrethroids, which are used for treatment of all approved long-lasting insecticidal nets (LLINs). The vast use of just one class of insecticide has led to the problem of resistance to insecticides in malaria vectors. One of the major malaria vectors in Odisha State is *Anopheles culicifacies* Giles. The aim of this study was to determine the resistance status of *An. culicifacies* to deltamethrin, a synthetic pyrethroid and other common insecticides used by the National Vector Borne Diseases Control Programme (NVBDCP) for indoor residual spraying in Odisha State.**

***Methods*: Mosquitoes were collected during April 2014 - June 2014 from 15 randomly selected villages in five *Plasmodium falciparum* endemic southern districts of Odisha State. The blood-fed wild caught 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.**

***Results*: Results indicated that *An. culicifacies* was resistant to all the three insecticides used in the malaria control programme in the five districts of Odisha State.**

***Interpretation & conclusions*: Resistance management strategy by appropriate rotation of different groups of insecticides including carbamates and incorporating a synergist with synthetic pyrethroids for treating mosquito nets should be considered for the control of malaria vectors in the area, especially where *An. culicifacies* is predominant. Periodical monitoring of susceptibility/resistance status of *An. culicifacies* to different insecticides is warranted.**

Key words *Anopheles culicifacies* - India - insecticide resistance - malaria - Odisha - vector

Use of insecticides for reducing populations of malaria vectors has been the main strategy for control of malaria in India. Currently, 12 insecticides belonging to four chemical classes are recommended by WHO Pesticide Evaluation Scheme (WHOPES) for indoor residual spraying (IRS)[1]. The current strategy to control malaria vectors in India mainly includes IRS with DDT/synthetic pyrethroids and use of long lasting insecticide nets (LLINs)[2,3]. *Anopheles culicifacies* (Diptera: Culicidae) is the widespread malaria vector species found throughout the rural and peri-urban areas, contributing to about 65 per cent of malaria cases in India[4]. In the beginning, indoor residual spraying with DDT was used for the control of this species.

But, gradually *An. culicifacies* developed resistance against DDT and it was the first mosquito species to develop resistance against this insecticide[5]. Soon after the introduction of malathion in the country in 1969, especially in DDT resistant areas, double resistance was reported in *An. culicifacies* in Gujarat and Maharashtra States in 1977[6]. In 1990s deltamethrin was introduced in the country for indoor residual spraying and treatment of mosquito nets. Consequently, reduced susceptibility to deltamethrin in *An. culicifacies* was reported for the first time from Ramnathapuram district, Tamil Nadu in 2002[7].

Studies have been carried out on the susceptibility status of *An. culicifacies* in different parts of Odisha State[8]. The studies carried out during 2004 in eight districts of the State reported that *An. culicifacies* was resistant to DDT in all the eight districts, to malathion in four districts (Mayurbhanj, Bolangir, Nuapada and Kalahandi) and showed a tendency of developing multiple resistance to DDT, malathion and deltamethrin in three districts (Bolangir, Nuapada and Kalahandi)[9]. An entomological study was conducted in ten southern districts of the State when LLINs were just distributed and it showed that in eight districts, the response of *An. culicifacies* to deltamethrin was under 'verification required' category indicating its tendency towards development of resistance to deltamethrin[8]. In view of this indication of tolerance of this species to the synthetic pyrethroid in the event of four to five years of field use of LLINs in the districts, a study was undertaken in five of the ten southern districts of Odisha State to verify the susceptibility/resistance status of *An. culicifacies* to deltamethrin and also to the other two commonly used insecticides *i.e.* DDT and malathion.

Material & Methods

The study was conducted in five southern districts of Odisha State *viz*. Rayagada, Nowrangpur, Kalahandi, Malkangiri and Koraput. *An. culicifacies* was tested against the diagnostic dosage of DDT, malathion and deltamethrin during April to June 2014. Dry summer (March-June), wet rainy (July-October) and dry winter (November-February) are the three prevailing seasons. The districts have been hyperendemic for malaria since many years[8]. *Plasmodium falciparum* is the predominant species, with a proportion of >90 per cent of the total malaria cases[8,10]. *Anopheles fluviatilis* and *An. culicifacies* are the two malaria vector species prevalent in the five districts[8,11]. The villages in the districts are situated on foothills or plains and are characterized by presence of perennial streams, rivulets, ponds and paddy fields, which are the major breeding habitats of the two vector species. Majority of the villagers live in huts made of mud walls with either thatched or tiled roofs. The walls of the hut are usually of seven feet in height, with a gap of 2 to 3 feet between the side walls and roof. The cattle sheds are kept close by human dwellings. Human dwellings are sprayed with two rounds of DDT or synthetic pyrethroids every year. In addition, long lasting synthetic pyrethroid (deltamethrin) treated mosquito nets (LLINs) have been distributed in these districts since 2009 in a phased manner.

One community health centre (CHC) from each of the five districts was randomly selected. In each CHC, three villages, representing the respective CHC with reference to the density of *An. culicifacies,* were randomly selected for collection of *An. culicifacies* to determine its susceptibility/resistance status[8].

Mosquito collections and susceptibility test: Female *Anopheles* mosquitoes were collected from cattle sheds and human dwellings in the study villages during early morning hours using mouth aspirator and flash light. The collected mosquitoes were transported to the laboratory at Vector Control Research Centre, Field Station, Koraput, Odisha, in a mosquito cage (one cubic foot) covered with a wet towel, identified to species based on morphological characters using a standard key[12] and separated according to their gonotrophic conditions. WHO kits were used to conduct the susceptibility test[3]. The temperature and relative humidity (RH) in the laboratory was maintained at 25 ± 2 °C and 70-85 per cent RH. Papers impregnated with DDT 4 per cent, malathion 5 per cent and deltamethrin 0.05 per cent were obtained from the University Sains Malaysia, Penang, Malaysia. The fully fed mosquitoes were exposed to the diagnostic dosage of the insecticides for one hour in 5 to 6 replicates, each replicate with 15 to 25 mosquitoes. Parallel controls were maintained for comparison (*i.e.* 2 to 3 replicates each with 15 to 25 mosquitoes)[3]. Number knocked down was recorded after one hour of exposure and after the exposure the mosquitoes were maintained in holding tubes with access to glucose food for 24 h at the same temperature and relative humidity. Mortality was scored after 24 h of holding. Since the control mortality in all the tests remained below 5 per cent, the test mortality was not corrected using Abbott's formula[13]. According to the WHO criteria[3], a corrected mortality of >98 per cent is

'susceptible', <90 per cent is 'resistant' and 90-98 per cent is 'verification required'.

Results & Discussion

A total of 135, 130 and 131 female *An. culicifacies* mosquitoes in Rayagada, 105, 110 and 113 in Nawarangapur, 105, 111 and 131 in Kalahandi, 111, 105 and 119 in Malkangiri and 111,111 and 112 in Koraput district were exposed to DDT 4 per cent, malathion 5 per cent and deltamethrin 0.05 per cent, respectively. The test mortality of this vector species against the three insecticides are given in the Table. The test mortality ranged between 11.4 and 15.3 per cent against DDT 4 per cent, 60.4 and 76.2 per cent against malathion 5 per cent, and 72.6 and 84.0 per cent against deltamethrin 0.05 per cent in these districts. The results showed that *An. culicifacies* was resistant to DDT, malathion and deltamethrin in all the five districts.

Under the modified plan of operation implemented in 1977, the study districts continued to receive DDT indoor residual spraying, since annual parasite incidence (API) was >2 in all the districts[14]. From 2001 onwards, DDT was replaced with synthetic pyrethroids (deltamethrin/alphacypermethrin/lambdacyhalothrin) for indoor residual spraying in five of the 11 CHCs in Rayagada district and in seven of the 13 CHCs (where API >10) in Kalahandi district. (State National Vector Borne Diseases Control Programme Office, Bhubaneshwar, personal communication). Insecticide treated mosquito nets (ITMNs) were introduced during 2001 to 2008 in some of the CHCs of the five districts. In addition, long lasting synthetic pyrethroid (deltamethrin) treated mosquito nets (LLINs) were distributed in these districts from 2009 to 2013 in a phased manner.

An. culicifacies was reported to be resistant to DDT but susceptible to malathion and deltamethrin[15] during 1995 in Koraput district. Subsequently, during 2010-2011 the response of this species to deltamethrin in Rayagada, Kalahandi and Malkangiri districts was found to be resistant, in Nawarangpur district was under the 'verification required' category and in Koraput district was susceptible[8] as per the guidelines of WHO, 2013[3]. Resistance to DDT and malathion in *An. culicifacies* was confirmed during the current study. In addition, the current study confirmed the development of resistance in this species to deltamethrin in the five southern districts of the State. *An. fluviatilis,* the other malaria vector in the study area was susceptible to DDT, malathion and deltamethrin[8,15]. However,

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

District	DDT 4%						Malathion 5%						Deltamethrin 0.05%					
	Number exposed		Number dead		TM (%)	Response	Number exposed		Number dead		TM (%)	Response	Number exposed		Number dead		TM (%)	Response
	T	C	T	C			T	C	T	C			T	C	T	C		
Rayagada	135	58	17	0	12.6	R	130	66	82	0	63.1	R	131	55	107	01	81.7	R
Nowrangpur	105	63	12	0	11.4	R	110	55	78	01	70.9	R	113	64	82	0	72.6	R
Kalahandi	105	63	13	0	12.4	R	111	67	67	0	60.4	R	131	55	104	0	79.4	R
Malkangiri	111	62	14	0	12.6	R	105	63	80	0	76.2	R	119	51	100	0	84.0	R
Koraput	111	64	17	0	15.3	R	111	62	74	0	66.7	R	112	56	86	0	76.8	R

T, test; C, control; TM, treated mortality; R, resistant

the susceptibility status of *An. fluviatilis* could not be assessed during the current study due to non-availability of adequate number of this species from the study area.

During the early part of 20th century, indoor residual spraying of insecticides, particularly DDT, was the key component of malaria control and was responsible for the spectacular reduction in malaria incidence[16]. Over time and with widespread use in agriculture, mosquito resistance to DDT had emerged[17]. Between 1961 and 1966, the malaria rate in India increased three-fold; DDT resistance was a partial cause for this[17,18]. Several studies have been conducted on vector susceptibility to insecticides in different parts of the country[18,19]. Resistance to DDT in *An. culicifacies* is widespread in the country[20-22] and to malathion in the States of Maharashtra, Gujarat, Tamil Nadu and Uttar Pradesh[6,23-25]. There are a few reports of decreased susceptibility in this vector to synthetic pyrethroids[7,9,26]. This species has developed resistance to DDT in 286 districts, to malathion in 81 and to pyrethroids in two districts in India[27]. A recent study conducted in four States (Andhra Pradesh, Odisha, Jharkhand and West Bengal) of India showed that *An. culicifacies* was resistant to DDT in 32 districts and to malathion in 14 districts, under verification required category in 10 districts and susceptible in eight districts. To deltamethrin this species was resistant in four districts, under verification required category in 11 districts and susceptible in 17 districts[28].

One of the limitations of the study was that parallel data could not be generated for *An. fluviatilis* due to its non-availability in adequate number. Considering operational implication, generation of data was not done up to sibling species level in *An. culicifacies,* which might be another limitation. The spread of insecticide resistance, especially pyrethroid resistance as indicated by the current study and also the studies conducted in other parts of the country, is a major threat for the vector control programmes, as it is being extensively used for IRS and impregnation of bed nets in India. In this context, preservation of pyrethroid susceptibility in target vector population should be the key priority in the choice of vector control methods at this hour. Insecticide resistance management needs to be considered for vector control, including the selection of insecticides for IRS. Using the same insecticide for multiple successive IRS cycles may not be recommended; it is preferable to use a system of rotation with different groups of insecticides including carbamates in the area where *An. culicifacies* is resistant to deltamethrin[29]. Rotations should start with the insecticides to which there is the lowest frequency of resistance. In the areas of high coverage with LLINs, pyrethroids may not be a good option for IRS, as this will add to selection pressure. Further, using nets impregnated with a synthetic pyrethroid together with a synergist would be a better option against pyrethroid resistant malaria vectors. Mixture nets such as this may have application against resistant mosquitoes, particularly those whose resistance is based on oxidative metabolism[30]. Since, monitoring insecticide resistance is a necessary element of the implementation of insecticide-based vector control interventions, regular monitoring of susceptibility/resistance status of the malaria vectors to commonly used insecticides, at least once a year or preferably every six months[3] is warranted particularly in *An. culicifacies* predominant areas to strengthen the evidence base for the effectiveness of ongoing vector control interventions.

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Conflicts of Interest: None.

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