Short Research Communications

Insecticide resistance and human blood meal preference of *Anopheles annularis* in Asom-Meghalaya border area, northeast India

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Although, the increased use of insecticide-treated nets (ITNs) and indoor residual spray (IRS) have made significant decrease in the number of malaria cases, but these efforts are subsided by the development and spread of insecticide resistance among major malaria vectors[1]. Currently, DDT and synthetic pyrethroids are used widely for IRS and ITNs throughout the malaria endemic countries including India, which insist unconditional need of regular monitoring of the insecticide resistance in mosquito vectors. *Anopheles* (*Cellia*) *annularis* Van der Wulp is widespread in Asia and recently emerged as an important vector of malaria in India and neighbouring countries[1–2]. In northeastern states, *An. annularis* is abundant and recently has been presumed to be an important vector of malaria in addition to *An. minimus* and *An. dirus*[2–3]. Continuous and indiscreet use of DDT for IRS has led to the development and spatial spread of physiological resistance among many efficient malaria vectors in India[4–6].

Furthermore, the use of synthetic pyrethroids in ITNs and long-lasting insecticidal nets (LLINs) has also led to the development of resistance in some known mosquito vectors[5–6]. Although metabolic mechanisms play a major role in conferring resistance, behavioural changes in the vector mosquito population might have an impact on the efficacy of the insecticides[7]. Insecticide resistance is a dynamic phenomenon and the resistance level among the mosquito species differ even between the nearby areas[8]. Hence, the extrapolation of insecticide resistance results from one geographical area to another may be inappropriate. In addition to monitoring insecticide resistance among wild caught *Anopheles* mosquitoes, human host preference and malaria parasite detection remain integral components in understanding the transmission dynamics. Such data provide vulnerable parameters for estimating transmission intensity and serve as a relative measure of the disease risk in an area of interest.

Anopheles annularis is primarily considered as a zoophilic mosquito, however, its host preference and incrimination data in many parts of India are still scanty and only a few systematic studies have been carried out previously[2, 9]. Identification of human host preference and vectorial status are useful in understanding the role of different *Anopheles* mosquitoes in malaria transmission at local level. The present study was carried out to collect information on DDT and deltamethrin susceptibility status, human host preference and possible role in malaria transmission of *An. annularis* mosquito along Asom-Meghalaya border in northeast India.

This study was conducted at five villages each in Chandubi and Rani areas (GPS location: 25° 52' 23" N to 91° 26' 36" E) along Asom-Meghalaya border area in Khasi hills during June–August 2011 (Fig. 1). Ecologically, Chandubi area has predominately mixed thicket and dense forest, whereas Rani area is relatively plain interspersed with precambrian residual hills covered with thin forest and settlement areas. Humid climate, vast paddy fields, irrigation drains and duck rearing ponds provide suitable environment for vector mosquito breeding. Adult *Anopheles* mosquitoes were collected inside the human houses using CDC miniature light-trap model 512 (John W. Hock Inc., USA), installed for at least 12 h (1800 to 0600 hrs). A total of four trappings were conducted at four randomly selected houses in each of the study villages. The mosquitoes landed on the wall, roofs, wooden pillars and other temporary structures including clothings and bicycles which were kept inside the houses were collected using hand held aspirators (John Hock, USA) and torch-light. The collected *Anopheles* mosquitoes were identified to species based on morphological characteristics.
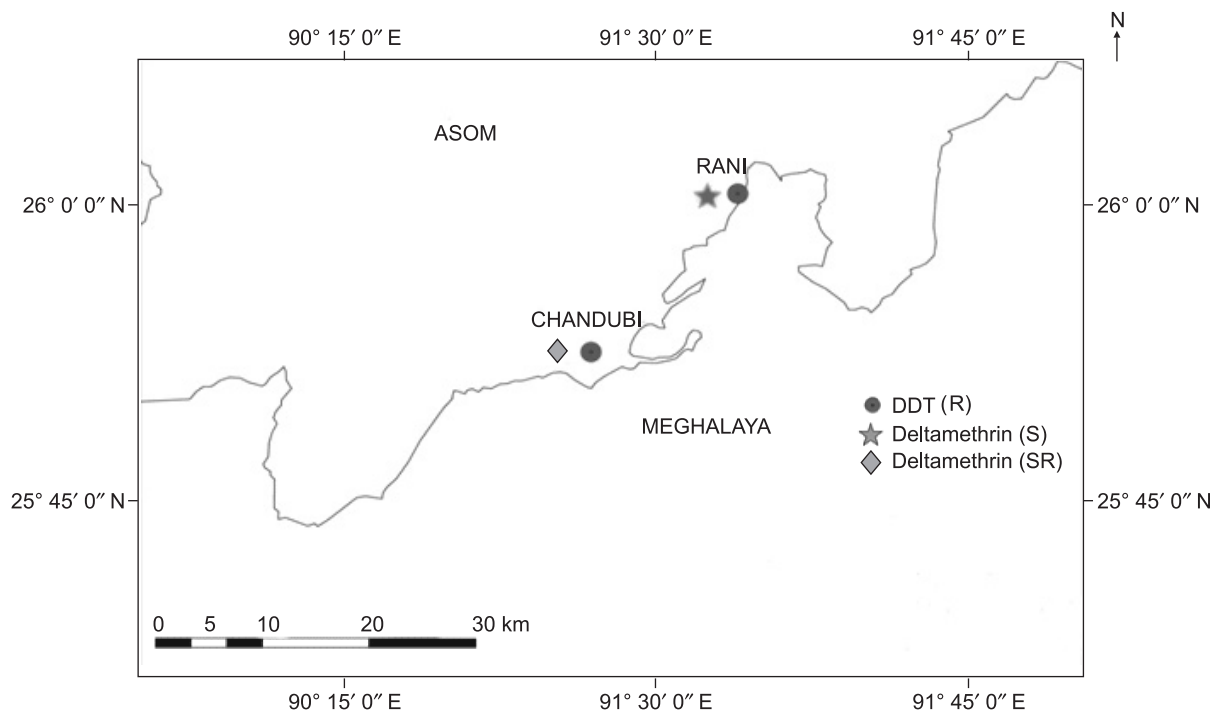
To monitor the susceptibility status against DDT and

Fig. 1: Study area indicating DDT and deltamethrin resistance in wild caught *An. annularis* mosquitoes.

deltamethrin, wild collected unfed adult female mosquitoes in the batches of 10–15 number per batch were exposed to 4% DDT and 0.05% deltamethrin-impregnated papers for 1 h. Knockdown times (KDT) were determined by monitoring the number of knockdown mosquitoes at every 10 min interval. Mortality was recorded after 24 h of exposure and corrected using Abbott's formula. All the study areas had a round of DDT IRS during April–May 2011. The behavioural resistance was estimated by comparing the number of *An. annularis* mosquitoes that were collected from unsprayed temporary structures and from those usually sprayed such as walls and permanent structures including pillars and roofs. Immediately after resistance assay, the selected mosquitoes were stored in 1.5 μl eppendorf tube in silica gel for molecular study. DNA of *An. annularis* mosquitoes was extracted using QIA amp DNA mini kit (Qiagen, Hilden, Germany) following manufacturer's instructions. PCR assay for human host preference, for *Plasmodium* parasite detection and nested PCR for *Plasmodium* species identification were carried out with primers described previously[10–11].

Anopheles mosquito density for both the study areas was expressed in per trap night density (PTND). Corrected mortality rates <80% indicated resistant >98%, fully sensitive and ranging between >80 and <98% indicated tolerant to an insecticide. Student's *t*-test was used to compare the knockdown rates among both the insecticides at different time intervals. KDT_{50} and KDT_{95} for the mosquito vectors were determined by log-probit method using Ldp Line computer programme. Fitment of probit was assessed using Chi-square test, where the overall significance of the multiple-tests was determined following Bonferroni procedure[12]. Human dwellings, resting collection among different landing sites was compared using Chi-square test following Yates correction.

Of the total, 534 known *Anopheles* vector species collected, *An. annularis* (23.8%) and *An. philippinensis/nivipes* (24.3%) were predominant with a PTND of 4 and 4.1, respectively. However, the efficient vector *An. minimus* was recorded in very low number (PTND = 1.4) during the study. The results of insecticide susceptibility tests on *An. annularis* against DDT and deltamethrin in Chandubi and Rani areas are given in Table 1. The results indicate that *An. annularis* was resistant to DDT in both the study areas as the corrected mortality recorded was 28.3 and 11.9% in Chandubi and Rani areas, respectively whereas, for deltamethrin, 97.7% mortality was recorded in Chandubi area and 98.1% in Rani area indicating that *An. annularis* was completely susceptible in Rani area. The DDT sensitivity level of *An. annularis* varied between both the study areas as the corrected mortality was found to differ significantly ($t = 2.7$; $p = 0.04$), whereas, in case of deltamethrin the difference was statistically insignificant ($t = 0.1$; $p = 0.9$). Knockdown percentage in *An. annularis* post 10 min exposure between

Table 1. DDT and deltamethrin resistance in *An. annularis* in Khasi hill region of Asom-Meghalaya border, northeast India

Insecticide	Location	N	KDT_{50}	KDT_{95}	χ^2 (p)	r	m	M
DDT (4%)	Chandubi area	60	299.6	12642.2	0.4 (0.9)	1	1	28.3
	Rani area	43	207.6	11540.5	0.5 (1)	1	0.9	11.9
Deltamethrin (0.05%)	Chandubi	43	8.2	34.6	0.6 (0.4)	1	2.6	97.7
	Rani area	54	8.8	53.7	1.4 (0.7)	1	2.1	98.1

N—Total number; KDT—Knockdown time (min); r—Correlation coefficient; m—Slope; M— % corrected mortality.

both the study areas was similar for both the insecticides ($t \geq 0.45$; $p \geq 0.51$).

Similarly, the KDT_{50} values for both DDT and deltamethrin did not differ between both the study sites ($t = 0.8$; $p = 0.4$ for DDT and $t = 0.6$; $p = 0.6$ for deltamethrin). Probit model used to estimate KDT_{50} and KDT_{95} values displayed normal distribution of percentage knockdown with time for both DDT and deltamethrin. The hand collection of *Anopheles* mosquitoes using aspirators revealed that after taking blood meal, *An. annularis* preferred resting on the roofs of the human dwellings and clothings put inside the houses as compared to the insecticide sprayed walls and pillars. The density of *An. annularis* resting on the probable insecticide unsprayed areas (85.2%) was significantly higher as compared to that resting on the sprayed areas ($\chi^2 = 57.8$; $p < 0.0001$; OR=33.4; 95% CI=12.3–90.8). No significant difference was observed between *An. annularis* collected among insecticide unsprayed areas and sprayed areas in both the study sites ($\chi^2 = 0.01$; $p = 0.91$; RR = 1.1; 95% CI =

Fig. 2: Human blood preference of wild caught *An. annularis* (Lane 1: 50 bp ladder; Lanes 2, 4 & 5: Negative for human blood; Lanes 3, 6 & 8: Positive for human blood; and Lane 7: Negative control).

0.70–1.83). Also, the total density of known *Anopheles* vector collected from insecticide unsprayed areas (77.7%) in the study was significantly higher than those collected from insecticide sprayed areas ($\chi^2 = 55.3$; $p < 0.0001$; OR = 12; 95% CI = 6.1–24).

A total of 38 blood-fed female *An. annularis* mosquitoes were tested for human blood preference, which showed that 8 (21.1%) were fed on human blood (Fig. 2). Overall anthropophilic index (AI) recorded at Chandubi and Rani areas was 23.8 and 17.6, respectively and did not differ statistically ($\chi^2 = 0$; $p = 0.95$; RR = 1.2; 95% CI = 0.6–2.2). The same 38 mosquitoes were also tested for the presence of *Plasmodium* parasite, which revealed only one specimen collected from Chandubi area was detected positive for *P. falciparum* (infection rate = 2.6). Mosquito resistance to at least one insecticide used for malaria control has been identified in 64 countries worldwide. WHO has strongly recommended that monitoring of insecticide resistance is a necessary element of the implementation of insecticide-based vector control interventions. Present results have demonstrated the development of physiological and behavioural resistance against DDT in *An. annularis* mosquitoes. Many studies have indicated that the development of metabolic resistance against DDT in India, however none has evidenced the behavioural avoidance to DDT[4–6, 13]. DDT is used in IRS since long and may be effective against some efficient malaria vectors, but behavioural avoidance might prevent mosquitoes to come in contact with DDT sprayed areas. Previous studies have indicated that potential malaria vector *An. minimus* and known JE vectors were completely susceptible to deltamethrin, while their susceptibility to DDT was considerably reduced[14–15].

Although, *An. annularis* was found to be susceptible to deltamethrin in Rani area, but cent percent mortality could not be achieved. The results indicate that *An. annularis* has developed low level of deltamethrin resistance in the study areas. The development of deltamethrin resistance in malaria vectors is a serious concern for control programme because synthetic pyrethroids are widely used in bednet impregnation and public health
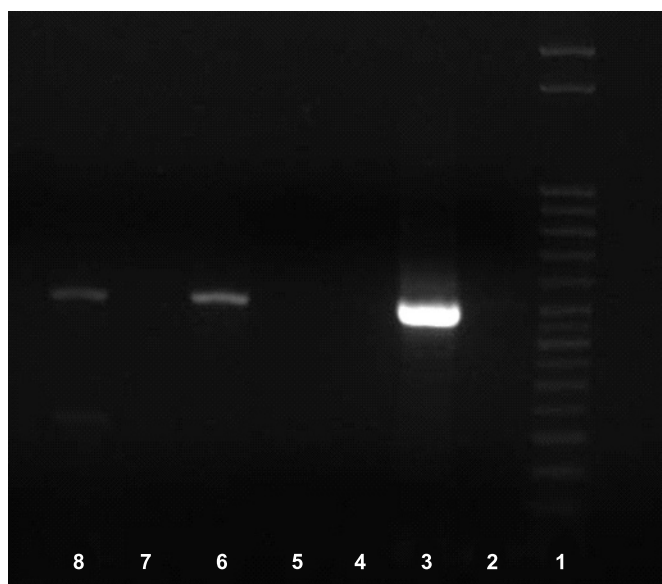
programmes to control multiple-resistant vectors[4–6]. Human host preference results suggest that a considerable number of *An. annularis* was found to fed on human blood, indicating that this supportive malaria vector might be shifting its feeding preference towards humans from animals. One specimen of *An. annularis* was detected positive for *P. falciparum* infection. A recent study conducted in Asom has shown that 14.3% of human blood-fed *An. annularis* mosquitoes were positive for *Plasmodium* infection[2]. Although, the present results do not claim to incriminate *An. annularis* as a malaria vector, but provide convincing evidences that it may be a vector of malaria in the study area.

The present study, although includes small sample size, but provides important information on vector control and malaria transmission in an area which is difficult to access and suffer ethnic conflicts frequently. Further, it augments the scanty available data on behavioural resistance of malaria vectors in India.

ACKNOWLEDGEMENTS

REFERENCES

1. Prakash A, Bhattacharyya DR, Mohapatra PK, Mahanta J. Role of the prevalent *Anopheles* species in the transmission of *Plasmodium falciparum* and *P. vivax* in Asom state, northeastern India. *Ann Trop Med Parasitol* 2004; *98:* 559–68.

2. Dhiman S, Bhola RK, Goswami D, Rabha B, Kumar D, Baruah I, *et al*. Polymerase chain reaction detection of human host preference and *Plasmodium* parasite infections in field collected potential malaria vectors. *Pathog Glob Health* 2012; *10*(3): 177–80.

3. Dev V, Sharma VP. The dominant mosquito vectors of human malaria in India. In: Manguin Sylvie, editor. *Anopheles mosquitoes: New insights into malaria vectors.* Croatio: In Tech. Rijeka 2013; p. 239–71.

4. 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.

5. 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:* 36–8.

6. Singh OP, Raghavendra K, Nanda N, Mittal PK, Subbarao SK. Pyrethroid resistance in *An. culicifacies* in Surat district, Gujarat, west India. *Curr Sci* 2002; *82*(5): 547–50.

7. Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: What are the implications for malaria control?. *Trends Parasitol* 2011; *27*(2): 91–8.

8. Kamau L, Vulule JM. Status of insecticide susceptibility in *An. arabiensis* from Mwea rice irrigation scheme, central Kenya. *Malar J* 2006; *5:* 46.

9. Alam MS, Khan MGM, Chaudhury N, Deloer S, Nazib F, Bangali AM, *et al*. Prevalence of anopheline species and their *Plasmodium* infection status in epidemic-prone border areas of Bangladesh. *Malar J* 2010; *9:* 15.

10. Mohanty A, Kar P, Mishra K, Singh DV, Mohapatra N, Kar SK, *et al*. Multiplex PCR assay for the detection of *An. fluviatilis* species complex, human host preference, and *P. falciparum* sporozoite presence, using a unique mosquito processing method. *Am J Trop Med Hyg* 2007; *76*(5): 837–43.

11. Johnston PS, Pieniazek NJ, Xayavong MV, Slemenda SB, Wilkins PP, Silva AJ. PCR as confirmatory technique for laboratory diagnosis of malaria. *J Clin Microbiol* 2006; *44*(3): 1087–9.

12. Finney DJ. *Probit analysis*, III edn. Cambridge, U.K.: Cambridge University Press 1971.

13. Mishra AK, Chand SK, Barik TK, Dua VK, Raghavendra K. Insecticide resistance status in *An. culicifacies* in Madhya Pradesh, Central India. *J Vector Borne Dis* 2012; *49:* 39–41.

14. Baruah K, Lal S. A report on the susceptibility status of *An. minimus* (Theobald) against DDT and deltamethrin in three districts of Assam. *J Vector Borne Dis* 2004; *41:* 42–4.

15. Dhiman S, Rabha B, Talukdar PK, Das NG, Yadav K, Baruah I, *et al*. DDT and deltamethrin resistance status of known Japanese encephalitis vectors in Assam, India. *Indian J Med Res* 2013; *138:* 988–94.

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