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Status of DDT and pyrethroid resistance in Indian *Aedes albopictus* and absence of knockdown resistance (*kdr*) mutation

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ABSTRACT

Background & objectives: Aedes albopictus is one of the vectors for dengue and chikungunya and emergence of pyrethroid resistance in this species could be of a major concern in controlling the vector. This study reports insecticide susceptibility status of *Ae. albopictus* to DDT and pyrethroids in some Indian populations and status of presence of knockdown resistance (*kdr*) mutations.

Methods: Three to four day old adult female *Ae. albopictus* collected from Delhi, Gurgaon (Haryana), Hardwar (Uttarakhand), Guwahati (Assam) and Kottayam (Kerala) were bio-assayed with DDT (4%), permethrin (0.75%) and deltamethrin (0.05%) impregnated papers using WHO standard susceptibility test kit. Mosquitoes were PCR-genotyped for F1534C *kdr*-mutation in the voltage-gated sodium channel (VGSC) gene. DDT and pyrethroid resistant individuals were sequenced for partial domain II, III and IV of VGSC targeting residues S989, I1011, V1016, F1534 and D1794 where *kdr* mutations are reported in *Ae. aegypti*.

Results: Adult bioassays revealed varying degree of resistance against DDT among five populations of *Ae. albopictus* with corrected mortalities ranging between 61 and 92%. Kerala and Delhi populations showed incipient resistance against permethrin and deltamethrin respectively. All other populations were susceptible for both the synthetic pyrethroids. None of the *kdr* mutations was detected in any of DDT, deltamethrin and permethrin resistant individuals.

Interpretation & conclusion: Ae. albopictus has developed resistance against DDT and there is emergence of incipient resistance against pyrethroids in some populations. So far, there is no evidence of presence of knockdown resistance (*kdr*) mutation in *Ae. albopictus*.

Key words *Aedes albopictus*; chikungunya; dengue; India; knockdown resistance; pyrethroid; voltage-gated sodium channel

INTRODUCTION

Dengue and chikungunya, the two arboviral infections transmitted by *Aedes* (Diptera: Culicidae) mosquitoes, have emerged as major public health problems around the world, particularly in tropical and subtropical countries including India[1-4]. *Aedes aegypti* and *Ae. albopictus* are two important vectors for these two arboviral infections. As no specific vaccine or drug is available for dengue and chikungunya infections, their control solely relies on the control of vector populations or reduction in human-vector contact. In recent times, pyrethroid based aerosols, liquidators, mats, mosquito coils and indoor space sprays are being widely used for *Aedes* control. In addition, synthetic pyrethroids have emerged as insecticides of choice for vector control because of their rapid knockdown effect, low mammalian toxicity and degradability in environment. This is the only class of insecticides recommended by World Health Organization (WHO) for treating mosquito nets[5]. In India, pyrethrum extract and malathion are used for fogging and focal space spraying during dengue and chikungunya epidemics to bring down the *Aedes* adult populations[6].

Emergence of pyrethroid resistance in *Aedes* is a serious threat to control chikungunya and dengue epidemics. Pyrethroid resistance in *Ae. albopictus* has emerged in various parts of the world[7–10], however, pyrethroid resistance hasn't been reported from India though resistance to DDT has been reported[11–15]. Recently, a *kdr* mutation (F1534C) has been reported in this species in high frequency in Singapore where use of permethrin for dengue control is very common[16].

DDT and pyrethroids act on the voltage-gated sodium channel (VGSC) of insects[17]. Broadly, in insects, two major mechanisms are known to confer resistance against these insecticides: (i) enhanced metabolic detoxification of insecticide which is the most common form of resistance mechanism due to either higher level of expression or presence of more efficient forms of enzymes, and (ii) reduced target site insensitivity resulting from non-syn-

onymous mutation(s) in VGSC gene, commonly referred as *kdr* (knockdown resistance) mutation. Such *kdr* mutation(s) are considered to have cross-resistance between DDT and pyrethroids[17].

Knockdown resistance is common occurrence in a wide array of insects including *Ae. aegypti,* where several mutations are reported[17]. Presence of such mutation in *Ae. albopictus* is poorly studied and only one mutation, i.e. F1534C, is reported so far in Singapore population[16]. The F1534C is known to confer resistance against DDT and permethrin in *Ae. aegypti*[18], however, such association has not been studied in *Ae. albopictus*.

Keeping in view of world-wide emergence of pyrethroid resistance and a reported *kdr* mutation in this vector, it was imperative to study the status of resistance and presence of possible *kdr* mutations, if any, in Indian *Ae. albopictus* populations. The present study is focused on assessment of current susceptibility status for DDT and pyrethroids in various *Ae. albopictus* populations and investigating presence of *kdr* mutations.

MATERIAL & METHODS

Mosquito collection

Aedes albopictus immatures (larvae and pupae) were collected from peri-domestic breeding sites and outdoor breeding sites of various locations from urban areas of Delhi, Gurgaon (Haryana), Guwahati (Assam), Kottayam (Kerala) and Hardwar (Uttarakhand), which were allowed to emerge into adult. Larvae/pupae were collected from at least 20 positive containers. F_1 progeny were obtained from larvae collected from Guwahati (Assam) and Kottayam (Kerala). Only one collection was performed from each study site between August and November 2012. Mosquito larvae were reared in laboratory in enamel basins with two litre dechlorinated water and were supplied with fish food till pupation. Pupae were transferred to bowl containing water and placed inside cloth cages (one cubic feet) for emergence into adult. Emergent mosquitoes were identified morphologically at species level and maintained with 10% glucose solution soaked in cotton pads.

Adult bioassay for susceptibility

Adult bioassays were carried out against DDT (4%), permethrin (0.75%) and deltamethrin (0.05%) using WHO standard susceptibility test kit. Twenty-five sugar-fed females (2–3 days old) of F_0 population (Delhi, Haryana and Haridwar) and F_1 population (Assam and Kerela) were used for each bioassay in three replicates and a corresponding control. Prior to insecticide exposure mosquitoes were transferred to the holding tube for one hour and then gently transferred to exposure tubes containing insecticide impregnated papers supplied by WHO Collaborating Centre, Universiti Sains, Malasiya. Mosquitoes were transferred to recovery tubes after one hour of exposure to insecticide papers and were provided access to 10% glucose solution soaked in cotton pads during recovery period. Mortalities were recorded after 24 h and the percent mortality was corrected, whenever required, by applying Abbott's[19] formula. All bioassays were carried out at 27±2°C with 70±10% relative humidity.

DNA isolation and kdr genotyping

DNA was isolated from resistant and susceptible mosquito (individually) gained from adult bioassay using method described by Livak[20] and stored at –20°C for further molecular studies. Genotyping of F1534C *kdr* mutation was done by an allele-specific PCR (AS-PCR) developed by Yanola *et al*[21] for *Ae. aegypti* with some modifications in primers. The list of primers is provided in Table 1. PCR conditions were same as adapted by Yanola *et al*[21]. In addition, partial domain II, III and IV of VGSC gene were amplified and sequenced targeting mutation sites S989P, I1011M, I1011V, V1016G, V106I, F1534C and D1794Y reported in *Ae. aegypti*. Partial domain II, III and IV of VGSC were amplified using primers aegSCF20 and aegSCR21 for domain II, aegSCF7 and aegSCR7 for domain III, and albSCF6 and albSCR8 for domain IV, designed by Kasai *et al*[16] for *Ae. albopictus*. PCR was carried out in a 25 µl reaction volume containing 0.625 units of AmpliTaq gold DNA polymerase (ABI), 0.2 mM each dNTP, 1.5 mM $MgCl_2$ and 0.5 µM each of the forward and reverse primers. The PCR conditions for amplification consisted of an initial heat activation step at 95°C for 3 min, followed by 35 cycles of 95°C for 30 sec, 54°C for 45 sec and 72°C for 30 sec with a final extension step at 72°C for 7 min. The PCR products were purified using QIA quick PCR purification kit (Qiagen Inc., Germany) as per manufacturer's instructions and directly sequenced using primers aegSCF3 for domain II, aegSCR22 and aegSCR8 for domain III, and albSCF7

Table 1. Primers used for F1534C genotyping (modified from Yanola *et al*[21])

Name of primer	Sequence (5'-3')
F1534-f1	gcgggcTCTACTTCGTGTTCTTCATCATATT
C1534-f1	gcgggcagggcggcgggggcggggccTCTACTTC GTGTTCTTCATCATGTG
CP-r	TCTGCTCGTTGAAGTTGTCGAT

In lower case sequence in short 6 bp-GC tail and 26 bp-GC long tail.

Table 2. Results of insecticide susceptibility test against DDT, deltamethrin (DEL) and permethrin (PER)

Localities	GPS coordinates of sample collection sites	Percent corrected mortalities*		
		DDT (4%)	DEL (0.05%)	PER (0.75%)
Delhi	28.61° N, 77.23° E	85	97	100
Gurgaon (Haryana)	30.73° N, 76.78° E	72	98	100
Hardwar (Uttarakhand)	29.96° N, 78.17° E	61	100	100
Kottayam (Kerala)	9.58° N, 76.52° E	85	100	96
Guwahati (Assam)	26.18° N, 91.73° E	92	100	100

*Number of mosquitoes exposed: Test=75; Control=25.

for domain IV[16] using sequencer 3730XL DNA analyzer (ABI). Sequence data were analyzed on Finch TV and aligned using ClustalW implemented in Mega 5.0.1[22].

RESULTS & DISCUSSION

Results for adult bioassay test carried out on *Ae. albopictus* from all five study sites using WHO's standard insecticide susceptibility test kit are presented in Table 2. High resistance against DDT was observed in Uttarakhand population (61% mortality) and Haryana population (72% mortality), whereas Delhi, Kerala and Assam populations showed tolerance (85–92% mortalities). Delhi population showed 97% mortality for deltamethrin and Kerala population showed 96% mortality against permethrin. All other populations studied were fully susceptible against both pyrethroids. The results are in conformity with earlier studies which showed DDT resistance in this vector species against DDT and pyrethroids in various populations from Maharashtra, Kerala, Jharkhand and Assam[11–15]. Susceptibility against synthetic pyrethroids suggests absence of selection pressure in *Ae. albopictus* populations studied. However, keeping in view indication of emergence of incipient resistance in Delhi and Kerala populations, regular monitoring of resistance against synthetic pyrethroid is essential for an efficient vector management. This is also important because resistance to pyrethroids in *Ae. albopictus* has been reported from several countries[7–10, 23] including neighbouring countries like Pakistan[8] and Sri Lanka[9].

Results of genotyping for F1534C *kdr* mutation by allele-specific polymerase chain reaction (AS-PCR) on 30 samples from Delhi and 20 from all other populations showed absence of this mutation. Further, sequencing of representative samples (five for each domain for each locality) did not reveal any non-synonymous mutation in the VGSC gene. So far, a single *kdr* mutation F1534C with high frequency has been reported in *Ae. albopictus* from Singapore only[16]. Regular use of permethrin in Singapore for the control of dengue over a decade has been attributed as a possible reason of selection of this mutation. This mutation has been reported to confer resistance against DDT and permethrin in *Ae. aegypti*[18], however, role of such mutation in *Ae. albopictus* has not been established. F1534C is one of the most common mutations reported in *Ae. aegypti* in different parts of world. Recently, authors have found high frequency of F1534C mutation in *Ae. aegypti* collected from Delhi which has been shown to confer resistance against DDT and deltamethrin[24].

The present study shows DDT resistance in *Ae. albopictus* and development of incipient resistance against synthetic pyrethroids in Delhi and Kerala which need verification. No *kdr* mutation was detected in the populations studied.

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REFERENCES

1. Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol* 2002; *10:* 100–3.
2. Gubler DJ. Resurgent vector-borne diseases as a global health problem. *Emerg Infect Dis* 1998; *4:* 442–50.
3. Gupta N, Srivastava S, Jain A, Chaturvedi UC. Dengue in India. Centenary review article. *Indian J Med Res* 2012; *136:* 373–90.
4. Krishnamoorthy K, Harichandrakumar KT, Krishna Kumari A, Das LK. Burden of chikungunya in India: Estimates of disability adjusted life years (DALY) lost in 2006 epidemic. *J Vector Borne Dis* 2009; *46:* 26–35.
5. *Pesticides and their application for the control of vectors and pests of public health importance*. VI edn. Geneva: World Health Organization 2006.WHOD CDSD NTDD WHOPESD GCDPPD 2006.1.
6. *Guidelines for clinical management of dengue fever, dengue hem-*

orrhagic fever and dengue shock syndrome. Delhi: National Vector Borne Disease Control Programme. Available from: *http://www.nvbdcp.gov. in/Doc/Clinical%20Guidelines.pdf* (Accessed on May 31, 2014).
7. Chuaycharoensuk T, Juntarajumnong W, Boonyuan W, Bangs MJ, Akratanakul P, Thammapalo S, *et al*. Frequency of pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Thailand. *J Vector Ecol* 2011; *36:* 204–12.
8. Khan HA, Akram W, Shehzad K, Shaalan EA. First report of field evolved resistance to agrochemicals in dengue mosquito, *Aedes albopictus* (Diptera: Culicidae), from Pakistan. *Parasit Vectors* 2011; *4:* 146.
9. Dharshini S, Vinobaba M, Jude PJ, Karunaratne SH, Surendran SN. Prevalence and insecticide susceptibility of dengue vectors in the District of Batticaloa in eastern Sri Lanka. *Trop Med Int Health* 2011; *39:* 47–52.
10. Kamgang B, Marcombe S, Chandre F, Nchoutpouen E, Nwane P, Etang J, *et al.* Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* in central Africa. *Parasit Vectors* 2011; *4:* 79.
11. Chakraborti S, Mourya DT, Gokhale MD, Banerjee K. Insecticide susceptibility status and enzyme profile of *Aedes albopictus* populations from different localities of Maharashtra state. *Indian J Med Res* 1993; *97:* 37–43.
12. Sharma SN, Saxena VK, Lal S. Study on susceptibility status in aquatic and adult stages of *Aedes aegypti* and *Aedes albopictus* against insecticides at international airports of south India. *J Commun Dis* 2004; *36:* 177–81.
13. Singh RK, Dhiman RC, Mittal PK, Dua VK. Susceptibility status of dengue vectors against various insecticides in Koderma (Jharkhand), India. *J Vector Borne Dis* 2011; *48:* 116–8.
14. Dev V, Khound K, Tewari GG. Dengue vectors in urban and suburban Assam, India: Entomological observations. *WHO South East Asia J Public Health* 2014; *3*(1): 51–9.
15. Dhiman S, Rabha B, Yadav K, Baruah I, Veer V. Insecticide susceptibility and dengue vector status of wild *Stegomyia albopicta* in a strategically important area of Assam, India. *Parasit Vectors* 2014; *7:* 295.
16. Kasai S, Ng LC, Lam-Phua SG, Tang CS, Itokawa K, Komagata O, *et al.* First detection of a putative knockdown resistance gene in major mosquito vector, *Aedes albopictus*. *Jpn J Infect Dis* 2011; *64:* 217–21.
17. Davies TG, Field LM, Usherwood PN, Williamson MS. DDT, pyrethrins, pyrethroids and insect sodium channels. *IUBMB Life* 2007; *59:* 151–62.
18. Yanola J, Somboon P, Walton C, Nachaiwieng W, Prapanthadara L. A novel F1552/C1552 point mutation in the *Aedes aegypti* voltage-gated sodium channel gene associated with permethrin resistance. *Pesti Biochem Physiol* 2010; *96:* 127–31.
19. Abbott WS. Method of computing the effectiveness of an insecticide. *J Econ Entomol* 1925; *18:* 265–7.
20. Livak KJ. Organization and mapping of a sequence on the *Drosophila melanogaster* X and Y chromosomes that is transcribed during spermatogenesis. *Genetics* 1984; *107:* 611–34.
21. Yanola J, Somboon P, Walton C, Nachaiwieng W, Somwang P, Prapanthadara LA. High-throughput assays for detection of the F1534C mutation in the voltage-gated sodium channel gene in permethrin-resistant *Aedes aegypti* and the distribution of this mutation throughout Thailand. *Trop Med Int Health* 2011; *16:* 501–9.
22. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011; *28:* 2731–9.
23. Ponce G, Rodriguez I, Flores A, Garcia S, Jimenez DJ. Frequencies of voltage-gated sodium channel *kdr* mutations in *Culex quinquefasciatus* and *Aedes albopictus* (Diptera: Culicidae) of northeastern Mexico. Austin, Texas: Annual Meeting of Entomologial Society of America 2013.
24. Kushwah RBS, Dykes CL, Kapoor N, Adak T, Singh OP. Pyrethroid-resistance and presence of two knockdown resistance (*kdr*) mutations, F1534C and a novel mutation T1520I, in Indian *Aedes aegypti*. *PLoS Negl Trop Dis* 2015; *9*(1): e3332.

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