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 India¹-⁴. *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 nets⁵. 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 populations⁶.

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 world⁷-¹⁰, however, pyrethroid resistance hasn’t been reported from India though resistance to DDT has been reported¹¹-¹⁵. 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 common¹⁶.

DDT and pyrethroids act on the voltage-gated sodium channel (VGSC) of insects¹⁷. 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-
ononymous 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.

Knockdown resistance is common occurrence in a wide array of insects including Ae. aegypti, where several mutations are reported. Presence of such mutation in Ae. albopictus is poorly studied and only one mutation, i.e. F1534C, is reported so far in Singapore population. The F1534C is known to confer resistance against DDT and permethrin in Ae. aegypti, 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. F1 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 fed females (2–3 days old) of F0 population (Delhi, Haryana and Hardwar) and F1 population (Assam and Kerala) were used for each bioassay in three replicates and a corresponding control. Prior to insecticide exposure mosqui-

<table>
<thead>
<tr>
<th>Name of primer</th>
<th>Sequence (5'-3')</th>
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<tbody>
<tr>
<td>F1534-f1</td>
<td>gcggggaCTACTTCTCGTGTTCTTCATCATATT</td>
</tr>
<tr>
<td>C1534-f1</td>
<td>gcgggcaagggcgggggggggggggggggggcTCTACTTCTCGTGTTCTTCATCATATG</td>
</tr>
<tr>
<td>CP-r</td>
<td>TCTGCTCGTGAAGTTTGCAGAT</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Localities</th>
<th>GPS coordinates of sample collection sites</th>
<th>Percent corrected mortalities*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DDT (4%)</td>
</tr>
<tr>
<td>Delhi</td>
<td>28.61° N, 77.23° E</td>
<td>85</td>
</tr>
<tr>
<td>Gurgaon (Haryana)</td>
<td>30.73° N, 76.78° E</td>
<td>72</td>
</tr>
<tr>
<td>Hardwar (Uttarakhand)</td>
<td>29.96° N, 78.17° E</td>
<td>61</td>
</tr>
<tr>
<td>Kottayam (Kerala)</td>
<td>9.58° N, 76.52° E</td>
<td>85</td>
</tr>
<tr>
<td>Guwahati (Assam)</td>
<td>26.18° N, 91.73° E</td>
<td>92</td>
</tr>
</tbody>
</table>

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

for domain IV\textsuperscript{16} using sequencer 3730XL DNA analyzer (ABI). Sequence data were analyzed on Finch TV and aligned using ClustalW implemented in Mega 5.0.122.

RESULTS & DISCUSSION

Results for adult bioassay test carried out on \textit{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\textsuperscript{11–15}. Susceptibility against synthetic pyrethroids suggests absence of selection pressure in \textit{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 \textit{Ae. albopictus} has been reported from several countries\textsuperscript{7–10, 23} including neighbouring countries like Pakistan\textsuperscript{8} and Sri Lanka\textsuperscript{9}.

Results of genotyping for F1534C \textit{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 \textit{kdr} mutation F1534C with high frequency has been reported in \textit{Ae. albopictus} from Singapore only\textsuperscript{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 \textit{Ae. aegypti}\textsuperscript{18}, however, role of such mutation in \textit{Ae. albopictus} has not been established. F1534C is one of the most common mutations reported in \textit{Ae. aegypti} in different parts of world. Recently, authors have found high frequency of F1534C mutation in \textit{Ae. aegypti} collected from Delhi which has been shown to confer resistance against DDT and deltamethrin\textsuperscript{24}.

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

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