The pyrethroid resistance status and mechanisms in *Aedes aegypti* from the Guerrero state, Mexico

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**Abstract**

Dengue is one of the most important vector-borne diseases worldwide and is a public health problem in Mexico. Most programs in dengue endemic countries rely on insecticides for *Aedes* control. In Mexico, pyrethroid insecticides (mainly permethrin and deltamethrin) have been extensively used over a decade as adulticides and represented a strong selection for insecticide resistance for dengue vectors in several parts of the country. We studied the type, frequency and distribution of insecticide resistance mechanisms in *Aedes aegypti* from six municipalities in the state of Guerrero selected on the basis of historically intense chemical control and a high risk for dengue transmission. *Ae. aegypti* eggs were collected from October 2009 to January 2010 using ovitraps. F1 adults, emerged from these collections, were exposed to permethrin, deltamethrin and DDT in WHO diagnostic tests and showed high resistance levels to both pyrethroids and DDT. This was consistent with the presence of increased metabolic enzyme activities and target site insensitivity due to kdr mutations. Biochemical assays showed elevated esterase and glutathione S-transferase activities in the six municipalities. The V1016I kdr mutation on the IIS6 domain of the sodium channel gene was present in an overall frequency of 0.80. A second mutation, F1534C on the IIIS6 domain of the same gene was also detected, being the first report of this mutation in Guerrero. The multiple resistance mechanisms present in *Ae. aegypti* from Guerrero state represent a warning for the efficacy of the pyrethroid usage and consequently for the success of the dengue control program.

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**1. Introduction**

Dengue fever (DF) and dengue haemorrhagic fever (DHF) are diseases of great importance globally on public health in terms of morbidity and mortality. Dengue is caused by a flavivirus with four serotypes and is transmitted primarily by the *Aedes aegypti* mosquito. Dengue affects tropical and subtropical areas and is endemic in most countries in Latin America. Data from the Pan American Health Organization indicate that the dengue incidence rate in Mexico ranged from 20 to 51 cases/100,000 inhabitants in 2009–2010 ([http://new.paho.org/mex](http://new.paho.org/mex)) [1]. Guerrero state regularly reports approximately 10% of all dengue cases in Mexico, and in 2009, the dengue incidence reached 139 cases/100,000 inhabitants. Acapulco was the municipality most affected in Guerrero state with 2742 cases in 747,925 inhabitants (CENAVECE [http://www.cenave.gob.mx/dengue/]) [2].

The prevention and control of dengue transmission in Mexico, as in many dengue endemic countries, focus on *Ae. aegypti* control and insecticides play a major role. Between 1950 and 1960, DDT was widely used in Mexico for indoor residual spraying and in 1998 still was used for malaria control in some regions of the country. Subsequently, the organophosphate (OP) malathion was extensively used as an adulticide for ultra-low volume (ULV) space spraying from 1981 to 1999. The OP temephos is still widely used as a larvicide. From 2000 to 2011, the major adulticide insecticide class used for *Aedes* control have been pyrethroids (PYR), either through residual spraying, impregnated mosquito nets, or space spraying [3]. During 2007, 60,944 kg of PYR active ingredient was used for vector borne disease control [4], demonstrating the widespread use of this class of insecticide throughout the country. Resistance to organophosphate and pyrethroid insecticides is now widespread throughout the global geographic range of *Ae. aegypti*.
and in several states from Mexico a rapid rise of a permethrin knock down resistance allele has already been reported [6]. Metabolic resistance and alterations in the target site of the insecticides are two of the most important insecticide resistance mechanisms. Metabolic resistance is conferred by alterations in the levels or activities of detoxification enzymes, predominately esterases, glutathione S-transferases and/or cytochrome P450s [7]. Structural changes in an insecticide’s target site, such as the voltage-gated sodium channel, can lower the affinity for the insecticide [7]. Cross-resistance between pyrethroids and DDT is frequently due to mutations on the voltage-gated sodium channel gene, and this mechanism is known as “knockdown resistance” or “kdr” [6,8,9,10]. Most resistance-associated mutations are found in segment 6 of domain II (IIS6) and domain III (IIIS6) of the sodiumchannel gene. In Ae. aegypti populations from Latin America, several mutations that correlate with resistance to DDT and PYR have been identified i.e. V1016I, I1011M, I1011V and F1534C [8,9,6,11,12]. The V1016I mutation is widely distributed in populations of Ae. aegypti in Mexico, and rapidly increased in frequency from 1997 to 2009 [6].

Here we report an analysis of pyrethroid resistance and its underlying mechanisms in Ae. aegypti from Guerrero state, Mexico.

2. Material and methods

2.1. Mosquito collection

Ae. aegypti eggs were collected transversally between October 2009 and January 2010, from 1092 ovitraps placed in six municipalities in Guerrero state (Fig. 1). These municipalities have been considered by the health authorities as high risk for the dengue virus transmission, due to their history of persistent transmission and high numbers of cases of dengue fever [2]. The sites collected were: Eight neighborhoods from Iguala (Chapultepec, Luis Quinte- ro, San José, Santa Cruz, Acatempa, Mirador, Vicente Guerrero and Tierra y Libertad) with a total of 188 ovitraps; four neighborhoods from Acapulco (Progreso, Renacimiento, Hogar Moderno and Benito Juárez) with a total of 560 ovitraps; two neighborhoods from Chilpancingo (Tatatildo and San Mateo) with a total of 80 ovitraps; four neighborhoods from Zihuatanejo (Primer Paso, Morelos, Huial and Emiliano Zapata) with a total of 160 ovitraps; two neighborhoods from Tlapa (Caltitlán and Jardín Niños) with a total of 64 ovitraps and; one neighborhood from Técpant de Galeana (Colonia Pri) with 40 ovitraps.

Eggs were hatched at the insectary of the Centro Regional de Investigación en Salud Pública (CRISP) and larvae were reared to adults at 25–27 °C, relative humidity 60–70% and fed on a diet of 10% sucrose solution. One to three days old adults were used for the insecticide susceptibility tests [13], biochemical assays and molecular analysis. Three reference mosquito strains were used in this study: the New Orleans susceptible strain, originally colonised by the U.S. Centers for Disease Control and Prevention (CDC), the laboratory strain 90, which is 10% permethrin resistant, and a strain collected by ourselves in Isla Mujeres, Quintana Roo, Mexico in January 2009, which was previously reported as resistant and called IMUS by Saavedra-Rodriguez et al. [9]. This strain (IMUS 2009) was colonised without insecticide pressure at CRISP, of which F11 generation was used for this study.

2.2. Determination of diagnostic doses of insecticides

As the WHO recommended discriminative dosage for permethrin susceptibility testing bioassays of 0.25% did not kill a single field individual mosquito in our first bioassays, we decided to...
increase the dosage based on one of our laboratory strain base-line (undertaken following the WHO recommendations [13]), which permethrin mortalities were not less than 90% using the WHO recommended discriminative dosage (thus called laboratory strain 90). Using a higher discriminative dosage would allow us to obtain resistance percentage figures and so to be able to compare results of the localities collected. For the three pyrethroids, base lines were established using six doses of deltamethrin and seven of permethrin which gave a range of mortalities between 10% and 99% and 2% and 99% respectively, with four replicates each. Data were analysed with the software EPA (Probit Analysis Program version 1.5) and Chi square analysis showed no significance between the observed and expected lines. The resulting diagnostic doses were: permethrin (0.816% concentration) and deltamethrin (0.034% concentration). Filter papers (Whatman #1 CH of 12 cm x 15 cm) were then impregnated with these insecticide percentages as described by WHO [13,14]. Four percent DDT was used to impregnate filter papers. All insecticides were technical grade; the pyrethroids were provided by Bayer de Mexico and DDT was obtained by Sigma.

2.3. Insecticide bioassays

Mortality with diagnostic concentration treated filter papers of the three insecticides was determined using WHO test kits against 1–3 day old mosquitoes [13,14]. At least 100 mosquitoes were used in each bioassay and if the mosquitoes were available, three replicates were done for each insecticide and locality. After an hour of exposure, mosquitoes were transferred to the holding tube and provided with a 10% sugar solution soaked cotton pads. Mortality was recorded after 24-h, and dead and surviving mosquitoes were frozen separately for molecular assays. Tests with papers impregnated as described by WHO [13] but without insecticide, were conducted in parallel and served as control.

2.4. Biochemical assays

To determine whether a resistance mechanism based on the metabolism of the pyrethroid and DDT insecticides could be involved in these mosquito populations, enzymatic levels were measured by biochemical assays compared with those of the susceptible strain. Protein concentrations were calculated according to Bradford [15] and esterase concentration, glutathione S-transferase (GST) activity and cytochrome P450 content were measured as described by Penilla et al. [16]. As the distribution of a normal population with a determined enzyme activity can be observed in a histogram of 47 mosquitoes data, batches of at least 24 females and 23 males of one-day-old mosquitoes were used by assay and were individually homogenized on ice in 200 μl of distilled water in a v-bottomed micro titer tube [16]. Esterase concentrations were quantified using three distinct substrates: α and β-naphthyl acetate and para-nitrophenyl acetate (pNPA). Glutathion S-transferase (GST) activity was measured using the chlorodinitrobenzene (CDNB) substrate (Sigma) and, cytochrome P450 content was determined using heme-peroxidase reactions. Absorbances at end points for the colorimetric assays and kinetics of 2 and 5 min for the pNPA and GST assays, respectively, were read in a Multiskan™ spectrophotometer (Thermo Labsystems). Enzyme activities or content/mg protein were calculated for all reference and field samples.

2.5. Detection of mutations in Ae. aegypti voltage- gated sodium channel

Genomic DNA was extracted from 100 survivor and dead mosquitoes (sample size was determined from the total of 10,179 pyrethroid exposed mosquitoes, at a 95% of confidence, 10% of sampling error and a resistance allele frequency of 0.49 previously reported [26]) of different pyrethroid bioassays using the Livak method [17]. The PCR primers Ae2021aF and Ae2021aR were used to amplify a 457 bp fragment of exons 20 and 21, encoding the subunit 6 of domain II of the sodium channel gene [12]; 77 out of 100 mosquitoes DNA amplified. Cycling conditions were 95°C for 5 min followed by 35 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s followed by a final elongation stage at 72°C for 10 min. To amplify exon 31 encoding the subunit 6 of the domain III, we used the PCR primers AaEx31P and AaEx31Q, which amplified a 350 bp fragment [12,18]. The conditions were 95°C for 5 min followed by 35 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s followed by a final elongation stage at 72°C for 10 min. PCR reactions were carried out according to the methods described by Harris et al. [12]. For the primer set AaEx31, 18 out of 100 genomic DNA tested, amplified.

To confirm the presence of mutations in its nucleotide sequence, purified PCR products (Qiagen™ PCR purification kit for sequencing) of exons 20-21 and exon 31 from eight mosquitoes (seven permethrin survivors and one deltamethrin survivor), were sequenced by Macrogen (Seoul, Korea). The sequences were then assembled and aligned using Lasergene (DNAsStar, Madison, WI).

2.6. Kdr genotyping

2.6.1. Hot oligonucleotide ligation assay (HOLA) for the detection of V1016I

For the 77 amplified PCR products, a HOLA assay was used to calculate the frequency of the V1016I mutation in the exon 20-21 as described by Rajatileka et al. [18]. For each allele, a 20 μl ligation reaction containing 3 μl of PCR product, 1X Ampligase™ buffer, 50 nM of each detector/reporter mix, and 0.05 U/μl Ampligase™ was set up. The reaction conditions for the hot ligation were 95 °C for 5 min followed by 25 cycles at 94 °C for 1 min, 62 °C for 2 min and 4 °C-hold. The sequence of the oligonucleotide reporter lle1016rpt and detector ValII1016dct and lle1016dct are shown in Table 1.

2.6.2. Tetraplex assay for the detection of F1534C

To test this assay as a diagnostic technique for detection of the F1534C mutation for these samples, a tetra primer PCR assay was used as designed by Harris et al. [12], using the primers AaEx31P, AaEx31Q, AaEx31wt and AaEx31mut. In this PCR, the flanking primers amplify a control band of 350 bp. Two internal allele specific primers give products of either 231 bp (wild type, phenylalanine allele) or 163 bp (mutant, cysteine allele) by forming PCR primer pairs with the flanking regions. Each PCR reaction contained 2.5 mM MgCl2, 0.4 mM each dNTPs, 0.5 μM each primer, 2.5 U taq polymerase, and 1% of the total genomic DNA (extracted

### Table 1

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Position</th>
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<td></td>
<td>ATTGTATGCTTGTGGTGTC</td>
</tr>
<tr>
<td>Ae2021aR</td>
<td></td>
<td>GCGTGGCCGATGTTC</td>
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<tr>
<td>AaEx31P</td>
<td></td>
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</tr>
<tr>
<td>AaEx31Q</td>
<td></td>
<td>GTGATGTCGAGTGAATGAGT</td>
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<tr>
<td>AaEx31wt</td>
<td></td>
<td>CCTCTACTTTGTGTTCTTCATCTT</td>
</tr>
<tr>
<td>AaEx31mut</td>
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<td>GCGTGAAGAACGACCCGC</td>
</tr>
<tr>
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<td>1016-1st</td>
<td>GCAAGGCTAAGGAAAGGTAATC</td>
</tr>
<tr>
<td>lle1016dct</td>
<td>1016-1st</td>
<td>GCAAGGCTAAGGAAAGGTAAT</td>
</tr>
<tr>
<td>lle1016rpt</td>
<td>1016-1st</td>
<td>CTGGCTACGGTGGAAGCAACAT</td>
</tr>
</tbody>
</table>

rpt = reporter, dct = detector. Ninety-six-well streptavidin plates (Sigma™ScreenTM) were used for SNP detection. Colour change was scored visually after incubating with 100 μl TMB solution (Roche® BM Blue Pod Substrate) for 5 min.

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from each of the eighteen mosquitoes that amplified) giving a total final volume of 25 μl. The cycling conditions were 95 °C for 5 min followed by 35 cycles at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, and a final elongation at 72 °C for 10 min. PCR products were visualized on a 2% agarose gel.

2.7. Statistical analysis

Statistical analyses were performed using SPSS 19.0 software [19]. Descriptive analyses of mortality were obtained from different exposures to insecticides. An analysis of variance (ANOVA) was conducted to compare the average mortality of all strains and the New Orleans reference strain. The statistical analyses included Levene's test for homogeneity of variance and descriptive analysis (mean, standard deviation, α 0.05). The enzymatic activity data of the field mosquitoes were compared using ANOVA, and Levene's and Dunnet's tests were used to compare means against those activities of the reference strain (α 0.05). The allele frequency of the V1016 mutation was calculated using the Hardy–Weinberg Equilibrium Calculator (http://www.changbioscien.com).

3. Results

3.1. Insecticide bioassays

Bioassays for insecticides were carried out using a range of mosquitoes from 102 to 1853 per locality. Results from the bioassays indicated that the mosquito populations tested from Guerrero state are highly resistant to both of the PYRs (permethrin and deltamethrin) tested. Mortalities varied between 9.8–15.7% and 8.7–45.2% for those exposed to permethrin and deltamethrin, respectively (Table 2) compared to 100% mortality in the susceptible New Orleans strain. The statistical analyses included Levene's test for homogeneity of variance and descriptive analysis (mean, standard deviation, α 0.05). The enzymatic activity data of the field mosquitoes were compared using ANOVA, and Levene's and Dunnet's tests were used to compare means against those activities of the reference strain (α 0.05). The allele frequency in survivors to pyrethroid was found in the 58 positive mosquitos, with an overall allele frequency of 0.0029 ± 0.0061, n = 320) (< 0.05) compared with that of the susceptible New Orleans strain (0.00045 ± 0.00028, n = 149).

3.2. Enzymatic assays

A total of 1551 mosquitoes from the different localities and the reference strains were assayed for the three enzymes. The median of enzymatic activity for each mosquito population compared with those of the susceptible strain are shown in Figs. 2–5. Esterase concentrations with the substrates a-naphthyl acetate and pNPA (Figs. 2A and 3) and GST activities (Fig. 4) were significantly elevated (P < 0.001) in all field mosquito populations when compared with those of the susceptible New Orleans strain (mean ± deviation standard for New Orleans (n = 149) = 0.00041 ± 0.0001 nmol a-naphthylacetate/mg prot; 0.496 ± 0.160 μmol pNPA/min/mg prot; 1.60 ± 0.76 mmol CDNB conjugated/min/mg prot). Mosquitoes from Acapulco had the highest mean esterase concentration with both substrates: 0.0011 ± 0.0011 nmol a-Naphthol/mg prot, 2.12 ± 2.03 μmol pNPA/min/mg prot, n = 282). While mosquitoes from Tlapa had the highest mean GST activity (8.08 ± 2.11 mmol CDNB conjugated/min/mg prot, n = 188). Mean cytochrome P450 contents reported in pmol P450/mg prot (Fig. 5) were significantly higher in mosquitoes from Acapulco (0.0029 ± 0.0061, n = 282), Iguala (0.0016 ± 0.0012, n = 320) (P < 0.001) and Zihuatanejo (0.0012 ± 0.0007, n = 376) (P < 0.05) compared with that of the New Orleans strain (0.00045 ± 0.00028, n = 149).

3.3. kdr genotyping

Six out of eight PCR products were successfully sequenced showing two mutations, V1016I and F1534C, three samples were sequenced for both mutations from which two (from Acapulco and Iguala) were homozygous resistant for both mutations and one (from Chilpancingo) was heterozygous resistant for F1534C mutation and homozygous resistant for V1016I mutation. The other three samples were homozygous resistant for F1534C.

The first mutation is the result of a valine to isoleucine substitution in the IIS6 domain, exons 20-21. The HOLA assay to detect the V1016I mutation (Fig. 6) was conducted on the 77 genomic DNAs (from both pyrethroid survivors and dead mosquitoes that amplified the exon 21-22), of which 58 were positive in the colorimetric reaction. We calculated the frequencies for the pyrethroid resistance allele in these 58 individuals, using the formula of Hardy–Weinberg Equilibrium (Table 3). The V1016I mutation was found in the 58 positive mosquitoes, with an overall allele frequency of 0.8 for the sample studied. Acapulco had 1.0 resistance allele frequency in survivors to pyrethroid.

The second mutation in the IIS6 domain (exon 31) is non-synonymous, in which a single base pair substitution changes phenylalanine to cysteine on the 1534 codon. From the six mosquitoes sequenced, all had the mutation, but five were homozygotes (two from Acapulco, one from Iguala, one from Zihuatanejo and one from Tepan) and one was a heterozygote (from Chilpancingo).

The PCR tetraplex technique was successfully applied in the samples from Guerrero. Three genomic DNAs extracted from survivors to pyrethroids from Acapulco, Iguala and Zihuatanejo, and that amplified the exon 31, were tested to determine the presence of the F1534C mutation. Results showed a band of 163 bp for the three samples (Fig. 7). The three samples were homozygous resistant (RR). This is the first time that the F1534C mutation has been found in Ae. aegypti from Guerrero, Mexico.

4. Discussion

Pyrethroid and DDT resistance was detected at high levels in Ae. aegypti collected from the six municipalities in the Guerrero state of Mexico. Resistance to pyrethroid insecticides for this species had been already reported during the last decade in several states in Mexico [6,9,20,21,24,26,27]. The mechanisms in Ae. aegypti correlating with the pyrethroid resistance also have been determined in those studies. They were based mainly on kdr and there are reports inclusive of its rapid allele rising [6,9,26,27]. We found that

<table>
<thead>
<tr>
<th>Municipalities</th>
<th>0.816% permethrin</th>
<th>0.034% deltamethrin</th>
<th>4% DDT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD mortality%</td>
<td>n</td>
</tr>
<tr>
<td>Iguala</td>
<td>1847</td>
<td>12.3 ± 15.3</td>
<td>1853</td>
</tr>
<tr>
<td>Acapulco</td>
<td>1135</td>
<td>9.8 ± 15.6</td>
<td>823</td>
</tr>
<tr>
<td>Chilpancingo</td>
<td>517</td>
<td>14.2 ± 13.4</td>
<td>309</td>
</tr>
<tr>
<td>Zihuatanejo</td>
<td>1225</td>
<td>11.4 ± 14.7</td>
<td>1025</td>
</tr>
<tr>
<td>Tlapa</td>
<td>412</td>
<td>15.7 ± 7.7</td>
<td>402</td>
</tr>
<tr>
<td>Tepan de Galeana</td>
<td>318</td>
<td>14.3 ± 23.9</td>
<td>312</td>
</tr>
<tr>
<td>IMUS 2009</td>
<td>307</td>
<td>28.9 ± 15.3</td>
<td>321</td>
</tr>
<tr>
<td>New Orleans</td>
<td>300</td>
<td>1000.0 ± 0.0</td>
<td>307</td>
</tr>
</tbody>
</table>

*: total number of tested mosquitoes, SD: standard deviation.

* Mean of mortality percentage significantly different to the New Orleans strain, P < 0.0001.
resistance to pyrethroids in the field mosquito populations from five municipalities of Guerrero was also mainly due to a kdr-based resistance mechanism.

Two non-synonymous mutations in the sodium channel, V1016I and F1534C, previously reported to be involved in resistance to pyrethroids, were detected in this study. The I1011M/V, which also was previously identified in the Mexican population of Ae. aegypti, was not investigated in this study because of its negative correlation to pyrethroid resistance [9]. The V1016I mutation has been already reported in Mexico in the states of Chiapas, Quintana Roo, Yucatan, Veracruz, Nuevo Leon [6] and in some localities of Guerrero [26,27]. We have found for the V1016I mutation a resistance allele general frequency of 0.8 for the state of Guerrero (based in the collection in different localities from five municipalities). We did not determined the resistance allele frequency for all the samples undertaken in bioassays, but correlation

Fig. 2. Box plots representing the esterase median activity (horizontal bar) and upper and lower quartiles for different Aedes aegypti populations from Guerrero using the substrates: (A) α naphthyl acetate and (B) β naphthyl acetate. The vertical lines show the full range of the data set. Black stars could mean outliers. Red stars show statistical differences among the field mosquitoes and resistant strain (IMUS = IMUS 2009) against the susceptible strain (P < 0.001) and for resistant strain and the susceptibility strain in (B) (P < 0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
of this mutation and bioassay results supporting the role of this mutation in conferring pyrethroid resistance has been previously reported [9]. The high number of dengue cases reported in some municipalities like Acapulco and Iguala in 2009–2010 could be explained in certain way by the high resistance allele frequency found for this mutation in the dengue vector.
The mutation F1534C was found in all six mosquitoes that were sequenced, with five homozygotes and one heterozygote. This mutation is very prevalent in the Cayman Islands, and is also found in Southeast Asia and in populations of *Aedes albopictus* from Singapore [12,28,29]. Recently this mutation was reported in *Ae. aegypti* in a DDT/permethrin-resistant strain (PMD-R) from Thailand [30], it has been also associated with pyrethroid resistance in *Ae. aegypti* from Vietnam and Cayman Islands [12,32], and it was shown to confer resistance to type I but not type II pyrethroids in cockroach sodium channel [31]. The phenotypic effect of having both F1534C and V1016I alleles is not yet known but the high frequencies of both of these alleles in the Caribbean suggests a selective advantage [12]. This study represents the first report of this mutation in Mexico. Analysis of the resistance allele frequency in these mosquito populations still has to be carried out in our laboratory in order to calculate the percentage of individuals with both mutations (V1016I and F1534C) and the resistance levels to pyrethroids that together and each mutation separately confers.

The same studies reporting pyrethroid resistance in Mexico also concluded that metabolic resistance mechanisms are involved, where esterase or cytochrome P450 activities have been found elevated [20,21,24]. Our results also suggest that pyrethroid resistance could be mediated by esterases-, GSTs-, and for mosquitoes from three municipalities by cytochrome P450s-based resistance mechanisms. Metabolism assays are necessary to be done for these field mosquito populations in order to confirm their contribution extent to pyrethroid resistance. In Brazil however, it was hypothesized that the high esterase activity detected with the pNPA substrate in some populations could be construed as indirect evidence of the role of esterases in pyrethroid resistance, because esterase activity increased with the introduction of PYR in adult mosquito control [23]. Studies in Cuba in a deltamethrin selected laboratory strain showed that esterase and GST enzymes were responsible for resistance to pyrethroids [22]. Elevated expression of GSTe-2 has been associated with DDT and, permethrin resistant *Ae. aegypti* strain, together with recently RNAi experiments, have demonstrated a role for Epsilon class GSTs in conferring resistance to pyrethroids [25]. In another study, it has been demonstrated that elevated activities of GSTs determined by biochemical assays with the CDNB substrate from *Ae. aegypti* populations of other municipalities from Guerrero, were not correlated with DDT metabolism [33], which suggests that those elevated GST activities may be more associated in pyrethroid than in DDT resistance.

Our results demonstrated the existence of PYR and DDT cross resistance with two target site point mutations, and multiple underlying enzyme activity-based resistance mechanisms. The use of PYR in Guerrero for dengue vector control dates back more than 10 years and they were widely used as adulticides for both indoor and outdoor ULV/residual spraying. As a result of recognizing the high levels of PYR resistance the Vector Control Program in Guerrero has switched to an organophosphate (chlorpyrifos) for ULV as adulticide for dengue vector control. Further studies should to be carried out to establish frequency and changes of these kdr mutations, changes in the metabolic mechanisms and the role of

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Fig. 5. Box plots representing cytochrome P450 median content (horizontal bar) and upper and lower quartiles for different *Aedes aegypti* populations from Guerrero. The vertical lines show the full range of the data set. Black stars could mean outliers. Red stars show statistical differences among the field mosquitoes and the susceptible strain (P < 0.001 and P < 0.05 for Zihuatanejo). IMUS = IMUS 2009. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 6. Acapulco mosquito samples from the HOLA assay: 1–6, survived mosquitoes to pyrethroid exposure; 18, heterozygous mosquito from the reference strain 90, V = valine, L = isoleucine, RR = homozygous resistant and RS = heterozygous susceptible.
Phenotype and kdr allele frequencies for the amino acid 1016 of the voltage-gated sodium channel of different Aedes aegypti populations from Guerrero and the IMUS 2009 resistant strain (survivors and dead) exposed to pyrethroids (permethrin and deltamethrin) in WHO tests.

<table>
<thead>
<tr>
<th>Municipality</th>
<th>Phenotype/bioassay</th>
<th>n sample</th>
<th>Genotype Kdr/HOALA assay</th>
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<td></td>
<td>Survivors</td>
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<td>RR Ile/Ile</td>
<td>RS Val/Ile</td>
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Fig. 7. Agarose gel (2%) electrophoresis of tetraplex PCR products for F1534C mutation. Permethrin resistant mosquito samples: 1 Acapulco, 12 Iguala, 14 Zihuatanejo, M, molecular marker; FF, control homozygous susceptible mosquito; CC, control homozygous resistant mosquito; FC, heterozygote.

Table 3

References


