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Mechanisms of Pyrethroid Resistance in *Aedes (Stegomyia) aegypti* from Colombia

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Abstract

In Colombia *Aedes (Stegomyia) aegypti* is the main vector of urban arboviruses such as dengue, chikungunya and Zika. This urban mosquito has a well-established capacity to develop insecticide resistance to different types of insecticides (pyrethroids, organochlorides, organophosphates), using multiple resistance mechanisms. An understanding of ongoing resistance mechanisms is critical to determining the activities of vector control programs.

In order to identify the biochemical and molecular mechanisms associated with pyrethroid resistance in Colombia, three laboratory-selected strains resistant to DDT, Propoxur and lambda-cyhalothrin, and 7 field-collected strains were evaluated. CDC bioassays were performed to measure the susceptibility status to pyrethroid type I (permethrin) and II (deltamethrin and lambda-cyhalothrin), and potential cross-resistance to different types of insecticides; organochlorine (DDT), carbamates (propoxur) and organophosphates (malathion). The enzymatic activity of esterases, glutathione S-transferases (GST) and P⁴⁵⁰ monooxygenases were biochemically determined. Frequencies of *kdr* mutations Val1016Ile and Phe1534cys were determined through real-time PCR. The Rockefeller strain of *Aedes (Stegomyia) aegypti* was used as the susceptible control.

The laboratory-selected strains “propoxur” and “lambda-cyhalothrin” and one field population (Medellín (BF) F₂) were resistant to all evaluated pyrethroids. Six of the seven field populations as well as the laboratory- selected “DDT” strain were resistant to permethrin. All the evaluated strains were resistant to DDT. Cross-resistance between lambda-cyhalothrin and propoxur was

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observed in the laboratory-selected strains; however, all field-collected strains were susceptible to propoxur and no evidence of malathion resistance was found. The main biochemical mechanism for resistance observed in the field-collected strains was related to the enzyme GST. Further, the frequencies of *kdr* mutations alleles associated with insecticide resistance were high and ranged from 0.02 to 0.72 for Ile1016 and from 0.44 to 0.99 for Cys1534. Strains with high frequencies of both *kdr* mutations were resistant to both type I and II pyrethroids. These results suggest that *Ae. aegypti* from Colombia have developed multiple resistance mechanisms associated with pyrethroid resistance; therefore a resistance management strategy against these field populations of *Ae. Aegypti*, incorporating these findings is strongly recommended.

Graphical Abstract

Keywords

Aedes aegypti; pyrethroids; resistance; insecticides; glutathion S-transferases; *kdr* mutations

1. Introduction

Mosquitoes of *Aedes* genus are vectors of at least 20 arboviruses that currently affect human health (Chouin-Carneiro et al., 2016; Conway et al., 2014; Gubler, 2002). Two species, *Aedes (Stegomyia) aegypti* (*Ae. aegypti*) and *Ae. (Stegomyia) albopictus* are considered the main vectors of dengue virus (DENV), chikungunya (CHIKV) and Zika virus (ZIKV) in urban areas (Karunamoorthi and Sabesan, 2013). Worldwide, Dengue virus is the most important arbovirus, causing an estimated 390 infections annually, and at least 12,500 deaths, most of these in children (Bhatt et al., 2012).

In Colombia, *Ae. aegypti* is considered the main vector of DENV, CHIKV and ZIKV (Mattar et al., 2015; Padilla, J.C.; Rojas, D.P.; Saenz, 2012; Parra et al., 2016). Control of *Ae. aegypti*, is executed by the Vector-Control programs of the local public health departments. For larval control, chemical insecticides such as temephos, growth regulators, and bioinsecticides derived from bacteria are used as larvicide. For the adult control mainly the organophosphorus (OP) malathion and the pyrethroids (PYRs) deltamethrin and lambda-cyhalothrin insecticides are employed in ultra low volume spraying (ULV) in public

spaces (Grisales et al., 2013; Maestre-Serrano et al., 2014; Roldan S Ardila, L Santacoloma, 2013).

However the use of malathion has decreased, due to the public's rejection of this insecticide for its strong odor, corrosive effect and possible associations with cancer risk in humans (Bonner et al., 2010; Dusfour et al., 2011; Sritharan et al., 2017). Currently, PYRs are the insecticides that are more frequently used. Pyrethroids are the only recommended by WHO for the treatment of mosquito nets, walls and different materials (WHO, 2006), because they are safe for close contact due to their lower toxicity to mammals. Additionally, they have a fast and persistent effect on mosquitos in low doses, and are odorless, making them ideal for use in ultra low volume fumigations (ULV) in public spaces (WHO/CDS/WHOPES/GCDPP, 2005).

However, pyrethroid resistance has been progressively documented in several countries, among them Colombia, hindering the actions of vector control in those regions where it has been detected (Moyes et al., 2017; Ranson et al., 2008). The enzymatic mechanisms involved in pyrethroid resistance include three of the major groups of detoxification enzymes, which are esterases, P⁴⁵⁰ monooxygenases and glutathione S-transferases (GST) (Enayati et al., 2005; Liu, 2015; Ranson et al., 2008).

Another pyrethroid resistance mechanism is the alteration of the target sites of the insecticide known as *kdr* "knockdown resistance". This mechanism of resistance is caused by mutations in the gene that encodes the voltage-dependent sodium channel of mosquitoes, and reduces the binding of the PYRs to its target site in the protein (Bregues et al., 2003; Saavedra-Rodriguez et al., 2007). In Latin America the Val1016Ile and Phe1534Cys mutations have been associated with pyrethroid resistance in *Ae. aegypti* (Aguirre-Obando et al., 2017; Alvarez et al., 2015; Brito et al., 2018; Francis et al., 2017; Maestre-Serrano et al., 2014; Saavedra-rodriguez et al., 2018; Saavedra-Rodriguez et al., 2007; Vera-Maloof et al., 2015).

In Colombia, the emergence of resistance to PYRs has been reported in several populations of *Ae. aegypti* (Instituto Nacional de Salud, 2014). Previous studies of pyrethroid resistance mechanisms in Colombia have evaluated either biochemical or molecular tests, but not both. The enzymatic mechanisms described include alteration in levels of esterases, P⁴⁵⁰ monooxygenases (Ocampo et al., 2011; Santacoloma et al., 2012; Santacoloma Varón et al., 2010) and glutathione S-transferases (Fonseca-González et al., 2011). Additionally, the presence of both *kdr* mutations (Val1016Ile and Phe1534Cys) has been reported in Colombia in the Caribbean region (Atencia et al., 2016; Maestre-Serrano et al., 2014). Recently a new mutation, Val1419Ile, was reported which was observed in similar frequencies with Val1016Ile; the presence of both mutations was associated with lambdacyhalothrin resistance in three populations of mosquitoes studied in the country (Granada et al., 2018).

This project, is the first reported study where all known enzymatic and molecular resistant mechanisms associated to pyrethroids in *Ae. aegypti* from Colombia were evaluated, in order to identify the main resistance mechanisms present in the country. As reference, we

used laboratory-selected strains derived from field-collected mosquitoes from Colombia to identify the selected mechanism after continuous pressure with a particular insecticide. Additionally, we evaluated the existence of cross-resistance among different classes of insecticides. These results may help to develop resistance management strategies to decrease the emergence of PYRs resistance in *Ae. aegypti* from Colombia.

2. Materials and methods

2.1 Study area

Field collected mosquitoes were obtained from different cities in Colombia: Giron (Department (State) of Santander), Buga, Palmira, Yumbo and Calineighbourhood “Paso del Comercio” (Valle del Cauca) and Medellin (Antioquia) during 2014-2015 (Figure 1). Mosquito samples from Medellin were collected in two areas, one with high history of ULV spraying with malathion (denominated in this study as Medellin AF) and the second from an area of low history of insecticide application (Medellin BF). We decided to divide them under the criterion of “ULV spraying frequency” because we wanted to see within the same city how the populations might vary in response to the frequency of the vector control interventions carried out by the vector control program of Medellin. All locations were selected because they had history of insecticide resistance and in each case, the samples were provided by the local secretary of health.

2.2 Experimental design

The laboratory experiments were carried out at CIDEIM (Centro Internacional de Entrenamiento e Investigaciones Médicas) located in Cali- Colombia, and under the approval of the Institutional Ethics Committee of Animal Research in Experimentation CIEIA/CIDEIM.

For the study, F₁-F₂ generation of field collected *Ae. aegypti* mosquitoes from seven locations in Colombia and three laboratory selected resistant strains that had been continuously selected (as described below) by treatment with DDT (F₃₀), Lambda-cyhalothrin (F₂₉) and Propoxur (F₂₉) were evaluated. The laboratory resistant strains were used as biological models that allow us to understand the evolution of the resistance phenomenon when they are exposed continuously to a same insecticide.

The selection process was performed using the methodology described by Ocampo et al. (2011). The selection process was carried out using the CDC bottle bioassay and exposing the adults to the insecticide concentrations, as described as follow. Each strain had repeated exposures to lambda-cyhalothrin (5 µg/ml × 10 minutes), DDT (150 µg/ml × 30 minutes), and propoxur (15 µg/ml × 40 minutes). These times/concentrations were selected at the beginning of the selection process as they resulted in 50% survival with these strains and were used throughout the selection process. Then, after the exposure, live mosquitoes were separated for egg production. Selections were carried out over two or three generations to allow the population to recover from any fitness limitations.

Aedes aegypti eggs and larvae from the field collection sites were transported to the CIDEIM laboratory and the colonies maintained under standard laboratory conditions

(Ocampo et al., 2011). Adult female mosquitoes between 1-3 days post emergence were independently evaluated with CDC bottle bioassays, biochemical assays and *kdr* mutation analysis.

2.3 Insecticide bioassays

2.3.1 CDC bottle bioassays—The susceptibility status to pyrethroid and cross-resistance to other insecticides was evaluated by bioassays using the standardized CDC methodology (Brogdon, et al., 2010). The technical grade insecticides were obtained from: SIGMA: deltamethrin (Lot SZ13C059XV), and from Chem Service: permethrin (Lot 3373000, N-12848-250MG), lambdacyhalothrin (Lot 3123900, N-12307-100MG), DDT mixture (p-p' and o,p' isomers), (Lot 3871600, N-11567-250MG) malathion (Lot 3494600, N-1246-100MG), propoxur (Lot 3050100, N-11128-250MG) and temephos (Lot 31716CO, N-10996-100MG). Concentration and diagnostic time (DT) previously reported for *Ae. aegypti* were re-evaluated with the new products using a Rockefeller strain (Fonseca-González et al., 2011; Ocampo et al., 2011; Santacoloma et al., 2012). The following concentrations and DT (resistance thresholds) were established for this study: Permethrin (21.5 µg/mL-45 min), deltamethrin (6.25 µg/mL-40 min), lambdacyhalothrin (6.25 µg/mL-40 min), DDT (2000 µg/mL-60 min), malathion (100 µg/mL-45 min), and propoxur (12.5 µg/mL-45 min). Most of the concentrations and DT corresponds with what was previously published except for DDT. The new lot of the DDT product required increasing the concentration from 150ug/mL to 2000ug/mL to kill the susceptible Rockefeller strain. We used this higher concentration to run de bioassays with all the study strains. Results were expressed as the percent of mortality at the concentration and diagnosis time (DT) established. The resistance threshold was identified using the susceptible strain (Rockefeller) as control.

The bottles were prepared following the protocol described by Brogdon, et al. (2010) using ethanol as solvent. Briefly, twenty female mosquitoes were introduced into a 250mL treated bottles with the corresponding concentration of insecticide. Each test consisted of four insecticide treatment bottles and one control bottle with ethanol only. Three replicates were conducted for each insecticide and mosquito strain. The number of dead or live mosquitoes was recorded at 5 min intervals over a 1hour period. Then all mosquitoes were transferred to plastic cup free of insecticide and containing a cotton ball soaked in a sugar solution; mortality was determined after 24 hours.

2.3.2 Larval bioassays—Larval bioassays were performed following WHO recommendations (WHO, 2005) using the concentration of temephos 0.012ppm (Instituto Nacional de Salud, 2014; Ocampo et al., 2011). Late third- and early fourth- instar larvae were used for each mosquito population. Four replicates per concentration were tested with 20 larvae per replicate. The mortality was evaluated 24 hours postexposure and the results were recorded as percentage mortality.

2.1. Biochemical assays

The activities of α - and β -esterases, glutathione S-transferase (GST) and P⁴⁵⁰ monooxygenases were measured using biochemical assays modified as previously described

(Penilla et al., 1998) and the protein concentrations were calculated according to Bradford (Bradford, 1976). Batches of 72 one-day-old mosquito females were used in the assay and were individually homogenized on ice in 200 µl of distilled water in a flat-bottomed titer microplate (Penilla et al., 1998).

Esterase activities were quantified using two distinct substrates: α- and β- naphthyl acetate. GST activity was measured using the chlorodinitrobenzene (CDNB) substrate and P⁴⁵⁰ monooxygenases activity was determined using a heme-peroxidase reaction. Absorbances at the reaction end point were determined using a Multiskan® spectrum spectrophotometer microplate reader. Enzyme activity/mg protein was calculated for all field and laboratory samples (strains); normality tests, descriptive statistics and ANOVA analyses were performed.

The median of enzyme activity for each strain were compared with the susceptible Rockefeller strain by Kruskal–Wallis non- parametric test ($P < 0.05$) and Dunnet's tests were used to compare the median against those activities of the reference strain (α 0.05) using SPSS 21 (IBM Corporation Armonk, NY, USA). The results of α- and β- naphthyl acetate and GST activity were reported as µmoles product formed / min / mg protein; the activity of P⁴⁵⁰ monooxygenases was reported as pmoles of cytochrome P⁴⁵⁰ / mg protein.

2.2. Detection of *kdr* mutations

2.2.1. Genotyping assays for Val1016Ile and F1534C mutations- DNA

extraction—The presence and frequency of *kdr* mutations were determined using the methodology previously described (Saavedra-Rodriguez et al., 2007). The DNA of 54 randomly selected adult female mosquitoes from each field population and laboratory strain was extracted and resuspended in 60 µl of 1× TE buffer (Tris-EDTA pH 8.0). The DNA was quantified in a NanoDrop 2000® spectrophotometer and stored at −20 °C.

2.2.2. Real-time PCR assay for the Val1016Ile and Phe1534Cys mutations—

Detection of the Val1016Ile mutation was performed using the primers Val1016f (GCGGGCAGGGCGGCGGGGGCGGGGCCACAAATTGTTTCCCACCCGCACCGG) or Ile1016f (GCGGGCACAAATTGTTTCCCACCCGCACTGA) and Ile1016r (TGATGAACCSGAATTGGACAAAAGC) as described by Saavedra-Rodriguez et al., (2007). DNA (2 µl) was amplified in a reaction volume of 20 µl. Each reaction contained 10 µl of SYBR® Green PCR Master Mix (Applied Biosystems 4309155), 0.2 µl of each primer (in ddH₂O to a final concentration of 100 picomoles/µl) and 7.6 µl of double distilled water (ddH₂O).

Detection of Phe1534Cys mutation was performed using the protocol described by Yanola et al., (2011). The primers Cys 1534f (GCGGGCAGGGCGGCGGGGGCGGGGCTCTACTTTGTGTTCTTCATCATGTG), Phe 1534f (GCGGGCTCTACTTTGTGTTCTTCATCATATT) and 1534r (TCTGCTCGTTGAAGTTGTCGAT) were used. The reaction mixture (20 µL) contained 2 µl of DNA, 10 µl of SYBR® Green PCR Master Mix (Applied Biosystems 4309155), 0.066 µl of Cys1534f primer and 0.2 µl of Phe 1534f and 1535r (in ddH₂O to a final concentration of 50 picomoles/µl) and 7.7 µl of double distilled water (ddH₂O) was added.

2.2.3. Allelic frequencies of Val1016Ile and Phe1534Cys—The allelic frequencies for each mutation were calculated according to methods described (Saavedra-Rodriguez et al., 2007). In brief, the Allelic frequency of Val1016 = $p = \frac{GG + (GA/2)}{2}$, Ile1016 $q = \frac{AA + (GA/2)}{2}$, Phe1534 = $p = \frac{TT + (TG/2)}{2}$ and Cys1534 = $q = \frac{GG + (TG/2)}{2}$. The Hardy–Weinberg equilibrium equation was carried out using the online calculator: <http://www.oege.org/software/hwe-mr-calc.shtml> (accessed 13 November 2017).

In order to determine the possible relationship between the allelic frequencies of Ile1016 and Cys1534 and the percentage of mortality for all PYRs and DDT insecticides evaluated, a correlation was computed using the value of the Pearson correlation coefficient (r) using Graphpad software prisma 6.0 (Moher et al., 2009).

2.2.4. Dilocus haplotype frequencies—The frequencies for each dilocus haplotype were calculated from a table 3×3 (Three 1534 genotypes × three 1016 genotypes) constructed with the nine potential genotypes and following the methodology as described (Vera-Maloof et al., 2015). The dilocus genotypes were classified as: Val1016/Phe1534, Val1016/Cys1534, Ile1016/Phe1534, Ile1016/Cys1534, Val1016/Phe-Cys1534, Val-Ile1016/Phe1534, Val-Ile1016/Phe-Cys1534, Ile1016/Phe-Cys1534 and Val-Ile1016/Cys1534. The frequencies for each dilocus haplotype Ile1016/Cys1534 (IC) “Resistant”, Val1016/Phe1534 (VF) “Susceptible”, Val1016/Cys1534 (VC), and Ile1016/Phe1534 (IF) were determined using the web tool “CubeX” (Gaunt et al., 2007) <http://www.oege.org/software/cubex/> (accessed 14 November 2017).

3. Results

3.1. Bioassays

The percentages of mortalities to the evaluated insecticides among the different *Ae. aegypti* strains are shown in Table 1 and Supplementary Material 1 (CDC bioassay figures). Most of the mosquito strains were resistant to permethrin, except mosquitoes from the Medellin (AF) F₂ and DDT F₃₀ strains. All laboratory-selected mosquito strains and only one of the field mosquito strain (Medellin (BF) F₂) were resistant to deltamethrin. Five strains were resistant to lambdacyhalothrin: two laboratory selected strains (Propoxur and lambdacyhalothrin F₂₉) and three field strains (Yumbo F₂; Giron F₁ and Medellin (BF) F₂). The lambdacyhalothrin F₂₉ strain recorded the lowest mortality rate (17.6%) to lambdacyhalothrin insecticide. Cross-resistance was observed between pyrethroids and carbamates in the selected-laboratory strains. Propoxur F₂₉ was resistant to all PYRs and DDT insecticide, but was 100% susceptible to malathion. Lambdacyhalothrin F₂₉-selected strain were resistant to all PYRs, DDT and propoxur, but was susceptible to the organophosphates malathion and temephos. All analyzed strains were resistant to DDT; in contrast, all strains were susceptible to malathion. Only one field-collected mosquito population (Palmira F₂) was resistant to temephos.

3.2. Biochemical assays

Mean values of enzymatic activity levels for each mosquito strains are shown in figure 2. The most common enzymatic mechanism of insecticide resistance observed was the increase

of the GST enzymes. The glutathione S-transferase activities were significantly elevated in seven out of ten strains evaluated (Dunnett test $P < 0.05$) (Figure 2). Only mosquitoes from Propoxur F₂₉ strain had high activity of GST in the laboratory-selected strains. Mosquitoes from Yumbo F₂ showed the highest average activity of GSTs, followed by Buga F₁.

When were compared with the susceptible Rockefeller strain, the activity of α - esterases was elevated in DDT F₃₀, Yumbo F₂ and Medellin (BF) F₂ mosquito strains, while the activity of β - esterases was elevated only in mosquitoes from the DDT F₃₀ strain (Dunnett test $P < 0.05$). P⁴⁵⁰ monooxygenases content was significantly higher in two out of seven field strains, the field-collected mosquitoes from Yumbo F₂ and Medellín (BF) F₂, (Dunnett's test $P < 0.05$) (Figure 2).

3.3. *Kdr* mutations

3.3.1 Detection of *kdr* mutations and allelic frequencies of Val101Ile and Phe1534Cys

—Both *kdr* mutations alleles, Ile1016 and Cys1534, were observed in all the evaluated strains (Table 2). Allelic frequencies of Ile1016 ranged 0.02-0.72, and Cys1534 ranged 0.44-0.99. Frequencies of Cys1534 were higher than Ile1016 in all samples. The Cys1534 allele was close to fixation in three strains (Lambdacyhalothrin F₂₉, Yumbo F₂ and Palmira F₂) with frequencies of 0.99.

Mosquitoes from the lambdacyhalothrin F₂₉ laboratory strain had the highest frequency of both mutations Ile1016 (0.72) and Cys1534 (0.99), while the highest frequencies in the field mosquitoes were detected in Medellin (BF) F₂ (0.71 and 0.82, respectively). Both strains were resistant to all PYRs and DDT. In contrast the laboratory strain DDT F₃₀ and field strain Medellin (AF) F₂ had the lowest frequency of Ile1016. The latter one had the lowest frequency of Cys1534 as well. Medellin (AF) F₂ was susceptible to almost all insecticides evaluated, except to DDT (Table 2).

Overall, the homozygous wild type Val1016/Val1016 genotype predominated in the evaluated strains. This genotype was found in 61% of the samples. While the homozygous resistant Ile1016/Ile1016 was found in 37% of the samples. The heterozygous Val1016/Ile1016 was absent in most of the samples. Only one heterozygous sample from the Propoxur F₂₉ strains was found. On the other hand, the homozygous mutant Cys1534/Cys1534 was more frequent in all samples (73%). While the homozygous for the wild type phenotype Phe1534/Phe1534 was found in three out of the eleven evaluated strains (Table 2).

Correlation analyses were carried out between percentage of mortality to PYRs and DDT insecticides and the frequencies of Ile1016 and Cys1534 alleles are shown in the Supplementary material 2 (Pearson analyses table). Negative correlation between percentage of mortality and frequencies of Ile1016 allele was found with permethrin (Pearson $r=0.765$; $P=0.006$) and lambdacyhalothrin (Pearson $r=0.709$; $P=0.014$) insecticides. For the allele Cys1534 the negative correlation was found only with permethrin insecticide (Pearson $r=0.762$; $P=0.006$).

3.3.2 Dileocus haplotypes frequencies—All mosquitoes evaluated were genotyped for both *kdr* loci. The nine dileocus genotypes (Three 1016 genotypes } Three 1534 genotypes) (Supplementary material 3 (Dileocus genotypes table)). The 36% of mosquitos evaluated expressed both mutations (double homozygous mutants). In contrast the double homozygous wild type (Val/Val + Phe/Phe) was absent in most of the samples. Frequencies of dileocus haplotypes analysis at loci 1,016 and 1,534 are show in the Table 3. The double homozygous Ile1016/Cys1534 (IC) haplotype was observed in higher frequency (>0.5) in the strains with higher resistance to PYRs (Lambdacyhalothrin F₂₉ Yumbo F₂, Girón and Medellin BF). While the Ile1016/Phe1534 (IF), was absent in all laboratory- selected strains and two out of the seven field- strains. These results are possibly due to the lack of homozygotes for both alleles in most populations (Table 3).

4. Discussion

This study has demonstrated that multiple mechanisms of PYRs resistance have developed in different Colombian *Ae. aegypti* field populations, namely, enhanced GST expression and mutations in the *kdr* gene. While, the laboratory- selected resistance strains (lambdacyhalothrin F₂₉, Propoxur F₂₉ and DDT F₃₀) were mainly associated with *kdr* selection rather than detoxification enzyme expression.

Almost all the populations (except Medellin AF) showed resistance to the PYRs type I insecticide permethrin, although this insecticide has not been reported in use for dengue vector control in Colombia (Maestre-Serrano, 2012; Maestre-Serrano et al., 2014). These results have also observed in other studies in Colombia in the Caribbean region (Maestre-Serrano et al., 2014), Casanare (Roldan S Ardila, L Santacoloma, 2013), Choco and Putumayo (Fonseca-González et al., 2011; Santacoloma Varón et al., 2010). The high frequency of permethrin resistance observed is comparable with the DDT resistance observed in the same populations. These results suggest a possible crossresistance between these two insecticides, potentially associated with the previous use of DDT (until the 90's) in malaria and dengue eradication campaigns (Santacoloma Varón et al., 2010). In Colombia, the phenomenon of widespread resistance to DDT has been widely documented; previous studies in insecticide resistance in the country demonstrated the presence of DDT resistance in *Ae. aegypti* in all the populations studied (Fonseca-González et al., 2011; Ocampo et al., 2011; Santacoloma Varón et al., 2010). We observed the same pattern, as all the field strains evaluated were highly resistant to DDT, suggesting that the DDT-resistance persists even though this insecticide has not been used for more than 26 years (Maestre-Serrano, 2012).

In Colombia, GST-based resistance had been associated with DDT resistance (Fonseca-González et al., 2011) and may also play a role in resistance to PYRs (Lumjuan et al., 2011; Maestre-Serrano et al., 2014). The biochemical assays showed that the most common enzymatic mechanism observed in the field populations was enhanced GST expression/activity. All the field populations with increased activity of GST were resistant to DDT and most of them were resistant to permethrin, with the exception of the Medellin AF population. Interesting, Medellin AF (High ULV spraying frequency Spanish “alta fumigacion”) has been continuously sprayed with the insecticide malathion; could this

protect or reverse PYRs resistance? The way in which GST confer resistance to PYRs has not yet been elucidated, but is believed that GSTs can help to protect against oxidative stress induced by PYRs, thanks to predominantly peroxidase activity in the presence of PYRs (Enayati et al., 2005; Lumjuan et al., 2007).

Although in our results the main enzymatic mechanism observed was GST, which was increased in almost all the field populations, previous studies in Colombia (that did not included GST analyses) observed both high esterases and P⁴⁵⁰ monooxygenases, that was associated mainly to a broad observed temephos resistance in several places in the country (Ocampo et al., 2011; Santacoloma Varón et al., 2010). Due to the previous findings, vector control programs in the country reduced the use of temephos and started to use other larvicides such as *Bacillus thuringiensis* or insect growth regulators. Additionally, due to malathion concern in health, the use of PYRs insecticides increased in the country. We hypothesize that this reversal in esterases and P450 monooxygenases activity could be associated with changes in the vector control program in the country.

We observed greater heterogeneity in terms of type II PYRs resistance. The resistance to deltamethrin resistance was observed only in one location and lambdacyhalothrin resistance in three localities. Although resistance to deltamethrin has been documented in the country, mosquitoes are most commonly found to be susceptible (Roldan S Ardila, L Santacoloma, 2013; Santacoloma Varón et al., 2010). This suggests that deltamethrin could still be effective to control mosquitoes from places where there is no resistance. In contrast the lambdacyhalothrin resistance has been commonly detected in Colombia (Granada et al., 2018; Instituto Nacional de Salud, 2014). The presence of resistance to lambdacyhalothrin could be associated to its use since the 1990's in Colombia in dengue and malaria campaigns (Fonseca-González et al., 2011; Roldan S Ardila, L Santacoloma, 2013; Santacoloma Varón et al., 2010).

The laboratory-selected strains were more homogeneous about PYRs resistance. The laboratory strains Propoxur F29 and Lambdacyhalothrin F29 were resistant to all the evaluated insecticides, PYR, DDT and propoxur, demonstrating that each strain developed cross resistance to other groups of insecticides different from those with which they were selected. However, the biochemical results showed that only the Propoxur F29 strain had a resistance mechanism based on high GST activities, while the *kdr* mutation study showed high frequencies in both mosquito strains, demonstrating both strains having a resistance mechanism mediated by *kdr* that confers cross resistance to PYRs and DDT.

Example of cross-resistance between carbamates and PYRs mediated by *kdr* mutations was observed in mosquitos *Ae. aegypti* from Santiago de Cuba that were selected with propoxur during 14 generations, frequencies of the Ile1016 allele increased from 0.033 in the original strain to 0.40, showing resistance to PYRs. The most parsimonious explanation offered by the researchers is that the Ile1016 allele is linked to a loci conditioned by resistance to propoxur and that selection with propoxur leads to a rapid increase of this allele linked to *kdr* mutations (Saavedra-Rodriguez et al., 2007).

In all mosquitoes evaluated, the Val1016Ile and Phe1534Cys *kdr* mutations were present. In our observations the allelic frequencies of Cys1534 were higher than those for the Ile1016 allele. This same pattern has been documented in other populations of *Ae. aegypti* from Venezuela where, the Cys1534 allele has been found almost fixed in several populations, with frequencies ranging between 0.35-1.00 (Alvarez et al., 2015). In the Guadalupe and San Martin Islands similar observation have been made, where the ranges of the Cys1534 were between 0.92-0.98 (Goindin et al., 2017); recently in Jamaica, this allele was determined to be fixed in all *Ae. aegypti* populations examined (Francis et al., 2017). In Colombia, previous reports showed that the Cys1534 frequencies ranged between 0.56 to 0.63 in *Ae. aegypti* from Antioquia, La Guajira and Meta (Granada et al., 2018).

The allele Ile1016 has registered a rapid increase and dispersion in *Ae. aegypti* populations in some Latin- America countries (Brito et al., 2018; García et al., 2009; Saavedra-Rodriguez et al., 2007; Vera-Maloof et al., 2015). These studies suggest that Val1016Ile mutation is easily selected (García et al., 2009; Vera-Maloof et al., 2015). This mutation was described for the first time in Colombia in 2014 in *Ae. aegypti* from the Caribbean region, with frequencies ranging between (0.09-0.35) (Maestre-Serrano et al., 2014). Later the allele Ile1016 was describing in three populations of Colombia, and was observed in similar frequencies as a new mutation *kdr* found, the Val419Ile. The presence of both mutations was associated with lambda-dacyhalothrin resistance (Granada et al., 2018).

Even though in all mosquitos samples both *kdr* mutants were observed, albeit with different frequencies, not all the mosquitoes were found to be resistant to PYRs. The correlation analyses suggests that in our samples, the high frequencies of both Ile1016 (>0.5) and Cys1534 could be involved with the permethrin and lambda-dacyhalothrin resistance, while the high frequency of Cys1534 alone were correlated with permethrin resistance. In a previous study in Colombia, a correlation was observed between high frequencies of Ile1016 and the resistance ratios (RR) to deltamethrin, cyflutrin and permethrin, but not to lambda-dacyhalothrin and DDT (Maestre-Serrano et al., 2014). In our study, the susceptible strains (Rockefeller and Medellin AF), had the lowest allelic frequencies for Cys1534 (0.44) and Ile1016 (0.02-0.04). Despite have both *kdr* mutations did not show any correlation with resistance to PYRs.

Our results may suggest that the presence of the Cys1534 mutation alone was not sufficient to generate resistance to permethrin, and it is necessary that both mutations be in high frequencies. Vera-Maloof et al., 2015 hypothesized that the Cys1534 allele appears rapidly in populations and its frequency increases much faster than Ile1016 and that resistance to PYRs requires the sequential evolution of these two mutations. Mutations in domains II and III are synergistic so that the double mutants Ile1016/Cys1534 (IC) have greater resistance to PYRs than the single *kdr* mutation (Brito et al., 2018; Saavedra-rodriguez et al., 2018; Vera-Maloof et al., 2015).

In Colombia, natural populations of *Ae. aegypti* have the ability to develop resistance to PYRs insecticides used in public health. The presence and combination of increased activity of GST and both *kdr* mutations associated with PYRs resistance, makes it necessary to

develop new strategies for vector management. It is important to maintain continuous monitoring of resistance when PYRs are used in vector control programs.

5. Conclusion

Pyrethroid resistance in *Ae. aegypti* from Colombia are mediated by multiple mechanisms. During the period of the study in which the samples of the field populations were collected (2014-2015), the principal mechanisms associated with PYRs resistance were high activity of the GSTs enzymes and high frequencies of both *kdr* mutations (Val1016Ile and Phe1534Cys). Resistance mechanisms can vary over time in response to insecticides applied by control programs. These mechanisms appear in response to the selective pressure exerted by the insecticides used, which is why we must constantly monitor these mechanisms over time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Aguirre-Obando OA, Martins AJ, Navarro-Silva MA, 2017 First report of the Phe1534Cys *kdr* mutation in natural populations of *Aedes albopictus* from Brazil. *Parasit. Vectors* 10, 160 10.1186/s13071-017-2089-5 [PubMed: 28347326]
- Alvarez LC, Ponce G, Saavedra-Rodriguez K, Lopez B, Flores AE, 2015 Frequency of V1016I and F1534C mutations in the voltage-gated sodium channel gene in *Aedes aegypti* in Venezuela. *Pest Manag. Sci* 71, 863–869. 10.1002/ps.3846 [PubMed: 24935645]
- Atencia MC, Pérez M. de J., Jaramillo MC, Caldera SM, Cochero S, Bejarano EE, 2016 Primer reporte de la mutación F1534C, asociada con resistencia cruzada a DDT y piretroides, en *Aedes aegypti* de Colombia. *Biomédica* 36 10.7705/biomedica.v36i3.2834
- Bhatt S, Gething P, Brady O, Messina J, Farlow A, Moyes C, 2012 The global distribution and burden of dengue. *NIH-PA Author Manuscr. Nat* 496, 504–507. 10.1038/nature12060.The
- Bonner MR, Williams BA, Rusiecki JA, Blair A, Beane Freeman LE, Hoppin JA, Dosemeci M, Lubin J, Sandler DP, Alavanja MC, 2010 Occupational exposure to terbufos and the incidence of cancer in the Agricultural Health Study. *Cancer Causes Control* 21, 871–877. [PubMed: 20155313]

- Bradford M, 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem* 72, 248–254. 10.1016/0003-2697(76)90527-3 [PubMed: 942051]
- Brengues C, Hawkes NJ, Chandre F, McCarroll L, Duchon S, Guillet P, Manguin S, Morgan JC, Hemingway J, 2003 Pyrethroid and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. *Med. Vet. Entomol* 17, 87–94. 10.1046/j.1365-2915.2003.00412.x [PubMed: 12680930]
- Brito LP, Carrara L, De Freitas RM, Bento J, Lima P, Martins AJ, 2018 Levels of Resistance to Pyrethroid among Distinct kdr Alleles in *Aedes aegypti* Laboratory Lines and Frequency of kdr Alleles in 27 Natural Populations from Rio de Janeiro, Brazil 2018.
- Brogdon William G., Chan A, 2010 Instrucciones para la Evaluación de la Resistencia a Insecticida en V mediante del Ensayo Biológico de la - 1–28.
- Chouin-Carneiro T, Vega-Rua A, Vazeille M, Yebakima A, Girod R, Goindin D, Dupont-Rouzeyrol M, Lourenço-de-Oliveira R, Failloux AB, 2016 Differential Susceptibilities of *Aedes aegypti* and *Aedes albopictus* from the Americas to Zika Virus. *PLoS Negl. Trop. Dis* 10, 1–11. 10.1371/journal.pntd.0004543
- Conway MJ, Colpitts TM, Fikrig E, 2014 Role of the Vector in Arbovirus Transmission. *Annu. Rev. Virol* 1, 71–88. 10.1146/annurev-virology-031413-085513 [PubMed: 26958715]
- Dusfour I, Thalmensy V, Gaborit P, Issaly J, Carinci R, Girod R, 2011 Multiple insecticide resistance in *Aedes aegypti* (Diptera: Culicidae) populations compromises the effectiveness of dengue vector control in French Guiana. *Mem. Inst. Oswaldo Cruz* 106, 346–352. 10.1590/S0074-02762011000300015 [PubMed: 21655824]
- Enayati AA, Ranson H., Hemingway J, 2005 Insect glutathione transferases and insecticide resistance. *Insect Mol. Biol* 14, 3–8. 10.1111/j.1365-2583.2004.00529.x [PubMed: 15663770]
- Fonseca-González I, Quiñones ML, Lenhart A, Brogdon WG, 2011 Insecticide resistance status of *Aedes aegypti* (L.) from Colombia. *Pest Manag. Sci* 67, 430–437. 10.1002/ps.2081 [PubMed: 21394876]
- Francis S, Karla SR, Perera R, Paine M, Black WC, Delgoda R, 2017 Insecticide resistance to permethrin and malathion and associated mechanisms in *Aedes aegypti* mosquitoes from St. Andrew Jamaica. *PLoS One*. 10.1371/journal.pone.0179673
- García GP, Flores AE, Fernández-Salas I, Saavedra-Rodríguez K, Reyes-Solis G, Lozano-Fuentes S, Bond JG, Casas-Martínez M, Ramsey JM, García-Reón J, Domínguez-Galera M, Ranson H., Hemingway J, Eisen L, Black WC, IV, 2009 Recent rapid rise of a permethrin knock down resistance allele in *Aedes aegypti* in Mexico. *PLoS Negl. Trop. Dis* 3 10.1371/journal.pntd.0000531
- Gaunt TR, Rodríguez S, Day IN, 2007 Cubic exact solutions for the estimation of pairwise haplotype frequencies: implications for linkage disequilibrium analyses and a web tool “CubeX.” *BMC Bioinformatics* 8, 428 10.1186/1471-2105-8-428 [PubMed: 17980034]
- Goindin D, Delannay C, Gelasse A, Ramdini C, Gaude T, Faucon F, David J-P, Gustave J, Vega-Rua A, Fouque F, 2017 Levels of insecticide resistance to deltamethrin, malathion, and temephos, and associated mechanisms in *Aedes aegypti* mosquitoes from the Guadeloupe and Saint Martin islands (French West Indies). *Infect. Dis. Poverty* 6, 38 10.1186/S40249-017-0254-x [PubMed: 28187780]
- Granada Y, Mejía-Jaramillo A, Strode C, Triana-Chavez O, 2018 A Point Mutation V419L in the Sodium Channel Gene from Natural Populations of *Aedes aegypti* Is Involved in Resistance to λ -Cyhalothrin in Colombia. *Insects* 9, 23 10.3390/insects9010023
- Grisales N, Poupardin R, Gomez S, Fonseca-Gonzalez I, Ranson H, Lenhart A, 2013 Temephos Resistance in *Aedes aegypti* in Colombia Compromises Dengue Vector Control. *PLoS Negl. Trop. Dis* 7 10.1371/journal.pntd.0002438
- Gubler DJ, 2002 The global emergence/resurgence of arboviral diseases as public health problems. *Arch. Med. Res* 33, 330–342. 10.1016/S0188-4409(02)00378-8 [PubMed: 12234522]
- Instituto Nacional de Salud, 2014 Instituto Nacional de Salud. Informe epidemiológico “Red de vigilancia de la resistencia a insecticidas de uso en salud pública en Colombia 2004–2014.” *Inst. Nac. Salud* 27.

- Karunamoorthi K, Sabesan S, 2013 Insecticide Resistance in Insect Vectors of Disease with Special Reference to Mosquitoes: A Potential Threat to Global Public Health. *Heal. Scope Int. Quarerly J.* 2, 4–18. 10.17795/jhealthscope-9840
- Liu N, 2015 Insecticide Resistance in Mosquitoes: Impact, Mechanisms, and Research Directions. *Annu. Rev. Entomol* 60, 537–559. 10.1146/annurev-ento-010814-020828 [PubMed: 25564745]
- Lumjuan N, Rajatileka S, Changsom D, Wicheer J, Leelapat P, Prapanthadara L. aied, Somboon P, Lycett G, Ranson H, 2011 The role of the *Aedes aegypti* Epsilon glutathione transferases in conferring resistance to DDT and pyrethroid insecticides. *Insect Biochem. Mol. Biol* 41, 203–209. 10.1016/j.ibmb.2010.12.005 [PubMed: 21195177]
- Lumjuan N, Stevenson BJ, Prapanthadara L. aied, Somboon P, Brophy PM, Loftus BJ, Severson DW, Ranson H, 2007 The *Aedes aegypti* glutathione transferase family. *Insect Biochem. Mol. Biol* 37, 1026–1035. 10.1016/j.ibmb.2007.05.018 [PubMed: 17785190]
- Maestre-Serrano R, 2012 World ' s largest Science, Technology & Medicine Open Access book publisher Susceptibility Status of *Aedes aegypti* to Insecticides in Colombia Pest Engineering, Farzana Perveen, Dr. (Ed.). 10.5772/31243
- Maestre-Serrano R, Gomez-Camargo D, Ponce-Garcia G, Flores AE, 2014 Susceptibility to insecticides and resistance mechanisms in *Aedes aegypti* from the Colombian Caribbean Region. *Pestic. Biochem. Physiol* 116, 63–73. 10.1016/j.pestbp.2014.09.014 [PubMed: 25454522]
- Mattar S, Miranda J, Pinzon H, Tique V, Bola??os A, Aponte J, Arrieta G, Gonzalez M, Barrios K, Contreras H, Alvarez J, Aleman A, 2015 Outbreak of chikungunya virus in the north Caribbean area of Colombia: Clinical presentation and phylogenetic analysis. *J. Infect. Dev. Ctries* 9, 1126–1132. 10.3855/jidc.6670 [PubMed: 26517488]
- Moher D, Liberati A, Tetzlaff J, Altman DG, Group P, 2009 Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement 2535, 1–8. 10.1136/bmj.b2535
- Moyes C, Vontas J, Martins A, Ng L, Koou S, Dusfour I, 2017 Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *PLoS Negl Trop Dis* 1–20. 10.1371/journal.pntd.0005625
- Ocampo CB, Salazar-Terreros MJ, Mina NJ, McAllister J, Brogdon W, 2011 Insecticide resistance status of *Aedes aegypti* in 10 localities in Colombia. *Acta Trop* 118, 37–44. 10.1016/j.actatropica.2011.01.007 [PubMed: 21300017]
- Padilla JC; Rojas DP; Saenz R, 2012 *Dengue en Colombia*, First, ed. Guías de Impresión Limitada, Bogota.
- Parra B, Lizarazo J, Jiménez-Arango JA, Zea-Vera AF, González-Manrique G, Vargas J, Angarita JA, Zuñiga G, Lopez-Gonzalez R, Beltran CL, Rizcala KH, Morales MT, Pacheco O, Ospina ML, Kumar A, Cornblath DR, Muñoz LS, Osorio L, Barreras P, Pardo CA, 2016 Guillain-Barré Syndrome Associated with Zika Virus Infection in Colombia. *N. Engl. J. Med* 375, 1513–1523. 10.1056/NEJMoa1605564 [PubMed: 27705091]
- Penilla RP, Rodriguez AD, Hemingway J, Torres JL, Arredondo-Jimenez JI, Rodriguez MH, 1998 Resistance management strategies in malaria vector mosquito control. Baseline data for a large-scale field trial against *Anopheles albimanus* in Mexico. *Med Vet Entomol* 12, 217–233. [PubMed: 9737593]
- Ranson H, Burhani J, Lumjuan N, Black WC, IV, 2008 Review Insecticide resistance in dengue vectors. *TropIKA* 1–12.
- Ardila Roldan S, Santacoloma L, H.B., 2013 Estado de la susceptibilidad a insecticidas de uso en salud Pública en poblaciones naturales de *Aedes aegypti* (Diptera: Culicidae) del departamento del Casanare, Colombia. *Biomedica* 33.
- Saavedra-rodriguez K, Maloof FV, Corey LC, Garcia-rejon J, Lenhart A, Penilla P, Rodriguez A, Sandoval AA, Flores AE, Ponce G, Lozano S, W.C.B., Iv, 2018 Parallel evolution of vgsc mutations at domains IS6, IIS6 and IIIS6 in pyrethroid resistant *Aedes aegypti* from Mexico 1–9. 10.1038/S41598-018-25222-0
- Saavedra-Rodriguez K, Strode C, Flores AE, Garcia-Luna S, Reyes-Solis G, Ranson H, Hemingway J, Black IV WC, 2014 Differential transcription profiles in *Aedes aegypti* detoxification genes after temephos selection. *Insect Mol. Biol* 23, 199–215. 10.1111/imb.12073 [PubMed: 24299217]

- Saavedra-Rodriguez K, Urdaneta-Marquez L, Rajatileka S, Moulton M, Flores AE, Fernandez-Salas I, Bisset J, Rodriguez M, McCall PJ, Donnelly MJ, Ranson H, Hemingway J, Black IV WC, 2007 A mutation in the voltage-gated sodium channel gene associated with pyrethroid resistance in Latin American *Aedes aegypti*. *Insect Mol. Biol* 16, 785–798. 10.1111/j.1365-2583.2007.00774.X [PubMed: 18093007]
- Santacoloma L, Chaves B, Brochero HL, 2012 Estado de la susceptibilidad de poblaciones naturales del vector del dengue a insecticidas en 13 localidades de Colombia. *Biomédica* 32, 333–343. 10.7705/biomedica.v32i3.680 [PubMed: 23715182]
- Santacoloma Varón L, Chaves Córdoba Bernardo, Brochero H, 2010 Susceptibilidad de *Aedes aegypti* a DDT, deltametrina y lambdacialotrina en Colombia. *Rev. Panamericana Salud Publica* 27, 66–73. 10.1590/S1020-49892010000100010
- Sritharan J, Demers PA, Harris SA, Cole DC, Peters CE, Villeneuve PJ, Sritharan J, 2017 Occupation and risk of prostate cancer in Canadian men: A case-control study across eight Canadian provinces. *Cancer Epidemiol* 48, 96–103. 10.1016/j.canep.2017.04.006 [PubMed: 28456092]
- Vera-Maloof FZ, Saavedra-Rodriguez K, Elizondo-Quiroga AE, Lozano-Fuentes S, Black IV WC, 2015 Coevolution of the Ile1,016 and Cys1,534 Mutations in the Voltage Gated Sodium Channel Gene of *Aedes aegypti* in Mexico. *PLoS Negl. Trop. Dis* 9, 1–22. 10.1371/journal.pntd.0004263
- WHO, 2006 PESTICIDES APPLICATION AND THEIR APPLICA For the control of vectors. *World Heal. Organ.* 1, 125 10.1111/j.1439-0310.2006.01165.x
- WHO, 2005 Guidelines for laboratory and field testing of mosquito larvicides. *World Heal. Organ.* 1–41. <https://doi.org/Ref:WHO/CDS/WHOPES/GCDPP/2005.11>
- WHO/CDS/WHOPES/GCDPP, 2005 Safety of Pyrethroids for Public Health Use. *World Heal. Organ.* <https://doi.org/who/cds/whopes/gcdpp/2005.10>
- Yanola J, Somboon P, Walton C, Nachaiwieng W, Somwang P, Prapanthadara L, 2011 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* 16, 501–509. 10.1111/j.1365-3156.2011.02725.x [PubMed: 21342372]

Highlights

- Resistance to pyrethroids insecticide was detected in *Ae. aegypti* from Colombia
- Pyrethroids resistance is mediated mainly by GST enzymes and kdr mutations
- DDT insecticide resistance may induce cross resistance to pyrethroids in Colombia
- Cross-resistance between propoxur and lambda-cyhalothrin selected strains was observed
- Changes in insecticide use in Colombia has modified reported resistance mechanisms

Figure 1.
Map of Colombia. Collection sites of *Aedes (Stegomyia) aegypti* used in the present study.

Figure 2.

Box-plots of the median enzymes activity: **A.** α and β esterases assay. **B.** GST assay. **C.** P450 monooxygenases assay. The horizontal bar in the box showed the median activity. The lines in the box denotes the upper and lower quartiles. *Aedes aegypti* strains with elevated enzymatic activity compared with the Rockefeller strain are marked with * (Dunnett test $P < 0.0001$).

Mosquito samples: 1. Rockefeller; 2. DDT F₃₀; 3. Propoxur F₂₉; 4. Lambdacyhalothrin F₂₉; 5. Buga F₁; 6. Yumbo F₂; 7. Palmira F₂; 8. Cali (PdC) F₂; 9. Girón F₁; 10. Medellín (BF) F₂; **11.** Medellín (AF) F₂

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Table 1. Bioassay results of the field collected and laboratory mosquito strains evaluated with seven insecticides.

Strains	Mean and standard deviations of mortality percentages to the Insecticides tested						
	Permethrin	Deltamethrin	λ -cyhalothrin	DDT	Propoxur	Malathion	Temephos
Laboratory-Selected strains							
Rockefeller	100 $\pm$ 1.2 n=130	100 $\pm$ 1.48 n=130	100 $\pm$ 1.11 n=142	90 $\pm$ 1.16 n=148	100 $\pm$ 0 n=124	100 $\pm$ 0 n=180	100 $\pm$ 0 n=80
DDT F ₃₀	87 $\pm$ 11.4 n=172	62.28 $\pm$ 29.2 n=117	87.8 $\pm$ 8.6 n=143	80 $\pm$ 9.0 n=47	73.1 $\pm$ 37.3 n=145	90.9 $\pm$ 15.7 n=81	100 $\pm$ 0 n=80
Propoxur F ₂₉	57.4 $\pm$ 24.6 n=352	73.8 $\pm$ 23.7 n=158	76.7 $\pm$ 6.1 n=141	58 $\pm$ 20 n=48	66 $\pm$ 30 n=167	100 $\pm$ 0 n=83	100 $\pm$ 0 n=80
Lamdcyhalothrin F ₂₉	45.6 $\pm$ 20.7 n=144	66.6 $\pm$ 23.4 n=134	17.2 $\pm$ 2.4 n=176	72.1 $\pm$ 5.5 n=68	59.8 $\pm$ 11.5 n=204	97.7 $\pm$ 3.3 n=180	100 $\pm$ 0 n=80
Buga F ₁	52.9 $\pm$ 15.9 n=217	92.6 $\pm$ 12.8 n=143	87.1 $\pm$ 7.4 n=117	28.1 $\pm$ 19.5 n=56	100 $\pm$ 0 n=125	87.2 $\pm$ 12.9 n=94	100 $\pm$ 0 n=80
Yumbo F ₂	65.7 $\pm$ 9.3 n=141	93.0 $\pm$ 9.8 n=114	79.8 $\pm$ 9.8 n=138	22.2 $\pm$ 9.9 n=56	99.2 $\pm$ 1.4 n=146	100 $\pm$ 0 n=106	100 $\pm$ 0 n=80
Palmira F ₂	76.6 $\pm$ 3.5 n=153	94.0 $\pm$ 4.8 n=297	91.9 $\pm$ 7.3 n=134	19.8 $\pm$ 14.3 n=48	100 $\pm$ 0 n=133	88.9 $\pm$ 15.7 n=110	43.7 n=80
Cali- (PdC) F ₂	71.6 $\pm$ 16.4 n=129	86.8 $\pm$ 11.6 n=105	91.5 $\pm$ 9.8 n=285	9.2 $\pm$ 8.3 n=50	82.5 $\pm$ 16.6 n=103	100 $\pm$ 0 n=115	100 $\pm$ 0 n=80
Girón F ₁	38.3 $\pm$ 21.2 n=190	81.5 $\pm$ 14.0 n=184	62.9 $\pm$ 9.6 n=143	37.6 $\pm$ 19.2 n=45	100 $\pm$ 0 n=128	86.7 $\pm$ 18.8 n=128	100 $\pm$ 0 n=80
Medellín (BF) F ₂	48.9 $\pm$ 15.0 n=169	51.0 $\pm$ 7.4 n=126	38.9 $\pm$ 15.2 n=139	0 $\pm$ 0 n=45	99.1 $\pm$ 1.6 n=142	100 $\pm$ 0 n=126	100 $\pm$ 0 n=80
Medellín (AF) F ₂	98 $\pm$ 2.01 n=143	89.9 $\pm$ 6.8 n=157	81.4 $\pm$ 9.5 n=131	42.3 $\pm$ 11.0 n=59	100 $\pm$ 0 n=135	92.5 $\pm$ 10.6 n=86	100 $\pm$ 0 n=80

n= sample size

Mortality percentages in shaded cells indicate that the mosquito population is resistant.

Mortality percentages in bold indicate that the mosquito population is susceptible.

Allelic frequencies of Val1016Ile and Phe1535Cys in laboratory-selected strains and field-collected strains of *Aedes (Stegomyia) aegypti* from Colombia.

GG Homozygote for Val1016 susceptible; GA Heterozygote Val1016/Ile1016; AA Homozygote Ile1016 resistant. TT Homozygote for Phe1535/Cys1534; GG Homozygote for Cys1534 resistant

* If $P < 0,05$ not consistent with Hardy Weinberg Equilibrium (HWE)

Table 3.

Frequencies of dilocus haplotypes analysis at loci 1016 and 1534.

Strains	Size of sample	Haplotypes frequencies			
		Val1016/ Phe1534 (VF)	Val1016/ Cys1534 (VC)	Ile1016/ Phe1534 (IF)	Ile1016/ Cys1534 (IC)
Laboratory-Selected strains					
Rockefeller	47	0.563	0.393	0.0	0.042
DDT F ₃₀	46	0.195	0.695	0.0	0.108
Propoxur F ₂₉	54	0.027	0.648	0.0	0.324
Lambdacyalothrin F ₂₉	54	0.009	0.268	0.0	0.722
Field-collected strains					
Buga F ₁	53	0.122	0.6132	0.0	0.264
Palmira F ₁	50	0.0	0.4694	0.010	0.520
Yumbo F ₂	50	0.0	0.280	0.010	0.710
Cali (PdC) F ₂	53	0.217	0.5377	0.047	0.198
Girón F ₂	53	0.056	0.3962	0.0	0.547
Medellín (BF) F ₂	51	0.107	0.1863	0.058	0.647
Medellín (AF) F ₂	52	0.567	0.4135	0.009	0.009
		Susceptible		Resistant	