Major Resistant Mechanism to Insecticides of Aedes aegypti Mosquito: a Vector of Dengue and Zika Virus in Vietnam

Khoa Pham Thi**, Hieu Ho Viet¹ and Hung Minh Nguyen¹

¹Center for Molecular Biology, Institute of Research and Development, Duy Tan University, Vietnam

Abstract

Aedes aegypti, a main vector of dengue fever, is very high density in some areas of Vietnam. These mosquitoes in some cities such as Hanoi (HN), Nhatrang (NT), Dongnai (DN), Kien Giang (KG), Daklak (DL), and Hochiminh (HCM) cities have been found high resistance to Pyrethroid group and DDT. Mosquito populations have been sensitive to malathion insecticide.

Identifying insecticide resistant characteristics by esterase enzyme electrophoresis showed that the first esterase appeared in pyrethroid insecticide resistant mosquito population and not recorded in Bora Bora strain.

Analysis of mutations in by multiplex PCR 1011 and 1016 codons encoding VAL and ISO in 20 and 21 exons showed that mutation in 1016 codons with two types VAL/1016/ISO and VAL/1016/GLY were found in the domain II of the sodium channel gene with 94 Aedes aegypti adult female mosquitoes after susceptibility test to alphacypermethrin. The results showed that no mutation in 1011 codon encoding ISO was observed. 14 samples collected from four provinces containing mutations in 1016 codons with two VAL/1016/ISO and VAL/1016/GLY types have been detected.

Introduction

In Vietnam, recent years diseases caused by mosquitoes: Dengue fever caused by Flaviridae virus and transmission main vectors of dengue fever Aedes aegypti, more and more increasing number of infected patients death, special in some large cities such as Hanoi, Nhatrang, Dongnai, Kien Giang, Bannmethuot, Daklak and Hochiminh(Zika patient) cities [1,7,8]. One of causes was development of insecticide resistance in mosquitoes - a challenge in the world. Genetic resistant characteristics of transmission main vectors of malaria, dengue, filariasis and trypanosomiasis is serious impediment to development in many tropical countries cities [2,8,10,11]. Natural selection occurs quickly. Mutation individuals were selected and conserved to increase resistant clones.

In Vietnam, since 1960, resistant characteristics in Culex quinque fasciatus, Aedes aegypti mosquitoes have been detected. In 1975, it was found that Anopheles epiroticus (Anopheles sundaicus), An.sinensis, An.vagus were resistant to DDT, permethrin, and deltamethrin insecticide cities [3,9,8]. Insecticide usage was a selection pressure to population and structure’s change. Insecticide resistant observation was performed by susceptibility assay and PCR method was used to detect the resistant level to find a control method of these disease by insect vector; a suitable method selected to waste avoid and pollution environmental were need.

Materials

Time of the study: from 9/2006 to 12/2009

Area of the study: Aedes aegypti larve’s were collected at some areas in Hanoi, Nhatrang, Buonmethuot, Bienhoa, Rachgia, Hochiminh, Hoabinh, and Ninhbinh cities.

Susceptibility assay of mosquito adults with six insecticides: Permethrin 0.75%, Alpha-cypermethrin 30 mg/m2, Lambda-cyhalothrin 0.05%, Deltamethrin 0.05%, DDT 4% and Malathion 5% have been performed in Department of Experimental Chemistry cities [4,7,11]. PCR analysis was carried out in Department of Biotechnology in National Institute of Malariology, Parasitology and Entomology (NIMPE), Vietnam.

Nucleotide sequencing was performed in Center of DNA Diagnostics, Institute of Biotechnology, Vietnam Academy of Science and Technology.

Methods

This is the last version of discriminative dose of insecticides for mosquitoes (Table 1)
Aedes aegypti larvae were collected from natural and personal pool field, classified according to classification table of Aedes in Vietnam cities [2,15].

- Larvae process in insect rearing with condition at 27 °C±2 °C and 80% ±10% Relative Humidity (RH), 10 hours/daylight. Larvae food was produced by NIMPE [2,7].
- Susceptibility assay was followed by WHO/CDS/CPS/MAL/98.12 [2,15] with six above insecticides.

- The susceptibility assay was conducted with female mosquito adult supplying with sugar solution.
- The number of mosquitoes for an assay is 100.
- The number of mosquitoes for a control is 20-40.
- Exposure time: the testing tubes were vertically kept under subdued light for 1 hour.
- Dead mosquitoes are counted after 10, 15, 20, 30, 40, 50, 60 minutes. After the exposure time, mosquitoes were transferred to holding-tubes supplying with sugar solution, and maintained in a climatic chamber for 24 hours at 25 °C±2 °C and 80% ± 10% RH.
- If the control tube had the mosquito mortality rate over 20%, the tube should be removed and again test conduct, if the mosquito mortality rate in control tube from 5 to less 20%, change by Abbott’s formula.

\[
\text{Mortality} = \frac{\% \text{ fact death mortality} - \% \text{ control death mortality}}{100 - \% \text{ control death mortality}} \times 100
\]

The percentage mortality after 24 hours is recorded.

**Result assesses:**
- Mortality from 98 to 100%: sensitivity.
- Mortality from 80 to 97%: might be resistance and need to confirm again

**PCR technique**

Mutation analysis of Kdr gene of Aedes aegypti in 1011 and 1016 codons by a method of Saavedra-Rodriguez K. et al, 2007 [12,13,14] showed in (Table 2).

**Table 2:** Mutation rates in 1011 and 1016 codons.

| Insecticide | Anophelines | Aedes aegypti | Culex quinquefasciatus |
|-------------|-------------|---------------|------------------------|
| DDT         | 4%          | 4%            | 4%                     |
| Fenitrothion | 1%          | 1%            |                        |
| Malathion   | 5%          | 0.80%         | 5%                     |
| Bendiocarb  | 0.10%       |               |                        |
| Propoxur    | 0.1%        | 0.10%         | 0.10%                  |
| Alpha-cypermethrin | 0.05% |         |                        |
| Bifenthrin  | 0.20%       |               |                        |
| Cyfluthrin  | 0.15%       |               |                        |
| Deltamethrin| 0.05%       | 0.03%         |                        |
| Lambda-cyhalothrin | 0.05%  | 0.03%         | 0.03%                  |
| Permethrin  | 0.75%       | 0.25%         | 0.25%                  |
| Etofenprox  | 0.50%       |               |                        |

*Half an hour exposure
Four hours exposure
Two hour exposure for Anopheline Larvae
0.1% Anopheline Larvae
Two hour exposure Anopheline Larvae

- Mortality less 80: resistance

Esterase enzyme electrophoresis by Green et al, 1990.

**PCR products were analyzed by 4% agarose electrophoresis in TBE 1X buffer in 45 minutes. DNA extraction kit for nucleotide sequencing was purchased from Fermantas. Nucleotide sequencing was performed by ABI-3100 sequencer.**

**Result:** Tool rates has Aedes aegypti larvae in some test provinces (from 2008 to 2009).

**In the North:** Investigating in Hanoi city with 4 districts, the result showed that vector-bone disease was found in test tools from 20% to 50%. Further, knowledge human about fresh environment to disease control is low, degradation environment.

In special, Tuliem district from March to July in 2007, Aedes aegypti was not found in test place, but Aedes albopictus was found. However, from March to May in 2009 we found Aedes aegypti larvae in water tool about 50% mortality rate (Table 3).

**Table 3:** Primer sequences used for PCR

| Mutation     | Primer                        | Size (bp) |
|--------------|-------------------------------|-----------|
| Val1011      | 5’-ATTGTATGCTTGTGGGTGACG-3’   | 24        |
| iso1011      | 5’-GGATGAACCGAAATTGGACAAAAGC-3’ | 40        |
| Val1011 reverse | 5’-CCAGGTGATTTACTAGATTTCCACCGACGG-3’ | 104   |
| Met1011      | 5’-GGATGAACCGAAATTGGACAAAAGC-3’ | 40        |
| iso1016      | 5’-CCAGGTGATTTACTAGATTTCCACCGACGG-3’ | 104   |
| Val1016      | 5’-GGATGAACCGAAATTGGACAAAAGC-3’ | 40        |
| iso1016 reverse | 5’-CCAGGTGATTTACTAGATTTCCACCGACGG-3’ | 104   |
| Gly1016      | 5’-GGATGAACCGAAATTGGACAAAAGC-3’ | 40        |
| Val1016 reverse | 5’-CCAGGTGATTTACTAGATTTCCACCGACGG-3’ | 104   |
| Gly1016      | 5’-GGATGAACCGAAATTGGACAAAAGC-3’ | 40        |

**Citation:** Thi KP, Viet HH and Nguyen HM. Major Resistant Mechanism to Insecticides of Aedes Aegypti Mosquito: a Vector of Dengue and Zika Virus in Vietnam. SM Trop Med J. 2016; 1(2): 1010. https://dx.doi.org/10.36876/smtmj.1010
Table 4: Tool rates had *Aedes aegypti* larvae in some provinces (from 2007 to 2008).

| Time     | Collecting area                          | No. water tool to test | No. tool had larvae | Tool rate (%) |
|----------|------------------------------------------|------------------------|---------------------|---------------|
| 4/2008   | Thanhxuan District, Hanoi City           | 50                     | 20                  | 40            |
| 6/2007   | Dongda District, Hanoi city              | 50                     | 19                  | 38            |
| 8/2007   | Hoangmai District, Hanoi city            | 50                     | 10                  | 20            |
| 5/2008   | Tuliem District, Hanoi city              | 50                     | 26                  | 50            |
| 6/2007   | Huu nghi commune, Hoabinh city, Hoabinh province | 50                     | 0                   | 0             |
| 8/2007   | Xuanmai commune, Luongson District, Hoabinh province | 50                     | 0                   | 0             |
| 5/2008   | Ninhbinh city, Ninhbinh province         | 50                     | 0                   | 0             |
| 5/2014   | Phatdiem commune, Kimson District, Ninhbinh province | 50                     | 0                   | 0             |

Table 5: Susceptibility test result with *Aedes aegypti* population (72nd mosquito generation) in Hanoi from 2008 to 2009.

| Insecticide                  | Tuliem | Thanhxuan | Dongda | Hoangmai |
|------------------------------|--------|-----------|--------|----------|
| Mortality (%)                | assess | Mortality (%) | assess | Mortality (%) | assess | Mortality (%) | assess |
| 1 permethrin 0.75%           | 80.0   | 51        | R      | 65.5     | R       | 79         | R      |
| 2 alpha-cypermethrin 30mg/m² | 83.0   | 90        | R/S    | 82       | R/S     | 96         | R/S    |
| 3 lambda-cyhalothrin 0.05%   | 63.0   | 74        | R      | 59       | R       | 59         | R      |
| 4 deltamethrin               | 97.0   | 64.0      | R/S    | 85.0     | R/S     | 62.0       | R      |
| 5 DDT 4%                     | 20.0   | 1         | R      | 8.0      | R       | 32         | R      |
| 6 malathion 5%               | 99.0   | 98.0      | S      | 98.0     | S       | 98.0       | S      |

Table 6: Tool rate had *Aedes aegypti* larvae in some provinces 2008.

| Time     | Collecting area                          | No. water tool to test | No. tool had larvae | Tool rate (%) |
|----------|------------------------------------------|------------------------|---------------------|---------------|
| 9/2008   | Nhatrang city, Khanhhoa province         | 100                    | 62                  | 62.0          |
| 8/2008   | Buonmethuot city, Daklak province        | 100                    | 76                  | 76.0          |
| 8/2008   | Hochiminh city                           | 100                    | 55                  | 55.0          |
| 8/2007   | Bienhoa city, Dongnai province           | 100                    | 47                  | 47.0          |
| 8/2008   | Rachgia city, Kiengiang province         | 100                    | 62                  | 62.0          |

Table 7: Susceptibility test result with *Aedes aegypti* population in Nhatrang city, Khanhhoa province and Banmethuot city, Daklak province.

| Insecticide          | Nhatrang 9/2008 | Daklak 9/2008 |
|----------------------|-----------------|--------------|
| Mortality after 24 hours (%) | assess          | Mortality after 24 hours (%) | assess |
| permethrin 0.75%     | 3.03            | R            | 14.58         | R            |
| alpha-cypermethrin 30mg/m² | 10.6            | R            | 83.0          | R/S          |
| lambda-cyhalothrin 0.05% | 10.2            | R            | 35.7          | R            |
| deltamethrin         | 8.08            | R            | 42.85         | R            |
| DDT 4%               | 2.0             | R            | 0             | R            |
| malathion 5%         | 98.0            | S            | 98.3          | S            |

Table 8: Susceptibility test result with *Aedes aegypti* population in Rachgia- Kiengiang, Bienhoa-Dongnai and Hochiminh cities.

| Insecticide          | Kiengiang 9/2008 | Dongnai 9/2008 | Hochiminh city 9/2008 |
|----------------------|------------------|----------------|-----------------------|
| Mortality (%)        | assess           | Mortality (%)  | assess                | Mortality (%) | assess |
| permethrin 0.75%     | 13.5             | R              | 6.6                   | R            | 16.0   | R      |
| alpha-cypermethrin 30mg/m² | 47.4             | R              | 55.1                  | R            | 16.6   | R      |
| lambda-cyhalothrin 0.05% | 39.3             | R              | 30.9                  | R            | 10.0   | R      |
| deltamethrin         | 37.7             | R              | 24.7                  | R            | 31.9   | R      |
| DDT 4%               | 7.0              | R              | 6.2                   | R            | 2.0    | R      |
| malathion 5%         | 98.3             | S              | 98.0                  | S            | 98.0   | S      |
Table 9: Genotype frequency of *Ae. aegypti* sensitive and resistance mosquitoes with pyrethroid (N=36) at district of Thanhxuan.

| Populations                  | Allene | Est-1 | Est-2 | Est-3 | Est-4 | Est-5 | Est-6 |
|------------------------------|--------|-------|-------|-------|-------|-------|-------|
| Sensitive Bora Bora          | a      | 0     | 0.81  | 0.69  | 0.46  | 0.92  | 1     |
|                              | b      | 0     | 0.19  | 0.31  | 0.54  | 0.08  | 0     |
| Resistance Thanhxuan district| a      | 0.42  | 0.73  | 0.625 | 0.645 | 0.625 | 1     |
|                              | b      | 0.58  | 0.27  | 0.375 | 0.355 | 0.375 | 0     |

*(Table 4) shows in some test place such as Hoabinh city and Xuanmai commune in Hoabinh province, Phatdiem commune in Ninhbinh province not were found *Aedes aegypti* larvae but had *Aedes albopictus* larvae.*

**(Susceptibility test with *Aedes aegypti* with some insecticides)**

*(Table 5) showed that *Aedes aegypti* at district of Thanh Xuan, Dongda, Hoangmai, Tuliemin Hanoi cities was resistant to permethrin and lambda-cyhalothrin, special highest resistant to DDT. It might be resistant to alpha cypermethrin, and test mosquitoes were sensitive to malathion. From Center to South*

*(Table 6) showed that water tool rate in some test cities were high such as Nhatrang city 62.0%, Daklak city 76.0%, Rach gia city of Kiengiang province 62.0%. Fresh environment knowledge, skill larvae to disease control is low, subjective. Special larvae in the roof of house and veranda.*

*(Table 7) showed that *Aedes aegypti* at Nhatrang city, Khanhhoa province and Buomethout city, Daklak were resistance with permethrin and lambda-cyhalothrin, special highest resistance with DDT. Might be resistance with alpha cypermethrin and test mosquito were sensitive with malathion.*

**Need a strategy to mosquito control active to prevent mosquito density, special in summer season.**

*(Table 8) *Aedes aegypti* population in test places was high resistance with pyrethroid and DDT. Mortality after 24 hours with DDT were highest in Hochiminh city (2.0%) and with alpha cypermethrin were highest in Hochiminh city.*

**Esterase electrophoresis result**

*Mutation identified result on kdr gene of *Aedes aegypti*: PCR reaction performed on specific primer at 1011 codon and 1016 codon to reproduction kdr fragment gene of individual from test place, alive and death after susceptibility test.*

**1011 and 1016 codon mutations.**

*Mutation analysis: Codon 1011 encoding ISO has mutation A to G to change ISO to VAL (ISO/1011/VAL), or A to G to ISO to MET (ISO/1011/MET). Codon 1016 encoding ISO has mutation G to A, to change VAL to ISO (VAL/1016/ISO), or T to G to change VAL to GLY (VAL/1016/GLY).*

*Codon 1011 has not mutation, has 14 mosquito samples (14, 89%) of 4/6 provinces (66.6%) codon 1016 has mutation with two heterzoge VAL/1016/ISO and VAL/1016/GLY (Table 9). Table 3 showed that has 9 samples-*Aegypti* from Hanoi city (7.50%) with 5 heterozygote mutation samples VAL/1016/GLY (55.5%), 1 homozygote mutation sample GLY/1016/GLY (11.11%), 1 heterozygote mutation sample VAL/1016/ISO (11.11%) and 2 mix heterozygote mutation samples VAL/1016/ISO and VAL/1016/GLY (Table 10). Khanhhoa province has 2 heterzoge mutation samples AL/1016/GLY (5.88%). Daklak has 1 heterozygote mutation sample VAL/1016/GLY (11.11%). Hochiminh city has 1 mix heterozygote mutation sample VAL/1016/ISO and VAL/1016/GLY (22.22%). Kiengiang and Dongnai provinces have not found mutation in 1011 and 1016 codons (Figures 1-4). Agarose gel electrophoresis of PCR products amplified 1011 and 1016 codons.*

Citation: Thi KP, Viet HH and Nguyen HM. Major Resistant Mechanism to Insecticides of Aedes Aegypti Mosquito: a Vector of Dengue and Zika Virus in Vietnam. SM Trop Med J. 2016; 1(2): 1010. https://dx.doi.org/10.36876/smtmj.1010
According susceptibility test result *Aedes aegypti* population in test places were high resistance with pyrethroid (permethrin, alpha-cypermethrin). Addition kdr mutation gene result showed that: *Aedes aegypti* population were resistance with insecticide, might be has many mutation sites on *Aedes aegypti* mosquito that 1011 and 1016 codons were one of mutation sites involved in insecticide resistance to pyrethroid.

**Result**

1. *Aedes aegypti* mosquito in test city were resistance against permethrin mortality rates of: 52.25% in Hanoi, 3.03% in Nhatrang city, 16% in Hochiminh city, 13.37% in Kiengiang province, 6.15% in Dongnai province and 14.58% in Daklak province. Resistance against lambda-cypermethrin mortality rates of: 57.7% in Hanoi, 10.2% in Nhatrang city, 10% in Hochiminh city, 39.3% in Kiengiang province, 24.75% in Dongnai province and 42.85% in Daklak province. Might be resistance against deltamethrin in Hanoi city: 82% mortality rate, high resistance with DDT mortality rates of13.75% in Hanoi city, 2.0% in Nhatrang city, 2% in Hochiminh city, 7% in Kiengiang province, 6.2% in Dongnai province and 0% in Daklak province and tolerant against alpha-cypermethrin mortality rates after 24 hours: 82.25% in Hanoi, 83% in Daklak province, 10.6% in Nhatrang city, 66.6% in Hochiminh city, 47.42% in Kiengiang province, 55.1% in Dongnai province.

2. Tools have larvae: water jar, jar, vase, broken piece of a bowl had *Aedes aegypti* larvae or roof of house, veranda.

3. PCR result showed that not mutation in 1011 codon. Mutation identify in 1016 codon with two VAL/1016/ISO and VAL/1016/GLY types. Total 94 mosquitoes tested and 14 samples had mutation. Almost mutations were heterozygote.

**References**

1. National institute of malarialogy, parasitology and entomology. Classification table of Anophelinae in Vietnam (mosquito-baby toss-larvae). 1987.

2. National institute of malarialogy, parasitology and entomology. Technical used in the control malarial. Publisher medicine. 1971; 95.

3. Nguyen Thi Bach Ngo, Do Thi Dien, Nguyen Thi Bich Lien, Nguyen Dinh An. Assess filariasis disease situation and susceptibility test of Culex quinquefasciatus and Culex vishnui with some insecticides at Kanhtrung commune, district of Khanhtrung commune, district of Khanhtrung commune, district of Kinhtho province. Journal of malaria and parasite diseases control. 2005; 6: 42-48.

4. Nguyen Thi Thanh, Bui Dinh Bai, Nguyen Van Ngo, et al. Malaria solve methods at Vancanh commune, where had drug resistance parasite, vector malaria Anopheles minimus, An. dirus outdoors. Proceedings of scientific research. Publisher medicine. 1992; 152-161.

5. Nguyen Tho Vien, Do Thi Hoang, Nguyen Van Ngo, et al. Malaria resistant mechanism to insecticides of Aedes Aegypti Mosquito: a vector of Dengue and Zika Virus in Vietnam, SM Trop Med J. 2016; 1(2): 1010. https://dx.doi.org/10.36876/smtmj.1010
9. Trinh Dinh Dat. Identify level and genetics characteristic with insecticide of Culex quinquefasciatus Say. Genetics and application. 1993; 24-29.

10. Vu Duc Huong, et al. Additional biomass leads some species of Culicidae mosquitoes transmit the disease is mediated in Vietnam. Proceedings of scientific research. Institute of malaria, parasite and entomology from 1991 to 1995. 1997; 2: 117-128.

11. Vu Duc Huong, et al. Additional control methods some species of Culicidae mosquitoes transmit the disease is mediated in Vietnam. Proceedings of scientific research. Institute of malaria, parasite and entomology from 1991 to 1995. 1997; 2: 129-138.

12. Brengues C, NJ Hawkes, F Chandre, L McCarroll, S Duchon, P Guillet, et al. Pyrethroid and DDT cross-resistance in Aedes aegypti is correlated with novel mutations in the voltage-gated sodium channel gene. Medical and Veterinary Entomology. 2003; 17: 87-94.

13. H Ranson, B Jensen, JM Vulule, X Wang, J Hemingway and FH Collins. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan Anopheles gambiae associated with resistance to DDT and pyrethroids. Journal of the Insect Molecular Biology. 2000; 9: 491-497.

14. Saavedra-Rodriguez K, L Urdaneta-Marquez, S Rajatileka, M Moulton, AE Flores, I Fernandez-Salas, et al. A mutation in the voltage-gated sodium channel gene associated with pyrethroid resistance in Latin American Aedes aegypti. Journal of the Insect Molecular Biology. 2007; 16: 785-798.

15. WHO. Test procedure for insecticide resistance monitoring in malaria Vectors, bio-efficacy and persistence of insecticides on treated surfaces, Document WHO/CDS/CPC/MAL/98.12. Geneva, Switzerland. 1998.