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Research Article
Detection of *Ace-1* Mutation in Temephos-Resistant *Aedes aegypti* L. in West Sumatra, Indonesia

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Abstract

Background and Objective: Dengue cases have increased while the spread is getting broader worldwide. Temephos has been frequently used to control the larvae of the *Aedes aegypti* L., the primary vector of dengue. The intensive use of this larvicide has given rise to resistance. This study aims to determine the susceptibility status of *Ae. aegypti* to temephos and examine the two mutations (F290V and F455W) that possibly occur in the *Ace-1* gene of *Ae. aegypti* from Salido Sub-District, IV Jurai District, Pesisir Selatan Regency. **Materials and Methods:** The susceptibility test was performed referring to a standard method of the World Health Organization, followed by a molecular test (polymerase chain reaction) and sequencing. **Results:** The results showed that the larvae of *Ae. aegypti* have been tolerant to temephos (0.012 mg L^{-1}) with a percentage of larval mortality of 91.67%. The sequencing analysis in the *Ace-1* gene revealed the absence of F290V and F455W mutation in temephos-resistant *Ae. aegypti*, but a point mutation was detected at codon 506. This mutation shifts the ACA codon to ACT, but still codes for the same amino acid, threonine. **Conclusion:** Our study indicates the presence of other resistance mechanisms in the major dengue vector of the Salido District. Implementation of the alternative population control strategy is required to prevent the temephos resistance further.

Key words: *Aedes aegypti* L., temephos, insecticide resistance, *Ace-1* gene, susceptibility test, dengue vector, mutation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dengue is a primary public health concern throughout tropical and sub-tropical countries worldwide[1]. As a mosquito-borne viral disease, dengue has been gradually expanding into previously dengue-free areas as a result of vector expansion and human movement[2]. *Aedes aegypti* is the primary vector of dengue[3] and since the 1970s, larvicide temephos has been widely used in controlling mosquito vectors in Indonesia[4]. Consequently, extensive and repeated use of the larvicide has manifested resistance development as reported in Indonesia[5-7] and also in several countries[8,9].

Insecticide resistance arises due to the development of resistance mechanisms. Two main mechanisms of resistance exist in pests: Target-site and metabolic. In the target-site mechanism, the binding sites of insecticides are mutated so that the binding affinity of insecticides is lost. In metabolic mechanism, over-expression of metabolic enzymes occurs, which neutralize insecticides before reaching the target site[10]. The AChE is an enzyme becoming the target of organophosphate insecticide including temephos. In several insects, two genes are described, *Ace-1* and *Ace-2*, coding for the two synaptic enzymes, AChE1 and AChE2, respectively[11]. The occurrence of target site alteration is due to the gene mutation. In the *Ace-1* gene, three mutations have been associated with acetylcholinesterase insensitivity in insects among them are G119S, F290V and F455W[12]. To date, the reports regarding F290V and F455W mutations in the *Ace-1* gene of *Ae. aegypti* from Salido has not yet been available. Further, this research investigated the resistance status and the presence of the point mutations that might occur in temephos-resistant larvae of *Ae. aegypti* from Salido Sub-District.

MATERIALS AND METHODS

Study area and sampling: This study was conducted from February to June, 2018. Samples were collected from Salido Sub-District, IV Jurai District, Pesisir Selatan Regency, West Sumatra, Indonesia. This area is located at coordinates 1°18'55.8" latitude-1°19'11.3" latitude and 100°33'46.5" East longitude-100°34'00.1" East longitude.

Aedes aegypti larvae were obtained from water reservoirs such as tubs or buckets, dispensers and others in the resident's houses. The identification of larvae (instar III) was carried out with the guidance of the Zootaxa 589 identification key book by Rueda[13] at the Animal Physiology Laboratory, Andalas University.

Susceptibility test: The susceptibility test procedure was performed according to WHO. First, the temephos with initial concentration of 6.25 mg L^{-1} was diluted to obtain several treatment concentrations: 0.000 (control), 0.003, 0.006, 0.012 and 0.025 mg L^{-1}. For each treatment, 20 third-stage larvae were transferred to the test glass containing 250 mL of each temephos solution. After 24 hrs, the mortality rate was evaluated. Populations with a mortality rate of <90% were considered resistant.

Molecular test: Detection of gene mutations employed the larvae of *Ae. aegypti* that were still alive after being treated with temephos (0.012 mg L^{-1}) or those categorized as resistant. The DNA extraction was performed using PureLink Genomic DNA mini kits (Invitrogen, USA). The amplification of the *Ace-1 Ae. aegypti* gene was done by Polymerase Chain Reaction (PCR) procedure. The primer used for the amplification was designed by Geneious software version 11.1.2 (Biomatters Ltd., Auckland, New Zealand) referring to the *Ace-1 Ae. aegypti* gene which originates from GenBank with the accession number of BK006052.1. The primers used for the amplification of the *Ace-1* gene primers are ACE F4 (5'-GTTTG GTGAA AGTGC AGGTG-3') and ACE R4 (5'-CATAG GTTGT GTTGA GCCCA-3'). The PCR procedure was performed under the following conditions: Denaturation step at 95°C for 5 min, 45 cycles of amplification (30 sec at 95°C, 30 sec at 61.3°C and 60 sec at 72°C) and an elongation step of 72°C for 5 min. The PCR products were stored at -20°C, followed by electrophoresis[14]. Sequencing was undertaken by Macrogen (Seoul, Korea).

Data analysis: The susceptible status of *Ae. aegypti* larvae population was determined based on WHO standards as follows: (1) Resistant: Larval mortality <90%, (2) Tolerant: The larvae mortality is 90-97% and (3) Susceptible: The larvae mortality is 98-100%. Lethal Concentrations (LC_{50}, LC_{90} and LC_{98}) were determined by probit analysis based on the percentage of larval mortality[15]. The sequencing results were analyzed using Geneious software version 11.1.2. The reference genes of *Ace-1 Ae. aegypti* standard was obtained from GenBank with the accession number of BK006052.1, as a comparison with the nucleotide sequences of the *Ace-1* gene *Ae. aegypti* in the Salido Sub-District.

RESULTS AND DISCUSSION

Table 1 represented the susceptibility status of *Ae. aegypti* to temephos in standard dose (0.012 mg L^{-1}) in

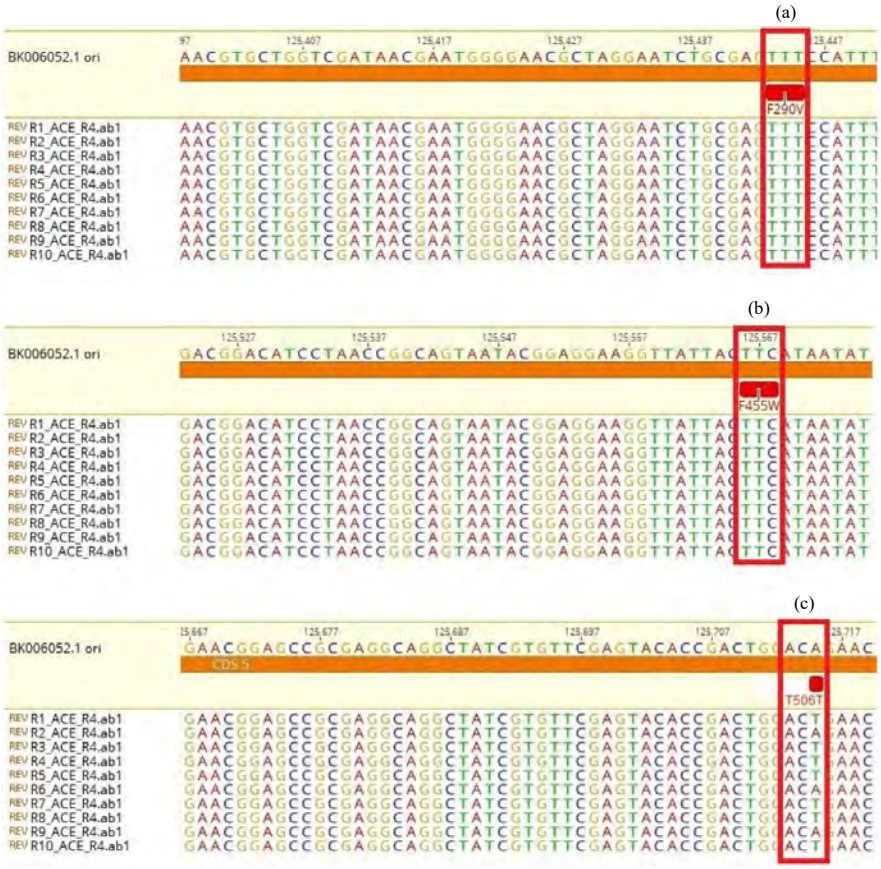

Fig. 1(a-c): Sequencing analysis of the *Ace-1* gene of *Ae. aegypti* from Salido District, (a) Absence of F290V mutation, (b) Absence of F455W mutation and (c) Presence of a silent mutation at codon 506 (T506T)

Table 1: Mortality rate and resistance status of *Ae. aegypti* to temephos from Salido Sub-District, Pesisir Selatan Regency, West Sumatra

		Mortality of *Ae. aegypti* larvae		
Temephos concentration (mg L^{-1})	N	Mean±SE	Mortality(%)±SE	Resistance status
Control	20	0±0.0	0±0.0	-
0.003	20	6.70±0.7	33.33±3.8	Resistant
0.006	20	11.83±0.6	59.17±3.0	Resistant
0.012	20	18.33±0.6	91.67±3.3	Tolerant
0.025	20	20±0.0	100±0.0	Susceptible

Table 2: LC_{50}, LC_{90} and LC_{98} values of insecticide temephos against *Ae. aegypti* larvae from Salido Sub-District, Pesisir Selatan Regency, West Sumatra

LC	Temephos concentration (mg L^{-1})±SE
50	0.005±0.0003831
90	0.011±0.0007022
98	0.015±0.0010904

Salido Sub-District is considered a tolerant category, whereas at higher doses (0.025 mg L^{-1}) the population is still susceptible to temephos.

The probit analysis aims to determine the lethal concentration (LC) value of temephos in controlling *Ae. aegypti* larvae in Salido District, as represented in Table 2. The data above shows the value of the lethal concentration of 50% (LC_{50} = The concentration of temephos required to control 50% of *Ae. aegypti* larvae population), the lethal concentration of 90% (LC_{90} = The concentration of temephos required to control 90% of *Ae. aegypti* larvae population) and lethal concentration of 98% (LC_{98} = The concentration of temephos required to control 98% of *Ae. aegypti* larvae population).

The length of the amplification product of the *Ace-1 Ae. aegypti* gene using ACE F4 and ACE R4 primers was 1,082 bp. There are no mutations in F290V and F455W but the silent mutation was found at codon 506 which ACA changed to ACT, but still coding for the same amino acid, threonine (Fig. 1a-c).

DISCUSSION

To prevent the dengue outbreak, temephos has been widely used in managing *Ae. aegypti* population in West Sumatra. Temephos has been applied as a larvicide for over 40 years in Indonesia[16] including Kanagarian Salido. Interestingly, this research showed that the larvae of *Ae. aegypti* in this area have not been categorized as resistant. Whereas, in another study area which is located in the same province, the population of *Ae. aegypti* has been considered resistant to temephos (0.012 mg L^{-1}) as reported in the village of Kapalo Koto and Gunung Pangilun in Padang[6] and Kanagarian Tanjung Bingkuang in Solok[17] with a mortality rate of 10, 61.7 and 78%, respectively. It was suggested that the difference in resistance status of *Ae. aegypti* is likely due to the level of selection pressure of insecticides gained by the mosquito larvae population, where the selection pressure in Kenagarian Salido is probably lower than the known resistant areas.

An insect population that receives frequent insecticide selection pressure will tend to develop a resistant population in a shorter period compared to an insect population that receives less insecticide selection pressure[18]. This theory has been illustrated by a study in the Minomartani Sub-District, Yogyakarta. Despite their proximity, the two study areas (RW_9 and RW_{10}) showed different levels of resistance. The mosquito population in the RW_9 area was categorized as susceptible. Perhaps, it is because the people infrequently use temephos as a larvicide and community larvae surveillance did not actively encourage people to take preventive strategies to break the life cycle of the mosquito. Meanwhile, the mosquito population in the RW_{10} area has a tolerant status where it is known that the inhabitant in this area often uses temephos as a larvicide[19]. This finding represents that the difference in the level of resistance is influenced by the application of the insecticides. Nonetheless, the factors that trigger resistance such as the use of insecticides on a wide scale, long-term use and high frequency still require attention[20].

One of the mechanisms underlying vector resistance to temephos is the insensitivity of the enzyme acetylcholinesterase (AChE) due to mutations in the *Ace-1* gene. In *Aedes aegypti*, only a few reports are available regarding mutations in the *Ace-1* gene that lead to enzyme insensitivity. The absence of F416V and mutation in temephos-resistant *Ae. aegypti* has been confirmed in Martinique[21] and Colombian population[9]. In the other study, the presence of F290V (phenylalanine→valine) and F455W (phenylalanine→tryptophan) mutations generating insensitivity in the AChE enzyme were reported in *Culex pipiens* and *Cx. tritaeniorhynchus*[22].

Based on the analysis of the *Ace-1* gene sequencing data in the Salido population, no mutations occur either in F290V or F455W. The absence of mutations in F290V showed similar results to the study on temephos-resistant *Ae. aegypti* in Martinique[21] and Colombia[9]. In the F290V mutation, one mutation is required to change the TTT (phenylalanine) to GTT (valine) while the F455W mutation requires two mutations to change the TTT (phenylalanine) to TGG (tryptophan)[12]. This situation could be described as a codon constraint, where *Ae. aegypti* has a different nucleotide sequence from other mosquito species such as *Anopheles gambiae* and *Cx. pipiens*[23]. An example is the 119th amino acid of the *Ace-1* gene (glycine). In *An. gambiae* and *Cx. pipiens,* the 119th amino acid is coded by the codon GGC, but in *Ae. aegypti* the 119th amino acid is coded by the codon GGA. Therefore, mutations at this point (G119S) are difficult in *Ae. aegypti*, because it requires two nucleotide mutations at codon AGA (glycine) to change to codon AGT or AGC (serine). The absence of mutations in F290V and F455W indicates that there is no change in the amino acid sequence resulting in no possibility of reduced, altered or lost function of the acetylcholinesterase enzyme.

As a highlight, this study found a silent mutation at codon 506 (ACA→ACT) in the *Ace-1* gene encoding amino acid threonine (T506T) which was also found in the study of Hasmiwati[5]. However, this mutation did no chance to cause AChE enzyme insensitivity, because there is no change in the amino acid sequence. Besides, codon 506 is not the AChE active site of *Ae. aegypti* so that it does not affect the sensitivity of the AChE enzyme.

This study represented that temephos has been less effective in the controlling larvae of the dengue vector in Kanagarian Salido. Although the resistance status of larval *Ae. aegypti* in Kanagarian Salido, District IV Jurai are still tolerant, regulation of temephos use requires a concern. The relatively long use of temephos can eventually lead to population control failure in *Ae. aegypti* due to the ineffective larval control[16]. To suppress the development of resistance further, alternative steps can be taken such as the use of bioinsecticides such as tobacco (*Nicotiana tabacum* L.)[24], soursop (*Annona muricata* L.)[25], citronella (*Cymbopogon nardus* L.)[26] and custard apple (*Annona reticulata* L.)[27]. The broad spectrum of natural enemies together with their ability to kill mosquitoes can become the candidates for the development of the control strategies against dengue vectors[28]. Besides, in insecticide resistance management, the main strategies considered for public health are rotations, mosaics (which involves the spatial alternation of two or more insecticides with different modes of action) and mixtures of insecticides[29].

CONCLUSION

The absence of two point mutations in the VGSC gene (F290V and F455W) indicates the possible presence of mutations or other resistance mechanisms in the Salido Kanagarian population. Considering the status of the dengue vector population in Salido that is no longer susceptible to temephos, further and thorough investigations regarding the mechanism of resistance are required. By understanding the mechanism of resistance, determining a program for population control of the dengue vector will be more definitive.

SIGNIFICANCE STATEMENT

In this paper, we confirmed the absence of the two well-known mutations (F290V and F455W) associated with target-site resistance in the *Ace-1* gene of temephos-resistant *Ae. aegypti* L. from Salido District, West Sumatra, Indonesia. Nonetheless, a silent mutation was present in codon 506 (ACA→ACT) which codes for the same amino acid threonine. This finding indicates the requirement for further detection of resistance mechanisms in *Ae. aegypti* from the Salido population that probably occur.

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