



DHF Prevention Efforts in Summersari Jember: Assessment of *Aedes aegypti* Resistance Status to Deltamethrin

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Abstract: This study aims to determine the resistance status of *Ae. aegypti* to the deltamethrin insecticide in Summersari Jember Village. A total of 100 2nd generation female *Ae. aegypti* were tested using the CDC bottle bioassay with a deltamethrin dose of 10 µg/ml and a diagnostic time of 30 minutes. Molecular detection using AS-PCR was also performed to detect *kdr* mutations at position 1016 of the VGSC gene. Mortality data was calculated using Ms.Excel with a confidence level of 95%. The molecular weight of the PCR product is calculated using a gel analyzer application. The results of the CDC bottle bioassay show that the *Ae. aegypti* population is still tolerant to the insecticide deltamethrin (91,25% in mortality rate). However, molecular analysis confirms the presence of a mutation at position 1016 of the voltage-gated sodium channel (VGSC) gene (homozygous mutation), which is known to be the primary target of pyrethroid insecticides. These findings indicate that although phenotypic resistance has not yet fully developed, the mosquito population has shown early indications of target-site insensitivity to deltamethrin, which has the potential to develop into resistance under sustained selection pressure. Further use of deltamethrin insecticide may potentially increase phenotypic resistance, namely a decrease in mosquito mortality after exposure to deltamethrin insecticide. Therefore, an integrated vector control strategy and routine monitoring of mosquito resistance status in the region are required.

Keywords: *Aedes aegypti*; deltamethrin; Jember; mutation; tolerant

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INTRODUCTION

The *Aedes aegypti* mosquito is one of the main vectors of dengue and Zika (Harapan et al., 2020). Transmission of these diseases occurs through the bite of female *Ae. aegypti* mosquitoes (I. Agustin et al., 2017). According to data from the Indonesian Ministry of Health, dengue fever cases in Indonesia were reported at the end of 2022 with a total of 143,000 cases, with the highest number of dengue fever cases in the provinces of West Java, East Java, and Central Java. There were 13,189 cases of DHF in East Java (Ministry of Health, 2024). In 2022, Jember had the second highest number of dengue fever cases with 781 cases (East Java Provincial Health Office, 2023).

Mosquito vector control is important to prevent dengue fever. One of the most common methods used to control *Ae. aegypti* mosquitoes is the use of insecticides. However, continuous use of insecticides over a long period of time and in excessive doses can cause a decrease in the sensitivity of *Ae. aegypti* mosquitoes to these insecticides. Resistant mosquitoes will have immunity to insecticides, rendering the use of insecticides ineffective for control (Irmayanti et al., 2013). Furthermore, adult mosquitoes that have become resistant will produce resistant offspring, resulting in an

increase in the proportion of resistant vectors in the population (Ikawati et al., 2015). On the other hand, data on mosquito resistance to insecticides is very important for determining policies on the use of insecticides as vector control. In the implementation of integrated vector control, vector control activities depend on the characteristics and conditions of the area, understanding of the bionomics of vectors and pests (including resistance to chemicals). Inappropriate use of insecticides in integrated vector control activities has the potential to increase disease cases in the area. The types of chemical insecticides commonly used for mosquito control include organochlorines, organophosphates, carbamates, and pyrethroids (Sutarto & Syani, 2018). Chemical insecticide products are often used for mosquito control, especially those containing active ingredients from the Pyrethroid group, such as deltamethrin, cypermethrin, deltamethrin, metofluthrin, and transfluthrin. These products are the choice of the community because they are easy to use, readily available, and the results can be seen immediately (Irmayani et al., 2013).

Vector resistance to insecticides can occur due to mutations in the form of nitrogen base changes in the target gene. An example is a mutation in pyrethroid insecticides, which is often associated with a mutation at point 1016 of the VGSC gene. The VGSC gene is the target site of pyrethroid insecticides in the nervous system. A mutation at this point can cause paralysis and death in mosquitoes (Naw et al., 2022). Several mutations that cause substitutions in domain II of the VGSC gene can lead to resistance to pyrethroids, one of which is a point mutation from valine to glycine at position 1016. This type of mutation tends to be limited to Southeast Asia, including Thailand, Indonesia, Vietnam, and Taiwan. Several regions in Indonesia that are known to have a decrease in the target site of the insecticide deltamethrin in *Ae. aegypti* mosquitoes (Suhartati et al., 2020) include North Sumatra Province, Jambi (Sunaryo & Widiastuti, 2018), Sleman (Mulyaningsih et al., 2018), Banda Aceh City (Isfanda & Riezky, 2019), Semarang Regency, Palembang (Ghiffari et al., 2013).

On the other hand, the susceptibility status of *Aedes aegypti* mosquitoes to deltamethrin insecticide in Jember Regency is not yet clearly known. Molecular evidence regarding the mutation status of the VGSC gene at the V1016 site has also not been clearly documented. Previous studies have only confirmed the resistance ratio of *Ae. aegypti* larvae to temephos larvicide, which is declared to be not yet resistant (RR95, <5) (Khaleyla et al., 2021). Therefore, this study aims to determine the resistance status of *Ae. aegypti* mosquitoes to deltamethrin in Summersari Village, Jember Regency, based on the CDC bottle bioassay and molecular testing using AS-PCR. This research is important for assessing phenotypic susceptibility and detecting molecular markers of resistance in *Aedes aegypti* mosquitoes from Jember. The results of this study are expected to be taken into consideration in integrated vector control, especially in Jember Regency. In addition, these data can also be used as part of national mapping of *Aedes aegypti* resistance patterns to deltamethrin in Indonesia.

METHOD

Research design

This study is an experimental study to assess the resistance status of *Aedes aegypti* mosquitoes from Summersari Village, Jember Regency to deltamethrin insecticide.

Sample collection and propagation

Larvae sampling was conducted systematically based on WHO guidelines (Table 1) from May to September 2023. Mosquito larvae and pupae were collected from 61 sites in Summersari urban village, Jember district. This number is based on the

minimum building requirements for sampling resistance tests according to (WHO, 2011). The number of buildings >500 with House Index >5%, the number of sampling points is at least 59. Data collection was conducted with a minimum distance of 100 m between sampling points. All potential mosquito breeding sites (inside and outside the house) were checked. Larvae and pupae found in breeding sites were hatched at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, University of Jember. Adult mosquitoes were identified using Illustrated keys to the mosquitoes of Thailand (Rattananarithkul et al., 2006). Since the number of larvae obtained from the field did not meet the minimum requirements for the CDC bioassay, they were multiplied until the bioassay was conducted on the second generation. Mosquito larvae are fed fish pellets and sugar solution for adult mosquitoes. Blood meal for adult mosquitoes was provided by placing rats into mosquito cages for 3 hours.

Table 1. Minimum number of building sample adequacy based on WHO 1999

Number of House in the locality	True House Index		
	>1%	>2%	>5%
100	95	78	45
200	155	105	51
300	189	117	54
400	211	124	55
500	225	129	56
1000	258	138	57
2000	277	143	58
5000	290	147	59
10000	294	148	59
Infinite	299	149	59

Resistance testing of *Ae. aegypti* mosquitoes with the CDC Bottle Bioassay method

The test was conducted on 100 female *Ae. aegypti* mosquitoes aged 3-5 days. The resistance test was conducted based on the protocol issued by the Centers for Disease Control and Prevention (Brogdon & Chan, 2012). A total of 4 test bottles (250 ml size) were prepared by pouring 1 ml of test solution (deltamethrin 10 µg/ml in acetone) evenly on the surface of the bottle. The test dosage is determined based on the resistance test recommendations issued by the CDC. One control bottle (250ml size) was filled with 1 ml of acetone. After filling the test solution, the bottle was allowed to stand for 24 hours with open conditions to evaporate the acetone and leave only the test solution in the test bottle. The test was conducted by placing 20 sugar-filled female mosquitoes into each bottle simultaneously. Observations were made by counting mosquito mortality every 5 minutes for up to 2 hours. The diagnostic time of the test was 30 minutes.

Mutation detection using the AS-PCR method

DNA isolation from mosquitoes was performed using the salting out extraction method. The DNA sample was then amplified using the AS-PCR method. A cocktail was prepared by mixing 12.5 ml of master mix, 8 µl of ddH₂O, 1 µl each of forward and reverse primers, and 1.5 µl of the isolated *Ae. aegypti* genome sample. The gene fragment was amplified by PCR using the forward primer Val1016G F (ACCGACAAATTGTTTCCC) and two reverse primers, Val R (GCGGGCAGCAAGGCTAAGAAAAGGTTAATTA) and Gly R

(GCGGGCAGGGCGGGCGGGGGCGGGGCCAGCAAGGCTAAGAAAAGGTAACTC) (Widiastuti et al., 2015).

The PCR conditions consisted of a pre-denaturation stage for 2 minutes at 94°C, a denaturation stage for 30 seconds at 94°C, annealing for 30 seconds at 55°C, and extension for 30 seconds at 72°C, with the final stage being a final extension for 2 minutes at 72°C, for a total of 35 cycles. The AS-PCR results were then visualized using electrophoresis.

Data analysis

Data analysis for CDC bottle bioassay was calculated based on the number of mosquito deaths at diagnostic time using Ms. Excel with a confidence level of 95%. The resistance status of *Ae. aegypti* mosquitoes was determined based on the following criteria:

- A. Mosquito mortality ≥98-100% declared susceptible
- B. Mosquito mortality 80-97% declared tolerant
- C. Mosquito mortality <80% declared resistant

The mortality of the control group was evaluated using the Abbott formula to ensure the validity of the test results (Brogdon & Chan, 2012). If the mortality of control mosquitoes is >10%, the test will be repeated. The following is the calculation of Abbot's formula:

$$\text{Abbot's formula} = \frac{(\text{mortality in test bottles [\%]} - \text{mortality in control bottle [\%]})}{(100\% - \text{mortality in control bottle [\%]})} \times 100$$

The molecular test results were analyzed based on the DNA bands produced by electrophoresis. The DNA bands that appeared had molecular weights of 60 bp and 80 bp and were categorized as heterozygous mutants (V/G), DNA bands with a molecular weight of 80 bp were categorized as homozygous mutants (G/G), and DNA bands with a molecular weight of 60 bp were categorized as homozygous wild types (V/V) (Setiawan et al., 2023). The molecular weight of the electrophoresis results was calculated using the Gel Analyzer application version 23.1.1.

RESULTS AND DISCUSSION

The results of the resistance test of *Ae. aegypti* mosquitoes using the CDC Bottle Bioassay technique showed that the mortality of test mosquitoes at the 30th minute reached 91.25% and 0% in control mosquitoes (Table 1). Thus, *Ae. aegypti* mosquitoes from Summersari Village, Jember Regency were declared tolerant to 10 µg/ml deltamethrin.

Table 1. Percentage mortality of *Ae. aegypti* mosquitoes

	Repeat 1 (number)	Repeat 2 (number)	Repeat 3 (number)	Repeat 4 (number)	Control (number)
Number of mosquito deaths	19	18	17	19	0
Average	18,25				0
Total average percentage mortality	91,25% (Tolerant)				0%
Mosquito origin	: Summersari Village, Jember Regency				
Test insecticide	: 10 µg/ml deltamethrin				
Control solution	: Aceton				
Diagnostic time	: 30 minutes				

The AS-PCR visualization shows that the sample binds to the Gly 1016r primer (molecular weight 80bp) (Pic 1). This indicates that a VGSC gene mutation has

occurred at codon 1016 with an amino acid change from valine to glycine in *Aedes aegypti* mosquitoes in Summersari Village, Jember Regency. The VGSC gene mutation in *Aedes aegypti* at codon 1016, with the amino acid change from valine to glycine, is also known as the V1016G gene mutation, where in this study a mutation with a homozygous allele (G/G) has occurred. *Ae. aegypti* mosquitoes with the homozygous mutant allele (G/G) are resistant to pyrethroid insecticides. This resistance will spread rapidly within a population because *Ae. aegypti* easily adapts to its environment. This resistance will be passed on to subsequent generations, so that over time the population will be dominated by insecticide resistance (Cahyati & Fitriani, 2020).

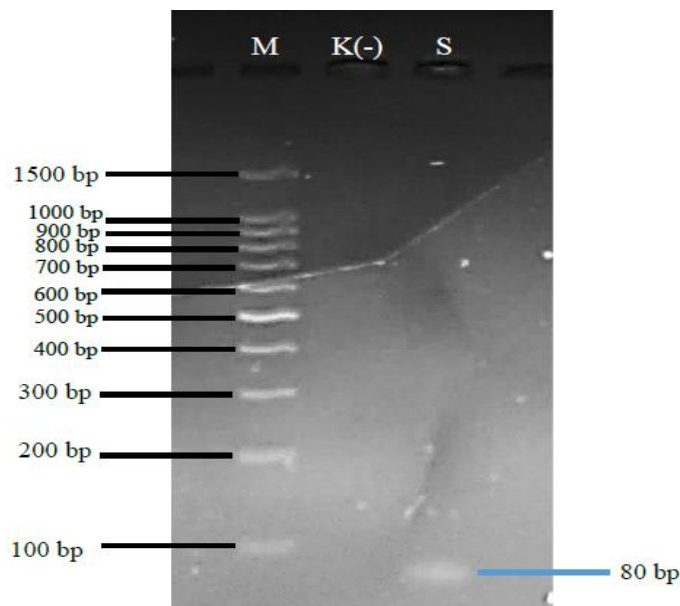


Figure 1. *Ae aegypti* samples from Jember attached to the 80 bp molecular weight primer

The resistance status of *Aedes aegypti* mosquitoes is influenced by various factors. According to (Daswito et al., 2022), there are three main factors that influence mosquito resistance: biological factors, which include mating, generation change, and mosquito avoidance behavior; operational factors, which include materials used, dosage, and rotation of insecticide use; and genetic factors, such as the number and frequency of resistant genes (Gen-R). This study has different results from *Aedes aegypti* mosquitoes in Banjarbaru City have developed resistance to deltamethrin insecticide (Ridha et al., 2018). It is known that the area is caused by the uncontrolled use of insecticides, thus causing resistance in *Aedes aegypti* mosquitoes. According to (Widiastuti et al., 2015), most *Aedes aegypti* mosquitoes in Klaten District were found to have heterozygous mutations in the second domain of the VGSC gene (V1016G) and only 18.2% (from 24 sample) had homozygous mutations in the 1016G allele, 59,1% had heterozygous mutation, and 22,7% has wild type. This VGSC (V1016G) gene is the target target of pyrethroid synthetic insecticides. *Aedes aegypti* from Banda Aceh City in Ateuk hero village where it is known that are still tolerant of deltamethrin 0.05% insecticide. This tolerant status is due to the operational use of deltamethrin which is only carried out occasionally (Fogging) (Isfanda & Riezky, 2019)

The results of this study are tolerant to deltamethrin insecticide 10 µl / ml, which means that the insecticide can still be used for fogging and mosquito repellent, but it is not recommended to use it repeatedly and excessively because with this tolerant status mosquitoes can develop faster and change their status to resistance. According to other researchers in the Research journal (Isfanda & Riezky, 2019) long-term and

repeated use of one class of insecticides (such as organophosphates, pyrethroids, or carbamates) can lead to the development of resistance in target insects. Alternatively, rotating the use of insecticides from different classes or increasing the dose can help reduce the likelihood of resistant insects developing. Decisions on mosquito resistance status also need to be made molecularly. Mosquitoes that are tolerant based on bioassay tests need to be subjected to molecular testing to confirm the makeup of the genes that play a role in eradicating the insecticide. The insecticide deltamethrin that enters the body will act on the VGSC gene located in the central nervous system. The mechanism of action of pyrethroid synthetic insecticides is mainly on the insect nervous system. The insecticide will inhibit the axons of ion channels resulting in a continuous action potential. Synthetic pyrethroids bind to voltage gated sodium channel (VGSC) proteins that regulate the pulse of nerve impulses. As a result, nerve impulses will be stimulated continuously and cause insects to experience hyperexcitation (anxiety) and convulsions (spasticity) (Ghiffari et al., 2013).

The results of field surveys conducted in Summersari Subdistrict found many larvae and pupae of *Aedes aegypti* mosquitoes in several sampling locations (Nihayah et al., 2025). The high density of *Aedes aegypti* larvae in the study area has the potential to contribute to the formation of a population tolerant to deltamethrin through increased population size and selection pressure due to repeated insecticide use. This condition may facilitate the survival of individuals carrying the *kdr* allele, including mutations at position 1016 of the VGSC gene, even though phenotypic resistance has not yet been clearly detected. Experimental studies show that high larval density conditions can reduce the phenotypic expression of resistance to pyrethroids through gene × environment interactions, even though resistance alleles are still present in the population (*Aedes aegypti*) (Grossman et al., 2018). This is also in line with statements from other researchers that mosquito resistance mechanisms in Indonesia are generally influenced by the use of insecticides, urbanization, and climatic factors (Ridha et al., 2025)

The finding that the *Aedes aegypti* population still shows tolerance to deltamethrin based on the CDC bottle bioassay test, but has mutated at position 1016 of the VGSC gene, indicates that target-site resistance mechanisms have begun to develop in the study area. Some factors that may contribute to this discrepancy include the low frequency of mutant alleles in the population, the dominance of heterozygous individuals, and the lack of development of other resistance mechanisms, such as metabolic detoxification. Therefore, these results indicate that a selection process is currently underway. This condition has the potential to increase the risk of phenotypic resistance in the future if insecticide selection pressure continues. In addition, the high density of larvae in the study area may facilitate the maintenance and spread of the *kdr* allele within the population. Therefore, the results of this study emphasize the importance of strengthening integrated resistance surveillance based on bioassays and molecular testing, as well as evaluating insecticide use strategies to maintain the sustainability of vector control programs. With the known status of *Ae. aegypti* mosquitoes to be tolerant to deltamethrin insecticide, other methods of mosquito control are needed as an effort to prevent DHF, so that mosquitoes do not develop resistance. Resistance patterns in Southeast Asia show a trend of increasing frequency of *kdr* mutations in *Ae. aegypti* populations. Several countries, such as Cambodia, Laos, Thailand, Vietnam, and Malaysia, have also reported mutations at the V1016G, S989P, and F1534C sites, which are strongly associated with resistance to deltamethrin and permethrin. However, several studies also indicate the presence of populations with a tolerant status that are not yet fully resistant, suggesting that the

evolution of resistance is gradual and influenced by insecticide selection pressure as well as local ecological factors.

An alternative method to replace the use of insecticides is integrated vector control, which involves various sectors. Integrated vector control is a control technique that combines various methods to eradicate mosquitoes, such as mosquito breeding site elimination, community involvement through larval monitoring volunteers development (Adrianto et al., 2023), biological control using natural enemies (Firmansyah et al., 2015; Aziz et al., 2021; Sari & Novela, 2020), and the use of tools such as ovitraps (Nihayah et al., 2025). The use of ovitraps containing tap water or neem gum water (*Azadirachta indica*) in Jember District is considered effective as a mosquito trap (Nihayah & Purwatiningsih, 2023). This approach emphasizes the integration of environmental, biological, and community-participation strategies to effectively and sustainably reduce vector populations, thereby lowering the risk of disease transmission, such as dengue fever (Ridha et al., 2018; Kementerian Kesehatan, 2024).

Based on findings that the *Aedes aegypti* population still shows tolerance to deltamethrin but has carried a mutation at position 1016 of the VGSC gene, further research is needed to evaluate the relationship between genotype and phenotypic response, determine the frequency of resistant alleles, identify the possible involvement of metabolic resistance mechanisms, and assess the role of ecological factors such as larval density in the dynamics of insecticide resistance development in the study area.

CONCLUSION

The results of the CDC bottle bioassay show that the *Aedes aegypti* population is still tolerant to the insecticide deltamethrin. However, molecular analysis confirms the presence of a mutation at position 1016 of the voltage-gated sodium channel (VGSC) gene, which is known to be the main target of pyrethroid insecticides. These findings indicate that although phenotypic resistance has not yet fully developed, the *Aedes aegypti* mosquito population from Jember Regency has shown early indications of target-site insensitivity to deltamethrin, which has the potential to develop into resistance under sustained selection pressure.

RECOMMENDATION

This study is limited to bioassay detection by counting the number of mosquito deaths, resistance test results that show tolerant status sometimes also found mutations in related genes. Therefore further research is needed to detect mutations in the VGSC gene of *Ae. aegypti* mosquitoes from Jember.

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