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GENETIC DETECTION OF AEDES AEGYPTI MOSQUITO RESISTANCE TO PYRETHROID INSECTICIDE

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ABSTRACT

Background: Aedes aegypti mosquitoes are the main vectors of dengue fever, which is endemic in tropical and subtropical countries, including Indonesia. Continuous vector control efforts have been carried out to reduce dengue cases. As a preparedness measure, a molecular approach such as VGSC gene detection is needed to identify the resistance of Aedes aegypti mosquitoes to pyrethroid insecticides. **Object:** This study aims to determine the results of VGSC gene detection as an indication of resistance in Aedes aegypti mosquitoes exposed to fenvalerate insecticide using the Real-Time PCR method. **Method**: This quantitative descriptive study used observational data analysis and the CDC Bottle Assay method, consisting of four test bottles and one control bottle. After the resistance test, mosquito samples were processed into suspensions for DNA extraction, followed by testing for DNA purity and concentration. VGSC gene detection was performed using the Real-Time PCR method, with results expressed as cycle threshold (CT) values. **Results:** The findings showed that out of four mosquito samples tested, two samples (R1 and R4) were positive for the VGSC gene, with CT values of 1.67 and 1.63, respectively, while the other two samples (R2 and R3) showed N/A results, indicating no VGSC gene detected. **Conclusion:** Needs follow-up and innovation from health workers and cross-sectors to increase the use of postpartum family planning. Based on these results, it can be concluded that 50% of Aedes aegypti mosquito samples showed the presence of the VGSC gene, while the remaining 50% did not.

Keywords: Aedes aegypti Mosquito, Pyrethroid, VGSC Gene, Real-Time PCR, CT Value

BACKGROUND

Dengue Hemorrhagic Fever (DHF) is an infection caused by the Dengue virus transmitted through the bite of Aedes aegypti and Aedes albopictus (Nurbaya et al., 2022). DHF is one of the most common infections found in various tropical and subtropical countries (WHO, 2022). The incidence of DHF is triggered by temperature, knowledge and habits, level of education, environment, and the habit of hanging clothes (Mentari, 2023). Data from the Ministry of Health of the Republic of Indonesia (2024) recorded a total of 186,324 DHF incidences with 1,120 deaths in Indonesia as of September 4, 2024. In East Java, there were 22,745 cases, while in Surabaya City at the beginning of 2024, 43 cases were recorded, increasing from 25 cases in February 2023. Dengue Hemorrhagic Fever (DHF) can cause various serious complications, such as a drastic decrease in platelets that triggers blood clotting disorders, plasma leakage, damage to vital organs such as the liver and kidneys, and Dengue Shock Syndrome (DSS), which can be life-threatening (Soedarto, 2012).

Vector control is necessary as an effort to prevent DHF, which can be carried out chemically through fogging and insecticide application. The use of insecticides is one of the commonly used measures in the community to break the chain of DHF transmission (Nurbaya et al., 2022). Commonly used insecticides include organochlorines, organophosphates, carbamates, and pyrethroids. Pyrethroid insecticides containing fenvalerate as the active ingredient are effective in controlling vectors by disrupting the central nervous system, producing a rapid knockdown effect, and having low toxicity to humans. Fenvalerate is neurotoxic because it binds to VGSC (voltage-gated sodium channel) proteins (Wahyuni et al., 2021). This binding causes VGSC to remain open, triggering depolarization of nerve cells that interferes with signal transmission, ultimately causing muscle paralysis and death in mosquitoes (Chahine, 2018). Resistance to insecticides develops due to continuous use, allowing mosquitoes to survive better. Resistance can reduce insecticide effectiveness and increase the risk of disease transmission by vectors (Ditjen P2P, 2018).

A 2019 study of 27 provinces in Indonesia reported that Surabaya showed a permethrin resistance ratio of 19 (moderate resistance) and deltamethrin of 9 (low resistance) (Silalahi et al., 2022). Qibtiyah (2022) revealed that Aedes aegypti mosquitoes developed tolerance to 0.05% cypermethrin insecticide in four districts of Malang Regency with mortality rates of: Turen 90%, Kepanjen 90%, Karangploso 96%, and Dau 100%, with an average of 94%. Research at Tanjung Emas Port, Semarang City, showed mutations indicating resistance genes to 0.05% cypermethrin insecticide at 80% (homozygous resistance) and 20% (heterozygous resistance) (Sukaningtyas, R., Udijono, A., & Martini, 2021).

Mosquitoes of the Culicidae family (Anopheles, Aedes, Culex) possess the VGSC gene that plays a role in the mosquito nervous system and serves as an insecticide target site (Clarkson et al., 2021; Maftukhah et al., 2022; Panjinegara et al., 2024). The VGSC gene encodes nerve membrane proteins for signal transmission, playing an important role in mosquito movement and flight. Detection of the VGSC (voltage-gated sodium channel) gene provides an overview of the resistance status of Aedes aegypti mosquitoes to pyrethroid insecticides because the target site of these insecticides is VGSC (Chahine, 2018).

This study used Aedes aegypti mosquitoes to test resistance to fenvalerate insecticide because Aedes aegypti is the main vector of DHF. Aedes aegypti is more commonly found in urban environments and has higher adaptability than Aedes albopictus, making Aedes aegypti more likely to transmit DHF (Narang & Khanuja, 2020). Aedes aegypti has characteristic black thoracic scutum with two white spots forming a lyre-shaped pattern, two white patches on the mesepimeron, a white line on the anterior midfemur, and white scales on the clypeus. Meanwhile, Aedes albopictus has a narrow white stripe on the thoracic scutum, a V-shaped white patch on the mesepimeron, a white line on the anterior midfemur, and no white scales on the clypeus (Engohang-Ndong & Rodriguez-Morales, 2022). Based on the above background, it is necessary to conduct research related to the resistance test of Aedes aegypti mosquitoes to fenvalerate insecticide, as it has not been widely studied, followed by VGSC (voltage-gated sodium channel) gene detection as a molecular resistance indicator using the Real-Time PCR technique.

This study aims to determine the results of the VGSC (voltage-gated sodium channel) gene as an indication causing resistance in Aedes aegypti mosquitoes due to exposure to fenvalerate insecticide using the Real-Time PCR technique.

RESEARCH METHODS

Description of Materials or Research Subjects

The research used Aedes aegypti mosquito samples obtained from the Center for Public Health Laboratory (BBLKM) Surabaya. A total of 100 Aedes aegypti mosquitoes were used, divided into four test vials and one control vial, each containing 20 mosquitoes per test vial. The sample criteria were female Aedes aegypti mosquitoes aged 3–5 days, as this age range is considered optimal, with the mosquitoes still strong and productive. The variable studied was the VGSC (voltage-gated sodium channel) gene, identified as an indicator of Aedes aegypti resistance due to exposure to fenvalerate insecticide using the Real-Time PCR technique.

Research Design

This study was classified as descriptive quantitative research based on observation of Aedes aegypti mosquito samples to determine the presence of the VGSC (voltage-gated sodium channel) gene in resistant mosquitoes due to fenvalerate insecticide exposure. The research was conducted from November 2024 to April 2025.

Research Procedure

The collection of Aedes aegypti mosquitoes and insecticide resistance testing using the CDC Bottleassay method was carried out at the Center for Public Health Laboratory (BBLKM) Surabaya. The test exposed mosquitoes to fenvalerate for 30 minutes to observe mortality and survival rates. Subsequently, molecular detection of the VGSC (voltage-gated sodium channel) gene was performed at the Molecular Biology Laboratory, Department of Medical Laboratory Technology, Poltekkes Kemenkes Surabaya using the Real-Time PCR technique. The test results were presented in tables and graphs to display the percentage of live and dead mosquitoes.

Instruments and Equipment

The research utilized the CDC Bottleassay apparatus for insecticide resistance testing, fenvalerate insecticide as the active compound, and a spectrophotometer NanoDrop for DNA quantification. DNA extraction was conducted using the Promega Wizard Purification Kit, and the Real-Time PCR assay used fluorescence-based detection with SYBR Green dye for amplification analysis.

Data Collection Methods

Data were collected through observation and direct molecular examination of the presence or absence of the VGSC (voltage-gated sodium channel) gene in Aedes aegypti mosquitoes. The data on mortality and survival rates from resistance testing, as well as the detection results of the VGSC gene, were presented in tables and figures, including pie charts and graphs, to illustrate molecular resistance patterns.

Data Analysis

The collected data were analyzed using descriptive statistics and visually presented in tabular and graphical forms. All data were organized and processed using Microsoft Excel to show percentages of mortality, survival, and gene detection outcomes.

Research Ethics

This research has obtained ethical approval from the Health Research Ethics Committee (KEPK) of Poltekkes Kemenkes Surabaya with approval number No.EA/3105/KEPK-Poltekkes_Sby/V/2024. Ethical principles were maintained throughout the study to ensure the proper handling and humane treatment of the mosquito samples.

RESULT AND DISCUSSION

Table 1 shows the results of the resistance test of Aedes aegypti mosquitoes using the CDC Bottleassay method conducted at the Center for Public Health Laboratory (BBLKM) Surabaya. From all mosquito samples exposed to fenvalerate insecticide for 30 minutes, a total of 55 mosquitoes died with an average mortality rate of 68.75%, while 25 others showed resistance with an average survival rate of 31.25%.

Table 1.

Resistance test results of Aedes aegypti mosquitoes using the CDC Bottleassay method with fenvalerate insecticide

Replication	Live Mosquitoes		Mosquito Mortality	
	Frequency	Percentage	Frequency	Percentage
Control	20	100%	0	0%
1	7	35%	13	65%
2	5	25%	15	75%
3	8	40%	12	60%
4	5	25%	15	75%

Table 2.

DNA quantification results of Aedes aegypti mosquitoes using a NanoDrop spectrophotometer

No	Sample	DNA Purity (λ260/ λ280 nm)	DNA Concentration (ng/µL)
1	Control	1,96	26,16
2	R1	1,99	9,21
3	R2	1,92	8,64
4	R3	1,99	12,03
5	R4	1,90	28,35

Table 2 shows the results of DNA quantification of Aedes aegypti mosquitoes using a NanoDrop spectrophotometer to assess DNA purity and concentration levels. All samples produced DNA purity values within the optimal range of 1.8–2.0, as well as DNA concentrations exceeding the minimum threshold of >5.00 ng/µL.

Based on Table 3, the detection results of the VGSC gene in four Aedes aegypti mosquito samples showed that two samples (R1 and R4) were confirmed positive or detected to have the VGSC gene, while the other two samples (R2 and R3) showed negative results or no gene detection. Thus, out of four detected samples, two samples showed the presence of the VGSC gene, which acts as a marker of resistance to fenvalerate insecticide.

Table 3.

Detection results of VGSC gene as a fenvalerate resistance gene in Aedes aegypti mosquitoes

No	Sample Code	Sample	Cq (Cycle quantification) Value	Description
1	KP	Positive Control	5,71	Positive
2	KN	Negative Control	N/A	Not Detected / Negative
3	R1	1	1,67	Positive
4	R2	2	N/A	Not Detected / Negative
5	R3	3	N/A	Not Detected / Negative
6	R4	4	1,63	Positive

Resistance testing of Aedes aegypti mosquitoes to fenvalerate insecticide using the CDC Bottleassay method was conducted to determine the level of mosquito susceptibility. Based on interpretation criteria, mortality rates below 80% are categorized as resistant, mortality between 80–90% as suspected resistance (tolerant), and mortality ≥98% as susceptible[6]. The resistance test results showed that all four mosquito samples had mortality rates below 80%, thus categorized as resistant. This finding is relevant to Anggraini (2023), who reported that Aedes aegypti mosquitoes in Binjai City were resistant to permethrin insecticide (pyrethroid group) with a mortality rate of 25%. This result serves as the basis for further molecular analysis to confirm the relationship between phenotypic resistance and VGSC gene mutations, particularly knockdown resistance (kdr) mutations that play a role in resistance to pyrethroid insecticides such as fenvalerate (Anggraini et al., 2023).

Fenvalerate acts by inhibiting ion flow through sodium channels (VGSC) in the insect nervous system, resulting in continuous nerve stimulation leading to hyperexcitation and death (Wahyuni et al., 2021). Resistance to fenvalerate can be detected through two mechanisms: metabolic resistance caused by increased activity of detoxification enzymes such as esterase, GST, and monooxygenase; and target-site resistance caused by VGSC mutations that reduce nerve sensitivity to insecticides (Adrianto et al., 2022).

Detection of the VGSC gene in Aedes aegypti mosquitoes resistant to fenvalerate began with DNA extraction. Dead mosquitoes were separated from live ones. Resistant mosquitoes were then placed in a freezer at −25.3°C for 1–2 minutes until completely immobilized to facilitate sampling. They were then transferred into an Eppendorf tube, homogenized, and mixed with PBS to form a homogeneous suspension (Ditjen P2P, 2018). DNA was extracted using the Promega Wizard Purification Kit to obtain pure DNA, which was then used as a template in PCR reactions to detect the VGSC gene related to insecticide resistance (Puspitaningrum et al., 2018).

After extraction, DNA quantification was carried out using a NanoDrop spectrophotometer. Assessment was based on UV absorbance of DNA at λ260 nm and protein at λ280 nm, with an ideal λ260/λ280 ratio between

1.8–2.0. DNA is considered good quality if the concentration is >5.00 ng/µL and the purity ratio is within that range, making it suitable for molecular analysis such as PCR (Aisyah et al., 2019). All samples were declared suitable since they had DNA purity within the optimal range and concentrations >5.00 ng/µL. Differences in DNA concentration and quality may be influenced by extraction methods such as DNA kit, CTAB, or spin column. Decreased concentration may also be caused by DNA degradation or exposure to high temperatures. Contamination by protein, RNA, or phenol residues may also affect the accuracy of DNA measurement (Parwito et al., 2024). For molecular analysis such as PCR targeting the VGSC gene, high-quality DNA and careful sample handling are required (Salamun, 2024).

Detection of the VGSC gene using Real-Time PCR employed the fluorescent dye SYBR Green, which specifically binds to double-stranded DNA and allows detection of amplification product accumulation based on fluorescence intensity. SYBR Green absorbs blue light and emits green light when bound to DNA. Unlike probe-based methods, SYBR Green can detect all amplification products without requiring specific probes, making it a common and effective signal marker in Real-Time PCR(Lestari et al., 2023).

The VGSC gene detection results showed that sample 1 (R1) and sample 4 (R4) produced CT values of 1.67 and 1.63, respectively. These values indicate the presence of the VGSC gene, with possible resistance caused by increased monooxygenase enzyme activity (Apriyani et al., 2025). Samples 2 (R2) and 3 (R3), which showed N/A results, indicate that the VGSC gene was not detected, possibly due to knockdown resistance (kdr) mutations that alter the structure of the target protein, reducing fenvalerate effectiveness. This result aligns with research by Sintya (2022), which reported a combination of KDR V1016G and V410L mutations in Aedes aegypti resistant to permethrin. This demonstrates that VGSC gene mutations play an important role in the development of resistance to pyrethroid insecticides, including fenvalerate (Sintya et al., 2022).

Cycle Threshold (CT) refers to the total number of cycles until the fluorescence signal crosses the threshold. VGSC gene detection is considered positive if the CT value appears before 40 cycles. Low CT values (R1 and R4) indicate early and high detection of the target gene, whereas high or N/A values (R2 and R3) indicate that the gene is undetected or very low. The positive control (CT 5.71) shows successful amplification, while the negative control (N/A) confirms no unwanted amplification, indicating successful PCR reaction (Ningsih et al., 2020). Positive results are indicated by a sigmoid-shaped amplification curve crossing the fluorescence threshold, showing rapid and consistent DNA amplification. CT values appearing early indicate high gene concentrations, while samples without sigmoid curves or not crossing the threshold are considered negative, as no specific amplification occurred and fluorescence signals remained flat (Lestari et al., 2023). Each curve reflects the presence and concentration of the target DNA, while variation in CT values between samples reflects variation in the number of target genes. Interpretation of these curves is crucial for determining the molecular resistance status of Aedes aegypti mosquitoes (Ningsih et al., 2020).

The results showed that mosquito resistance mechanisms may vary among populations depending on genetic selection factors. VGSC gene detection using PCR in Indonesia remains limited, therefore, further research is needed to expand understanding of molecular resistance in Aedes aegypti. Routine monitoring of resistance genes is essential for early detection and informed vector control decision-making. This study has

limitations as it only detects the presence of resistance genes; thus, it should be continued with methods such as sequencing to identify specific mutations.

CONCLUSION

The detection of the VGSC gene in resistant Aedes aegypti mosquito samples was caused by increased monooxygenase enzyme activity, while the absence of the VGSC gene in other samples was due to knockdown resistance (kdr) mutations that alter the structure of target proteins. Thus, two samples showing the presence of the VGSC gene serve as molecular markers of resistance to fenvalerate insecticide.

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