



Original Article

Molecular detection of VGSC and Ace-1 mutations in *Aedes aegypti* (Linnaeus) from Dumai Seaport, Indonesia

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ABSTRACT

The geographic distribution of mosquitoes, including *Aedes aegypti* (Linnaeus), the primary vector of dengue, is strongly influenced by international and intercity transportation. This study aimed to assess insecticide resistance status and to identify mutations in the VGSC (Nav) and Ace-1 genes in *Ae. aegypti* populations collected in Dumai port, Indonesia. Adult female mosquitoes were tested for resistance to permethrin, deltamethrin, and malathion using WHO standard bioassay methods. Point mutations were detected through PCR amplification and sequencing. The results showed resistance to pyrethroids (permethrin and deltamethrin), while susceptibility to malathion was maintained. Three kdr mutations (F1534C, V1016G, and S989P) were identified; however, no Ace-1 mutation (G119S) was detected. These findings highlight the importance of continuous resistance monitoring, particularly in high-risk transportation hubs, to support effective vector control strategies.

Key words: *Aedes aegypti*, insecticide resistance, mutation, pyrethroids, VGSC

INTRODUCTION

Aedes aegypti (Linnaeus) mosquitoes are the major vectors of a number of arboviruses of global importance, such as dengue, chikungunya, Zika, and yellow fever (Leta et al. 2018; Dusfour et al. 2019). Global travel, urbanization and climate change that promote the habitat expansion of *Ae. aegypti* mosquitoes are also aggravating the dissemination of such diseases (Yadouleton et al. 2022). Due to intense human mobility and goods transfer, seaports are considered to be important points of introduction for both *Ae. aegypti* mosquitoes and the viruses they carry (Barzon et al. 2021).

Dumai International Seaport in Indonesia serves as a major hub for global trade, particularly in palm oil exports, with substantial passenger and vessel traffic that increases the risk of introduction and dissemination of arboviruses and their vectors. Importantly, the port is located in Dumai Timur Subdistrict, an area consistently reporting the highest incidence of dengue hemorrhagic fever (DHF) in Dumai City. In 2022, Dumai recorded 168 DHF cases across ten primary healthcare

service areas (DKK 2023). More broadly, Riau Province has shown an increasing trend in dengue morbidity in recent years, placing Dumai within a wider high-risk regional context (Riau DKP 2022). This epidemiological profile highlights Dumai as a high transmission setting where vector control interventions are intensively implemented.

The port is divided into two separate parts: the operational zone and an industrial zone. In the industrial zone, pest control firms regularly spray insecticides in and around warehouses and processing plants, and not infrequently they are seemingly inadequately documented. The operational area (which includes passenger terminals and offices) is under the care of the Port Health Office, which adheres to national policy for insecticide treatment. These differences in insecticide use practices could impose different selection pressures on local mosquito populations. This turns Dumai into a prime seedbed for the introduction and spread of arboviral diseases.

Vector control is still the primary method for preventing arboviral diseases in endemic areas, and the

pyrethroid insecticides, deltamethrin and permethrin, are the most frequently used (WHO 2022). But the extensive application of pyrethroids results in the selection of insecticide resistance in populations of *Ae. aegypti* across the world including Indonesia, where resistance to pyrethroids such as deltamethrin and permethrin have been reported (Juache et al. 2022). This resistance reduces the effectiveness of vector control programs, particularly in high risk areas such as seaports. In Dumai, possible selection pressures for a high differentiating pattern of resistance might come from both operational and industrial areas due to potential different types or frequencies of insecticide application in both environments.

The genetic mechanisms of insecticide resistance in *Ae. aegypti* include mutations in key genes such as the voltage-gated sodium channel (VGSC) gene, responsible for knockdown resistance (kdr), and the acetylcholinesterase-1 (Ace-1) gene, associated with resistance to organophosphates (Mutero et al. 1994; Martinez-Torres et al. 1998; Sternberg & Thomas 2018). Despite global awareness of insecticide resistance, detailed genetic studies of resistance mechanisms in seaport environments are limited, particularly in Indonesia. Research on resistance mechanisms in Dumai, specifically considering the impact of different insecticide usage in the port's two zones, is scarce. Understanding these genetic factors is crucial for informing effective, localized vector management strategies.

This study aims to assess the insecticide resistance status of *Ae. aegypti* populations at Dumai International Seaport to deltamethrin, permethrin, and malathion. Additionally, we investigate the presence of resistance associated mutations in the VGSC and Ace-1 genes. By considering both the operational and industrial zones in Dumai, this research will offer a comprehensive analysis of how varying insecticide usage in these areas impacts mosquito resistance. Insights from this research are expected to inform evidence-based, localized vector control strategies tailored for high risk international seaport settings.

MATERIALS AND METHODS

Study sites

The study was carried out from March to September 2024 in the Dumai Seaport, located in Riau Province, Indonesia (01°26'50"N – 02°15'40"N; 101°0'38"E – 101°43'33"E). The northern coastal area of Dumai City is characterized by lowland plains, while the southern region consists of higher elevation areas adjacent to Bathin Solapan District.

Collection and rearing of mosquitoes

Aedes spp. eggs were collected using ovitraps deployed in various buildings across the seaport over a five-day period in 2024. A total of 534 ovitraps were placed both indoors and outdoors, particularly near potential breeding sites such as vegetated areas. The ovitraps were lined with filter paper following the protocol of the Ministry of Health (2018). The ovitrap index (OI) was 26.59%. Collected eggs were reared under laboratory conditions, and species identification was conducted at the adult stage using morphological keys to ensure that only *Ae. aegypti* were included in subsequent analyses.

Insecticide bioassays

The insecticide impregnated papers were supplied by the WHO Collaborating Centre at the Vector Control Research Unit, Universiti Sains Malaysia. These papers were treated with diagnostic concentrations recommended for *Ae. aegypti*: 0.75% permethrin (a type I pyrethroid), 0.05% deltamethrin (a type II pyrethroid), and 0.5% malathion (an organophosphate). Control groups were exposed to papers treated with silicone oil only (control group), without any active insecticide.

Insecticide susceptibility was assessed following standard WHO bioassay protocols. For adult assays, batches of 25 non-blood-fed female *Ae. aegypti* (F2 generation, 3–5 days old) were tested per tube using WHO susceptibility test kits, with four exposure tubes and two control tubes per assay. Mosquitoes were exposed to insecticide-treated papers for 60 minutes, and knockdown was recorded at 5-minute intervals during the first 30 minutes and at 10-minute intervals thereafter. After exposure, mosquitoes were transferred to holding tubes and maintained under ambient conditions with a 10% sucrose solution for 24 hours. Mortality was assessed 24 hours post-exposure following the protocol of the Ministry of Health (2018), and surviving individuals were subsequently processed for molecular analysis.

Table 1. Primer sequences used for PCR amplification of mutations in VGSC and Ace-1 genes. Primer designs were adopted from Hidajat et al. (2020) and Satoto (2019)

Gen	Primer	Sequence
VGSC Domain II Segmen 6	AaSCF1	AGACAATGTGGATCGCTTCC
	AaSCR4	GGACGCAATCTGGCTTGTTA
VGSC Domain III Segmen 6	AaSCF7	GAGAATCGCCGATGAACCTT
	AaSCR7	GACGACGAAATCGAACAGGT
Ace 1	AceF	CGATAACGAATGGGGAACG
	AceR	TCAGAGGCTCACCGAACACA

Molecular analysis

Genomic DNA was extracted from individual mosquitoes using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA extracted was used as a template for PCR amplification of the genes VGSC and Ace-1 associated with insecticide resistance. The PCR assay for each target gene was conducted separately in 0.2-ml PCR tubes using GoTaq® Green Master Mix (Promega), nuclease-free water, gene-specific forward and reverse primers. The primers were selected from Satoto (2019) and Hidajat et al. (2020) as shown in Table 1.

The IIS6 and IIS6 regions of the VGSC gene were PCR amplified. We amplified the target fragment harboring the mutation site of interest for the Ace-1 gene using the corresponding gene-specific primers. PCR amplification was performed with an initial denaturation at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 15 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s and a final extension at 72 °C for 10 min.

The PCR products were analyzed by agarose gel analysis on agarose prepared in 1× TBE or TAE buffer with loading dye and DNA ladder. The amplified products were visualized on gel documentation system. Further sequencing was performed on products with the expected amplicon size.

Sequencing of the PCR products was carried out by PT Genetika Science (Jakarta, Indonesia) using the BigDye® Terminator v3.1 cycle sequencing kit. Sequencing was performed on an ABI PRISM® 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Forward and reverse reactions were conducted with the corresponding PCR primers.

Data analysis

Mosquito populations were classified as resistant or susceptible according to the WHO criteria: a mortality rate of 98–100% is considered susceptible, 90–97% suggests possible resistance, and < 90% indicates confirmed resistance. The knock down times for 50% (KdT_{50}) and 95% (KdT_{95}) of the tested mosquitoes were calculated using the log probit model.

All sequence analyses and editing were conducted using the BioEdit Sequence Alignment Editor v7.2.3. Field collected sequences were aligned with reference sequences associated with VGSC and Ace-1 gene mutations. Specifically, sequencing results were aligned with reference sequences AB914689 and AB914690 for the IIS6 region, AB914687 and AB914688 for the IIS6 region, and AJ621915 and KJ504172 for the Ace-1 region, with all reference sequences obtained from GenBank.

RESULTS

Insecticide resistance status of *Ae. aegypti* populations

A total of 534 ovitraps were deployed throughout the study area, of which 142 were positive for *Aedes* eggs, resulting in an overall ovitrap index (OI) of 26.59%. Positive ovitraps were recorded in both the operational and industrial zones. The OI was higher in the industrial zone (34.91%; 74/212) than in the operational zone (21.12%; 68/322) (Figure 1).

The insecticide susceptibility status of adult *Ae. aegypti* populations from Dumai International Seaport is presented in Table 2. According to the WHO susceptibility criteria, mortality rates of 98–100% indicate susceptibility, 90–97% indicate possible resistance requiring confirmation, and mortality below 90% indicates resistance. Both the operational and industrial populations were resistant to deltamethrin and permethrin, with mortality rates ranging from 15% to 39%. In contrast, mortality following exposure to malathion was 100% in the operational population and 98% in the industrial population, indicating susceptibility in both populations. The corresponding KdT_{50} and KdT_{95} values are also presented in Table 2.

Resistance associated mutations in the VGSC and Ace-1 genes

The alignment results revealed point mutations at two target sites in *Ae. aegypti* samples from both the seaport and industrial populations. A total of 8 samples, consisting of 4 *Ae. aegypti* from each population, were analyzed for mutations in the VGSC IIS6 gene. At position 989, all samples exhibited a substitution of serine (TCC) with proline (CCC). Additionally, at position

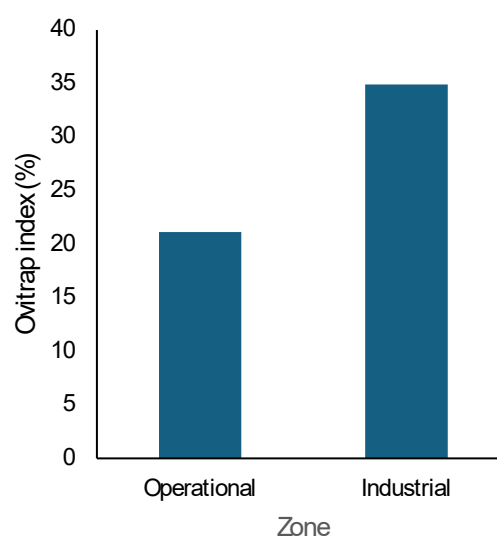


Figure 1. Ovitrap index (%) in the operational and industrial populations.

1016, a substitution of valine (GTA) with glycine (GGA) was observed in all samples (Figure 2). Amplification and sequencing were successfully performed on all specimens.

Sequencing analysis of 8 *Ae. aegypti* samples (4 from the seaport population and 4 from the industrial population) revealed a point mutation at position 1534 of the VGSC IIS6 gene in 3 samples from each population. This mutation resulted in an amino acid substitution from phenylalanine (TTC) to cysteine (TGC) (Figure 3).

A 384 bp fragment of the Ace-1 gene was successfully amplified from four *Ae. aegypti* samples from each of the seaport and industrial populations. Sequence alignment showed no mutations at the target

site 119. All samples displayed a homozygous wild type genotype (GGC) indicating no genetic variation at this site in the analyzed fragment (Figure 4).

DISCUSSION

This study presents the first investigation into the insecticide resistance status of *Ae. aegypti* populations from Dumai Port, a key international seaport and national point of entry (PoE) in Indonesia. As a significant hub for trade and travel, the port area presents a unique setting for assessing vector control challenges, particularly in relation to the development and spread of insecticide resistance.

The results of the bioassays showed that populations of *Ae. aegypti* from industrial and operational areas

Table 2. Mortality rates of adult *Aedes aegypti* 24 h after exposure to insecticides

Population	Insecticide	N	KdT ₅₀ (min)	KdT ₉₅ (min)	Mortality	Status
Operational	Deltamethrin	100	79.91	119.18	39	R
	Permethrin	100	82.78	120.86	23	R
	Malathion	100	23.81	59.6	100	S
Industrial	Permethrin	100	83.53	115.08	15	R
	Deltamethrin	100	82.09	112.76	25	R
	Malathion	100	38.94	63.5	98	S

Control mortality was 0% in all bioassays, therefore no correction using Abbott's formula was required.

R: resistant; S: susceptible; KdT₅₀: Knockdown time for 50% of the population; KdT₉₅: Knockdown time for 95% of the population.

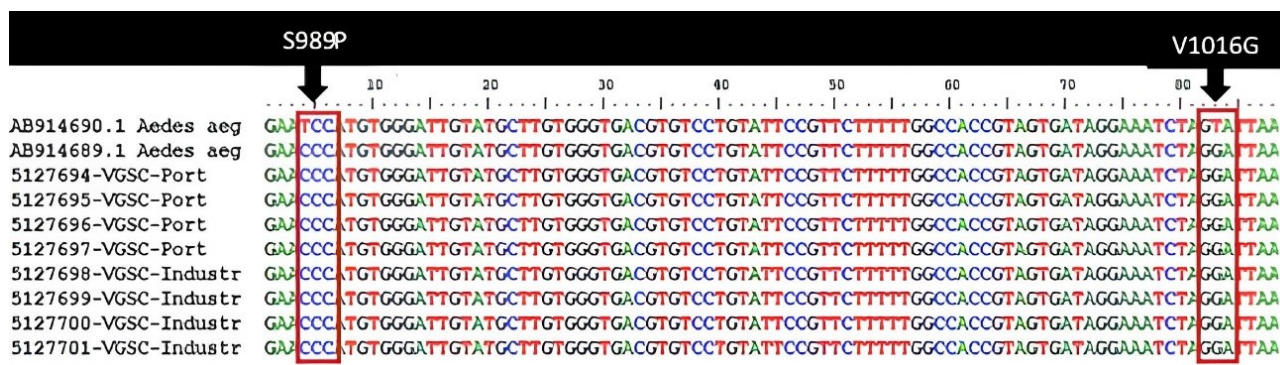


Figure 2. Alignment of VGSC IIS6 gene sequences in *Aedes aegypti* from seaport and industrial populations.

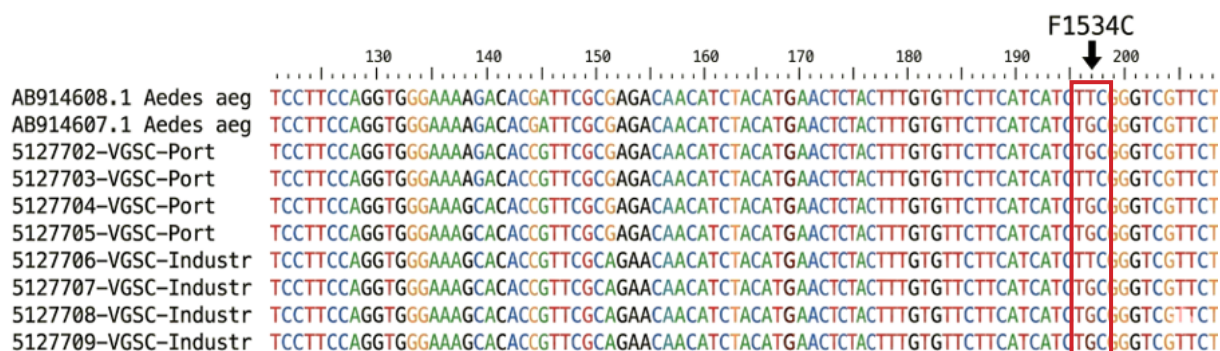


Figure 3. Alignment of VGSC IIS6 gene sequences in *Aedes aegypti* from seaport and industrial populations.

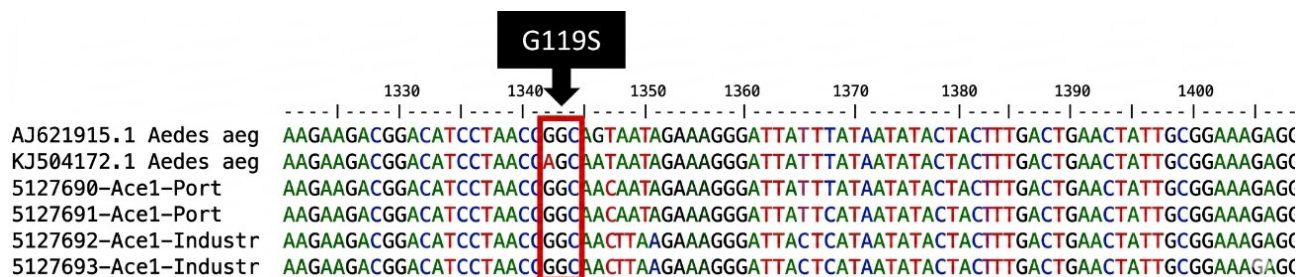


Figure 4. Alignment of Ace-1 gene sequences in *Aedes aegypti* from seaport and industrial populations.

remain susceptible to malathion, an organophosphate insecticide. The observed susceptibility could be due to the long period of discontinuation of malathion use in Dumai Port where insecticide has not been used continuously for more than a decade. Reduced exposure to malathion may lead to reduced selection pressure and maintenance of susceptibility of mosquito populations. Studies have shown a significant association between the intensity and duration of insecticide use and resistance (Leandro et al. 2020; Machani et al. 2020). Thus, the continued efficacy of the insecticide on *Ae. aegypti* populations in the study site may be associated with the non-routine use of malathion. Thus, the absence of routine use of malathion may explain the continued effectiveness of this insecticide against *Ae. aegypti* populations in the study site.

Conversely, *Ae. aegypti* populations from both study sites were resistant to permethrin (type I) and deltamethrin (type II). The finding suggests that resistance is not confined to a single subclass of pyrethroids, but is spread across the pyrethroid group. This pattern is of particular concern because resistance to both of the major classes of pyrethroids could compromise the effectiveness of pyrethroid-based vector-control interventions. Prolonged exposure to pyrethroid insecticides may be related to the resistance to concurrent application observed in this study. Vector control operations at Dumai International Seaport have been relying solely on pyrethroid insecticides like permethrin and deltamethrin since malathion was stopped in 2014, which has resulted in continued selection pressure on local populations of *Ae. aegypti*. Pyrethroids are also widely used by private pest control operators to control pests in warehouses, offices and other port related facilities. The repeated application of insecticides with similar mode of action on more than one pest control in concerted environment may impose a high selection pressure which may lead to development and persistence of pyrethroid resistance in local populations of *Ae. aegypti* (Dusfour et al. 2019; Moyes et al. 2021).

The widespread use of pyrethroids in public health and domestic applications can compromise the success

of rotation strategies when rotation is confined to chemicals from the same class. This is especially true for high risk entry points such as Dumai International Seaport where insecticide exposure is likely to be repeated. Resistance to the two major classes of pyrethroids could compromise common interventions like space-spraying and household insecticides (WHO 2022). These results highlight the need for regular resistance monitoring and the use of insecticides with different modes of action, in addition to integrated vector management strategies, to ensure effective vector control (Devillers et al. 2023).

Mutations in the voltage gated sodium channel (VGSC) gene provide confirmation of a genetic basis for pyrethroid resistance. Three major mutations, S989P, V1016G, and F1534C, were detected, all of which are well known to act synergistically to produce pyrethroid resistance pyrethroids (Hirata et al. 2014; Moyes et al. 2021; Uemura et al. 2024). These mutations are associated with knockdown resistance (kdr) and their detection is of concern because they may lead to a reduced effectiveness of pyrethroid-based control strategies.

From an ecological perspective, the appearance of resistance shows *Ae. aegypti* adaptive response to environmental stressors, especially to pesticide pressure. The genetic structure of resistant populations is strongly shaped by the interaction between gene flow, demographic parameters and the severity of insecticide administration (Devillers et al. 2023). Both genetic diversity and population structure need to be included in integrated vector control techniques to address this increasing challenge. Moreover, the application of insecticide rotation schemes and the formulation of a nationally coordinated vector control policy could considerably improve the long term success of mosquito control programs in Indonesia.

One particularly disturbing aspect of this study is that these changes are detected in *Ae. aegypti* populations from the international shipping terminal at Dumai Port. This raises major concerns for the potential development and spread of resistance alleles within the framework of international trade and travel.

But resistance could spread faster through human-mediated gene movement, particularly in sensitive areas such as international shipping ports. Ongoing monitoring and genetic characterisation of resistance alleles in mosquito populations is critical to prevent the global spread of pesticide resistance (Ur et al., 2021). Hence, molecular surveillance at the main entrances such as Dumai Port should be improved to reduce the potential public health threats of resistant of *Ae. aegypti* populations.

There are a number of limitations to this study that should be noted. Although mosquito eggs were collected from a large number of ovitraps distributed throughout the study area, only a limited number of samples were taken for molecular analysis. Given the limited flight range of *Ae. aegypti*, this may not be representative of the genetic diversity of mosquito populations within the study sites.

However, samples were collected from both operational and industrial zones to represent variation in environmental conditions and insecticide exposure. Future studies are needed to characterize the distribution and frequency of resistance associated mutations with larger sample size and broader spatial coverage. Nevertheless, this study provides important baseline data on insecticide resistance in a high-risk international seaport setting.

CONCLUSION

The *Ae. aegypti* population from Dumai Port exhibited resistance to pyrethroids (permethrin and deltamethrin) but remained fully susceptible to malathion. The detection of VGSC mutations, particularly F1534C, S989P, and V1016G, likely contributes to the observed pyrethroid resistance. Conversely, the absence of the Ace-1 G119S mutation aligns with the continued susceptibility to malathion. These findings underscore the importance of routine resistance surveillance and support the strategic use of malathion in vector control programs, particularly at international entry points where preventing the dissemination of resistant vectors is critical to public health.

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